Ebola Virus Haemorrhagic Fever

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Proceedings of an International Colloquium on Ebola Virus Infection and Other Haemorrhagic Fevers held in Antwerp, Belgium, 6-8 December, 1977

INTRODUCTION

The present volume contains the full proceedings (papers and discussions) of the International Colloquium on Ebola Virus Infection and other Haemorrhagic Fevers, organized in Antwerp, Belgium, 6-8 December 1977.

This three-day meeting was co-sponsored by the World Health Organization and the Prince Leopold Institute of Tropical Medicine and received additional support from the Belgian Ministry of Public Health, the Ministry of Education, and the National Foundation of Scientific Research.

The purpose of the colloquium was to present and discuss all the information available on Ebola Virus. Its Proceedings may well remain for some time a standard reference work on the subject, while illustrating at the same time the large gaps in our knowledge concerning the virus and the disease it causes.

An effort was also made to include information on other haemorrhagic diseases, with due attention to their public health aspects.

The help of Dr. P. Brès from the World Health Organization in elaborating the programme is much appreciated. My special thanks go to Mr. G. Roelants, Librarian of the Prince Leopold Institute, for his assistance in preparing the typescript.

S.R. PATTYN

ACKNOWLEDGEMENT

PREFACE

When Marburg disease appeared for the first time in 1967, the scientific world was struck by the suddenness and brutality of the viral assault. The fear was great that such a new type of infectious disease could disseminate and quickly become a new and frightful world health problem.

Very fortunately and maybe for non well explained reasons, the incident remained located and sporadic.

When the events of South Sudan and North Zaire were again reported in the summer of 1976, the fear became even greater because the very spectacular and sensational description of the situation in Yambuku was difficult to explain and the risk for rapid spread was not to be overlooked.

Many industrialized countries which have regular relations with the countries involved in the epidemic had to take some position about the possible importation of cases.

Fortunately enough, the skill and energetic intervention of a certain number of experts were able, not only to confine the cases to the region where they occurred, but also to learn quite a lot about the causes of the epidemic development.

I would like to pay a special tribute to the Centers of the U.S.A., the U.K., France, South Africa and Belgium, who worked very cleverly on the identification and behaviour of the new "Ebola" virus.

As early as one year after the events, this International Colloquium was organized in Antwerp. The participation to this meeting of the most knowledgeable and skilled experts from the whole world has made it possible not only to clarify the various events and their succession and evolution in the field but also to make definite on the virus involved and provide scientists and public health authorities with a clear picture of the characteristics of the etiologic organism and its epidemic genius and also lift a side of the veil on the other mysterious and dangerous haemorrhagic diseases such as Lassa, Congo, Marburg or Ebola fevers.

I recognize the high scientific value of the contribution of the various speakers and am especially thankful to Prof. Dr. S.R. Pattyn and the Management of the Prince Leopold Institute of Tropical Medicine in Antwerp for their scientific work and also for the very efficient organization of this Colloquium.

The World Health Organization should also be mentioned for its continuous concern and support for these problems.

The Department of Public Health of Belgium has been glad to be able to support initiative and wishes to express gratitude to all participants who made it possible to know more about these mysterious and dangerous new diseases.

Prof. Dr. S. HALTER,
Secretary General of the
Ministry of Public Health
and Family Affairs,
Brussels.

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INTRODUCTORY REMARKS

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During the past 20 years or more, there have been a number of severe outbreaks of viral disease apparently "new" to medicine. Good examples are the initial outbreaks of Kyasanur Forest disease, O'nyong nyong fever, Bolivian haemorrhagic fever, Marburg and Ebola fevers. To some extent their recognition can be accounted for by improved communications, both general and medical (through WHO and other international channels), but in almost every case where an explanation has been advanced, these outbreaks have been attributed to intrusion into or interference with previously little frequented areas, because of population pressure and/or agricultural developments. With population throughout most of the world continuing to increase markedly despite all efforts at control, and with an estimated 460 million people in the world with insufficient to eat for good health (F.A.O., 1975), these pressures are unlikely to diminish during the remainder of this century at least - and it seems reasonable to expect further outbreaks of severe viral diseases (some of them "new").

Were these diseases really new ? It seems unlikely. If not, why were they recognized when they were ? The 1956 outbreak of Kyasanur Forest Disease was recognized mainly because of attention drawn to it by a high monkey mortality, but investigated and identified primarily because of the proximity of the Virus Research Institute, Poona. Similarly, O'nyong nyong fever, except perhaps for the scale of the epidemic, might well have been dismissed as yet another outbreak of dengue-like disease without serious consequences, had not the Virus Research Institute, Entebbe, been ready and able to investigate it. The original Bolivian haemorrhagic fever outbreak was brought to notice (and to investigation by the Middle America Research Unit) because of its severity. Marburg fever received the fullest investigation from the start because it first occurred in highly developed countries, and affected laboratory workers. The Ebola outbreaks were brought to notice because of their great severity, because they were spreading rapidly among hospital staff, because the whole health services of the epidemic areas appeared to be in jeopardy, and because there appeared to be risk (widely reported in the local and world Press) of much wider spread.

We can conclude that unless "new diseases" which occur in relatively remote areas cause large or severe epidemics, or affect hospital staffs, or occur in the "parish" of a virus research institute, they are unlikely to be investigated or their cause discovered. There are probably a number of candidate viruses for "new" diseases in the International Catalogue of Arboviruses.

The emergence of a previously latent zoonosis is probably usually due to a change in the ecology of its maintenance cycle or to changes in the ecology of neighbouring areas. New contacts with man, or changes in virulence, may be induced by increases in the population of maintenance hosts or related species and/or by establishment of the infection in a new maintenance host. The larger the scale of man-made environmental changes and the more they involve areas little frequented by man, the greater must be the probability of emergence of a zoonosis ("old" or "new"). Intrusion of agriculture, particularly of food crops attractive to rodents, into previously underdeveloped areas obviously increases the hazard of rodent maintained infections; extensive food storage inadequately rodent-proofed has a similar effect; irrigation or other water developments (including those that reduce the salinity of surface waters, e.g. the emergence of West Nile fever in the Camargue due to a vast increase in Culex modestus following rice growing) increases the hazard of mosquito-borne infections; and the introduction of large domestic mammals (especially cattle) into new territory may enhance the risk of tick-borne infections.

Clearly the first priority for early recognition of potentially dangerous outbreaks must be to educate the health and administrative authorities, particularly in the tropics, of the need for some form of surveillance and reporting of outbreaks of acute febrile disease (particularly in hospital personnel) in all new agricultural ventures involving intrusion into underdeveloped territory. This need not be elaborate and need not involve expensively trained staff-policemen, foremen, teachers, or villagers can be given simple but clear instructions. A small but well-trained team with limited investigational facilities should keep reporting under review, assess and advise on communicable disease problems as they become apparent and, when an incident requires investigation, it should be able to go to it with minimum delay carrying all necessary equipment. The team should have a first call on any available microbiological laboratory resources. It should be equipped, well trained and disciplined to collect specimens safely from cases or corpses of dangerous infectious diseases, trained to make an

epidemiological assessment, able to institute emergency control measures with such local support as is available and to advise on seeking appropriate assistance from within or outside the country when necessary. It must be capable of sending specimens properly refrigerated, packed according to international regulations and with adequate prior arrangements, to a reference laboratory. The receiving laboratory must be furnished with the fullest details of the outbreak, and of the patient(s) from which the material was collected. The fullest co-operation of airline staff, customs officials, etc., has to be arranged in advance, by telephone or telex if necessary. It is therefore wise if accurate information about the outbreak is issued officially at the earliest possible moment to minimize the otherwise inevitably inaccurate press reporting which may greatly increase the difficulties of sending and handling specimens and cause unnecessary public alarm.

Outside expert assistance may be needed in the control and investigation of such outbreaks. The nature of the help required will vary with circumstances but if the infection is a highly dangerous one, only a well-equipped and welltrained team should be sent. The team must not only be expert and well trained but needs an able, experienced and tactful leader; and it must be selfsufficient in terms of immediate medical care for its members, equipment (including protective clothing, containers, field sterilizers, etc.), materials, and camping equipment, electricity generators, fuel, etc., if necessary; and it must carry adequate supplies to enable it to effectively equip the local hospital and health authorities to control the outbreak. This implies formidable logistic problems, the most important of which are communications, transport and the dissemination of information.

The Ebola epidemics exposed many of these problems and we have learned a great deal from them and will learn even more at this meeting. I hope that when the next serious epidemic ("new" or "old") occurs, we will be able to show that we have profited from these lessons.

SECTION I : EBOLA VIRUS INFECTION

1. Clinical Aspects

CLINICAL ASPECTS OF EBOLA VIRUS INFECTION IN YAMBUKU AREA, ZAIRE, 1976.

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SUMMARY

Observations of six active cases and of a retrospective survey on 265 probable or serologically confirmed cases of Ebola virus infection in Zaire,1976, area are presented. Predominant symptoms of the disease included profound prostration, fever, headache, myalgia, arthralgia, abdominal pain and sore throat.

The most frequent signs were diarrhea, vomiting, oropharyngeal lesions, cough and conjunctivitis. Bleeding occurred in 70 percent of all cases, mainly from the gastrointestinal tract. Proteinuria was uniformly present. Skin rash was seldom reported among black skinned patients. Central nervous involvement was evident in some cases. Abortion occurred among 23 percent of 82 pregnant women who had the disease. Ten cases of possible neonatal Ebola virus infection occurred, but were not definitely elucidated.

The first accurate clinical description of Ebola virus infection (EVI) is found in the report of Dr. Ngoy Mushola from Bumba, who stayed at Yambuku hospital from September 1976, 15th to 19th : "the illness is characterized with a high temperature of about 39°C, hematemesis, diarrhea with blood, retrosternal abdominal pain, prostration with "heavy" articulations, and rapid evolution death after a mean of 3 days".

Subsequent observations of acute cases and of a retrospective survey on more than 250 probable and serologically confirmed cases are presented in this paper.

METHODS

Six patients were questioned and examined briefly during the acute stage in villages by survey team physicians during preliminary investigations at the end of October 1976. Information on other cases was obtained in villages of the affected region by six physician-led teams working with local nurse interpreters. Data were obtained as part of a retrospective epidemiological study in NovemberDecember 1977 using standardized forms. Family members provided information on fatal cases. Case and infection criteria were as described under surveillance and containment(1).

One International Medical Commission (ICM) member documented independently 136 fatal cases between November 1st and 9th, 1976. Concordance of findings in these two surveys was excellent.

RESULTS

Interviews were completed on 231 probable cases, and 34 individuals who were found to have Ebola virus antibodies by immunofluorescence. The frequency of symptoms and signs in these groups is shown in tables 1 and 2 respectively.

TABLE 1

PERCENTAGE OF SYMPTOMS PRESENTING IN PERSONS(1) WITH EBOLA INFECTION AND CONTROLS IN ZAIRE, 1976,

Symptom	Death Probable cases		Positive Titer	
	N	%	n	%
Fever	231	98.1	34	58.8
Headache	210	96.2	34	58.8
Abdominal Pain	201	81.1	34	50.0
Sore Throat	207	79.2	34	32.4
Myalgia	206	79.1	34	47.1
Nausea	178	66.3	30	33.3
Arthritis	193	53.4	34	38.2
Other (2)	42	50.0	23	26.1

(1) Age I or >.
(2) Anorexia, chest pain-pleuritis, chills, tinnitus, vertigo.

n = Total respondents.
% = Positive yes/respondents.

TABLE 2

PERCENTAGE OF SIGNS PRESENTING IN PERSONS(1) WITH EBOLA VIRUS INFECTION IN ZAIRE, 1976.

Symptom	Death (Probable Cases)		Positive Titer	
	N	%	N	%
Bleeding	223	77.6	34	17.6
Diarrhea	228	78.5	34	44.1
Oral-Throat Lesions	208	73.6	34	26.5
Vomiting	225	64.9	34	35.3
Conjunctivitis	208	58.2	34	35.3
Cough	208	35.6	34	17.6
Abortion	73	24.7	9	11.1
Edema	193	4.1	34	0
Jaundice	191	5.2	34	0
Other (2)	141	3.5	33	3.0

(1) > 1 year only.
(2) Amennorhea, dark urine, paralysis, polyuria, rash, dysarthria, hiccoughs, hyperhidrosis, lymphadenitis, paralysis, polyuria, rash.

The onset of illness was often sudden with progressively more severe frontal headache soon spreading occipitally. Fever was almost invariably present from the beginning as was weakness. Myalgia appeared very early, often from the first day of illness. It was reported as cervical and low back pain radiating into the legs. Arthralgia of the large joints was also very common from the beginning. Severe generalized disease became very soon apparent. Patients presented a typical lethargic, expressionless face with deep set eyes. There was often a complete loss of appetite and a rapid weight loss subsisting for a long time in those who recovered from the disease.

After 2 to 3 days of increasing severity of illness, gastrointestinal symptoms developed in most patients. Abdominal pain, including cramping, usually preceeded diarrhea (three or more liquid stools for one or more days) and/or vomiting and persisted nearly always until death. The stools were initially watery and clear, but turned black or contained red blood in 66.2% of all cases with bleeding history.

Vomiting was somewhat less common than diarrhea, usually starting after the diarrhea had begun. Hematemesis with red blood or "vomito negro" were reported in 42.8% of those with bleeding history.

Sore throat occurred in most patients and was often reported in association with a sensation of a "ball" in the throat. Severe dysphagia was reported in some cases, and is probably due to pharyngeal or swollen tissues in the throat. Oral-throat lessions were a typical feature of the disease, especially in fatal cases. They were present as fissures and open sores, especially on the lips, and appeared after 3 to 4 days of illness. Typical herpetic oral lesions were observed in a few patients by one physician. A grayish patchy exudate was noted on the soft palate and oropharynx in one instance. About a quarter of the fatal cases had oropharyngeal hemorrhage, mainly gingival bleeding. The cough was dry and appeared to be associated with oral-throat lesions rather than with lower respiratory tract pathology. Chest pain was rarely reported by both relatives of fatal cases and convalescents. Conjunctivitis was present in more than half of the patients, was non-purulent and sometimes complicated with subconjunctival bleeding.

Bleeding manifestations are listed in table 3. The gastrointestinal tract was the most common site of bleeding, and hemorrhage occurred more often among fatal than non-fatal infections. Bleeding started on days 3-5, and varied from melena and slow oozing from gums to brisk hemorrhage from multiple sites in fulminant cases. Hematuria was not reported.

TABLE 3

BLEEDING MANIFESTATION OF PERSONS (1) WITH EBOLA VIRUS INFECTION, ZAIRE, 1976.

Manifestation	Death (Probable Cases)		Positive IFA	
	n	%	n	%
Melena	210	66.2	33	15.2
Hematemesis	222	42.8	33	6.1
Mouth-Gingival	215	25.6	33	0
Vaginal	108	20.4	24	4.2
Epistaxis	216	16.7	33	0
Injection Sites Scarification	197	6.6	33	3.0

(1) : > 1 year only.

Skin rash or desquamation were rarely mentioned by relatives of fatal cases or by survivors in this black skinned population, and were seldom observed by IMC-physicians in the field. Other less common or rare symptoms include edema, jaundice, tunnitus, vertigo, amenorrhea, dark urine, oliguria, polyuria, dysarthria, hiccoughs, hyperhidrosis and lymphadenitis. In some cases central nervous system involvement was observed, including terminal hemiplegia and psychobehavior.

The only clinical laboratory test done on patients admitted to Yambuku Hospital was urinary protein. This was reported as uniformly positive and was used as a major diagnostic criterion by the nursing sisters early in the epidemic.

The duration of symptoms and signs among persons with hemorrhagic fever is given in table 4. Data for the "convalescent" group were by far the most reliable. Illness among fatal cases ranged from 1 to 15 days with a strong unimodal peak of 6 to 8 days.

Fifty nine percent of persons with antibodies had one or more symptoms, the most prominent being fever, headache, abdominal pain, myalgia, diarrhea and athralgia Many more persons who had been in contact with fatal cases reported symptoms but had no Ebola virus antibodies. Bleeding and oral-throat lesions particularly were more common in fatal cases than among survivors. The convalescent period took 1 to 3 weeks in most cases, but in some survivors recovery Was very slow and could last up to 5 weeks. Convalescent patients were marked by profound prostration and weight loss. Non-specific symptoms such as headache and weakness only slowly disappeared. In at least two survivors psychotic behaviour was observed up to two months after recovery from the disease. They both showed character changes with confusion, anxiety, restlessness and aggressive behaviour.

TABLE 4

DURATION OF SYMPTOMS AND SIGNS OF PERSONS (1) WITH EBOLA VIRUS INFECTION

Symptom & Sign	Deaths Probable Case Mean Duration + SE		Convalescents Mean Duration + SE	
Fever	7.2	(.28)	6.8	(1.48)
Headache	7.3	(.29)	5.5	(1.04)
Sore Throat	6.5	(.31)	10.7	(2.57)
Abdominal Pain	5.9	(.30)	7.9	(2.11)
Myalgia	7.1	(.30)	5.7	(1.01)
Arthralgia	6.5	(.32)	9.9	(2.08)
Bleeding	3.5	(.20)	9.3	(2.95)
Diarrhea	4.9	(.24)	7.5	(1.75)
Oral Throat Lesions	5.5	(.32)	5.3	(.64)
Vomiting	4.0	(.25)	3.9	(1.17)
Conjunctivitis	5.1	(.40)	6.3	(1.51)
Cough	6.9	(.57)	10.0	(3.98)

(1) > 1 year of age.

Table 5 summarizes the findings in six active cases observed in villages around Yambuku during a preliminary survey in October 1976 by ICM physicians. Four of these were virologically or serologically confirmed cases. All the patients were in their seventh to nineth day of illness. They all exhibited a typical appearance of the disease with expressionless face and profound prostration. A relative bradycardia was found in patient n°1. Patient n°2 complained of severe epigastric pain radiating to the back suggesting pancreatic involvement, vomited and had intractable hiccoughs. He had enenthema on the hard and soft palate. Half of the patients suffered from chest pain and gingival bleeding. The only survivor was seen at the nineth day of her illness and showed a less severe course of the disease. She was a serologically confirmed case.

Abortion occurred among 25 percent of 73 pregnant women who died. Two of nine pregnant survivors also aborted. Ten infants were born to mothers who died of EVI. All of these children died in turn within 19 days. These cases of possible neonatal Ebola virus infection had few symptoms. Seven were said to have had fever but bleeding was infrequent. In the absence of virological and pathological data it was not possible to decide whether these represented actual cases of neonatal infection.

DISCUSSION

The clinical features of Ebola virus infection as seen in this outbreak are virtually indistinguishable from those seen in the related Marburg virus infection (2,3).

If anything, the evolution of EVI appeared to be more inexorable and less variable than hemorrhagic fever due to the Marburg agent. Skin rash appeared to be less common than in Marburg disease, but was uniformly present among white patients (4,5) and was frequent during the EVI outbreak in Sudan6. This difference may be due to the use of mainly retrospective data. Oropharyngeal lesions and sore throat were far less frequent in Marburg disease.

In contrast to observations made simultaneously in Sudan, the Zaire illness had less respiratory symptoms, a shorter clinical course and a higher fatality rate. Whether this was due to differences in virus virulence per se, route of infection (injection or person-to-person), or to host and ecological variables such as climate (relative humidity) is not known.

TABLE 5

SYMPTOMS AND SIGNS OF 6 CONFIRMED OR PROBABLE CASES OF EBOLA VIRUS INFECTION AS OBSERVED BETWEEN THE 7TH AND 9TH DAY OF ILLNESS IN YAMBUKU AREA,ZAIRE, 1976.

Case	Sex	Age	Outcome (1)	Fever	Headache	Nausea	Abdominal pain	Myalgia	Anorexia	Sore throat	Chest pain	Arhtralgia	Diarrhea	Bleeding	Oral-throat lesions	Gingival bleeding	Vomiting	Conjuvitis
1	M	25	+	+	+	+	+	+	+		+	+	+	+			+	+
2	M	32	+	+	+	+	+	+	+	+	+		+	+	+	+		
3	F	36	+	+	+	+	+	+	+	+		+	+	+	+	+	+	
4	F	40	+	+	+	+	+	+	+	+		+	+	+	+	+	+	
5	M	30	+	+	+	+	+	+	+	+							+	+
6	F	30	+	+	+	+			+		+		+					

Other symptoms:
1: Bradycardia
2: Enanthema-Hiccoughs-Dyspnoea
5: Dark urine
6: Cough, Weight loss

Though far from proven, we suspect that consumptive coagulopathy (disseminated intravascular coagulation) and pancreatitis were major features of the syndrome. Death appeared to be preceeded by shock.

Early in the disease symptomatology is non-specific making diagnosis very difficult until more similar cases appear and the severe hemorrhagic syndrome becomes apparent. Even then, differential diagnosis with other hemorrhagic fevers such as Lassa fever can be clinically impossible, although bleeding occurs 7 less frequently in Lassa fever

There is a need for prospective clinical studies with appropriate controls (patients admitted with fever) and thorough investigations on the virology, haematology and biochemistry of EVI. This could give clues for diagnosis and management of the illness, Rapid diagnostic methods would be of invaluable help to control outbreaks.

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CLINICAL ASPECTS OF EBOLA VIRUS DISEASE AT THE NGALIEMA HOSPITAL, KINSHASA, ZAIRE, 1976

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The epidemic of Ebola Virus Disease (EVD) which had been in progress in Yambuku in northern Zaire for some time had already taken a heavy toll in lives among the staff at the Yambuku Mission Hospital when the first Belgian nun, a midwife, became ill on September 14th and died on the 19th. The second of the three Belgian nurses, Sister M.E. (Kinshasa Case 1), aged 42 years, became ill 4 days later and she was transferred to Kinshasa as lack of staff caused the hospital to close. Accordingly, she left Yambuku in the company of a nursing colleague, Sister E.R., and a priest, Father A.S. They travelled by road to Bumba where they spent the night at the local convent and the next day flew by scheduled flight of Air Zaire to Njili, Zaire's International Airport near Kinshasa, from where they took a taxi to the Ngaliema hospital where she was admitted on 25 September. Sister E.R. continued to nurse Sister M.E. who died September 30th. Sister E.R. (Kinshasa Case 2), aged 56 years, became ill with similar symptoms 8 days later and died on October 14th. Two days earlier Zaire nurse, M.N. (Kinshasa Case 3) aged 23 years, employed at the Ngaliema Hospiral who had nursed Sister M.E. also became ill (Fig. 1).

Examination of liver tissue taken post-mortem from Sister M.E. resulted in diagnosis of Marburg-like disease at the Institute for Tropical Medicine in Antwerp, Belgium, and a request was issued by the Zaire Government for specific Marburg convalescent plasma.

A supply, accompanied by a physician, arrived from Johannesburg, South Africa, on 16 October. By then it had been established at the Center for Disease Control Atlanta, Georgia, USA, that the virus, now known as Ebola virus, although morphologically similar to Marburg virus, was antigenically different. It was therefore believed unlikely that the Marburg plasma would be of benefit but two units (500 ml) were nevertheless administered intravenously to Nurse M.N. on on October 16th. However, her condition continued to deteriorate and she died on October 20th.
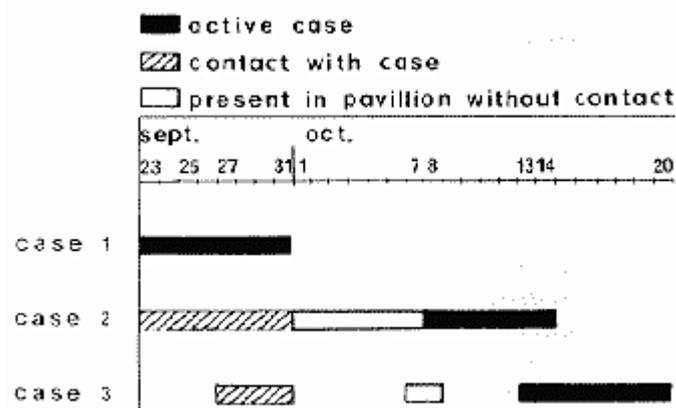
Fig. 1 Interrelationships between 3 fatal cases of Ebola Virus Disease in Kinshasa, Zaire, 1976.

Clinical features. These are summarized in fig. 2. The early symptoms include fever, headache, myalgia, diarrhoea and vomiting, which are non-specific and may not indicate the serious and highly

lethal nature of the infection. The characteristic triad of features which leave little doubt about the diagnosis, namely haemorrhage, rash and severe sore throat occur later during the course of the infection at a stage when its progression to death may be irreversible.

The rash in these three cases was morbilliform and started on the front of the trunk on day 5 or 6, spread to the back, buttocks and limbs on the following day and disappeared the day after.

On examination the throat of Case 1 showed reddening in the early stages which progressed over the next few days to severe, red, oedematous, tender swelling of the soft tissues which caused great difficulty in swallowing.

In Sister M.E., severe dysphagia as well as dyspnoea resulted from the swollen tissues at the back of the throat which was preceded by intense reddening of the tongue and throat on day 3 when she also had conjunctival injection.

Haemorrhage was manifested in Case 1 by oral and conjunctival petechiae on day 4 of illness, haematemesis and melaena from day 5, gingival bleeding on day 7, and bleeding injection sites on day 8. Case 2 had melaena from day 6. Case 3 had one slight haematemesis with fresh blood on day 7, susbsequent vomitus being free of obvious fresh or altered blood. On day 8 she had some melaena, and large echymoses developed, especially over pressure points such as elbows and shoulders. She also manifested marked swelling of the face and upper extremities on day 7 of illness. Urinary output at this stage was good and in balance with fluid intake.

Case 1 exhibited erythematous swelling of the vulva. All three patients were mentally alert until shortly before death. Anxiety was very marked in Case 3.

Laboratory findings. Laboratory tests were done in Case 1 (fig. 2) but, to safeguard the health of laboratory personnel, no tests were done by the hospital laboratory on the other two patients. However, the International mission (IMC) established some basic laboratory services within the confines of Pavillion 5 and some tests could therefore be done on Case 3 in the later stages of illness.

In none of the three patients were blood counts done sufficiently early to establish whether a marked leukopenia occurs in EVD as it does in disease (MVD)(3,4,5). Platelet counts were unexpectedly normal, unlike those found in MVD.

In case 3, mild clinical jaundice was diagnosed on the basis of yellowed sclerae on the day before death. Although not noted in the clinical record, jaundice may also have occurred in the terminal stages of Case I where a total serum bilirubin of 59,8 micromol/l (3,5 mg/100 ml) was established. Raised SGOT and SGPT levels which were established in only one patient indicated the occurrence of liver damage.

The only laboratory evidence of the probable occurrence of disseminated intravascular coagulation (DIC) was obtained from Case 3 in whom tests for fibrinogen degradation products were done which were shown to be present in increasing amounts.

Ebola virus was isolated from blood of all three patients i.e. from Case 1 on day 6, from Case 2 on days 3 and 6, and from Case 3 on day 3. In addition, formalinized liver tissue showed, by electron microscopy, the presence of virus 2 particles resembling those of the Marburg group of viruses.

Fig. 2 Clinical and laboratory data of three fatal cases of Ebola Virus Disease in Kinshasa, Zaire, 1976.

Treatment.

Case 1. When Sister M.E. was admitted to hospital, the aetiological agent responsible for the epidemic was still undetermined and typhoid was high on the list of differential diagnoses. Sister M.E. was therefore put on cotrimoxazole which was later changed to chloramphenicol and penicillin. Vitamin KI was given daily from day 4 onwards and she received blood transfusions on days 5, 7 and 8. Intravenous fluid therapy was started on day 5. in spite of the regression of some symptoms, i.e. dysphagia and vomiting, her condition progressively deteriorated and in spite of supportive treatment in the form of adrenalin and hydrocortisone on day 8 she died shortly thereafter.

Case 2. In view of the increasing suspicion that a viral agent was responsible for the epidemic, an antiviral preparation, 'Virustat' was given to this patient. She, like Case 1, was given aspirin as an antipyretic. Gamma globulin was administered on days 3 and 5, and chloramphenicol was started on day 3. Enterovioform was used in an effort to control the diarrhoea which was a major feature in this case. Intravenous fluid replacement was started on day 6. The patient's condition deteriorated; she was given hydrocortisone on day 6 and she died on day 7.

Case 3. The first three days of this patient's illness were marked by nothing more specific than pyrexia and fatigue. A provisional but unconfirmed diagnosis of malaria was made at one of the hospitals she visited and she was eventually admitted to her own ward at the Ngaliema hospital where antimalarial therapy was continued. On day 4 her oral fluid intake dropped to negligible levels necessitating intravenous therapy. When she was admitted to hospital it became known that the cause of the epidemic was a Marburg-like virus. Consequently on day 4 she was given 2 units (500 ml) of Marburg convalescent plasma. Valium was given to relieve her anxiety and chloramphenicol was also administered.

The severe sore throat was greatly relieved by the sucking of ice cubes. In view of the favourable outcome gained with two Marburg patients treated with anticoagulation(5) heparin treatment was started on day 6 in anticipation of DIC when the patient had developed all the classical symptoms of EVD but clinical haemorrhage had not yet become evident. Heparin was prescribed as a continuous infusion at the rate of 16 000 U daily on day 6 (with a loading dose of 2 000 U) increased subsequently to 30 000 U/24 hours. There were problems with the intravenous apparatus and for prolonged periods of time, intravenous therapy was intermittent, resulting in unsatisfactory anticoagulation as evidenced by the PTT which had not significantly increased. Although haemorrhagic phenomena developed these were not severe in terms of actual blood loss.

On day 7, the pulse rate increased sharply; the patient complained of substernal pain and developed a gallop rhythm with a pulse rate of 136 beats/minute. She was digitalized which resulted in a slowing of the heart-rate but she died that night, possibly as the result of a diffuse myocarditis.

DISCUSSION

The clinical features of these three cases of EVD are virtually indistinguishable from those seen in MVD. The high case fatality rate might be reduced in intensive care facilities where the necessary expertise and equipment are available for handling cases with the problems associated with extensive disseminated intravascular coagulation.

Anticoagulation is still a controversial subject as far as its benefits in this kind of case is concerned. In South Africa two out of three MVD patients with laboratory and/or clinical evidence of DIC were given very carefully regulated and monitored prophylactic heparin treatment, and both recovered(5). The full-blown case with severe DIC who is already depleted of clotting factors should not be anticoagulated but given replacement therapy in the form of fresh frozen plasma.

Although the white cell counts were found to be normal, none were done at the onset of illness and the leukopenic phase was probably missed.

One of our three patients had an elevated serum bilirubin level in the terminal stages and slight jaundice of the sclerae was noted in a second case.

It should be stressed, however, that clinical jaundice is not a common observation and, when present, is very slight in spite of the profound hepatic damage which may occur.

Although it was shown in vitro that the Ebola virus was antigenically different from the Marburg virus this did not necessarily imply a lack of crossimmunogenicity. For this reason Case 3 was given the benefit of the doubt, and plasma containing Marburg antibody to a titre of 1:64, obtained from a South African nurse, was administered, but the patient, not unexpectedly, failed to respond.

SUMMARY

A hospital-based outbreak of Ebola Virus Disease (EVD) occurred in Kinshasa, capital of Zaire, following the arrival of a sick nursing sister from the northern epidemic area. Her travelling companion, and a Kinshasa nurse who cared for her after her arrival in Kinshasa, also became ill and all three patients died after illness lasting 7 to 8 days. The illness was characterized by fever, headache, myalgia, diarrhoea, vomiting and later on a morbilliform rash, sore throat and haemorrhagic phenomena.

The clinical picture closely resembled that of Marburg virus disease (MVD) but the causative agent which is morphologically indistinguishable from the Marburg virus, is antigenically different. It was not unexpected, therefore, that Marburg convalescent plasma, administered to the last Kinshasa case, did not result in a favourable outcome of the illness.

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AFRICAN HAEMORRHAGIC FEVER IN THE SOUTHERN SUDAN, 1976: THE CLINICAL MANIFESTATIONS

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The clinical description of African Haemorrhagic Fever in the Sudan was obtained from a variety of sources. In Maridi, clinical details of patients were obtained both from active cases, and hospital records and by interviewing recovered cases or close family members. In Nzara, the descriptions were based entirely on retrospective interviews.

Of the total 280 cases occurring in the area, adequate clinical descriptions were obtained from 183. Information was collected on a questionnaire form designed prior to the investigation utilizing the symptomatology described in previous outbreaks of Marburg virus disease.

For the purpose of this paper a case has been defined as at least 2 days fever plus either gastrointestinal symptoms, chest pain or haemorrhagic remarkations and a clear history of contact. In Nzara where apparent primary cases were occurring, the clinical definition demanded haemorrhagic features.

The Onset of the Disease

Initially the disease presented as a progressive febrile, 'flu-like' illness. Earliest complaints included a fever, usually between 100 and 102ºF, severe headache and generalised myalgia in all cases. The course of the illness was steadily progressive. Within a few days patients became progressively more ill, exhibiting a uniform appearance with deeply sunken eyes and a fixed expressionface described as "mask like" or "ghost like". During the early stages of illness, patients, especially in Nzara, presented at the hospital, where were given injections of chloroquine and antibiotics as outpatients.

Other early symptoms included a dryness, or soreness of the throat which occured in 63% of all cases. This appeared to make the individual disinclined at or drink but was only rarely described as painful. Chest pain was an other early and frequent symptom occurring in 83% of cases, sometimes severe and commonly described in the lower costal areas occasionally clearly pleuritic and associated with a dry cough.

These initial symptoms became progressively more severe over the first four or five days, by which time most patients had presented themselves to the hospital. They appeared toxic, complained of severe headache and myalgia.

The myalgia often made patients reluctant to be examined. Muscles were tender to palpation, joint movements were painful and many patients complained especially of lower back and lumbo-sacral pain.

Gastrointestinal Symptoms

Gastrointestinal symptoms were a prominent feature of this outbreak.

Usually starting towards the end of the first week but occasionally as early as the second or third day patients commonly developed gastrointestinal symptoms. The most frequent was diarrhoea which occurred in 81% of cases. It began abruptly and lasted for about seven days in those who survived. Vomiting occurred less frequently (in 59%), starting after the onset of diarrhoea.

Accompanying the diarrhoea and vomiting many patients described colicy abdominal pain and developed a profound anorexia.

Cutaneous Manifestations

In Maridi, a number of acute cases were observed to develop a rash around the 5th to 7th day and we consider that most cases do exhibit cutaneous manifestations. Despite the large number of retrospective interviews in our patients, 52% reported either a noticeable rash during the illness or subsequent desquamation. The rash, was rather measles like, papular or maculo-papular and predominantly seen on the upper arms, flexor surfaces of the forearms and upper legs. Desquamation

when it occurred, took place some 10 to 14 days after onset of disease and appeared in the same sites but also especially on the palms of the hands and soles of the feet.

Haemorrhagic Features

Haemorrhagic manifestations were both a characteristic feature and a prognostic indicator in Maridi and Nzara. Virtually all of the fatal cases had visible blood loss (91%), whilst 71% of all documented cases had haemorrhagic features.

The most frequent and often severe manifestation was gastrointestinal haemorrhage, which occurred in 59% of cases and 86% of fatal cases. This took the form either of watery diarrhoea with fresh blood, malaena stools, or vomiting of fresh blood.

Whilst gastrointestinal haemorrhage was the most common expression of the haemorrhagic diathesis, bleeding was noted in a variety of other situations. Bleeding from the nose, mouth and gums was the second most frequent, occurring in 50% of fatal cases in Nzara and was often severe. Subconjunctival haemorrhages were commonly described. Vaginal haemorrhage was reported and a few patient had cutaneous haemorrhages. Haematuria appeared unusual.

Haemorrhagic features occurred after about the 5th day of illness and reached a maximum on the 10th day. The severity of the disease appeared to be directly related both to the extent and the severity of the haemorrhagic symptoms.

Central Nervous System

Symptoms referable to the central nervous system appeared to be frequent in this outbreak. Neck stiffness was reported especially in the more severely ill Spinal fluid obtained in these early cases was reportedly clear macroscopically.

Many patients exhibited bizzare behaviour - patients tended to abscond from hospital and behaved in an inappropriate manner, stripping off their clothes and wandering about the hospital in a confused state. One patient developed a terminal hemiplegia whilst a further patient was readmitted following recovery with overtly psychotic symptoms.

Physical Examination

Physical examination presented few characteristic features. The most characteristics was the general appearance of the patient, even in the early stages of the disease. The drawn, mask-like features, sunken eyes and loss of skin turgor came to be recognized as characteristic often supplemented with dry mucous membranes and oral fissuring. Patients both resisted and resented physical examination.

The posterior pharyngeal wall was injected. Neck stiffness was demonstrable in several of the more severely ill individuals.

The abdomen was soft and neither the liver nor spleen were palpable although tenderness was observed in the epigastrium and below the right subcostal margin. Jaundice was not observed.

The Course of the Illness

Severe cases demonstrated a relentless deterioration. Death most commonly Occurred on the 9th day although ranging from 2 days to 21 days after the onset The majority of deaths occurred predictably in severely ill patients usually exhibiting most of the classical features of the disease with severe haemorrhagic features. However, death also occurred in individuals convalescing from the infection. These deaths were sudden and unexpected. Recovered cases suffered a prolonged convalescence, often with continuing headache and profound lethargy, continuing for up to several months after the acute illness.

Mortality

The overall mortality in the Sudan outbreak was 51%. This mortality was deduced from the total number of cases considered to be due to AHF on clinical and epidemiological grounds. In Maridi where a higher proportion of clinical cases had serological evidence of infection the overall mortality was 54%.

In Maridi there was little evidence to indicate any alteration in mortality as the outbreak progressed. In Nzara, however the mortality may have been higher at the start of the outbreak falling from 88% in July to 62% in August and 38% in September (Table 1). However the numbers, especially in July, are small.

TABLE I

MORTALITY BY MONTH OF INFECTION IN NZARA

	July	August	September	October
Deaths	7	13	14	0
Total	8	21	37	4
Per Cent	88	62	38	-

Comparison of Maridi and Nzara

The absence of serological confirmation in the majority of surviving patients in Nzara has lead us to compare the clinical features of cases in the two outbreaks (Table 2). Both the time of onset of the various clinical features and their prevalence suggest that the two groups were consistent. The only evident differences were the frequency of chest pain, vomiting and cutaneous manifestations, all of which could be explained by the retrospective nature of the Nzara surveys. Haemorrhagic manifestations, severe symptomatology and mortality were consistent in the two groups.

TABLE 2

CLINICAL SYMPTOMS - MARIDI AND NZARA, SUDAN, 1976

Symptoms	Frequency (183 cases)		
		Maridi	Nzara
Fever	100%	100	100
Headache	100%	100	96
Chest Pain	83%	87	76
Diarrhoea	81%	84	76
Vomiting	59%	70	43
Dry Painful Throat	63%	63	62
Rash or Desquamation	52%	64	35
Cough	49%	60	33
Bleeding (any)	71%		
Melena	59%		
Bleeding (recovered cases)	48%		
Bleeding (fatal cases)	91%		

Asymptomatic Cases

The available evidence both in Nzara and Maridi, suggests that very mild or asymptomatic cases occurred. The sero-positive cases detected in the Nzara Cotton Factory and subsequently followed up indicate that half had experienced no illness over the past year whilst the others had had relatively mild febrile illnesses. Similarly, in Maridi, close contacts of cases were found to have serological evidence of infection but no history of recognizable illness.

DISCUSSION

The differential diagnosis of haemorrhagic fever especially in individual patients presents considerable difficulties in rural African populations. In an individual patient, the clinician must consider bacterial causes such as meningococcal septicaemia, septicaemic plague and relapsing fever: protozoal causes including malaria and trypanosomiasis as well as a variety of viral infections of which yellow fever, Marburg virus disease, Lassa fever and now African haemorrhagic fever are the more important.

However, when a cluster of cases occurs with a prodromal febrile illness followed by a haemorrhagic diathesis in a high proportion of cases and when transmission from person to person is observed amongst close contacts of cases especially when involving hospital staff, the possible aetiological candidates are considerably reduced. In Africa, Lassa fever, Marburg virus disease and now African Haemorrhagic Fever appear the most likely causes.

These three diseases present many features in common. The prodromal illness, vomiting, chest pain and rash are almost identical in Lassa and AHF. The pharyngitis in Lassa is commonly pronounced and the conjunctivitis severe and associated with periorbital swelling in contrast to AHF, where both the pharyngitis and conjunctivitis are rarely severe. Diarrhoea also occurs in both infections, although of greater severity in AHF.

The difficulties in clinical differentiation between AHF and Lassa are even greater when AHF is compared to Marburg virus disease. The symptomatology of the Sudan outbreak is substantially the same as that described in the two outbreaks of Marburg except for the frequency of chest pain which was rare in the previous outbreaks of Marburg. However chest pain was also uncommon in the Zaire outbreak of AHF.

The laboratory facilities available in Nzara and Maridi, or for that matter any similar hospital in rural Africa, provide little assistance in the diagnosis apart from the exclusion of alternative aetiologies. Few laboratory investigations were carried out in the Sudan outbreak but it is not anticipated that the findings would differ from the detailed studies carried out in the two previous outbreaks of Marburg.

The clinician practising in the rural hospital therefore has to rely on the clinical and epidemiological information available. Suspicion would then set in motion a train of events including the collection of appropriate samples for sophisticated virological investigation, the possible use of disease specific immune sera based perhaps more on an epidemiological assessment than the clinical features and the institution of personal, institutional and community measures to contain transmission.

The mobilization of adequate logistics to permit the collection, transport and processing of sophisticated virological samples presents numerous difficulties in remote areas of rural Africa. The subsequent low isolation rate obtained from Sudan material and the apparent transience of detectable antibodies further complicate an adequate investigation of such outbreaks which by their very nature tend to arise in remote-areas with limited resources. These technical and logistic questions remain of paramount importance to the physician practising in remote rural populations as well as to those responsible for epidemiological surveillance and public health in countries at risk.

ISOLATION, MONITORING AND TREATMENT OF A CASE OF EBOLA VIRUS INFECTION

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In the second half of 1976 specimens from a serious outbreak of haemorrhagic fever in Zaire and the Sudan 1 were sent to highsecurity laboratories in Belgium, England and the United States of America where a distinctive virus was isolated which was subsequently designated Ebola . On 5 November 1976 one of the investigators at the Microbiological Research Establishment in England, accidentally pricked his thumb while transferring homogenised liver from a guinea-pig infected with this new virus5. In accordance with standard safety protocol he immediately removed the glove and immersed his thumb in hypochlorite solution then squeezed it tightly. There was no bleeding and careful examination with a hand lens failed to reveal a puncture mark. He was kept under surveillance and on the sixth day became ill.

Shortly after midnight on 11 November his temperature rose to 37.4ºC. During the early morning he complained of nausea and central abdominal pain but there was no headache or myalgia. About 14 hours after onset he was seen at the Microbiological Research Establishment, where a blood sample was taken for direct electron microscopy and guinea-pig inoculation. He was then transferred to the high-security infectious diseases unit at Coppetts Wood Hospital in London and admitted directly into a Trexler negativepressure plastic isolator(6,7).

On arrival he felt physically exhausted and complained of anorexia, nausea and constant central abdominal pain. There were no other symptoms. His temperature was 38ºC with a relative bradycardia. He was alert and did not seem to be particularly ill. Apart from slight abdominal tenderness there were no other abnormal findings. Since it appeared highly probable that the illness was due to infection with Ebola virus, treatment was started that same evening, 20 hours after onset of symptoms, with human interferon, which had been prePared by stimulating peripheral lymphocytes with Sendai virus Interferon was given by intramuscular injection in a dose of 3 million units every 12 hours for 14 days.

The following morning his temperature had returned to normal and he was free from symptoms, but later in the evening his temperature rose again to 39º C.

His appetite remained poor but no other symptoms developed. By this time direct electron microscopy of his blood had revealed Ebolalike virus particles. In view of this finding it was thought advisable to give the patient convalescent serum obtained from people who had recovered from the illness in Africa. Treatment of this serum to ensure safety presented serious problems. The closely related Marburg virus has been shown to persist in the body for several months after the acute illness, though it has not been shown in the circulating blood. Marburg virus is relatively resistant to heat but is inactivated in serum maintained at 60ºC for 60 minutes9,,* The Ebola convalescent serum was therefore treated at this temperature for 60 minutes to ensure safety. The serum was also tested for HB_s Ag and HB_s Ab because carriers are common in many parts of tropical Africa. 450 ml serum was given by slow intravenous infusion over a period of four hours from 1.30 a.m. on 13 November, commencing 47 hours after onset of illness. Blood samples were taken at frequent intervals to ascertain virus and antibody levels.

There was no obvious change in the clinical condition of the patient until the fourth day of illness, 14 November, when an erythematous maculo-papular rash was noted over the chest wall. About mid-day he had a sudden violent bout of shivering followed by a sharp rise in temperature to 40oC. This was accompanied by nausea, retching and a single episode of vomiting. Since admission he had been constipated but at this point he had a loose bowel action. His mental state began to change and over the next 24 hours there was striking deterioration in concentration and memory. Protein was detected in his urine and persisted thereafter until the fever subsided. Over the next 72 hours, when the fever was at its height, there was severe malaise and extreme weakness. Profuse watery diarrhoea developed and continued for two days accompanied by persistent vomiting. The rash spread to all parts of his body and ultimately became confluent. There was no bleeding into the skin or mucous membranes. The throat was inflamed and a few small patches of thrush were detected. The abdomen was slightly distended but there was no tenderness or guarding. He was mildly dehydrated and urinary output was falling. Metoclopramide was prescribed for the vomiting and Lomotil for the diarrhoea.

On the sixth day of illness, 16 November, a further 330 ml of convalescent serum, pretreated in the same manner, was infused and followed by Hartmann's solution to correct dehydration. Next day his urinary output fell to its lowest volume of 830 ml despite adequate fluid replacement and a satisfactory blood pressure. At this point his appetite began to return; vomiting and diarrhoea became less frequent and ceased after the 18 November. Swallowing proved painful and examination showed extensive candidiasis of the throat, which responded to treatment with amphotericin B lozenges. The erythematous stage of the rash began to fade on 19 November leaving staining over the limbs on the same day he complained of stiffness of the small joints of his hands and to a lesser degree of the wrists and knees. The oliguria and proteinuria present at the height of the illness could have been attributed to deposition of immune complexes in the kidney, especially in view of the transient arthral gia at the end of the acute stage, but these features were recorded in severe cases during the original Marburg outbreak, when no serum was given.

After 20 November his general condition improved. His fever subsided to a low level, his energy began to return, and there was dramatic improvement in his interest and ability to concentrate, though he could barely recollect the acute phase of his illness. The joint symptoms did not persist. The temperature returned to normal on 22 November but there was a further flicker of fever on the next two days after which the temperature remained normal. Output of urine was normal by 23 November. Subsequently he made an uneventful but slow recovery over 10 weeks. At the end of the acute stage he had lost a considerable amount of weight which he regained slowly during convalescence. The rate of growth of hair slowed during the acute illness and during convalescent cence there was considerable loss of hair from his scalp. There were no other clinical complications.

In the early stage of the illness facilities were not available for conducting haematological or biochemical studies safely, so efforts were concentrated on establishing the virological diagnosis; in the late stage of the illness when provision had been made for routine tests, they were not required for the management of the patient, though they proved useful for assessing the extent of the damage during convalescence(10). Fortunately, there was no bleeding and the use of prophylactic heparin was not considered to be necessary. Electrocardiograms taken during the acute stage were normal though the amplitudes of the T-waves were lower than in a recording made on the 27 January during convalescence. Blood urea, and sugar concentrations and liver function were normal during convalescence. The HB_s Ag and HB_s Ab tests on blood were negative. The result of a chest radiograph was normal. During the early period of convalescence the haemoglobin level and white blood cell counts were depressed and did not fully recover until 8 February 1977, three months after the onset of illness. Bone marrow depression was shown during the original outbreak of Marburg disease and was attributed to the activity of the virus. interferon also causes bone marrow depression affecting the stem cells of the granulocytes(11-13) and synthesis of haemoglobin(14). Furthermore, interferon causes immunodepression(15) and may have contributed to the severity of the thrush. Once the haemoglobin and white blood cell levels had returned to normal the patient was subjected to plasmaphoresis and a total of seven units of plasma were taken between 16 and 25 February 1977.

It is not possible to assess the value of interferon and convalescent serum from experience with one patient. While the course of the illness was milder than expected from reports elsewhere, the pattern and duration of symptoms were not modified. Although there was no obvious clinical improvement after treatment, there was a striking fall in the level of circulating virus. On the first day of illness a blood sample was found to contain 10(4.5) guinea-pig infective units/ml; on the day after starting treatment with interferon there was no change in the amount of virus, but on the next day after the infusion of serum, the level in the blood dropped to 3-10 guinea-pig infective units/ml and remained at this level until the viraemia disappeared on the ninth day of illness, before the temperature had returned to normal. The second infusion of serum had no effect on the amount of virus. Since there is known to be a time lag before interferon produces an effect on virus levels it is not possible to assess the relative effectiveness of the two preparations in clearing the blood.

Before the infusion of serum the fluorescent antibody titre in the patient's blood was 1/2; after the infusion of 450 ml convalescent serum with a fluorescent antibody titre of 1/128-1/256, circulating antibody was detected in the patient's blood at a titre of 1/16. This was consistent with the dilution of the convalescent serum. Circulating antibody remained at this level until the tenth day when the titre increased to 1/32 and gradually rose to a maximum titre of 1/128 by day 34. After plasmaphoresis the level dropped to 1/32 and fell further to a titre of 1/16 on 5 May 1977.

The patient was nursed in a Trexler negative-pressure plastic isolator within a high-security section of the Hospital throughout the acute stage of his illness and during convalescence until certain clearance tests proved to be negative for Ebola virus. Air pressure within the isolator was maintained below atmospheric and extracted air was drawn through a HEPA (high efficiency particle arrester) filter before being discharged above roof level. All supplies were introduced through an entry port

without breaking the air seal. Infected material was removed in a similar manner into plastic bags which were sealed to prevent contamination of the surroundings. Dry waste was destroyed by incineration within the high-security area; liquid waste was pretreated with 1% Hycolin, a cresolic disinfectant, before being boiled. Doctors and nurses had access to the patient but were separated physically by a plastic film barrier.

Once the acute stage had subsided it was decided to take specimens for clearance tests at weekly intervals and it was arbitrarily agreed that three negative sets of cultures from throat swabs, blood, urine and faeces would be an acceptable standard for discharging the patient from isolation. After two sets of specimens had been shown to be free from virus the patient was removed from the isolator and transferred to a high-security room equipped with airfiltration and facilities for the safe disposal of excreta. He remained there pending the results of the third set of clearance specimens. Altogether he had spent 32 days in the isolator.

The contents of the isolator were removed and destroyed by incineration or packed for autoclaving. The room and the interior of the isolator were then fumigated with formaldehyde and left for 24 hours, after which the canopies were dismantled and destroyed by burning. The room was refumigated with formaldehyde and sealed for 24 hours. The staff undertaking these tasks wore full protective clothing and biological respirators.

When the tests of the third set of specimens proved to be negative the patient was discharged home. In view of previous experience in Germany with Marburg virus(16), a sample of semen was taken on day 39 and found to contain 3-10 guinea-pig infective units/ml. However this discovery was not thought to justify further isolation, especially as the patient fully appreciated the implications. Semen was positive again on day 61 but negative on days 76, 92 and 110.

The Trexler negative-pressure plastic isolator and the techniques used for the disposal of waste proved to be effective in preventing spread of Ebola virus from the patient to attendant staff and to the general community. Of the 24 nurses who were directly concerned in the care of the patient, six became ill with acute respiratory infections, which lasted on average two days. Four of the five doctors looking after the patient developed a 'flu-like' illness with some gastrointestinal symptoms. At onset these illnesses caused concern but the problems invariably resolved within two or three days and antibody studies later showed no evidence of Ebola virus infection among either medical or nursing staff.

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DISCUSSION

A.W. Woodruff : None of the speakers have mentioned any deafness, as has been reported in some cases of Lassa fever; this could be a clinical difference between Lassa and Marburg-Ebola. Secondly, has one of them noticed in the pharynx a membrane which could be confused with a diphteritic membrane, as we observed in one of our patients with Lassa in London ?

M. Isaacson : Personally I have seen one case, but although she had very swollen tissues and a rather dirty-looking pharynx, I would not have confused it with a diphteritic membrane. The sore throat was very intense, the patient did complain also of the feeling of a painful lump in the throat, in fact it was subjectively the most worrying feature in this patient.

P. Piot : Deafness was not observed but tinitus was, and this may indicate involvement of the nervous system. We saw one patient with a greyish patchy exsudate on the soft and hard pallates but there was no membrane.

T.E. Woodward : My experience years ago was with patients with Korean Epidemic Hemorrhagic fever. I was struck by certain similarities and I want to ask about the blood pressure. I saw that the pulse became rapid in almost all patients, while myocarditis was mentioned by all speakers. In 1952 in Korea, it was noticed that if too much fluid is given it can be bad rather than good. In fact there was absolutely no integrity of the vascular system and fluid actual ly leaked into the tissues including the myocardium. It seemed that the careful titration of epinephrin did maintain the circulation for a while, but too much epinephrin was bad, too much fluid was bad and even if more than two units of serum albumin were given in an attempt to bolster the circulation, that too would pour too much fluid into the vascular system. I just wondered about how well these patients tolerated intravenous fluid ?

N. Isaacson : Case no 3 that we treated in Kinshasa, as I mentioned, did exhibit oedema: her face was oedematous, her upper extremities were oedematous, how much of this was due to fluid retention or to cardiac failure we don't really know. The renal output seemed to be reasonable. We were faced in Kinshasa, as well as in all the other parts where the disease occurred, with really a lack of laboratory facilities. This is perhaps one of the problems that has become most prominent for all of us who had to deal with it. We had patients there who needed both isolation plus sophisticated care with all the facilities that are required and we just did not have them. With a situation of intervascular coagulation and of possible impending cardiac failure, of possible myocarditis, we required electrocardiography, we required anticoagulation facilities, we required all the necessary Laboratory facilities and we did not have them. This made our work extremely difficult, I don't think this patient got adequate care. The blood pressure remained normal throughout.

L. Eyckmans : It is clear from the presentations of this morning that the hemorrhagic component is a very bad prognostic sign, but was actual blood loss contributive to death or was it only a bad symptom ?

M. Isaacson : At least in one of our cases I think blood loss was a feature. Our last cases had limited blood loss, nothing very serious.

D.P. Francis : There is a tremendous amount of intratissular and diarrheal loss though, and some of the patients, especially those with diarrhoea, appeared just like cholera patients with deep-set eyes and the typical skin. There is a lot of fluid loss.

F. Dekking : Dr Emond, how do you disinfect the isolator after use ?

R.T.D. Emond : We spray the inside of the isolator with 1% hypochlorite solution, Leave it twenty-four hours, then wash it out thoroughly and put the isolator back into use. If the patient had a dangerous infection, we destroy it, and the only occasion on which we actually did this was in the particular patient described. We sealed the room where the isolator was, we fumigated the room with formaldehyde generated by heat, put heat generators also inside the isolator and ran the pumps so that formaldehyde was drawn through the filters. We left it all for twenty-four hours and then wearing protective clothing and respirators we dismantled the canopy. The canopy was in fact too large to go through the opening of any of the incinerators we had, so rather primitively, we dug a hole in the field and burned it. I cannot think of any safer way of disposing of it. The filters were dismantled, sealed, autoclaved, incinerated and replaced by fresh ones. This is a technique we have used, and I'm willing to change my way if someone can suggest anything better.

M. Isaacson: What would be wrong with disinfecting the isolator for example with ethylene oxide and re-use the canopy ?

R.T.D. Emond : The total cost for this particular episode was so enormous, that the canopy was negligeable in it. If you consider that all the scientific staff in Porten were put off work and under surveillance, that our hospital with 160 people was put out of action, and that a great many community phi,,,--' cians were involved, the total cost must have run into 100.000 or 200.000 pounds. The price of the envelope is about 900 pounds Sterling.

M. Dietrich : Did you ever consider to use peracetic acid ? What was done with the waste coming out the isolator ?

R.T.D. Emond : Peracetic acid is used for the disinfection of these envelopes, The problem with it is that it is unstable, it has to be freshly prepared and we have preferred to use hypochlorite for ordinary routine purposes. I would not be prepared to put a fresh patient into an envelope in which we had nursed a patient with an Ebola, Marburg or Lassa virus infection. I think it is much wiser to destroy these envelopes. All dry waste was stored in plastic bottles inside the isolator until the bottles were full, treated with disinfectant for twenty-four hours, then removed in sealed bags and disposed of by boiling.

K.M. Johnson : Nobody asked what the action mechanism of passive antibody in a systematic disease like this could be. Why was it deemed necessary, even in the beginning to quarantine medical staff that was protected by the bed isolator ?

R.T.D. Emond : In Great Britain as in other countries, there was considerable at the reports which were coming from Africa about this new disease. The equipment at that time, although it had been used on a considerable number of occasions, had never been used in any really serious infection. Thirdly, one of the children of the patient developed a mild fever and it was thought it was possible that it was going to spread within the family grouping. Taking all things into consideration, it was thought advisable that the staff should be asked to go into voluntary quarantine which they agreed to do.

2. PATHOLOGY. VIRUS MORPHOLOGY. TAXONOMY.

HUMAN PATHOLOGY OF EBOLA (MARIDI) VIRUS INFECTION IN THE SUDAN

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In 1967 cases of a hitherto unknown hemorrhagic fever occurred in Marburg. Pathology of Marburg disease could be investigated well enough to become informed about specific organ damage and subsequent clinical syndrome. In Sudan an outbreak of a hemorrhagic fever similar to Marburg virus disease occurred. In contrast to 1967, local circumstances and logistics in the Sudan did not allow elaborate routine nor scientific tests. Thus, only limited material was to be investigated leaving many questions unanswered.

MATERIALS AND METHODS

Postmortem biopsies were performed in two cases only to very limited extent. Biopsies of liver, heart, lung, spleen, kidney, and brain preserved in glutaraldehyde were processed by routine methods for histology and stained by Hematoxylin-Eosin, Prussian Blue, Ladewig, and Sudan-III-stain. Electron microscopic investigations were performed on EPON embedded material under standard conditions.

Peripheral blood smears of 11 patients were available at different stages of disease. Bone marrow aspirates of two patients could be evaluated. Blood smears and bone marrow aspirates were stained by panoptic staining May-Grunwald-Giemsa (Pappenheim).

RESULTS

Histology :

Liver: There was moderate hyperemia and marked edema in the center of the lobules with atrophy and dissociation of the liver cell cords in this area. In the periphery of the lobules the liver cells were loaded with fat droplets, as shown by Sudan III staining. Within the terminal plate - rarely in other areas of the lobule - individual or small groups of liver cells had undergone eosinophilic degeneration or necrosis. The portal tracts were enlarged and rather intensely infiltrated with lymphoid cells, histiocytes and - less intensely - plasma cells and eosinophils, focally interspersed with basophilic medullary junction.

Spleen: There was marked hyperemia and cellular depletion of the red pulp, and marked atrophy of the lymphoid follicles.

Myocardium (left ventricle) : There was a general, rather proteinaceous edema of locally varying degree of the interstitial connective tissue with focal and rather inconspicuous accumulations of inflammatory cells, similar to those found in the interstitial connective tissue of the liver and kidney.

Lung: The alveoli were focally dys- or atelectatic. The alveolar walls were generally and moderately thickened, due to an increase in cellularity and the deposit of fibrinoid material within and adjacent to the surface of the alveolar wall.

Brain: In the very small fragment of topographically not definable brain tissue no evidently pathological alterations could be recognized.

Studies of heart, lung, spleen, and kidney by electron microscopy did not show any virus particle or inclusion bodies.

A comparison of our histopathological observations with those previously made in a case of Ebola virus infection by Prof. Gigase at the "Institut de Médecine Tropicale 'Prince Leopold' at Antwerp , suggests almost identity as far as the parenchymal lesions are concerned. Furthermore, the liver lesions in both cases correspond well with the liver lesions which have been described in Marburg virus infections. The same can be stated with regard to the kidney, spleen, and heart lesions. The histopathology of the liver differs quite clearly from the histopathology observed in other virus diseases, e.g. infectious hepatitis and yellow fever. However, a differentiation from infections caused by Arena viruses (Lassa fever, Bolivian hemorrhagic fever, Argentinian hemorrhagic fever) appears to be difficult - if not impossible - by means of light microscopy only.

Hematology:

Slides of peripheral blood were available, taken at random at 20 different days of 11 patients. Considerable changes of the peripheral blood cells were seen. In some cases there was slight anisocytosis and occasionally small percentage of schistocytes. Evidently leukopenia existed at the beginning of the disease with increasing cell counts later predominantly of granulocytes. The most prominent finding was a shift to the left in the granulocytes, and up to 33% pseudo-Pelger forms. Very large cells with dark blue cytoplasm were identified as activated lymphocytes or lymphoblasts - also named "virocytes". Platelets were markedly decreased in some cases. The almost characteristic finding of pseudo-Pelger and so-called "virocytes", as well as an increase of granules (probably remnants of nuclear decay), mainly in areas bordering the terminal plate of the lobule. The Kupffer cells were enlarged and they contained small granules of dark brown or black, iron-negative pigment. A few small granuloma enclosing fragments of Schistosoma eggs were also seen.

Electron microscopy: Hepatocytes showed enlarged mitochondria without cristae containing coarse granules or being empty. Many empty vacuoles indicating fat droplets in cytoplasm were seen. The spaces of Dissé were considerably enlarged. Frequently the plasmatic membranes of hepatocytes were not recognizable. Microvilli were absent. Inside the cytoplasm of altered hepatocytes meander like inclusions of 1 microm as well as single filamentous particles were found. However, inclusion bodies of nucleocapsids with regular formation were not present. The extracellular space contained virus particles and nucleocapsids not to be distinguished from Marburg virus particles.
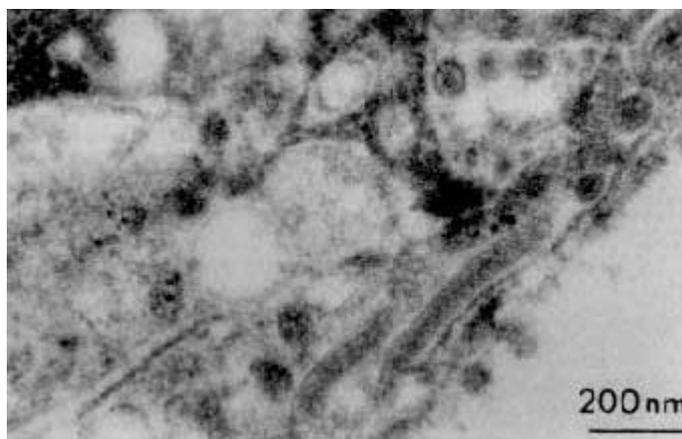
Fig. 1. Ebola (Maridi) virus in human liver, 60.000 x

Kidney: The glomerula were inconspicuous. The epithelial cells of the tubules, particularly of the proximal portion of the nephron, exhibited varying degrees of granular, hydropic, and fatty degeneration and - focally necrosis and desquamation. The Bowman's space at the glomeruli and the lumen of the tubules were irregularly filled with amorphous proteinaceous precipitate. -ocal cellular infiltrations - analogous to those found in the portal tracts 'if the liver - were seen around blood vessels, particularly at the corticolymphocytes had its peak between the 6th and 10th day, though these features could be observed in smears from 3rd to 24th day of illness.

A 28 years old patient, who died at day 8 of his illness, showed at the 3rd day leukopenia, thrombocytopenia, 8% pseudo-Pelger, and 12' atypical lymphocytes. One day later leukopenia and thrombocytopenia were found again. There was a marked shift to the left of the granulocytes, and also a finding of 30' pseudo-Pelger forms.

The bone-marrow aspirate showed normal to increased cellularity. Red blood cell precursors were seen with little morphological changes only, such as atypical mitoses. Megakaryocytes were not at all decreased in number, and did not exhibit significant alterations morphologically. There was an increase in monocytes and plasma cells, as well as in eosinophiles to be expected in inhabitants of an area with high risk of parasitic diseases. However, the granulocyte precursors showed major alterations as described in detail. There were blocked mitoses, an 1 . increased number of necrotising cells, and a marked vacuolisation of granulocytic precursors, predominantly myelocytes and promyelocytes.

Some of the granulocyte precursors contained fragmented or very bizarre nuclei or twin nuclei.
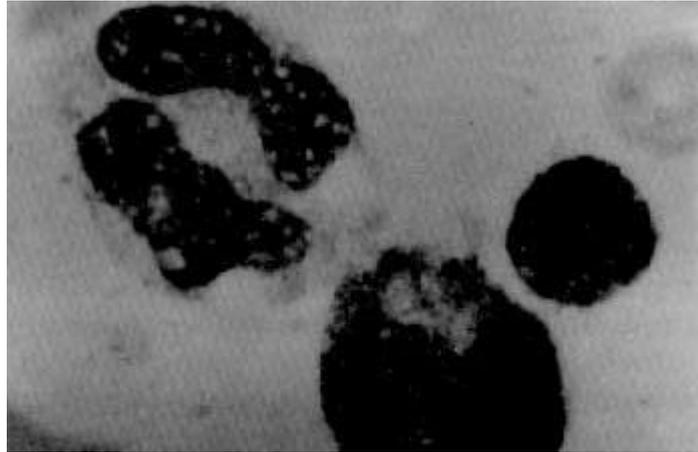
Fig. 2. Bone marrow: twin nucleus and vacuolization of metamyelocyte.

Further morphologic peculiarities were seen in storage cells having phagozised decay and greenish inclusions, which could not be identified.

By morphological means only it is impossible to draw definite conclusions concerning the pathogenesis of the hematological changes. However, the marked alterations of the granulocyte precursors may suggest direct effect of virus or virus particle. The normal, at least not decreased megakaryocyte count and the peripheral thrombocytopenia may be correlated with peripheral sequestration of platelets, but is not sufficient to state this mechanism of thrombocytopenia. All described hematological alterations are very similar, if not identical, to those observed in cases with Marburg virus disease.

DISCUSSION

The description of pathologic anatomy of the Marburg virus disease shows many similarities with the results above(1). In addition, clinical features, morphology of virus, and hematological investigations parallel each other in both diseases, Marburg as well as Ebola (Maridi) virus disease(2). Thus, it may be permissible to use the information learned by the Marburg virus disease for understanding pathogenesis of clinical symptoms that occurred in Ebola (Maridi) virus infection. Unfortunately, mortality in Ebola (Maridi) infection was considerably higher than in Marburg virus disease. This could mean that, despite the similarity of morphological features and some similarities in the clinical course, Ebola (Maridi) virus infection may effect different target organs, as f.i. myocardium, than in Marburg disease. Therefore, more informations on pathology are needed urgently in order to improve possible clinical treatment for better survival.

SUMMARY

Histo-pathological studies of biopsy material from two cases in Sudan by light and electron microscopy showed lesions in liver, heart, lung, spleen, and-kidney, most similar to the observations in Marburg virus disease. Furth more, peripheral blood smears and bone-marrow aspirates showed morphological alterations and quantitative changes of peripheral blood cells, that can be used to assist in the diagnosis of suspected hemorrhagic fever. Unfortunately they are inconclusive to explain the pathogenesis of some clinical symptoms, specifically the cause of the bleeding tendency.

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PATHOLOGY OF EBOLA VIRUS INFECTION

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So few specimens of tissue from fatal cases of Ebola virus disease have been available for pathologic study that no description can be considered representative, and any analysis of pathogenetic mechanisms should be recognized as speculative. No report of gross pathologic findings is available and specimens for histopathologic study have consisted only of liver tissue from three cases in Zaire (sent to CDC by the WHO Field Team), and liver, spleen, and kidney tissues from two cases in the Sudan (sent to CDC by Dr. D.S. Ridley, Hospital for Tropical Diseases, London, and Dr. D.I.H. Simpson, London School of Hygiene and Tropical Medicine). Clinical pathology information will be presented by other contributors to this Colloquium.

Because of this paucity of tissue and lack of information of Ebola virus pathology it will be necessary to draw upon findings from Marburg virus studies carried out after the 1967 and 1975 episodes. Findings from 1967 primarily derive from the papers by P. Gedigk, H. Bechtelsheimer, G. Korb, and H. Jacob 1,2,3 ; pathologic findings from the single fatal case in South Africa in 1975 are not formally available but some observations are included here based upon a set of histologic slides sent to CDC by Professor J.H.S. Gear and his 4 colleagues of the South African Institute of Medical Research, Johannesburg (4).

Ebola Virus Infection

The three liver specimens from confirmed Ebola virus disease cases in Zaire were remarkably similar. There was fatty change and necrosis of hepatocytes and Kupffer cells (Figures 1,2). This necrosis was focally distributed throughout lobules, in some cases involving single cells and in other cases extending from central veins to lobular peripheries (Figures 3,4). The sequence of hepatocyte necrosis involved an initial cytoplasmic eosinophilia, then a shrinking, darkening and dissolution of nuclei (nucleoclasia). Intact cells with hyalinized cytoplasm and ghostlike nuclei apparently remained in place for some time; but finally rarifaction, swelling and cytolysis occurred, leaving large amounts of karyorrhectic debris in situ. Considering the extent of this necrosis, there was remarkably little inflammatory infiltration into sinusoids. There were large numbers of hepatocyte mitoses, but often binucleate cells underwent the same necrotic changes as they were entrapped by expanding foci of infection.

Extraordinary large and vivid intracytoplasmic eosinophilic inclusion bodies were present in hepatocytes in two of the three Zaire cases; further study showed that these represented an extreme and that large numbers of smaller and less distinct inclusions were present in all three cases (Figures 5,6). Because smaller inclusions had indistinct margins and an eosinophilic color matching the early hyaline change of the cytoplasm of infected hepatocytes, they could only be identified with certainty in rather normal cells. Inclusion body stains helped in this identification, but as in most acute hepatocellular infections, the presence of large numbers of Councilman-like bodies in areas of necrosis was a complicating factor. This matter has practical importance since histologic identification of Marburg/Ebola virus inclusions, in the presence of Councilman-like bodies, could have presumptive diagnostic value in laboratories where virus isolation or immunofluorescent methods are not available. The identity of the virus inclusions in the three Ebola cases was confirmed by thin-section electron microscopy of formalin-fixed liver tissue (Figure 7). Although preservation was poor, the inclusions found within the cytoplasm of hepatocytes were clearly made up of massed tubules which were identical to the internal constituent (? the nucleocapsid) of virus particles (Figure 8). These structures were indistinguishable from those found in Vero cell cultures infected with Ebola or Marburg viruses; similarly they were indistinguishable from hepatocyte inclusions in human, monkey and guinea pig(5,6,7) infections caused by Marburg virus . In the same three Zaire liver speci mens, large number of virus particles were found in all of the extracellular spaces (sinusoids, spaces of Disse and areas of necrosis); these were indistinguishable from Ebola virus particles in cell culture and Marburg virus particles in cell culture and in vivo (Figure 9). No Aiver necrosis was evident in the very small tissue specimens collected from fatal cases of hemorrhagic fever in the Sudan. There was some necrosis and calcification in tubules and glomerular tufts of kidney and there were necrotic cells in the spleen specimens. However, because no information was received about the course of disease nor laboratory

confirmation of the diagnosis, it is prudent to put off interpretation of this exceptional sparing of the liver.

Marburg Virus Infection

Comparison of the liver lesions of the Zaire Ebola cases with those of Marburg infections in 1967 and 1975 indicates precise similarities. At this point, further comparisons are impossible, but a brief review of Marburg disease pathology may be of value. In the fatal Marburg virus infection in South Africa in 1975, hepatocellular necrosis was the most pronounced pathologic finding (Figures 10, 11). The focal necrosis was similar to that in the Zaire Ebola cases, but the damage seemed almost synchronous, that is, large numbers of hepatocytes seemed to have been caught at the time of death of the patient in a similar state of early cytoplasmic hyalinization and eosinophilia without frank dissolution (Figures 12, 13). Small inclusions and Councilman-like bodies were identified in this liver tissue (Figures 14, 15). There were necrotic cells in other organs, but not in numbers like those in the liver. In the kidney there was tubular necrosis and in glomerular capillaries there were multifocal fibrin thrombi characteristic of disseminated intravascular coagulopathy (DIC) 8 (Figure 16). There was also pulmonary edema and an effusion of macrophages into alveoli (Figure 17). It is hoped that a detailed pathologic description of this case will be published soon by South African pathologists.

Because study of the Marburg virus disease of 1967 was so comprehensive, the findings have special comparative value(1,2,3). Gross pathologic findings included evidences of hemorrhagic diatheses into skin, mucous membranes, soft tissues, visceral organs and into the stomach and intestines. There was swelling of spleen, lymph nodes, kidney and especially brain. Microscopically, focal necroses were found in many organs, most conspicuously in the liver, lymphatic system, testes, and ovaries. Liver necrosis, identical to that described in Ebola virus infection, was especially prominent, and as in the latter there was no favoring of any particular zone of lobules. Necrotic foci grew by expansion. Inclusion bodies were prominent, as were Councilmanlike bodies and basophilic karyorrhectic debris. Liver biopsies from convalescent patients indicated rapid regeneration coinciding with the decline of serum transaminase levels. Lymphoreticular organ changes included necrosis of 1) lymphoid follicles, 2) the red pulp of spleen, and 3) the medulla of lymph nodes. An eosinophilic "thrombic" debris was left in situ as a result of this necrosis. There were histologic evidences of hemorrhagic diathesis in many organs. In the brain there was a diffuse panencephalitis with glial nodule formation, perivascular lymphocytic cuffing, and evidence of interstitial edema.

Pathogenesis

The pathophysiologic alterations which make Marburg and Ebola virus infections so devastating have not been studied systematically. The cause of the hemorrhagic diatheses was searched for in tissues from the fatal Marburg cases in 1967 but no vascular lesions were identified. The increase in vascular permeability, associated reduced effective circulating blood volume, interstitial edema in visceral organs and brain, and DIC may all stem from the liver necrosis and renal tubular necrosis. It is not clear how the shock syndrome in this disease relates to activities of pharmacologic mediators of capillary permeability and complement split products. Whatever the underlying mechanism, it was concluded after the 1967 Marburg episode that DIC and cerebral edema played an essential role in the fatal outcome of infection. The magnitude and rapidity of the destructive events of Marburg or Ebola infection would predict that the terminal DIC/shock syndrome would be most difficult to deal with and this is the case in fact. The success in South Africa in saving two Marburg patients with heparin and supportive treatment and the success in the United Kingdom in saving one Ebola patient with convalescent plasma does not tell us too much about the pathogenetic mechanisms of these hemorrhagic fevers, but they do remind us of the regenerative capacity of the liver and kidney tubules.

Differential Pathologic Diagnosis

Although no pathognomonic lesion has been found which would permit certain histopathologic diagnosis of Marburg/Ebola infections, some pathologists considered that the overall pattern of lesions is unique and distinguishable from 1,2 yellow fever, infectious hepatitis, and other well-known infections. Others deem the differential diagnosis extremely difficult, especially in the context of examination of tissues from a single case and in a setting where Lassa Fever must also be considered. In any event, it is clear that pathologic examination is not a substitute for etiologic diagnosis(8), but in areas without full laboratory facilities, histologic diagnosis may be as important as it has been for many years in yellow fever diagnosis. The recent production by Drs. Y. Robin and J. Renaudet (with the support of the WHO) of an excellent 35mm transparency set with accompanying text, entitled 'La Fièvre Jaune -- histopathologique positif et differentiel', provides an important new resource for the differential pathologic diagnosis of hemorrhagic fevers in the African setting where Lassa fever, yellow

fever, Crimean hemorrhagic fever, typhoid fever, infectious hepatitis, leptospirosis, and other infectious diseases occur. This slide set is complemented by the recent description of the pathology of human Lassa fever by Winn and his colleagues(9,10), and recent consideration of the differential diagnosis of hemorrhagic fevers in 11 Africa (from the viewpoint of Lassa fever) by Monath and Casals (11).

Pathology in Experimental Animals

After the 1967 Marburg virus episode, several attempts were made to develop animal models for further study of the pathology and pathogenesis of the infection. Monkeys (Cercopithecus aethiops), guinea pigs, and hamsters were found to be most valuable(6,7,12,13). After serial passage of the virus in guinea pigs, infection was invariably lethal; the disease was marked by a swollen and friable liver and spleen. Microscopically, these changes coincided with focal liver necrosis and congestion, hemorrhage and destruction of lymphoid elements in the spleen. In addition to similar liver and spleen lesions, meningitis and hemorrhagic vascular lesions of the brain parenchyma were most characteristic of the hamster disease. Severe hepatocellular necrosis and lymphoreticular necrosis marked the fatal disease in monkeys. In each of these three species, hemorrhagic diatheses were found, and although the pathogenetic characteristics of the human hemorrhagic fever were not reproduced precisely, similarities were such that the models would likely have been of value in testing prophylactic or therapeutic regimens (e.g. passive or active immunization, interferon, or chemotherapeutic agents). However, little was done with these Marburg infection models except for the descriptive studies.

Investigation of Ebola virus infection in experimental animals has been extremely limited so far. In guinea pigs, pathologic studies carried out at the Microbiological Research Establishment at Porton Down by Bowen and his colleagues 14 and at the CDC 15 on virus from Zaire and from the Sudan indicated that focal liver necrosis was the most prominent lesion and that splenic white pulp necrosis and some -lymph node necrosis also occurred. As had been true of Marburg virus in studies done after the 1967 episode, the guinea pig liver disease varied in severity from a progressively destructive lethal course involving most hepatocytes when serially passaged virus was used, to an apparently self-limiting infection with calcification of necrotic hepatocytic foci when unpassaged virus was used. In a similar way, Ebola virus from Zaire seemed more capable of producing progressive hepatitis, and virus from the Sudan more often caused arrested, calcified lesions. Correspondingly, more guinea pigs survived infection with the Sudan virus. In these preliminary studies virus inoculum dose was not carefully controlled so no inference may be drawn as to virulence differences of the various isolates, but such studies will be done.

Ebola virus has not yet been studied in hamsters, and results of pathologic study of monkeys study of monkeys inoculated at Porton Down (for appraisal of interferon sensitivity of the virus) are not yet available.

In conclusion, it would seem that the pathologic alterations found in Ebola virus infection of man and experimental animals are similar to those in Marburg virus infection. Even in the absence of comprehensive comparative studies, it seems safe to draw upon our experiences with Marburg virus and to immediately   direct our concern to developing the means for Ebola virus prophylaxis and treatment. The opportunity for a comprehensive study of the pathologic alterations in fatal human Ebola virus infection has been lost in the extremely difficult and hazardous circumstances in the field, but our discussion of the means of medical intervention can proceed, nevertheless, based upon presumed pathophysiologic characteristics of the disease.

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This is not a comprehensive listing of the Marburg virus pathology literature. Extended bibliographies are found in references 1, 2, 3, 6, 7, and 8 and many other papers containing pathologic observations are included in the book Marburg Virus Disease (edited by Martini, G.A., and Siegert, R.) Springer-Verlag, New York, 1971.

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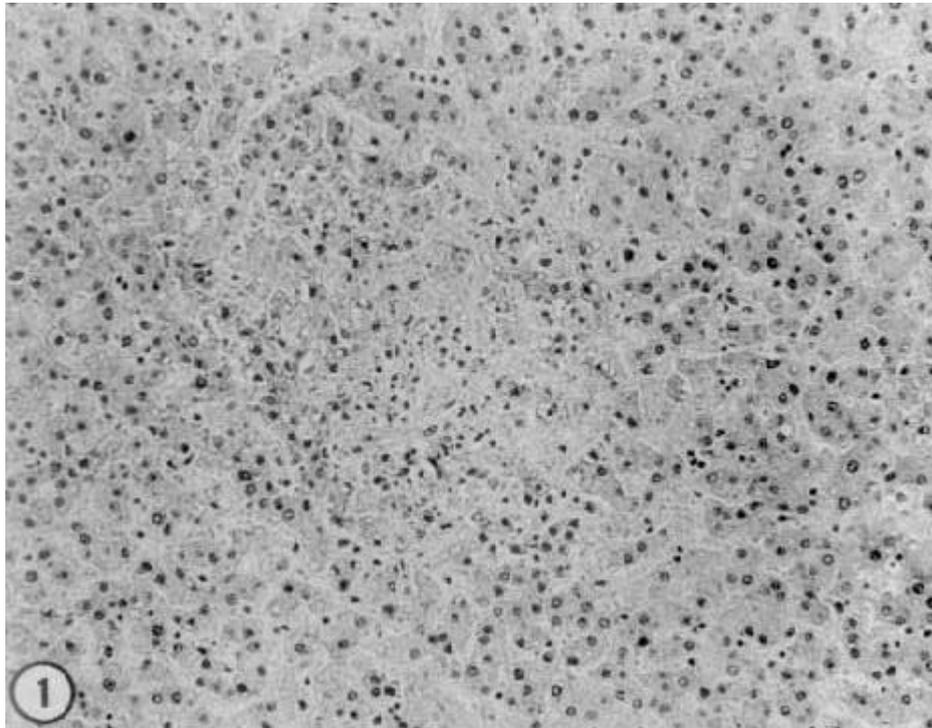
Fig. 1. Ebola virus infection, Zaire. Liver. Focal hepatocellular necrosis with karyorrhexis, but little interstitial inflammation. In this case the severe damage to hepatocytes is not marked by any architectural disarray. Hematoxylin and eosin; X 110.
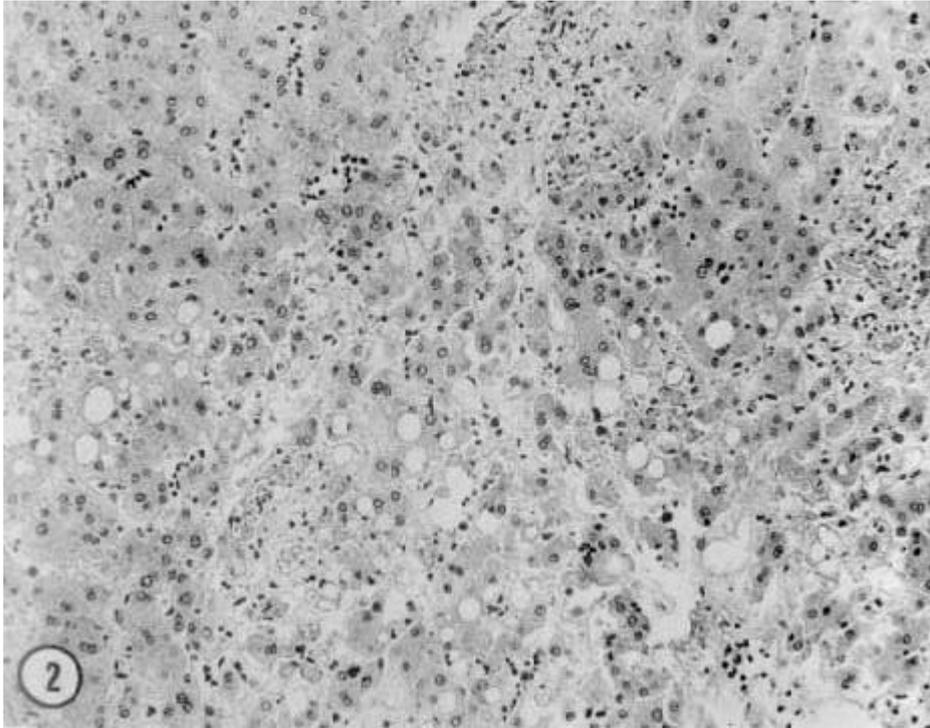
Fig. 2. bola virus infection, Zaire. Liver. Focal necrosis with fatty change and modest inflammatory response. In this case massive lobular structural changes were associated with the infection. Hematoxylin and eosin; X 110.
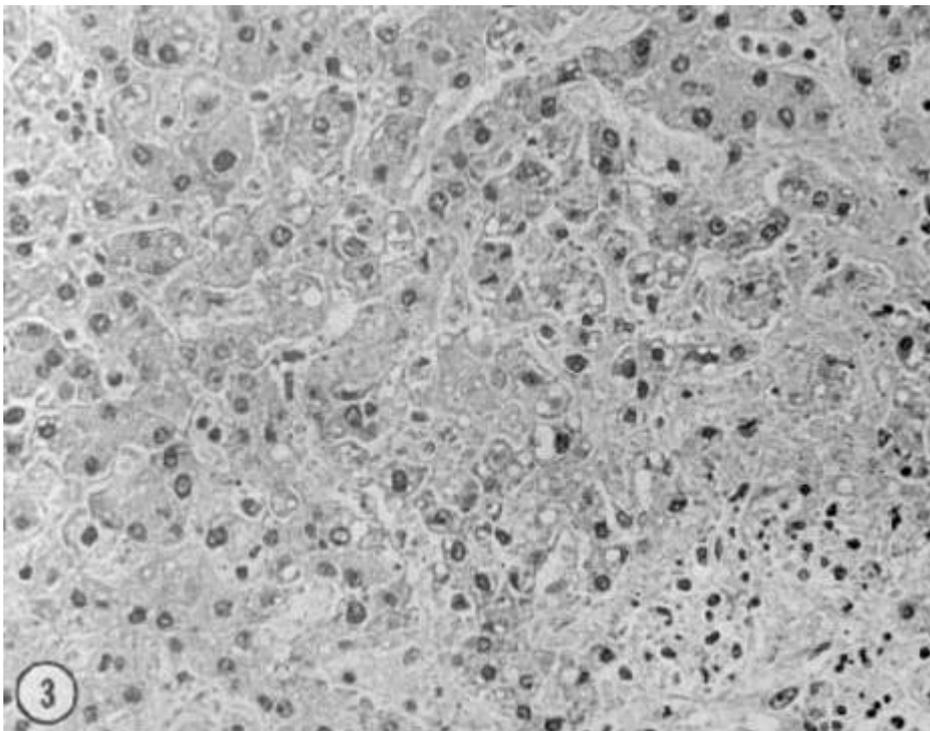
Fig. 3. Ebola virus infection, Zaire. Liver. Hepatocellular necrosis expanding from foci, marked in this case by zones of cells which are intact but undergoing eosinophilic cytoplasmic change, pyknosis, and dissolution of nuclei. Hematoxylin and eosin; X 250.
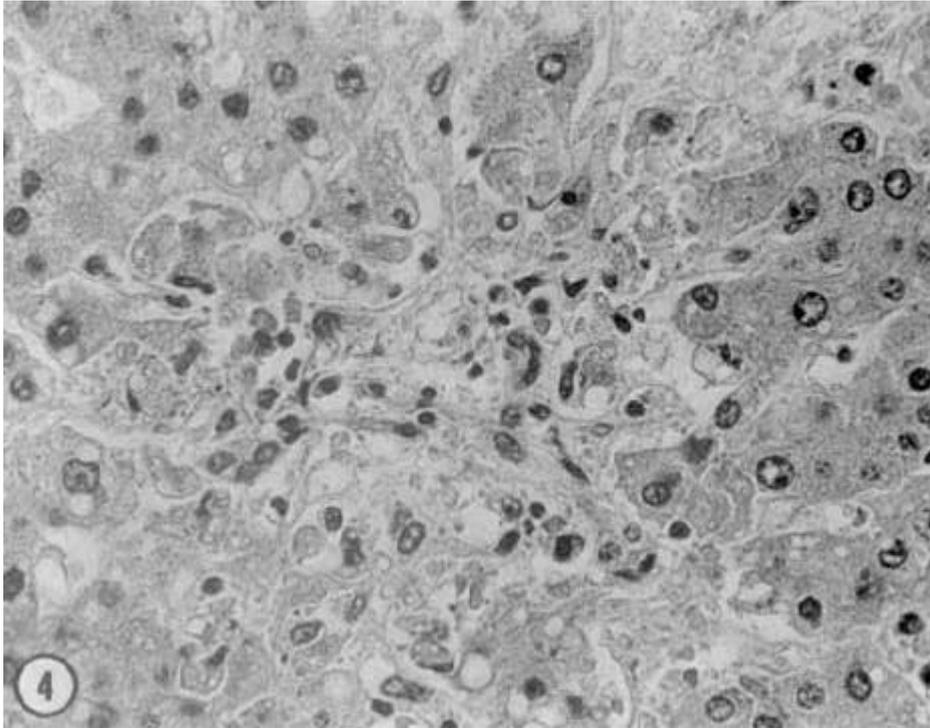
Fig. 4. Ebola virus infection, Zaire. Liver; same case as in Figure 3. A discrete focus of necrosis illustrating the paucity of inflammatory infiltration. Hematoxylin and eosin; X 375.
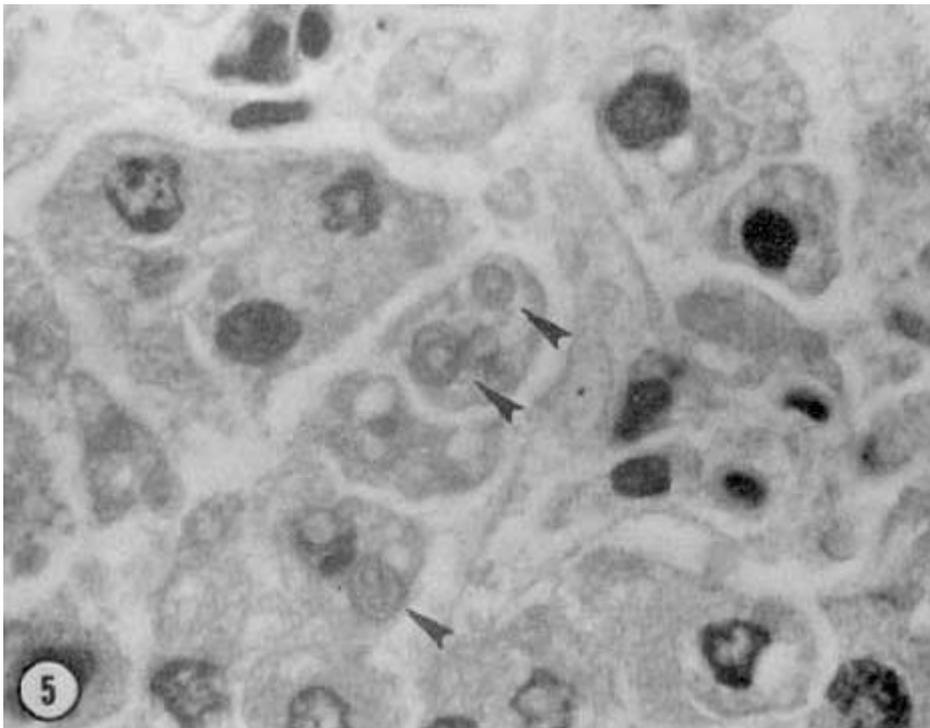
Fig. 5. Ebola virus infection, Zaire. Liver. Intracytoplasmic inclusion bodies (arrows) can only be discerned from Councilman-like bodies when found within intact hepatocytes. Hematoxylin and eosin; X 1400.
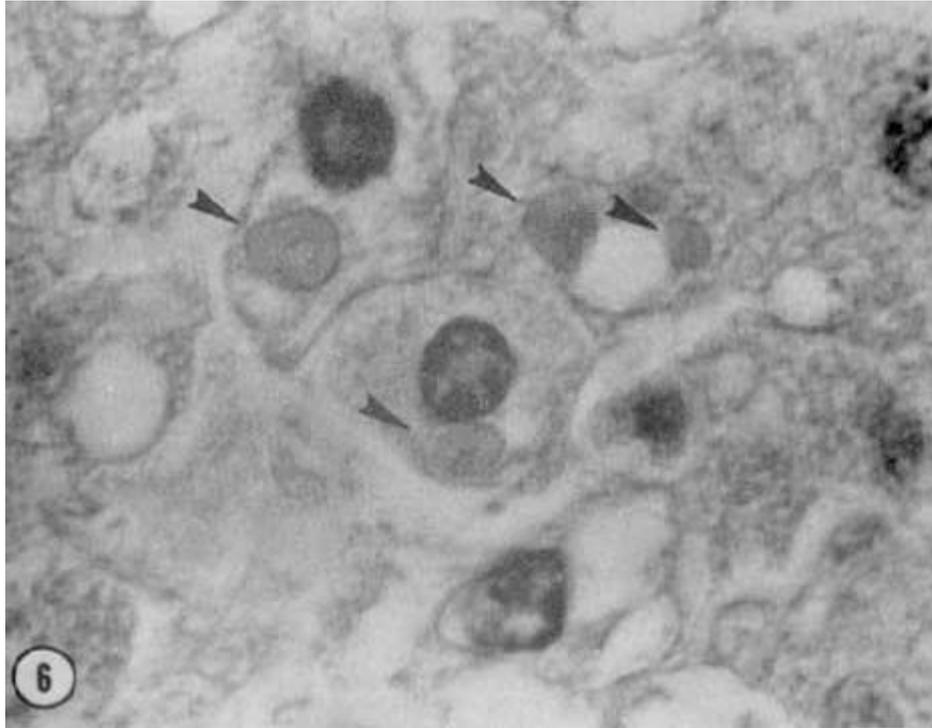
Fig. 6. Ebola virus infection, Zaire. Liver. Inclusion bodies (arrows), which are magenta in color when stained by hematoxylin and eosin, were not rendered more discernable from Councilman-like bodies by any of the several inclusion stains tried. Hematoxylin and eosin; X 1600.
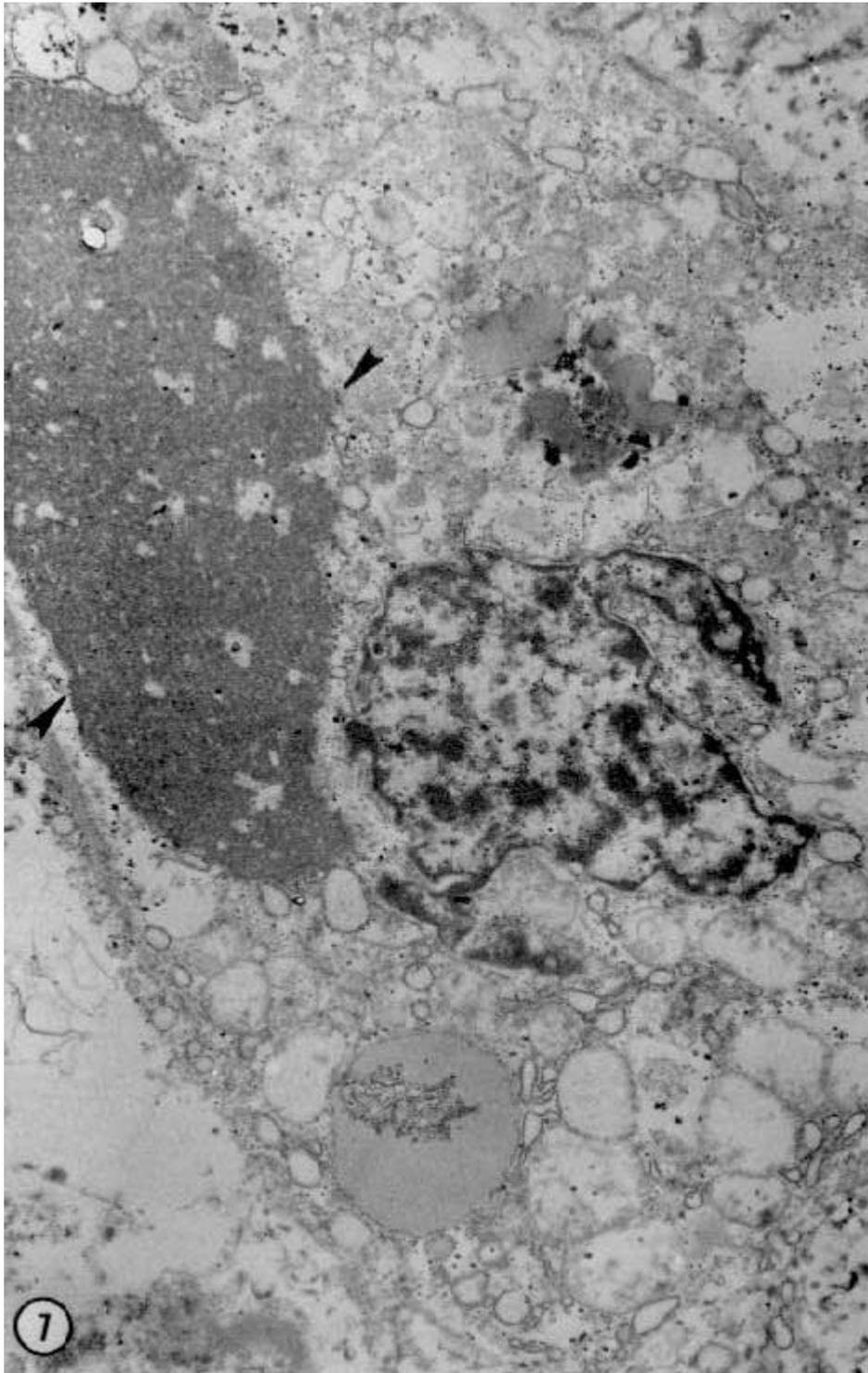
Fig. 7. Ebola virus infection, Zaire. Liver; formalin-fixed tissue processed for electron microscopy. Identity of inclusion bodies (arrows) was confirmed as being identical to structures found in Ebola virus infected cell cultures. Thin section; X 11,000.
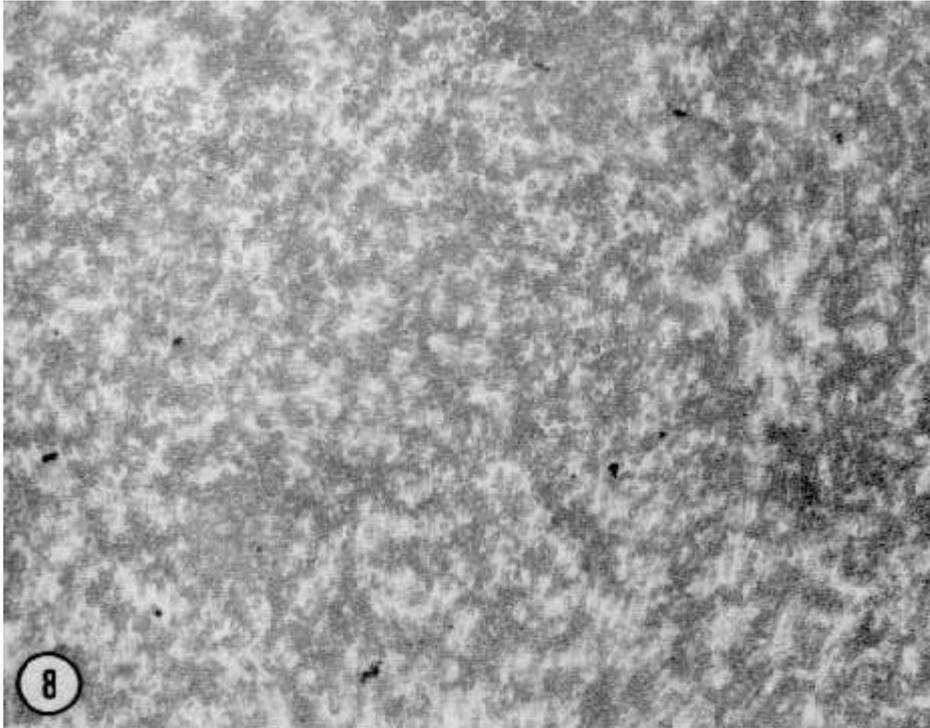
Fig. 8. Ebola virus infection, Zaire. Liver; formalin-fixed tissue processed for electron microscopy. At higher magnification inclusions such as that illustrated in Figure 7 were found to consist of cylindrical structures in an amorphous matrix. Thin section; X 46,000.
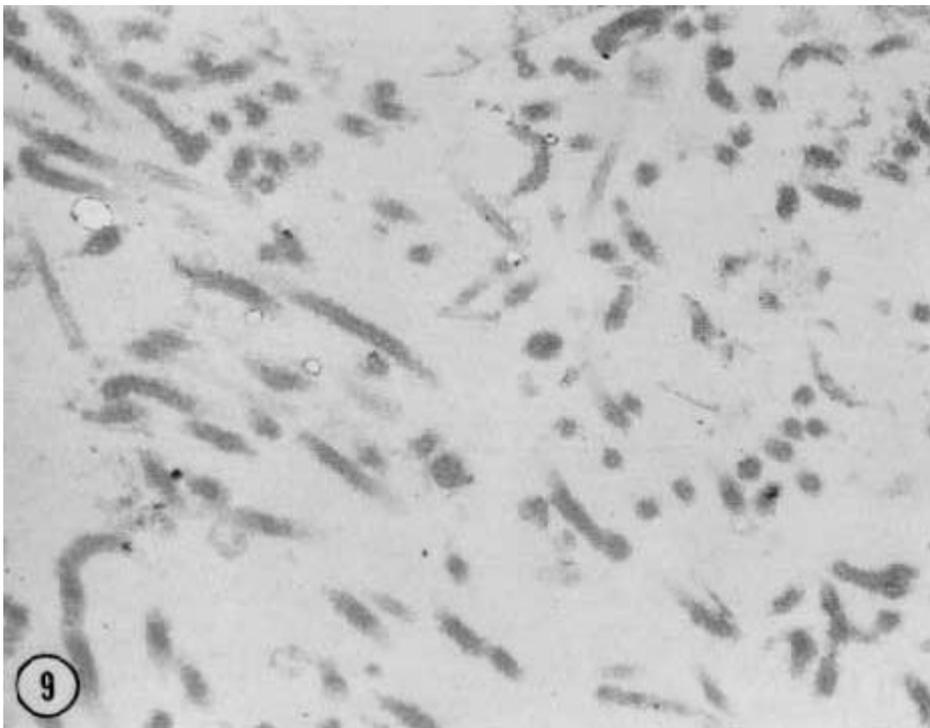
Fig. 9. Ebola virus infection, Zaire. Liver; formalin-fixed tissue processed for electron microscopy. Large numbers of virus particles within a distended sinusoid in an area of severe hepatocellular necrosis. Thin section; X 40,000.
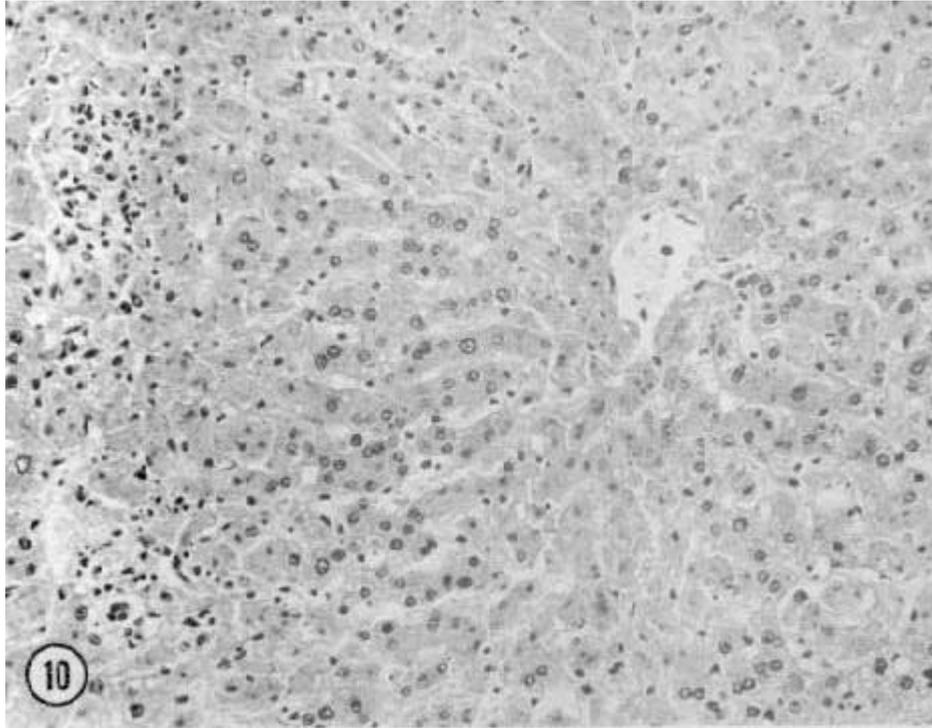
Fig. 10. Marburg virus infection, South Africa, 1975. Liver. Focal hepatocellular necrosis marked by cytoplasmic eosinophilia, nuclear pyknosis and nuclear dissolution. Hematoxylin and eosin; X 250.
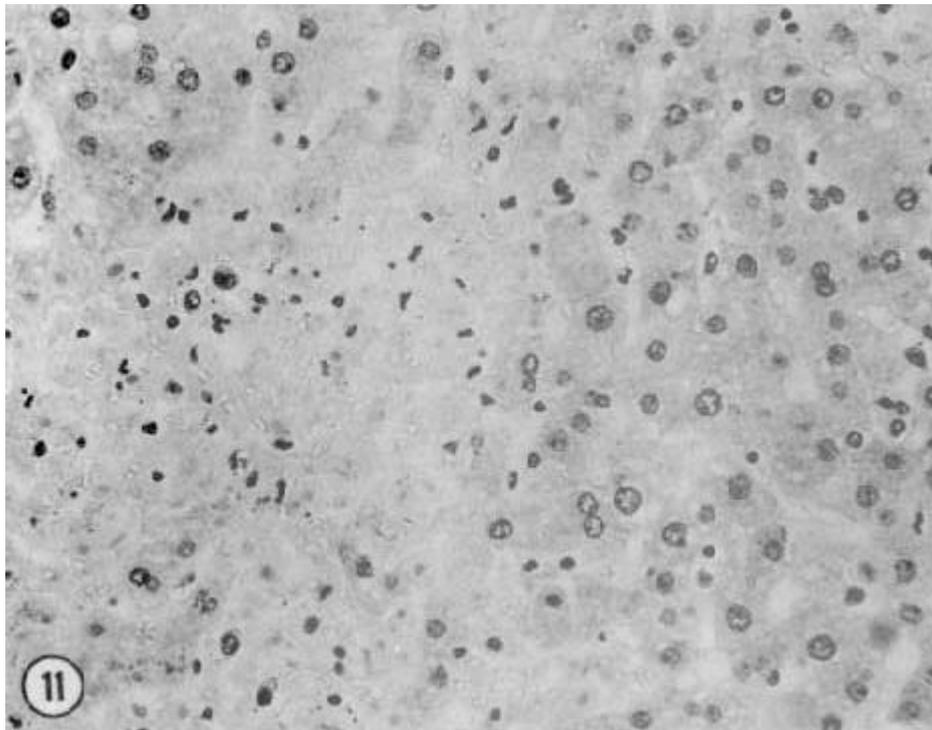
Fig. 11. Marburg virus infection, South Africa, 1975. Liver. A focus of infection in which total dissolution of liver cells has left only acellular debris. Hematoxylin and eosin; X 250.
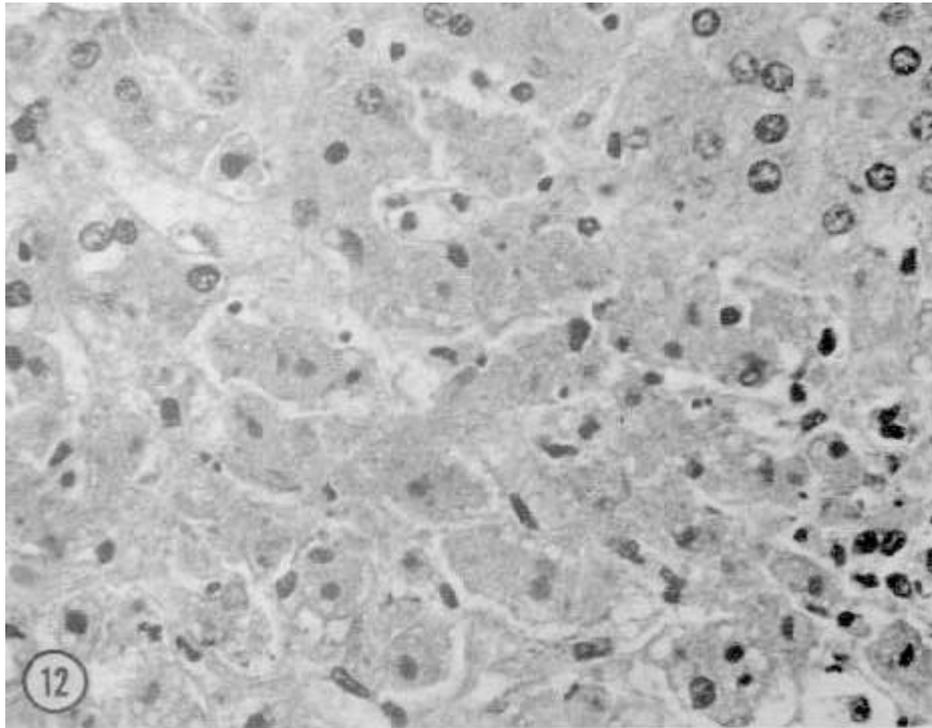
Fig. 12. Marburg virus infection, South Africa, 1975. Liver. Hepatocellular necrosis in this area is marked by nearly synchronous reduction of cells to intact forms with only pyknotic or ghostly remnants of nuclear profiles. Hematoxylin and eosin; X 350.
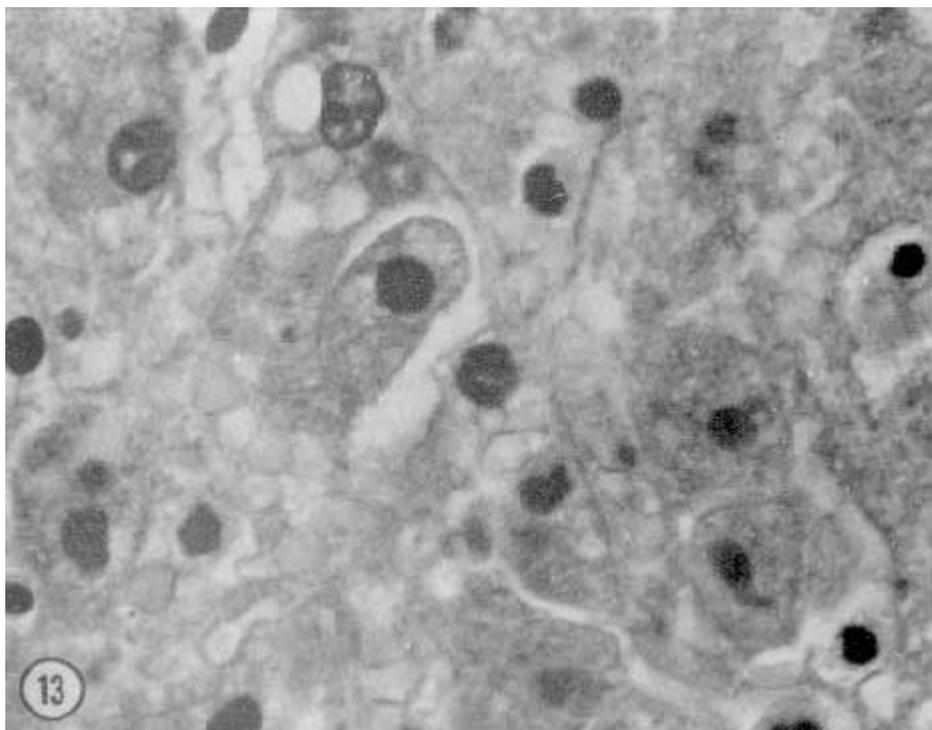
Fig. 13. Marburg virus infection, South Africa, 1975. Liver. Higher magnification showing pyknosis (center) and nuclear dissolution (lower right) in intact hepatocytes. Hematoxylin and eosin; X 1400.
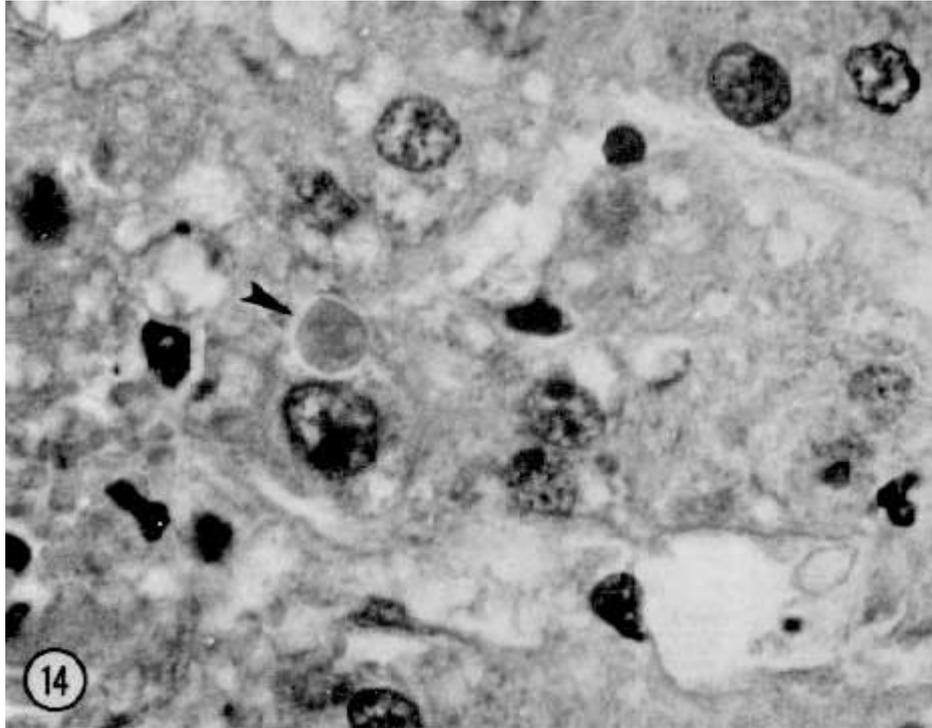
Fig. 14. Marburg virus infection, South Africa, 1975. Liver. Inclusion body (arrow) within the cytoplasm of an intact hepatocyte. Hematoxylin and eosin; X 1400.
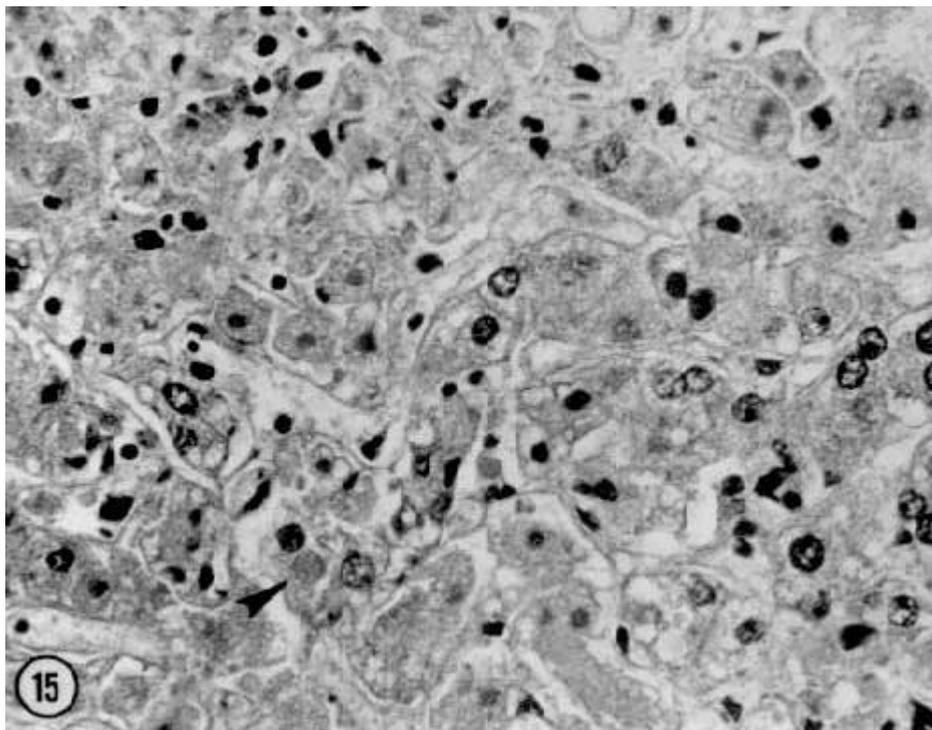
Fig. 15. Marburg virus infection, South Africa, 1975. Liver. The identification of inclusion bodies (arrow) is extremely difficult with a background of extensive necrosis in which Councilman-like bodies are often produced. Hematoxylin and eosin; X 350.
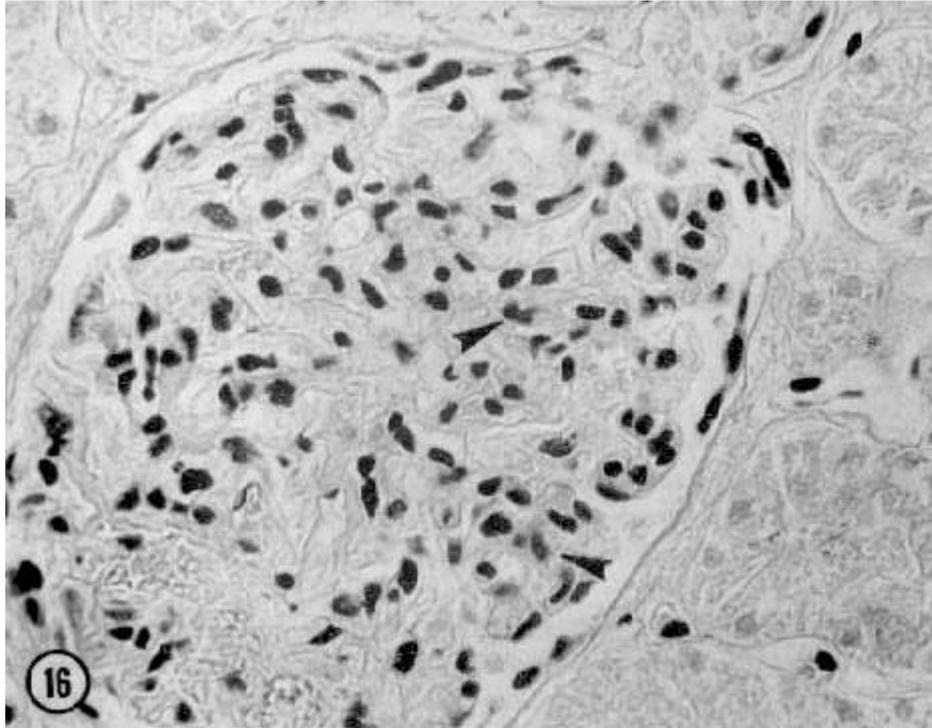
Fig. 16. Marburg virus infection, South Africa, 1975. Kidney. Multifocal fibrin thrombi (arrows) in glomerular capillaries, characteristic of disseminated intravascular coagulopathy. Hematoxylin and eosin; X 450.
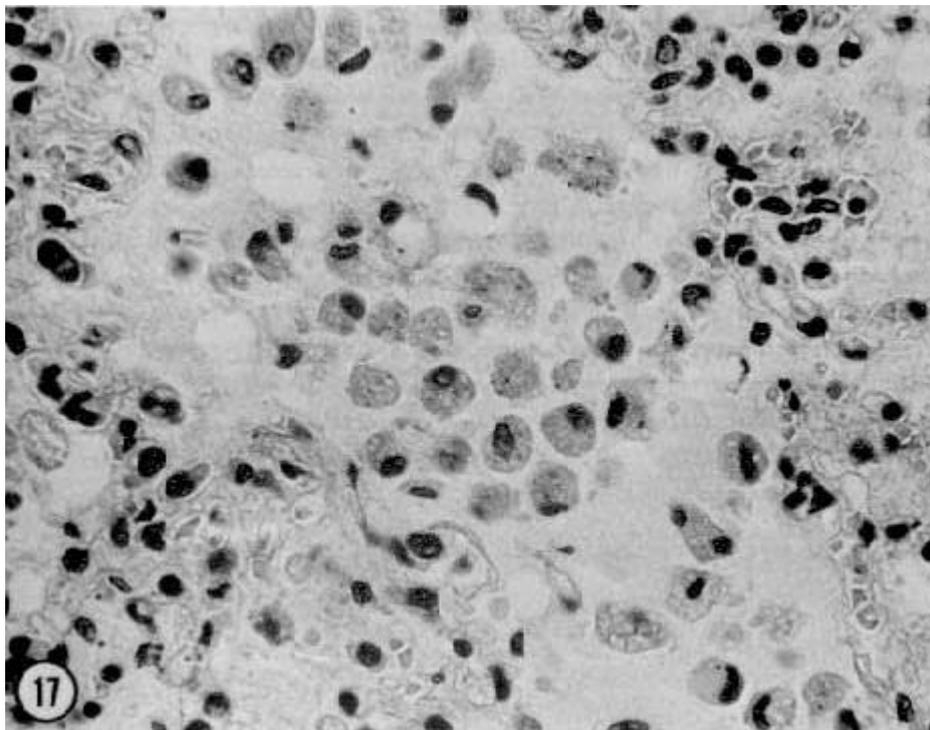
Fig. 17.
Marburg virus infection, South Africa, 1975. Lung. Effusion of macrophages into alveolar space was associated with pulmonary edema (complicated by Candida infection). Hematoxylin and eosin; X 450.

EBOLA AND MARBURG VIRUS MORPHOLOGY AND TAXONOMY

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In 1967, in an era when it was felt with a degree of savoir-faire that every possible morphologic form of pathogenic viruses had already been visualized, the first electron microscopic observations of Marburg virus were absolutely hair-raising. Despite the temperate choice of terms used in the literature, the same sense of wonder was felt in each of the laboratories involved as they found the bizarre filamentous agent. Following the 1967 disease episode, Marburg virus morphology and morphogenesis were studied in detail in several countries by negative contrast and thin-section electron microscopy after propagation in cell cultures, and in the organs and body fluids of humans, monkeys, and guinea pigs (1-7).

Electron microscopic studies following the 1975 Marburg virus infections in South Africa were limited to cell culture preparations and liver specimens from the single fatal case (CDC and South African Institute for Medical Research, Johannesburg). Ebola virus morphology studies have been concentrated on human liver tissue (8) (CDC and Prince Leopold Institute of Tropical Medicine, Antwerp), guinea pig tissues (9) (Microbiological Research Establishment, Porton Down) and cell culture preparations (8,9,10) (all laboratories). After careful comparison of morphologic and morphogenetic details of the viruses isolated in the three disease episodes, it seems clear that they are indistinguishable and only separable by the antigenic properties described elsewhere in this Colloquium. Minor differences in structure which have been noted can be attributed to variations in 1) the condition of virus preparations, 2) the nature Of background material, 3) the effects of fixatives used for biohazard containment, and 4) the methods used for electron microscopic preparation in various laboratories. Therefore, the following morphologic and morphogenetic descriptions are meant to be generally representative and illustrations of details are taken from studies with viruses from both Marburg episodes and with Ebola virus from Zaire and the Sudan.

<u>Virus Morphology</u>

In their native state, Marburg and Ebola virus particles are pleomorphic, appearing in negative contrast preparations as either long filamentous forms or "U"-shaped, "6"-shaped, or circular forms (Figures 1,2,3). The virus particles are composed of an internal helical structure which is presumed to be the nucleocapsid, a unit-membrane envelope, and a surface projection layer (Fig.4), Particles are approximately 80nm in diameter and extremely variable in length. Lengths of Marburg virus particles measured at CDC in 1967 ranged randomly between 130 and 2600nm. Peters and his colleagues 4, however, found that a median length of Marburg virus particles was 665nm and that longer particles appeared to occur in multiples of this length. In 1967 Marburg virus particles as long as 8,000nm were found, and this year Ebola virus particles up to 14,000nm were measured. The shorter and circular-shaped or '6"-shaped particles predominate in tissues and body fluids of man and experimentally infected animals, and in cell cultures there are more anomalous particles containing bizarre windings of nucleocapsid strands, more branched particles, and more very long particles (Figures 5,6). In a serial harvest series of Ebola virusinfected Vero cell culture supernatant fluids, the proportion of the various particle shapes and lengths did not vary between 1 and 4 days postinfection.

The virus particle surface projection layer is composed of spikes about 10nm long; the character of the spikes is significantly affected by fixation and staining conditions. The envelope layer is clearly formed of host cell membrane and along the length of the filamentous particles this envelope is rather closely apposed to the nucleocapsid. The envelope is distended over the wound nucleocapsid of "6"-shaped and circular-shaped particles, and over terminal windings of nucleocapsid which are common at one end of long particles. The envelope layer is often incomplete over the terminal bleb of long particles; this probably results from avulsion of virus particles rather than neat pinching off at the end of the budding process. Envelope blebbing can occur elsewhere along the length of the filamentous particles, probably as a result of osmotic shock during preparation for electron microscopy. The nucleocapsid structure is complex; it consists of a dark (stain penetrated) central axis, 20-30nm in

diameter, surrounded by a light helically wound capsid with a diameter of 40-50nm and a cross-striation interval of 5nm, The outer edge of the nucleocapsid often appears fuzzy and thick so that in intact particles the separation between the main nucleocapsid layer and the envelope is often indistinct. Peters and his colleagues 4 defined another "intermediate layer" beneath the envelope. Particles occur which have a uniform diameter of 80nm and surface projections but no internal structure; in other cases long particles may contain segments of the internal structure and often the envelope diameter is reduced beyond the ends of the internal structure or between segments.

Variations in negative contrast methods can affect the appearance of virus particles and this can add some confusion to virus identification. Virus particles from fresh unfixed cell culture supernatant preparations exposed to negative contrast media for short times at near neutral PH (as with droplet methods with phosphotungstate or silicotungstate stains) are usually unpenetrated and appear as smooth membrane-bound forms with surface projections. In some cases fixation of fresh virus (gluteraldehyde or formaldehyde) seems to stabilize the viral envelope so that the same forms are obtained. Caution must be exercised in distinguishing these unpenetrated particles from the normal microvillous projections of plasma membranes common in many cell cultures. When the negative contrast method favors stain penetration, the resolution of the unique nucleocapsid structure makes identification of Marburg/Ebola viruses unquestionable. In a recent study at CDC, Ebola virus in Vero cell culture supernatant fluid was fixed with 0.5% gluteraldehyde for 1 hour and subjected to several negative contrast techniques in order to determine which methods yielded the most clearly identifiable particles. We found that all methods in which stain exposure was extended, PH was lowered (as with uranyl acetate staining - PH 4.5), or there was exposure to a lipid solvent (as with pseudoreplication from an agar surface with a formvar film cast in ethylene dichloride), nucleocapsids were well resolved and identification was certain. The glutaraldehyde fixation slightly obscured cross-striation detail of nucleocapsids; in comparison, the formaldehyde fixation schemes used in 1967 seem to have left fine structural details intact. In any case, there is no reason for carrying out negative staining on viable organisms. Finally, in this same Ebola virus study some further insight was gained into the practical sensitivity of negative contrast electron microscopy in a diagnostic setting. When we diluted daily harvests of Vero cell culture supernatant fluids fourfold with gluteraldehyde (final concentration 0.5") in water, and then ultracentrifuged these preparations at 25,000 PRM for one hour, virus particles were easily found even in 24 hour postinfection specimens, and extraordinary numbers of particles were present in all subsequent specimens. In this case the virus inoculum had been passaged in Vero cells previously, so high MOIs and rapid growth rates were obtained, but in a diagnostic setting a search for Particles in inoculated Vero cell cultures should start at the same time immunofluorescence testing begins -- that is at 24 to 48 hours.

Virus Morphogenesis and Cytopathology

The ultrastructural events involved in Marburg and Ebola virus morphogenesis and the associated changes in host cells have been examined in human liver tissue, in guinea pig organs, in monkey organs (so far only Marburg virus), and in cell cultures 3,4,6,7,8,9. in each case the viruses have been shown to be constructed from preformed nucleocapsids, which develop within cytoplasm, and envelopes which are added via budding through plasma membranes (Figure 7). Surface projections are inserted in the viral envelope at the bud site. The budding process is not seen often in human or experimental animal tissues, partly because of the convolutions of host cell membranes in relation to plane of section and the asynchrony of infection. Budding is seen commonly in infected cell cultures; the apparently regulated formation of simple filamentous particles contrasts with the violent plasma membrane deformations involved in envelopment of pleomorphic particles. The avulsion of plasma membrane at the termination of the budding process is not like the usual pinching off of other enveloped viruses.

Nucleocapsid formation and accumulation in cytoplasm leads to massive inclusion bodies, both in vivo and in cell cultures. Early condensation of nucleocapsids occurs in amorphous or granular matrices, and most young inclusions consist of variable proportions of matrix (? constituent ribonucleoprotein and nucleic acid) and filamentous nucleocapsids (Figure 8). Judging from the daily harvest series of Ebola virus-infected Vero cells, these inclusions seem more likely to be the "factories" for nucleocapsids going on to form virus particles rather than accumulations of leftover constituents; that is, these inclusions appear early -- at the time when virus particle formation is starting. In Ebola virus-infected Vero cells the median length of nucleocapsids (with entire lengths in plane of section) associated with these early inclusions was 750nm. Variations in inclusion body structure have been seen commonly, but there is still no understanding of their nature. For example, some early inclusions consist of masses of 50 to 60nm spheres (Figure 9). Some late inclusions consist of crystalline arrays of nucleocapsid cylinders, and others consist of amorphous dense material (Figures 10,11). In one case (Marburg virus of 1975) infected cells accumulated extremely dense flat sheets of unrecognizable

material. Perhaps all the late inclusions represent anomalous variations in the condensation of viral constituents, but adequate studies have not been done.

The cytopathic change in tissues and in cultured cells infected with Marburg or Ebola viruses is striking, especially because cells are not arrested in late stages of the common terminal pathway of cytonecrosis. The progression of cel destruction has been shown to be similar in cell culture (for example, in an Ebola virus-infected Vero cell harvest series) and in vivo (for example, in monkey liver; 7). Infection processes, of course, form a continuum, but for convenience 4 stages can be distinguished. In the first stage of infection, virus particle budding and inclusion body formation occurs without apparent effect upon the morphologic appearance of cell organelles. Large amounts of virus are formed in this stage (Figures 7,10). In the second stage of infectio virus particle budding and inclusion body formation continue in cells with dilated endoplasmic reticulum, beginning intracytoplasmic vesiculation, and mitochondrial damage (loss of cristae and swelling) (Figures 12,13,14). In the third stage of infection, virus production ceases as the breakdown of cytoplas mic organelles and associated endophagocytosis (lysosomal response) continues. In this stage there is a change in cytoplasmic and nuclear density -- the alternate pathways to cell death appear as condensation or rarification. In the fourth stage of infection, destruction of membrane systems, including the nuclear and plasma membranes, reduces cells to debris (Figures 15,16). The progression of infection to this last stage is extreme with the two viruses. The degree of dissolution of infected cells in the liver of monkeys inoculated wit Marburg virus is so pervasive that focal sites contain only the vestiges of cellular structure. In Ebola virus-infected Vero cells the progression of infection in individual cells is rapid; between 48 and 96 hours postinfection, when more and more cells are still becoming infected; there is no shift in the proportion of cells observed in late versus ealy stages of infection, and ther is no build-up of intact dead cells as would be the case with most other viral infections.

Overall, the morphologically visible events in Marburg and Ebola virus infections at the cellular level seem as devastating as the effects of infection at the clinical level.

<u>Virus Taxonomy</u>

At the time of the initial characterizations of Marburg virus, morphologic similarities with rabies, vesicular stomatitis and other rhabdoviruses were noted(4,5,6). In the years since then, the isolation of many more viruses with physicochemical and morphologic characteristics very similar to the prototype rhabdoviruses has led to a more precise definition of the taxon, the Rhabdoviridae family. At the same time the differences in construction of the rhabdoviruses and Marburg and Ebola viruses have become more widely appreciated

Physicochemical characterization data must be comprehensive if they are to be of value for taxonomic consideration, but most properties of Marburg and Ebola virus remain untested, suggestive, or unconfirmed. Our lack of progress has been due entirely to the biohazards involved in viral biochemistry laboratories.

If the available physicochemical data were examined at this time in an objective context by the International Committee on Taxonomy of Viruses (ICTV), it is likely that all taxonomic considerations would be deferred. However, we have an immediate need for a nomenclature which will avoid the perpetuation of competing terms -- this need has been made quite clear in the past year. Although the names of the two viruses, Marburg and Ebola, are settled into general use, nomenclature for the "group" is confusing. For example, use of the term "African hemorrhagic fever" to describe the disease syndrome caused by the two viruses may have clinical value, but the term does not sit well as a virus genus or family designation. Alternately, the term "tuburnavirus", as advanced by Simpson and Zuckerman(11) as a taxonomic designation, has not been submitted to the ICTV for consideration by the virology community. In response to this situation the ICTV has asked its Vertebrate Virus Subcommittee (F.A. Murphy, chairman) to undertake a study of the matter. Toward this end, the Rhabdovirus Study Group (F. Brown, Animal Virus Research Institute, Pirbright, chairman) is solic iting, from working virologists, virus characterization data and opinions regarding nomenclature. Dr. Brown would welcome all experimental data and personal opinions. If the data warrant construction of a new taxon this will be done, but in any event the matter of nomenclature will be settled democratically and as soon as possible.

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This is not a comprehensive listing of the Marburg virus pathology literature. Extended bibliographies are found in references 4,5,6 and 7 and in the book Marburg Virus Disease (edited by Martini, G.A., and Siegert, R.) Springer Verlag, New York 1971.

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Fig. 1. Ebola virus. Unfixed diagnostic specimen from first Vero cell passage, showing elongated particle shape, but no internal tail. Sodium phosphotungstate; X 90,000.

Fig. 2. Ebola virus. Glutaraldehyde fixed particle from Vero cell culture supernatant; particles up to 14,000nm long were found in such preparations. Uranyl acetate; X 28,000.

Fig. 3. Ebola virus. Glutaraldehyde fixed particles from Vero cell culture supernatant; typical "6-shaped" configuration such as was common in blood of Marburg virus infected animals. Uranyl acetate; X 66,000.

Fig. 4. Ebola virus. Unfixed diagnostic specimen from first Vero cell passage, showing cross-striations of internal helical structure and surrounding envelope layer. Sodium phosphotungstate; X 156,000.

Fig. 5. Ebola virus. Glutaraldehyde fixed particle from Vero cell culture supernatant; there was more evidence of branching in such preparations than previously found. Uranyl acetate; X 43,000.

Fig. 6. Ebola virus. Glutaraldehyde fixed particle from Vero cell culture supernatant; elaborate windings of the internal structure occurred within the envelope blebs of many particles. Sodium phosphotungstate; X 39,000.

Fig. 7. Ebola virus. Vero cell culture, day 2; virus particles budding from plasma membrane (arrows) with "nucleocapsids" in cytoplasm. Thin section; X 37,000.

Fig. 8. Ebola virus. Vero cell culture, day 2; inclusion body (arrows) consisting primarily of amorphous matrix within the cytoplasm of a cell in an early cytopathic state. Thin section; X 9,000.

Fig. 9. Marburg virus. Vero cell culture, day 3; uncommon configuration of intracytoplasmic inclusion body in which 50-60nm spheres occur at the edges of an amorphous matrix. Thin section; X 39,000.

Fig. 10. Ebola virus. Vero cell culture, day 2; this most typical inclusion body configuration, consisting of precise cylinders in an amorphous matrix, is present in an otherwise normal cell. Thin section; X 19,000.

Fig. 11. Ebola virus. Vero cell culture, day 4; high magnification of the cylindrical structures which form most inclusions and constitute the internal structure of virus particles. Thin section; X 789000.

Fig. 12. Ebola virus. Vero cell culture, day 3; starting cytopathology marked by mitochondrial swelling and destruction while virus particle production continues.Thin section; X 13,000.

Fig. 13. Ebola virus. Vero cell culture, day 4; early cytopathology with mitochondrial destruction associated with massive cytoplasmic replacement with viral material. Entire plasma membrane involved in virus budding. Thin section; X 16,000.

Fig. 14. Ebola virus. Vero cell culture, day 4; high magnification of the surface of cell illustrated in Figure 13, showing nascent budding along whole plasma membrane as supplied by massive cytoplasmic infection. Thin section; X 46,000.

Fig. 15. Ebola virus. Vero cell culture, day 2; late cytopathology with organelle destruction and destruction of plasma membrane (top). Thin section; X 37,000.

Fig. 16. Ebola virus. Vero cell culture, day 4; terminal cytopathology marked by nuclear and cytoplasmic rarifaction, organelle destruction and frank dissolution of the plasma membrane. Thin section; X 16,000.

DISCUSSION

S.R. Pattyn : what about the name Toroviruses that has been proposed some time ago ?

F.A. Murphy Dr.Almeida proposed that name in 1970 or so, and nothing never happened. The I.C.T.V. will never involve itself in the names of the viruses per se and it seems to me that the terms Marburg, Ebola are entrenched. We need a family or genus term, but it should come from people who are working in the field and the Taxonomy Committee is there only to see that democracy is respected. Each of the common virus names has come out a different way and any one who has got a good name should advance it.

G.A. Eddy : It is possibly a little early to name this group of viruses, if indeed it is a group, someone should first Zook at the virion polypeptides.

F.A. Murphy : In considering the rhabdovirus study group, I would guess that they wouldn't want to do very much in a formal taxonomic matter, until more is known about the proteins and the nucleic acid. They might be willing to echo a nomenclature which seems to be needed now.

P. Brès : I would discourage the use of the term African Haemorrhagic Fever which may be rather confusing because this would include yellow fever. For reasons of symmetry you would have to say American Haemorrhagic Fever which would include Argentinian and Bolivian H.F. as well. This has been discussed with some people and we thought that Ebolavirus Haemorrhagic Fever would better describe the syndrome.

J. Casals : Is there enough virus in the blood of a patient so that you could do the same procedure with blood serum, and have a diagnosis within six hours maybe ?

F.A. Murphy : It was done in Germany on Marburg virus. Another thing that is much quicker, the Liver itself of the case in South Africa, 1975, fluoresced so brightly and specifically that the results could have been available in hours.

T. Muyembe : Why is the name Ebola jirus and not Yambuku ? Ebola is a river and was not involved in the epidemics.

F.A. Murphy : That's a long story. The people who were there at the time, not any individual, had the privilege of naming the virus. That name came out of a lot of discussion and I think if any one doesn't like the name now it's almost too late to discuss it.

77 nm.

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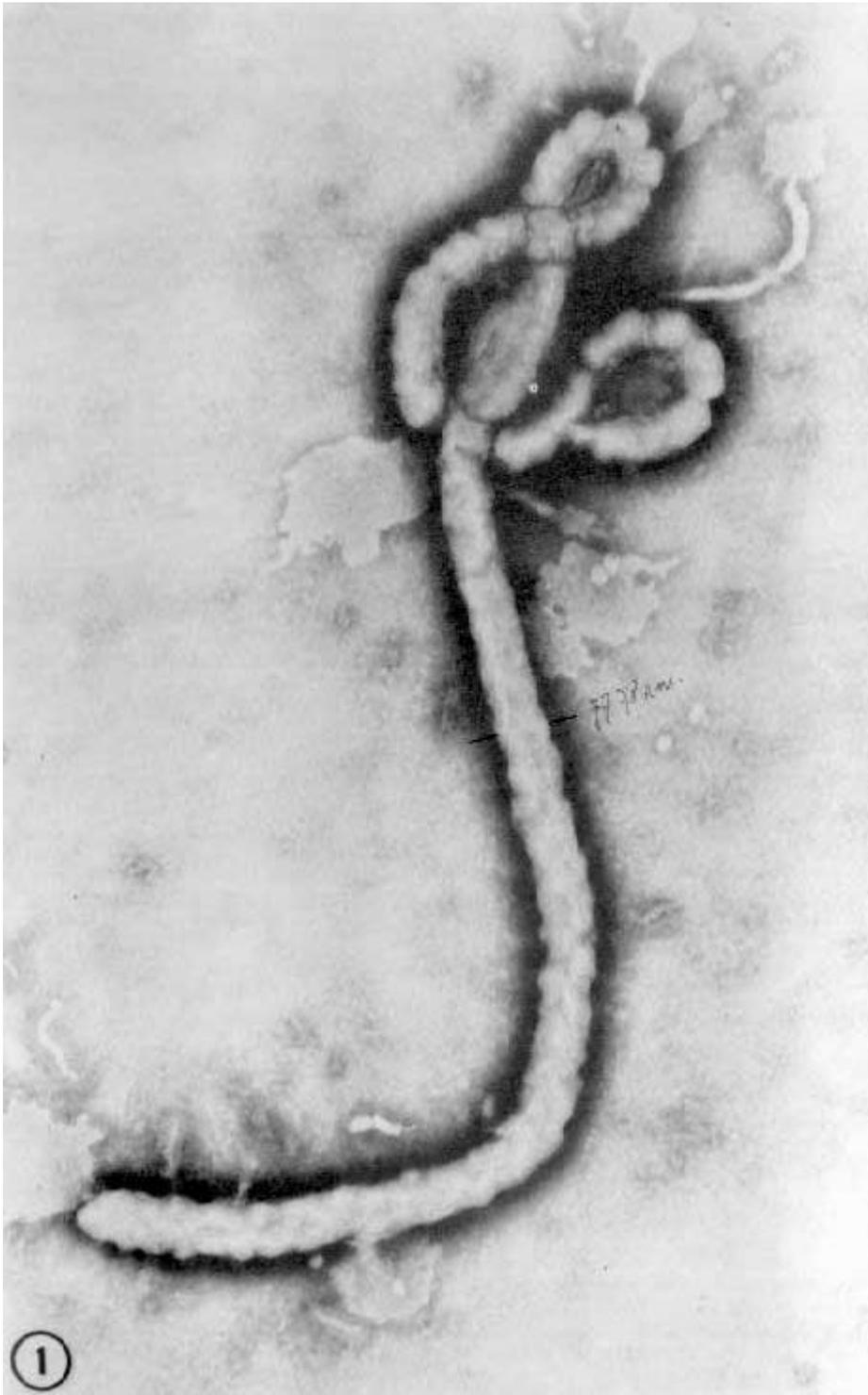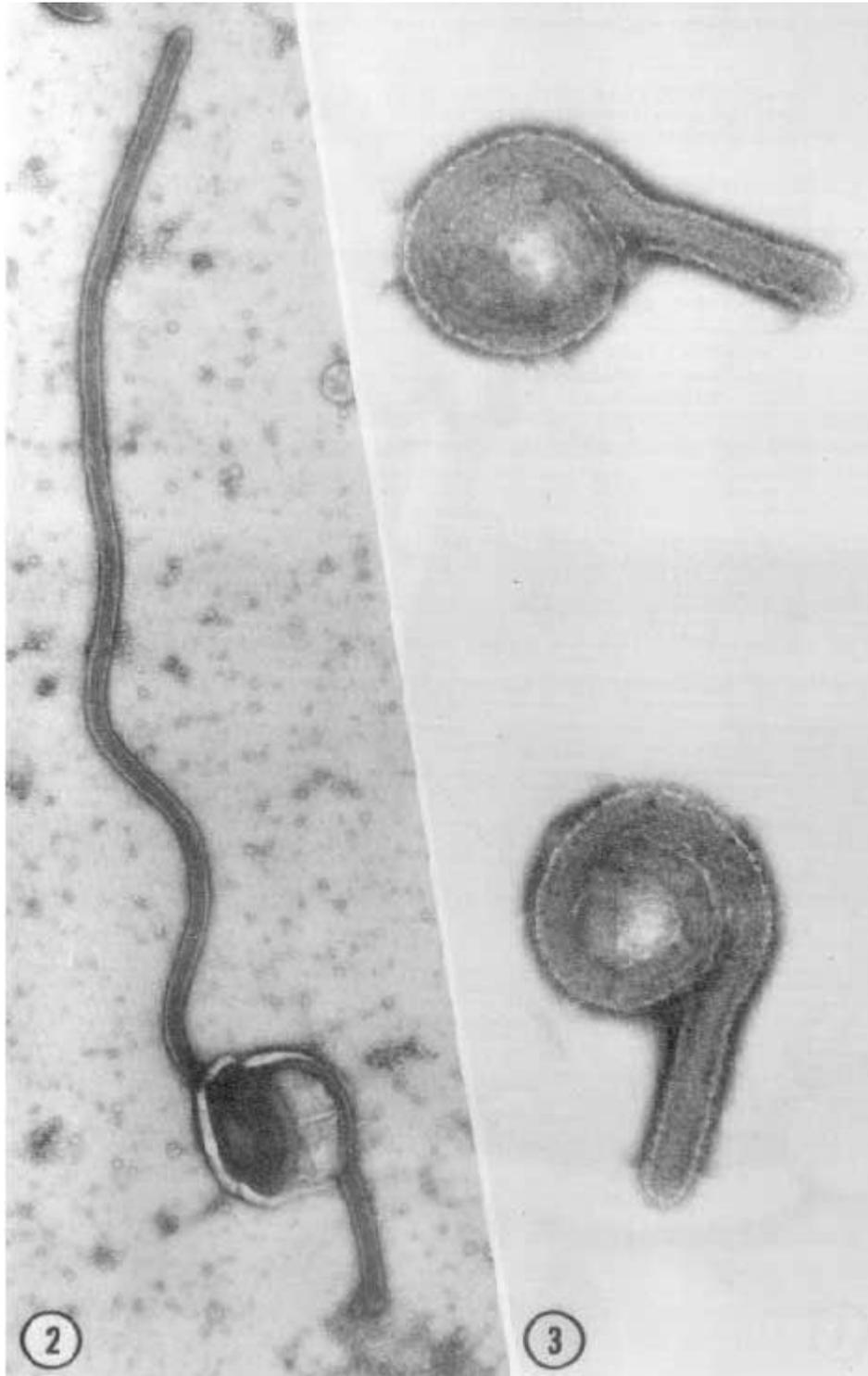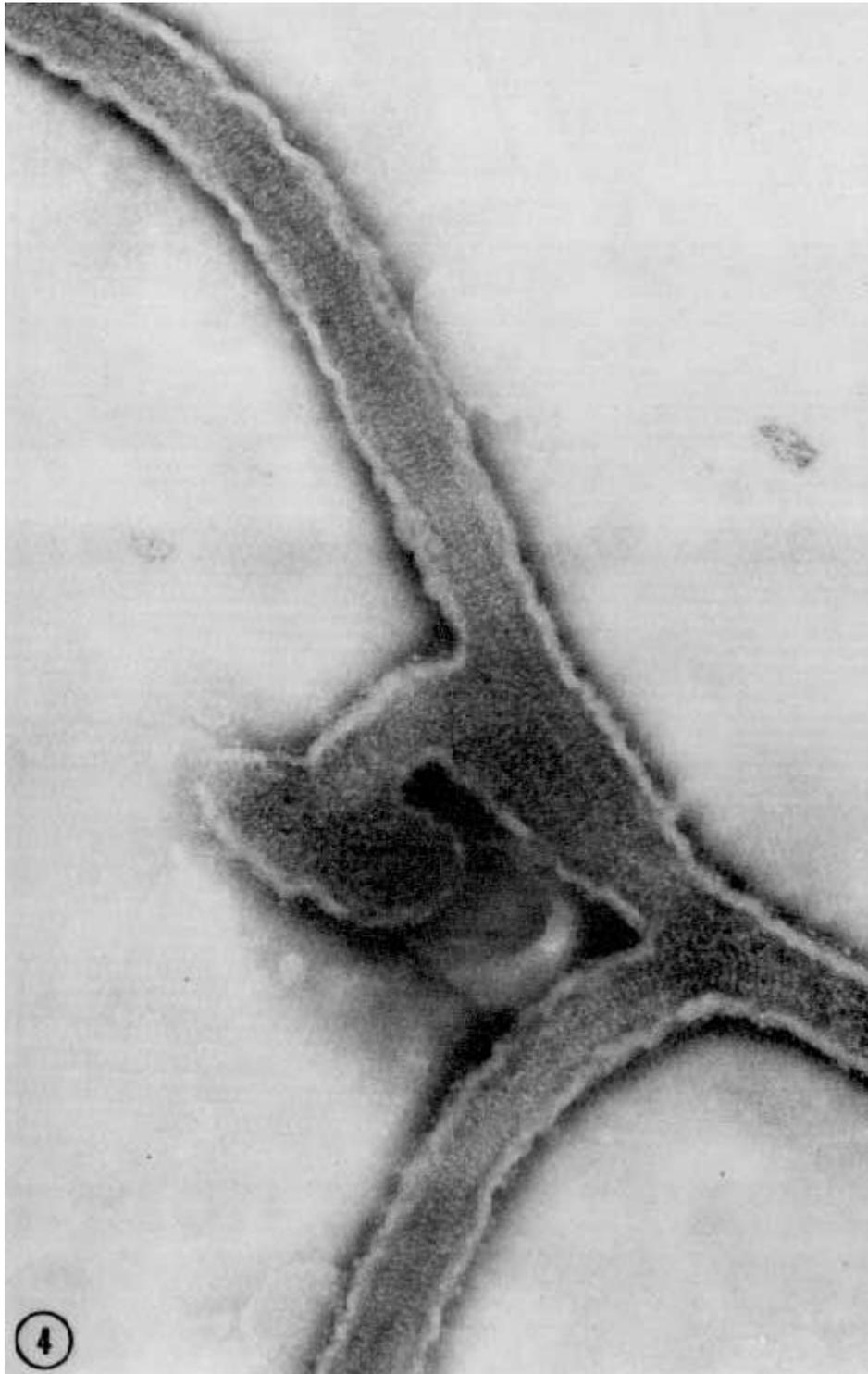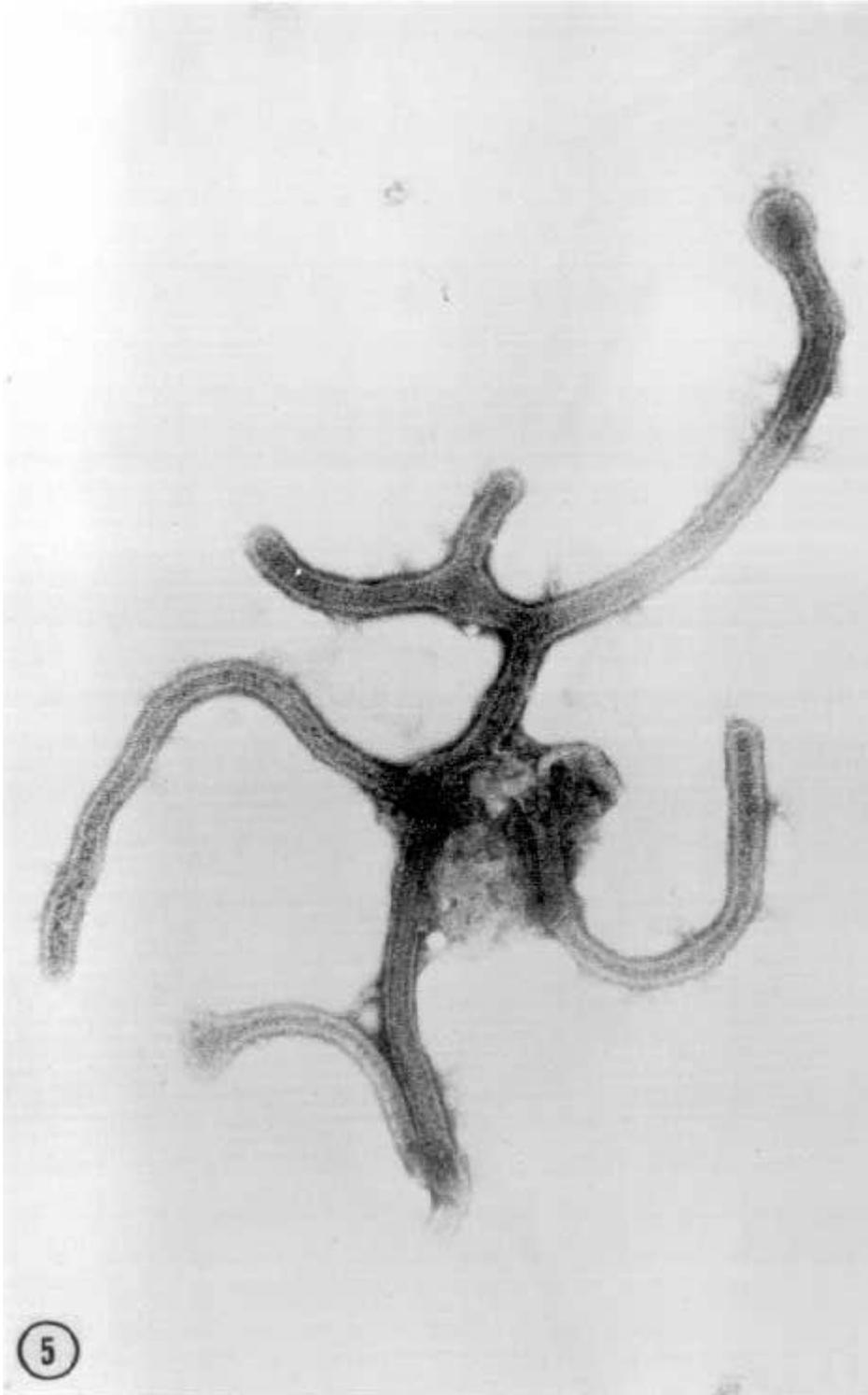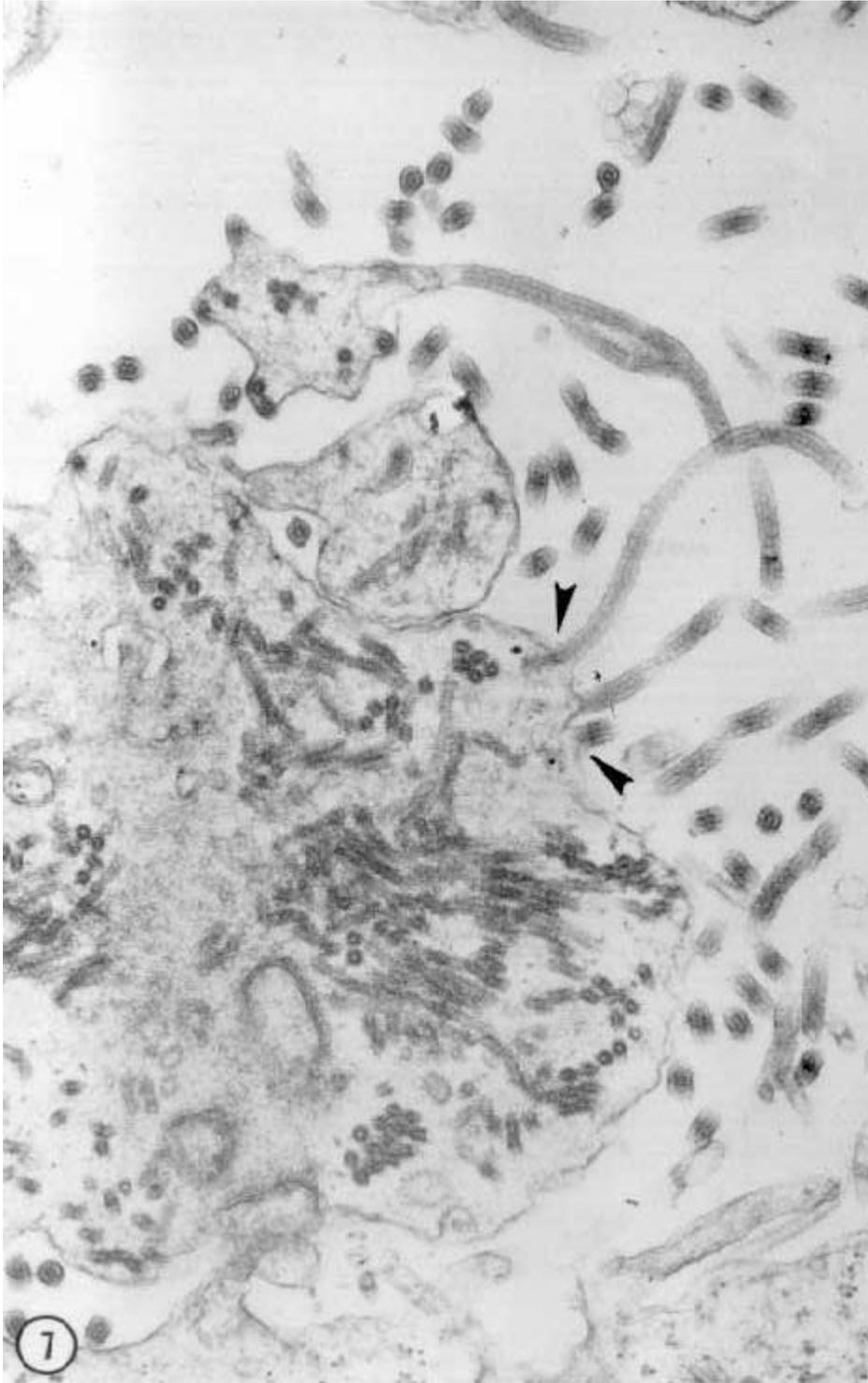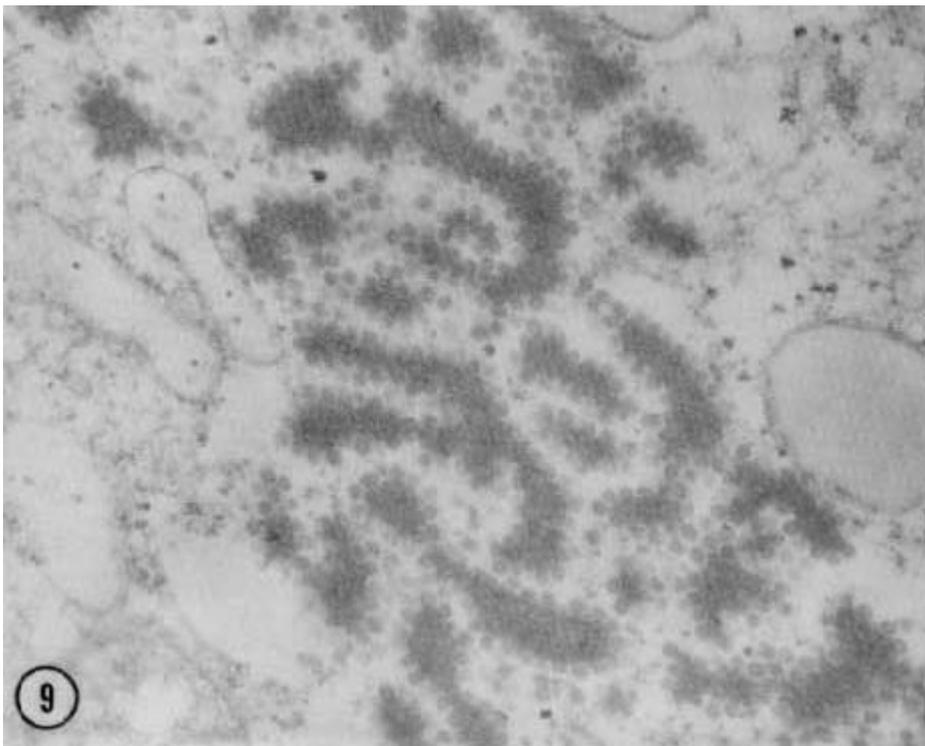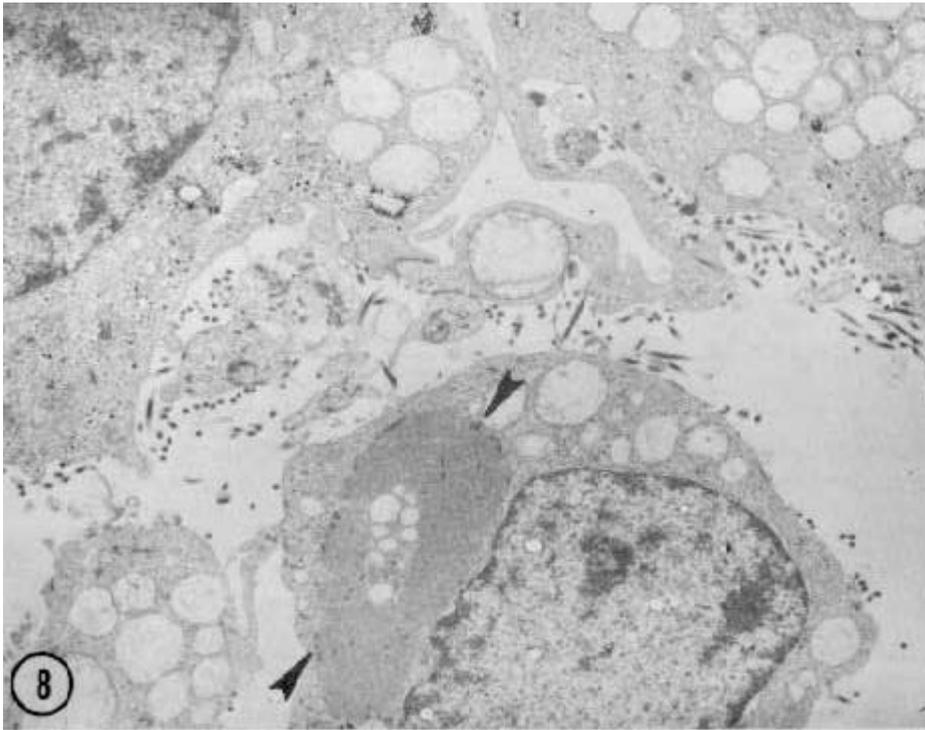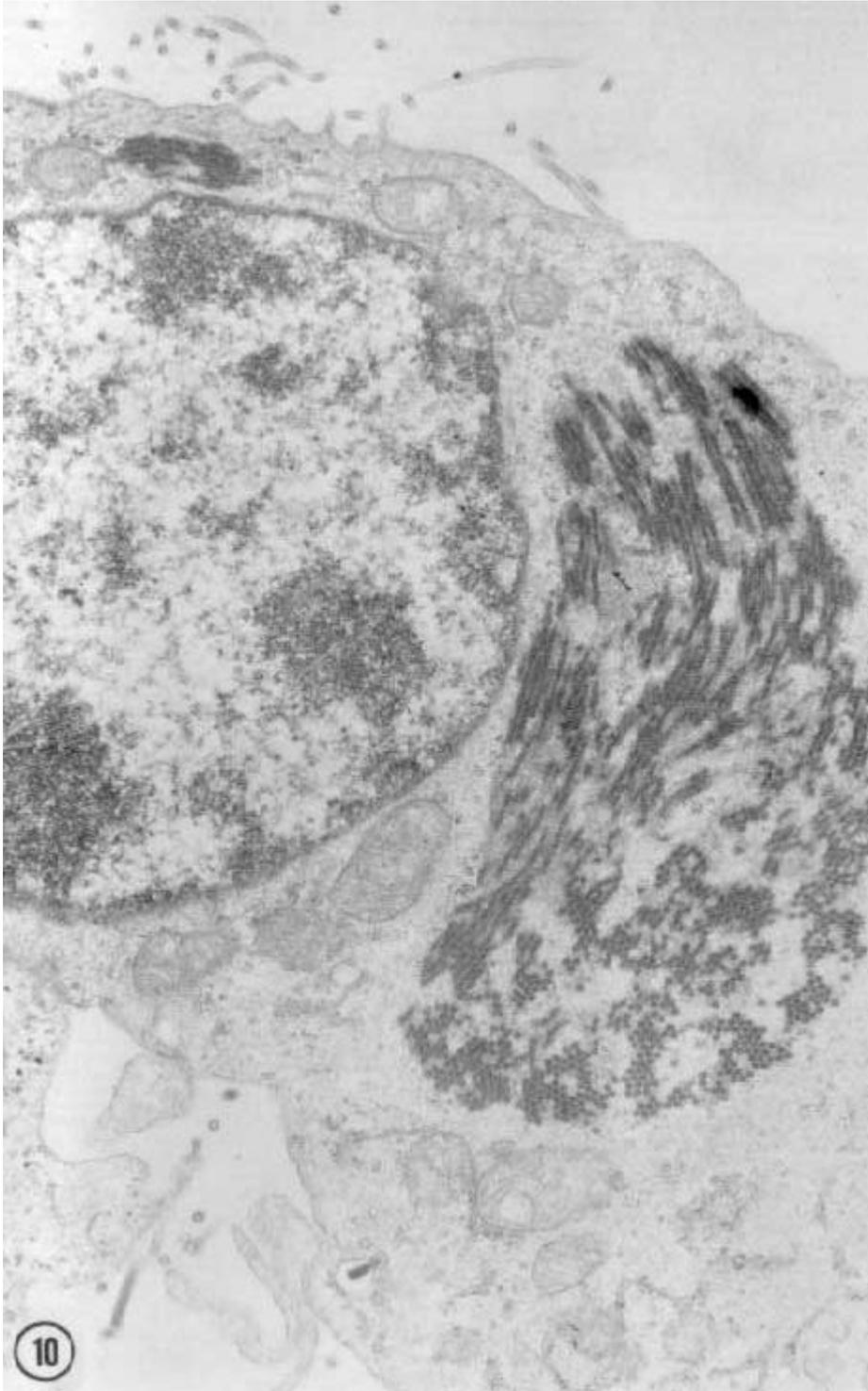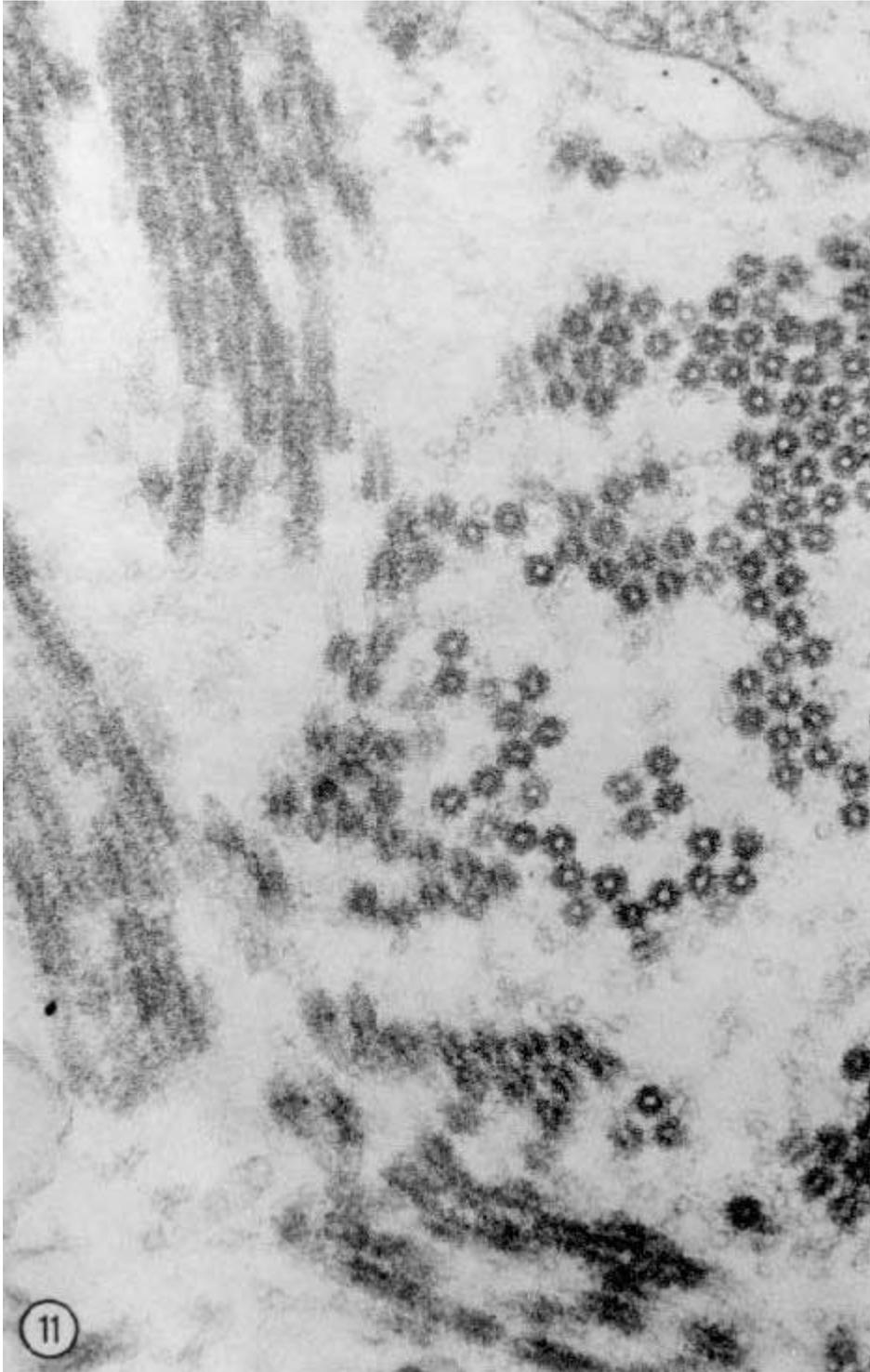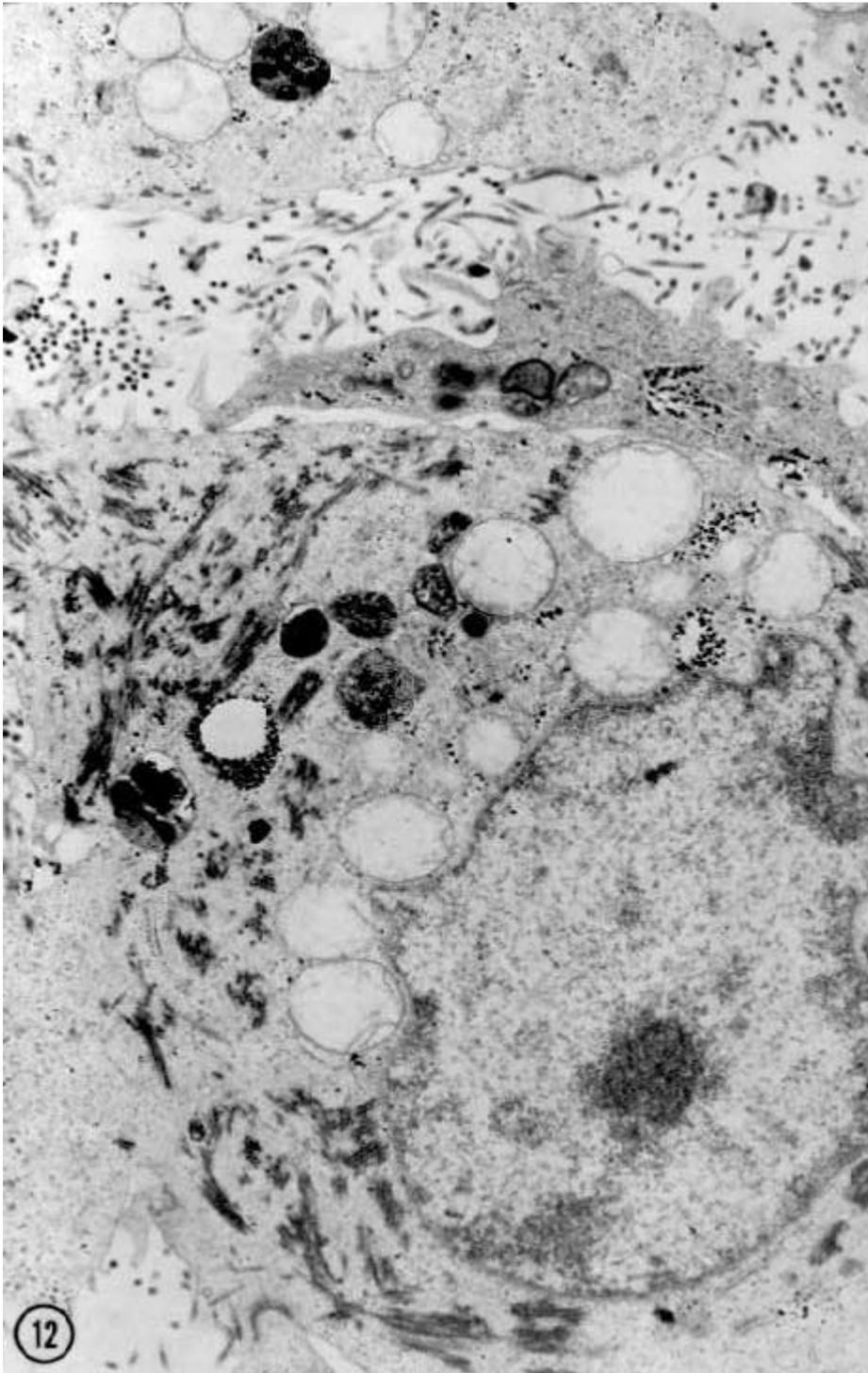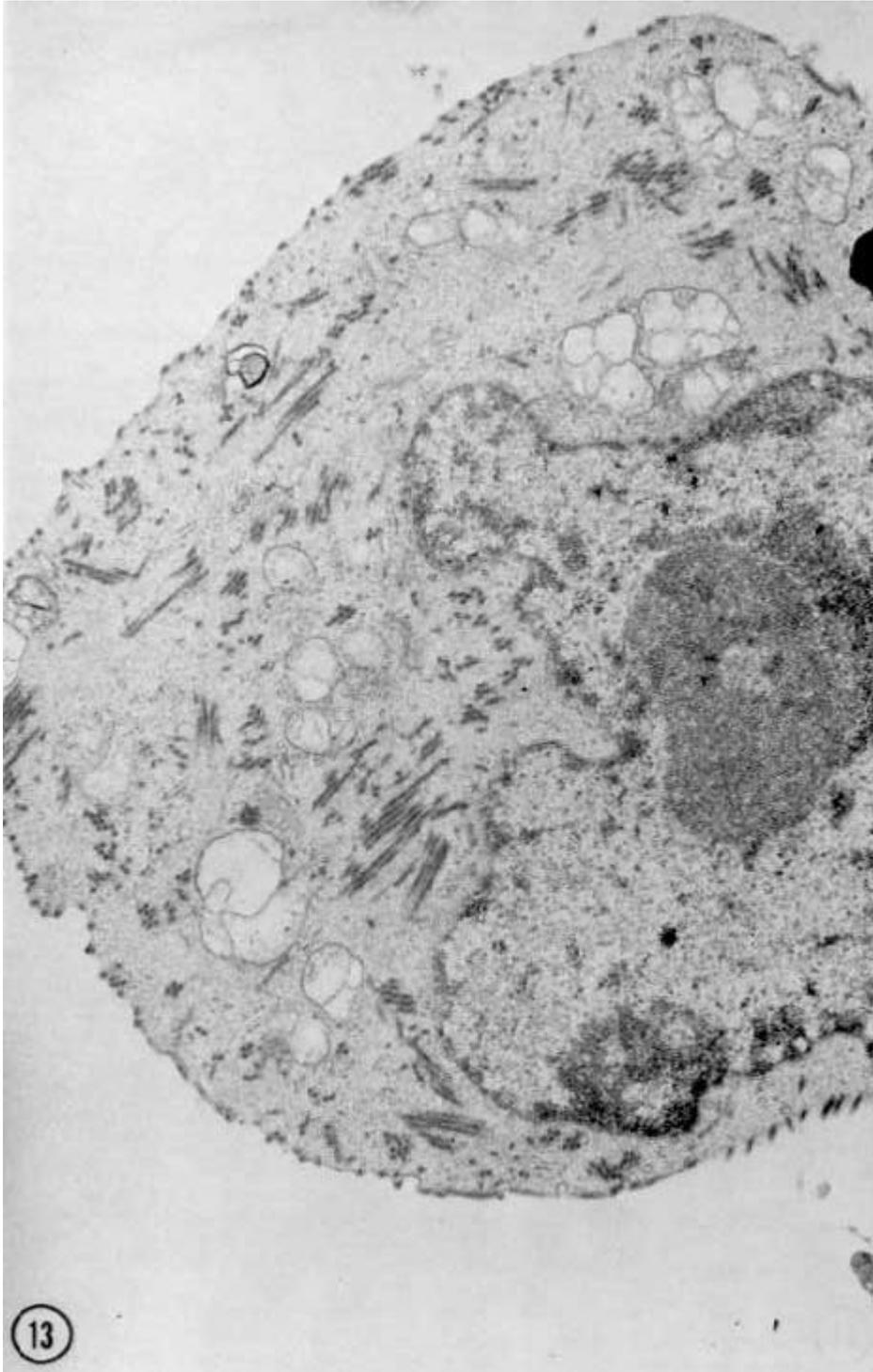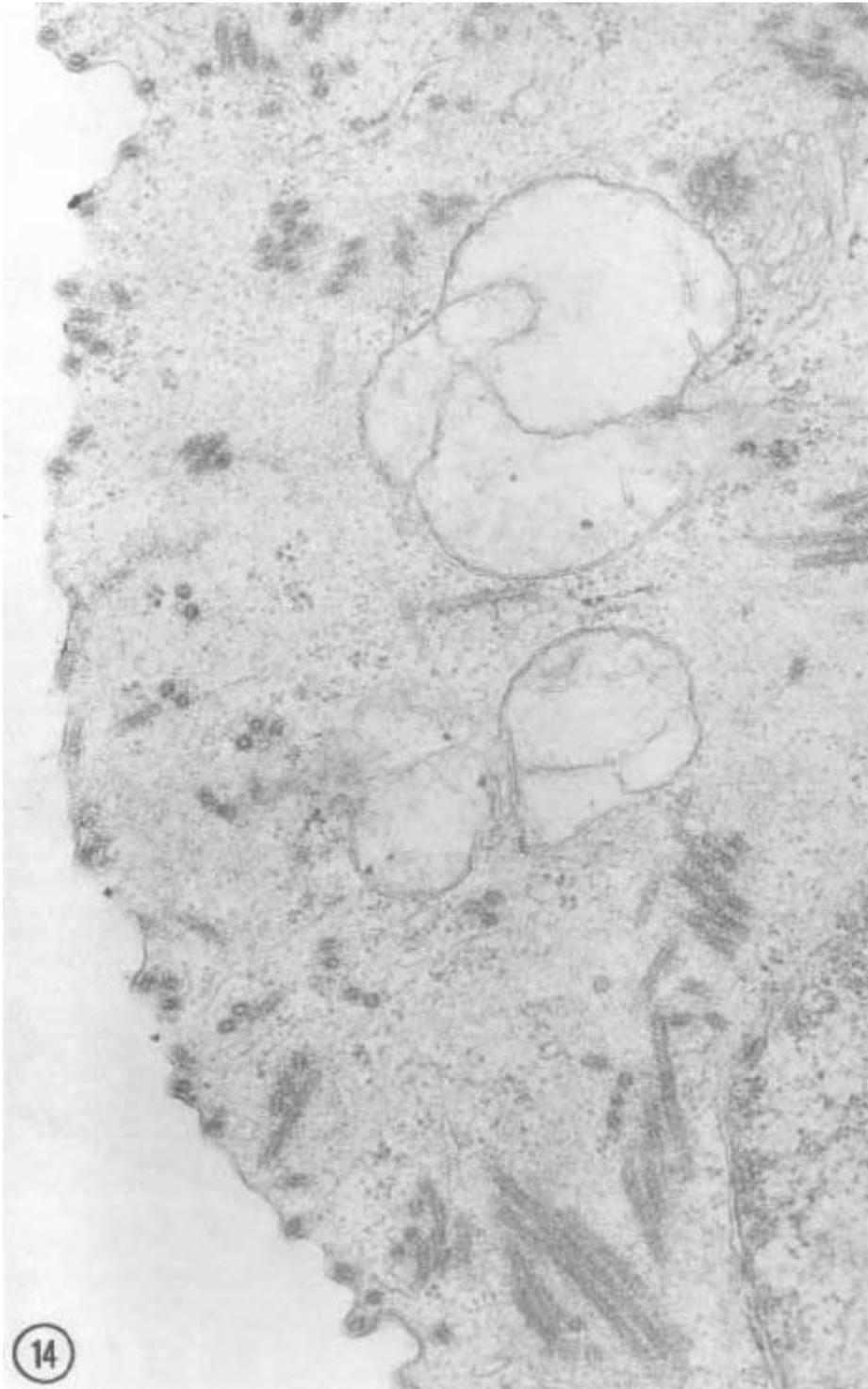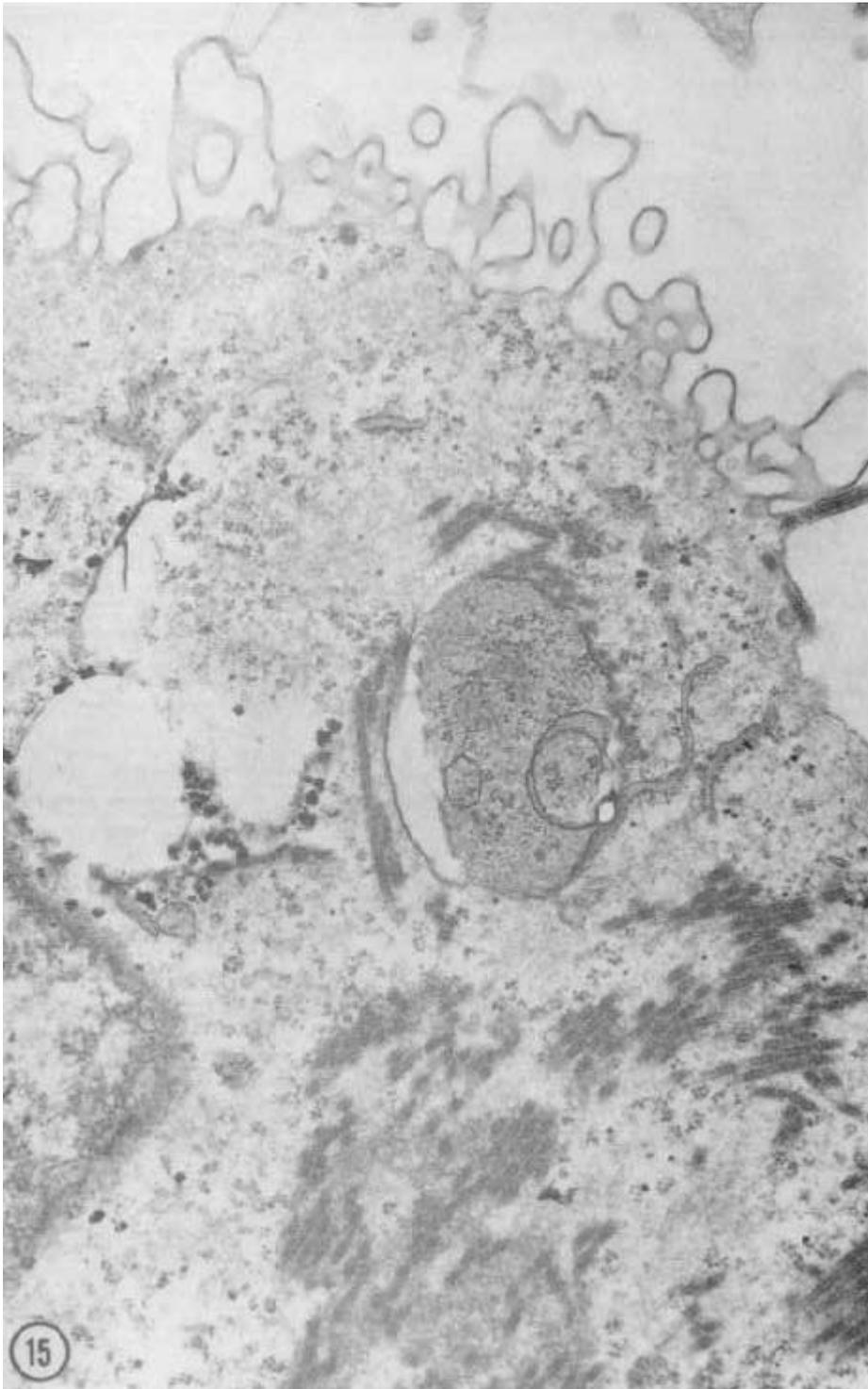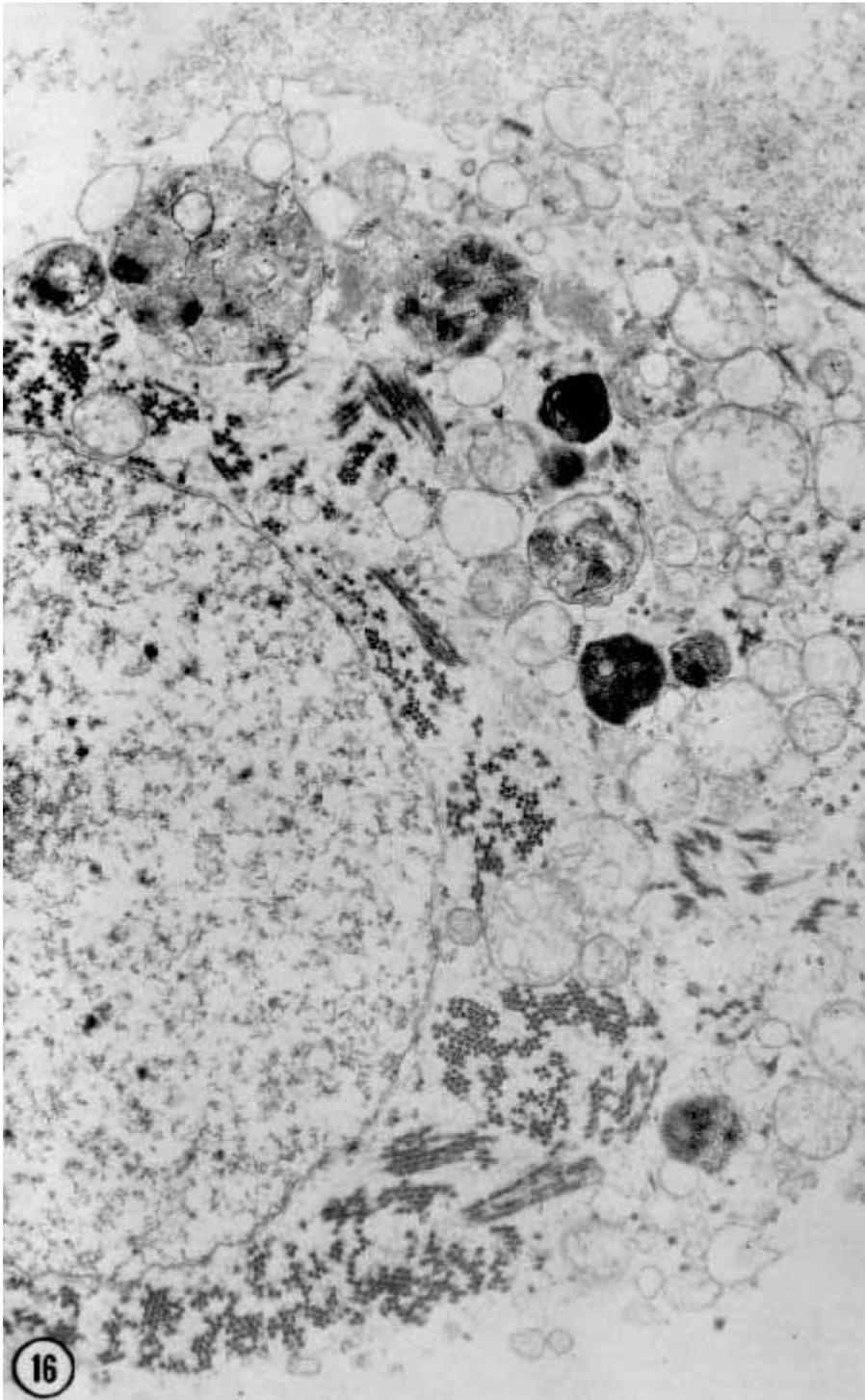
3. LABORATORY DIAGNOSIS

VIROLOGICAL DIAGNOSIS OF EBOLA VIRUS INFECTION

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It is impossible to consider the virological diagnosis of Ebola virus infection loose from the diagnosis of haemorrhagic fevers in general. The clinical picture of the disease indeed is too nonspecific to allow any hypothesis as to which virus may be responsible for any given case. At present, only the geographic origin of a specimen may give some indication as to the identity of the viruses involved (table 1). However, data of this nature may change with time and each case may represent the first occurrence of a known virus in a geographic area where it had not been encountered or even represent an entirely new virus as was the case with Ebola virus. It is for this reason that, when we received the first sample of the Zaire Haemorrhagic Fever epidemic of 1976, we decided to inoculate it on 3 different substrates in order to cover the maximum number of possibilities (table 2).

TABLE 1

VIRUS ETIOLOGY OF HAEMORRHAGIC FEVER RELATED TO GEOGRAPHIC ORIGIN

Africa	Lassa Marburg Ebola (Congo)a Yellow fever
Asia	Korean H.F. Dengue Kyasanur F.D. Congo
Europe (Krim)	Congo
S. America	Junin Machupo

a: Although numerous strains of Congo virus have been isolated in Africa, mostly from arthropods, the virus has never been identified as a cause of H.F. in that Continent.

TABLE 2

FIVE SUBSTRATES FOR VIRUS ISOLATION FROM CASES OF HEMORRHAGIC FEVER

Newborn mice (I.C.)	arboviruses
Weanling mice (I.C. + I.P.)	arenaviruses
Vero cells	arboviruses arenaviruses tuburna viruses

TABLE 3

TECHNIQUES USED IN THREE LABORATORIES WHO ISOLATED AND RECOGNIZED THE
EBOLA VIRUS FROM THE 1976 EPIDEMIC

	Substrates	Signal for positivity	Time in days	Identification
Antwerp	Newborn mice IC	dead	4-5	N.P
	Weanling mice IC+IP	dead	7	N.P.
	Vero cells	CPE (complete)	11	E.M. (U.S.)
Porton	Newborn mice IC+IP	dead	5-9	N.P
	Guinea pigs	fever	4-7	E.M. liver (U.S.)
		dead	12	
	Vero cells	CPE (partial)	6-7	E.M. (f.t.)
ATL	Vero cells	CPE (partial)	3	E.M. (f.t.) IF serol. ident.

    CPE cytopathic effect.
    N.P. not performed.
    f.t.   floating technique.
    E.M. electron microscopy.
    U.S. ultra thin sections.
    IF   immunofluorescence.

Where the weanling mice and newborn mice died respectively on the 5th and 7th days it became apparent that the virus involved was most probably not Lassa virus since the latter as a rule is not pathogenic for newborn mice.

Since material was forwarded to the laboratories of Porton Down and CDC Atlanta, it is worthwile to compare the techniques used in the 3 laboratories as available from published evidence 1,2,3 (table 3). All three laboratories characterized the Marburg like agent in their Vero cell cultures by electron microscopy, either by the simple and rapid floating technique or in ultra-thin sections of the infected cells.

Furthermore three laboratories also recognized the virus in ultrathin sections of liver tissue when this became available.

Finally the laboratory in Atlanta was able to perform the serological characterization of the virus within 24 hours of its isolation by the application of indirect immunofluorescence technique2.

CONCLUSIONS

From these results a number of important conclusions can be made for future rapid virus diagnosis of hemorrhagic fevers (table 4).

TABLE 4

CONCLUSIONS

    1. Continue to inoculate s.m. from cases of H.F.
    2. Use low passage level of Vero cells.
    3. Cell culture medium may be important.
    4. Do not wait for CPE to do E.M. in emergency : daily E.M.
    5. Consider E.M. of the patients serum.
    6. Guinea pigs for highly contaminated specimens.
    7. I.F. for rapid serological identification.
    8. I.F. for virus detection.
    9. Autopsy material also in glutaraldehyde.
    10.Research optimum diagnostic strategy to allow rational prevention and protection.

1. We think that it is still necessary to inoculate newborn mice with diagnostic material from cases of H.F. Since no clues can be obtained concerning the nature of the virus responsible for a new case or

a new epidemic of H.F. Indeed, many arboviruses especially flaviviruses do not multiply in Vero cells and even if they do they not necessarily produce CPE.

2-3. Not all passage levels of Vero cells are equally susceptible to some viruses while the culture medium may also be important . The Vero cells used in the Atlanta laboratory were of a lower passage number than those in Antwerp, and after inoculation with Ebola virus they show a cytopathic effect much sooner. It is thus advisable that Vero cells with a lower passage number be used. In our laboratory a complete CPE was observed in Vero cells maintained in a succinate/succinic acid buffered serumless medium.

4. Electron microscopy has become an indispensable tool for rapid virus diagnosis certainly in this field. It is not necessary to wait for the appearance of a CPE, to examine the supernatant in the E.M. for the presence of virus. In an emergency it can be considered to perform E.M. daily on a drop of the supernatant.

5. Direct E.M. examination of the patients serum should be done. As far as I know, this has not yet been tried and might offer the most rapid diagnosis possible.

6. The guinea pig is necessary only when highly contaminated specimens are submitted. This procedure is analogous to what is done to isolate other fastidious organisms from highly contaminated material as is done for leptospira (through inoculation into hamsters), borreliae (in mice) Mycobacterium tuberculosis and Veterans disease bacteria (in guinea pigs).

7. If E.M. is becoming at long last the standard procedure to recognize morphologically a virus in the same way the Gram stain does in diagnostic bacteriology, the indirect immunofluorescence in virology on its turn is becoming the equivalent of the slide agglutination test in bacteriology, as was beautifully illustrated by Johnson and coworkers when they found that Ebola was serologically different from Marburg(2). This implies that laboratories specializing in the diagnosis of H.F. should have at their disposal the necessary antisera to identify the possible agents.

8. I.F. could as well be applied to look for antigens in inoculated tissue culture and perhaps even in human specimens.

9. The experience with Ebola virus has clearly shown that autopsy material also may reveal rapidly the virus group to which the responsible virus belongs. Therefore it will be necessary to provide those who collect specimens with a fixative suitable for E.M.

10. Finally it will be necessary to set up the optimum strategies to cover all possible H.F. diagnoses. For this purpose additional research into some of the above mentioned aspects will be necessary.

I would like to emphasize that all this is only useful, if for the different H.F. their mode of transmission is known and the mode of excretion of the virus, so that a rapid diagnosis allows rational preventive and protective measures to be taken. But these matters are intimately intermingled : more information on transmission and contamination will only become available if rapid and relatively simple diagnostic procedures are available.

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SOME OBSERVATIONS ON THE PROPERTIES OF EBOLA VIRUS

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Ebola virus, morphologically a twin to Marburg virus in electron micrograph was found to be immunologically distinct by fluorescent antigen and antibody techniques (FA).

The number and variety of observations we have been able to make have been severely limited by restricted facilities in our currently functional Class IV Laboratory. Also, because work was done in occasional moments stolen from our primary task of providing sero-epidemiological support to the field team in Zaire, most of these observations are incomplete.

All attempts in the CDC laboratory to isolate viruses from human material submitted from Zaire have been made in Vero cell cultures. Cytopathic effect (CPE), while not dramatic or diagnostically definitive, nevertheless occurs (1). Harvests of cell cultures which show CPE all contain fluorescent antigen. However, when very small amounts of virus were inoculated, fluorescent antigen was not observed until 9 days later, at which time CPE was not present. This finding indicates that CPE alone cannot be used as an end point to diagnose Ebola virus infection in field samples inoculated into cell cultures.

We had difficulty in producing CF antigen in cell cultures to both Marburg and Ebola viruses. High virus concentrations are required, and since the homologous systems for Marburg showed that FA is far more sensitive than CF (2), we used the FA test exclusively.

Mr. E. Bowen of the Microbiological Research Establishment, Porton, provided us with a Sudanese strain of Ebola virus which was isolated in a guinea pig (GP), and convalescent human sera from Sudan survivors. Results of cross-FA tests on human antisera from the Sudan and Zaire and antigens prepared from Sudan and Zaire Ebola strains are shown in Table 1. In most instances homologous titers were twofold to fourfold higher. The same pattern of antibody response occurred with single-injection GP immune sera as depicted in Table 2.

We tried to determine whether Ebola virus is sensitive to interferon (IF) *in vitro* in a study carried out in consultation with Dr. T. Merrigan, Stanford University Medical Center, who kindly provided the human IF. A single screening test with various doses of human IF and Vero cell cultures was performed.

TABLE 1

IMMUNOFLUORESCENT ANTIBODIES IN HUMAN SERA, ZAIRE AND SUDAN STRAINS OF EBOLA VIRUS

Human Sera	immunofluorescent antigen	
	Zaire str.	Sudan str.
Zaire 1	1024 (x)	256
Zaire 2	16	16
Sudan 164	64	256
Sudan 2	16	64
Normal	< 4	< 4

(x) Reciprocal of endpoint serum dilution.

TABLE 2

IMMUNOFLUORESCENT ANTIBODIES IN GUINEA PIG SERA, ZAIRE AND SUDAN STRAINS OF EBOLA VIRUS

		Zaire Str.	Sudan Str.
Zaire (Guinea Pig)	1	512 (x)	256
	2	1024	256
Sudan (Guinea Pig)	1 (xx)	64	64
	2	64	256
	3	64	128
	4	32	128
Marburg (Guinea Pig)		4	4
Normal (Guinea Pig)		< 4	< 4

(x) Reciprocal of endpoint serum dilution.

(xx) This animal was infected in Porton and its serum was sent to CDC.

Maintenance medium (E-MEM with 2% fetal calf serum) with and without IF was placed on the cultures 24 hours before they were inoculated with virus. Media were removed, and one precalculated virus dose was inoculated into replicate tubes. CPE was used as an end point for VSV-infected cultures and fluorescent antigen for Ebola-virus cultures. Results shown in Table 3 reveal that VSV was 100 times more sensitive than Ebola virus to IF. Obviously these results are not definitive. A more suitable cell culture system for IF assay such as the HR 202 human diploid line should be tried.

TABLE 3

IN VITRO SENSITIVITY OF EBOLA VIRUS TO HUMAN INTERFERON

Virus	Dose	Interferon Dosage and Virus Growth			
	TCID 50	10	100	1.000	10.000 IU
VSV	50	+	-	-	-
Ebola	10	+	+	+	-

+ = Virus grew. - = Culture protected.

In vivo attempts to challenge Ebola-immune GP's with a high dose of Ebola or Marburg virus failed because we were unable to reliably kill 100% of animals with either agent regardless of dose. However, Ebola-immune GP's challenged with Marburg virus did develop FA titers to both viruses, which is useful for testing unknown field specimens for both agents using a single serum.

We have been unable to inactivate Ebola virus completely in infected Vero cells with ultraviolet light. Usually about 50% of the tubes inoculated with an "inactivated" cell suspension are positive.

Finally, in this parade of negative data, all of our attempts to neutralize Ebola virus with *in vitro* test systems have so far been unsuccessful. Table 4 summarizes three different approaches. We suspect that such will continue to be the case until we can purify virus suspensions and separate specific proteins to use as antigen or infective material, a procedure which must await the availability of our new maximum containment "suit" laboratory and distant horizons.

SUMMARY

Ebola virus is morphologically identical to and immunologically distinct from Marburg virus. Vero cell cultures have been used to isolate virus, and fluorescent antigen and antibody techniques have been used to confirm isolates and test strain differences. A twofold to fourfold higher titer was seen in the homologous antibody response pattern in sera from convalescent humans and immune GP's between the Zaire and Sudan strain of Ebola virus. VSV was 100 times more sensitive to interferon in vitro than Ebola virus. Preliminary attempts to neutralize Ebola virus have been unsuccessful.

TABLE 4

EBOLA VIRUS - IN VITRO NEUTRALIZATION ATTEMPTS

A	CPE Vero Cell Cultures – Serum Dilution		
	G.P. Immune Serum	Homolog. FA Titer	Serum Dilution 1:4, 1:16 vs 1,000 TCID50
	Eb-Zaire	1024	No neutralization
	Eb-Sudan	64	
	Marburg	1024	
	Normal	< 4	
B	Fluorescent–Focus Inhibition Test		
	G.P. Immune Serum	Human FA Titer	Serum Dilution 4 – 16 – 64 – 256
	Eb-Zaire 1	1024	25-30 foci
	Eb-Zaire 2	256	15 foci
	Eb-Sudan	64	15-20 foci
	Normal	< 4	15-20 foci
C	Precipitation of Virus-Serum Complexes with Staphylococcal Protein A.		
	Virus + Diluent + Staph. A		
	Virus + Eb. Human + Staph A	CPE Observed in All Cell Cultures at the Same Time.	
	Virus + Normal Human + Staph A		
	Virus + Eb. Human + Staph B		
	Virus + Diluent Only		

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VIROLOGICAL STUDIES ON A CASE OF EBOLA VIRUS INFECTION IN MAN AND IN MONKEYS

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INTRODUCTION

On the 5th November 1976 an investigator at the Microbiological Research Establishment accidentally pricked his thumb through a protective rubber glove whilst inoculating guinea pigs with material containing Ebola virus (1,2,3). He developed an illness which was clinically similar to Ebola haemorrhagic fever cases seen during the July to November outbreaks which occurred in the Western Equatorial province of the Sudan and in the adjacent Equateur Region of Zaire (4,5).

Virological investigations were carried out on a blood sample collected about hours after he became feverish six days after the accident. Further studies were also made on specimens of blood collected daily during the acute phase of the illness and on blood, urine, throat swabs, faeces and seminal fluid collected during the convalescent phase. Although monkeys had not been implicated in transmission of the disease in Africa, it was thought important to infect keys experimentally with Ebola virus to determine whether they were susceptitible to infection, to define the pathogenesis of the infection and to determine whether this would provide a useful experimental model for evaluating methods therapy for use in human Ebola virus infection.

RESULTS

Human Studies. The first specimen of blood collected on the 11th November, hours after the patient became feverish was examined by electron microscopy and virus particles were seen which were similar to those of Ebola virus. Guinea pigs which had been inoculated with this blood specimen developed a febrile illness and electronmicroscope examinations of their blood and tissues collected on the fifth and sixth day post inoculation showed particles which were again similar to those of Ebola virus.

Studies on blood collected during the acute phase of the illness showed that highest levels of virus in the blood ($10^{4.5}$ guinea pig infectious units/ml) were recorded on the first and second days of the illness. Following treatment with interferon and convalescent serum the level dropped to $10^{0.5}$ guinea pig infectious units/ml and remained at this level until the viraemia was undetectable on the ninth day from onset of illness (table 1). No virus was isolated from throat swabs, faeces and urine collected between days 14 and 27. Ebola virus was, however isolated from specimens of seminal fluid collected on days 39 and 61. Samples of seminal fluid collected on days 76, 92, 110 and 128, and two further specimens collected at three monthly intervals thereafter were negative.

After the infusion of 450 ml of convalescent plasma (fluorescent antibody titre of 1/128-1/256) on the second day of illness antibody levels of 1/16 were recorded in the patient's blood from days three to nine. This increased to 1/32 on day 10 and gradually increased to a fluorescent antibody titre of 1/128 by day 34. The patient was then subjected to plasmaphoresis between 16th and 25th February 1977. A total of seven units of plasma was taken which resulted in the fluorescent antibody level dropping from 1/128 to 1/32. A specimen of blood collected on the 5th May 1977 had a fluorescent antibody titre of 1/16 while a specimen collected on the 9th November almost one year from the onset of illness had a fluorescent antibody titre of 1/8.

Transmission of Ebola virus infection to the monkey. In this patient's case it was not possible to assess the relative effectiveness of either interferon or serotherapy in the treatment of the infection. We therefore considered it important to try to establish a suitable animal experimental model system to evaluate this type of therapy and preliminary studies were carried out in rhesus (Macaca mulatta) and vervet (Cercopithecus aethiops) monkeys.

Virus inoculum. The original source of virus was human acute-phase blood prototype strain E718 from the Zaire outbreak which was sent to us by Professor Pattyn. The virus inoculum was a

suspension of guinea pig liver taken during the late febrile stage of the disease in the third guinea pig passage. The monkeys were sedated and inoculated intraperitoneally with 0.4ml of virus suspension and the dose of virus calculated by parallel intraperitoneal titrations in guinea pigs and expressed as guinea pig infectious units (G.P.I.U.)/ml. Monkeys received 10^3 and 10^4 (G.P.I.U.).

Clinical Observations. Monkeys became febrile on the third day after infection with temperatures ranging from 40ºC - 40.6ºC. (table 2). The pyrexia persisted until the terminal stage of the infection when the temperature became subnormal. Maculo-papular skin rashes involving the forehead and face, the medial aspects of the fore and hind limbs and the chest developed in all rhesus monkeys between the 4th and 5th day, fading slightly before death which occurred between the 5th to 8th post-inoculation day. No animal survived the infection but neither of the two vervet monkeys infected developed skin rashes. Two of the monkeys had diarrhoea and all monkeys lost about 10 per cent body weight. Virus was first detected in the blood on the second day reaching maximum virus titres of 10 5.5 to 10 6.5 /ml on the 4th and 5th day.

Necropsy was carried out on all monkeys shortly after death and various tissues removed for virological studies and histopathology. Virus infectivity titrations showed high titres of Ebola virus in most of the organ tissues examined. It was not possible to determine whether some of these organs contained Ebola virus because of the high concentration of virus present in the blood. Further details of the virological studies and histopathology will be published elsewhere.

TABLE 1

Day of Sample and Test from Onset of illness	Details & Remarks	Activity of Circulating Antibody as F.A. Titre	Recovery of Infective Virus as Guinea Pig I.P. Infective Unitsper ml or gram of Sample Tested	
			Positive	Negative
1	-	-	Blood, 10^4.5	
2	Before transfusion of 450ml convalesc. Plasma	Lte 1/2	Blood, 10^4.5	
3	11am, 6pm, 11pm	1/16	Blood, 10^0.5	
4	-	1/16	Blood, 10^0.5	
5	-	1/16	Blood, 10^0.5	
6	-	1/16	Blood, 10^0.5	
7	am pm	1/16	Blood, 10^0.5	
8	-	1/8	Blood, 10^0.5	
9	-	1/16		Blood
10, 11, 12, 13	-	1/32		Blood
14, 16, 20	-	1/64		Blood, Faeces, Urine and Throat Swab
23, 27	-	Not done		Blood, Faeces, Urine and Throat Swab
34	-	1/128		Blood
39	-	Not done	Seminal Fluid 10^0.5	
61	-	1/128	Seminal Fluid 10^0.5	Blood, Urine
76	-	1/128		Blood, Urine, Seminal Fluid
92, 110, 128, 219, 310	-	Not done		Urine, Seminal Fluid

TABLE 2

RECTAL TEMPERATURE (ºC) AND VIRUS CONCENTRATION IN THE BLOOD (ML)

Monkey	Dose	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Total Weight Loss
Rhesus 6 Wt 3.686 kg	10^4	38.6 $10^{0.5}$	38.9 $10^{2.5}$	40.4 $10^{4.5}$	40.0 $10^{5.5}$	Moribund 34.5 $10^{4.5}$				255 g
Rhesus 7 Wt 3.629 kg	10^4	38.1 $10^{0.5}$	38.7 $10^{2.5}$	40.2 $10^{4.5}$	39.8 $10^{6.5}$	39.5 $10^{5.5}$	Moribund 34.5 $10^{5.5}$			340 g
Rhesus 12 Wt 3.289 kg	10^3	38.8 $10^{0.5}$	38.8 $10^{2.5}$	39.6 10^4	40.6 10^5	39.8 10^5	39.6 $10^{6.5}$	39.8 $10^{6.0}$	Found Dead	396 g
Vervet 1	10^4	38.6 10^{05}	38.9 10^2	39.6 $10^{4.5}$	40.2 10^6	39.7 10^6	Moribund 34.5 $10^{5.5}$			N.D.
Vervet 3	10^4	38.2 $10^{0.5}$	38.9 $10^{1.5}$	40.1 10^4	40.6 $10^{6.5}$	39.7 $10^{6.5}$	Killed 36.1 $10^{5.5}$			N.D.

DISCUSSION

Details of the clinical illness in man following accidental laboratory infection with Ebola virus has been described fully by Emond et al. 5 . Virus was detected early in the disease, $10^{4.5}$ G.P.I.U./ml being detected on the first day of illness. No detectable change in the levels of circulating virus was evident on the day following initiation of interferon therapy. However within twelve hours of administering 450ml of Ebola immune plasma the viraemia level had fallen to $10^{0.5}$ G.P.I.U./ml. This much reduced level of circulating virus persisted throughout the acute stages of illness and virus became undetectable on the 9th day of illness.

Emond et al. (5) were unable to draw any definite conclusions as to the value of either interferon or immunotherapy. As both were administered together their respective merits cannot be assessed. There is no doubt that viraemia levels were dramatically reduced soon after the administration of immune plasma, but the patient's clinical condition deteriorated despite the low virus levels in the blood.

The discovery of virus in the semen was not unexpected since Marburg virus is known to persist in seminal fluid for several weeks after infection (6,7). Marburg virus has also been recovered from the anterior chamber of the eye two months after the onset of illness (8).

The disease produced in rhesus, vervet and squirrel (Saimiri sciureus) monkeys following infection with Marburg virus closely paralleled the illnesses caused in man (9). Fever, severe weight loss, anorexia, skin rash and haemorrhage were common factors. Ebola virus similarly produces an illness in monkeys which resembles the disease in man. The rash in rhesus monkeys is very much more marked than in vervets which often have no obvious rash at all. As with Marburg, Ebola causes a uniformly fatal illness in monkeys and this animal is considered to be eminently suitable for studying the pathogenesis and possible treatment of these unique virus infections in more detail.

SUMMARY

Ebola virus was detected in high titre in the blood of a patient at the onset of illness. Administration of interferon had no immediate effect on circulating virus levels but the additional administration of immune plasma reduced circulating virus levels dramatically within 12 hours. Virus was detected in seminal fluid for up to 61 days after the onset of illness.

Experimental infection of rhesus and vervet monkeys produced a uniformly fatal illness. The course of disease resembled that found in man with weight loss, anorexia, fever haemorrhages and skin rash being frequently seen. Viraemia was obvious within two days of infection and persisted until death which occurred between days 5 and 8.

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DISCUSSION

P . Brès : Mr. Bowen Just mentioned that in the monkey experiments no virus was found in the bile nor in the feces. I think this is of enormous importance for future control measures in human cases. Would Mr. Bowen give us more details and tell how many samples were examined ?

E.T.W. Bowen : Only three fecal samples were examined, we would like to do a few more before we would be categoric about stating that the virus was not excreted in the feces.

P. Brès : Was the blood on the skin lesions also positive ?

E.T.W. Bowen : We did not take any blood from the skin lesions.

J.G. Breman : I am very interested in seeing the high virus titer in the pancreas of the monkeys, this correlates with our clinical impression that the patients were suffering from pancreatitis. This also correlates with the German observations and one observation in South Africa where a prolonged amylasemia and amylasuria was observed. I would like to know if there are related observations in autopsy studies. Secondly, the lung positives I think are also very important. You will recall from the clinical presentation that in the Sudan there were more predominant pulmonary symptoms than in Zaire and this then may mean that this was a very important mode of spread.

J. Casals : Dr. Webb, you have shown in your serological crosstesting a systematic, consistent difference between the homologous and heterologous titers observed between the viruses in Sudan and those in Zaire. I don't know whether in your own mind that constitutes enough of a difference to talk about different strains or varieties of the same virus. In that case we would have to consider the two epidemics as two separate outbreaks of similar diseases.

P. A. Webb : I think one should remember that the fluorescence test after all is qualitative and not really quantitative, based on interpretation of degrees of fluorescence. I think that until we have a neutralization test, we can't say.

E.T.W. Bowen : We have done cross protection tests in guinea-pigs, the viruses certainly crossprotect.

C.E. Gordon Smith : You will recall in the 1967 Marburg outbreak, there was one case with seminal infection and transmission; it must have been at a rate of something like 1 in 20 and here we have one out of one studied. We also, in the South African case, had a persistent infection of the eye. Someone is going to have to explain why no such persistent infections have led to further outbreaks of the disease in the Sudan or Zaire.

4. EPIDEMIOLOGY

THE EPIDEMIOLOGY OF EBOLA HAEMORRHAGIC FEVER IN ZAIRE, 1976

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INTRODUCTION

The epidemiological investigations attempted to describe the outbreak of Ebola haemorrhagic fever (EbHF) by its distribution in time, in geography and amongst persons. Factors related to spread were also studied. These included possible modes of transmission, the incubation period, secondary attack rates and related risk factors. Serological surveys were undertaken to find evidence of prior Ebola virus disease in the area and asymptomatic infections occurring during the epidemic The cause of the epidemic (1)was searched for by attempts to find the index case and evidence of Ebola virus in some animal and insects (2).

DESCRIPTION OF THE EPIDEMIC AREA

The epidemic focus was in north-central Zaire. It was located in and near the Yambuku Mission, in the Yandongi collectivity (country) of the Bumba Zone of the Equateur Region (Figure 1). This collectivity has about 35 000 persons and the Bumba Zone has about 275 000 persons. Half of the population is less than 15 years of age. Over 75% of the population lives in forest villages of less than 5 000, most in small localities of fewer than 500. The area forms part of the Zaire river basin and is essentially tropical rainforest. The Zaire river forms the southern boundary of the zone and effectively separates geographically the most northern sectors from the remainder of the country. The major ethnic group is Budza and Lingala is the principal language.

Traditionally, the people are hunters and have contact with a wide variety of wild animals. Cash crops are palm oil, rice, some coffee and cocoa. Malaria, filariasis, measles, pneumonia, amoebiasis, bacillary dysentery and goitre are common. There is some poorly controlled movement of palm oil, rice and other staples out of the Equateur into the Central African Empire and the Sudan. These are exchanged for luxury items such as cloth, utensils, transistor radios and other implements of modern technology.

The mission was established in 1935 by Belgian missionaries near Yambuku, a small isolated village 100 km north of Bumba, the administrative capital of the zone. The mission developed a large local following over the next four decades and was involved in education, agricultural development, animal raising, social service and health programmes, as well as religious activities.

By 1976, before the epidemic, the hospital had 120 beds and a medical staff of 17 directed by a Zairian paramedical assistant. Included in the medical staff were three Belgian nursing nuns. The hospital outpatient department drew its clientele essentially from the Yandongi collectivity population, but others from within and even outside the Bumba zone were attracted by the relatively good supply of medicines. Between 6 000 and 12 000 patients per month came to the outpatient clinic for general medical care.

Five syringes and needles were issued to the nursing staff each morning for use at the outpatient department, the prenatal clinic and the inpatient wards. These syringes and needles were sometimes rinsed between patients in a pan of warm water. At the end of the day they were sometimes boiled. The surgical theatre had its own ample supply of instruments, syringes and needles which were kept separately.

FIGURE 1

AFRICA

SCALE 1:15,400,000 OR 248 MILES TO THE INCH
STATUTE MILES

KaLōmēYēng
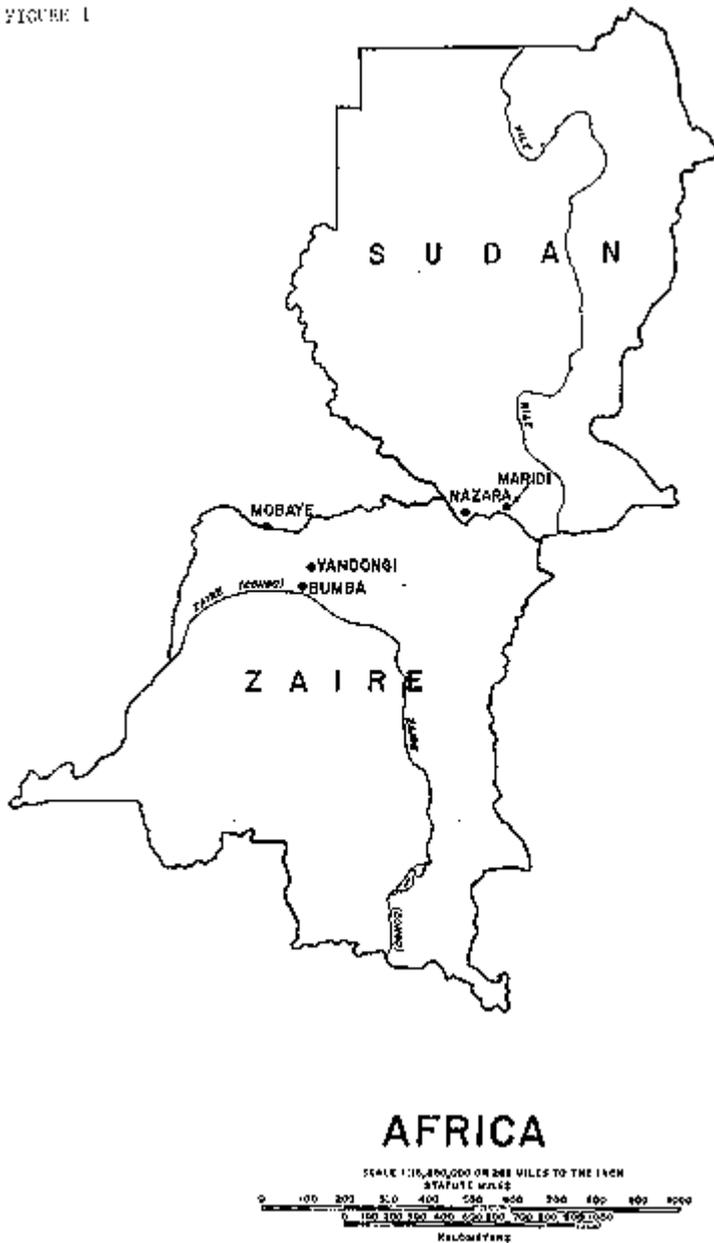
DEFINITIONS AND METHODS

A probable case of EbHF was a person living in the epidemic area who died after one or more days with two or more of the following symptoms and signs, occurring between 1 September and 5 November 1976: headache, fever, abdominal pain, nausea and/or vomiting, and bleeding; usually a probable case either had an injection or contact within the three preceeding weeks with a probable or proven case of Ebola virus infection and clinically could not be assigned another diagnosis. A proven case of EbHF was a person from whom Ebola virus was isolated or visualized by electron microscopy or who had a fluorescent antibody (IFA) titer of at least 1:64 to Ebola virus within three weeks after onset of symptoms. An Ebola virus infection was deemed to have occurred in persons who had a similar IFA antibody titer, but who reported no illness during the period 30 August to 15 November 1976.

A possible case was a person with at least 24 hours of headache and/or fever, with or without other signs and symptoms, who had contact with a probable or a proven case of EbHF within the previous 3 weeks. These cases were treated with antimalarial drugs, antibiotics and antipyretics to exclude

diseases common to the area. Persons reporting such symptoms retrospectively were bled and their sera were tested for Ebola virus antibodies.

Any case of fever with bleeding, regardless of outcome, reported to the Ministry of Health (MOH) from any part of Zaire was also regarded as a possible case and every effort was made to establish a diagnosis by virological or pathological means.

Infants born to probable cases of EbHF were called neonatal cases if they died within 28 days of birth.

A primary contact was any person having direct face-to-face contact with a probable or a proven case (sleeping in the same room, sharing meals, caring for patients, preparing a cadaver for burial, touching the body at a funeral, etc.). Contact was required from two days prior to onset of symptoms to death or clinical recovery of the patient. The surveillance interval for primary contacts was 21 days from the last such contact. Secondary contacts were persons having face-to-face contact with a primary contact.

Case investigations were performed by six physician-led teams working with nurseinterpreters and standardized pre-coded forms. The forms had questions on clinical as well as on epidemiological features. Controls were chosen from the same village as a probable case. They were matched as far as possible with cases by sex and age and a member of the same family was chosen if available. One part of the study was done in a restricted zone of 21 villages near Yambuku before the six teams began.

A family was defined as persons using the same kitchen, claiming the same person as the family head, living in contiguous dwellings and sleeping in the village during the time an active case occurred in the family unit.

A case was considered to have acquired his disease by injection if, in the three weeks preceding symptom onset, he received an injection by any medical practitioner in the epidemic area and had no primary contact with a probable or proven case. Person-to-person transmission was designated when a probable case had face-to-face contact with another case within three weeks prior to symptom onset without history of injection receipt. Transmission was classified as both possible if the case had both an injection and face-toface contact with another case within three weeks of symptom onset and one transmission type was not likely by history.

Hospital records were reviewed for the period January 1974 to October 1977. Outpatient records were not kept.

One village with a high attack-rate was studied in greater depth to gain better insight into transmission patterns and subclinical infection. An investigative team mapped every house in the village and censused all cases occurring during the epidemic and remaining residents. Sera were drawn from as many residents as possible.

Serum specimens were taken from family and non-family primary contacts who reported febrile illness during the epidemic, and were possible cases, from other residents of 8 villages where cases occurred, and from residents of 4 villages near the epidemic area where no cases occurred. These were screened for Ebola virus indirect immunofluorescent antibodies (IFA) using a method previously described (4) .

RESULTS

Time. The first known case, a 44-year old male instructor at the Yambuku Mission school, came to the Mission hospital on 26 August 1976 with a febrile illness felt due to malaria. He was given an injection of chloroquine at the dispensary. The fever dropped and remained normal over the next four days but rose to 39.2° on 1 September. The typical syndrome evolved from that day and he died on 8 September with severe haemorrhage.

From I September to 24 October there were 318 cases resulting in 280 deaths the epidemic peaked during the fourth week and then receeded somewhat more gradually over the next 5 weeks (Figure 2). Date of symptom onset was not available for about 10% of cases.

Place. Fifty-five villages of less than 5,000 persons had cases. All infected villages in the epidemic area were within 60 km of Yambuku. This area includes about 100 villages. The larger towns of Abumombazi and Bumba, about 100 km to the north and south, respectively, had imported cases, as did Kinshasa, 1 100 km to the south-west. The large majority of affected villages were along roads running east and west of Yambuku, along which were located more villages than the north-south road. Forty-three of 73 villages in the Yandongi collectivity were affected. This collectivity had an attack rate of 8.0 cases per 1 000 persons.

The epidemic spread relatively slowly in the epidemic area. Within the first two weeks after onset of the epidemic cases were occurring no further than 30 km from Yambuku. Almost another two weeks passed before a sick nursing sister was evacuated to Kinshasa. it was over a month until cases were

imported into Abumombazi and Bumba. The mean duration of active disease was 26 days per locality and ranged from 1 to 55 days.

At the Yambuku Mission Hospital, where all staff members contacted patients or instruments used for treating patients, 13 of 17 hospital employees acquired the disease and 11 died.

Person. All ages and both sexes were affected. Females predominated, mainly in the age groups 5-14 and 15-29 (Table 1). Age-sex specific attack rates, using Yandongi collectivity population as denominator, shows adult females with the highest attack rates (Figure 3). Convalescents were all adults, except for one child of 8 years of age.
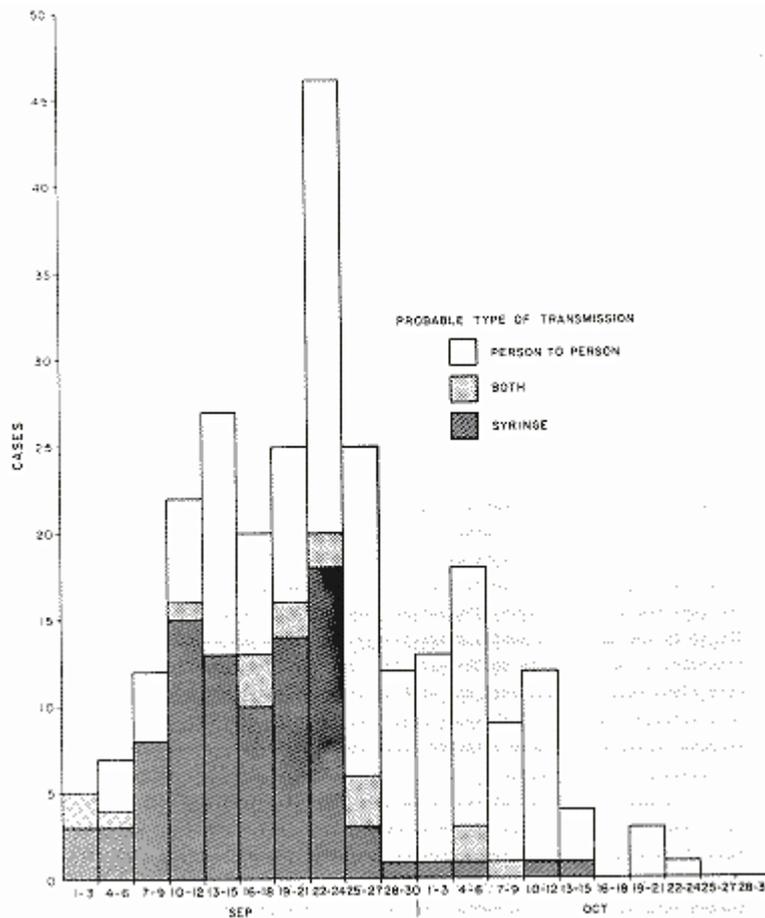
Fig.2 Cases of Ebola Hemorrhagic Fever, by day of onset, Equateur Region, Zaire, Africa, Sept. 1 - Oct. 30, 1976

TABLE 1

AGE AND SEX DISTRIBUTION OF EbHF CASES, ZAIRE, 1976

	Male		Female		Total	
Age	n	%	n	%	n	%
Newborn & Infant	10	3.1	14	4.4	24	7.5
1-14 yrs.	18	5.7	22	6.9	40	12.6
15-29	31	9.7	60	19.0	91	28.8
30-49	57	17.9	52	16.4	109	34.3
50 or >	23	7.2	26	8.2	49	15.5
Unknown	2	0.6	3	0.9	5	1.6

Total	141	44.2	177	56.0	318	100
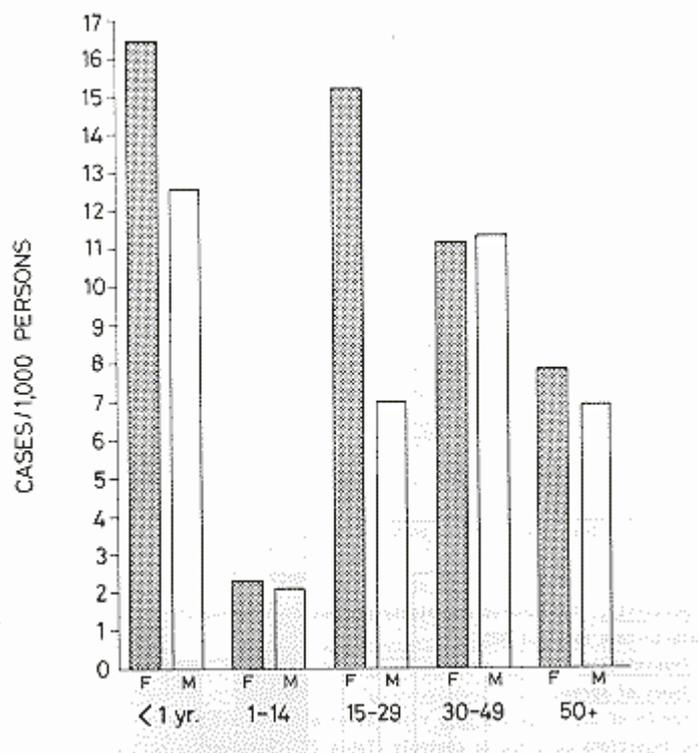
Fig. 3 Attack rates of Ebola Haemorrhagic Fever by age and sex, Yandongi collectivity, Bumba Zone, Equateur Region, Zaire, 1976.

Mortality, Two hundred and eighty persons died during the epidemic, a death-to-case ratio of 88%.

Transmission

Types of spread

For 85 of 318 cases the only risk factor elicited was receipt of one or more injections compared to controls (Table 2). These were almost all given at the outpatient service or on the general medical wards at the Yambuku mission hospital. Less than 1% of the controls had contact with the hospital during the epidemic (p 0.0001). History of injection receipt away from the Yambuku mission hospital occurred in only 2 instances. One case had an injection at the dispensary in Kwaédza, near several villages where cases were occurring. Another case was alleged to have received an injection at the Modjambuli dispensary out of the epidemic zone, and known to be closed at the time.

TABLE 2

DISTRIBUTION OF CASES OF AFRICAN HEMORRHAGIC FEVER IN ZAIRE BY TRANSMISSION TYPE

	Cases		Survivors	
Transmission type	No	%	No	%
Injection	85	26.7	0	0
Person-to-person	149	46.9	30	78.9
Both possible	43	13.5	4	10.5
Unknown	30	8.9	0	10.5
"Neonatal"	11	3.5	0	0
Total	318	100.0	38	99.9

One hundred and forty-nine persons acquired their disease following contact with patients. These contacts occurred, for the most part, at the villages after injected patients returned home in September and when family and friends came to visit these sick persons at the hospital.

In 43 instances, cases could have acquired disease either by injection or contact. Eleven cases were possibly "neonatal" infections. The remaining cases lived in the epidemic area and had probable case or hospital contact. However, the details were not specific enough to class in the three other groups.

All survivors were infected by person-to-person contact, excepting four where both transmission types were possible and four where the transmission type was unknown (Table 2). Seventeen persons, whose home village was not Yambuku, had contact with cases at Yambuku. It is possible that this group had an injection without reporting this to the family.

The distribution of age and sex by transmission type in the 21 village stud is shown in Table 3. Twenty-two females in the 15-29 year old group acquired their disease by injection compared to only two males in this group.

Those acquiring the disease by contact had a variety of close associations with possible cases (Table 4). However, the only type of contact more associat with the disease, when compared to controls, was aiding in the delivery of a pregnant woman with EbHF. Several persons had multiple contacts before becoming ill, especially medical personnel working at the Yambuku mission hospital.

Figure 2 shows onset of cases throughout the epidemic by transmission type. During the first two weeks of the epidemic over two-thirds of the cases were acquired by injection. This type of spread almost completely terminated when the mission hospital closed at the end of September.

Other factors, such as exposure to foods, domestic and wild animals, and travel outside the epidemic zone did not appear to be related to disease transmission.

TABLE 3

AGE AND SEX DISTRIBUTION OF 145 EBOLA HAEMORRHAGIC FEVER CASES IN 21 VILLAGES CLOSE TO YAMBUKU ACCORDING TO TRANSMISSION TYPE

Age Group	Transmission Type				Total	
	Injection		Case Contract			
	Male	Female	Male	Female	Male	Female
< 15 yrs.	3	6	4	3	7	9
15-29 yrs.	2	22	16	16	18	38
30-49 yrs.	7	7	21	13	28	20
50 or > yrs.	7	2	6	8	13	10
Total	19	37	47	42	66	79

TABLE 4

FACTORS ASSOCIATED WITH PERSON-TO-PERSON SPREAD CONTROLS FOR CASES AND FAMILY

Risk factor	P to P cases		Family controls		P
	No	% Yes	No	% Yes	
Touched case	126	85.7	91	83.5	n.s.
Attended funeral	126	85.7	98	85.7	n.s.
Care for case	119	70.6	84	71.4	n.s.
Slept in same room	116	69.0	86	66.3	n.s.
Prepared cadaver	116	87	57.5	n.s.	
Aided in delivery of child of sick patient	104	18.3	74	9.5	p<0.001

n.s. = not significant.

Secondary attack rates

Five consecutive transmission generations of EbHF cases were documented. Sporadic, apparently spontaneous, probable cases simply were not recorded. When "family" was defined as all persons living in contiguous housing and sharing common eating facilities, attack rates for secondary case generations one through four never exceeded eight percent. However, when 92 families affected in the 21 villages surveyed along an east-west axis close to Yambuku were examined, contact infection rates were 16.7, 3.6 and 9.0 percent in three successive generations. Moreover, there were marked differences in secondary contact transmission related to both sex of the primary case and blood and marital relationships within households. The secondary attack rate was 27.3% among spouses, between brothers and sisters and between parents and children but only 8.0% to all other relatives. Among the high risk relatives 10 of 60 (17%) contacts became ill when the primary case was male and 27 infections occurred among 75 such contacts (36%) of female primary cases. Although the precise factors involved were impossible to determine, direct care of cases and intimate family contact, including sexual intercourse, possibly were the important variables.

Within all family units, the overall secondary attack rates were generally low amongst members who contacted cases acquiring their disease by both injection and by person-to-person transmission (Table 5). There is a significant difference (p 0.1) in secondary attack rates subsequent to contact with an "injected" case compared to others. However, there is no difference in attack rates caused by persons of different subsequent infection generations, all having secondary attack rates of less than 5%.

Spread within villages

Approximately one-third of the villages affected had one case and another third had between 2 and 5 cases (Table 6). Only two villages had more than 30 cases, Yambuku and Yandongi, the nearby administrative capital of the collectivity. The mean number of cases per village was 5.

Incubation period

The mean incubation period for cases due to injection was 6.3 days following the first injection (Figure 4). This ranged from I to 15 days. The incubation period for person-to-person spread was 9.5 days when the first day of contact was considered date of infection acquisition. This is imprecise as infection could have occurred at any time during the contact period, which at times covered up to 3 weeks. There were 17 instances where only 1 or 2 days of person-to-person contact was documented; the mean incubation period of these cases was 6.3 days, with a range of 1 to 21 days.

In one well-documented case a single contact occurred within 48 hours of symptom onset in that contact.

TABLE 5

FAMILY CONTACT ATTACK RATE BY GENERATION OF ILLNESS

Generation	No. Families of Cases	No. Family Exposures	No. Subsequent Cases	Attack Rate (%)
1x	61	498	38	7.6
2	62	459	20	4.4
3	18	117	3	2.6
4	5	29	1	3.4
TOTAL	146	1103	62	5.6

(x) persons acquiring disease by injection; subsequent generations acquired disease by person-to-person contact.
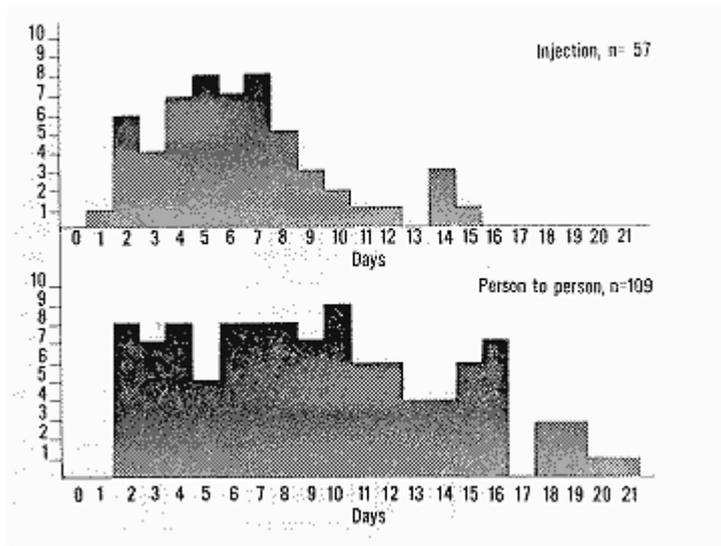
Fig. 4. Time of onset of Ebola Haemorrhagic Fever by transmission type
(after initial contact with source) Zaire, 1976.

TABLE 6

DISTRIBUTION OF CASE NUMBERS IN VILLAGES

Number of Cases	Number of Villages	% of Villages	Cumulative %
1	17	30.9	30.9
2 - 5	18	32.7	63 .6
6 - 9	12	21.8	85.4
10 -14	4	7.3	92.7
15 -19	1	1.8	94.5
20 -29	1	1.8	96.3
30+	2	3.7	100.0
Total	55		
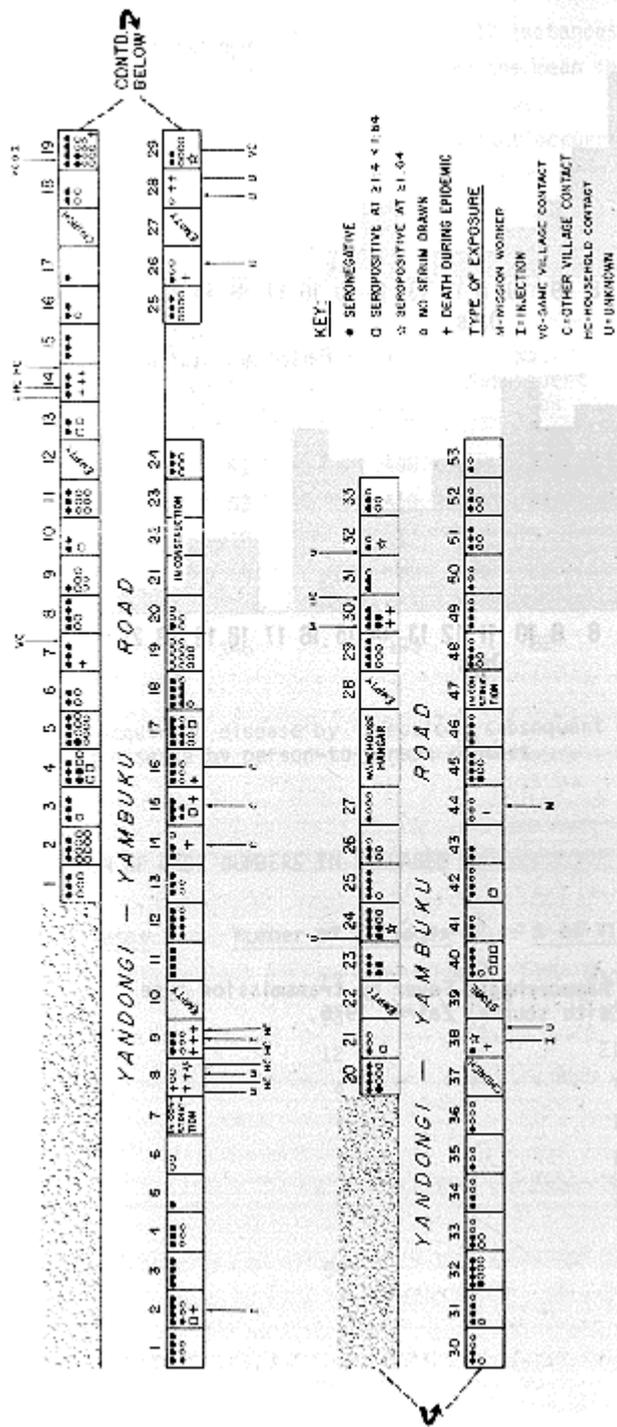
Fig 5. Ebola Haemorrhagic Fever, by household with individual inhabitants, Yamolembia, Zaire, September - October 18, 1976

Epidemic Disease in the Village of Yamolembia I.

This village, located five kilometers from Yambuku, was chosen for detailed analysis of disease transmission. The town was mapped and a door to door census revealed that 415 persons were resident in 71 households prior to the epidemic (Figure 5). Between 4 September and 18 October 1976, 24 persons developed probable or confirmed cases of EbHF. The first case was a 27 year old man who received an injection at YMH outpatient clinic on 29 August. Within six days, four more persons with a shitory of injection at the Yambuku Mission Hospital became ill. During this same period two nurses, a medical assistant and a catechist contracted the disease. These persons had all been in frequent contact with patients at the hospital, but had not received injections. By mid-October 15 additional villagers

had sickened, 12 of these secondary close contact with patients in this or other villages. Seven of these contacts occurred in the home of a neighbour, and three were contacts of sick relatives in other villages. Information on three cases was lacking.

Ten cases were among males, 14 among females. Adults of 15-45 years were most commonly affected. Only two persons survived, both contact infections. Cases occurred in 15 of the 71 households (21%) with 98 members. Four had secondary cases and one other had more than one case but could not be documented sufficiently to arrive at a conclusion as to transmission mode. There were six secondary and three tertiary cases giving transmission rates of 7.2% and 4.0%. Households with cases were scattered through the villages and no pattern of disease transmission other than very close patient contact was established.

In December 1976 and January 1977, sera were sought from as many people as possible. A total of 236 serum samples were obtained. Three persons, two of them in clinically non-infected households, who had not had symptoms during or since the epidemic, were found to have Ebola virus IFA titres of at least 1:64. All three had experienced contact with fatal cases. Extrapolating to the entire population two more silent infections might be expected. Thus it appeared that 29 people (7%) in the village had been infected, clinical illness ensued in 83% with an infection mortality rate of 76%.

Source of the epidemic

Although the first case began his symptoms six days after receiving an injection in Yambuku, it is of interest to follow his movements in the threeweek period preceeding his illness.

This person had been on a touristic visit to Mobaye-Bongo Zone in the northern part of the Equateur Region from 10-22 August 1976 with six other mission employees travelling in a mission vehicle. During this period he visited a number of larger towns (Abumombazi, Yakoma, Katokoli, Wapinda) along the road from Yambuku to Badolité which is very close to the Central African Empire frontier. He never arrived at Badolité because a bridge was out of use a few kilometers before Badolité. On his return to Yambuku on 22 August, he bought some freshly killed, and some smoked, antelope, and another traveller in the group bought some freshly killed, and some smoked, monkey from a local market along the route. His wife dried and stewed the antelope upon his return and this was consumed by his entire family. He had no contact with the monkey after his return to the village. The only possible animal contacts at the household were with two ducks. This person left to work in his nearby banana plantation a few days following his return. No unusual event occurring during the trip or in the fields upon his return was reported to the patient's wife.

During the search through the hospital records from January 1974 to the beginning of the epidemic, there was only one case resembling EbHF. This person was an adult male who was hospitalized on 20 August 1976 with "epistaxis and diarrhoea". The person left against medical advice on 30 August; he could not be found despite an active search during the investigations.

DISCUSSION

The cause of the epidemic was not identified until six weeks had passed and over 95% of cases had occurred. This was due to the similarity of clinical Ebola virus disease (3) to yellow fever (5), Lassa fever (6) and to a lesser extent to typhoid fever (7), one of the earliest diagnoses made by local investigators (8).

However, none of the patients responded who were treated with chloramphenicol. Jaundice was not an important clinical feature of the disease. All five of the expatriates who died had been vaccinated against yellow fever. Lassa fever has a higher frequency of pulmonary symptoms and bleeding is less common. Person-to-person spread is very uncommon and spread to a third generation is rare. Much of the early confusion could have been resolved if specimens would have been promptly collected and sent directly to the appropriate laboratory.

The epidemic curve resembled a common source infection and the peak occurred after 24-28 days had passed. The people in the community had already associated the mission hospital with the epidemic and had stopped coming to the outpatient department. Several patients with EbHF left the hospital for their villages in the second fortnight of September. The hospital staff, themselves severely affected by the epidemic, closed the hospital at the end of the month. This essentially stopped injection-transmitted disease and the epidemic shortly terminated.

The geographical distribution of cases was related to the hospital catchment area and the customs of the people. The epidemic zone was restricted to being quite near the mission hospital because the villagers in that area, during the beginning of the rice harvest, did not appear to stray too far from home. Several of the cases returned to their parents' homes when they became ill and these villages were for the most part also close to the hospital. The exportations of cases out of the epidemic zone to Bumba and Kinshasa illustrate how special circumstances can endanger large population centres. In

both of these instances injected persons had begun symptoms before leaving Yambuku. Because they were of the more favoured class they had the means to travel swiftly out of the epidemic zone hoping to find effective treatment at a larger medical centre. With the rather constant clinical presentation, incubation period and strict quarantine measures in the large cities, it became easy to identify cases and limit spread rapidly.

The women in the epidemic area were most severely affected. The group of women in the child-bearing age, between 15 and 29, went to the active prenatal clinic at the hospital. Although there are no data from hospital outpatient records to compute the rates of attendance by each age and sex group compared to the general population, other data show that adult females were more frequently hospitalized than males. Adult females also traditionally care for the sick. When death occurs, they are charged with washing and dressing the body for burial. When birth occurs in the village, it is the women of this group who assist the traditional midwife, especially when a complication occurs. Girls from 5 to 14 have taken on many of the duties of older females and are assumed to have also been in closer contact with cases while caring for them.

The contention that transmission by contaminated syringe and needle was the major mode of spread is based on good histories from at least 85 families that persons received injections within the three week period prior to illness onset whereas less than 1% of unaffected family members received injections. It is known that Ebola virus viraemia exists for at least 13 days past symptom onset and haematogenous spread of this disease has been confirmed (8). The manner in which the syringes and needles were cleaned and the technique for giving injections in this setting was conducive to transmission by this means.

Spread by close contact, mainly at the villages, could have been through the vehicle of contaminated body fluids (blood or excreta) entering open cuts or scratches. Although a sore mouth and throat were frequently reported, this was not often associated with cough or other respiratory signs which might have promoted spread.

Although it could not be completely ruled out, animals and insects appeared to have no role in transmission during the epidemic (2). Most families in the epidemic zone had the same type of contact with wild and domestic animals and those with cases did not differ from others. Although most adult men in the epidemic zone were hunters, the first known case was not.

The difficulty of person-to-person transmission of Ebola virus along with the high mortality rate indicates that the agent is probably an animal virus or has some other source in nature. The Marburg outbreak in 1967 was associated with Cercopithecus aethiops monkeys imported from Uganda and the illness produced has been popularly called "Green Monkey disease" (9). This is misleading as these groups of monkeys associated with the 1967 epidemic had an extremely high mortality rate while grouped in animal care areas at the laboratories which may indicate the monkeys contracted the disease from another source. Furthermore, these monkeys had contact with 48 species of other wild animals while en route from Africa to Europe (11). No investigations of the other animal species were done. Serologic studies done in East Africa among animals captured in the wild showed that several different primate species had antibodies to Marburg virus but the specificity of the test used has since been questioned. No source could be identified for the outbreak of Marburg virus disease occurring in South Africa in 1975 although the first of three cases received an "insect bite" a week before the disease onset (13).

The source of this epidemic, although not determined exactly, probably originated in the Sudan. The Sudan epidemic began in late June and was already known to have extended to the important regional centre of Maridi by August (11) Travel is not extensive between the Bumba Zone and the Sudan or the Central African Empire. However, many of these international exchanges are illegal and details are difficult to get. It was found that someone could travel from the infected zone in Southern Sudan to Bumba Zone in four days, well within the incubation period.

The origin of the cases in the Sudan is still obscure. This epidemic was also associated with a hospital. It is very likely that if these hospitals would not have served as "amplifiers" and disseminators, the epidemics may not have come to the attention of health authorities. The best way to ensure rapid diagnosis and necessary control measures is to develop an awareness of this disease among peripheral health workers, provide them with instruction and materials for taking specimens and proper equipment for protecting themselves if an outbreak of this or similar disease should occur.

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DISCUSSION

Bastian : In your presentation you mentioned some customs, can we have some more information on the customs to which you referred ?

J.G. Breman : The custom that at this time of the year, which is the rice harvest, people stayed very close to the epidemic area. Distant travel outside the zone, except in the well-noted and well-studied instances, did not occur. That epidemiologically is really the most important factor.

J.A. Bryan : How many midwives were there in the area and did everyone of these people become ill or none of them ?

J.G. Breman : In Yambuku interestingly enough, there were three midwives, all of them working at the hospital during the time of the epidemic, none of them had clinical symptoms and none had Ebola virus antibodies. In the village of the fifteen or so instances where there was a relation to a birth, and there were several stillborns which were not counted as neonatal disease, about half of the midwives there, were infected.

K.M. Johnson : I think the kind, intensity and duration of contact were important in the transmission. Are there any indications from the answers to the non-quantitative set of questions that were asked to the people, to define those individuals within the families who were at a considerable higher than 5% risk of getting the infection ?

J.G. Breman : There were two studies. One performed late October early November in a group of villages along the east-west road, and the second study beginning late November when people would not recall as well. Data on age, sex. names corresponded in over 90% of instances. In the early study it was found that when spouses were compared to parents and children or sibs, the secondary attack rates were upwards of 20%. When males were compared to females, the secondary attack rate was of the order of 16% for males and 27% for females, indicating that just asking if someone was a member of the family was not enough to define this high-risk group.

S. van Nieuwenhove : I just want to add something to the question of the slowing down of the epidemic. Normally burial involves a lot of physical contact with the corpse. But as time went on the population became very suspicious and did not touch the corpses any more, not even to bury them. That is probably why person-to-person transmission in the end slowed down very quickly.

A.A. Arata : The question that we still have not come to is, I think, where did the virus really come from ?

J.G. Breman : Retrospectively, what else could we have done or done differently in our investigations ? We had a long list of animal contacts, domestic and wild, but most of them were animals that were commonly seen by all. But there are a few very unusual species and varieties. It might have been helpful to find out if someone had this type of contact. Undoubtedly with the low contact rate and the high mortality rate this is probably a zoonosis.

I.W. Pinkerton : Was any information obtained from older people, as to the past occurrence of such epidemics ?

J.G. Breman : A lot of interviews early on were carried out with village elders, specifically regarding past epidemics of this type of syndrome. It was so unique to the villagers that they easily could reply. People over sixty years old had never seen anything like this. A good portion of these were bled as were the sisters who had been at the Mission for more than twenty or thirty years and none of them had antibodies.

C.E. Gordon Smith : Would you expect to detect antibodies from far distance infections ?

J.G. Breman : if that would have been twenty or thirty years ago, I don't know, but Dr. Isaacson brought us some titer from Marburg with a 1/8 titer after art acute in 1975 that certainly could have been detected.

K.M. Johnson : I don't think that anybody knows what the endpoint is by any method in terms of disappearance of antibodies. A small sample of people infected with Marburg virus in were rebled and retested in our laboratory in late 1975 early 1976 all are still positive titers were low but they all could be recognized as having antibodies. I suspect that we will find that the majority of survivors from epidemics will still be positive for a number of years. If you wanted to go to twenty or thirty years, I would hesitate clearly to make any statement at the moment.

THE HAEMORRHAGIC FEVER OUTBREAK IN MARIDI, WESTERN EQUATORIA, SOUTHERN SUDAN

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The epidemic was brought to our attention on the 26th of September, 1976, by the Director-General for Laboratories, Khartoum. We flew to Maridi, with the idea of a highly infectious disease - probably viral - characterized by a high mortality rate (40 - 50%) especially among the hospital staff, possibly Lassa fever.

On arrival in Maridi we found a really grave situation. There were over 30 cases in three hospital wards, most of them seriously ill, a few in coma, some were recovering. A few patients were left neglected, a large number of nursing staff, in panic, did not show up for work.

We examined some of the patients. All gave the same history of sudden onset with high fever followed by severe headache, myalgia, gastro-intestinal upset (vomiting or diarrhea or both) - chest pain, cough with dryness of the throat.

All patients were given antimalarial treatment without improvement and then anti-typhoid drugs during the second week. Some of them developed haemorrhagic manifestations, such as epistaxis, haemoptysis, haematemesis, malaena leading to deterioration in their general condition and cachexia. Some patients developed signs and symptoms of general nervous system involvement going into a state of convulsions, coma and death.

The epidemic started on the 6th of August 1976, when a student from Nzara arrived in Maridi en route to Juba. He was suffering from a severe febrile disease, and becoming increasingly ill was admitted to Maridi Civil Hospital. He died a week later. His brother and nephew, after burrying him in Maridi returned to Nzara. The brother then became ill and died in Nzara, the nephew also fell sick, went to Juba for treatment, flew to Khartoum and died in Omdurman on 30th August.

Meanwhile in Maridi a nurse, a hospital cleaner and a hospital messenger all developed identical symptoms and were admitted into various wards of the Hospital. Hospital contacts of these patients, seeded the disease around Maridi town - leading to one of the most tragic hospital outbreaks that ever occurred in the recent history of Medicine.

Position	Total in Hospital	Infected	% Infected	Deaths
Doctor	2	1	50%	1
Office Personnel	11	5	45%	1
Medical Assistants	6	6	100%	5
Nurses	53	14	26%	5
Student nurses	95	39	41%	22
Cleaners	39	6	15%	2
Miscellaneous	24	5	21%	5
Total	230	76	33%	41

The outbreak started by Mid-August and ended by the last week of November. The peak was during September.

Month	Cases	Deaths
August	10	3
September	111	56
October	84	53
November	23	5
Total	228	117

We collected specimens for virological studies :

blood, throat swabs, urine, C.S.F. These were transported by air to Khartoum and then to Porton-Down, England, where the etiologic agent was isolated. The following instructions were issued as primary measures for the control of the outbreak :

1. Very strict quarantine measures and isolation of suspected cases.
2. The application of strict aseptic techniques in handling specimens for investigation.
3. Minimize the unnecessary handling of patients by either the hospital staff or their relatives.
4. The wearing of gowns, masks, gloves by all hospital staff attending these wards.

With the institution of these measures the number of cases started to drop.

It appeared then that Maridi hospital acted as an amplifier of the disease without which the disease would have died out naturally.

The disease had originated in Nzara towards the end of June 1976 in three employees of the local cotton factory. The total number of cases in Nzara was about 70 with 33 deaths (mortality 47%).

The big question still remains as to the origin of the responsible virus.

EBOLA FEVER IN THE SUDAN, 1976 : EPIDEMIOLOGICAL ASPECTS OF THE DISEASE

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ABSTRACT

A large outbreak of haemorrhagic fever (subsequently named Ebola Fever) occurred in the Southern Sudan between June and November 1976. There was a total of 284 cases; 67 in the source town of Nzara, 213 in Maridi, three in Tembura, and one in Juba. The outbreak in Nzara appears to have originated in the workers of a cotton factory. The disease in Maridi was amplified by transmission in a large, active hospital in that town. The overall mortality rate was 53". Transmission of the disease required close contact with an acute case and was usually associated with the act of nursing a patient. The incubation period was between 7 and 14 days. Although the link was not well established, it appears that Nzara could have been the source of infection for a similar outbreak in the Bumba zone of Zaire.

In June and July 1976, a severe viral haemorrhagic fever erupted in Nzara in Western Equatoria Province of Southern Sudan. From Nzara, the disease spread to Tembura, Maridi, Juba, and Khartoum in the Sudan and also involved the Bumba zone of northern Zaire where a large outbreak followed.

METHODS

Investigation of the outbreak in Sudan involved finding all of the cases and recording pertinent information. Several sources were used to find as many cases as Possible: 1) Hospital records, 2) visiting homes of patients and ferreting out other cases or their contacts, 3) searching house-to-house in the infected areas for additional cases, and 4) contacting local chiefs for information.

A case was defined as: 1) anyone having the symptoms of fever and headache lasting for at least two days with the addition of gastrointestinal symptoms (diarrhea or vomiting) or chest pain; or 2) diagnosed by a physician in a hospital. Using this definition, a total of 284 cases occurred in Sudan between June and November, 1976 -- 67 in Nzara, 213 in Maridi, three in Tembura, and one in Juba.

THE ORIGIN OF THE OUTBREAK - NZARA

The origin of the outbreak was Nzara, a small town with clusters of houses scattered in the dense woodlands bordering the African rain forest zone. The total population of the area within 10 miles is estimated to be about 20,000 most of whom live in mud-walled thatchedroofed houses surrounding the town proper. The main employer in Nzara is a large agricultural corporation which has 2,000 employees, half of whom work in a large cotton factory in the town. The corporation has excellent records of employee absenteeism which facilitated the epidemic investigation. A small hospital is operative in Nzara, but at the time of the outbreak the facilities were limited and few patients were admitted.

Discussions with local people and a review of the factory records for the previous two years did not reveal any fatal haemorrhagic disease in Nzara until late June or early July, 1976. At that time, one or two factory workers per week started dying of haemorrhagic disease and subsequently their families or friends who cared for them would manifest the same symptoms. By the first week in September, six factory workers and 25 of their contacts had developed the same syndrome and 21 had died. Of the six factory employees, five worked in one specific end of the cotton factory. Extensive discussions with

friends and families of these workers did not reveal any possible link between them except the factory. None had cared for any pre-existing cases of the disease nor were they ill prior to onset when they might have received an injection with a contaminated needle, nor did they have any known contact with monkeys or any other wild animals. Their houses were widely scattered over the area and their social circles were very different.

With their only link being the cotton factory, the investigation for an animal reservoir of infection was concentrated in Nzara and specifically in the cotton factory itself. The results of this investigation will be reported elsewhere.

TEMBURA

The outbreak in Nzara continued on until late October infecting a total of 67 people of whom 31 (46%) died. Before the outbreak spontaneously died out, cases were exported to two neighbouring areas. One was Tembura, a small town 160 km to the north where an ill woman went to be nursed by her family. Subsequent to her death, the three women caring for her died of the same haemorrhagic disease. No subsequent cases were discovered in the area despite active searching.

MARIDI, JUBA, AND KHARTOUM

The other exportation was to Maridi with a very different outcome. Maridi, a town with an estimated population of 10,000 with another 5,000 people in its environs, is located about 180 km east of Nzara (between Nzara and the regional capital of Juba). The hospital in Maridi, in contrast to the one in Nzara, is an actively practising hospital with a large staff. It serves as a teaching hospital where student nurses are taught patient care. With its large staff, (total 230), it served as an ideal centre for the large hospital associated outbreak that followed the introduction of the disease from Nzara. After Maridi was infected, four cases (one from Nzara and three from Maridi) were transferred to the Regional Hospital in Juba. In late September, three of these cases were flown to Khartoum (1,200 km north) where two died. Fortunately, only one secondary case in a nurse from Juba occurred as a result of these exportations.

MORTALITY

In total, 284 cases occurred with 151 deaths (mortality rate -53ol). All but four of the cases were in Nzara and Maridi. In these two areas, the clinical disease appeared identical and the mortality rate (46% in Nzara and 54% in Maridi) very similar. Moreover, the mortality rate stayed relatively constant through the five months of the outbreak, during which approximately 15 generations of person to person disease occurred. Monthly mortality ranged from 63% in October to 40% in November with the previous month's rates falling somewhere between. The mortality rate by age and sex showed similar, but insignificant, variations. For children, teenagers and adults it was 44%, 39%, and 56% respectively while the overall mortality rate for males was 56% and females, 48%.

CONTRAST OF NZARA AND MARIDI

The similarity of the clinical disease and its mortality in Nzara and Maridi is contrasted with the type of outbreaks in the two locations. The Nzara outbreak was centred in factory workers and spread to their families. In Maridi, the hospital served both as the focus and amplifier of the infection. After two separate importations from Nzara, (the major one during the last week of July and another during the first week of September), Maridi hospital workers became ill and they were in turn hospitalized. Those caring for them were then infected and the cycle repeated itself. The difference between Nzara and Maridi is best exemplified by examining where patients most probably became infected. Few patients (26%) were even hospitalized in Nzara and they seldom stayed more than a few days which decreased the chance of infection within the hospital. But in Maridi, almost three quarts of patients were hospitalized and often for over two weeks. As a result, Maridi hospital was a common source of infection (46% of cases) whereas the Nzara hospital was not (3% of cases).

The outbreaks that followed in both instances reflected the initial seeding of infection in each locale. The outbreak in Maridi was larger (213 cases) and accelerated rapidly after the initial importation within the hospital, whereas the Nzara outbreak was smaller (67 cases) and more sustained. The age specific attack rates reflect these differences. The Maridi attack rates were higher (overall 14.2 cases per 1,000 population compared to 3.4 cases per 1,000 in Nzara) and the cases were primarily adult males reflecting the predominance of male staff in the hospital (75%). In contrast, Nzara had equal attack rates in adults and had a moderate number of teenage male cases. These teenage cases were all associated with a single chain of transmission that links Nzara to Maridi and Juba.

TRANSMISSION

The transmission of viral haemorrhagic fever in both outbreaks was similar, requiring intimate contact with a previous case. There was seldom any problem, in determining the source of infection for a case. Usually, the possible source (or sources) was well known to the patient being investigated. This is well documented in Maridi where sources were determined for all but five of 203 investigated patients, This contrasts and reemphasizes the uniqueness of the Nzara outbreak where 14 of 67 (21%) cases had no contact. Nine of these were employees of the cotton factory and possibly represent the initial introduction of the disease into the human population of the area.

Once in humans, transmissions from one person to the next were not rapid. It required close and usually prolonged contact with an acutely ill patient. Transmission did not seem to occur via the airborne route. To better define the pattern of transmission, we took a small sample of 17 highly-infected households and studied the type of contact that resulted in spread of infection. In this group, all secondary cases had slept in the same room and all secondary cases had touched the primary case during his/her acute disease. However, touching and sleeping in the same room had a relatively low attack rate (23%), whereas actually nursing of the case greatly increased the chances of becoming infected (81%). This requirement for close contact explains the lack of cases in children who, although sleeping in the same room, did not become infected. No casual infections in passersby or in contacts of primary contacts were observed.

HOSPITAL SPREAD

From these observations. it is evident that nursing of a patient was almost a requirement for becoming infected (39 of 44 instances). Therefore, a hospital should be, and was, an ideal environment in which to transmit disease. At least one third of the staff of Maridi hospital had disease and 41 staff members died. All of the 6 medical assistants were infected and 41% of the student nurses. Before the disease was recognized, most wards of the hospital had haemorrhagic patients in them and at the height of the epidemic, the hospital was in chaos. In total, 93 of Maridi's 213 patients acquired their disease in the hospital. Most of these (72) were hospital staff infected during their duties. At least six others were patients who were infected by contact or injection with infectious material from nearby acutely ill patients. Fifteen additional people, probably received their infection as visitors of infectious patients in hospital. All of them were involved in the care of an acutely ill patient during their visit to the hospital.

SECONDARY ATTACK RATES IN THE COUNTRY

Once out of the hospital and into the community, the disease spread in a similar manner, but did not have the large substrate on which to feed that the hospital provided. We studied thirty-six families with 38 primary cases and listed contacts that resided in the same house (Table 1). As can be seen from the table, the original 38 cases had 232 contacts of whom 30 (13%) developed subsequent disease. Similar rates (14% and 9%) were observed in the subsequent generations giving an overall secondary attack rate of 12%. These results document the relatively slow rate of spread of this disease.

TABLE 1 SECONDARY ATTACK RATES IN FAMILY CONTACTS OF CASES, MARIDI - 36 FAMILIES.

Generation	Cases	Contacts	Secondary Cases	Secondary Attack Rate
1	38	232	30	13%
2	30	126	18	14%
3	18	54	5	9%
4	5	39	0	-

INCUBATION PERIOD

Since most cases had had prolonged contact with their source case, it was difficult to make accurate measurements of incubation periods. However, 11 cases were discovered in whom relatively short exposure times were documented and subsequent onset of disease accurately recalled. From these cases, and the lack of any documented extremely short or long generation times, we concluded that the incubation period was usually between 7 and 14 days.

CONTROL

The control of this outbreak in the Sudan relied on the classic public health Principles of identification and isolation. The outbreak in Nzara died out spontaneously. However, the outbreak in Maridi required intervention. Strict barrier nursing was established initially in early October, 1976 and reinforced with addition al disposable isolation equipment in mid-October. In early November, surveillance teams were established to search house-to-house in Maridi and any patients discovered were placed in a specially constructed isolation ward. With time, the surveillance was expanded to include most of Western Equatoria Province. The last known case in Sudan occurred on November 20, 1976.

LINK BETWEEN SUDAN AND ZAIRE

Despite extensive efforts, the exact link between viral haemorrhagic fever in Nzara, Sudan and Bumba Zone, Zaire, remains undetermined. There is no doubt that there is extensive traffic bringing commercial goods to and from the two areas. In fact, we interviewed one truck driver in Nzara who personally escorted an early case in Nzara to hospital on July 24, 1976 with the help of two other friends. The two other friends both developed disease respectively on the 1st and 3rd of August,1976 and died 10 days later. The driver left the day after aiding his friend and arrived in Bumba 4 days later. He stated he had no disease and that he knew of no one who travelled with him who had become ill. It is very possible that several other people who had had similar close contact with sick patients travelled from Nzara to Zaire during the course of the outbreak in Nzara. One of them may have become ill and set up a secondary chain of infection in Zaire.

CONCLUSIONS

Ebola fever is a newly recognized disease with many similarities to two other haemorrhagic diseases (Lassa and Marburg fevers) that are known to spread from person to person 1-4. All three diseases, although caused by different agents, are clinically similar, have high mortality rates, and are all spread among humans by close contact with infected effluvia from acutely ill patients. The most likely sources of infectious virus responsible for this person to person transmission are blood (or blood containing excreta) or urine. Respiratory spread has been postulated in one outbreak of Lassa 2 but since large numbers of secondary cases are unusual for all of these diseases, the frequency of aerosolized virus is probably low. Additional late spread has been documented via semen in Marburg disease (3).

Because of the relatively insufficient mode of spread, most outbreaks of these diseases have undergone few generations and have been short lived. However, the outbreak in Maridi is an example of the potential of these diseases to proliferate in fertile soil. The obvious means of preventing such a disaster is to recognize cases early and establish barrier nursing. Considering that 30% to 71% of patients with these diseases have haemorrhagic manifestations (3,5), it is not difficult to recognize a cluster of epidemiologically related cases. Once recognized, the diseases are not difficult to contain.

Any hospital in the tropics or any hospital receiving patients from the tropics should be on constant alert for haemorrhagic signs in febrile patients. Once cases are recognized, the following procedures should be followed :

1) Collect specimens (blood or tissue) for diagnosis. (The World Health Organization will arrange for proper shipment of specimens to high security laboratories).

2) Establish a surveillance system to identify other cases of influenza like disease with or without haemorrhagic manifestations.

3) Establish barrier nursing with gowns, gloves, and masks (or better, respirators).

    a. Disinfect patients' excreta with a proper agent like formaldehyde,

    b. Laboratory specimens, if taken at all , must be extremely cautiously dealt with.

4) Identify contacts and perform daily temperature surveillance. Once temperature rises, patients should be isolated.

5) Once the diagnosis is made, convalescent plasma should be dispatched to the area.

With such a simple system, these diseases can be identified, isolated, and patients properly treated. From the Lassa story, it is evident that Lassa fever is far more prevalent than 6 previously thought . The same could be equally true for Marburg and Ebola fevers. As we become more aware we will, no doubt, identify more outbreaks of these diseases and as this happens we will see that large outbreaks

are indeed rare and can be prevented with simple precautions. However, if such precautions are not taken early, as was the case with the first outbreak of viral haemorrhagic fever in Sudan, these diseases can spread far and wide across international borders. The hospital, especially the referral hospital, is the site where such outbreaks can either be recognized and halted, or unrecognized and disseminated. With them rests the responsibility for stopping the spread of these dangerous diseases.

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THE NZARA OUTBREAK OF VIRAL HAEMORRHAGIC FEVER

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The detailed epidemiology of African Haemorrhagic Fever in the Southern Sudan has been dealt with by Francis (this report). This brief account is intended to supplement this with some details of the Nzara outbreak especially where differences were observed. The investigation in Nzara was carried out retrospectively and, as it appeared probable that Nzara was the original focus of the disease in the Sudan, particular attention was paid to the possible routes of introduction of the disease into the human population.

THE AREA OF THE OUTBREAK

Nzara is a township in Yambio District, Western Equatoria, of some 20,000 people clustered around an extensive agricultural organization and Cotton Manufacturing Factory employing a total of 2,000 staff. The town is partly composed of brick built houses in labour lines and surrounded by densely inhabited areas with mud and thatched tukals, housing the majority of the town's population.

Ecologically, the area lies close to the central African rain forst zone. It is rich agricultural country with extensive teak plantations and fruit orchards. Much of the area is dense woodland with areas of secondary forest. The civil disturbances of the last two decades had caused many people to leave the area, but since the early 70's the area has become rapidly repopulated with subsequent reclamation of forested areas for subsistence farming as population pressure increases.

THE ORIGIN OF THE OUTBREAK

The original cases of haemorrhagic fever occurred amongst employees of the Nzara Cotton Factory; the first case (YuG) fell sick on June 27th and died in hospital on July 6th after an illness lasting for nine days. Both the hospital records and descriptions obtained from close relatives described an illness with features indistinguishable from those of subsequent cases. During his illness he was nursed in hospital by his brother (YaG) who fell sick one week after the death but survived. Examination of serum from the family contacts and also the surviving brother were negative. They lived some six miles out of Nzara on the old Maridi road. This first case had been a store keeper working in the cloth room of the Cotton Factory.

The second case (Bz) who died on July 14th, also in hospital, worked in the cloth room of the Cotton Factory. Following his death his wife, his only surviving relative, also died. Both were reported as having suffered a severe haemorrhagic illness.

The third and most important case in Nzara (PG) worked alongside these previous two males in the cloth room in the Cotton Factory. He fell sick on July 18th and after several short stays in the hospital died on July 27th. He lived approximately one mile south of the Cotton Factory in a densely populated area.

These three men working in the Cotton Factory had remarkably little social contact; they lived in quite separate areas around the township and behaved in quite different manners. The first two cases were described as quiet unremarkable people whilst the third case (PG) was involved in a variety of enterprises within the town. His home was next to a shop run by a merchant (MA). He helped in the shop acting as an interpreter for visiting tradesmen, including those from Zaire and often ate with the family. Two brothers staying in the household were close personal friends of his. They were involved with a local jazzband and in addition PG has a number of close girl friends. Following his illness and eventual death, there was an eruption of cases involving the MA household, the family of their servant and six women who had closely attended him whilst sick. From this single case 69% of all Nzara cases and 87% of deaths could be traced. Also from this the MA family provided the first introduction of cases to Maridi and Tembura and very possibly the route of introduction to Zaire through lorry drivers who stayed in the household and travelled between Northern Zaire and Western Equatoria for the purpose of trading in beer, cigarettes and light machinery. Thus the majority of cases occurring in the Nzara area were traced to this single infection. Direct man to man transmission was subsequently observed through six generations of cases.

OTHER CASES IN NZARA

In addition to this predominant cluster of cases a series of other cases occurred in Nzara township between July and early October 1976 in which no direct contact could be established with previous cases. This aspect of the epidemiology appears quite distinct from the pattern observed in Maridi.

Of these apparent primary cases three worked in the cloth room at the Cotton Factory; two in the adjacent weaving section; one in maintenance and two were agricultural officers employed by the corporation. In only one of these cases (AR) whose onset of disease was in mid September was there any evidence of positive serology despite the fact that six survived their infections.

THE COTTON FACTORY

Apart from the two agricultural employees both living to the south of Nzara, the main focus of infection appeared to be the Cotton Factory; predominantly the area around the cloth room and weaving sections. Presumably, either transmission was occurring direct from person to person following its original introduction into this population and behaving quite unlike the subsequent epidemiology pattern observed both in Nzara and Maridi or alternatively transmission was being effected within the factory from an animal source.

The Cotton section of the Factory employs some 480 staff. Another 500 also worked in surrounding buildings concerned with the manufacture of cotton seed cake, cotton seed oil and soap. The Cotton Factory is housed in a single large building divided into various sections. Raw cotton is taken in at one end and is processed within the same building to the finished cloth. The cloth room and store are adjacent to the weaving section at one end of the building and employed 24 staff. From this one area 4 deaths (16%) occurred and a further 5 cases were seropositive in November. Thus, 37% would appear to have been exposed to infection in sharp contrast to the remainder of the factory staff (Table 1). These findings are also in sharp contrast to the serology both of suspect cases, in whom only 16% were positive, and close family contacts in whom only 2% were positive. In a follow up survey of all cases found to be seropositive in the factory in November 1977, half of these cases gave no history of previous illness over the preceding 12 months whilst the other 50% gave history only of a minor febrile episode.

Examination of the cotton factory showed that rat infection was especially prevalent and in addition the roof housed considerable populations of bats. There was no evidence that there had been any recognizable epizootic in either of these populations. Limited numbers of both rats and bats were collected for virological studies and the results are awaited with considerable interest.

The Cotton Factory also has a small clinic room where minor illness in the factory employees are treated. The clinic is run on a daily basis by a nurse employed by the corporation. She treats predominantly minor illnesses and injuries. Injections of chloroquire and occasionally antibiotics are given in this dispensary.

A definite history of previous injection was not obtained in any of the surviving cases detected clinically during the survey.

TABLE I

SEROLOGICAL SAMPLING IN VARIOUS GROUPS IN NZARA

Group	No collected	+ve	%
1. Probable cases	27	4	16
Possible cases	10	2	20
Total	37	6	16
2. Close household contacts	41	1	3
3. Immediate neighbours	18	0	-
4. Cluster samples around the home of YuG	19	0	-
5. Employees of the Cotton Factory:			
Weaving room	55	2	4
Spinning room	28	1	4
Cloth room	17	3	18
Drawing in room	9	1	11

Of the Cotton Factory employees found seropositive in November, approximately 50% would have had injections at the dispensary for their minor febrile illnesses. A careful enquiry revealed no evidence of co-primary cases occurring related to syringe passage.

The high proportion of seropositivity amongst the Cotton room employees in sharp contrast to the low prevalence of antibodies both in clinically suspect cases and in close contacts of known cases and deaths is strongly suggestive of continued transmission within the Cotton Factory, beyond the period of the epidemic occurring in Nzara. The contrast in serological results in Maridi and Nzara plus the decline observed in antibodies titres between November and January would appear to suggest that demonstrable antibody responses are a rather transient phenomenon.

If this is so, then it would support the view that those cases found seropositive in the Cotton Factory had been infected more recently than cases occurring in the township and discovered during epidemiological surveillance.

The available evidence suggests that the Cotton Factory was the predominant source of infection and that a series of apparently primary cases occurred in this population. In most cases whilst in the one notorious, incident of case three (PG) the infection appeared to lead to at least six generations of cases in Nzara alone. The question of syringe passage with regard to transmission from this case must also be considered. Two nurses were involved in this outbreak and both died. They visited patients, including the original case (PG) in their home and were known to have given injections. It is quite conceivable that the friends and neighbours of this case might have received prophylactic injections at the time of the original illness or subsequence to his death.

VIRAL HAEMORRHAGIC FEVER IN THE SUDAN, 1976 : HUMAN VIROLOGICAL AND SEROLOGICAL STUDIES

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SUMMARY

Two further strains of Ebola virus were isolated from acute phase sera collected from acutely ill patients in Maridi hospital during the investigation in November 1976. Antibodies to Ebola virus were detected by immunofluorescence in 42 and 48 patients in Maridi who had been diagnosed clinically but only in six of 31 patients in Nzara. The possibility of the indirect immunofluorescent test not being sufficiently sensitive is discussed.

19% of Maridi case contacts in hospital and in the local community had antibodies. Very few of them gave any history of illness indicating that Ebola virus can cause mild or even subclinical infections. 37% of the cloth room workers in the Nzara cotton factory appeared to have been infected suggesting that the factory may have been the prime source of infection.

INTRODUCTION

An outbreak of viral haemorrhagic fever in Nzara and Maridi townships of southern Sudan in the second half of 1976 affected nearly 300 people, 150 of whom died from the infection. The virus responsible, since named Ebola, was first isolated from patients in October 1976 and was shown to be identical to virus strains isolated during a simultaneous epidemic occurring in northern Zaire (1,2,3).

A brief description of the Sudanese outbreak was outlined by Simpson et al. (4); Smith et al. (5) gave details of the principal clinical features; Francis et al. (6) gave a detailed account of the epidemiological aspects; while Ridley et al. (7) described the histopathological changes found in two fatal cases.

This paper describes additional virus isolation attempts on acute human material and post mortem specimens collected by the joint WHO/Sudanese investigation team and serological studies on human sera collected from convalescent patients, case contacts and members of the local populations of Nzara and Maridi who were not believed to have been exposed to infection with Ebola virus during the outbreak.

MATERIALS AND METHODS

Acute samples: Blood, throat swabs, and urine samples were collected within five days of the onset of illness from eight acutely ill patients in Maridi Hospital isolation wards. All eight patients were febrile, grossly dehydrated and displayed many of the clinical features described by Smith et al. (5) .

In the isolation wards and when handling samples, team members wore protective clothing and biological respirators as suggested by Simpson . Blood samples were collected using disposable syringes and needles and immediately injected into vacutainer tubes. Throat swabs were placed in sterile saline in screw-capped polypropylene serum tubes (Nunc); urine samples were collected from bed-pan samples in similar polypropylene tubes. Specimen containers were washed in 2% formol saline solution, and placed in plastic bags for removal from the isolation area. Swabs, needles and syringes were immersed in 2, formol saline, placed in plastic bags inside a bucket and taken for

immediate incineration. Protective clothing was removed on leaving the isolation area; disposable items were incinerated, reusable gowns were decontaminated by boiling.

After clotting, blood was centrifuged in sealed vacutainer tubes in the field laboratory and serum aspirated off into polypropylene tubes. All acute samples were stored in liquid nitrogen prior to dispatch to the Microbiological Research Establishment, Porton.

Post-mortem samples. Small pieces of liver, spleen, heart, lung, kidney and bone marrow were collected in polypropylene tubes and stored in liquid nitrogen.

Convalescent plasma. Aliquots of immune plasma were collected using a modified plasmapheresis technique. As no centrifuge was available, plasma and cells were separated by gravity only over a 2-3 hour period.

Sera from contacts . Blood samples were collected using disposable syringes and needles before centrifugation in sealed vacutainer tubes. In Nzara samples were collected in the cotton factory and in homesteads around the township; in Maridi samples were collected in the hospital grounds, schools and surrounding homesteads. After centrifugation sera were stored in polypropylene tubes and stored at – 5ºC. For dispatch they were placed in liquid nitrogen and stored at the Microbiological Research Establishment at –20ºC until tested.

Laboratory studies. All manipulations involving potentially infectious material were carried out in the Maximum Security Laboratory at the Microbiological Research Establishment.

Virus isolation attempts. Ten per cent suspensions of post mortem tissues were prepared in a pH 7.2 phosphate buffered saline containing 0.75% bovine plasma albumin (Armour Fraction V), penicillin and streptomycin. Tissue suspensions, acute serum samples, urine and throat swab eluates were inoculated into cultures of an African green monkey cell line (Vero) and Dunkin-Hartley strain guinea pigs following the procedures described by Bowen et al.

Immunofluorescent Technique. The indirect immunofluorescent test was similar to that described by Wulff and Lange (9) . Vero cells were prepared in 25 cm2 plastic flasks (Nunc) and maintained in Leibovitz medium (LI5) containing 2% feotal calf serum at pH 7.2-7.4. Each flask was inoculated with 1 ml of Ebola virus as a 10% guinea pig liver and spleen suspension. The medium was changed on the second day to reduce toxicity caused by using guinea pig tissue. As indicated by daily immunofluorescence antibody (IFA) testing of replicate preparations, flasks were incubated at 37ºC until 30-40% of the cells had become infected, usually at 6-7 days.

For slide preparation media was first decanted off the infected cells and the cell sheet washed three times in a pH 7.27.4 phosphate buffered saline (PBS). The cell sheet was detached with 2 ml of 0.2% trypsin in versene; the cell suspension was removed and diluted in an equal volume of PBS containing 3% calf serum to inactivate the trypsin/versene activity. The suspension was centrifuged at 1000 rpm for five minutes, the supernatant discarded, and the cells resuspended in PBS. This procedure was repeated twice and the cells were finally suspended in PBS containing 0.2% of bovine serum albumin to a concentration of approximately 5 x 10^5 cells/ml. The suspension was seeded into the wells of polytetrafluoroethylene coated glass slides each having 12 x 6 mm wells. Each well received one drop of cell suspension. The slides were allowed to dry under an ultra-violet lamp for 20 minutes followed by ten minutes fixation in chilled acetone.

Sera were examined for the presence of Ebola virus antibodies by an indirect immunofluorescent method (9). Duplicate dilutions of all test sera were made in PBS and sera were screened at dilutions of 1:4 and 1:8. Known human positive and negative sera were included as controls and PBS controls similarly used. All test and control sera were screened on both infected and uninfected slides. Diluted sera were placed in slide wells and held in a moist chamber at 37ºC for 30 minutes and then washed in PBS for 5-10 minutes. Slides were then dried in air and a drop of Fluorocein labelled rabbit anti-human IgG conjugate (Wellcome) at dilutions of 1:20 to 1:25 applied to all wells. The slides were again held at 37ºC in a moist chamber for 30 minutes and then washed three times in PBS for 2-4 minutes on each occasion and finally rinsed in distilled water for 30 seconds. Slides were then dried in air and examined under a Reichert Fluorvar microscope using an HBO 500w mercury vapour burner. Sera were only accepted as positive if clear fluorescence was observed at dilutions gte 1:8. Sera which were positive only at a dilution of 1:4 were regarded as equivocal and graded as negative.

RESULTS

Virus isolation. Two strains of Ebola virus were isolated from acute human sera from two patients in the Maridi isolation wards - one from a 12-year-old schoolgirl (collected on 5 November 1976) who died three days later; the other, collected on 8 November 1976, was from an 18-year-old male student who later recovered. The isolations were made in guinea pigs inoculated intraperitoneally. The guinea

pigs developed a febrile illness five days after inoculation; passage of guinea pig blood to further guinea pigs produced a similar febrile illness but a fatal infection was only produced in guinea pigs after five passages. The isolates were later successfully cultured in Vero cells and identified as strains of Ebola by immunofluorescence.

No isolates were obtained from other patients' sera or from any of the throat swabs, urines or post mortem tissues. Virus particles were, however, visualised by electronmicroscopy in liver sections from both fatal cases.

Serology. One hundred and four surviving Maridi patients were identified. All of them had been diagnosed as suffering from Ebola fever on clinical grounds alone. 35 of these patients were members of Maridi hospital staff and the remaining 69 patients were Maridi residents, some of whom were relatives of hospital staff. 30 of the 35 surviving hospital staff were bled for serological studies. 25 of them (83%) had detectable IFA antibodies. Only 18 of the 69 patients who were not hospital staff were willing to be bled. 17 of these patients had detectable antibodies. Altogether 42 of the 48 patients diagnosed clinically as having been infected and who were bled had antibodies against Ebola virus (Table 1).

Among the probable and possible case contacts hospital staff were considered to be among those most at risk. 159 members of the staff had given no history of severe febrile illness during the epidemic. Sera from 64 of these staff members Were tested for Ebola virus antibodies. The results are shown in Table 2. Seven staff members, including four nurses, a cleaner, a toilet cleaner and a water carrier had evidence of infection. None of the three doctors attending sick patients had antibodies.

One hundred and two members of the Maridi population who had been in contact with known cases of infection were tested. The majority of them were close family contacts and several had helped to nurse sick relatives during their illnesses. 22 case contacts had demonstrable antibodies (Table 3). Close questioning revealed that nine of these close contacts had some evidence of febrile illness without manifesting severe disease. 29 members of a Maridi school were tested as a control group. None of them was thought to have had any contact with a known or suspected case of Ebola virus disease. However, three schoolboys had antibodies. None of them had any history of recent illness.

In Nzara 37 surviving patients diagnosed clinically as having had Ebola fever were identified and 31 of them were bled for antibody studies. Only six (19%) had demonstrable antibodies and none had antibody levels greater than 1:32 (Table 4). Among close family contacts only one person out of 78 who were tested had detectable antibodies although five of these contacts had an antibody titre of 1:4 which not accepted as positive.

TABLE 1

RESULTS OF IFA TESTS ON CONVALESCENT PATIENTS' SERA

	Number of survivors	Number tested	Number positive
Hospital staff			
Medical Assistant	1	1	1
Nurses and midwives	9	8	7
Student nurses	17	14	11
Cleaners	4	4	3
Miscellaneous staff	4	3	3
Total	35	30	25
Non-hospital staff			
	69	18	17
Overall total	104	48	42

TABLE 2

RESULTS OF IFA TESTS ON SERA FROM MARIDI HOSPITAL STAFF CONTACTS

	Number bled	Number positive
Doctors	3	0
Nursing staff (including tutors, midwives, theatre staff)	35	4
Drivers	5	0
Cleaners	11	1
Toilet cleaner	1	1
Water carrier	1	1
Laboratory assistant	1	0
Messenger	3	0
Gardeners/carpenters	4	0
Totals	64	7

TABLE 3

RESULTS OF IFA TESTS ON SERA FROM MEMBERS OF THE MARIDI POPULATION

	Number bled	Number positive
Case Contacts	102	22
No known contact	29	3
Total	131	25

TABLE 4

RESULTS OF IFA TESTS ON SERA FROM NZARA CONVALESCENT PATIENTS AND FAMILY CONTACTS

Number of survivors	Number bled	Number positive
37	31	6
Number of contacts bled	78	1

Sera from 109 cotton factory workers were tested (Table 5). Seven members of the staff had antibodies with the largest number, three, in the cloth room and adjacent store. Three workers in the weaving section had antibodies while only one member of the 28 spinning section staff who were bled was positive. Antibody levels ranged from 1:16 to 1:64 and none of the seven workers gave a history of any recent illness.

TABLE 5

RESULTS OF IFA TESTS ON SERA FROM NZARA COTTON FACTORY STAFF

Department	Number bled	Number positive
Cloth room and store	17	3
Drawing-in section	9	1
Weaving section	55	2
Spinning section	28	1
Totals	109	7

DISCUSSION

No really satisfactory serological test has yet been devised to detect and quantify antibodies against either Marburg or Ebola viruses. Early workers (11,12,13) used complement fixation tests to detect antibodies to Marburg virus following the outbreak in 1967. They used crude antigens derived from guinea pig and monkey tissues but found them to be unreliable because they lacked specificity. Malherbe and Strickland-Cholmley (14) compared antigens derived from animal tissues with those prepared in monkey kidney cell cultures and found both types to be unsatisfactory. Slenczka and Wolff (15) and Slenczka et al. (16) found that antigens derived from chronically infected Vero cells were much more satisfactory but still had doubts about their activity.

Neutralization tests have been carried out in guinea pigs and tissue culture systems but have proved costly in animals, cumbersome, time consuming and generally very unsatisfactory. Siegert and Slenczka (13) and ourselves have had some success with neutralization tests in Vero and BHK$_{21}$ cells based on counts of intracytoplasmic inclusion bodies but the results were difficult to reproduce. The problem is that no really obvious cytopathic changes can be readily visualized in any of the cell culture systems used so far although Johnson et al. (2) have claimed that some cytopathic changes are discernible in Vero cells. However these workers, like ourselves, have based their serological results on immunofluorescent tests. Immunofluorescence with Marburg virus was described in BHK$_{21}$ by Carter and Bright (17) but recent work on Marburg and Ebola has been based on the indirect immunofluorescent technique described by Wulff and Lange (9) for use with Lassa virus. The method is more sensitive than complement fixation and appears to be quite specific. It certainly distinguishes between Marburg and Ebola viruses (2). Wulff and Conrad (18) believe that the method could be used successfully for survey work since, at least with Marburg, antibodies can be detected five years after infection by this method.

The diagnosis of Ebola virus infection was confirmed by the indirect immunofluorescence test in 42 of 48 Maridi patients who had been diagnosed clinically. These results were confirmed in several patients by a parallel test kindly carried out at CDC, Atlanta, by Dr. Patricia Webb. There can be little doubt that the other six patients, in whom no antibodies could be detected, had been infected with the same virus since they all had febrile illnesses accompanied by haemorrhages and symptoms as described by Smith et al. (5). The lack of detectable antibodies in 25 of 31 Nzara patients was surprising and disappointing especially as all of them had had illnesses clinically indistinguishable from proven cases of Ebola fever. The Nzara patients were bled at times ranging from six to 17 weeks after the onset of their illnesses. It may be that their antibody levels may have fallen below those detectable by immunofluorescent testing. In view of earlier results with Marburg virus this possibility seems unlikely. However, antibody levels in proven cases of Ebola infection in Maridi did fall quickly. 18 of 23 patients bled in November 1976 and again in January 1977 had distinct falls in antibody levels ranging from 2- to 5- fold. Later antibody levels were often detected at the lowest acceptable dilution of 1:8. Two patients actually became negative. These results could possibly be explained if the immunofluorescent test was directed towards detecting IgN antibodies alone. Unfortunately the test was specifically directed to detect IgG and therefore no satisfactory explanation for the lack of detectable antibodies can be advanced. Obviously further work needs to be carried out on developing more sensitive test systems.

The finding of Ebola virus antibodies in 25 out of 131 (19%) Maridi case contacts and in seven of 64 hospital staff contacts (19%) indicates that Ebola virus can cause milder illnesses and even subclinical infections. It also supports the view advanced by Francis et al. 6 that Ebola virus is not readily transmissible from person to person except where there is close and prolonged contact with a patient suffering from severe disease. Clinical observation of cases occurring late in the epidemic showed that frank haemorrhagic manifestations were less apparent. There may have been some attenuation of Ebola virus resulting from continuing passage in man. Observation in guinea pigs infected with isolates obtained later in the illness may confirm this.

The antibody results on sera collected from the Nzara cotton factory staff indicate that nine of the 24 staff (37%) of the cloth room and adjacent store were infected. The levels of antibody, ranging from 1:16 to 1:256, indicate a fairly recent infection although the seven members of staff who were bled in November and were shown to have been infected gave no history of illness. These results point very strongly to the cotton factory having been a prime source of infection. The results of virus isolation attempts and serological studies on rodents and bats collected in the factory are awaited with interest.

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DISCUSSION

A.W. Woodruff : Can I ask our colleagues about their own quarantine ?

D.I.H. Simpson : We have in fact more or less put ourselves in quarantine and had no contact with patients for at least ten days before we left the area, on the 20th of November. The lost case was known to have occurred on the 22nd of November, we didn't have contact with that. We did go into quarantine somewhat enforced and somewhat reluctantly for another fortnight after that.

G.A. Eddy : I am interested in the apparent disappearance of antibodies. This has some importance for epidemiologic studies and also for the collection of immune plasma: . Might it not be possible to have an exchange of sera between your place and Atlanta for instance, so that we can get a little better confirmation of this apparent disappearance of antibodies ?

D.I.H. Simpson : This in fact has already been done in several cases and there is a reasonably good correlation between the results which were carried out at Porton with the ones that were carried out in Atlanta. I should point out of course that all our tests have been carried out with the Zaire 718 strain and it is possible, although I think it is not all that conclusive, that the fact that we are using a Zaire strain to test Sudanese patients my also influence the results somewhat.

J.G. Breman : There are two alarming points in this epidemic, I think both of them have some lessons for us. The first is that Dr. Babiker said the epidemic first came to his attention on the 30th of September. Yet by the 30th, at least as I recall, 75 cases with 3O or 40 deaths had occurred. Can you explain what the delay was there and relate this to possible quicker action in the future ? The second point is the fact that in the Sudan at Least ten generations of cases had occurred before the epidemic died out. In many instances you did a beautiful job in showing five or six generations traced early. Working at epidemics, Looking at monkey parks with a computer, we found that you need a 12.5% secondary attack rate to continue an epidemic without it dying out. With this secondary attack rate on our side so high with close family contacts and on your side actually very high in the first and second generation, this is very alarming compared to, say, Lassa fever rates where it is unusual to have a second and third generation. When you work at the data of only seropositive cases compared to death is there any decay in mortality rates throughout the epidemic which would then account for what happened ? By month there was apparently no difference in mortality.

E.T. Babiker : The outbreak became alarming when it was introduced into Maridi hospital. It became evident to the Minister of Health that there was an alarming situation when there was a large outbreak among the medical staff and when the medical doctor fell ill and was transported to Khartoum. Meanwhile between August and the end of September the medical doctor had a few cases continuously and tried to investigate these, collecting blood for typhoid and sending it to Khartoum, receiving the result or not. In this way it took six or seven weeks to confirm or exclude this infectious agent in such a distant place far from Laboratory facilities. This accounts for the delay. We also asked ourselves if those outbreaks occurred in the t I think this historical information is probably meaningless because the noes are that the outbreak is probably not a new disease. But I think if would not have spread to Maridi and Yambuku hospitals, no one would have remembered it. There has never been any outbreak like these before, because -amplifying forces have not been there : a needle, an amazing remarkable social structure or a newly established teaching hospital where these kind of outbreaks can happen. You need this sort of secondary amplifiers to get a recognizable outbreak and I think even that the 70 cases of the Nzara outbreak, it remained Limited to that locality, would have gone undetected.

Van Nieuwenhove : During the epidemic in Maridi, 13.914 doses of typhoid vaccine were administered. The hospital material for such a Large number of vaccinations nations was certainly insufficient while the sterilization procedures the same as in Yambuku. Could not therefore injections have played an equally important role in the Sudan as in Yambuku ? The cases we had in Sudan have actually been traced back to the I source. There were no cases which could not be explained. Most of the a had had contact with other cases during the incubation period. We saw the same thing in Yambuku, it's not because someone has had contact with a case that necessarily this contact was the source of the infection. In Sudan the death rate for hospitalized patients went up from 25% in August, to 44,6% in September and 70% in October. The vaccination campaign was started on the 25th of September, I find such a rise in death rate very strange.

P. Brès : The vaccination programme was not completed, perhaps only 114 of it was completed. As Dr. Daoud said, man to man transmission was well evidenced and the decrease of cases was a result of the use of protective clothing. When these ran short, there was a booster effect, and this illustrates the efficiency of the protective clothing. As you know, most of the victims were the not well-trained student nurses in the hospital and these were eliminated from the second phase. it is dangerous to stick too much to the figures if you don't know what was behind them.

D.P. Francis : We did out very best to administer the typhoid and gamma globulin injections that were brought in early before the diagnosis was known. But actually very few doses were given, a lot of them in the hospital staff because they were at high risk and could obtain the necessary needles and syringes. Since they had some knowledge they did not shape this material with any active cases on the ward.

A.W. Woodruff : Among 13.500 injections given, under the circumstances one would expect that afterwards there would be a fair incidence of hepatitis B infection.

E.T. Babiker : This number of vaccinations is absolutely incorrect. We have not been thinking very much in terms of injection transmission in the Maridi outbreak. I don't think the magnitude of the outbreak in Maridi is compatible with injections. it could be for a few field cases but I think the main transmission was by person to person transmission. As to the hepatitis cases, we have not any.

J. De Smijter 1 do not agree that one would see many cases of hepatitis after even when it is massive. In a survey in Mali we found evidence of past hepatitis B in 80% of the population. In Zaire we found 60 to 70%, this means t hat more than 90% of the population has immunity against hepatitis B. In that case I would not expect to see many cases of hepatitis B after vaccination.

D.P. Francis : As has been said, the immunization programme in Maridi was very limited. Contrary to the situation in Yambuku, there was no prenatal clinic nor a well baby clinic to serve as an amplifier. I think there was no needle contact between well and ill people. There were people infected in the hospital, admitted initially for various diseases, who were treated in the hospital, including injections, and who five to seven days later came down with usually a fatal disease. There are six definite Ebola cases nosocomially acquired which I presume were needletransmitted. ,-,'a do not have any other documentation of people who came to the hospital prior to their disease and who received injections. Some of these no doubt aid occur in Nzara where nurses did private practice carrying needles around. The worst people in washing their hands between patients ape physicians. In a country that has a dollar per patient per year to spend on medicine, the thought of providing plastic gloves for every febrile patient is just not possible.

M. Isaäcson : Maridi hospital was a large teaching hospital and it was noted that the main victims were the student nurses. In teaching hospitals, through out the world student nurses are in the unfortunate position of frequently having to do the most unpleasant duties and these are the people who are least trained and least experienced to deal with this safely. In future nurses training there should be a great deal of emphasis on safety precautions. Particularly in Africa any pyrexia of unknown origin should be regarded potentially a lethal viral haemorrhagic fever and should be treated and handled as such. In addition, when there is any suspicion of a lethal haemorrhagic fever being dealt with, one of the most important approaches is to have constant supervision and guidance from someone who is skilled in the protection of hospital staff and in the protection against hospital cross infection.

R.A. Coutinho : I can understand that people panicked since they realized that the treatment which was given in the hospital was not very successful. As far as I can say from my experience in Africa, people would then go to the witch doctor. I didn't hear that, but I could imagine that the witch doctors were giving some treatment and possibly that they played a role. I wonder if anyone inquired about this.

J.G. Breman : There was one cluster of nine cases in Zaire which was interesting in that eight of these were women who received scarifications all over the body following the death of a child with whom another was in contact. All of these women died about the same time, but they did have contact as well as this very interesting practice. Another practice which I felt was even more important was a type of purgative which is used throughout Africa for a variety of treatments, but here it was done in a very interesting way. In this little group it was done with a thin bamboo reet put into the rectum and then the others would blow material through this reet. The same reet was used for all the women. Of course this could have incised the intestinal mucosa and certainly implicates blood products or stool as a vector.

CONTAINMENT AND SURVEILLANCE OF AN EPIDEMIC OF EBOLA VIRUS INFECTION IN YAMBUKU AREA, ZAIRE, 1976

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SUMMARY

Containment measures in the epidemic zone of Yambuku Equatorial Province, Zaire, 1976, included quarantine of the entire Bumba zone and prohibition of all traffic between affected villages. Ebola Virus transmission was interrupted by the closure of Yambuku hospital with cessation of giving injections, by isolation of patients in their villages and by a change in funeral habits. Protective clothing was used efficaciously by high risk individuals such as medical staff.

Surveillance was based on door-to-door surveys by ten locally engaged teams under supervision of an epidemiologist. It covered the whole epidemic zone and a buffer zone. More than 500 villages and more than 30.000 families were investigated. Two physician-led teams investigated the area between Yambuku and Southern Sudan, without finding any case. Logistic support was a major problem throughout the whole campaign.

At the beginning of September 1976, medical staff of Yambuku Mission hospital (consisting of Zairian and Belgian nurses) faced an exceptionally lethal epidemic of an unknown hemorrhagic fever which did not respond at all to any available treatment and which seemed highly contagious. This paper deals with the consecutive measures for containment and surveillance during and after the epidemic in Yambuku area.

FIRST FIELD INVESTIGATIONS

On demand of the medical assistant in charge of the Yambuku hospital, the Chief medical Officer of the Bumba zone arrived in Yambuku on September 15th. he stayed in Yambuku until September 19th making the first accurate clinical and epidemiological study of the new disease and noting the rapid spread of the epidemic along the roads to the villages of the region. His report points out that there was no strict isolation of the patients in Yambuku hospital and that some of the patients escaped to go back and die in their villages where they were buried near the houses and even, sometimes, within the house. No specific containment measures were taken. It was suggested that patients be hospitalized, that burials be made in public cemeteries and that water be boiled before consumption.

Then the news of the death of the Belgian missionary midwife was received on the 19th of September, the Ministry of Health decided to send to the epidemic area a team of two physicians. They arrived in Yambuku on September 23rd when a total of 32 cases had been counted by the missionaries. The report of this second group of investigators evoked the possibility of an atypical yellow fever or of some undetermined arbovirus disease. But, based on the results of the serological tests (Widal), it was eventually concluded that the outbreak was a fulminating epidemic of typhoid fever in a non-vaccinated population". A typhoid vaccination campaign was prescribed for all medical personnel and

the population at risk. One acutely ill Belgian nurse and two other missionaries accompanied the team to Kinshasa where they were hospitalized at Ngliema hospital. From a blood specimen of the ill sister, a Marburg-like virus was identified on October 13th.

Meanwhile, the medical staff of Yambuku hospital and the missionaries went on collecting information on the spread of the epidemic with the registration of the number of cases, number of deaths, date of admission at the hospital and date of death. These data, which proved to be very helpful for later investigators, were communicated daily to Kinshasa through the Catholic missions' radio network. When two other missionaries died in Yambuku and the disease apparently irresistibly extended further, a panic arose among the hospital staff and the local population.

Considering this situation, the Ministry of Health decided to send to the affected area a second epidemiological mission. This team arrived in Bumba on October 4th 1976, where in cooperation with the local medical and administrative authorities, it was decided to establish severe quarantine measures to protect the town of Bumba (50.000 inhabitants) which is an important port on the Zaire river and a very busy commercial centre. The entire Bumba zone was quarantined and all traffic between the city and the rest of the country prohibited by the use of army controlled roadblocks. In Bumba two quarantine camps were established ; near the hospital, one building was reserved for the isolation of 43 students and their contacts evacuated from Yambuku high school and a military camp was evacuated for the isolation of possible patients and contacts. Two active cases, who had fled from Yambuku, were strictly isolated in a house and, immediately after death on October 6th, buried by team members wearing protective clothing. Furthermore, health education sessions were organized in different parts of Bumba. When the team reached Yambuku on October 6th, the hospital had closed down; all patients had left, disseminating the disease in villages as far away as 50 km from the mission. Individual protective material, consisting of gowns, gloves and masks, and disinfectants were distributed to the medical staff and the principles of barrier nursing explained. Strict isolation of patients and affected villages was recommended, together with the establishment of roadblocks on the ways to Yambuku and Yandongi.

This team noted the predominant role of the hospital in the dissemination of the disease and the explosive character of the outbreak, suggesting that several Contaminations had occurred simultaneously. Yellow fever or typhoid were discarded and the possibility of Lassa fever was evoked. Early after their return to Kinshasa on October 9th, the virus responsible for the outbreak was identified.

FURTHER INVESTIGATIONS AND SURVEILLANCE BY THE INTERNATIONAL MEDICAL COMMISSION (IMC)

An International Medical Commission was formed on October 18th, 1976, under the direction of the Minister of Health of Zaire to confront the outbreak which, according to the reports received from the affected area, was reaching about 270 cases by the middle of October. For the first time, one and a half months after the beginning of the epidemic, enough resources in man-power, equipment and organization were put together to fight the disease. Equipment for individual protection and the performance of strict barrier nursing was, at last, available in adequate quantity.

First field investigations by the IMC.

A subgroup of the commission, composed of 6 physicians, left Kinshasa for Bumba by air on October 19th. A landrover was transported with them in a C-130 of the Zairian Air Force, together with protective material and dry ice for preserving specimens for virological or serological investigation. The objectives of this short-term investigation were :

1. Determination of the geographical extension of the epidemic, the actual acti vity of the outbreak and institution of measures designed to interrupt the epidemic chain.
2. Identification of survivors of the infection and evaluation of the possibili ty to immune plasma collection from them.
3. Evaluation of the necessity and logistic needs for the installation of a treatment and investigation base in the epidemic area.

In Bumba, control measures were discussed with medical and administrative authorities. Collective containment consisted in the establishment or reinforcement of road blocks on roads leading in and out of all villages in the Bumba zone. The entire infected zone was placed in quarantine until 6 weeks after the death of the last case. All traffic to and from Bumba by air, road, railway and the Zaire river was suppressed, and all passengers and cargo transportation along the roads from Yambuku was prohibited. Military and local authorities were in charge of these road blocks. A one-day survey by road was then conducted by 4 subteams to determine the importance of the outbreak in number of cases, both past and active, the number of affected villages, the geographic spread of the epidemic and to look for possible

convalescents. A brief stop of about 15 minutes was made in every village, where local authorities were asked for number of cases and deaths, date of first and last death in the village and the presence of active cases. If active cases were present, at least one patient was seen and questioned by the epidemiologist but without having close contact. Clinical features were noted. All data were registered on forms made by team members using standardized definitions (see addendum) . 69 villages within a perimeter of about 50 km around Yambuku were visited. Apparently the outbreak was, at the time of this survey, diminishing. One subteam investigated the neighbouring villages near Yambuku and Yandongi where, according to the missionaries' reports, most victims had occurred. The 2 physicians of this team were protected by disposable gowns, gloves, surgical paper masks, hoods, plastic bags serving as boots and motor-cycle goggles when examining patients. All protective clothing, except the goggles, was burned on the spot, immediately after each examination. This subteam took blood samples for virus isolation of 9 active cases, 5 of whom died within the following days, and identified 5 possible and 1 confirmed convalescents from whom serum specimens were obtained.

After a crude check of their haemoglobinemia (Tallquist method and Unopet), two convalescents were convinced to accompany the team to Kinshasa to become the first donors of hyperimmune plasma,

Some days later, a Zairian Air Force helicopter was available for visiting remote areas from which no direct information was gathered by the missionary surveillance system. In this way, the epidemic zone could be correctly delineated.

Methods recommended for disease control included rapid burial of cadavers with their clothing impregnated in sodiumhypochlorite or formalin, together with all potentially infectious items. The mourning ceremonies were to be limited and all direct contact with the cadaver or excreta be avoided. Houses were to be disinfected if disinfectants available, and in several cases huts were burnt down by the population. The mission hospital was disinfected by fumigation with formaldehyde, matrasses removed and burnt or left in the sun for several days. Floors and walls were scrubbed and disinfected. Furniture and all items were left in the sun for disinfection.

Logistic support was a major problem throughout the whole operation in the Bumba zone, especially during the preliminary surveys described above. Communication between Yambuku, Bumba and the commission in Kinshasa was provided only through the catholic missions network. Considerable delays occurred and many radio messages to Kinshasa were badly received, or not at all. The field-team did not get any message from the IMC in Kinshasa and demands for air transport back to Kinshasa were left unanswered, causing difficulties in preserving the specimens for virus isolation at appropriate temperature because of a very limited stock of dry ice. Local transport by road was often problematic because of the rainy season and the IMC-team, owning only one landrover, depended on vehicles provided by the catholic missions of Bumba and Yambuku. Moreover, there was a general shortage of fuel in the area. Suitable accommodation in Bumba and Yambuku was provided through the kindness of the local catholic missions.

The Commission's survey in North eastern Zaire (figure 1).

Two physician-led teams, with vehicles, were flown to north-eastern Zaire on November 1st to find a link between the epidemics in the Sudan and in Yambuku. Visits were made to health units, cities and villages; interrogations were made regarding outbreaks of severe hemorrhagic disease during the previous 9 months. They did not find either isolated cases nor foci of hemorrhagic fever in the area nor along the Sudan and Central African Empire border during a two-week search. Although no recent or past cases were found, one of the epidemiologists observed regular traffic between Southern and Haut-Zaire and the Bumba zone which can be reached in four days from Nzara (Sudan), where a similar outbreak started in July.
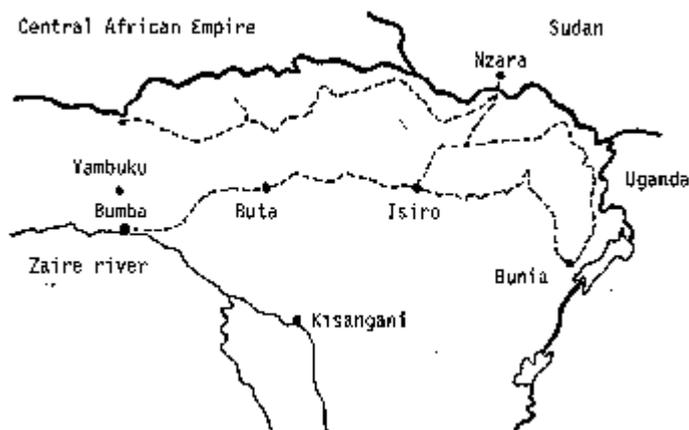
Figure 1 : Itineraries of two survey teams in Haut Zaire

The organized surveillance programme.

Following the preliminary survey of the International Commission, the setting up of containment and surveillance in the epidemic zone around Yambuku and in a buffer zone about 20 kms around this area was given top priority.

The programme was based on the following principles :

1. Intensive search of active cases by door-to-door surveys in each village in the affected area.
2. Organization and reinforcement of containment measures in the villages.
3. Appropriate health education.
4. Detection of possible convalescent cases of disease.

At the beginning of November, two main bases with modern radio equipment, fuel and food depots and lodgment facilities were established at Yambuku and Ebonda, a village on the Zaire river 12 km west of Bumba. There were three peripheral bases with fuel depots, supplied by helicopter, at Abumombazi, Tshimbi and Yalosemba. An Air Force helicopter was available in Bumba for transport of goods and supervision of the peripheral bases and diesel stocks.

Ten teams were formed, eight of them were put at the disposition of the IMC by several organizations (State owned and catholic and protestant mission hospitals , the National Campaign for the Eradication of Smallpox, the National Campaign against Sleeping Sickness, PL2 Plantation Company), and two teams were recruted at Bumba. Each team was composed of a paramedically trained teamleader, an auxiliary and a driver - all living in the Equatorial Province of Zaire. In total, 38 persons participated in the campaign. Each team was issued with the following protective clothing for 50 examinations (masks, gloves, boots, gowns, hoods, goggles and respirators); forms, clipboards, pens and paper; geographical maps; basic camping material (mosquito nets, oil lamps, torches, blankets, sheets, water containers, cooking equipment); food (C-rations); disinfectant (sodiumhypochlorite); basic medication to treat villagers (aspirin, chloroquine, tetracyclin, antidiarrheal) first-aid kit for team members.

All the teams took part in a day of training in Ebola hemorrhagic fever and investigation technique. A basic instruction document, including standardized data collection forms, was given to each participant. The training programme covered general information on the epidemic, differential diagnosis of the disease, instructions for personal protection, the principles of isolation and quarantine and a discussion on the themes for action in sanitary education. An epidemiologist directed the teams' first day in a village to elucidate investigation techniques.

At the first visit to the village, the population was assembled around the village chief and the reason for the visit and current state of the epidemic was explained. The accent was then put on isolation and quarantine measures, should cases occur. Many teams went further and explained the dangers of infections and advised the construction of isolation huts.

During a subsequent house to house survey the name of the family head and the number of persons in each family were recorded. Data on past cases were collected. Suspect cases were not examined. Medicines were given to them and arrangements were made for isolation in the village. Work reviews were held daily at Yambuku and Ebonda and physicians were sent to follow up on suspect cases and to bleed candidate convalescent cases. Every village was visited a second time in much the same way as

the first visit, with the accent on the control of fever cases. A third rapid survey was made in which village chiefs were asked whether any new suspect cases had occurred.

The covered area and the circuits of the teams are traced on figure 1; 550 villages and 34,000 families or approximately 170,000 persons have been visited by the surveillance teams. The last active case was found to have died on November 5th 1976 in Bongolu, 38 km east from Yambuku. Several cases of febrile illness were found by the teams and all responded to antimalarial treatment.

Based on these facts, and continuing the surveillance for another 4 weeks, the lifting of quarantine on 16 December 1976 was advised, that is, six weeks after the date of the last death. The Yambuku Hospital was reopened around mid-November.

Under supervision of an epidemiologist, passive surveillance was continued in the epidemic area till the end of January when the plasmaphoresis programme was stopped. Village leaders were regularly asked for suspect cases and the hospital records were daily controlled by a physician of the IMC. Much emphasis was put on limitation of injections and on the proper way to sterilize syringes and needles and on the maintenance of sterility.

On February 2nd 1977, the death of a suspect case of hemorrhagic fever was reported at Yambuku hospital which caused much concern among hospital staff and population. The patient was isolated, nursed and buried by convalescents from the epidemic according to the instructions issued by the IMC, but, unfortunately, no specimens were taken for virus isolation. Subsequent investigations by an IMC epidemiologist, who arrived in the area one week after the patient's death, did not reveal any additional cases but were inconclusive as to the etiology of the disease.

DISCUSSION

Containment measures in the epidemic zone of Yambuku consisted mainly in closure of Yambuku mission hospital, strategic isolation of patients and villages and protection of high risk individuals with special equipment and information.

The isolation of villages and the prohibition of all traffic between the villages by roadblocks proved to be very efficient and were surprisingly well respected by the population. These measures certainly prevented further spread to unaffected villages which were particularly vigilant when there were cases in adjacent villages. Many elder people remembered containment measures as dictated during past smallpox epidemics and made them apply. At the end of the epidemic isolation of patients was more or less practised in the villages and, occasionally, burials were performed soon after death without preparing the cadavre (washing, touching, dressing, vigil) but this, especially the last, was very hard to accept for the family. In some instances, huts of the deceased were burnt down. It is nearly impossible to evaluate the impact of these measures.

Protective clothing, mentioned earlier, was worn systematically by investigators during contact with suspect cases. Team-members safely examined several

virologically confirmed patients with this equipment, including disposable surgical masks, respirators not being available at that time. At the moment, information on virus shedding is very limited and thus far, virus has only been isolated from the blood and seminal fluid 1. There is an urgent need for virological studies on the presence of Ebola virus in different sites of the body, in order to introduce rational containment measures.

It was shown that a rapid organization of systematic active surveillance is possible, even in areas with limited resources in skilled manpower and technology, providing basic logistic problems are resolved. Transportation was a major requirement during the whole campaign, and lack of vehicles and fuel was the main obstacle at the beginning of the activities.

Very valuable use was made of existing health service facilities such as mobile teams of the Smallpox eradication campaign, the sleeping sickness control programme, and students from a paramedical school. Only active surveillance can inform authorities with serious data on the status of the outbreak and when to lift quarantine which should not be ended until at least twice the incubation time has passed since the last case has occurred.

It is recommended that countries at risk for highly mortal hemorrhagic diseases have an efficient surveillance system and minimum equipment for sample collection and shipment to high security laboratories. International collaboration appears to be necessary for containment of such outbreaks.

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ADDENDUM

DEFINITIONS USED DURING FIELD INVESTIGATIONS IN THE EPIDEMIC AREA OF
YAMBUKU

a) PROVEN CASE : patient manifesting an acute clinical syndrome and in whom the virus had been isolated and/or had specific antibodies.

b) PROBABLE CASE : person having had three days of headache, backache, fever,abdominal pain, nausea and/or vomiting, as well as hemorrhage, with no other diagnosis and not responding to treatment. It is essential that there should have been a contact with another case of Ebola virus infection or a history of injection.

c) POSSIBLE CASE : patient with at least 24 hours of fever and headache, with no other diagnosis and not responding to treatment, having had a contact with a probable or proven case during the previous three weeks or a history of injection.

CONTACT : a person having had direct face-to-face contact with a), b) or c), that is, having slept in the same room, having taken meals together and having cared for a patient or prepared the body, either two days before the beginning of symptoms, during illness or immediately after death.

CONTAINMENT AND SURVEILLANCE OF A HOSPITAL OUTBREAK OF EBOLA VIRUS DISEASE IN KINSHASA, ZAIRE, 1976.

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The clinical aspects of Ebola Virus Disease (EVD) in three nurses were described in a separate paper (1).

When the diagnosis of Marburg or Marburg-like disease was made, hospital acquired infection had already occurred in one nurse. At the time there were reports, later confirmed, of many people having become ill and died of this disease in the Equateur region of northern Zaire and there were then no known cases of recovery.

The health authorities were greatly concerned and a state of panic was imminent among sectors of the public.

The main thrust of the International Medical Commission's (IMC) efforts was therefore directed at containment of the disease in this city with a population of about 2 million people and the country's main international airport. These activities consisted on the one hand of the institution of containment measures aimed at preventing further hospital cross infection and on the other of a large scale surveillance programme of contacts and investigation of reported cases of suspect EVD.

CONTAINMENT MEASURES

From the moment of arrival of the two nuns and the priest at the Ngaliema Hospital, Kinshasa, Zaire, on 25 September 2976, some precautionary measures were taken to prevent spread of infection. Pavillion 5 in which they were accommodated has a number of single rooms with toilet and shower ensuite. All of these rooms surround an open courtyard which promotes good ventilation. Barrier nursing was introduced from the start and cotton gowns and cotton masks were worn when attending the patient. These were later replaced by disposable gowns and masks but as supplies were inadequate the gowns and the disposable plastic overshoes were hung up outside the door of the patient's room for re-use. It is noteworthy that Sister E.R. (Case 2) did not wear protective clothing when attending her patient. The disinfectant in use was 'Dettol' (chlorxylenol) which is known to have a narrow antibacterial spectrum and is unknown to have viricidal properties. When nurse M.N. was admitted on 13 October, quarantine was imposed on all the staff of Pavillion 5 but all other patients were either discharged or moved to other wards as they had not been directly exposed to the EVD cases. The staff was accommodated in Pavillion 5, together with a large number of contacts of the sick nurse. On the 16 October, the following additional precautions were introduced and existing ones modified to reduce further the risk of virus transmission :

1. Sufficient supplies of protective disposable clothing were procured to enable single use of items only. These included Balaclava type helmets which cover the whole head leaving only the face exposed. Full face respirators were obtained which provided airtight isolation of the exposed part of the face with air exchange through a filter canister which was at least 99,98% ef fective against 0,3 micron particulate matter at a flow rate of 85 liters per minute. The respirators were assigned to individuals and marked with their names. Staff members were urged not to share or exchange respirators under any circumstances in view of the potential danger of a face piece becoming internally contaminated with virus by a wearer during incubation period should he/she become infected. Goggles, to be worn with a disposable surgical mask, were issued to some staff members as there were insufficient respirators at first. Special attention was paid to the correct method of removing protective clothing after leaving the patient's room so that the potentially contami nated surfaces never touched the

bare hands or face or uncontaminated mate rials and equipment. The gloved hands were first rinsed with sodium hypo chlorite, after which the respirator was removed and sponged off with sodium hypochlorite. The Balaclava helmet and overshoes were pulled off with gloved hands and the gown, which had cuffed sleeves, and the gloves were then peeled off in one single operation, Wearing a fresh pair of gloves, the gown was folded with the exposed surface inwards. All these items were discarded directly into a makeshift incinerator and burnt. The respirators were replaced in their containers which were also marked with the individuals' names.

2. The use of the hospital autoclaving and incineration facilities, at some distance from Pavillion 5, was rejected as this would have necessitated transport of virus-contaminated material with potential danger to other parts of the hospital. At first an open fire was used in the pavillion courtyard but this was soon replaced by an improvised incinerator constructed from a large oil drum. The drum was covered with wire mesh to prevent dissemination of the larger burnt particles. All disposable equipment and other waste, excluding excreta, were burnt immediately on leaving the patient's room, enclosed in plastic bags. A large galvanized iron bath was placed on bricks in the courtyard, a fire]it underneath, and this was used to boil all non-disposable instruments, utensils and linen (Fig. 1). All these items were soaked in hypochlorite prior to boiling. The virus was assumed to have a sensitivity to heat similar to that of Marburg virus (2).

3. The patients used their own toilets until the terminal day or two of illness. Initially the toilets were flushed without special precautions. Later, it was recommended that use of the toilet was followed by pouring in half a liter of undiluted household bleach (sodium hypochlorite) and that flushing was to be delayed until the next time it was used. The concentration of the local product could not be ascertained but subsequently various products were obtained to enable a standard hypochlorite solution of known concentration to be made up. Excreta can be more effectively treated by autoclaving or closed incineration when the necessary facilities are available. When Case 3 could no longer leave her bed, bedpans were used and treated in a similar manner and then boiled.

4. 'Dettol' (chlorxylenol), in use in Pavillion 5, was replaced by sodium hypochlorite as a 1% or 4% solution (the latter in the presence of much organic matter) and by an iodophor disinfectant in a concentration yielding 450 ppm available iodine (3). These disinfectants were recommended in view of their known viricidal properties.

5. After the death of the first two patients their bodies were removed and the rooms locked up. Following the death of the third patient all three rooms were fumigated with formaldehyde vapour on four successive days. The rooms were entered with full protective clothing, the mattresses removed and burnt. Floors, walls and furniture, which were still blood-stained, were scrubbed and disinfected. Sphygmomanometers and stethoscopes were dismantled and the parts either boiled or soaked in a hypochlorite solution. Finally, all items were left in the sun for several days.

6. Disposal of bodies was carried out by wrapping them in cotton sheets impreg nated with a phenolic disinfectant. The fully wrapped body was sealed inside two large, heavy-duty plastic bags and then placed in a wooden coffin. Contact of relatives and friends with the body was strictly prohibited. The prepared body was however released for the funeral service and immediate burial. After the death of the last patient, the staff remained in voluntary isola tion in Pavillion 5 for a further 21 days. Morale, which was at a very low ebb when the IMC arrived, was rapidly restored by active participation of commission members in their activities in the pavillion, and by the pro vision of reading matter and other recreational materials. Some of the IMC members were close primary contacts of EVD and many were secondary contacts. Although it would not have been feasible for the IMC to carry out their activities while in strict isolation its members avoided un necessary contact with the general public and generally did not use public transport, nor visited restaurants or shops.

7. A negative pressure medical containment bed isolator was provided by the Canadian government. It was accompanied by a nurse specialist trained in assembling the unit and in its operation and the handling of patients in these isolators. The isolator was to be used in the event of a further case presenting in the Kinshasa chain of infection. Although no further cases occurred it served as an invaluable morale booster as hospital staff members could be assured of nursing a patient with little danger to themselves. Also, they would not be subjected to further quarantine. Several Zaire nurses and physicians as well as IMC members were trained in the use of the isolator.

8. As there was a certain degree of overcrowding in Pavillion 5, another pavillion was requested to be vacated in order to house all those contacts who were not needed for nursing or cleaning duties. These contacts, and those subsequently traced and brought in by the surveillance teams, were accommodated in Pavillion 2 in cohorts according to their last day of contact. Segregation between cohorts was strictly enforced in self-contained rooms with private bathrooms, toilets and verandahs, and in suites of rooms for the larger cohorts. Their size varied from 1 to 8 people. The contacts were given prophylactic malaria treatment and their temperatures were taken twice daily. A fever lasting for three consecutive readings was considered an indication for transfer to Pavillion 5 as a suspect case of EVD until proven otherwise. Although a few people developed pyrexias, these proved to be transient and transfers to Pavillion 5 were not necessary. Cohort isolation, commonly practised in neonatal nurseries, was adapted to this situation as it was believed that the occurrence of further cases among the primary contacts was almost inevitable. This would have imposed further hardships on the whole group if they were isolated as one large group with free intermingling. With cohorts formed according to date of last contact, it was anticipated that cohorts could be released in their entirety at the end of the 21 day period, enabling the preparation of their quarters to receive new contacts. Should one of the contacts in a cohort become ill, only the members of that cohort would require extension of the isolation period. Morale in Pavillion 2 was at first also very low, and aggravated by the boredom of the children. The provision of toys for the children improved matters. There was one pregnant woman in the group and she gave birth to a healthy infant while in quarantine. Mother and infant were separated from their cohort and isolated together for the remaining period.

Fig. 1 The courtyard of Pavillion 5 in which the AHF patients and exposed staff were isolated. Note the make-shift incinerator, boiler and nurses in full protective clothing including full face respirators.

SURVEILLANCE

By the time a diagnosis of Marburg-like disease had been made, it was no longer vital to trace the contacts of Cases 1 and 2 during their journey from Yambuku to the hospital in Kinshasa as the likely maximum incubation period had already elapsed without secondary cases having been reported.

Case 3, Nurse M.N. of the Ngaliema Hospital, had numerous local contacts and a surveillance program was established. The surveillance team comprised 4 medical practitioners and 4 health inspectors with drivers. Its function was to trace and classify contacts, bring in primary contacts to Pavillion 2 for quarantine, register and visit the secondary contacts, and check out reports of cases of suspicious illness. Initial problems posed by lack of transport and breakdown of telephone services were relieved by the provision of WHO vehicles and the establishment of radio communication. Primary contacts were defined as persons having had face to face contact with confirmed or suspect cases, or who had stayed in their house or eaten at the same table at any time during or since the 48 hours preceding onset of illness (4).

Secondary contacts were defined as persons having had a similar relationship to primary contacts. On 12 October Nurse M.N. visited the Foreign Affairs Ministry in order to finalize arrangements for an

overseas study visit. She spent several hours waiting in the company of numerous unidentified strangers. The following day she became ill with severe headache and it is possible that she had been infective for one or more days. On 14 October she became feverish and went by taxi to the Mama Yemo Hospital which is the principal hospital of Kinshasa, serving a population of approximately 2 million people. Here she was examined, and blood was taken for malaria microscopy. She was then referred to the isolation hospital but was not admitted. Nurse M.N. proceeded, still by taxi, to the University Hospital where she was once more examined, and then sent home. The following day she was scheduled to go on duty but was instead admitted as a patient to her own ward in the Ngaliema Hospital where she died 5 days later.

Despite her presence in a crowded government department, and in equally crowded emergency rooms in several hospitals where examinations and laboratory tests were done without precautions, no secondary cases developed from these contacts. Among her close contacts were a 14 year old girl who ate from the same plate and a young man who shared a bottle of soft drink with her on the first day of symptoms. This provides further evidence for the belief that the Ebola virus is not highly infectious and requires very close contact, probably with blood or secretions, for its transmission.

Thirty seven primary contacts of Case 3 were traced and quarantined. The quarantine period was based on the then estimated maximum incubation period of 16 days as seen in Case 3. As few data were available at the time about the incubation period, it was decided to err on the safe side and prescribe a quarantine period of 21 days. From the 37 primary contacts of Case 3, a further 274 secondary contacts were traced to 44 addresses. A considerable number of both primary and secondary contacts could not be traced, either because their identity was unknown, or their address was unknown or incorrectly supplied. The concept of secondary contact was later discarded when it became clear from data collected in the epidemic area of the northern Equateur region that secondary contacts were at no risk of being infected unless and until they themselves became primary contacts.

The team investigated 15 false alarms which included cases of typhoid, amoebiasis, viral hepatitis, a fatal case of acute pulmonary oedema, and a case of carbon monoxide poisoning.

DISCUSSION

The preventive measures practised during this outbreak were dictated by our personal experience and that of others during previous outbreaks of actual or suspected outbreaks of Marburg disease, Lassa Fever and nosocomial infections in general (3,5,6,7).

It became evident in Kinshasa, and later in the northern Zaire epidemic area, that airborne dissemination of virus did not play a major role, if any, in the transmission of the disease.

It appears that the observation of the basic principles of aseptic technique or barrier nursing are probably effective in breaking the chain of crossinfection. However, this requires constant supervision and staff members who are well versed in these principles. It is emphasized that protective clothing nay itself become a dangerous source of infection if it is carelessly taken off and discarded. During the Kinshasa outbreak, therefore, meticulous attention was paid to safe disposal techniques. Formalin should not be used in an attempt to disinfect excreta as it has very poor penetration power and tends to coagulate the surface, sealing viable virus inside.

Of some concern was the low morale and despondency of the staff in quarantine after the death of Case 2 and the onset of illness in one of their colleagues, Case 3. The psychological reactions of both patients and staff during epidemics with high mortality when little can be done in the way of either prophylactic or curative therapy deserves some attention. A team spirit was therefore actively cultivated and the isolated hospital staff responded dramatically to the various measures to achieve this.

Active cognizance by the Zaire Minister of Health and the IMC was taken of their plight and the sacrifices they were willing to make, and they were kept it all times fully informed about IMC activities and-events related to the epidemic outside the hospital. Recreational needs also received constant attention.

SUMMARY

Following the introduction of Ebola Virus Disease (EVD) into Kinshasa a local hospital-based chain of infection developed which ended with the infection of a local nurse at the Ngaliema Hospital.

Thirty seven primary contacts of the latter patient were isolated in cohorts according to the last day of contact, and no further cases of EVD developed. 'he surveillance teams which identified and traced these contacts also visited 174 secondary contacts and investigated a number of reports from Kinshasa and surrounding districts Of suspicious cases of illness or death, none of which were shown to be related to EVD.

Other containment measures included the use of disposable protective clothing together with respirators, viricidal disinfectants such as sodium hypochlorite and an iodophor, and careful disposal of excreta and other contaminated materials and equipment. Staff members involved in nursing and cleaning duties were instructed in the correct procedures to prevent auto- and cross infection.

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DISCUSSION

G.A. Eddy : In view of the low level of infectivity of Ebola virus, would you go through the rigorous containment practives, if you had to do this all over again ? Would you be as concerned as you obviously were, about spreading the infection to nursing and medical staff ?

M. Isaacson : This is a question that has engaged us on numerous occasions, and it is extremely difficult to answer. My personal opinion is that perhaps we ape overdoing things a little bit, on the other hand I also believe that we cannot afford doing less than the maximum precautions that are available. We cannot do it ethically, we cannot do it scientifically. So I would say that if I would be consulted again, I would indeed recommend the same procedures, although I personally might perhaps take certain liberties, I certainly would not recommend anything less than we have recommended during this outbreak.

S.R. Pattyn : I think we cannot afford to do less until we know more about virus excretion.

R.E. Shope I wonder whether there was any consideration given to the

possibility that arthropods perhaps mechanically, might transmit the infection

and if any arthropod precautions were taken.

M. Isaacson : Arthropod transmission was certainly considered but it was quite clear that, had this virus been arthropod transmitted, and knowing Zaire and its arthropod population, we would have had thousands of cases and not just a few hundred There was no evidence whatsoever for arthropod transmission. Therefore we did not take any specific precautions.

H. Bijkerk : Has there been any indication of a transmission by sexual contact, in view of the patients with positive seminal fluid for quite a long time ?

K.M. Johnson : Of the twenty people who survived this disease, I think 9 of them were males and they, in turn, during convalescence did not transmit the disease to any sexual partner. I also would like to make one other comment. With the total numbers that we heard about it must seem to much of this audience, a year afterwards, like an

consulted again, I would indeed recommend the same procedures, although I personally might perhaps take certain liberties, I certainly would not recommend anything less than we have recommended during this outbreak.

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. Johnson : Of the twenty people who survived this disease, I think 9 of them were males and they, in turn, during convalescence did not transmit the disease to any sexual partner. I also would like to make one other comment. With the total numbers that we heard about it must seem to much of this audience, a year afterwards, like an awful lot to do about maybe something that isn't so big. I don't think however that anyone who was there, could have at the time failed to appreciate how little was known and what the potentials were in both town and country and I cannot recall anyone think at the time that any of the measures, any of the efforts that were expanded to prevent the continuance of the chain in the capital city and to thoroughly document how in the rural areas this epidemic had ended, were superfluous. I don't think there was anybody in Zaire who felt that too much work had been done. In the Bumba zone for several months following the official government lifting of the quarantine, the commercial airlines still refused to fly, the people who ran the river boats between Kisangani and Kinshasa still wouldn't pull in to Bumba to take off cargo and the people of Zaire still retained a very great sense indeed of horror and anxiety about this whole happening.

CONTAINMENT AND SURVEILLANCE OF THE EBOLA VIRUS EPIDEMIC IN SOUTHERN SUDAN

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INTRODUCTION

The epidemic of viral haemorrhagic fever now known to be due to a Marburglike virus to which the name Ebola has been given, occurred in three districts - Nzara, Maridi and Tambura of Western Equatoria Province, Sudan. The affected province borders Zaire and the Central African Empire. It has thickly forested and fertile land inhabited by about 200.000 people who mainly belong to a Bantu tribe called Azande, some of whom reside across the border in Zaire and the Central African Empire.

The official news of the epidemic was received by the Regional Ministry of Health and Social Welfare on September 15, 1976 through a telegram sent by the Medical Officer of Maridi Hospital. Before this date rumours of the epidemic had dominated the town of Juba, where two suspected cases (both school masters) had been flown in from Maridi and admitted to hospital. It was later discovered from records that the first case had occurred in Nzara about the end of June 1976. Between this date and the time medical intervention was initiated on October 23, 1976, the epidemic had spread to Maridi and then to Tambura killing the majority of those affected.

Three medical investigatory teams visited the affected towns between 14 September and 4 October 1976. Each of them reported similar impressions as to the possible causative agent: they suspected a viral cause with yellow fever and smallpox being highly probable but also typhoid fever.

Except for spraying with DDT in Maridi, Yambio and Nzara and limited vaccination against yellow fever, none of the recommended control and preventive measures were carried out because nobody was made to stay and implement the measures in the affected areas.

I arrived in Maridi October 23, 1976. Apart from Maridi, the situation had improved in Nzara and in other parts of the Yambio and Tambura districts. The shortage of medical and public health personnel in the area was a big constraint and this was aggravated by lack of facilities such as transport and protective clothing. The telegram service was out of order and road communication closed to and from the Western Equatoria Province. Communication between Juba and Maridi was maintained by a radio-telephone set belonging to ACROSS, a voluntary agency.

Two landrovers were functioning but the hospital had no fuel. The town had a poor and small water pump, often out of work for lack of fuel and had no electricity supply.

There was one Medical Officer in charge of the hospital and two sanitary overseers responsible for the public health services of the town. The majority of hospital nurses had fled to hide from the disease. On this very day there were only three nurses at work during the afternoon shift and none during the night. There were seven in-patients suspected of the disease with two runaways. The hospital had closed down as far as normal routine work was concerned. Only patients suspected of the epidemic disease were admitted to wards which were used disorderly, that is without specific quarantine regulations being applied. Relatives of in-patients moved freely in and out of the hospital and the dead were handed to them to bury. Cases outside hospital were cared for by traditional healers.

In Maridi the administration had formed a Committee to assist in the control of the epidemic. Through this Committee communication with the public was maintained and the construction of a quarantine area some 20 yards away from the hospital premises was ordered. However, this building could not be finished because the workers became scared and could not be persuaded to complete the construction. There was great fear among the public which deserted the town.

I brought from Juba a limited supply of cotton gowns, masks, caps, bedsheets, blankets, some antibiotics, gamma globulin, infusions and disposable syringes. Hospital supplies by this time were exhausted.

FIRST STEPS

The following measures were taken.
1.   Creation of an office from which all control activities would be directed. This was quickly established with the help of the local administration and the Voluntary Agencies (ACROSS, German Caritas and Kock's Road Company). This office was referred to as the Epidemic Control Centre for the Western Equatoria Province.

2. Establishment of contact and communications with the various categories of people I was to work with and education of the public on how to behave during the epidemic period.

Many of the Health Personnel were demoralized and had deserted their work following the death of some of their colleagues. I tried to console them and promised to solve most of their professional difficulties through the provision of better working facilities (protective clothing, shoes, gloves, masks, caps, disinfectants, etc.) and support to their families by providing free food and medical care. Most regained their morale and courage and were able to cooperate with me until the end of the epidemic.

I had good cooperation with the local authorities through which I managed to meet and talk to the traditional rulers chiefs and elders of the people. I was provided with interpreters from English into the local dialects and a hand microphone to announce to the public rules and regulations for the control of the epidemic.

ENFORCEMENT OF STRICT BARRIER NURSING

On completion of the two quarantine blocks, with nurses duty shed and a fence with locked gate, patients were moved from the hospital wards and the hospital closed, except for the Medical Officer's office, out-patient clinic, stores and kitchen. Non-medical personnel of the hospital were instructed to remain at home and the number of nurses and cleaners on duty was reduced to 8-10 per shift.

Barrier nursing techniques were demonstrated to nurses and public health personnel using the limited supply of protective clothing and other facilities available. The WHO team that came to Maridi to investigate the epidemic, helped in these demonstrations. Nurses were instructed not to remain unnecessarily long with patients and never to eat food in the quarantine block or to drink water from cups used by the patients. All disposable materials including patients' mattresses and bedsheets were destroyed following the death or discharge of patients. The non-disposable items were disinfected in formalin solution and the staff were instructed not to take home any items such as masks, gloves and caps.

Relatives of patients were not allowed to enter the quarantine area. The quarantine gate remained locked except to allow the health personnel on duty to go in and out. The dead were not handed to relatives but buried by public health staff. For some time this was resented by the relatives. The public health staff including ambulance drivers wore protective clothing while on duty. This team was responsible for collecting sick people from their homes and taking them to hospital and for collecting dead bodies from the quarantine block for burial.

Quarantine measures. There was no known measure to protect the public. There was a greater chance of infection from close and long contact with patients as evidenced by the high rate of Infection among nurses and family contacts. Therefore, the only way to save the public was to remove the source of infection, the infected and sick people, from the community as quickly as possible.

Chiefs were to report to the control office any of their subjects falling ill, their whereabouts and any deaths. Social gatherings were suspended and the market regulated to avoid crowding. Funeral rituals were forbidden. Notices and circular letters were written in the majority of the local dialects and distributed to all towns and road stations. The public was advised to remain calm, to restrict their movements only to vital activities, to report to health authorities or chiefs when any of them fell sick and never to run away or hide from health personnel.

We knew that there were more cases hidden in homes than those brought to hospital. Because very few in-patients survived the disease, and because health staff were also affected and killed by the disease, panic had arisen resulting in the running away of some hospital patients, and hiding of those who should have been brought to the hospital.

At the beginning, I employed volunteers - most of them school boys, to secretly spot homes where cases were hiding or where deaths had occurred. This method yielded good results, more cases were discovered which we were able, with some difficulty, to remove to quarantine. Two weeks later we decided to increase the number of detectors and to work openly. At little cost more students were employed, funds were provided by WHO. They were to search a larger area, the town and its immediate surroundings. The detection teams were put under the supervision of three public health officers who had been sent from Yambio, Juba and Khartoum, and two sanitary overseers from Maridi. The town was divided into blocks to be allocated to team members. By then a rule had been passed by us through the local administration giving team members the right of access to every house. They were required to check the health of every family member and register sick people or deaths and their close contacts. Literally every house was checked, and the Epidemic Control Office sent an ambulance to collect patients and take them to the hospital.

Case detectors roamed the town on foot, on bicycles or by car. Case detection and collection were not always easy. Some patients or their relatives resisted orders for removal to the hospital. In a few instances patients were moved from one house to another and police assistance had to be called for.

The result of the intensive search for cases was dramatic. In two weeks, Maridi town was almost depleted of cases. During the next three weeks few cases appeared among family or close contacts of cases who were in our registers for follow-up.

Surveillance Activities. During our search for cases and recent suspicious deaths in homes, all family or close contacts of such cases were registered and instructed not to leave their residence until two weeks after the date of last contact with a case or a dead body. They were visited and checked at home twice a week for a fortnight.

For distant towns, stations and other rural settlements, instructions were sent to the responsible people in those places, to report to the Epidemic Control Office any persons falling sick or dying in their areas. Surveillance teams under public health officers covered those areas in cars or on bicycles. Actually by the time the incidence of disease was highest in Maridi, the epidemic had subsided in the rest of the province (Nzara, Tambura, etc.) but nevertheless, we had to cover those areas during surveillance visits. Movement of people, vehicles and aircraft in, out and within the province remained forbidden.

The most distant place to which the epidemic spread from Maridi was Manikakara 20 miles west, but there the disease remained localized to five cases of whom two died; and from Nzara the furthest point was Tambura 130 miles away, where a lady patient exported the disease to her family out of whom three were affected and all died with no further spread. These two distant spots were checked twice during the time of our work in the epidemic area.

Before the Epidemic Control team withdrew on December 7, 1976, the public health personnel of the province were instructed to continue surveillance activities for six months after the disease was declared eradicated on December 15, 1976. I myself visited the province again twice, in March and May 1977, no cases were reported since November 22, 1976.

There were some difficulties in carrying out surveillance. The area to be covered was very large. The province is 225 miles long and 50-100 miles wide. Parts of it are mountains and some of the feeder roads had bridges broken or overgrown with shrubs and grass. Rural settlements are very scattered. Azande live in small clans whose homes are often 1-3 miles apart. Shortage of fuel also restricted our movements.

If this Ebola virus infection had the epidemiological features of smallpox infection, I do not think we would have contained the epidemic in view of the acute shortage of manpower and supplies and insufficient logistic support.

COLLECTION OF MAMMALS AND ARTHROPODS DURING THE EPIDEMIC OF HAEMORRHAGIC FEVER IN ZAIRE

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A survey team of the International Commission, stayed in the Yambuku epidemic area between November 1, and November 9, 1976. By then, the haemorrhagic fever outbreak was nearing its end.

One aim was to search for cases and convalescent patients.

The other purpose was to make a first investigation on the natural conditions susceptible to have favored the transmission and to collect mammals and arthropods for virus-isolation attempts. The hasty organization fixed by the circumstances for this ecological investigation makes the results unfortunately overmodest.

Prospected area. At that time, the mode of contamination of the first known

patient, who had made a travel in the forests of Mobaye-Bongo zone, but also received a perenteral injection at Yambuku Hospital a few days before his fatal illness, already appeared conjectural. Therefore, it was decided that the first ecological investigations would concern Yambuku, epicentre of the epidemic, and some other places within a radius of ten to twenty kilometers (Yalikonde, Yahombo, Yalikenga, Bolisa westward, Yamolembia, Yandongi and Yamunzwa eastward and part of Yamikeli on the Bumba-Yandongi road). All these inhabited places, except Bolisa, were more or less recently affected by the epidemic (last deaths on October 28 and November 3, respectively in Yahombo and Yamikeli).

Collecting methods.

1. Insects.

- *Culicidae* :

The circumstances prohibited the organization of man-baited catches. Mosquitoes were caught by glass tubes from resting sites inside houses and in outbuildings. Five to ten minutes were given to each visited compound, which were inspected by night or in the early morning. The knock-down method was also used.

- *Cimicidae* :

Bedbugs were collected from the raphia slats of the beds or, after shaking, from the soil.

2. Mammals .

- Monkeys and mean-sized animals

Cartridges were distributed to some hunters and animals were shot in the nearby forest.

- Rodents

Trapping proved very disappointing, partly due to the lack of a sufficient number of traps (Chauvency's type). Therefore wild and domestic species were mainly hand caught by villagers to whom disposable gloves were supplied.

- other mammals :

They were generally shot. Seven bats were net caught.

Organ samples from a dead cow, sera from ten pigs and one cow were collected.

Virological sampling. Insects were pooled by species. Bedbugs, slightly anaesthetized, were introduced into Nunc tubes and put in dry ice.

As most mammals were dead when submitted, these were not bled but spleen, liver, kidney and heart were pooled in individual pools.

Entomological survey results.

1. *Culicidae*

The mosquito prevalence in the houses was surprisingly low. From 61 compounds inspected only 3 *Anopheles funestus* Giles, 2 *Culex pipiens fatigans* Wiedemann, 8 *C. cinereus* Theo. and 5 *Mansonia africana* (Theo.) were caught. The presence in the houses at night of *C. cinereus*, chiefly an ornithophilic species, results from the poultry frequently admitted to sleep there.

Knock-downs, carried out in six compounds, were negative.

No virus was recovered from this small number of mosquitoes.

All villagers testified that mosquito biting activity was then very low in and around the dwellings, also mosquito nets were not in use.

Aedes aegypti (L.) was worth special attention since Kunz and Hofmann had shown that Marburg virus, multiplies in it after intrathoracic injection although this does not prove *A. aegypti* transmits the disease in nature.

The prevalence of *A. aegypti* was assessed in Yandongi, the most urbanized place and therefore the most favorable for its multiplication. 28 compounds were inspected for larvae. Breteau index was 3.6; house and container indices were 2.3. These figures are low and, well under those generally observed during yellow fever epidemics. Other species found were *Culex p. fatigans*, *C. cinereus* and *C. nebulosus* Theo.

No insecticide had been applied during the last months in the ward selected for this inspection. Low *A. aegypti* indices are usual in wet equatorial countries of Africa, where domestic water storages are generally small.

2. Ceratopogonidoe

Culicoides were copiously biting in the early morning and at the end of

the afternoon.

3. Cimicidae

Numerous bedbugs were found in many houses but not in all. They all belong to the species *Cimex hemipterus* (Fabricius).

Though many authors tried in the past to incriminate bedbugs in the transmission of various diseases, either through bites or through feces (1), all evidence was generally negative or inconclusive. However, recently, some selvatic species of bedbugs were shown to be vectors or reservoirs of arboviruses (Kaeng Khoi, Fort Morgan and Bijou Bridge viruses). Still more recently, in Senegal, Hepatitis B virus was detected in *C. hemipterus* collected from bedding and kept alive without a blood meal for 30 days (2).

In the Yambuku area,, 818 nymphs and adults of *C. hemipterus* were collected from Yalikonde, Yahombo, Yandongi and Yamonzwa in houses or wards more or less recently affected by the epidemic.

No virus was recovered.

Mammal survey results.

1. Monkeys

Antibody surveys on Marburg virus in Uganda (3) had pointed to *Cercopithecus* monkeys, especially *C. aethiops* (L.), to be involved in the natural cycle of the disease.

Efforts were made to collect specimens from these. However very few monkeys were shot, because the hunters' guns were generally locally made and our cartridges frequently returned intact though having been percussed.

Monkeys were abundant in the area and are frequently used as food.

The species we have registered are all chiefly forest-dwellers :

Cercopithecus ascanius schmidti Matschie, which seems to be common (a small troop was seen), *C. nictitans* (L.), *C. pogonias grayi* Fraser, *C. neglectus* Schlegel, *Colobus guereza* Rüppell.

Cercopithecus aethiops which is chiefly a savanna-dweller was not observed, 4 although this species is recorded from this part of Zaire by Schouteden (4)

No behaviour anomaly or abnormal mortality had been observed by the hunters among the forest monkeys during the last months.

5 *Cercopithecus* were collected : *C. ascanius* (2), *C. nictitans*, *C. pogonias*, *C. neglectus*.

No virus was recovered.

2. Rodents

1. Domestic rodents

The black rat, *Rattus rattus* (L.), was the single domestic rodent. *Mastomys natalensis* (Smith) was not found in the family compounds. It seems that, according to a process ascertained in other parts of Africa (5,6) *Mastomys* has been evicted by the common rat.

R. rattus were caught in Yambuku catholic mission, Yahombo, Yalikenga and Yalikonde. They were especially numerous in the latter village. Rice corps frequently stocked in dwelling-rooms were very attractive for them. No abnormal mortality was noticed among these domestic rodents during the epidemic.

The organs of 30 *R. rattus* were grouped into pools.

No virus was obtained.

2. Wild rodents

On the whole, 88 wild rodents were caught in the nearby forest.

Muridae :

69 *Praomys*, among which *P. tullbergi minor* (Hatt) seems to be predominating, 5 *Thamnomys rutilans* (Peters), 1 *Hylomyscus sp*., 1 *Lemniscomys striatus* (L.), 1 *Lophuromys a sikapusi* (Tem.). Squirrels :

2 *Funisciurus sp*. and in addition, 6 unidentified specimens subsequently collected.

Dormouses :

3 *Graphiurus sp.*

No virus was recovered.

Wild rodents are occasionally eaten by the villagers, especially the giant gambian rat (*Cricetomys emini*) (which does not appear in our collections), never the black rat.

3. Other mammals or small vertebrates

Likewise, no virus was obtained from some other wild animals: 2 duikers *Cephalophus monticola* (Thünberg), 1 giant bat *Hypsignathus monstrousus* (Allen), 1 lizard *Agama sp.* and the 7 unidentified net-caught bats.

Organs or sera of cows and pigs gave also negative results.

CONCLUSION

During the outbreak, the mosquito biting activity was low in and around the dwellings and *A. aegypti* was very scanty. This does not favour the role of an arthropod in the transmission. *Culicoides,* by their mere abundance, can be excluded as vectors.

No virus was recovered from more than 800 bedbugs collected. However, this does not exclude definitely the role of bedbugs in intra-family contaminations. Although monkeys are quite common in the Yambuku area forest, only 6 could be shot. From 147 mammals collected in the area, no virus was recovered. No abnormal mortality or behaviour anomaly was observed among the wild fauna and the domestic rodents during or just before the epidemic. However, there is a need for serum surveys for Ebola antibodies in wild animals.

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APPROACHES TOWARDS STUDIES ON POTENTIAL RESERVOIRS OF VIRAL HAEMORRHAGIC FEVER IN SOUTHERN SUDAN (1977)

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SUMMARY

This study was carried out in Nzara, W. Equatorial Province, Sudan, (in January-February, 1977) following the protocol agreed upon at the meeting at the London School of Hygiene and Tropical Medicine, 4-5 January 1977.

Mammals having the highest possible contact with humans in the Nzara cotton factory and agricultural environs where primary cases were noted were studied (Table 1). Although primates have been suggested as reservoirs, these were not collected because of the difficulties involved in collecting adequate samples in such a short period.

Organ and tissue samples were collected, frozen in liquid N_2 and sent to Porton Down, U.K., for virological and serological studies on 13 February, 1977 (Table 2). Voucher specimens of the animals collected, preserved in formalin, were sent at the same time to the British Museum (Natural History), London, for confirmation of field identifications.

Data on sex, age and habitats (including human contacts) of all potential hosts were recorded to be correlated with virological and serological results. As the virology and serology are not yet available, the paper emphasizes the rationale used in establishing a survey for potential reservoirs of a disease of unknown aetiology.

INTRODUCTION

As we have yet to receive information on material delivered to the Microbiological Research Establishment concerning the isolation and/or serological identification of Ebola virus from mammals within the Southern Sudan area, we nave tried to organize this paper as a general protocol for research for unknown reservoirs in disease situations such as occurred in Southern Sudan.

The first consideration should be given to the apparent ecology of the disease. If an insect vector, such as a mosquito, was involved we might have expected a large number of primary cases. In this instance, there were few primary cases which resembled the situations observed in the case of Lassa virus and other known rodent-borne viral haemorrhagic diseases, or even for that matter bacterial diseases such as plague and tularemia, so that an arthropod such as a tick or a mite is not excluded.

Because the initial concern suspected Marburg virus, it was suggested that primates would be logical reservoirs. However, in the case of Marburg, inoculation with the virus in primates was highly, if not inevitably, fatal suggesting that they were not a prospective reservoir. On the other hand, rodents had tolerated initial Marburg inoculations. Initial studies on other unknown virus reservoirs were, in a sense, rather easy to conceive as in the case of Lassa, once recognized as an arenavirus, it was expected to be associated with rodents because of its similarities to Argentinian and Bolivian haemorrhagic fevers (1,2,3,4,5,6).

It was decided therefore that the most common mammalian species, with the highest contact rates to man in the Nzara area, would be collected. Bats and rodents heavily infested the area of the cotton factory (Figures 1 & 2) in which the primary cases were noted, and field rodents were collected in the areas surrounding where the primary cases lived in and about dwelling compounds (Figure 3). (7,8, 9).

As bats live a long time, we feel that we collected animals that would have been alive during the period of the epidemic. Life expectancy of rodents is much shorter, about 10-12 months, (varying by size, species, habitat, population levels and from year to year. Our collections were made in mid-January to mid February (1977), 8-9 months after the initial outbreak so the adults should have been young during the outbreak. Considering potential antibody levels in the animals we collected, we aged all mammals as age structure is important in antibody and isolation studies during post-epizootic

analysis. Too often this is not considered when working with non-human mammalian species. In general, therefore, we feel that we collected a high percentage of mammals assumed to be alive in the early and acute stages of the epidemic.

TABLE 1

NUMBER AND SPECIES OF MAMMALS AND OTHER VERTEBRATES COLLECTED IN SUDAN (1977) AS POTENTIAL RESERVOIRS OF EBOLA VIRUS. IDENTIFICATION OF THE MAMMALS HAS BEEN CONFIRMED BY THE BRITISH MUSEUM (NATURAL HISTORY) AND REGISTRATION NUMBERS OF VOUCHER SPECIMENS ARE LISTED

Taxon	Total Number Collected	British Museum Insectivora (Shrews) (Natural History) Registr. Numbers of Voucher Specimens
Insectivora (Shrews)		
Crociduva flavescans	7	B.M.(N.H.) 1977 661-667
Chiroptera (Bats)		
Epomophevus anupus	2	B.M.(N.H.) 1977 595-596
Micropterus pusillus	2	B.M.(N.H.) 1977 597-598
Taphozous mauvitanius	12	B.M.(N.H.) 1977 599-608
Sootoecus hirundo	12	B.M.(N.H.) 1977 609-618
Scotoecus hindei	2	B.M.(N.H.) 1977 619-620
Tadarida (Mops) nanula	8	B.M.(N.H.) 1977 621-625
Tadarida (Mops) trevori	140	B.M.(N.H.) 1977 626-660
Rodentia (Rodents)		
Thamnomys (Gramomys) avidulus	3	B.M.(N.H.) 1977 3240-3242
Lermiscomys striatus	10	B.M.(N.H.) 1977 3243-3251
Aethomys sp.	4	B.M.(N.H.) 1977 3252-3255
Rattus rattus	103	B.M.(N.H.) 1977 3256-3267
(1)*Mastomys (Praomys) natalensis*	180	B.M.(N.H.) 1977 3268-3295
Lophuromys sikapusi	6	B.M.(N.H.) 1977 3296-3300
Cricetomys gambianus	2	B.M.(N.H.) 1977 3301-3302
Cryptomys ochraceocinereus	1	B.M.(N.H.) 1977 3303
Other		
Toads	1	(unregistered)
Lizards	4	(unregistered)
TOTAL	501	137 voucher specimens were deposited in the B.M.(N.H.) - 66 bats 64 rodents 7 shrews and 5 batraciens.

(1)   In other parts of Africa this nominate species is certainly a species complex based on cytotaxonomic studies which could not be carried out in the Sudan because of time and facilities.

TABLE 2

SERA AND ORGAN SPECIMENS COLLECTED FOR VIROLOGICAL AND SEROLOGICAL STUDIES ON AFRICAN VHF

a.	Sera	423	
b.	Liver spleen and kidneys	501	(pooled by individuals)
c.	Testes	197	(Collected based on recorded retention of Ebola virus in this tissue in an accidental

			human case)
d.	Salivary glands (bats only)	120	(Bats only; as these tissues are good sites of bat-borne viruses)
e.	Other	3	
	TOTAL	1.244	(From 501 vertebrates)

METHODS

All positive traps were soaked in hypochloride solution each day. Trap sacs (as used for plague studies where fleas are involved) were not used because of possible urine contamination. Baits were made of local grains, mostly maize, boiled with vegetable oils or tinned fish, the latter of which proved more suitable.

Fig. 1. Exterior of Nzara cotton factory where primary cases of African haemorrhagic fever were working. Bat roost (Tadarida (Mops) trevori) was in attic of last unit on the right.

Fig, 2. Interior of store room in Nzara cotton factory where primary cases worked. Droppings and urine can be seen at the corners of the walls.

Fig. 3. Vegetational characteristics of the dwelling compound now abandoned according to tribal tradition of the primary case who worked in the cotton factory store room. His tomb is in the foreground.

Fig. 4. Outside working area in Nzara where mammals were bled and organs collected.

Only the two of us (Arata and Johnson) handled animals during removal from traps, dissection, taking of blood, sera and organs, and disposal. Others assisted in setting and collecting traps. Gowns, visors, masks, gloves, etc., were used whenever possible and dissection and organ collection were done outside in open air with breeze (sometimes too much) (Figure 4). Only primitive sterilization was available (boiling of instruments in pressure cookers on Primus stoves, boiling clothes and gowns daily in large local kettles over open fires, burning of all disposable items especially syringes, gloves, masks, caps, etc.: all animal carcasses were incinerated with petrol in oil drums).

> Voucher mammalian specimens were preserved and sent in formalin (10% + 1-2% glycerine) to the British Museum (Natural History), London. Each was numbered individually in the field after dissection to cross reference ecological condition of collection sites and the estimated age (juvenile, sub-adult or adult) of individuals. Also, as the fauna of this area of Africa on the forest savannah belt is poorly known, accurate determination by species, and occasionally by genus was difficult in many instances under such field conditions (e.g. rodents and bats are specifically identified by tooth form and skull structures, meristics, colour variants, etc., which must be compared to museum collections). British Museum (Natural History) numbers are provided for representatives of each of the mammalian taxa studied (Table 1).

Good local facilities, as were available, were provided: all equipment arrived, albeit late. Khartoum provided a good supply of liquid N_2 during the study and the local authorities and population were excellent in collaboration.

ECOLOGICAL OBSERVATIONS

The area about Nzara, Western Equatorial Province, where the epidemic is presumed to have begun is on the northern level of the Congo forest in a transitional zone of derived Guinea savannah - the vegetation and fauna demonstrate this. The bats collected (personal communication - J. Hill, British Museum (Natural History) indicate that this faunal component represents a southern sylvatic element with few savannah species involved. In fact, our collections provide northern records for several mammalian species, and Mr.Hill noted that the known range of *Tadarida (Mops) trevori*, expanded by our collection, closely paralleled the distribution in Sudan and Zaire, the area of the epidemic. This bat species was collected from a large roof colony in the cotton factory directly over the storeroom where the primary cases worked (Figure 2) and which was laden with faeces and urine. Several bats were collected in the room on the desk of the unfortunate primary case. Although conjecture only, it is interesting.

On the other hand, we collected few true savannah rodents (possibly due to the short time). However, *Arvicanthis*, a grassland form, was absent in our collections but has been reported as being common north of Nzara. *Mastomys*, which reaches the northern limits of its distribution in this area, was the most common rodent encountered, but our collections were not in sylvatic areas but only where human cases were noted, all of which were highly "cut-over" areas. *Mastomys* will live with *Rattus rattus*, and both were taken in traps set in several areas side by side (10,11).

As in many areas from east to west Africa, there is increasingly derived transition between the Guinea savannah and the original forest zones to the south providing adequate and enlarging habitats for mammals adapting to human environmental changes and making new links between sylvatic and domestic cycles.

It should also be noted that human modification of environment, especially for agricultural purposes which require the greatest land usage, while eliminating certain "wild species" selects for others, which, partially freed from natural predators, may achieve very high populations. This results in potentially greater contact with man thereby enhancing the transmission of adaptable pathogens through "favoured" reservoirs and vectors to man. Numerous small mammalborne diseases show these characteristics. Agricultural practices related to increasing human populations clearly show this phenomenon in both insect vectors, and vertebrate reservoirs of numerous diseases. Occasionally, the reverse might be true. However, we would suggest that as this phenomenon increases, and as surveillance and diagnostic techniques improve and become more widespread we may expect to discover "new" diseases which may show epidemic rather than endemic characteristics.

SUMMARY

Until our samples for serological and viral isolation studies have been examined, we have nothing further on the virological side to report regarding possible Ebola reservoirs, other than what we did in Nzara and how and why we did it. However, this exercise has made us reconsider the design of reservoir studies. Above all, there is the need for taxonomic and ecological relationships, the latter relating to man and his use of the environment vis-a-vis the suspect reservoirs. Suspect reservoirs, for control purposes, are those most directly in contact with man, even if they are secondary reservoirs or amplifying hosts from an unknown sylvatic focus. Often medical zoologists working in poorly known areas have ascribed to a "catch all and analyze all" philosophy. However, there are places in the world (like Nzara) where time and resources are very limited and one could not afford (in three weeks) to do a general faunistic survey. Although experience and a certain amount of intuition are required, some better approaches should be made:

(a) Is the reservoir survey to be serological ? Do we do the survey at the time of convenience of the investigator (university or governmental) or based upon the known or presumed ecological structure of the rodent population ? For example, the percentage of adults and juveniles may fluctuate widely throughout the year in temperate zones and in most dry tropics. The wet tropics differ considerably characterized by more modest fluctuations with fewer young susceptibles in any one period of the year due to more balanced annual reproduction.

(b) In temperate zones there are fewer potential species of reservoirs (and vectors) but most are linked with high seasonal reproductive cycles that tend toward epizootic patterns as they are species/density dependent. Dry tropics show similar patterns, but in the wet tropics, with a wealth of species, it is different. 5 Species are abundant but the number of individuals of each species are generally lower in number with greater seasonal stability producing a "buffering" effect, thus providing an enzoonotic expression of disease in the animal population. Of course, as mentioned above, this balance can be easily shifted if the ecological conditions are changed, especially by rapid land use schemes.

ACKNOWLEDGEMENTS

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DISCUSSION

S.R. Pattyn : Are there any results on the virological examination of these specimens ?

E.T.W. Bowen : We have tested to date approximately one hundred of these small mammals. Our problem is that it would be bad practice to carry out these primary isolation studies while working at the same time with the virus itself. We have to fit it in with our own experimental work. We are in fact mainly a diagnostic laboratory and we get requests for Lassa fever studies, Marburg studies, Ebola studies, so we have to fit all this in. I do apologize for the slowness in processing but we should get it through within the next six months. We tested about a hundred I think, mainly rats, and a few bats and so far all are negative.

J.G. Breman : Testing 1.200 laboratory specimens for virus seems to be an enormous job. Wouldn't it be reasonable to start with serum surveys? Perhaps this is difficult because of the need for specific species conjugates.

A.A. Arata : I think there is something that many laboratory oriented people do not recognize very well about animal populations. Collecting animals in a given place at a given time, can produce about 75% juveniles and about 25% adults resulting from a very high reproduction rate during that period of time Collecting the same population in the same location at another moment could produce 90% adults and only 10% juveniles. Therefore, the understanding of an animal population is extremely important in the design of the collection, and whether one is going to start with isolation or looking for antibodies. In certain populations in Central Asia, all the reproduction is concentrated at one period of the year. There is estivation in the summer, hibernation during the winter and all animals ape born within a nice little one or two months' period. In the dry tropics, there is a tendency for the same situation. In the wet tropics, rodent populations tend to have a very equitable reproductive Pate, so that there is always a small number of susceptibles in the population and never a massive group. One system tends toward an epidemic, epizootic situation, the other will tend toward an enzootic or endemic situation. It is extraordinarily important that those who collect and consider this type field studies try to obtain some idea of the ecology of the species that they are dealing with.

K.M. Johnson : I agree with everything you said. But, by and Large, it is probably true that when Looking for something in nature, it is more easy, on a numerical basis, at any time to find evidence of antibodies against infection than it is to isolate the agent itself in the individual animal. Although there are exceptions : some of the arenavirus infections in their specific rodent reservoirs are a good example.

A.A. Arata : I would agree and this is why from these 500 animals, there were sera, and the Laboratory would start with the serology before the isolation attempts. But again, if you deal with older animals you look for the serology, if you happen to be at an epizootic, then you should concentrate on the younger animals and do isolation because there would not be enough time for antibody formation.

RESULTS OF EBOLA ANTIBODY SURVEYS IN VARIOUS POPULATION GROUPS

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METHODS

Antibodies to Ebola virus were measured by the indirect fluorescent technique (IFA) (1). Virus infected monolayers of Vero cells were trypsinized when 20-40% of cells were infected and the suspension of cells was exposed to ultraviolet light (1200-1400 microwatts HS/CM2) for 15 minutes. Cells were dropped onto wells in teflon templated slides, fixed in acetone for 10 minutes then frozen at –60ºC until used. Our original purpose was to identify convalescent persons who might be suitable as plasma donors. Thus IFA tests were initially done in Zaire, and serum samples were sent on to Atlanta for confirmation. Eventually a total of 39 persons was judged to have Ebola antibodies. 33 of these were originally detected in Zaire, and only one serum scored as positive there failed to be confirmed in Atlanta. Thus we believe that use of the IFA method is practical in the field if electricity and a freezer, dry ice or liquid nitrogen are available. Use of microscope with a halogen light source and epicentric illumination simplified technical problem in setting up for work

RESULTS

The results of Ebola IFA on human sera done to date are shown in Table 1. Initial work done on persons who were ill or had close contact with fatal Ebola cases in the Yambuku region suggested that such antibody was uncommon but uniformly of at least 1:64 titre when present. Seventeen percent of persons who experienced an acute illness after such contact had such antibodies. Subsequent extension of the serosurvey, however, disclosed that a few persons with or without contact with a case of hemorrhagic fever and no symptoms also were positive. Additionally there were small numbers of individuals whose area had low titres of IFA to Ebola virus. Additional positive sera were found in villages where no fatal case had occurred. These individuals had not visited cases in other villages nor the Yambuku mission hospital. Four of the 5 persons with titres of 1:64 or greater were rebled in June, 1977 and three of them were still positive for Ebola antibodies. One further high titered and two low titered sera were collected by Dr. Peter Piot in early 1977 during a retrospective evaluation of a fatal case of possible hemorrhagic fever in Libela (38 km south of Yambuku).

In January 1977 a small epidemic occurred in Northern Bosogo (Uganda). Twenty three patients were affected, 10 persons died. Signs and symptoms included high fever, headache, sore throat, cough and pharyngitis, pneumonia. Three patients had a maculo papular rash over the abdomen, 2 cases developed diarrhoea, melena and haematemesis. One serum of a female taken 16 days post onset of fever showed Ebola IFA titre of 1:4, an inconclusive but probably negative result.

Serological results on human sera collected from convalescent patients, case contacts and controls during the Ebola outbreak in Nzara and Maridi townships of Southern Sudan in the second half of 1976, are indicated in Table 1 2 with the permission of E.T.W. Bowen and colleagues

Sera from two "control" groups also were tested. We found 6 of 243 sera obtained in 1975 from northern Rhodesia had IFA, one to a titre of 1:32. Samples from 200 Panamanian San Blas Indians were tested with results shown in Table 1.

Our conclusions from these data are as follows

1. The mortality rate of Ebola infection in Zaire was high; very few sur vivors were detected when specific antibodies were used as the criterion.
2. Asymptomatic infection was unusual but did occur.
3. An IFA titre of 1:64 was a good, but not a perfect index of past infection.
4. Ebola virus may be endemic in Zaire, as shown by persistent antibodies in a few persons with no apparent case contact during the epidemic.
5. Another method for measurement of typespecific antibodies is needed. To date virus plaques under agar have not been observed, and neutralization of virus-induced cytopathic effects has

been hampered by the virus breakthrough phenomenon even when serum "accessory factor" was added.

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TABLE 1

EBOLA IFA ANTIBODIES IN HUMAN SERA FROM SELECTED GEOGRAPHIC REGIONS

	Area	Number of Samples tested	Total number of positive anti-Ebola IFA sera	IFA titre 1:64	IFA titre 1:32 - 1:8	IFA titre 1:4
Zaire	Epidemic area	984	78 (8%)	38 (4%)	22 (2%)	18 (1.8%)
	Control area*	442	21 (5%)	5 (1%)	11 (2.5%)	5 (1.1%)
	Libela	36	3 (8%)	1 (2.7%)	0	2 (5.5%)
Sudan	Maridi	214	71 (33%)			
	Nzara	218	14 (6.4%)			
	Control area**	29	3 (10%)			
Uganda	N. Bosoga	1	1			
Rhodesia	Northern zone	243	6 (3%)		2	4
Central America	Panama	200	4 (2%)	1		3

* Villages (Yaeto, Likau, Bokoy, Nbengbe, Yandongi Moke) which had no fatal cases of hemorrhagic fever

** 29 members of Maridi school who had no history of recent illness and had not been in contact with known or suspected cases of Ebola Virus Disease

DISCUSSION

J. Casals : You have mentioned that there were difficulties with the neutra lization test with Ebola virus, we know that this happens with Lassa. What the type of difficulty that you have ?

G. van der Groen : The difficulty with the neutralization test is that were unable to produce plaques with Ebola virus, so we had to neutralize CPE in Vero cells. However, the virus in the tubes with the neutralization mix breaks through 1 or 2 days after CPE appears in the controls. Perhaps complement was to be added, but this remains to be done.

J.G. Breman : You haven't said it but I suppose your interpretation is that there are false positive serologic results. Of the 38 convalescents 20 had illness, 4 had no illness and no contact with patients, the other 14 had contact but no illness. None of the titers of less than 32 anywhere had symptoms nor contact. What exactly does the positives in the San Blas Indians at a titer of 64 mean ? How does this affect future epidemiologic studies ? As I recall, Dr. Casals screened some of the people that had recovered from Lassa fever against a number of different antigens and did not find any Oro reactivity with Lassa fever. I don't know if that is applicable here but it may be something that could be done in the future as long as we rely on the FA-test.

G. van der Groen : I think that is a priority now, we have so many serologic results and we must confirm these by a method completely independent of thisne. How to interpret the high positives, or the 1/4, 1 have no idea. In Zaire we found 5 positives, one with a high titer, in a village outside the

epidemic area. These perhaps were asymptomatic infections. The lower titers could be the result of an epidemic that occurred in the past, although we do not know how long antibodies remain in the circulation. Dr. Simpson told us about the rapid decrease of antibodies in some of the people he investigated. We had the same phenomenon with a plasma donor; nine months after the acute diseases : practically undetectable. This man gave a lot of plasma, how much this influenced his antibody titer I do not know.

K.M. Johnson : it is becoming clear, to us at least, that the more work you do with the FA-test the more interesting, the more complicated and the more biologically sloppy the results become. I would urge very great caution in making any kind of final interpretation of what you have just heard. My own guess is that most of the reactions that are 64 or greater, really do represent specificity. I cannot explain how a Panamanian Indian can have antibodies to Ebola virus. I don't think these are real antibodies. Of course if these are not, it means that any others in a given serum may not be as well. It is clear that we must have an alternative and a much more specific method with which we can answer these questions. Several facts suggest endemicity of Ebola in Zaire. Of the five individuals who were bled and found positive at 1/64 or greater and had never any contact with the hospital or with a fatal case, four were found again six, seven months later and rebled. They were all still positive. Titers here and there might be twofold lower but they were all still positive. So at least we measured the right people the first time. Secondly of a group of people, largely plasma donors, rebled at the same time, in other words anywhere from about seven to eight months after illness, only one now has apparently no detectable antibody, and I'm not yet sure whether we got the right specimen. I'm beginning to believe that the virus may in fact be endemic in Zaire.

LOGISTICS IN EPIDEMIOLOGICAL INVESTIGATIONS (ABSTRACT)

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The first investigative team that went under emergency conditions to the Sudan in October 1976, from London and from Nairobi, encountered many difficulties in transport and communications. When a second team went from London in January 1977 many of these difficulties had been resolved but nevertheless some problems arose, particularly in communications.

DISCUSSION

S.R. Pattyn : From our experience in Zaire we composed two lists of equipment. The first one enumerates the items necessary to investigate an epidemic for a short time and to bring to the laboratory, in the best conditions, the specimens necessary for diagnosis. The second one contains more and larger equipment and is intended for a longer stay, with more people in an epidemic area to help in its containment, surveillance and study. These lists certainly should be studied extremely carefully because indeed the smallest details are of the utmost importance once you are on the spot.

C.E. Gordon Smith : I would just like to restress the communication problem. It is the only part of this exercise I took part in, sitting in London having phone calls from various places and trying to keep track of the operation. We had the situation of our people in the Sudan, at risk of their own health and communications were really extremely bad. The very few occasions on which we were able to make contact at all was through Nairobi and in &bout a 45 minutes telephone call about two questions were answered, somewhat. This was prolonged by the fact that every time we stopped to wait for an answer, the telephone operator in Nairobi pulled out the plug and we had to start again all the way from London to get the answer to the next question. We know that not very far away from these people there was radio communication but it was not possible to obtain diplomatic clearance to communicate across the border with the other team. This would have facilitated a great many things but, for reasons which I don't clearly understand, this was not possible. I think that everything possible must be done on any future occasion to provide adequate communication systems particularly by radio.

D.P. Francis : A suggestion was made at one time at CDC that any emergency medical team should be preceded by an administrative, logistic officer to find out the problems about customs, addresses, transportation, etc. To avoid situations where one finds himself on a 'plane without one's supplies, and has to fly all the way back. To have such a person would be worthwhile.

K.M. Johnson : Particularly if he speaks a lot of different languages ! I think you are right and there might be a number of short-cuts that might be envisioned in this.

Arevshatsian : Was there any communication between the two teams in Zaire and the Sudan during their investigations ?

K.M. Johnson : I am not sure who was really trying hardest to send a message to the other group, but the answer is no. When the group in Zaire had pretty clear evidence, and this will probably have been about two weeks after they had arrived in the country, that syringe and needle in that situation were of some significance there was an attempt to pass this information to the team in Sudan. It was done in what might be called bilateral channels. We never knew whether that team heard the word and in fact it turned out that it did not. The two teams knew before they went into the respective countries that there was need to have this kind of communication. In Zaire it took us quite a long time to have any radio-communication between Kinshasa and the Yambuku area, but when it was established we did in fact end up with one extra set and the theoretical capacity to be able to use that set in Sudan. For reasons that are not quite clear, it never quite came off.

P. Brès : I agree that there were very important questions for which exchange of data would have been extremely useful. One was the case fatality ratio, we spent evenings trying to know what the other team could have found and whether we were right in the interpretation. When we discovered ten possible generations we would have liked to know whether the other team had also observed this. What happened in the region between the two foci ? What was the situation for relief if a member

of the expedition came down with the disease ? Exchange of information would have been very much appreciated. But we have to take into account the contingency and if two countries are not willing to allow free exchange of communications through private radio systems, nothing can be done about it.

K.M. Johnson : I would think that if you want to talk about advanced logistics it is fair to raise the question as to, whether or not, particularly from the standpoint of an organization like WHO., you are going to include in your base package the radio equipment, and how many. There is no doubt that for the group in Zaire that turned out to be a very negative factor in terms of what happened in a timeframe and in the investments that were done.

C.E. Gordon Smith : If the technical ability in the sense of a transmitter and someone who can operate it is provided at the beginning, then the possibility exists of using it and this is important for internal purposes within the country, quite apart from whether it is then also possible to communicate internationally. But the time scale is such that if you don't get the radio into the equipment at the beginning, it will never get there in time to be useful. I also believe that the problem about communication between Sudan and Zaire would have been solved had there been a long enough time for the rather slow diplomatic processes to work. If this had been asked for, right at the beginning, the problem would have been solved. And the impetus to solve it would exist if the radio existed but, in the absence of the radio, one puts off actually making the necessary arrangements.

A. Fabiyi : It might be advisable right now to request WHO. at this moment that it should make all the arrangements with the different governments to obtain advanced clearance for communication in cases of emergencies which we are discussing.

K.M. Johnson : Including the kind of equipment and the frequencies to be used.

5. PLASMAPHERESIS PROGRAMS

PLASMAPHERESE DANS LE FOYER EPIDEMIQUE DE YAMBUKU, ZAIRE

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Le plasma de convalescent est le seul traitement spécifique a opposer a un nouveau virus pathogène. Au cours d'une épidémie due a un tel virus, le dépistage des convalescents et la récolte de leur plasma constitueront donc une priorité. Mais, si la plasmaphérèse est une opération aisée en milieu hospitalier, son exécution dans une zone tropicale isolée (Yambuku, par exemple) devient, par contre, complexe, En effet, c'est au niveau du foyer épidémique que doit se déroulér la plasmaphérèse si]'en veut recueillir rapidement un nombre important d'unités de plasma. Les problèmes a resoudre sont alors nombreux : - acheminement du matériel a la fois lourd et fragile sur de longues distances.fourniture d'une énergie électrique d'intensité suffisante.

MATERIEL UTILISE POUR LA PLASMAPHERESE A YAMBUKU

- centrifugueuse réfrigéré
- matériel de prélévement (sacs plastiques, aiguilles, etc…)
- congélateur pour la conservation du plasma. réfrigérateur.
- générateur d'électricité d' une puissance de 10 k.w.a.

A ce materiel nécessaire pour la collecte des plasmas, il faut ajouter celui d'un laboratoire de sérologie virale pour la mise en évidence des anticorps spécifiques dans le plasma des convalescents (technique sérologique utilisée à Yambuku : immunofluorescence indirecte) et celui d'un laboratoire d'hématologie pour le contrôle et la surveillance des donneurs de plasma.

Le transport de ce matériel se fit par avion de Kinshasa à Bumba. De Bumba à Yambuku, les instruments les plus fragiles furent acheminés par hélicoptere, le reste par camion ou land-rover sur cent kilometres de pistes défoncées par les pluies.

La salle de prélèvement du plasma, les laboratoires d'hématologie et de sérologie virale étaient installés dans les pavillons du petit hôpital de la mission. L'alimentation électrique était fournie par le générateur propre a la mission et celui transporté depuis Kinshasa.

PRELEVEMENT DU PLASMA - CONTROLE DES DONNEURS

Douze convalescents donnèrent régulièrement leur plasma pendant cette opération de plasmaphérèse. Certains convalescents ne furent pas retenus : sujets trop jeunes ou trop âgés, femmes enceintes.

Au début de la convalescence, le faux des anticorps spécifiques chez ces e donneurs de plasma atteignait 1/256 . Quatre mois plus tard, il s'abaissait à 1/64 e . La récolte du plasma était alors arrêtée. Le rythme des prélèvements était d'une séance par semaine par donneur, au cours de laquelle deux unités de plasma étaient recueillies (une unité de plasma = 250 a 300 ml). Ce rythme de prélèvement n'entraina aucune réaction fâcheuse chez les donneurs.

Chaque convalescent admis comme donneur de plasma fit l'objet au début
- d'un examen clinique comprenant : appréciation de 1'état général, auscultation pulmonaire et cardiaque, prime du pouts, de la tension artérielle et de la température.
- recherche de sucre et albumins dans les urines.
- d'un examen hématologique comportant numération globulaire, formule sanguine, hématocrite, recherche de parasites sanguicoles.

L'hématocrite minimum exigé pour la prise de plasma fut 30% chez la femme et 35% chez 1'homme. Tous les donneurs enfermaient des microfilaires de *Loa Loa* et de *Dipetalonéma perstans* dans leur sang. Par contre, les autres parasites sanguicoles (trypanosome et plasmodium) ne furent jamais observ6s. De même, les recherches de l'antigène HB$_s$ et du virus Ebola dans le sang de chaque donneur intent négatives (recherches effectuées par le C.D.C. d'Atlanta).

Pendant la durée du programme de plasmaphérèse, chaque donneur de plasma reçut régulièrement nivaquine, vitamines, comprimés de fer ainsi qu'un supplément de nourriture le jour des prélèvements.

Par leur connaissance de la langue locale et des mentalités de la population les soeurs de la mission catholique furent précieuses, en particulier pour vaincre au d6but les réticences des convalescents a donner leur plasma.

DEROULEMENT CHRONOLOGIQUE DU PROGRAMME DE PLASMAPHERESE

La plasmaphérèse débuta a Kinshasa le 25 octobre 1976 sur deux convalescents évacués sur la capitale a cet effet. Les quatre premières unités prélevées sur ces personnes furent expédiées en urgence en Angleterre où elles permirent la guérison d'un technicien du Microbiological Research Establishment (Porton Down - Salisbury) accidentellement contaminé par le virus Ebola.

Le 16 novembre 1976, le programme de plasmaphérèse reprenait sur les lieux de l'épidémie a Yambuku où la totalité de la Commission Médicale Internationale s'était transportée. 11 se poursuivit jusqu'au 25 janvier 1977. Au total, 201 unités de plasma furent récoltées.

CONCLUSIONS - REFLEXIONS

En présence d'une épidémie due a un virus nouveau, il importe que la plasmaphérese démarre le plus tôt possible. Le plasma de convalescent récolté est ensuite administré aux nouveaux malades qui peuvent apparaitre dans la population autochtone mais également parmi les membrès de la Mission Médicale luttant contre l'épidémie. En l'absence de plasma protecteur, ces derniers n'auront a leur disposition, comme seuls moyens de protection, que le port de blouses, masques et gants afin d'éviter tout contact direct avec le malade.

En fait, de multiples facteurs tendent a freiner le démarrage de la plasmaphérese. L'épidémie de fièvre hémorragique de Yambuku a éclaté début septembre 1976. La plasmaphérèse n'a pu seulement débuter que le 25 octobre 1976 alors que l'épidémie était sur son déclin (dernière victime le 7 novembre 1976). Les causes de retard sont les suivantes :

-   temps pour que l'épidémie soit connue des autorités sanitaires et l'alerte donnée,
-   attente de l'isolement et de l'identification de l'agent viral responsable. Mise au point de l'antigène nécessaire pour le dépistage sérologique des convalescents.
-   mise en place des personnes et du matériel au niveau des foyers épidémiques. C'est a ce niveau qu'un gain de temps appr6ciable peut être réalisé a la condition que la mission médicate étudiant l'épidémie soit autonome dans ses moyens de transport et de télécommunication ...

Signalons enfin 'intérêt pour les laboratoires travaillant sur ces nouveaux virus de posséder quelques unités du plasma protecteur correspondant afin de pouvoir traiter toute personne contaminé accidentellement au cours d'expérimentation.

SUMMARY

Collection of plasma from convalescent persons is an early task during epidemics caused by new virus. It states problems when the epidemic focus lies in rural lonely area :

-   problem to convey the heavy and brittle implements to a far point,
-   problem to supply enough electric energy. Plasmapheresis requires annexed laboratories: laboratory of viral serology to reveal and to titrate specific antibodies in the plasma
-   laboratory for haematologic examination of plasma donors.

Twelve convalescent persons supplied regularly plasma at the rate of two units weekly. A total of two hundred and one units of plasma was obtained between November the second, 1976, and January the twenty fifth, 1977

PLASMAPHERESIS MEASURES IN SUDAN

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During investigations on the Ebola outbreak in Southern Sudan during the hight of the epidepic, it was felt necessary to start collecting immune plasma from patients who had recovered from the disease. Patients for consideration were selected on clinical grounds alone as there were no serological facilities available to examine the antibody status of each patient. Plasmapheresis measures were extremely limited because there were no supplies of electricity and in any case no centrifuge was available and refrigeration facilities were inadequate.

During the epidemic period in October and November 1976, 51 convalescent patients were bled for immune plasma in Nzara and Maridi, 500 ml of whole blood was removed from each patient using Traverol transfusion equipment and Mixed with acid-citrate dextrose solution. Each aliquot was then allowed to settle by gravity alone for a period of 3 hours. Plasma was then removed and red cells were returned to the patients' circulation.

Each aliquot of plasma was frozen as quickly as possible at -10ºC before being transhipped either to the United Kingdom or Germany for testing. Ten of the 51 aliquots were negative for Ebola antibody by immunofluorescent testing but the remaining 41 samples contained antibody at levels varying from 1/8 to 1/256.

A second WHO team left for the Sudan in January 1977 equipped with electricity generators, two refrigerated centrifuges and adequate -20ºC deep freeze storage space. In addition, this team carried a complete supply of plasmapheresis equipment. Within 2 weeks the second team had collected 103 units of immune plasma from convalescent patients in Maridi. All the units of plasma were transhipped by air freight to the United Kingdom where they were tested for Ebola antibodies and for Hepatitis B surface antigen and surface antibody by fluorescent antibody techniques and by radio-immuno assay respectively. Twenty three units of plasma contained no detectable Ebola virus antibody, 12 had antibody levels of 1:4; 33 levels of 1:8; 17 levels of 1:16; and 18 had antibody levels 1:32. Seven units had detectable Hepatitis B surface antigen and 20 had evidence of Hepatitis B surface antibody.

EVALUATION OF THE PLASMAPHERESIS PROGRAM IN ZAIRE

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The therapeutic value of human convalescent plasma (passive-antibody) in the treatment of patients with highly lethal viral diseases remains generally unproven. Reports on its successful use are mainly anecdotal. One instance where it was thought to be of value in the treatment of a laboratory acquired arena virus infection has been published (1). A laboratory worker developed Lassa fever while conducting experiments with animals infected with the virus. Five hundred ml of plasma with a titer of 1:64 by complement fixation (CF) was administered on the 10th day of illness, after which marked clinical improvement was noted; in 24 hours temperature returned to normal and viremia dissappeared. Virus was recovered from throat washings 4 days after plasma was administered and from the urine on days 5 and 22 after plasma therapy. Antibody was not demonstrated until approximately 7 weeks after onset of illness. The authors concluded that the administration of plasma with high titer of antibody was beneficial in aborting the disease.

Eddy et al. (2), using an experimental monkey model, have described the use of immunoglobulin in the prophylaxis and treatment of another arenavirus infection, Bolivian hemorrhagic fever. They demonstrated protection against development of initial clinical illness following Machupo virus infection in rhesus and cynomologous monkeys by using Machupo immunoglobulin of human origin given either before or shortly after virus inoculation. The finding must be viewed with caution, however, because some survivors developed severe neurological signs one to two months after virus inoculation and died 4-6 days thereafter. The data suggested that neurological sequellae were associated with high doses of immunoglobulin rather than intermediate or low doses. Although the efficacy of therapy remains undefined, in the face of the devastating epidemic in Zaire of a new virus disease with such an alarmingly high fatality rate, one early priority was to identify survivors with high levels of antibody and to obtain plasma which might be used for treatment of future patients and team members who might become infected during the investigation. The success of this program hinged upon rapid identification of donors, and the Critical determinant was the ability to test for fluorescent antibody (FA) in the field. This capability permitted bypassing the logistic logjam of shipping specimens to the base laboratory, in this case, to the U.S.A., and the inevitable delay before results could be returned to the field.

The first units were obtained in Kinshasa from three convalescent patients who had been transported there for plasmapheresis. Ten days after the first unit was obtained in Zaire, the efficacy of its use was tested in a laboratory worker accidentally infected with Ebola virus. The outcome was clinically gratifying and this gave impetus to a major plan to obtain more plasma by transferring operations to Yambuku, the epicenter of the disease. Dr. Courtois has told you how that program was carried out.

RESULTS

Table 1 shows the number of donors and units per donor obtained. Note that over half of the 201 units collected were obtained from six individuals.

The first and last units obtained from each donor were titrated for FA in the Atlanta Laboratory, and only four units from three donors, had titers of less than 1:64 by the indirect FA technique.

Tests run simultaneously on serial bleedings from six people disclosed that peak titers ranging from 1:256 to 1:1024 occurred 1-2 months after onset of disease. One to two months later most donors had experienced some decrease from peak titers. Seven months after onset of illness, all of the 13 persons tested still had antibody titers of 1:64 or higher.

All donors were found to have microfiliarial parasitemia. Tests for hepatitis B surface antigen (HB_s Ag) on the first and last units from each donor were negative.

TABLE 1

SUMMARY OF COLLECTION PATTERN, EBOLA ANTIBODY-CONTAINING HUMAN
PLASMA, KINSHASA AND YAMBUKU, NOVEMBER, 1-JANUARY 25, 1976-1977.

Donors	Units/Donor	Total Units
14	1 – 4	31
4	5 – 9	31
3	10 – 14	35
6	15 – 21	104
26		201

Units with IFA titer gte 1:64 - 197

Ebola immune plasma was given to one team member suspected of having acquired an Ebola infection. The cause of his illness has not been determined; however, Ebola infection was ruled out. The decay curve of passive antibody in the individual is shown in Table 2. After 500 ml of plasma with an antibody titer of 1:256 was administered, circulating antibody of 1:32 was noted for approximately two weeks, with decreasing titers and disappearance by three months.

TABLE 2

DECAY CURVE OF PASSIVE ANTIBODY ADMINISTERED TO INDIVIDUAL WHO DID NOT HAVE EBOLA VIRUS INFECTION

Day after plasma	Indirect FA titer
2	32
4	32
7	32
16	16-32
32	8
43	8
73	2
87	< 2

DISPOSITION AND PROCESSING OF PLASMA

Currently, plasma is stored in the frozen state (-20º) in Yambuku, Kinshasa, Johannesburg, and Atlanta. No definite decision has been made regarding further processing. Tentative evidence suggests that the following problems may influence the final decision :

1. Antibody given in the native unaggregated state intravenously might provide maximum effect.
2. Known uptake and half-life of ordinary gamma globulin which is aggregated and cannot be given intravenously (i.v.) is shorter than for unaggregated antibody (3,4).
3. The yield of antibody in gamma globulin is definitely a function of the starting plasma volume.
4. Methods for preparing gamma globulin suitable for i.v. use are still experimental in the U.S.A. Such procedures involve at least 30% further loss in antibody content as well as shortening the half-life *in vivo (3,4)*
5. Long-term storage of antibody at refrigerator rather than freezer temperatures is highly desirable for stability.

One possible approach to all of these problems would be simple lyophilization of plasma either in pools from individual donors or in a master pool from a few selected donors. Pooling of plasma would provide a uniform product as far as antibody titer is concerned and would permit better evaluation in a treatment program, but pooling would increase the risk of contamination by hepatitis B virus, since the radioimmunoassay (RIA) technique is not infallible. Some of these problems will be addressed in a

later section of the colloquium. A final tribute must be paid to those Zairois people who, in the face of this terrifying disease, still gave their blood in the hope that others might be saved.

SUMMARY

Two hundred and one units of convalescent plasma containing Ebola virus antibody were obtained from 26 donors. Most of the units had titers of 1:64 or higher. All units were HB$_s$ Ag negative, but all were found to contain microfilaria. The units are being stored at -20°C in four different localities. Plans for future processing are discussed. The key factor in the success of this program was the ability to detect antibody-positive persons in the field by using partially inactivated antigen slides and a fluorescent microscope.

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DISCUSSION

A. Fabiyi : In our efforts to collect plasma for Lasso, we have always measured the blood protein Level before we bled any individual. in order to relieve donors from further dangers, instead of going to an area where facilities are not available, efforts are made to transport them from one area to another, a few hours by Jeep, to a base where all the facilities for the plasmapheresis are available. We were just informed that the presence of microfilaria in the plasma is no contraindication for the use of it. What happened to the team member to whom plasma was given ? Were there any problems in that regard ?

K. Isaäcson : The patient has been given heated plasma, so I don't think the microfilaria posed any problem.

J. De Smyter : where is the convalescent plasma being kept now ? Will some be processed into gammaglobulin or do you consider this wasteful ?

K.M. Johnson : With respect to the plasma obtained in Zaire, Dr. Webb indidated the places without specifying exactly how many ml in each. It is presently distributed in the original plasma state. The question of further processing of the plasma in order to stabilize the antibody over a long period of time. and lots of other problems, is very complex and will be discussed later on.

J.G. Breman : Dr. Simpson, of the 46 positive donors at the first bleeding how many were included in the 59 donors that Dr. Draper had in his group and of those 59 how many persons were negative that were positive in the first group ?

D.I.H. Simpson : They were all, I think, included in the second round and I think only two had become negative.

J.G. Breman : However, there were 23 units in the second bleeding of the 101 taken that were negative.

D.I.H. Simpson : They did not all have two units taken off. When I say 23 units, some of them were from patients who were not patients at all, but who had been included in Dr. Draper's list because the hospital thought that these were cases, they were not the documented cases that we had.

T. Muyembe : How much plasma was obtained in Zaire, and how much of it was left in Zaire ? What are the indications for its administration ? We frequently have alarms, which are perhaps false alarms, but one is tempted in these circumstances to administer the plasma.

D. Courtois : in Yambuku 201 units of plasma were obtained. Dr. Johnson will be able to tell where they are now. The problem of the indication of its administration is extremely delicate, for it is very rare and should be used accordingly. it should be administered in the early days of the disease. Clinical diagnosis of the disease is difficult and can only be made in an epidemic context. We have been faced with this problem for one of the team members in Yambuku. It took us 3 days to make a decision. And we had no precise and rapid diagnostic criteria. In the future there will be many false alarms and frequently the decision will have to be bade at a distance. The only good criterium is the epidemic context. The decision on an isolated case will always be rather unscientific.

K.M. Johnson : in the case of the Zaire plasma, the agreement with the Minister of Health at the time was that half of it was destined to stay in Zaire and subfractions of the other half were to be kept both in Europe, somewhere, and in the United States, when a final decision is made on the type of processing, if any. The amount obtained represents at the moment a unique pool of antibody. We had that one chance, we seized it and it is very clear today that you couldn't go back and do it again no matter how much money anybody would put into it. So there is a very great concern that this antibody be kept in the state that will best preserve it for the longest possible time so that it can be used where it is needed.

P. Brès : in this connection, it should be noted that plasma cannot be administered to haemorrhagic fever patients before the etiologic virus has been antigenically identified. Remember that the plasma from Johannesburg given in Kinshasa did not do any good because the two viruses were antigenically different. This means that in the future morphological diagnosis of an Arena or an Ebola-like virus based on electron microscopy will not be sufficient but that the agent needs to be antigenically identified which may take another eight or fifteen days.

K.M. Johnson : I couldn't agree with you more, the question of a rapid highly specific viral diagnosis is not just an academic game.

6. PROBLEMS AND FUTURE NEEDS

DISSEMINATED INTRAVASCULAR COAGULATION

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In a dynamic circulatory system, acceleration of the blood coagulation sometimes followed by a secondary activation of the fibrinolytic system, develops in a hypercoagulable state, which can lead to an increased consumption of platelets and of some clotting factors. These changes, which may occur rapidly or at a more chronic pace, can induce an abnormal bleeding, small-vessel obstruction and single or multiple organ dysfunction; even latent forms exist. As well the clinical picture as the pattern of laboratory results most often are complex and change by the hour as the background situation worsens or improves (1,2).

The term disseminated intravascular coagulation (D.I.C.) is not entirely correct but came into use when it was recognized that, in both experimental and clinical conditions, accelerated fibrin deposition in various organs, e.g brain, kidney, lung, could be a result of defibrination; it is thought likely that in normal conditions fibrin is continuously being formed-on the vascular wall and lysed at a slow rate. The acceleration of this normal condition is termed D.I.C., which is not a disease in itself; it is a pathophysiological reaction with many apparently different aetiologies, and any discussion of diagnosis and management must first examine the underlying mechanism as they affect man.

THE EXPERIMENTAL MODEL

Numerous clinical conditions are known in which D.I.C. can occur (Table 1) many of the detailed studies on the pathogenic mechanism involved have been made by necessity in laboratory animals, particularly in the generalized Schwartzman reaction in the rabbit.

The intravenous injection of thrombin, thromboplastin, some snake venoms, or successive injections of Escherichia coli endotoxin can induce the entire spectrum of D.I.C. in animals. The former three examples are direct triggers which activate the coagulation cascade at several intermediate stages, leading to thrombin generation, fibrin deposition, platelet consumption with tissue damage, and concomitant (reactive) fibrinolysis. Endotoxins and antigen-antibody complexes cannot activate the coagulation sequence directly but will do so only when they link with certain mediators and then may work only by damaging tissues and relaxing a direct trigger.

TABLE 1

CLINICAL CONDITIONS WHICH CAN BE ASSOCIATED WITH DISSEMINATED INTRAVASCULAR COAGULATION

Acute forms :
- o amniotic fluid embolism
- o septic abortion
- o fat embolism
- o incompatible blood transfusion
- o oligaemic shock
- o snake bite
- o massive trauma

Subacute forms
- o abruptio placentoe
- o eclampsia
- o acute hepatic failure
- o burns
- o cardiac surgery
- o dissecting aortic aneurysm

- o haemolytic uraemic syndrome (Gasser-syndrome)
- o head injury
- o heat stroke
- o respiratory distress syndrome
- o severe haemolytic disease of the newborn
- o thrombotic thrombocytopenic purpura
- o numerous infections (Aspergillosis, Ebola, Dengue, Malaria, psittacosis, Rocky mountain spotted fever, septicaemia to various microorganisms, typhoid fever, yellow fever)

Chronic forms
- o hydatiform mole
- o pre-eclampsia
- o dead foetus syndrome
- o giant haemangioma (Kasabach-Merritt syndrome)
- o neuroblastoma
- o carcinomatosis
- o decompensated liver cirrhosis
- o organ transplantation
- o splenectomy
- o siccle-cell disease
- o purpura gangrenosa
- o malignant hypertension
- o disseminated lupus erythematosus

PATHOGENESIS IN MAN

Three major types of cell injury may activate the coagulation system in man (3):

1. injury or damage to the endothelial cell (e.g. in many microbial illnesses, antigen-antibody complexes), which by impeding prostacyclin formation (PG12) favours platelet deposition against the vascular wall; in addition any exposure of collagen to the circulating blood activates the coagulation factor XII (Hageman factor) and subsequently the coagulation cascade through the intrinsic pathway,

2. tissue injury (e.g. in diffuse malignancy, abruptio placentae), which releases tissue thromboplastin and in the presence of factor VII activates the extrinsic coagulation system, and

3. red cell (e.g. malaria or incompatible blood transfusion), leucocytes or platelet injury, which increases the availability of phospholipid, a component needed for both the intrinsic and extrinsic coagulation systems.

The outcome of these different initiating mechanisms is the formation of the serine protease factor X, which converts prothrombin to thrombin in the circulating blood. In case the activated coagulation factors cannot be rapidly cleared by the liver, a widespread fibrin deposition due to free thrombin activity may follow, and fibrinogen derivatives, as well as fibrin monomers formed due to excessive enzyme-induced proteolysis. A secondary fibrinolytic response to fibrin deposition results from activation of the plasminogen activator from endothelium. The plasmin thus formed can exaggerate the hypofibrinogenaemia and generates fibrin-(ogen) degradation products (FDP). Some Of the larger FDP (fragments X and Y) have a direct antithrombin action and the smaller D and E fragments interfere with fibrin formation by inhibiting its polymerisation; smaller polypeptides also inhibit some platelet functions.

Coagulation components besides fibrinogen which are normally consumed during the coagulation process are f.i. prothrombin, factor V and factor VIII. Platelet counts drop as aggregation occurs and as platelets are incorporated in microthrombi and to exposed collagen.

The amount of fibrin deposited in tissues is generally highest in the smallest vessels but may be influenced by the local rate of blood flow and the activity of local clearance mechanisms. The effects of fibrin deposition are those of ischaemia of the organ concerned (e.g. renal cortex, lung, pituitary, adrenals, liver, brain, spleen, skin, gastrointestinal tract ...), varying from minor hypoperfusion to infarction. Passage of red cells through fibrin strands can also cause red blood cell distortion and

fragmentation and subsequent haemolysis (microangiopathic haemolytic anaemia). Damaged red cells may also release ADP causing further local platelet aggregation.

Once the praecursor of the Hageman factor (XII) is being converted, it can activate directly or indirectly three other proteolytic enzyme precursors present in plasma : prekallikrein (resulting in the formation of the potent vasodilator bradykinin), complement (the first component) and the already mentioned plasminogen system.

LABORATORY RECOGNITION OF D.I.C.

Diffuse intravascular coagulation present both the haematologist and clinician with three dilemmas. First the dilemma of clinical suspicion, then the dilemma of timely recognition by laboratory tests and finally with the dilemma of whether and how to treat the condition.

Clinical suspicion of the existence of D.I.C. is often low down on the list of differential diagnoses in any peculiar clinical situation. By the time the appropriate laboratory tests are carried out and reported, the picture is already confused by previous antibiotic and steroid therapy, fluid replacement and often blood transfusion : D.I.C. is indeed not a static state - it changes by the hour for better or for worse. Laboratory tests must provide rapid results for sensible decisions. Not one test is diagnostic; collating several results may give a pattern compatible with D.I.C. at the time the blood samples were taken.

Two dogmatic statements can be made : (a) only absolute normality of all tests to be described excludes D.I.C. at time of testing and (b) the results of one set of tests giving abnormal results can be misleading and repetition at frequent intervals is therefore essential.

The tests which give results within one hour are valuable and useful; tests which take several hours are of little use as a guide to diagnosis or treatment of the acute clinical problem, but can give valuable information on the more rare forms of chronic D.I.C.

Tests giving a result within one hour

1. Packed cell volume : a centrifuges blood sample may reveal anaemia, jaundice or intravascular haemolysis.

2. Blood film : the classical appearance of fragmented or distorted red cells (Burr-cells) are most often seen in acute cases and suggest subacute D.I.C. when present; Burr-cells suggest that fibrin was deposited in the kidney or that carcinomatosis exists; artificial heart valves also cause fragmentation of red cells. Distorted red cells have a low sedimentation rate except when the fibrinogen level is very low.

3. Platelet count : in the presence of a normal platelet count D.I.C. is less likely or exists in a compensated form. Mechanical particle counters should not be relied on as they also count "debris" or dust that circulates in D.I.C.; phase contrast microscopy is therefore to be recommended.

4. Plasma thrombin clotting time : especially when made more sensitive by dilution of the thrombin to give a clotting time in normal plasma of 16-18 seconds (circa 2.5 units thrombin/ml) is a simple test measuring depletion of fibrinogen or the presence of fibrin(ogen) degradation products which interfere with the proteolytic action of thrombin or the rate of fibrin polymerisation.

5. Plasma reptilase clotting time : this snake enzyme (Available as a laboratory reagent from Pentapharm, Bazel.) is also sensitive to FDP but is not affected by heparin which not infrequently is used in these circumstances.

6. Fibrinogen level : a rapid estimation of fibrinogen is very useful as this level is often drastically reduced in overt decompensated D.I.C. Rapid methods based on the polymerisation time are less sensitive to fibrin(ogen) degradation products (F.P.T.-Dil (Fibrinogen Polymerization Time Test, A. Christiaens Pharmaceuticals S.A., Brussels)) (4). The fibrinogen titre, using doubling dilution of citrated plasma while not strictly scientific, shows linearity when compared with other methods (unless the fibrinogen drops to less than 0.5 g/1) and provides reliable and reproducible results in unskilled hands.

7. The one stage prothrombin time : is a simple and useful confirmatory test as it measures depletion of factor V and fibrinogen but is also sensitive to fibrin(ogen) degradation products and can therefore be is leading.

8. Specific assay for factor V : has a confirmatory nature.

9. Ethanol gel or protamine sulphate precipitation test : thrombin splits two fibrinopeptides (A and B) from fibrinogen leaving fibrin monomers which form soluble macromolecular complexes with fibrinogen (mol.wt.+ 1.000.000). Alternatively when fibrin or fibrinogen is broken down by plasmin the two largest fragments (X and Y) are still thrombin clottable and

can form large soluble molecule complexes with fibrinogen or fibrin monomer. These macromolecules can produce a gel when 70% ethanol is added 6. The complexes dissociate in the presence of protamine sulphate 7 so that fibrin monomers now polymerize to fibrin and precipitate. The ethanol test is the more reproducible one and suggests that monomer-complexes or monomer-X-Y-complexes exist, and therefore that thrombin is being generated at the time of testing.

10. Assessment of antithrombin III - thrombin complex : this approach is based on the finding that the antithrombin III molecule may be altered when it complexes with thrombin and a neoantigen is being exposed. A simple latex test (8) is presently being evaluated at a larger scale and provided its sensitivity and specificity are high enough and relate well with diffuse intravascular thrombosis, a simple method for its detection would become available.

INTERESTING BUT TIME CONSUMING TESTS

Fibrin(ogen) degradation products (FDP) in serum : the larger fragments (X,Y,D,E) formed when fibrinogen or fibrin is degraded by plasmin still retain the antigenetic sites of the fibrin(ogen) molecule; the smaller fragments subsequently broken down not. Therefore the four larger fragments can be detected in serum by various immunoassay techniques in serum, after clottable fibrin has been removed and in vitro lysis prevented. These tests do not differentiate fibrin degradation products from those of fibrinogen and are timeconsuming. The most commonly used techniques are the tanned red-cell haemagglutination inhibition immunoassay (Wellcome kit HA-14) (9,10) and two forms of agglutination tests (Wellcome kit HA-13, Thrombo-Wellcotest) (11,12).

In addition, fibrinogen degradation products have a brief life and can disappear from the circulation in a few hours after an isolated episode of D.I.C.

Specific assay for factor VIII is reliable only in this context when a twostage assay is performed which again is neither simple nor rapid.

OTHER, LESS USEFUL AND MOST OFTEN TIME-CONSUMING TESTS

Factor VIII-antigen : one knows that the factor VIII molecule loses its coagulant activity during activation but retains its immunological reactivity as can be demonstrated using serum. In plasma the coagulant part and the factor VIIIantigen part are usually present in a 1:1 ratio in the factor VIII molecule. In D.I.C. the coagulant fraction drops and the factor VIII-antigen rises (13).

Fibrinopeptide A : is being split off from the A alpha chain of fibrinogen by thrombin and can be measured; would a simple assay exist it would be highly interesting to diagnose incipient D.I.C. (14)

ß2-thromboglobulin : is a platelet-specific protein released when platelets are damaged and can be measured by an immunoassay (15).

Platelet factor 4 : is a heparin inhibiting activity released when platelets are damaged. Its assay is not very sensitive except by an immunotechnique which is time-consuming.

Plasminogen : its level can be lower when the secondary lysis is intense; this information is therefore confirmatory only.

DECOMPENSATED, COMPENSATED AND OVERCOMPENSATED D.I.C.

Three main patterns of laboratory results can be obtained in D.I.C. : decompensated, compensated and overcompensated intravascular coagulation (16,17).

TABLE 2 (according to Sharp, 1977) (18)

PATTERN OF LABORATORY RESULTS TO BE EXPECTED IN D.I.C.

	Decompensated	Compensated	Over-compensated
Platelet count	Reduced (lte 100.000 µl)	Normal or reduced	Normal
Plasma thrombin time	Prolonged ++	Prolonged +	Prolonged + or +-
Plasma Reptilase time	Prolonged ++	Prolonged +	Prolonged + or +-
Fibrinogen level	Reduced	Normal	Raised
Fibrin(ogen) degradation product level	Raised ++	Raised +	Raised +
One-stage prothrombin time	Prolonged	Normal	Normal

Activated partial thromboplastin time	Prolonged	Normal	Shortened
Factor VIII level	Reduced	Normal	Raised
Ethanol gel test	Positive	+-	+-

There is no doubt when the "<u>decompensated pattern</u>" is found that D.I.C. exists in the patient. The problem is still whether these results signify a past recovering episode, a progressing state, or a developing defect; thus the need for sequential testing can an evaluation be made accurately.

The <u>compensated pattern</u> is common and difficult to interpret; it may indicate a mildly abnormal proteolytic activity or ongoing D.I.C. This pattern may precede an explosive episode of D.I.C. within a few hours or days (19). Some consider these results as highly suggestive for early D.I.C. and a call for anticoagulation; in the opinion of many investigators they point the way to further sequential testing.

The <u>over-compensated form</u> is often considered as a "hypercoagulable state"; it may occur in case of recovery of D.I.C. or as an indication of build-up of abnormal proteolytic activity. Its significance in the time sequence of D.I.C. can only be judged in the light of previous results in the clinical picture.

PRINCIPLES OF MANAGEMENT

In view of the diversity of the primary conditions in which D.I.C. can occur, the dynamic nature of the process and its occurrence in acute or chronic forms, only general principles of treatment can be stated.

Two basic rules of management can be made (a) treatment of the underlying condition and supportive treatment come first expansion of blood volume, if depleted and in case of shock, control of acidosis and electrolyte imbalance are of prime importance (20); (b) in the presence of serious bleeding and a laboratory pattern of acute haemostatic failure, replacement of the depleted blood components : fresh dextran 70 or plasma protein fraction to expand the blood volume. In case of many acute self-limiting D.I.C. as in abruptio placentae no further treatment is neccesary once the uterus is evacuated.

In case the underlying condition is not known, if the clinical situation deteriorates and/or if the laboratory tests indicate a worsening situation in spite of supportive or replacement therapy, heparin therapy must seriously be considered. Opinion is still divided on this matter but continuous intravenous heparin administration seems to have been life-saving in certain situations:

1. in amniotic fluid embolism when the diagnosis is quickly recognized. Without further delay, a bolus of 10.000 U heparin should be given intravenously,
2. in mismatched transfusion "rapid heparinisation" may prevent severe renal damage,
3. in cancer patients with multiple metastases, continuous D.I.C. can be reversed by heparin and stop distressing haemorrhage,
4. in acute (promyelocytic) leucaemia heparin may stop the intravascular coagulation and reduce bleeding particularly when also platelets are given to correct a thrombocytopenia,
5. in septic abortion retained products in-utero should be eliminated and heparin treatment started immediately in septicaemie. Antibiotic therapy is the first treatment in association with heparin,
6. in D.I.C. due to severe heat stroke and purpura gangrenosa (or fulminans) and prior to induced labour in the dead foetus syndrome heparin was shown to be at least beneficial.

The continuous intravenous heparin infusion must be carefully monitored to minimize the bleeding risk. Probably due to released anti-heparin platelet factor 4 many patients require a high initial dose (e.g. 3.000 U/h) but it is safer to start with 1.000 U/h and increase the dose depending on the results of laboratory monitoring.

Inhibitors of fibrinolysis as aminocaproic acid, tranexamic acid or aprotinin are likely to do more <u>harm</u> than good. Clinical experience with streptokinase or urokinase is mainly limited to the haemolytic uraemic syndrome and purpura gangrenosa.

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ADDENDUM

Blood collection for the ethanol gelation test or protamine sulphate test

Blood is collected with a minimum of stasis from an antecubital vein through a 18 gauge, disposable needle, discarding the first 2-3 ml. Nine parts of blood are collected into a precooled lusteroid tube, containing one part of 0.1 M sodium citrate solution. The contents are immediately thoroughly mixed, kept on melting ice, and centrifuged (3.000 r.p.m., 1.200 g) for 30 min. at 4ºC within one hour. The platelet-poor plasma is transferred to another lusteroid tube, kept at + 4ºC, and the tests carried out within 2 hours after venipuncture

Performance of the ethanol gelation test

0.5 ml of plasma is transferred to a glass tube (10 x 80 mm). After incubation for 3 min. in a waterbath at 20ºC (in order to obtain temperature equilibrium), 0.15 ml of 50 percent ethanol solution (96 percent ethanol diluted with distilled water) is added with blow out pipette. The tube is shaken thoroughly in order to assure adequate and rapid mixing, and then left undisturbed. After exactly 10 min. the tube is tilted once slowly to the horizontal position and thus inspected. The results are recorded as follows - plasmas showing a gel are considered as positive - plasmas containing no grossly visible particulate matter, and plasmas showing discrete granules are considered as negative.

Performance of the protamine sulphate test

0.4 ml of platelet-poor fresh citrated plasma is transferred to a glass tube (10 x 80 mm). After incubation for 3 min. in a waterbath at 37ºC, 0.04 ml of 1 percent solution of protamine sulphate in 0.15 NaCl is added. After 3-5 min. the tube is examined visually and the result estimated as :
negative : no change, or some cloudiness

positive : + to +++ depending whether a small precipitate, conglomerated precipitate, fibrin strands or solid clot are formed respectively.

DISCUSSION

P. Brès : Are the ""helmet cells"" constantly found in cases of DIC and do they appear at the very beginning or at the threatening stage of DIC ?

M. Verstraete : They are not constantly found and they are not necessarily present at the beginning of the phenomenon. The first evidence of DIC is the decrease of platelets, of fibrinogen, and of factor

V and the presence of fibrine monomers. It can be detected with a simple test, called the ethanol gelation test, all what is needed is some ethanol.

L. Eyckmans : Blood smears can be made in most field conditions. If platelets are not observed in a smear, is this sufficient to diagnose DIC ?

M. Verstraete : When the blood was collected properly, the complete absence of platelets in a situation where intravascular coagulation is suspected, is highly indicative, I would say.

T.E. Woodward : Why was a question mark placed after heparin under therapy ?

M. Verstraete : Heparin has been given in a number of conditions where DIC was presumed but not proven. Heparin in a bleeding disorder is potentially dangerous, if it is not due to DIC. When DIC is proven, heparin has to be given under certain conditions. It should be given after administration of fibrinogen: both have to be combined. Not fibrinogen first because the coagulation system is activated. Adding fibrinogen to it, results in its precipitation. Thus as soon as heparin administration is started, start the fibrinogen infusion.

M. Dietrich : You also put a question mark after "antifibrinolytic agents". I think indeed antifibrinolytic agents are absolutely contraindicated in this condition since they may increase the DIC.

M. Verstraete : If the intravascular coagulation is induced by gram-negative sepsis or by viruses or by antigenantibody complexes in these specific indications, there is no point whatsoever to inhibit fibrinolysis which is the natural defence line in the body to dispose of these fibrine monomers and fibrine threads. So the message is quite clear, don't do it, because it is harmful.

THE DEVELOPMENT OF A VACCINE AGAINST AFRICAN HEMORRHAGIC FEVER

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INTRODUCTION

In addressing the subject of the preparation of a vaccine against Ebola or Marburg viruses, it might be instructive to consider the experience with vaccines against other hemorrhagic fevers. In terms of human immunizations, however, such data are very limited. Indeed, the experience of the Argentine workers with an attenuated Junin virus used against Argentine hemorrhagic fever represents the only significant human immunization trial against a severe hemorrhagic virus disease (1).

While the vaccine used in Argentina was attenuated, there are some experimental animal data which suggest that an inactivated vaccine may be satisfactory The advantages of an inactivated vaccine, from the developmental standpoint, are so numerous, that we must first consider that type of immunogen in any vaccine program. The purpose of this paper is to describe briefly the experience in our laboratory with passive and active immunization of rhesus monkeys against Bolivian hemorrhagic fever (BHF) using the etiologic virus, Machupo virus, in experimental challenge. Further, we will discuss the feasibility of developing a vaccine against Ebola or Marbrug viruses.

IMMUNIZATION AGAINST HEMORRHAGIC FEVERS

Part of our concern about inactivated vaccines arose from data, some of which were presented in an earlier publication (2), suggesting that Machupo virusinfected monkeys passively protected against hemorrhagic disease with specific immune globulin were more likely to suffer late neurologic complications than monkeys treated with lower doses of globulin. We have recently expanded these data and the results are shown in Table 1.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on the Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

TABLE 1

OCCURRENCE OF LATE NEUROLOGIC DISEASE IN PASSIVELY PROTECTED MACHUPO VIRUS-INOCULATED MONKEYS

Immune globulin dose(x) ml /kg	Hemorrhagic disease/ Total (Deaths)	MDD (xx)	Neurologic disease/ Total (Deaths)	MOO
1.5	0/8 (0)	-	7/8 (5)	50
0.5	0/3 (0)	-	1/3 (1)	42
0.15	4/8 (2)	26	0/6 (0)	-
None	3/3 (3)	19	- -	

(x) Monkeys inoculated with 1000 PFU Machupo virus, 4 hr later received indicated dose of immune globulin (human origin).
(xx) Mean day of death.

The data represent a composite of two passive immunization experiments as compared to our broader experience in untreated control monkeys. In both instances the monkeys were inoculated

subcutaneously with Machupo virus followed 4 hr later by the indicated dose of human immune globulin administered intramuscularly. Those which developed acute hemorrhagic disease became ill within a week after virus inoculation and died approximately 3 weeks after virus challenge. The monkeys protected with immune globulin exhibited a different response. Although there were some hemorrhagic signs and deaths in the lowest immune globulin dosage group, most of the monkeys, including all in the two highest dosage groups, exhibited no hemorrhagic disease.

Nevertheless, several of these monkeys developed late neurologic disease without hemorrhagic signs and without detectable viral antigen in their tissues when examined by immunofluorescence. There was a clear relationship between immunoglobulin dose and the development of late neurologic disease.

It is important to emphasize that the neurologic phenomenon was distinct from the hemorrhagic disease. The neurologic signs of illness appeared abruptly and the monkeys died within 4-5 days, or recovered. There were none of the typical hemorrhagic signs in the controls as described previously for Bolivian hemorrhagic fever in monkeys (3,4), and the pattern of acute neurological deaths were clinically dissimilar from the chronic neurological sequellae of the disease in monkeys (3,4).

Despite this complicating aspect of an immunologic imbalance resulting from passive antibody protection, we found that an experimental, inactivated, cellculture grown, BHF vaccine was safe and efficacious for monkeys. Data in Fig. 1 show the antibody responses of monkeys given inactivated BHF vaccine and challenged 3 weeks later with Machupo virus. The monkeys to be vaccinated were divided into two groups: one received 0.3 ml of vaccine and the second received 3 ml. Following the single dose of vaccine the monkeys were challenged 3 weeks later. Both groups of monkeys mounted relatively brisk antibody responses following challenge, despite the low levels or absence of detectable antibody prior to challenge. These responses should be compared to antibody patterns of control monkeys which may occasionally survive long enough to develop neutralizing antibodies (4). Antibodies in unvaccinated monkeys do not usually appear until between days 21 and 28 after virus challenge. Only one of the monkeys in this experiment became ill. It was viremic through day 17 of the study and did not develop antibody until day 21. The monkeys died 38 days after challenge.

These data suggest that immunity against hemorrhagic disease can develop in the absence of detectable neutralizing antibody and that an inactivated vaccine can induce an effective immune response. Although one of the monkeys suffered a relatively late death with neurologic manifestations, his overall disease course was not particularly different from that of approximately 20% of Machupo virus-infected control monkeys as reported previously (4), and shown in Table 1.

DEVELOPMENT OF AN EBOLA VIRUS VACCINE

If it is possible to immunize against arenavirus hemorrhagic fevers with inactivated vaccines, it would seem feasible to immunize also against Ebola or Marburg viruses with specific inactivated immunogens. The major problem would be to find the appropriate combination of virus strain and acceptable substrate to yield a sufficient concentration of viral antigen. Our data with inactivated Venezuelan encephalitis vaccine 5 indicate that a concentration of approximately 9.5 log 10 of alphavirus virions per milliliter may be required if an inactivated vaccine is to induce an adequate antibody response to an inactivated viral product. Nevertheless, lower concentrations may be effective through the use of repeated inoculations. Electronmicrographs of Vero cells infected with Ebola virus (6) suggest a large amount of viral antigen production. It would therefore seem feasible to obtain adequate antigen concentrations approaching immunogenic levels in substrates acceptable for vaccine use.

The scheme shown in Figure 2 describes the general procedures that might be used to prepare an inactivated Ebola virus vaccine. The substrate for this would be selected from among the limited number available, i.e., diploid fetal human lung, diploid fetal rhesus lung, diploid fetal cercopithecus lung or perhaps primary chick embryo cells. Most of the procedures shown are standard 5 with respect to those outlined in referenced vaccine production methods Although Figure 2 indicates the use of formalin as an inactivating agent, this may not be a realistic choice. Betapropiolactone might be used, but the recent development of the psoralen compounds for vaccine inactivation holds considerable promise (7). In summary, we believe that the production of limited quantities of an inactivated vaccine against either Marburg or Ebola viruses is a distinct, realistic possibility within the next 2 or 3 years. While the difficulty of preparing a sufficiently potent product in an acceptable cell culture system may prove to be a formidable obstacle, the preliminary indications suggest that it will not be impossible.
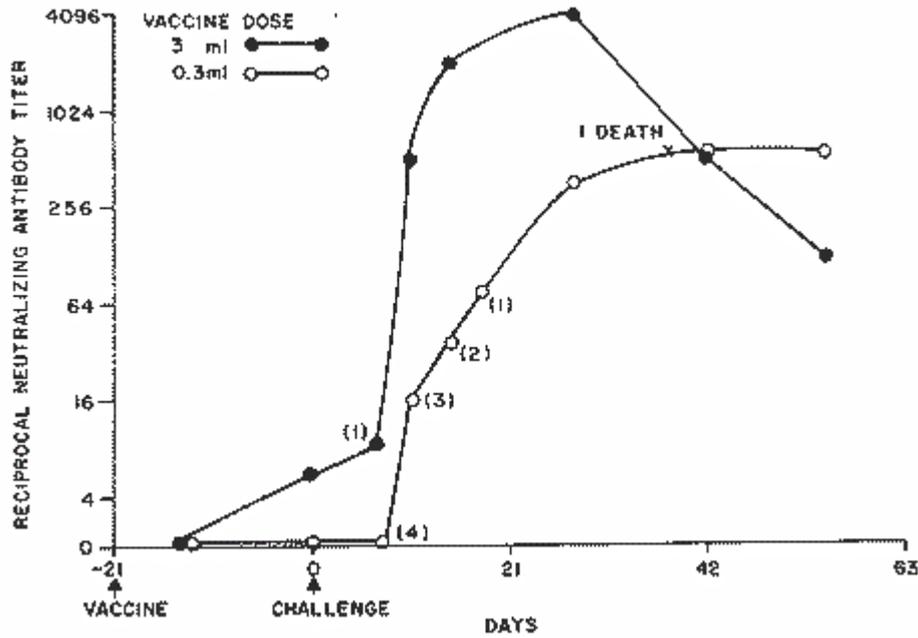
fig. 1 : Monkeys inoculated with one dose of inactivated BHF vaccine were challenged with 1000 PFU of Machupo virus 21 days later (day 0). The points indicate the geometric mean antibody response for groups receicing either 3 ml or 0.3 ml. Numbers in parentheses indicate the number viremic (n=4 per group) on the day tested.
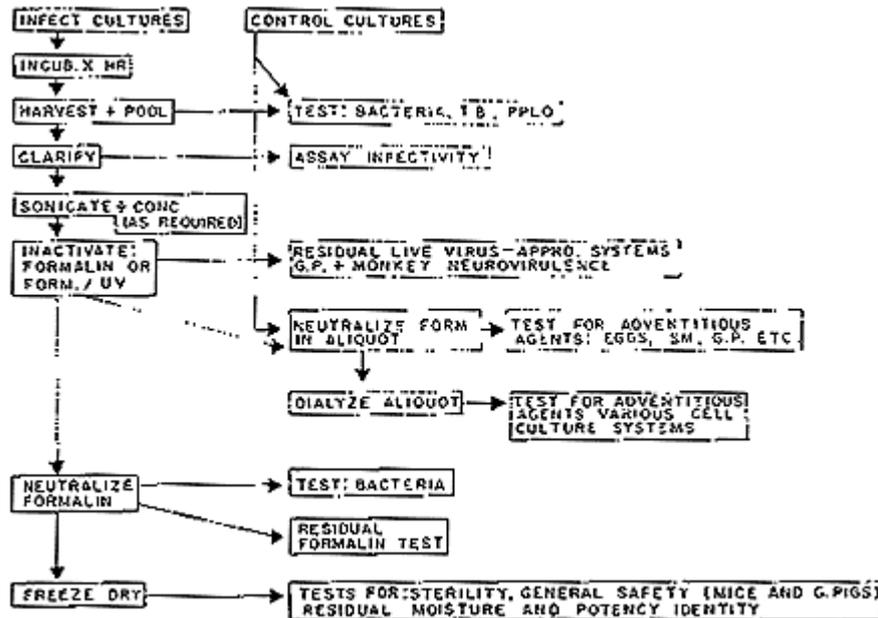
fig. 2 : A projected plan for the production of an experimental lot of Ebola vaccine. This plan assumes that the virus seed stock has been characterized and certified and that an acceptable cell culture substrate has been selected.

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DISCUSSION

A.W. Woodruff : I think that it is important that this meeting expresses concern at the lack of more effort along the lines for producing a vaccine against these infections. It is now 9 years nearly since Lassa virus was isolated. It 's been constantly causing trouble to public health authorities, physicians and health authorities generally and we still have no possible sight of a vaccine.

R.A. Coutinho : who should be vaccinated and why ?

G.A. Eddy : in the first place, laboratory personnel working with these viruses. With respect to Lassa, for instance, there is a real need for imunizing medical personnel in hospitals in the field where there is a great risk of acquiring this infection. And finally, for eventual national emergencies.

J. Casals : I want to make some comments on the use of immune plasma in the treatment of these hemorrhagic fevers. As Dr. Russeb mentioned, first of all we have to know whether it helps or not. Then whether it does any harm. In the case of Lassa fever, up to a certain point we knew how many people had been given the plasma and out of 5 or 6 one followed a course which was harmful. The plasma was given very late in the course of that disease but at the same time came from an individual who, for all we know, may have had virus in his blood. Another question that has been raised too is when to give this plasma . It seems to me there is going to be a lot of wastage. In the case of Lassa, it has been given almost to as many people who needed it as to people who just had a sore throat and simply because they had been in Sierra Leone or Nigeria, created a panic. Faced with a very acutely ill patient, is plasma to be given or not ? We hear a lot of immune complexing and the damage that it can produce. Is there any evidence that following the administration of plasma either in Argentinian haemorrhagic fever, where practically 300 individuals were treated with it, or in Lassa or in Ebola fever ?

J.I. Maiztegui : In Argentina in a period of about twenty years, the endemoepidemic area has extended so much that today it covers over 100.000 km2 with a population at risk of approximately 1.200.000 people. To our present knowledge, the ecology of the reservoir is such that the only possibility for control is the development of a vaccine. In Argentina the first research priority is the development of a vaccine. It was mentioned that clone 3 had been successfully used in 636 volunteers, however I think that the follow-up of these people was not well conducted. It was also said that laboratory workers who received this experimental vaccine were protected. Let me remind you that in the laboratory where this was done, people were handling mainly the attenuated strain and not pathogenic strains, of Junin virus so that this is not conclusive evidence. As to the convalescent plasma, we do not have any evidence of immune complexes in AHF. More than 10 autopsy cases were specifically investigated for this with negative results. Of the 18.000 reported cases of AHF, 60-70 % were confirmed thus there are 10.000 to 12.000 proven cases of AHF. Probably 6.000 or 7.000 of these received convalescent plasma in the acute stage of the disease. In my personal experience, I have never had an acute accident. We did a controlled trial that took four years and will be published shortly, which shows that plasma we gave 500 ml, is indeed useful. We are also concerned about the conservation of convalescent plasma and the search for better preparations. However, I would not dismiss so quickly the possibility that whole plasma is useful. Dr. Johnson raised the question of its action mechanism. It may seem that the active principle of the plasma are the antibodies. However I want to remember that in lymphocytic choriomeningitis the neutralizing antibodies in the acute stage are complement dependent. Some of the physicians in

Junin are convinced that whole plasma is superior to gaumaglobuines. I do not know if the analogy with Ebola is valid but convalescent plasma in AHF is useful. A last comment, I would strongly recommend to give convalescent plasma to anybody who has to work in the laboratory with this virus or has to handle patients with this disease.

THE EFFECT OF INTERFERON ON EXPERIMENTAL EBOLA VIRUS INFECTION IN RHESUS MONKEYS

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INTRODUCTION

The potential use of interferon preparations for the treatment of viral infections is receiving increasing attention, for example for the treatment of recurrent herpes keratitis, infection with herpes viruses in immunosuppressed patients and more recently for persistent infection with hepatitis B virus. Infection with Marburg or with Ebola or with Lassa viruses presents an acute lifethreatening situation. The case of a patient in London with Ebola virus infection who recovered after treatment with human leucocyte interferon and convalescent serum (1) stressed the need to investigate the effect of interferon on the course of this infection in an experimental nonhuman primate model.

The properties and mode of action of interferon. Interferons are small molecular weight glycoproteins which inhibit the replication of a wide range of animal viruses and are released from animal cells after induction. Interferons differ from other less specific inhibitors on the basis of the following criteria: their activity against many unrelated viruses, they are usually species specific but without tissue specificity, interferons are not toxic to cells and act via an intracellular mechanism requiring functional host cell metabolism and finally interferons are usually stable at pH 2. Interferons are not antigenic in the homologous species but specific antibodies can be obtained by immunization of heterologous hosts.

The chemical structure of interferons is not known but all contain polysaccharide residues, although the latter do not play a measurable role in antiviral activity. The human leucocyte interferon is a stable molecule known to contain at least one disulphide bond.

Interferon does not prevent adsorption or cell penetration by the virus but blocks viral replication at some point during assembly. The primary site of action of interferon appears to be on the cell membrane where it interacts with an interferon specific receptor system. The receptor is near the thyrotrophin, gonadotrophin, cholera and diphtheria toxin receptors, and in man it may be controlled by chromosome 21. The receptor system consists of a binding site made of gangliosides and an activator site consisting probably of glycoproteins. An interferon specific modification of the cell membrane components is required as a primary step for antiviral activity. The cells produce an inhibitory protein and other products including a protein kinase, a nuclease and other proteins. The inhibitory protein apparently interferes with the translation of viral mRNA at the ribosome inhibiting the synthesis of virus specific protein.

In addition to the antiviral effect, interferon preparations can decrease cell replication and may inhibit tumour growth, inhibit the replication of intracellular organisms such as rickettsia and protozoa, alter the sensitivity of cells to hormones and toxins, inhibit humoral antibody formation and cell mediated immunity but may enhance phagocytosis and cytotoxicity of T-lymphocytes. Some of these effects may be due to biologically active impurities in the interferon preparations.

Simply stated, therefore, the interferon system is part of the normal host defence against virus infection. Interferon is synthesized in response to infection and actively secreted from the cells. When interferon reacts with the membrane of uninfected cells an antiviral state is activated within the cells. All these properties make the clinical application of interferon particularly attractive for prophylaxis or for treatment early in the course of infection, although it may be effective therapeutically when the pathogenesis of the disease is dependent on slow and continued replication of the virus.

EXPERIMENTAL DESIGN

Animals. These were young adults rhesus monkeys (Macacca mulatta) of either sex weighing between 3 and 4 kgms. The monkeys were anaesthetised by intramuscular injection of ketamine hydrochloride for sampling of blood and for inoculation. Rectal temperature was recorded daily.

Virus inoculum. The virus was the prototype strain E 718 isolated from the patient who died in Zaire from Ebola haemorrhagic fever during the epidemic in 1976. The virus was passaged by intraperitoneal inoculation into DunkinHartley guinea pigs weighing 250 gms. The inoculum was prepared as a 10% suspension in phosphate-buffered saline containing 0.75% bovine albumin from a guinea pig liver at the third passage of the virus. The suspension was diluted and the dose of virus was calculated by titration in guinea pigs and expressed as guinea pig infective units. The monkeys received 10^4 guinea pig infective units in 0.4 ml by intraperitoneal inoculation.

Human leucocyte interferon. Interferon was produced by induction with Sendai virus of leucocytes in suspension culture obtained from buffy coats of human blood donations at the Central Public Health Laboratory, Helsinki. The specific activity of the final preparation was $2x10^6$ units/mg protein. The monkeys received $3x10^6$ units of interferon intramuscularly once daily.

Treatment schedule. Two of the rhesus monkeys received interferon for 2 days before infection and the treatment was continued daily thereafter. Two monkeys were inoculated with the virus suspension and interferon was administered 1 hr later and daily thereafter. The third group of monkeys was given interferon when fever developed on the third day after inoculation with the virus. A group of 3 other rhesus monkeys was inoculated with the virus only and these served as controls.

Sampling of blood and tissues. Samples of blood were obtained daily by femoral venepuncture. Biopsies were not carried out during life in view of the risk of uncontrolled haemorrhage. Autopsy was carried out on all monkeys shortly after death. Portions of the following organs were removed for examination: lungs, heart, liver, spleen, adrenals, kidneys, intestinal tract, mesenteric lymph nodes and testes. Bile and faeces were also collected for virus titration.

A 10% suspension of each tissue, bile and faeces was prepared for virus titration in guinea pigs.

Tissues were also fixed in 10% buffered neutral formalin and embedded in paraffine wax. Sections cut at 5 microm and stained with haematoxylin and eosin. Selected sections were stained by Verhoeff-van Gieson method, by Machiavello's technique for inclusion bodies, by the periodic acid - Schiff and Gordon and Sweet's methods and with Mallory's phosphotungstic acid.

RESULTS

Clinical observations. There were no marked differences in the clinical course of infection between the monkeys treated with interferon and the controls. Yet an impression was obtained that in the interferon treated group the introduction of measures to combat disseminated intravascular coagulation, such as anticoagulant therapy and replacement of platelets and coagulation factors and fluid replacement may have prolonged survival. The monkeys became febrile on or about the third day after infection and the pyrexia persisted until death. Maculo-papular skin rashes with petechiae developed in all the animals on the 4th or 5th day and remained until death, although in 3 of the monkeys treated with interferon the rash was fading by the 6th or 7th day. The time of death of the monkeys in the different groups is shown in Table 1, and it is noted that survival may have been somewhat prolonged in 3 of the animals treated with interferon.

TABLE 1 DAY ON WHICH DEATH OCCURRED

Day	Controls	Interferon -2 days	+1 hr	+3 days
5	+			
6	+	+		
7	+			++
8		+	+	
9			+	

TABLE 2 TITRE OF EBOLA VIRUS IN BLOOD (Log 10/ml)

Time in days after inoculation

Rhesus Monkeys	1	2	3	4	5	6	7	8
Controls	<0.5	2.5	5;4.5	5.5	4.5	-		
	<0.5	2.5	54.5	6.5	5.5	5.5		
	<0.5	2.5	4.0	5.0	5.0	6.5		
-2 Days	<0.5	<0.5	3.5	5.5	5.5	6.5	lte 6.5	
	<0.5	<0.5	1.5	5.5	6.5	7.5	-	
Interferon +1 Hr	<0.5	<0.5	1.5	3.5	2.5	3.5	6.5	6.5
	<0.5	2.5	3.5	6.5	2.5	3.5	5.5	-
+3 Days	<0.5	2.5	4.0	2.5	6.5	6.5		
	<0.5	1.5	5.0	6.5	6.0	6.5		

Viraemia . The titre of Ebola virus in the blood samples collected daily from each monkey was calculated by parallel titration in guinea pigs. The titres are shown in Table 2. Viraemia was not detected on the second day after infection in 3 out of 4 monkeys treated with interferon, whereas viraemia was evident in the 3 control monkeys and in the 2 monkeys treated with interferon 3 days after infection.

Findings at autopsy. Petechial and maculo-papular skin rashes of variable severity were present involving the forehead, face and cheecks, the medial aspects of the limbs and the chest. Visceral petechiae were present in all the animals. Peritonitis was evident -in-mo-st monkeys and intestinal haemorrhages and mucosal ulceration were found in many of the animals. The mesenteric lymph nodes were enlarged and haemorrhagic. The liver was pale and mottled. Petechiae and small haemorrhages were found on the pleural and cut surface of the lungs. Congestion and inflammatory changes were found in the tunica vaginalis and tunica albuginea of the testes.

There were no differences between the gross pathological findings between the treated and untreated animals.

The histological lesions in rhesus monkeys infected with Ebola virus have been described in detail elsewhere (2). Briefly, the findings in the present series were numerous small foci of parenchymal cell necrosis distributed randomly throughout the lobules of the liver. Areas of coagulative necrosis of variable size were common in some of the animals. Single or multiple intracytoplasmic inclusion bodies ranging in size from 5-25 microm were found in hepatocytes. These inclusion bodies stained a bright red colour by Machiavello's method. In the more severely affected livers, thrombosis occurred in some of the central and portal veins. There was little cellular infiltration in the portal tracts. Subcapsular haemorrhages were present. Necrosis of lymphoid follicles and depletion of lymphocytes, which were widespread in some monkeys, were found in the spleen and lymph nodes. Multiple small haemorrhages were present in the medulla and cortex of the adrenal glands. The kidneys were severely affected with widespread haemorrhages, fibrin thrombi in the glomeruli and tubules and cellular and fibrin casts were present in the tubules and collecting ducts. Cellular infiltration was absent. The histological changes in the intestines ranged from congestion and focal haemorrhages in the submucosa and serosa to severe haemorrhagic necrotising enteris with necrosis of Peyer's patches. The lesions in the lungs varied in their severity and included thrombosis of vessels, small foci of necrosis of the alveolar septae, oedema, intra-alveolar haemorrhages and areas of coagulative necrosis. The histological changes in the testes consisted of haemorrhages between seminiferous tubules and acute inflammatory changes with necrosis in the tunica vaginalis and tunical albuginea.

There were no consistent differences in the nature or severity of the lesions between the monkeys treated with interferon and the untreated animals.

DISCUSSION

Vaccines against Marburg, Ebola and Lassa viruses have not yet been developed and there is no specific therapy for these serious life-threatening infections. The interferons and antiviral chemotherapy should, therefore, be explored as an alternative to immunization, even for limited field application in any future localised outbreaks. Bowen, et al. have shown (3) the illness induced in rhesus

and vervet monkeys by infection with the Zaire strain of Ebola virus was very similar to the disease which occurs in man. Our preliminary findings with a small number of rhesus monkeys treated prophylactically with human leucocyte interferon and at the time of infection are not discouraging. Clinically, survival appeared to be enhanced and an impression was gained that life-support measures to combat disseminated intravascular coagulation and fluid replacement in the animals treated with interferon would have favoured recovery. Viraemia was delayed and there is obviously a need to establish the optimal dose and duration of treatment with interferon either prophylactically or early in the course of the infection. Combination therapy with infusion of convalescent serum (when available) is also being explored in the experimental rhesus monkey model.

At present, the availability of interferon is very limited. However, several chemical inducers of interferon have been investigated. Polyinosinic polycytidylic acid (Poly 1. Poly C), a double-stranded RNA, is a potent inducer of interferon in mice and has been found to be effective against various virus infections both prophylactically and therapeutically. It is, however, a poor inducer of interferon in man and in nonhuman primates and it does not induce any detectable serum interferon. This may be related to the presence of an enzyme in the serum of primates which hydrolyses and inactivates Poly I. Poly C.

Poly I. Poly C has been complexed with poly l-lysine and carboxymethyl cellulose (Poly. ICLC) by Levy, et al. (4). This stabilised derivative is partially resistant to hydrolysis by primate serum and induces the formation of several thousand units of interferon per ml. of serum in primates. This compound has been tested in non-human primates against several serious virus diseases. Thus, Poly. ICLC was effective prophylactically in simian haemorrhagic fever. When this compound was given to monkeys 6 hours after a large inoculum of yellow fever virus, about 75% of the treated animals survived and developed good serum antibody titres, in contrast to deaths of all the untreated monkeys (5). The use of Poly. ICLC in experimental Ebola virus infection in nonhuman primates is being explored, since it can induce high levels of serum interferon with few major toxic side-effects.

Finally, there is an urgent need to investigate the use of chemical antiviral agents both for the prophylaxis and treatment of these serious virus diseases. For example, ribavirin, a synthetic nucleoside analogue of guanosine, has been shown to be active in vitro against a wide range of both DNA and RNA viruses. Bivavirin appears to be particularly effective in vivo against influenza A and B and parainfluenza, all RNA viruses, and studies of such antiviral drugs in experimental Ebola virus infection are being planned.

SUMMARY

Rhesus monkeys were treated with human leucocyte interferon prophylactically and after experimental infection with the Zaire strain of Ebola virus. Viraemia was delayed and clinically survival appeared to be enhanced. There were no consistent differences in the pathological changes and the outcome of the infection beween the animals treated with interferon and those not receiving interferon. Nevertheless, there are clear indications for further investigation on the use of interferon alone and in combination with serotherapy, and for the use of interferon inducers.

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DISCUSSION

G.A. Eddy : I would like to ask Dr. Zuckerman if he measured the titers of interferon that resulted following inoculation in these monkeys. I would also like to point out our experience using

interferon inducer Poly 1:c which was a total failure. In our control monkeys inoculated with Machupo virus we found that the untreated monkeys which received no interferon inducer developed high titers of interferon on about day three or four, which is about four days prior to the onset of viremia or illness. This rather discouraged us from pursuing interferon with respect to Arena viruses. There is obviously plenty of interferon before the monkeys were even sick or had viremia.

A.J. Zuckerman : The levels of interferon in these monkeys are being assayed at the moment. As far as your second comment is concerned, you are right but there may be a very important difference between an endogenous and an exogenous interferon. We are trying to differentiate between the two, because we suspect that in the rhesus monkeys after infection interferon is produced anyway but the critical question is whether we measure endogenous or exogenous.

J. De Smyter : I don't think it will make much sense to administer interferon to an animal or to a person with circulating interferon. Thus it will be very important to know this spontaneous titer of interferon in this disease in monkeys. This still does not mean that exogenous interferon would be useless. Perhaps the disease could be avoided by giving interferon or an inducer after exposure i.e. after an accident like that which occurred in Porton Down. As far as there is a possible comparison with rabies, in this infection it is possible in monkeys to stop the infection if interferon or an inducer are given immediately after exposure, the production of interferon may even be the mechanism of action of post-exposure vaccine in rabies. So there could be a useful post-exposure immediate administration of interferon.

A.J. Zuckerman : I agree with you entirely. What we are trying to do in fact in this experiment is to mimic conditions in the field, one group has preexposure prophylaxis, the second immediate post-exposure prophylaxis and the third is comparable to a patient with a febrile illness. The impression was gained that if interferon is going to be used at all, it has to be used either prophylactically or immediately after exposure rather than after the full symptoms have developed.

GROWTH OF LASSA AND EBOLA VIRUSES IN DIFFERENT CELL LINES

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SUMMARY

Twenty two cell lines - VH 2, 8625, IgH-2, A6 TH-1, ICR-2A, Tb-1Lu, Calomys, BHK, PtK 1, PtK 2, *Aedes pseudoscutellaris*, *A. albopictus*, *A. aegypti*, Indian muntjac, PK 15, MOCK (ATCC), Vero, Rd and FHM - were infected with Ebola virus and Lassa virus.

In the reptile, amphibian, fish and Aedes cell lines tested no multiplication of both viruses was observed, except for Lassa where a very weak positive IFA occurred in both rattle snake and viper spleen cell lines.

For Lassa, cytopathic effect was obtained in Indian muntjac and Vero cells. In 8625, VSW, BHK, PtK 2, PK 15 and RD, Lassa could only be detected by indirect fluorescence antibody test (IFA). For Ebola cytopathic effect occurred in bat, marsupial, deer, dog monkey and human cell lines. In rat, marsupial (PtK 2) and pig cell lines Ebola was only detected by IFA.

Ebola virus shows a broader cell line susceptibility than Lassa.

INTRODUCTION

Until now only African green monkey kidney cell line (Vero) was used to isolate and assay Lassa (4) and Ebola viruses (1,2,3). The present study intends to find a more sensitive assay system in cell culture for Lassa and Ebola viruses.

MATERIALS AND MIETHODS

Virus. Lassa virus, Josiah strain pool 800593, second passage Vero 98, TCD 50/ml = 10^7 and Ebola virus Mayinga strain, pool 800590, second passage on Vero 98, TCD 50/ml on Vero = 10^6.6 were used. All work with Lassa and Ebola was carried out in CDC's maximum security laboratory.

Cell cultures. Details regarding the twenty two cell lines used are given in Table 1. Stock cultures were grown in 150 cm2 plastic tissue culture flask. For experiments tube cultures were inoculated.

TABLE I
VERTEBRATE AND INVERTEBRATE CELL LINES USED IN THE STUDY

Cell line	Obtained from ATCC (1)	Culture medium	Temp. of incubation (ºC)
1. VH-2	CCL 140	BME=NEAA in Hanks BBS (2)	+ FBS 10% Room
2. 8625	Collins lab	MEM (double conc.AA and Vitam)	+ CS 10% Room
3. VSW	CCL 129	BME (2)	+ FBS 10% Room
4. IgH-2	CCL 108	BME+NEAA in Hanks BSS	+ FBS 10% 36 ± 1
5. A 6	CCL 102	NCTC 109 (3)	+ FBS 15% Room
6. TH-1	CCL 50	BME	+ FBS 10% Room
7. ICR-2A	CCL 145	Leibovitz's L-15 (2)	+ FBS 10% Room
8. Tb-1 Lu	CCL 88	MEM+NEAA+sod.pyruvate+ Earles BSS+ HCO 3 (0.85g/1)	+ FBS 10% 36 + 1
9. Calomys		MEM+NEAA+sod.pyruvate+ Earles BSS+ HCO 3 (0.85g/1)	+ CS 10% 36 ± 1
10. BHK	Dr.Taylor	(CDC) MEM+NEAA+sod.pyruvate+ Earles BSS+ HCO 3 (0.85g/1)	+ FCS 10% 36 ± 1
11. PtK 1	CCL 35	MEM+NEAA+sod.pyruvate+ Earles BSS+ HCO 3 (0.85g/1)	+ NCS 10% 36 ± 1
12. PtK 2	CCL 56	MEM+NEAA+sod.pyruvate+ Earles BSS+ HCO 3 (0.85g/1)	+ FCS 10% 36 ± 1
13. A. pseudoscutellaris		Mitsuhashi & Maramorosch (2)	+ FBS 20% Room
14. A. albopictus	CCL 126	Mitsuhashi & Maramorosch	+ FBS 20% Room
15. A. aegypti	CCL 125	Mitsuhashi & Maramorosch	+ FBS 20% Room
16. Indian muntjac	CCL 157 Ham's F 10 (3)	+ FBS 20% 36 ± 1	
17. PK 15	CCL 33	MEM+NEAA+sod.pyruvate+ Earles BSS+ HCO3- (0.85g/1)	+ NCS 5% 36 ± 1
18. MDCL	Pfau	MEM+NEAA+sod.pyruvate	+ CS 10% 36 ± 1
19. MDCK	CCL 34	Lactalbumin hydrolysate (0.5%)+Earles BSS+ HC03- (0.85g/1)	+ NCS5% 36 ± 1
20. VERO 98	CDC	MEM	+ CS 10% 36 + 1
21. RD	Dr.J.Esposito (CDC)	MEM+double conc.AA+vit+ Hanks BBS	+ FBS 10% 36 + 1
22. FHM	CCL 42	MEM	+ CS 10% 36 + 1

1. ATCC American tissue culture Collection with corresponding tissue culture number ced salt solution; Abbreviations used BME: Basal medium Eagle; NEAA: Non Essential amino acids; BSS: Balan MEM: Minimum Essential Medium; FBS: Foetal bovine serum; CS: Calf serum; NCS: New born calf serum; FCS: Foetal calf serum.
2. Purchased commercially by GIBCO
3. Purchased commercially by microbiological Associates

Infection of cell lines. Supernatants of cultures growing in monolayers were decanted and the tubes refreshed with maintenance media (= culture media with 2% serum). For Aedes pseudoscutellares Laibovitz L-15 was used as a maintenance medium.

Three tubes were each inoculated with 0.1 ml 10 $^{-1}$ and 10^{-3} dilutions (in maintenance medium) of Ebola and Lassa, for each cell line investigated. Each time Vero cells were inoculated as controls. Infected cells were investigated daily on occurrence of CPE and on the 5th and 14th day cells were screened on presence of antigen by indirect fluorescence antibody test (IFA). Cells were scraped off and suspended in 0.2 ml supernatant. After addition of 0.2 ml phosphate buffered saline a mutispot slide is filled with cell suspension drops.

After drying and fixation in aceton (10') IFA is done as previously described (5).

RESULTS AND DISCUSSION

Results are summarized in Table 2.

Reptile, amphibian, fish and *Aedes* cell lines supported no multiplication of both viruses except for Lassa where a very weak positive IFA occurred in both rattlesnake and viper spleen cell lines.

With Ebola a cytopathic effect occurred in Vero 98 (395), PtK 1 (3,7), Tb I Lu (3,10), MOCK (ATCC) (7,10), Indian muntjac (10,11) RD (8,13) and MOCK (10, no CPE). Figures between brackets indicate first appearance (days post inoculation) of CPE in 10^{-1} and 10^{-3} inoculated cell cultures. The CPE with Tb 1 Lu was very pronounced and the easiest to observe.

With Lassa a cytopathic effect occurs only in Vero 98 (4,4) and Indian muntjac (10,12) cell lines.

The ease in detection of Lassa antigen by IFA decreased in the following order: RD, Vero 98, PK 15, BHK, Indian muntjac, PtK 2. With RD cells, very bright fluorescence occurred in the presence of Lassa antigen.

Ebola antigen detection by IFA was the best in Vero 98 and decreased in the following order: PK 15, RD, Calomys, MOCK (ATCC) Tb 1 Lu. PtK 1, Indian muntjac, PtK 2, MDCK (pfau), BHK.

Finally we can state that for both viruses Vero 98 is still the most sensitive cell line. The characteristic CPE in Tb 1 Lu for Ebola and the pronounced fluorescence in RD cells with the IFA test for Lassa can be helpful.

ACKNOWLEDGEMENTS

TABLE 2

CYTOPATHIC EFFECT AND POSITIVE IFA OF LASSA AND EBOLA IN DIFFERENT CELL-LINES

Cell-Lines		Ebola		Lassa	
		-1	-3	-1	-3
1. VH-2	Viper	-	-	-	-
2. 8625	Rattlesnake	-	-	C1O F7	-
3. VSW	Viper spleen	-	-	F14	
4. IgH-2	Iguana	-	-	-	-
5. A6	Toad	-	-	-	-
6. TH-1	Turtle	-	-	-	-
7. ICR-2A	Frog	-	-	-	-
8. Tb-lLu	Bat	CF4	-	-	-
9. Calomys	Rat	F5	F14	-	-
10. BHK	Hamster	F10	-	F10	F10
11. PtK1	Marsupal	CF5	-	-	-
12. PtK2	Marsupal	F14	-	F4	
13. Aedes ps		-	-	-	-
14. Aedes albopictus	-	-	-	-	
15. Aedes aegypti	-	-	-	-	
16. Indian muntiac	Deer	C10 F5	-	CF14	CF14
17. PK15	Pig	F8	F8	F6	F6
18. MOCK (pfau)	Dog	CF14	-	-	-
19. MOCK (ATCC)	Dog	CF6	CF14		
20. Vero 98	Monkey	CF5	CF5	CF5	CF5
21. RD	Human	C7F4	CF14	F4	F4
22. FHM	Fish	-	-	-	-

CF cytopathic effect and positive FA on indicated day. C only cytopathic effect on day tested. F. positive FA on day tested. negative result.

If at the indicated day FA is positive and CPE occurs later, then the first day at which CPE occurs, is indicated as the number behind C. f.e. ClOF5 : at day 5 FA was positive and CPE occurred on the 10th day.

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ATTEMPTS TO CLASSIFY UNGROUPED ARBOVIRUSES BY ELECTRON MICROSCOPY

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It is a general biological rule that each morphological virus group contains numerous serotypes. Therefore the Marburg virus group for which at present only two different viruses are known: Marburg and Ebola, may be expected to contain more viruses with the same morphology.

There is a possibility that such viruses may be present among the "ungrouped" arboviruses. Indeed numerous viruses are isolated in arbovirus laboratories all over the world. The general procedure is isolation of virus by intra-cerebral inoculation of newborn mice, after which the isolate is tested for ether and/or desoxycholate sensitivity and a haemagglutinin prepared. If haemagglutinin is not present or if antigenic relationship with known arboviruses is not detected, the new virus is classified as "ungrouped". The morphology of such isolates is generally not studied. There is possibility that other members of the MarburgEbola virus group might be present among ungrouped arboviruses. We therefore started a study of the morphology of some of these viruses. Because there is a definite advantage to work with tissue culture harvests, all viruses were also inoculated into Vero cells. Preliminary results are given in the present paper.

MATERIAL AND METHODS

Viruses. Ungrouped arboviruses listed in table 1 not producing a haemagglutinin were investigated.

All viruses were received in the freeze dried state, except Tettnang which was a frozen mouse brain suspension. Upon receipt the viruses were rehydrated and inoculated intra-cerebrally into newborn mice and in Vero cells.

ELECTRON MICROSCOPY

a. Floating method. A carbon coated grid was deposited on a drop of virus sus pension for one minute, the grid was then blotted with paper, floated on a drop of stain for 3 to 5 seconds and blotted again. The following stains have been used: phosphotungstic acid at 0.5, 1 and 2%, pH ranging from 6.4 to 7.1 and uranyl-acetate 0.5, 1 and 2%.

An alternative technique is to put a drop of virus suspension on a grid, let it dry and float the grid on a drop of stain for 30 seconds and finally blot it with filter paper.

b. Immune-electron microscopy. The method used is a modification of the one described by Anderson and Doane I for identification of rotaviruses. Serum-agar mixtures containing noble agar 1% in phosphate buffered saline and antisera diluted 1/50 or 1/100 depending upon their neutralization titers are pipetted into disposable microtitre plates, (sealed with sterile adhesive tape, these can be stored at 4° until use). A drop of the virus suspension to be examined is put on the agar containing homologous antiserum. A grid is floated on it and incubated at 37ºC for 80 minutes. Grids are then removed and floated for 3 seconds on 2% P.T.A. pH 6.8. Both pialoform and carbon coated grids were used.

c. Pseudo-replica technique. The method used is a modification from Scharp (2) Agarose 2% is prepared in petridishes. A block of about one square cm is cut and mounted on the edge of a glass slide. A drop of virus suspension is put on the surface of the agarose and allowed to dialyze and evaporate until the surface is completely dry, this usually takes about 10 minutes. Two or three drops may be applied. Two drops of formvar are then added and left for a few seconds and excess formvar is removed by keeping the slide vertically against blotting paper. When the film is dry, the edges of the block are cut with a sharp knife. By dipping the block gently into stain, a film is released. A grid mounted on a metal rod is pushed onto the floating film, through the stain and turned out of it. Excess film and stain are removed by rotating the edge of the rod against a blotting paper. Uranylacetate in 0.5 and 2% concentrations and P.T.A. in concentrations of 2 to 4% pH 6.8 were used. Electron microscopic observations were done at an accelerating voltage of 80v.

TABLE 1 UNGROUPED ARBOVIRUSES INVESTIGATED

Name	Abrv.	Isolation	Origin	Mice	Vero	EM
Bangoran	BGN	Culex	Dakar	+	-	-
Bangui	BGI	Man	Dakar	+	-	
Bobaya	BOB	Trudus	Dakar	+	+	+
Bimbo	BBO	Euplectus	Dakar	+	-	-
Gomoka	GOM	Anopheles	Dakar	+	-	-
Gossas	GOS	Tadaria	Dakar	+	-	
Kannamangalam	KAN	Bird	Yaru	+	-	-
Keuraliba	KEU	Tetara Kempi	Yaru	+	-	-
Kolongo	KOL	Euplectus	Yaru	+	-	
Kowanyama	KOX	Anopheles	Yaru	+	-	-
Landjia	LJA	Bird	Dakar	+	-	-
Le Dantec	LD	Man	Yaru	+	-	-
Minnal	MIN	Culex	Yaru	+	-	
Ouango	OUA	Bird	Yaru	+	-	-
Oubangui	OUB	Culex	Dakar	+	-	-
Salanga	SAL	Athomys M.	Dakar	+	-	-
Sandjimba	SJA	Bird	Dakar	+	-	-
Sebokele	SEB	Tick	Dakar	+	-	
Tanga	TAN	Anopheles	Yaru	+	-	
Tataguine	TAT	Culex	Yaru	+	-	
Tembe	TME	Anopheles	Yaru	+	-	-
Thottapalayam	TPM	Shrew	Yaru	+	-	-
Toure	TOU	Tetara Kempi	Yaru	+	-	-
Trinity	TNT	Mosquito	Yaru	+	+	+
Upolu	UPO	Bird	Yaru	+	-	-
Wongorr	WGR	Aedes sp.	Yaru	+	-	
Yata	YATA	Mansonia	Yaru	+	-	+
Yogue	YOG	Bat	Yaru	+	-	-
Zinga	ZGA	Mansonia	Dakar	+	+	+
An B 4268			Dakar	+	+	-
An Br 1398d			Dakar	+	-	-
An B 4289			Dakar	+	-	-
An Y 1444			Dakar	+	-	-

TABLE 2 COMPARISON OF EM TECHNIQUES ON A SELECTED SAMPLE OF VIRUSES

Virus Tested	Titre TCD 50ml	Floating Method	Serum-In-Agar	Pseudo-replication
Bobaya (TC)	10^6	-	-	+
Zinga (TC)	10^5.7	-		+
Trinity (TC)	10^7	-		+
Yata (MB) -		+		
Keuraliba (MB)		+		+
VSV (TC)	10^6.7	+		+
MB (TC)	10^3.5	-	+	+
Chik. (TC)	10^6	-	+	+
SF. (TC)	10^6.2	-	+	+

SI. (TC)	10^6.7	-	+	+
YF. (MB)	10^5.2	-	+	+
WN. (MB)	10^5	-	+	+
BUN. (TC)	10^7.2	-	+	+
Polio 1 (TC)	10^4.7	-	+	+
Polio 3 (TC)	10^5.7	-	+	+

TC = tissue culture harvest

MB = mouse brain harvest

2. Results of Electron Microscopy.

1. Ungrouped Arboviruses producing CPE in Vero cells. Table 2 shows the viruses producing CPE in Vero cells. One virus, Trinity, did not produce CPE when the hydrated freeze dried virus was inoculated. A newborn mouse brain harvest however, inoculated into Vero cells produced CPE and the virus could be repeatedly passed in Vero cells.

2. Results of Electron Microscopy

   a. We first investigated those viruses producing CPE in Vero cells. Although VSV, used as a model and harvested from Vero cells (titer 10^6.7 TCD 50/ml) could be readily detected by the floating techniques described, none of the following viruses could be detected in the electron microscope, by this technique : (Vero titer: 10^7 TCD 50/ml), Trinity. (Vero titer: 10^6 TCD 50/ml), Bobaya. Vero titer: 10^5.7 TCD 50/ml), Zinga. Vero titer: 10^4.5 TCD 50/ml), AbB 4268.

   b. Immune electron microscopy. To test this method known arboviruses were investigated. Virus-antibody aggregates were detected as indicated in table 2. Aggregates of virus particles were surrounded by a diffuse electron-transparent halo of antibody. These complexes were sometimes mixed populations of empty and full particles. Similar complexes were identifiable when antisera and viruses were added on 1% noble agar, but not when the viruses were inactivated by 2.5% glutaraldehyde at 37oC for 30 minutes. Although the titer of the virus used (MIB) was very low (10^3.5 TCD 50/ml), we were able to detect virus-antibody aggregates but the empty particles outnumbered the full ones. When viruses and antisera were mixed in a parafilm, incubated at 37ºC for 30 minutes, no complexes were identifiable. Since antisera against ungrouped viruses were not available, this technique could not be applied to them.

   c. Pseudoreplica technique. By this method all the viruses indicated in table 2 were detected: enteroviruses, a rhabdovirus (VSV), Bunyamwera, flaviviruses and alphaviruses. Three ungrouped viruses harvested from Vero cells could be visualized : Bobaya, Zinga and Trinity. They are spherical viruses with an average diameter of 90-100 mm resembling Bunyamwera virus. Two viruses, Yata and Keuraliba that do not propagate in Vero cells have been visualized in a membrane filtered mouse brain suspension and identified as rhabdoviridae, average dimensions of 65 x 180 nm. Several attempts to visualize other ungrouped arboviruses prepared from mouse brain remained negative.

   d. Comparison of the sensitivity of the antiserum-in-agar and the pseudo replica techniques. A suspension of Sindbis virus (10^6 TCD 50/ml) was diluted 1/4, 1/16, 1/164, 1/256 and examined by both techniques. Results were as follows :

VIRUS VISUALIZED BY

	Serum-in-agar	Pseudo replication
pure (10^6)	+	+
1/4	-	+
1/16	-	+
1/64	-	+
1/256	-	-

The pseudoreplica technique thus seems to be more sensitive. It also allows an easier and more rapid screening of the grids and produces much clearer pictures for morphologic study.

DISCUSSION

It is not surprising that the floating technique did not allow to detect any viruses except VSV because of the relatively low titers of the suspensions that could be tested. It is often stated in the literature that to detect virus particles by this technique, these should be present in concentrations of more than 10^7 or 10^8 particles/ml.

The serum-in-agar technique appears to be more sensitive than the floating method and viruses of titers 10^5 TCD 50/ml could be visualized. In fact, this method could serve as a rapid typing method as well provided the corresponding antisera are available. Besides, it offers the advantage that several viruses can be screened in one microtiter plate and that these plates can be stored at 4ºC for several months without deterioration.

It seems however that the most sensitive method is pseudoreplication. We have been able to detect the viruses shown in table 2 by applying both one and three drops on the agarose surface, despite the relatively low titers of some of them. However, AnB 4268 virus, producing a low titer in tissue culture cells (10^4.5 TCD 50/ml) was not visualized. Pseudoreplication of ungrouped arboviruses harvested from mouse brain was unsuccessful except for two rhabdoviruses.

Among ungrouped arboviruses, Trinity, Bobaya, Zinga and AnB 4268 multiply in Vero cells producing CPE.Bobaya, Trinity and Zinga have been classified as Bunyawera viridae and Yata and Keuraliba as rhabdoviridae.

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DISCUSSION

P. Brès : Dr. El Mekki, has Le Dantec virus been examined ?

A. El Mekki : Not yet, it is on our programme. But there is another interesting virus : Wanowrie, isolated in India from the brain of a patient who died in Sri Lanka from a disease which, as far as the clinical history goes, resembles very much a hemorrhagic syndrome.

J. Casals : Dr. van der Groen, I wonder whether you tried the CER cell line. It proved the most satisfactory cell line to work with a number of viruses and particularly Congo-Krimean hemorrhagic fever virus which gives no CPE and no plaques or with great difficulty, however the virus replicates extensively without visible CPE but it is really very good for immunofluorescence.

G. van der Groen : We tried different cell lines not so much to find a more sensitive one, but also to obtain an indication concerning a possible virus reservoir or transmitter. These are continuous cell lines and I know one cannot conclude from the sensitivity of cell lines that the corresponding animal would be the reservoir, but it gives some indication.

SECTION II OTHER HAEMORRAGIC FEVERS

MARBURG VIRUS DISEASE

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INTRODUCTION

Marburg Virus Disease is a hemorrhagic fever first described in 1967 when laboratory workers became ill after contacts with monkeys imported from Uganda to Marburg, Germany. Therefore synonyma are also Green Monkey or Vervet Monkey disease. In Marburg and Frankfurt, Germany, and in Belgrade, Jugoslavia, 31 patients got infected, of whom 7 died. Six of all patients were secondary cases (1).

TABLE 1 MARBURG VIRUS DISEASE

1967	Marburg and Frankfort/GERMANY Belgrade/YUGOSLAVIA Infected 31; died 7 (secondary 6)
1975	Johannesburg/S.A. Infected 3; died 1 (secondary 2)

In 1975 a patient died in Johannesburg, South Africa, from Marburg Virus disease that he acquired in Rhodesia, evidently while travelling. Two secondary 2 cases occurred, who recovered completely (2).

MORPHOLOGY AND TAXONOMIC STATUS

The virus particle size was measured 75 nm x 650 nm approximately It is a virus with high complex structure related to viruses of vesicular stomatitis and rabies. The taxonomic status is not clearly defined. It was proposed to include it to the rhabdo-virus group by morphological criteria. At the present time it is still unclassified (4).
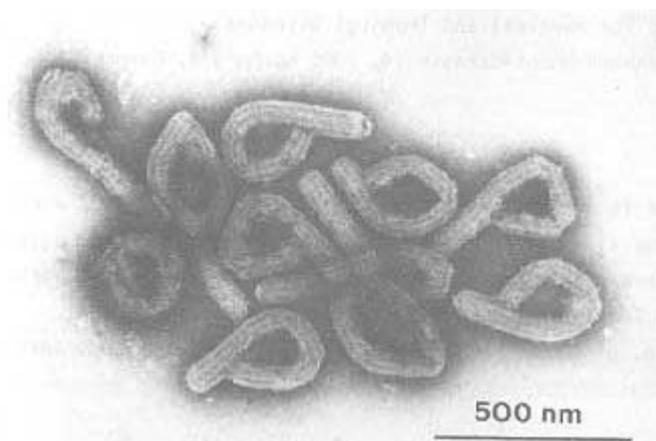

Fig. 1. Marburg Virus from VERO cell culture medium,
Negative staining 50 000 x
Courtesy of W. Slenczka and D. Peters, 1977 Marburg and Hamburg.

TRANSMISSION

In Marburg, Frankfurt, and Belgrade it became clear that either contacts with blood or organs of infected monkeys (Cercopithecus aethiops) or contact with already infected patients led to infection.

TABLE 2

MARBURG VIRUS DISEASE

clinical symptoms:	fever, myalgia, vomiting, diarrhoea, maculopapular symptoms: rash, bleedinq tendency, conjunctivitis, other
laboratory:	leukopenia, morphol. changes, thrombocytopenia, elevated GOT, GPT, decreased protein, other
histology:	cell necroses (multiple organs), inclusion bodies
clinical diagnoses:	hepatitis, myocarditis, pancreatitis, bronchitis, diagnoses: kidney failure, hepatic failure, encephalitis, other

The case in Johannesburg raised the possibility of being infected by a sting as concluded from patient's history. This could not be proved yet. Therefore, transmissions by to date unknown vectors remain possible. The two other cases were secondary.

RESERVOIR

Though monkeys from Uganda were the first known animals to be infected, the natural reservoir is still unknown. Epidemiological studies in monkeys trapped in Uganda and in monkeys' trappers indicated possible infection being present in a natural reservoir in Uganda (5). Further studies on monkeys in South Africa could not support this conclusion (6). However, animal inoculation was possible in a number of species, e.g. in guinea pigs, suckling mice, monkeys of different species, suckling hamsters, as well as in Aedes aegypti.

CLINICAL FEATURES

Incubation time is 4 to 9 days. Evidently, the virus spreads rapidly to most organs of the body inducing major dysfunction.

TABLE 3

MARBURG VIRUS DISEASE

Agent:	virus particle size 75nm x 650nm
Taxonomic status:	rhabdo virus-like
Transmission:	1 organs, blood (or contaminated materials) from vervet monkeys
	2 blood, contact from infected patients (nosocomial inf.)
	3 tick bite ?
Incubation:	4-9 days
Virus association:	two months (sperms, eye fluid)
Mortality:	approximately 25%

Headache, myalgia and fever occur followed by vomiting, conjunctivitis, a maculo-papular rash, enanthema, and enlarged lymph nodes. Watery diarrhoea occurs most frequently. In case of recovery symptoms improve over a period of several weeks. Reconvalescence requires up to several months. In more severe cases hemorrhagic disorders occur. Most remarkable are hemorrhages from the gastro-intestinal tract connected with poor prognosis. Other symptoms are mental disturbances, hyperaesthesia, and myelitis. Considerable bradycardia may be one of the symptoms in the beginning except in fatal cases during agony, where tachycardia is prevailing. 25% of the patients observed up to date died. Thus, the mortality is comparable with the mortality from Lassa fever.

LABORATORY FINDINGS

Laboratory parameters indicate the multiple organ damage. Leukopenia followed by leukocytosis is an almost typical feature, usable for diagnosis. Differential blood count demonstrates shift to the left of

the granulocytes, as well as pseudo-Pelger cells, and atypical lymphocytes with activated nuclei, plasma cells or lymphoblasts (7). Considerable thrombocytopenia may cause bleeding tendency. In some cases laboratory parameters may suggest dissiminated intravascular coagulation with subsequent kidney failure (8). Elevated GOT and GPT levels indicate severe liver damage. ECG changes are comparable with myocarditis or other damage of the myocardium.

An interesting fact is that the association with active virus material can be observed for a long time. In one case, who recovered, virus particles could be detected in sperma. In this case there was evidence that the patient infected his wife by sexual intercourse . A secondary case showed that even two months after the disease was observed, virus particles could be isolated on the eye-chamber fluid (2)

PATHOLOGY

Postmortem sections showed that almost in all organs of corpses with Marburg virus focal necrosis could be detected by routine histology. Damage of the parenchyma of the kidneys suggested tubular deficiency. Hemorrhagic diathesis and plasma cellular infiltration could be observed in various tissues. Cerebral damage as described in panencephalitis with glial nodules may be present.

DIAGNOSIS

Diagnosis of Marburg virus disease may be difficult in a single case, but less difficult in an epidemic outbreak. Patient's history as well as typical rash and other clinical symptoms, especially hemorrhages, may lead to suspect Marburg virus disease. Furthermore, morphology of peripheral blood smears and other hematological parameters including platelet count may assist the diagnosis.

TABLE 4

MARBURG VIRUS DISEASE

diagnosis:	history, source of infection, clinical symptoms, antibody formation (IF), characteristic changes peripheral blood cells, direct virus morphology blood; organ biopsy, inoculation guinea-pig; VERO cell culture
treatment:	symptomatic, life support questionable: interferon, convalescent serum

Rapid confirmation may be possible by immune fluorescence methods. Direct examination of blood or organ biopsies can be done by electron microscopy. Cytopathic effect in Vero cell culture or the detection of virus from guineapigs inoculated with blood from a diseased patient will give evidence of the diagnosis.

TREATMENT

Symptomatic care of these patients is necessary. This includes balance of fluids and electrolytes, as well as treatment of hemorrhagic disorder which, of course, must be defined by laboratory parameters exactly. It is questionable whether convalescent plasma will prevent severe disease or, in any way, improve the status of the patients. The use of interferon seems to be experimental at the time being.

PREVENTION

To prevent the occurrence of Marburg virus disease cases is impossible, because it is not known where the natural reservoir is located. However, in case of a suspected case of Marburg virus disease, preventive measures have to be taken in order to protect attending personnel from infection. It seems that routine isolation procedures may be sufficient. However, the occurrence of secondary cases in the Marburg virus infection in Johannesburg in 1975

teaches us that secondary infection may occur despite these measures. More strict isolation facilities have been used for reverse isolation of patients with susceptibility to infection, e.g. in the case of bone-marrow transplantation. Plastic isolation systems have been used (10). Therefore, also in isolation of cases with hemorrhagic fever the use of plastic isolation systems with negative pressure and completely tied plastic bag should be safer, and will avoid any quarantine procedures for attending hospital staff". Materials from the patients have to be processed with specific care! Therefore, any laboratory investigations including the definite diagnosis has to be done under strict regulations of safety.

Consequently, only few institutions all over the world can deal with such cases under optimal conditions.

SUMMARY

Marburg virus disease is a hemorrhagic fever, up to date unclassified. Mortality of the disease is 25% approximately. Despite the fact that the first outbreak was caused by contact with monkeys, the natural reservoir is still unknown. Transmission may occur by contacts with infected patients. Animal experiments showed that even Aedes aegypti could be a transmitter. Important to know that association with the virus or virus particle may last for several months.

Treatment is supportive only, because specific treatment is not known. Special attention has to be given to strict isolation procedures as necessary in patient care and for diagnostic laboratory investigations.

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DISCUSSION

M. lsaäcson : The Johannesburg-Marburg index case is of course the only Marburg case known that has been acquired in nature. The patient complained of some kind of sting or bite that he acquired one week prior to onset of illness. This was a very painful lesion. We did a rather thorough epidemiological investigation, and we in fact were able to identify the tree on the roadside under which the patient sat when he was stung or bitten by an unidentified agent. We proceeded to tear the roadside bank apart literally. The only sign of life we found from the entomological point of view was hundreds of spiders. We collected all the spiders we could find and took these to the laboratory. About half of these have now been investigated at the CDC in Atlanta as well as in our Laboratory in Johannesburg and have yielded no results. I think that these are the only comments I want to make on this particular case.

T.E. Woodward : Does the fact that we heard nothing about changes in the blood vessels mean that there is no vasculitis present ? Do you have any clue from findings in the gastro-intestinal tract why there is diarrhoea and intestinal bleeding ?

M. Dietrich : Around the vessels there were, as far as I remember, some cellular infiltrates, no pure vasculitis as such. The same holds true for the GI tract.

LASSA FEVER : HISTORICAL OVERVIEW AND CONTEMPORARY INVESTIGATION

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HISTORY

Science is a tapestry woven from remnants by workers linked by principle and the disparate ways of interhuman communication. The grand design is hidden from each weaver until chance and the accumulated fabric reveals itself in dramatic form. In 1934 and 1935 a new virus was recovered from monkeys, humans, and mice (1,2,3). It was named lymphocytic choriomeningitis virus of mice (LCM). For more than three decades this virus remained a morphological and biological orphan, albeit a profound model for much of modern immunological theory in the field of immune tolerance (4), until Dalton et al. revealed its unique configuration marked by the virionic incorporation of variable numbers of host-cell ribosomes in 1968 (5). Meantime the virus of Argentine hemorrhagic fever (AHF) named Junin was isolated in 1958 (6), and found to be immunologically related to another virus called Tacaribe which had been recovered from bats in Trinidad by workers of the Rockefeller Foundation perusing studies on the ecology of vector-borne viruses of the tropics (7).

In 1963 workers from the U.S. National Institutes of Health based in Panama isolated the causative agent of Bolivian hemorrhagic fever, a disease indistinguishable from AHF, and found that it shared antigens with Junin and Tacaribe viruses (8). Machupo virus induced chronic tolerant infection in its natural rodent reservoir host *Calomys callosus* (9) which was similar to the pattern of infection from LCM in *Mus musculus* elucidated by Traub in the 1930's (10,11).

Within months of the report of the morphological characteristics of LCM in 1968, Murphy et al. (12) demonstrated that Machupo virus shared these unique structural properties. Thus was born the family of viruses known as arenaviridae (arenosus = sandy, to mark the unique ribosomal entrapment in varions) (13).

In the 1960's Frame at Columbia University sought and received the collaboration of Rockefeller Foundation personnel, by now integrated into the facultY of Yale University School of Medicine, in screening the families of medical missionaries in Africa for indigenous viral infections. This cooperation resulted in the isolation of Lassa virus in 1969. The original virus strain was obtained from the blood of a missionary nurse from Jos, Nigeria.

She was the fourth patient in a nosocomial transmission chain originating from an obstetrical patient residing in Lassa, Nigeria, who sought treatment in Jos for a septic abortion 14,15). When the virions of Lassa virus were found to be indistinguishable from LCM, Machupo, Junin, and other arenaviruses, it was evident that Lassa fever was likely to prove to be rodent-transmitted.

In 1970 another Lassa outbreak occurred in the hospital at Jos. There were 28 cases and 13 deaths. Ratttus Ratttus and Mus musculus were found in and near houses of patients but none yielded Lassa virus, and retrospective analysis of this epidemic revealed that transmission of infection had occurred directly in the hospital (16). Although gastro-intestinal hemorrhage similar to that characteristic of the South American arenaviral hemorrhagic fevers (SAHF) was observed in some patients, unique complications such as pleural effusion, deafness and acute "nephritis" were recorded (17).

In 1972 Lassa fever hospital "outbreaks" were recognized in Liberia and Sierra Leone (18,19). During investigation of the latter, several Lassa virus isolates were made from mastomys natalensis, a rodent found in man-disturbed biotopes and human dwellings throughout most of sub-saharan Africa (20). The general rule of arenavirus biology was confirmed. Mortality rates in these "epidemics" were high (36-38%), but village sero-surveys done in Sierra Leone using a complement fixation test revealed that six percent of the population had experienced Lassa virus infection (19). A similar study in the region of Jos, Nigeria, employing a neutralization test which has not proven to be reproducible, showed that 13 percent of persons had Lassa virus antibodies (21).

More recently a fluorescent antibody method has been employed to measure anti-Lassa antibodies (22). By this technique, Lassa virus has been documented to occur in humans in most of West Africa including Ivory Coast, Ghana, Senegal, Guinea, Gambia, Upper Volta, Mali and the Central African Empire (23,24).

But *Mastomys* rodents are present in much of Africa. Why is Lassa fever a West African disease ? Although nature's pattern is unclear, there are clues. A virus antigenically related, but probably not identical, to Lassa has been isolated from mastomys in Mozambique (25). Elucidation of its pathogenetic properties and geographic distribution may help unravel the mystery. In addition Mastomys natalensis rodents bearing three distinct numbers of chromosomes have been reported from Africa (26). West African animals have 32 and 38 chromosomes (26), while those in southern Africa have 32 or 36 chromosomes. The latter forms have been documented to be distinct biological species (27). Time and work will tell us whether these clues are relevant.

Simple biological and virological parameters, however, suggest that the pathogenesis of Lassa virus infection in man is distinct from that of Junin and Machupo viruses. The South American diseases are characterized by a bleeding diathesis, low and intermittent viremia and a long interval between onset of symptoms and appearance of humoral antibodies (9). Virus is rarely detectable in urine and other clinical complications are unusual. Lassa fever, in contrast, is marked by prolonged viremia, a variety of major clinical manifestations and the simultaneous presence of both virus and specific antibodies in the blood during the second week of illness (28,29). Available data suggest that severe Lassa fever may have an immunopathological etiology. Finally, Junin and Machupo viruses have rarely been transmitted directly from person to person (9), whereas Lassa virus has produced several notable outbreaks based on this mode of transmission (16,18).

CURRENT INVESTIGATION

Examination of hospital records in eastern Sierra Leone between 1972 and 1975 revealed that clinical Lassa fever was almost certainly endemic to this region (30). Indeed, hospital records and the published literature (31,32) strongly suggested that Lassa fever had been described clinically from this area as far back as 1956. These considerations led to the organization of a multinational prospective study of Lassa fever in Sierra Leone which was initiated in February 1977, to address a variety of questions:
1. What is the spectrum of human response to Lassa virus infection ?
2. What is the true mortality rate of Lassa virus infection ?
3. What are the clinical consequences of Lassa virus infection in Children ?
4. How contagious is Lassa fever in an endemic setting ?
5. How can Lassa fever be diagnosed in less than 24 hours ?
6. Do Lassa fever patients have circulating antigen-antibody complexes ?
7. Is Lassa fever an immunosuppressive disease ?
8. Do passive anti-Lassa anti bodies have a role in treatment of Lassa fever ? Experimental work in monkeys with Machupo virus suggests that antibodies may be life-saving (33). Trials among Lassa patients are inconclusive (34).
9. What are the infection-competition dynamics among Mastamys and other "peridomestic" rodents which lead to human infection ?
10. What is the relation of mastomys chromosome type to chronic Lassa virus infection and excretion ?

A field laboratory has been established in Kenema for immunological work and for processing materials for virus assay at the Center for Disease Control in Atlanta. To date work has focused on febrile patients admitted to three hospitals near Kenema, although village-based studies of both human and rodent populations are planned.

From February through July 1977 we examined 263 hospitalized adults with fever. Lassa fever occurred continuously and 143 of these patients were deemed to have the disease based on virus isolation, a four-fold or greater increase in antibodies to the virus, or an initial titer of at least 1:1024 by the indirect fluorescent technique (IFAT). Males and females were equally affected and the mortality rate was about 20% although further laboratory work is required before the numbers are made precise.

No single symptom or sign was significantly correlated with laboratory-diagnosed Lassa fever. Combinations of findings such as pharyngitis, conjunctivitis and bleeding, however, were definitely predictive in the minority of patients in whom they occurred. Major complications, none of which were seen in more than 5% of Lassa fever cases, included deafness, abortion, pleural effusion, pericarditis, orchitis and iridocyclitis.

A search for Lassa fever among hospitalized children was begun in October. To date, nine cases have been confirmed, although it is already clear that this diagnosis accounts for a much smaller fraction of serious febrile disease in children than among adults.

Human serosurveys in villages of eastern Sierra Leone are in progress. Early returns suggest that prevalence of anti-Lassa antibodies varies widely but may reach 40 percent. At least one instance of

asymptomatic Lassa infection has been documented. Despite the high prevalence of infection in hospitals we have observed only a single case of the disease which appeared to have been acquired through contact with a Lassa patient.

Promising preliminary results have been obtained in rapid diagnosis of Lassa infection among patients admitted prior to the appearance of specific antibodies. Epithelial cells are obtained from the conjunctiva using a curette, placed in a small amount of buffered saline, and acetone fixed in wells of teflon-templated microscope slides. Lassa-specific antigen has been found in a minority of such cells from several independently confirmed cases using the IFAT. We regard the ability to make early rapid diagnosis as crucial to scientific assessment of the value of passive antibody or any other method in the treatment of Lassa fever, in addition we are evaluating specific IgM antibody in the diagnosis of Lassa fever.

SUMMARY

Lassa fever is clearly the most significant arenavirus disease of man in terms of morbidity, mortality, and its potential for person to person transmission. Unlike the arenaviral hemorrhagic fevers of South America, an array of serious clinical complications occurs which, together with the early appearance of antibodies in the presence of viremia, suggests that an immunopathological mechanism may be important in Lassa fever.

Accumulating evidence strongly suggests that Lassa virus infection may lead to a complete spectrum of host response in man, and that in fact this disease is neither as lethal nor as contagious as originally thought. Until much more is known, however, prudence requires that Lassa fever or even suspected cases of it be managed with maximum attention or containment and protection for medical and paramedical personnel.

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DISCUSSION

A.W. Woodruff : Since we had the first patient some six years ago that indicated that there was this focus of Lassa fever in Sierra Leone, we naturally kept an eye open for other cases of Lassa fever from that area and for other viral infections that might present as pyrexias among people coming from West Africa. We have not found any Lassa antibodies, nor Ebola or Marburg, but a fascinating array of other virus infections have turned up in these people. We have in addition to 5 cases of Dengue and 2 of Chikungunya, 4 in which there was only O'nyong-nyong antibody present. These came from Nigeria, Ghana, possibly Sierra Leone. This is, I think, the first evidence of this infection in Nigeria. Ntaya has turned up four times and is of some interest, there has not previously been any clinical record of symptoms associated with it. The involvement of the central nervous system, in at least two of these patients, raises the possibility that it my have neurotrophic propensities. Although Zinga has only been recovered from the Central African Empire, it seems possible to acquire it in Southeastern Nigeria. Perhaps the most fascinating was a Le Dantec virus antibody which appeared not in a person who had come from Africa but who had been bitten by an insect when unloading a cargo in Britain and had developed encephalitis and later Parkinson's disease which would be related to that infection.

P. Brès : O'nyong-nyong was an epidemic virus which appeared some fifteen years ago in East-Africa and then disappeared. But Chikungunya was very widespread, and it is mostly very difficult to

make a difference between 0'nyongnyong and Chikungunya. So if the diagnosis of O'nyong-nyong is certain, I think this is worth being published.

LASSA FEVER VIRUS ANTIBODIES IN MASTOMYS NATALENSIS CAUGHT IN DIFFERENT PARTS OF NIGERIA

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INTRODUCTION

In 1972, Monath et al. (1) reported the isolation of Lassa virus from the tissues of Mastomys natalensis during an epidemic of Lassa fever in a community in Sierra Leone. The majority of the virus isolates were from rodents caught in homes occupied by Lassa fever patients. The role of this rodent as a reservoir was confirmed by studies in Nigeria (2), when animals were collected between November and December 1972, and March and April, 1973, during the early dry season and the beginning of the rainy season. There was no evidence of an epidemic of Lassa fever during the periods of rodent collection. Most of the epidemiological surveillance for Lassa have been through serological evaluation of antibodies in man; there are no known studies of antibodies in sera of *Mastomys natalensis* (Wolf, et al. (1977) in a recent article in the Bulletin of WHO 55(4): 441-444, reported on an arenavirus closely related to Lassa virus from *Mastomys* in south-east Africa.). The present report is based upon the results of serological evaluation of CF antibodies in sera of Mastomys natalensis collected in several parts of Nigeria.

MATERIALS AND METHODS

During studies on the ecological distribution of *Mastomys natalensis* carried out by one of us (Dobrokhotov), attached to the WHO Arbovirus Vector Research Unit stationed in Enugu, Anambra State of Nigeria, sera samples were taken. Areas surveyed included NerPanskshin, Pankshin in Plateau State and Azare village 15 km apart in Bauchi State; Kaduna in Kaduna State and Mamu River Forest Reserve near Enugu in Anambra State. The rodents were bled in the field and sera placed in refrigerated plastic vials with screw caps, and transported to the Virus Research Laboratory in Ibadan. This report is based on 104 *Mastomys* sera: of these 29 were from Ner-Pankshin, 28 from Pankshin, both in Plateau State; 22 were from Azare-Gadau and 25 from AzareDalli in Bauchi State. Other sera from Anambra 10 and Kaduna 15 states were negative.

Complement Fixation Test (CFT)

Sera were inactivated at 60ºC for 30 minutes in the same vials in which they were transported to the Laboratory. None of the vials was opened until after inactivation.

Antigents for the CF test were supplied by the Center for Disease Control (Atlanta, USA), as lyophilized betapropiolactone (BPL) inactivated Vero tissue culture propagated Lassa virus. The control antigen consisted of uninoculated Vero tissue culture cells treated similarly as the Lassa virus antigen.

The CFT was conducted according to the micro-method of Weinbren as modified by the Virus Research Laboratory (3). Initially, the one hour and overnight incubation periods were used, however, the overnight incubation consistently gave reproducible results, and was used in subsequent tests. All the sera positive in the screening tests, as well as those with anti-complementary reactions were later titrated.

RESULTS

The results of the Lassa virus CF antibody tests on *Mastomys natalensis* sera are shown in Table 1: 56 sera from other rodent species from the same localities: *Tatera, Myomys, Rattus, Arvicathus, Lemniscomys*, the hedgehog, *Erinaceus*, and shrew, *Crocidura* were negative for Lassa CF antibodies.

Each serum was tested at least three times, and the reciprocals of serum dilutions reported here represent the average of the titres of these tests.

The titres of positive M*astomys* sera from Ner-Pankshin ranged from 1:4 to 1:256: eleven (37%) of 29 sera were positive, with 4 showing high CF titres (1:32 - 1:256). Ten of the total sera tested were anti-complementary.

In Pankshin none of the *Mastomys* sera was positive, but 5 were anticomplementary. *Mastomys* sera from Azare-Gadau were all negative for antibodies to Lassa virus. One sera of 25 (4%) from Azare-Dalli was positive with a titre of 1:8.

DISCUSSION

In humans, generally the presence of CF antibodies in the circulating blood is an indication of a recent virus infection. In Lassa fever, however, CF antibodies could only be detected 20 or more days after the onset of the disease. There is also evidence of persistent antibodies four to five years after either infection or exposure to the virus (3).

TABLE 1

RESULT OF CF ANTIBODY STUDIES ON SERA OF MASTOMYS NATALENSIS FROM SEVERAL PARTS OF NIGERIA

CF TITRES X

Source				Number positive					
	Total	4	8	16	32	64	128	256	
Plateau State:									
Ner-Pankshin	18(a)		22	3	2	1	0	1	37
Pankshin28(b)	0	0	0	0	0	0	0	0	
Bauchi State:									
Azare-Gadau	22	0	0	0	0	0	0	0	0
Azare-Dalli	24	0	1	0	0	0	0	0	4
Total	92	2	3	3	2	1	0	1	11.5

a) includes 5 anti-complementary.
b) includes 5 anticomplementary.
x) reciprocals of serum dilutions, giving at least a 3+ fixation in CF tests.

In addition, infected human serum has been shown to have high concentration of the virus (4,5). Virus has been isolated from human blood 19 days after onset and from urine 32 days after onset (5,6,7,8). According to Walker et al. (9), experimental infection of neonatal mastomys did not cause any clinical disease or pathological lesions despite the presence of virus in the blood, lymphnodes, liver, spleen, lung, brain, urine and throat secretions throughout a 74 day study. Infected adult Mastomys also remained normal but had virus in many organs, the virus persisting for 103 days at the termination of the experiment. Lassa virus CF antibodies were also demonstrated in infected animals.

Serological surveys for Lassa virus CF antibodies have been carried out among hospital personnel in different parts of Nigeria. In the Plateau and Kaduna States covering Ner-Pankshin, Pankshin, Vom, Birkin Ladi, Jos and Zonkwa the highest positive rate (9.8%) occurred in the Ner-Pankshin and Pankshin areas, whereas other areas showed an average positive percentage of 5.3% only (10). Furthermore, epidemiological investigations of a Lassa fever outbreak which occurred in a road company camp in the Pankshin area in 1976 showed that 15.5% of those bled for antibody evaluation had Lassa antibodies. There was, however, no evidence of clinical Lassa virus infection during the period of investigation (11). On the other hand, during the 1974 Lassa outbreak in Onitsha, Anambra State, none of the human and animal sera tested was positive for Lassa CF antibodies, although 3 cases of Lassa fever infection were confirmed by virus isolation and/or antibody studies (12). Finally, out of a total of 8 Lassa virus strains isolated from tissue pools and blood specimens obtained from *Mastomys natalensis,* five strains were from Ner-Pankshin and two from Vom in the Plateau State and only one

from the Bauchi area. These virus isolations correspond very closely with the antibody findings reported in this paper.

The Pankshin area of the Plateau State may be considered a highly endemic zone for Lassa fever in the state, as evidenced by the high level of antibodies in humans and *Mastomys*, as well as the high rate of virus isolation from *Mastomys* in that area.

There is no doubt that a combination of surveillance in man and in mastomys using serological and isolation methods coupled with study of ecological distribution of the rodents would yield valuable information delineating the distribution of Lassa fever disease in Nigeria. Such knowledge would lead to proper surveillance, and proper selection of study areas and the planning and execution of adequate and proper control measures.

Apparently, there are two different species within the "*Mastomys natalensis*" complex: one (dark colour) is largely commencal, while the other (brown colour) tends to be free ranging. It has also been confirmed that certain populations have 32 chromosomes and others 36, with no apparent hybrids. Studies are being carried out to try to correlate morphological and cytotaxonomic characters. At the time of these studies (end of rainy season) both forms occurred in houses. Antibodies have been found among both forms and among males and females. Antibody titres were higher in adult animals than in young ones.

SUMMARY

A survey for Lassa virus complement fixing (CF) antibodies was carried out on the sera of rodents and shrews trapped from different parts of Nigeria. Eleven of 29 *Mastomys natalensis* (37%) trapped at Ner-Pankshin, Plateau State, and 1 of 25 (4%) trapped in Azare Dalli, Bauchi State, were positive for Lassa fever virus CF antibodies. All other small mammal sera were negative. The results of the antibody surveillance in these rodents correspond with the findings of previous virus isolation attempts in mastcmys and with human antibody surveillance in different parts of Nigeria.

ACKNOWLEDGEMENT

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SOUTH AMERICAN HAEMORRHAGIC FEVERS

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ANTECEDENTS

In 1953/54 epidemic outbreaks of Argentine Haemorrhagic Fever were discovered in the north-west of Buenos Aires Province. This humid Pampa is the richest farming land of Argentine, the climate is temperate and the average annual rainfall is about 1,000mm. The geographical extension was formerly recognized as 16,274 km 2 with 268,049 inhabitants in 1958. At the present time it covers more than 100,000 km2 with a population of more than 1,000,000 people (1).

Bolivian Haemorrhagic Fever was first observed around San Joaquin, in Agua Clara, in September of 1959, in Yutiole in July 1960, in Yoatre in August and in Las Moscas in December. In May 1961 the first cases appeared in San Joaquin and Barranquita.

Another epidemic outbreak was also reported in Orobayaya 115 km to the East of San Joaquin. The island was abandoned by its 600 inhabitants. In 1962 there were only 15 persons at Orobayaya. At the present time after rodent control was initiated in 1964 in San Joaquin, new cases were found in other human settlements far from the first focus such as Magdalena, La Cayoba, Chaco Lejos, Rio Negro, Fortaliza (Fig. 1).

ETIOLOGY

Junin virus is the etiologic agent of Argentine Haemorrhagic Fever and was isolated in human clinical cases independently by Parodi et al. (2) and Piroski et al. (3) in 1958-59.

Machupo virus is the etiologic agent of Bolivian Haemorrhagic Fever and the initial isolation was on May 19, 1963, in a typical clinical human case by Johnson (4). It was also isolated from the wild rodent Calomys Callosus in San Joaquin (5).

Fig. 1 Geographical distribution of Bolivian Haemorrhagic Fever outbreaks.

TABLE 1
BIOLOGICAL CHARACTERS OF MACHUPO, LATINO AND JUNIN VIRUSES

NATURAL HOSTS	
Machupo & Latino Viruses	Junin Virus
Calomys Callosus	Calomys Musculinus Calomys Laucha (Hesperomys) Akodon Arenicus (A. Azarae) Akodon Obscurus Akodon ArenicoZa Oryzomys Flavescens WS musouzus Cavia Sp.
EXPERIMENTAL HOSTS	
Saguinus Geoffroyi Macaca MuZata Macaca Fascioularis Cavia Porcella C-13 (strain)	Cavia Porcella Mus musculus Calomys Musculinus

Biological properties. Intracerebral (Table 1) inoculation of infant hamsters and mice is the more adequate method for isolation of Machupo virus; both develop neurologic signs and die 6-15 days later. In low dilutions infected suckling hamsters frequently do not exhibit illness or death. The survivors appear stunted and develop antibodies. In adult hamsters and mice illness was rarely observed. *Calomys callosus* is the critecine rodent naturally infected with Machupo and Latino viruses in enzootic foci. No other cricetidae such as *Oryzomys, Zygodontomys, Holochilus* and *Nectomys* or *Muridae* as *Mus musculus, Rattus rattus, Rattus norvegicus, Echimydae* as *Proechimys* and *Marsupials* were positive for virus isolation.

Calomys callosus, above 9 days of age, inoculated with Machupo virus gives a "split response" (6).

In type A, occurring in 50% of inoculated animals, the animals are "immunotolerant", with persistent viraemia, little or no neutralizing and immunofluorescent antibodies, viruria, splenomegaly microcytic-hyprochromic anaemia and reduced fertility. In type B animals are "immunocompetent"; there is clearance of viraemia, presence of N, CF and immunofluorescent antibodies, minimal or no viruria, and no anaemia or splenomegaly.

In suckling *Calomys*, Machupo virus induces a viraemic immunotolerant infection. Latino virus produces chronic but non-tolerant infection. Apparently it only infects suckling hamsters. In the guinea-pig, Machupo virus produces little or no viraemia and there is no evidence of haemorrhagic lesions (6).

Marmosets develop anorexia, tremors, shock and die 8-20 days postinoculation. Virus multiplies in many tissues except in the brain. Histological lesions were cortical necrosis in lymph nodes, reticular hyperplasia with lymphoid depletion in the spleen.

In Rhesus monkeys, the virus causes a severe illness consisting of two clinical phases (7). After 6-7 days incubation all animals develop fever conjunctivitis, depression, anorexia, diarrhoea, clonic spasms, nasal haemorrhagic discharge, erythematous facial rash. Dehydration is maximal on 18-19th day, just before death occurring in 80% of the monkeys. In 20% of the monkeys surviving this first clinical phase, neurological signs develop after 26 to 40 days: intentional tremors, nystagmus, incoordination, paresis and coma. The pathology shows moderate to severe encephalitis with vasculitis.

In *Cavia porcella*, Junin virus produces a fatal disease resembling Argentine Haemorrhagic Fever in man. The animals develop fever on 5-8th day. Death occurs in hypothermic shock on the 11th to 15th day. Viraemia persists until death. The virus is located in the principal organs and is excreted with urine and saliva. Pathological findings are macroscopic and microscopic haemorrhagic lesions, in subcutaneous tissues, haemorrhagic lesions in the stomach wall, the small and large intestine, adrenals, lymph nodes, lung, peritoneum and thoracic cavity. Bone marrow shows moderate to massive necrosis. Homologous immune serum given 24 hours before or 5 days after infection, protects 55% of the guinea pigs.

Guinea pigs infected with XJ CL3 strain develop an inapparent infection detectable by the appearance of antibody. The virus is detected in spleen and lymph nodes only. Complementfixing and neutralizing antibodies appear after about 20 days and protect the guinea pigs against challenge with the pathogenic XJ strain (9). Tacaribe virus inoculated into guinea pigs induces resistance to high doses

of the XJ Junin strain. When suckling Mus musculus are infected with Junin virus they develop typical viral encephalitis, mortality is 95-100%. Adult mice are resistant.

According to Sabattini et al. (10) field studies have been made indicating that *Calomys musculinus* is a natural host for Junin virus, exhibiting persistent viraemia with virus excretion in urine and saliva, without disease symptoms.

EPIDEMIOLOGY AND ECOLOGY

From 1958 through 1974 about 16,000 cases of Argentine Haemorrhagic Fever were diagnosed on clinical grounds. From 1965 to 1974 an epidemiological unit was established at Pergamino (3,000 km2, 75,000 inhabitants). In 1959, 3,075 patients were studied, whose diagnosis was confirmed by CF tests and a few virus isolations (1). Striking seasonal occurrence of the disease remains centered in the humid "pampa" of the Buenos Aires Province, about 200 km west of the capital city, where maize is the principal and intensively cultivated crop. The maize is harvested in April, May and June with peak incidence in May. The disease is four times more prevalent in males than in females and occurs especially in rural workers. The wild fauna of the area is composed of several species of Cricetidae and Muridae. *Calomys musculinus* and *Calomys laucha* are the principal reservoir host of Junin virus, both of them maintain a persistent and inapparent infection with prolonged viraemia and virus in saliva.

Bolivian haemorrhagic fever occurred from 1959 through 1962, on the island of Orobayaya, Province of Itenez, and in the communities surrounding San Joaquin. The population of these epidemic areas was about 4,500-5,000, there were an estimated number of 470 cases with a 30% mortality. Cases occurred chiefly among adult males who had recently been working on small farms. There also seemed to be a marked seasonal tendency with peaks in July-August. Because of the panic caused by the epidemic, most of the 600 inhabitants of Orobayaya fled to Magdalena by the middle of 1962, resulting in a sharp decrease in the incidence of cases in the Itenez Province. San Joaquin city with 3,000 people, located in Mamore province, became the principal active epidemic center until 1964. In the second semester of 1962 cases began to occur among residents who had not gone outside the town; age-sex groups were more uniformly affected, and there was evidence that Machupo virus was active within San Joaquin itself (11).

From January 1, 1963 through May 30, 1964, 778 suspected cases of haemorrhagic fever were hospitalized in San Joaquin, of whom 122 died. Of 656 survivors 336 of acute and convalescent serum samples were tested for complement fixing antibodies with Machupo virus and 282 samples were positive (84%), suggesting that 650 persons hospitalized had haemorrhagic fever with a fatality rate of 19%.

In 1963 a significant difference occurred between the south and north halves of San Joaquin with an incidence of 90% in the south. There was also a higher risk in warm houses. Mild or subclinical infections were rare. The peaks of the epidemic curve were not related to the rainy or dry season and seemed to depend on other factors.

In 1964 the northern half of the city showed more virus activity, meaning that the infection spread very slowly, needing a year for moving to the north.

We observed evidence of direct person to person transmission but this mechanism is not the common mode of transmission.

The epidemics were located in the immensely flat plain known as the "Llanos de Mojos" or "pampas" at an average altitude of 240 mt. which extends to the east of the Cordillera de los Andes. Rising about 2 mt. above the level of the pampas are the "alturas", some of which are covered by dense forest or by scattered low bushes. During the rainy season when the pampas may be flooded, these alturas can best be described as islands. The human settlements have been located on the border of the alturas. The climate is tropical. Temperature is about 25-32ºC, annual rainfall about 1500-2000 mm. The population in Itenez and Mamorb Provinces is 10,000 inhabitants. The principal activity is cattle raising and the harvest of corn, rice, yucca, bananas, cultivated in chacos on the alturas. The prevailing vegetation consists of open grassland and savanna interspersed with islands of climax and semideciduous forest.

Kuns (12) describes two types of grasslands. The "Bajios" or marsh which are low-lying grassy plains covered by a few inches of surface water during the first five months of each calendar year. Savannas occupy higher sites, namely the alturas, and are maintained by annual burning. The farms are usually located on the periphery of the fore islands overlooking the grass covered bajios, and in this ecological nest little villages and isolated settlements have been established where the epidemics of haemorrhagic fever have been observed.

In the vicinity of San Joaquin and in other special ecological settlements previously described, we find the habitat of various genera of rodents (table 2) *Calomys Callosus* is a pastoral species occurring in scrub forest when the canopy is sufficiently open to permit the growth of grass.

TABLE 2

HABITAT PREFERENCES OF RODENTS CAPTURED SURROUNDING SAN JOAQUIN, BENI, BOLIVIA

	Marsh	Savanna	Fields Fallow	Brush Forest	Forest	Houses
Calomys Callosus		+	+			+
Holochilus Brasiliensis	+		+		+	
Mus Musculus						+
Oryzomys Bicolor				+	+	+
Oryzomys Capito				+	+	
Oryzomys con color				+	+	
Oryzomys Subflavus				+	+	
Oryzomys Migripes					+	
Proechimys Guyannensis			+	+	+	
Rattus Rattus			+			+
Zygodontomys Lasiurus			+	+		

The study of the rodent fauna in ten localities during a survey of a large geographic area shows that *Calomys* prosper only on grasslands. Ectoparasites collected from wild and domestic mammals and from humans were species grouped and inoculated in suckling hamsters and mice. No machupo virus was isolated from 803 pools. Three of the most common species of bats, *Myotis nigricans*, *Molossus major* and *Eumops sp*. were negative for virus isolation. Only in *Calomys callosus* has Machupo virus been found in endemo-emidemic areas of Beni, Bolivia. Antibodies against Machupo virus in other rodents and marsupials in endemic and non endemic areas of B.H.F. were negative.

Rodent control was therefore initiated with traps and poisons: approximately 3,000 *Calomys* were estroyed in this manner. The rodent control program was followed in about fourteen days by a dramatic reduction in the incidence of human cases of haemorrhagic fever, first in the east part of the city and second in the entire town when the control was extended to the west sector of San Joaquin.

At the present time the epidemiologic pattern of surveillance has been extended to Yacuma and Moxos provinces of Beni. Two brigades permanently inspect the region, trap rodents and in certain circumstances use poisoned baits when the Calomys populations are increased. They check the size of spleen, notifying the authorities if they find them longer than 2 cm. Since 1971 less than fifty cases were notified in new places.

OTHER TROPICAL FEVERS

Uruma Fever. In the last months of 1954 and the first semester of 1955 a prolonged outbreak of febrile illness with devastating effects and high mortality appeared in the Okinawan colonist settlement of Uruma, a place located 80 km northeast of Santa Cruz city. Of about 400 Okinawan pioneers, 192 got sick with Uruma Fever, an attack rate of 47.6%. There were 15 deaths, mortality rate 7.8. Uruma virus was isolated, but according to Schaeffer (13), this virus was responsible for only 15% of cases.

Haemorrhagic Exanthem of Bolivia. In May of 1967 an epidemic of haemorrhagic exanthematous disease appeared in recruit soldiers that were sent from the Altiplano area (4,000 mt. above sea level) to a stationary training camp of Riberalta (183 mt. of altitude). The clinical picture was characterized by pronounced haemorrhagic manifestations: petechieae, ecchymosis and vesicles, bleeding from the gums, gastro-intestinal and conjunctival haemorrhages and hematuria. Mild temperature of 38-39ºC. After two weeks, 21 soldiers who had pronounced symptomatology were returned to active duty. There was one fatal case. Laboratory investigations were negative and the provisional diagnosis was Haemorrhagic vesicular syndrome following black fly bites (14).

Yungas Haemorrhagic Fever. In 1969 a new epidemic outbreak appeared among road workers residing in a camp in La Asunta, 150 km from La Paz. The clinical picture resembled B.H.F. but specimens were not collected. Four men died, a liver sample taken by viscerotomy, revealed pathological lesions not characteristic of yellow fever, but rather similar to those of B.H.F.

I brought a summary description of these syndromes to alert researches and to promote investigations in tropical areas of South America.

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CRIMEAN - CONGO HEMORRHAGIC FEVER

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INTRODUCTION

Crimean hemorrhagic fever was first described as it occurred in Crimea in 1944 by a team of Soviet investigators led by M.P. Chumakov. The disease reappeared in the same area in the course of the following year and was shown by work in human volunteers to be caused by a filterable agent present in larvae and adult forms of a tick, *Hyaloma marginatum marginatum*, as well as in the blood of patients during the febrile stage; for various reasons the virus was not maintained serially in laboratory animals and was lost. Subsequent events in the characterization of -he disease were: the use of newborn mice as experimental animal which led to the isolation and continuous maintenance of the virus in the laboratory (1967); the demonstration that CHF and Congo viruses, the latter isolated in 1956, were serologically indistinguishable (1969); and the demonstration that viral strains from Central Asia, European USSR and Bulgaria were similar (1970), thus establishing a single disease entity instead of two or more.

The virus. CCHF virus is placed in the family *Bunyaviridae* even though few of the properties required for inclusion in the family are known for the virus. CCHF virus is morphologically and in size, 90100nm, like a bunyavirus; it contains RNA, possibly singlestranded; and it is inactivated by lipid solvents and detergents. Additional properties required for inclusion in the Bunyaviridae have not been reported for the virus: RNA in 3 or 4 segments with a total molecular weight of 6 x 10^6 daltons; helical symmetry of the nucleocapsid; presence of at least 3 polypeptides of which 2 are glycopeptides and located in the envelope. Since, in addition, CCHF virus is not part of the Bunyamwera supergroup its position in the Bunyaviridae is that of "other possible members", not in the *Orthobunyavirus* genus.

The antigenic characterization of the virus is, on the other hand, far better established than its position in the International Committee on Taxonomy of Viruses (ICTV) scheme. Exhaustive and continued efforts by hemagglutination inhibition (HI), complementfixation (CF) and agar gel diffusion and precipitation (AGDP) tests have shown the virus to be antigenically related to no other viruses except: to Hazara with which it constitutes the CCHF group, Table 1; and possibly to Nairobi sheep disease (NSD), Tables 2 and 3. The latter relationships is thoroughly intriguing, as it involves also Ganjam and Dugbe viruses, and should be carefully investigated as it might result in the creation of a second or third genus in the Bunyaviridae; unfortunately restrictions by the Department of Agriculture, U.S., preclude such study in our laboratory.

TABLE I

Results of HI and CF Tests with CHF-C Group Viruses

		Antigen, 8 units					
		CHF-C					
		IbAr 1200		Ug 3010		Hazara	
Test no	Serum	HI	CF	HI	CF	HI	CF
1	Man, convalescent CHF	80 (a)	-	-	-	40	-
	Man, convalescent Congo fever	20	-	-	-	0	-
	Mouse, immunized,CHF-C, JD 206	160	-	-	-	20	-
	Mouse, immunized, Hazara (serum A)	640	-	-	-	1280	-

	Sheep, presumed natural exposure to CHF-C or a related virus	640	-	-	-	80	-
2	Mouse, immunized, CHF-C, IbAr 10200	640	256	640	256	80	16
	Mouse, immunized, CHF-C, Ug 3010	160	256	640	256	20	16
	Mouse, immunized, Hazara (serum B)	160	8	160	8	320	128

a) Reciprocal of serum titer; 0, no inhibition at serum dilution 1:10, lowest used.

TABLE 2

SEROLOGICAL RELATIONSHIP BETWEEN CCHF AND NAIROBI SHEEP DISEASE (NSD)
VIRUSES (From : Dr. F.G. Davies, Kabete, Kenya)

-	Antigen NSD		
Mouse ascitic fluid	CF	IF	IHA (a)
NSD	128 (b)	640	10240
NSD (Gangam)	64	320	2560
Dugbe	4	20	< 20
Hazara	trace	20	320
CCHF (IbAr 10200)	0	10	320
Bhanja	0	0	<20
T-176 (c)	4	20	320
Palyam	0	0	<20
Ephemeral Fever	0	0	<20

a) Indirect hemagglutination with tannic acid treated sheep erythrocytes.
b) Reciprocal of serum titer.
c) Virus isolated from *Amblyoma cohaerens*.

TABLE 3

SEROLOGICAL RELATIONSHIP BETWEEN CCHF AND NAIROBI SHEEP DISEASE (NSD)
VIRUSES BY COMPLEMENT-FIXATION TEST

Antigen		Mouse serum or ascitic fluid			
		NSD	Ganjam I 3159	Ganjam I 619	Dengue 1
CCHF	Drozdov	0	0	0	
	U3010	4/2	16/4 - 4/2	4/2	0
	IbAr 10200	0	0 - 0	0	0
	JD 206	0	0		0
Hazara			trace	0	
DGK			0	0	
Uukuniemi			0	0	
Nyamanini			0	0	
Lanjam		0	0	0	0
WEE		0	0	0	0
Normal			0	0	

Reciprocal of serum titer/reciprocal of antigen titer;
0, no fixation at dilutions 1:2 of serum and 1:2 of antigen.

Antigenic comparison of strains. CCHF virus has a wide geographic and ecologically varied distribution, as will be seen later. By analogy with some other arboviruses it could be anticipated that such distribution might be reflected in antigenic differences; thus far this has not been the case.

Investigation of antigenic differences among strains of this virus has been limited by technical reasons; the only methods generally applicable until now have been CF and AGDP. Serological methods that could detect small but reproducible strain differences are the plaque reduction (PR) neutralization and the kinetic HI tests, neither of which is generally applicable to all strains of the virus. CCHF virus replicates poorly or not at all in most cell lines tried with one exception, CER cells, and in all instances with no visible CPE under fluid medium. Plaque formation by some but not all strains has been reported in LLC-MK2, CV-1 monkey cell line and African green monkey kidney primary cultures; such plaques are usually very small, delayed in appearing and require skilled technical handling. HA antigens are not easily prepared with most strains, require special conditions in carrying out the test and the titers at best are in the order of 1:50 to 1:100.

Replication of the virus in CER cells, a hamster kidney cell line of somewhat dubious parentage (Smith et al.(1) , is excellent but again with no CPE or plaque formation; the use of foci of infection detected by immunofluorescence (IF) and their neutralization by immune sera may lend itself to quantitative serological comparisons, as may also be done by titration of sera by indirect IF; no results are yet available.

An illustration of the type of cross-reactions among isolates of the virus observed by CF is shown in Table 4, in which isolates from widely separated areas were compared; they are nearly identical.

TABLE 4
COMPLEMENT-FIXATION TEST
Cross reactions among six strains of Congo-Crimean hemorrhagic fever virus

Antigen, strain	serum					
	3010	10200	10248	7620	206	Drozdov
Congo Ug 3010	**128/512**	256/1024	256/512	256/1024	128/512	128/512
Congo IbAr 10200	128/256	**256/256**	256/256	256/256	128/256	128/256
Congo IbAn 10248	64/512	256/512	**256/1024**	128/512	128/512	128/512
Congo IbAn 7620	128/256	512/256	256/256	**512/512**	256/128	256/256
Congo Pak JD 206	128/512	256/512	128/512	256/512	**128/512**	256/512
Crimean HF, Drozdov	128/1024	256/1024	128/1024	256/1024	128/1024	**256/1024**

Reciprocal of serum titer/reciprocal of antigen titer.

World distribution. A notable feature of this virus and of the disease it causes is its wide geographic distribution which encompasses the three continents of the Old World and three faunal regions: Palearctic, Oriental and Ethiopian.

As far as is known natural infection with the virus causes disease in man only; infection of lower animals has been abundantly documented by serological surveys that have included domestic animals-- cattle, sheep, goats, camels--but no disease has been reported in them.

In the accompanying map, Figure 1, are shown the distribution of the virus' activities :

Fig. 1. World distribution of Crimean-Congo Hemorrhagic Fever Virus

DV   - disease, virus
D   - disease, no virus
V   - virus, no disease
A   - antibodies, man
Aa   - a., lower animals

1) Disease confirmed by virus isolation and development of antibodies in survivors has occurred in Bulgaria, USSR (Crimea, Rostov-on-Don, Astrakhan, Armenia, Azerbaijan, and the Central Asian Repubiics of Kazahk, Uzbek, Turkmen, Kirgiz and Tadzhik), Pakistan, Uganda and Zaire. Laboratory infections are not included.

2) Disease, clinically diagnosed, unconfirmed by virus isolation has been reported in Yugoslavia.

3) Virus in the absence of naturally acquired disease has been isolated in Senegal, Nigeria, Central African Empire, Kenya and Ethiopia.

4) Antibodies in man, not associated with disease, have been reported in Yugoslavia, Turkey, Iran, India, Nigeria and Egypt.

The antibodies in Yugoslavia were, in our estimate, questionable; about 15 sera presumably containing CF antibodies when tested in that country were sent to our laboratory where they were found to be negative or non-specific.

Antibodies in Turkey were detected in the course of a seroepidemiological survey conducted at YARU (Serter, Casals, and Buckley, 1975, unpublished observations). About 1100 sera were tested by HI of which 26 were positive: 18 at dilution 1:20, 6 at 1:40, and one each at 1:80 and 1:160. By PR test, 8 of these 26 sera were positive of which 7 had a titer of 1:10 and one a titer of 1:40 against 80 PFU; see Table 5.

TABLE 5

ANTIBODIES AGAINST CCHF VIRUS DETECTED IN SEROLOGICAL SURVEYS
CONDUCTED AT YARU

Origin	Year collected	Species	Number	All sera	Test Selected sera				
				AGDP	AGDP	HI	CF	N	PRN
Iran	1972-73	Man	351	48	30/55	5/55	0/38	0/15	
		Sheep	728	280	77/105	68/105			
		Goat	135	48					
		Cattle	130	23					
		Camel	157	0					
		Small mammal	274	8					
Turkey	1974	Man	1074			26			8
West Africa	1973-75	Man	636				1		

Antibodies in Iran, were first reported by Chumakov and Smirnova (2) in sheep and cattle. In a survey conducted in our laboratory (3) the results shown in Table 5 were noted; antibodies were found in sheep, goat, cattle and a few small mammals. The antibodies in man, detected by AGDP open to question by ourselves as being to a great extent nonspecific; but there is little doubt that at least 5 persons had significant and specific titers by HI.

Antibodies in man were reported in India (4) by AGDP test; 9 of 633 human sera were positive, originating in Pondichery and Kerala States (Southern India). The same authors also reported antibodies in lower animals.

Neutralizing antibodies in man have been reported in Nigeria 5 by means of a mouse intracerebral test; 24 of about 250 persons had a log neutralization index of 1.5 or greater. This seems to be a very high proportion of positives by this test, particularly in view of the fact that no reports are available of natural disease.

Antibodies in lower animals but not in man, have been reported from other parts of the world, mainly by AGDP test, which test in our estimate is not without problems of specificity. The data have been assembled by Hoogstraal (6). Two bats, species not identified, of 19 were positive in an area of the French Pyrenees near the Catalan border; 6 of 687 cattle and 15 of 48 sheep in Hungary had antibodies; about 10% of 233 cattle were positive in Afghanistan (7,see 6) ; a number of sheep, goat and camel as well as 1 human sera were found positive by CF in Egypt (Darwish et al., 1977 unpublished). Antibodies in lower animals, particularly in sheep and cattle, have also been detected in areas in which the virus has been isolated.

The Disease. From descriptions by USSR and Bulgarian authors, it is evident that CHF is a truly hemorrhagic fever in which acute loss of blood is often a life threat. This conclusion has been borne out in a recent nosocomial episode in Pakistan, March 1976, in which there were 6 secondary cases; the index and 5 secondary cases had severe hemorrhages which may have been the cause of death in 3 of the 4 fatal cases.

The clinician with undoubtedly the most first hand experience with this disease as it occurs in the Soviet Union, based on observation of more than 150 cases in the Rostov-on Don area, is Dr. E.V. Leshchinskaya; she has on two occasions given the members of the US Hemorrhagic Fevers Delegation to the USSR the benefit of her knowledge. The following description is based on her reports.

The disease is generally acquired as the result of infection by a tick-bite or, in fewer instances, by contagion. The incubation period is between 2 and 7 days; the onset is sudden, acute, with fever, chills, severe muscular pains,

Intense headache, vomiting and pain in the epigastric and lumbar regions. Hemorrhages develop from the 3rd to the 5th day of onset and appear in the skin-from petechiae to purpura--and from mucous membranes, nasal, gingival, gastric, intestinal, urinary and uterine; the patients are in critical condition at this time. Physical examination reveals marked lassitude and somnolence, at other times altered consciousness and irritability; there is dry tongue with blood crusts, injected conjunctiva and numerous skin hemorrhages, many at the sites of injections. The pulse is slow to begin with but with blood loss soon becomes fast and feeble, along with lowered blood pressure and dull heart sounds, all indicating impending shock and vascular collapse. Palpation of epigastric region is very painful; liver and spleen are often enlarged. Leucopenia and thrombocytopenia are usual. Death occurs in from 5 to 30% of cases, usually on days 7 to 9 from onset. Autopsy reveals numerous hemorrhages in many organs and often large amounts of blood in stomach and intestines. In favorable cases, the temperature falls between the 10th and 20th day, hemorrhages diminish and the patient improves; the convalescence is long, from 2 to 4 weeks, with a tendency to fatigue and weakness. Loss of hair may occur.

In addition to the severe cases, there occur also according to Leshchinskaya, mild forms with a 2 or 3 days febrile course and no hemorrhages.

The disease in Africa has been reported in 12 cases occurring between 1956 and 1965 (8); one of the patients died 8 or 10 days after he left the hospital with gastric hemorrhages, caused by a gastric ulcer. No association with tick bites was reported in any of the patients; some had contracted the disease as a result of laboratory exposures.

The existence of inapparent infections with this virus has been questioned or denied by some Soviet investigators, on the grounds that they had found antibodies only in persons who had had the disease. On the other hand, Bulgarian investigators (9) in a survey by CF and AGDP of 3012 persons found antibodies in 156 who came from endemic areas and none in 586 originating in non-endemic areas; since not all the positives had a recollection of having had the disease, this was taken as evidence for subclinical infection. As mentioned above, antibodies were detected in man in Nigeria, Turkey, Iran and Egypt; however, in most of the positives there was no way of ascertaining whether they had had overt infections, severe or mild, in the past.

There is need for improvement in the tests used for seroepidemiological surveys with this virus. Some of the neutralization test results reported are open to question due to the known non-specific neutralizing activity of normal sera from many anwmal species, including man, on the virus (10). The majority of surveys

reported have been done by CF and AGDP tests; even in persons who had an overt infection there is a marked loss of positives within 3 or 4 years from onset, particularly among those who had a mild disease

The use of IF for antibody surveys must be considered. Twenty-nine sera from as many persons who had been diagnosed as cases of CCHF in Bulgaria, were kindly supplied to us by Dr. V.E. Vasilenko; they were tested by indirect IF in our laboratory using as antigen CER cells infected with CCHF virus, strain IbAr10200 with the result shown in Table 6. The sera were tested only at dilution 1:4, except 2 that were titrated and had titers, respectively, 1:32 and 1:64. Assuming that the patients resided in the endemic areas, the possibility of boosting exposures after their initial disease cannot be excluded as an explanation for the long-lasting antibodies.

TABLE 6

ANTIBODIES IN PERSONS RECOVERED FROM CCHF IN BULGARIA DETECTED BY IMMUNOFLUORESCENCE. SERA COLLECTED IN 1973 SUPPLIED BY DR. S.M. VASILENKO

Years after onset	Number	Positive	Negative
18-19	8	5	3
6-9	11	9	2
1-5	9	8	1
?	1	1	

Sera tested at dilution 1:4 only.

Epidemiological Considerations. The association of CHF with ticks has been established since the 1944-45 outbreaks in Crimea. After 1967, due in great part to the use of the newborn mouse as experimental host, great progress has been made not only on the characterization of the virus but on the epidemiology of the disease.

1. Biological properties. Certain biological properties of the virus have epidemiological significance. As far as it is known, the virus in nature causes disease in man only; there are indications, however, that infection of cows and sheep may be accompanied by a febrile reaction. The virus has been isolated from naturally infected cattle, goat and hedgehogs; and hares (7). Experimental inoculation of the virus by peripheral routes to 2 month old calves and 2-to 2 1/2 month old lambs results in viremia from 1 to 10 days after injection, in the absence of disease .12). Hare (Lepus europaeus) after intracardiac or intramuscular inoculation also develop viremia for a similar period with titers up to $10^{-4} / 0.02$ cc, again with no disease (13).

The recognition of CHF foci with very large numbers of birds, rooks, heavily parasitized by ticks including species closely connected with the disease in man, led first to the assumption that these birds may be a reservoir of the virus. However, in spite of numerous attempts CCHF virus has never been isolated from birds, some heavily infested with ticks from which virus was isolated; nor have

antibodies been found in birds with one questionable exception. Furthermore, experimental inoculation of rooks and other birds did not result in viremia nor antibody development.

Naturally acquired antibodies have been reported on numerous occasions in domestic animals, chief among them cattle, sheep, goat and camel; also in a number of wild animals including halre, tolai hare (*L. capensis*) and jird.

2. Isolations from arthropods. Attempts to isolate CCHF virus from mosquitoes in the Soviet Union and elsewhere have been uniformly negative. Although Causey et al. 14 isolated a strain from 1 to 377 pools of Culicoides species, the evidence is that insects have no part in the cycle of maintenance of the virus in nature.

Ticks. A remarkable feature of CCHF virus is the large number of tick species from which it has been isolated; in this respect, the virus is unique among tick-borne arboviruses, none approaching it, as stated by Hoogstraal (6) who compiled the data, in the "diversity of the reservoir-vector species linked with it and the numerous ecological environments in which it circulates". As shown in Table 7, CCHF virus has been isolated from 25 different species or subspecies including one from which no actual isolation was made but which was found to contain virus antigen by IF.

TABLE 7

TICKS FROM WHICH CCHF VIRUS HAS BEEN ISOLATED

(From Dr. Harry Hoogstraal, 1977)

Argasidae	Argas persicus	Eurasia
Isodidae	Amblyomma variegatum	Africa
	Boophilus annulatus	Eurasia
	Boophilus decoloratus	Africa
	Boophilus microplus	Eurasia
	Dermacentor daghestanicus (x)	Eurasia
	Dermacentor marginatus	Eurasia
	Haemaphysalis punctata	Eurasia
	Hyalomna anatolicum	Eurasia
	Hyalomna asiaticum	Eurasia
	Hyalomna dedritum	Eurasia
	Hyalomna impeltatum	Africa
	Hyalomna impressum	Africa
	Hyalomna marginatum marginatum	Eurasia
	Hyalomna marginatum rufipes	Africa
	Hyalomna marginatum turanicum	Eurasia
	Hyalomna nitidwn	Africa
	Hyalomna truncatum	Africa
	Ixodes ricinus	Eurasia
	Rhipicephalus bursa	Eurasia
	Rhipicephalus pulchellus	Africa
	Rhipicephalus pumilio	Eurasia
	Rhipicephalus rossicus	Eurasia
	Rhipicephalus sanguineus	Eurasia
	Rhipicephalus turanicus	Eurasia

(x) No isolation, detection in salivary glands by immunofluorescence.

An important epidemiological question concerns survival of the virus in ticks. Experimental studies by Lee and Kemp (15) with *Hyalomma m. rufipes* fed on calves; by Zgurskaya et al. (16) with *H.m. marginatum* fed on hare and hedgehogs; and by Kondratenko (17) with *H.m. marginatum, R. rossicus* and *D. marginatus* fed on suslik give evidence of transstadial survival and, even more important, transovarian transmission in these ticks. The fact supports the view that ticks are, not only the vector but a reservoir as well, perhaps not the only one but capable of maintaining the virus activity in certain foci for long periods of time. Once an individual tick is infected the virus seems to persist in it for the arthropod's life time, which given the life expectancy of the tick, is more than sufficient to carry the virus from an epidemic season to the next.

3. Natural cycle of the infection. Most ticks epidemiologically and virologically associated with CCHF virus infection in Eurasia are two-or three-host species (6). The greater the number of vertebrate hosts parasitized, the greater are the chances of maintaining and amplifying the virus and transmission

to man. Immature forms of these ticks, larvae and nymphs, stay on the same host, usually birds or small mammals; on detaching they molt to adults on the ground. The adult attaches to larger animals, cattle, sheep or man; the females oviposit and the egg develops into larvae which will then attach to ground feeding birds, hare or other smaller mammals. Since birds are not susceptible to the virus, their role is to amplify the number of ticks and to disperse them.

The vector most commonly associated with disease transmission in Central Asia is H. anatolicum anatolicum; in the European part of the USSR, *H. marginatum marginatum*; and in Bulgaria, the latter and also *Ixodes ricinus.*. There has been little opportunity to investigate the question in Africa, for lack of disease.

H.m. marginatum is an efficient reservoir of the virus (Hoogstraal, 1977, in preparation). Among mammals, the hare (*L. europaeus*) is considered an important reservoir in the European USSR and Bulgaria; in Asia its role may be played by hedgehogs, gerbil, and the tulai hare (*L. capensis*).

4. Disease incidence. There seems to be no central epidemiological bureau in the USSR that reports the nation-wide annual incidence of the disease.

In Crimea the disease occurred in 1944 and 1945 with a total number of about 200 cases; in the subsequent 25 years cases occurred sporadically, not annually and were few in number. From 1970 to 1974 no cases were seen, in spite of the fact that the virus was repeatedly isolated from ticks (18). In the Strakhan area, about 150 cases in all were reported between 1955 and 1969; in the Rostovon Don district, 321 cases were registered between 1963 and 1969 but very few in later years (19). The incidence in the Central Asian Republics of the USSR is difficult to document with accuracy; there have been numerous anecdotal reports but little overall coverage. it appears that few, if any, cases of the disease have been observed in the USSR in recent years (Gaidamovich, 1977, personal communication).

In Bulgaria, 717 cases were observed from 1953 to 1965 (20); in the period between 1968 and 1973, about 130 cases were reported (21).

Twelve cases of Congo virus disease have been described in Africa, between 8 1956 and 1965, of which one was fatal and 5 were laboratory acquired (8).

The most recent outbreaks of the disease have occurred in Pakistan in 1976, one in January-March in the northern part of the country involving 14 persons; and the second in March and April involving 17 cases, in the south-west in the proximity of Quetta (Burney, 1977, personal communication).

5. Transmission to man. Man acquires the disease in two different ways through a tick bite or contact or by contagion.

The great majority of cases are due to infection after a tick bite; occasionally the tick may not have actually bitten but the patient squashed it between his fingers as a means of self protection. This type of infection occurs in persons who have outdoors occupations, farmers, dairy maids or woodsmen.

A substantial number of cases of CHF have been acquired by contagion, particularly through contact with the blood of patients with hemorrhages, either in the home or in hospitals. The disease appears to be more severe following this type of exposure than after tick bite, probably due to the large mass of virus penetrating the body of the victim either through mucosae, inhalation, cuts or abrasions. The Soviet literature has abundant descriptions involving hospital personnel or relatives and friends, particularly in Central Asia, in which 5, 6 or more secondary cases developed following close contact with a bleeding index case. In Bulgaria (20), in a period of 13 years, 42 cases with 17 deaths occurred in medical and nursing personnel. During the JanuaryMarch, 1976 episode in north Pakistan, 6 secondary cases resulted from exposure to the index case, with 3 deaths, including a surgeon who operated on the index case; 7 tertiary cases were either moderately severe or mild, with no deaths.

Another source of contagion is in the laboratory, by exposure to infected materials and either i nhalation or mucosae absorption; Badalov et al. (22) describe the infection of a laboratory assistant who died following heavy exposure to the contents of a broken centrifuge tube. Laboratory infections have also been reported in Africa.

Exposure to the blood of infected animals, particularly cattle and sheep, during slaughtering or wool shearing has led to severe, often fatal infections. Chumakov et al. 7 describe an occurence in 1973 in which three farmers who skinned a sick cow were taken ill, hospitalized and died 6 to 8 days later.

Specific Diagnosis. After Butenko, in 1967, introduced the use of newborn mice intracerebrally inoculated with blood from the patient, virus isolation is easily accomplished. In a series of cases, Butenko et al. (23) reported 40 isolations from 47 blood samples taken between days 1 and 7 from onset, and 4 isolations from 10 samples taken from day 8 to 12. The highest titers of virus in the blood with an LD50 of $10^{-4.5}$/0.02ml, were between days 1 and 5 from onset.

Antibodies have been routinely detected by CF and AGDP tests, and perhaps lately, by IF; the neutralization and plaque reduction tests are not satisfactory. CF and AGDP antibodies are

observed (11) first between 11 and 15 days from onset and remain positive with diminishing titers for at least 3 to 5 years.

Unresolved Questions. As with other viral hemorrhagic fevers a high priority problems to resolve concerns rapid, early diagnosis. Even with current methods the time required for specific diagnosis might possibly be shortened; testing pressure smears of brain tissue from mice inoculated with suspect material or CER cells in chamberslides similarly inoculated by IF may well shorten the time required by from 1 to 3 days.

The fastest way to make a diagnosis would be by detecting the virus or its antigens in clinical specimens. Considering the high titer of virus in the blood between days 1 and 5, reportedly $10^{6.2}$ LD50/ml, it may be possible to detect it by means of IF or radioimmunoassay.

A highly sensitive test for detection of neutralizing antibodies requires urgent development, not only for seroepidemiological surveys, but also for the determination of possible antigenic differences among strains of this virus. Conceivably studies of subvirionic particles might help in resolving the question of antigenic differences; however, the extent to which techniques for purification, fractionation, and polynucleotide analysis can be used may be curtailed by the risk involved in studies of the kind with this agent.

There are undoubtedly many other unresolved questions with CCHF virus, particularly in epidemiology and treatment; we have only pointed those in fields with which we are more familiar.

ACKNOWLEDGEMENT

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DISCUSSION

J. McCormick : Dr. Casals, if we are looking in an acutely ill patient for fluorescent antibodies against this organism, how early should we expect to see it ?

J. Casals : Antibodies seem to appear by the eighth, ninth, tenth day. The Soviet studies indicate that they rise for the first month and then gradually go down so that by the end of the third, fourth year they are still positive. Those are by complement fixation and by agar gel diffusion. I must say that following exposure, virus circulates in the blood until the tenth day at least.

RICKETTSIOSES: CURRENT PROBLEMS OF CLINICAL INTEREST

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At least one rickettsial disease is present in most countries whenever attempts have been made to identify them. Rats, fleas and crowded populations, particularly in port cities, favor the presence of murine typhus fever. Its close relative, epidemic louse-borne typhus fever, has ravaged humans during wars, famine or in poverty and now exists in the cold environments of Europe, Africa, the Middle East, the Asian subcontinent, Latin and South America. Louseborne typhus fever remains an important cause of morbidity and mortality where environmental and socio-economic conditions allow proper interplay between the microbe, susceptible populations and the vector. During 1976, a total of 8,065 cases with 106 deaths were reported to WHO with principal foci in the highlands of Central Africa including Burundi, Rwanda and Southern Uganda. A few cases were reported from Nigeria and only two from Algeria. Bolivia, Ecuador and Peru in South America reported a total of 223 cases with eight deaths. Ethiopia has reported no cases since 1971. (Weekly Epidemiological Record, World Health Organization, Geneva, 8 July 1977, pp. 221-223). Patients with Brill-Zinsser disease are the reappearing ghosts of typhus fever and serve as human reservoirs of *Rickettsia prowazeki*.

Rocky Mountain spotted fever in the United States and Sao Paulo typhus in Brazil are the most significant and clinically severe types of the tick-borne group. There has been a steady increase in the number of patients with Rocky Mountain spotted fever in the United States. For many years, approximately 500 cases occurred annually with a mortality of about 20 percent prior to the availability of antibiotics. After introduction of specific therapy in 1948, the number of reported cases decreased to a low of about 200 in 1959, 1960, and 1961. The decrease was probably influenced by the widespread use of broad spectrum antibiotics early in the illness and under-reporting.

Since 1969, there has been a gradual increase in annual incidence with approximately 900 cases reported in 1975 and 1976, and a cumulative total of 1,102 for the forty-ninth week of 1977, ending December 11, (Morbidity and Mortality Weekly Report. Center for Disease Control, U.S. Department of Health, Education and Welfare, P.H.S. December 16, 1977, vol. 26, n° 50). Transformation of farms into housing developments and recreation in wooded areas probably accounts for the added exposure to infected ticks particularly in the South Atlantic States where the largest number of cases occur.

In the Eastern Hemisphere and elswhere, the tick-borne rickettsioses are milder and known variously as fièvre boutonneuse, South African, North Asian, Kenya, Indian, Siberian, Queensland tick-typhus and others. Mite-borne rickettsialpox with its rodent reservoir is meddlesome rather than important.

Scrub typhus fever (tsutsugamushi disease) occurs in Japan, China, Australia, the Southern Islands, Malaya, Indonesia, Burma, Thailand and the Asian subcontinent. It is mite-borne with a rodent reservoir and can be mild or lethal.

Q fever is the lone rickettsial infection unassociated with an exanthem which is mild unless manifested in the chronic forms of hepatitis or endocarditis which are become increasingly clinically significant.

Unlike smallpox, said to be on the verge of extermination, human rickettsial diseases will persist because rickettsiae and their animal-vector host have adapted to a firmly established existence with man as an accidental sporadic victim. Small mammals become inapparently ill and rickettsiae persist indefinitely in their tissues. Ticks, fleas and mites survive their infections and often transmit the agent transovarially. Only lice die of typhus infection. It remains unclear whether *R. mooseri* (murine typhus) may mutate to the characteristic *R. prowazeki* by passage through lice. In contrast to variola with its distinctive vesicle, patients with typhus and recurrent typhus often escape diagnosis which can make them a continuing source of infection. It is conceivable that patients with other rickettsial diseases, such as Rocky Mountain spotted fever, murine typhus and Q fever, may experience recurrent infections of the Brill-Zinsser pattern. This type of recrudescence has not been reported.

Physicians must learn to accept that sporadic cases of rickettsial diseases will occur and they must learn to identify and treat them, based on suspicion and good clinical judgement, aided by laboratory

tests. Judicious use of vaccines and other control measures should help limit them to a sporadic existence.

An excellent review (1) and the comprehensive statistical reports of the World Health Organization, describe well the global presence of rickettsial diseases which will not be recounted.

The orientation of this paper is clinical. Patients seriously ill with rickettsia] diseases recover promptly with proper supportive measures and specific effective antibiotics. Unfortunately, delays in making an early specific diagnosis often allow unabated vascular and tissue changes which lead to death or other sequelae.

To be described are: 1) the clinical findings of an early case and the pathophysiologic abnormalities and management of a patient severely ill with Rocky Mountain spotted fever which is the prototype of severe rickettsial infection and 2) a method of confirming the presence of rickettsiae in human skin as early as the third febrile day of Rocky Mountain spotted fever.

ROCKY MOUNTAIN SPOTTED FEVER

Illustrative Case Report of Severe Rocky Mountain Spotted Fever (2,3)
A previously healthy 4-1/2 year old boy was hospitalized in May because of fever and rash of about eight and five days, respectively. Despite symptomatic treatment and intramuscular penicillin given four days before admission, the child's condition deteriorated. The rash began as "little pink-red spots" on the forearm which the mother assumed to be measles.

The child lost considerable weight because of anorexia, mild diarrhea and vomiting which occasionally produced coffee-ground material. Generalized malaise, frontal headache, fever of 102° to 106° and rash persisted.

As the child's condition worsened, the eruption spread to the upper arms, legs face and trunk; it was most extensive on the arms and legs. Two days before hospitalization, the rash assumed the appearance of blood blisters.

Because of her son's increasing lethargy, the mother sought medical attention. In hospital, multiple laboratory tests showed: normal cerebro-spinal fluid with negative Gram stain; no evidence of pathogenic bacteria in fluid from a skin lesion; hemoglobin 12.8 gm.%; 10,500 white blood cells/cu mm with a marked left shift; serum sodium 115 mEq/liter. Before referral, the child was given 2 grams of ampicillin intravenously.

Examination on the eighth day of illness revealed a toxic, lethargic, semistuporous child responsive only to painful stimuli. A generalized petechial and confluent hemorrhagic eruption, which did not fade on pressure, was prominent on his trunk, arms and legs, with involvement of the palms and soles.

Findings included temperature 102.8 F, pulse 160, blood pressure 90/60, marked facial edema, photophobia moderate conjunctival injection and normal fundus. Tonsils were hypertrophied. Fresh and dried blood appeared on the teeth and gingivae. Nuchal rigidity was minimal, the liver moderately enlarged and spleen not palpable. Moderately severe peripheral edema and cyanosis of the left great toe, with poor capillary filling, were noted.

There was no history of measles or known recent exposure; his measles immunization status was unknown. At home, all siblings were well except for a 10 year old half-brother who was moderately ill with fever and rash.

When questioned specifically, the mother stated that four days before the onset of illness she had removed an engorged tick embedded behind the child's left ear.

Laboratory Values. Hemoglobin 12.0 gm.%, hematocrit 40%, white blood count 10,000/cu mm, pharyngeal smear and culture - no pathogens isolated, platelets 16,000, prothrombin time and partial thromboplastin time - prolonged, fibrinogenvery low; serum electrolytes: sodium 112 mEq/liter, chlorides 82 mEq/liter, carbon dioxide 19 mEq/liter, potassium 4.4 mEq/liter, blood urea nitrogen 35mg%, serum albulin 1.8 gm.%; initially, urine was not obtainable. Disseminated intravascular coagulopathy (DIC) was assumed to be present based on the hemorrhagic diathesis involving the skin and gums, the hemogram and serum clotting values.

Treatment. On hospitalization, the child was monitored in an intensive care unit, and within two hours was treated with chloramphenicol given intravenously. General supportive management included oxygen therapy, corticosteroids and judicious administration of dextrose and isotonic saline, given intravenously, sufficient to support the circulation, maintain urinary output and avoid induction of further tissue edema. Heparin was not given.

Course. The child's blood pressure remained stable during the first twentyfour hours. He remained semi-stuporous, agitated and critically ill. The rash became more confluent and hemorrhagic.

Within forty-eight hours of hospitalization, the clinical diagnosis of Rocky Mountain spotted fever was presumptively confirmed serologically by the finding of Proteus OX-2 agglutinins in the titer of

1:160. Cultures of the pharynx, blood and cerebrospinal fluid were negative. Penicillin was discontinued. Treatment with chloramphenicol, corticosteroids and fluids led to defervescence in thirty hours.

The child responded by the third hospital day (eleventh day of illness), when the electroencephalogram showed evidence of diffuse encephalopathy.

One week later, a second electroencephalogram showed improvement corresponding with the patient's good progress. After seventeen days of hospitalization, he was discharged afebrile, without rash, and with only a slight personality change. The convalescent serologic titers were: Proteus OX-2 1:640 and complement-fixation for Rocky Mountain spotted fever 1:128. The electroencephalogram was normal in one year when the child was clinically well.

Case Report of Rocky Mountain Spotted Fever During First Week.

During early May, 1976, a 4 year old girl who lived in a rural wooded area in Maryland developed fever with irritability, vomiting and headache. For four days the headache worsened, she felt chilly without actual chills and each day the temperature exceeded 103ºF in the afternoon with slightly lower morning levels. Increasing fever with prostration and myalgia prompted the mother to seek medical attention.

When examined on the fourth febrile day, the child was restless and complained of frontal headache; temperature 104.5º, pulse - 110, blood pressure 90/70, injected conjunctivae, normal lung findings, heart normal size, no murmurs, slight hepatomegaly, spleen palpable 2.0 cm. below costal margin, genitalia normal. Examination of extremities and CNS normal.

Rash: A distinct pink macular exanthem involved the palms, soles, forearms, legs, thighs and buttocks with a few individual lesions on the trunk. The macules were irregular in outline, measuring 3 to 6 mm. in diameter and faded on pressure.

These findings plus the history of removal of a tick from the scalp a week prior to onset strongly suggested Rocky Mountain spotted fever.

Laboratory findings were: Hemoglobin 14.0 gms., hematocrit 40, WBC 6,350 cu mm., differential normal, slight albuminuria, serum electrolytes, BUN, glucose normal. Two male guinea pigs were inoculated each with 4.0 ml of whole blood intraperitoneally, blood serum was saved for serologic tests and a full section of a macular skin lesion was excised for identification of rickettsia.

Based on clinical manifestations, chloramphenicol was given orally with an initial dose of 50 mg/kilo body weight and similar daily doses divided equally every six hours. A high calorie diet rich in protein was used.

Within thirty-six hours the headache and irritability abated, the-child appeared stronger and the temperature reached normal levels in 3.0 days. With defervescence, the rash, which did not progress, was faint and antibiotic treatment was stopped.

Initial serum specimens were negative for antibodies when tested by the Proteus OX-19 (Weil-Felix), complement fixation (C.F.) and rickettsial microagglutination tests (M.A.) Later specimens obtained on the twelfth and twentyfirst days of illness were positive as follows: W-F:160, 320; C.F.:32, 64; and M.A.: 16, 32, respectively. Guinea pigs developed febrile reactions and after severall transfers of blood to other animals, rickettsiae were identified in several tissues by smear; serologic tests were positive. Figure 1 shows fluorescent stained *Rickettsia rickettsii* in the skin lesion taken on the fourth day.
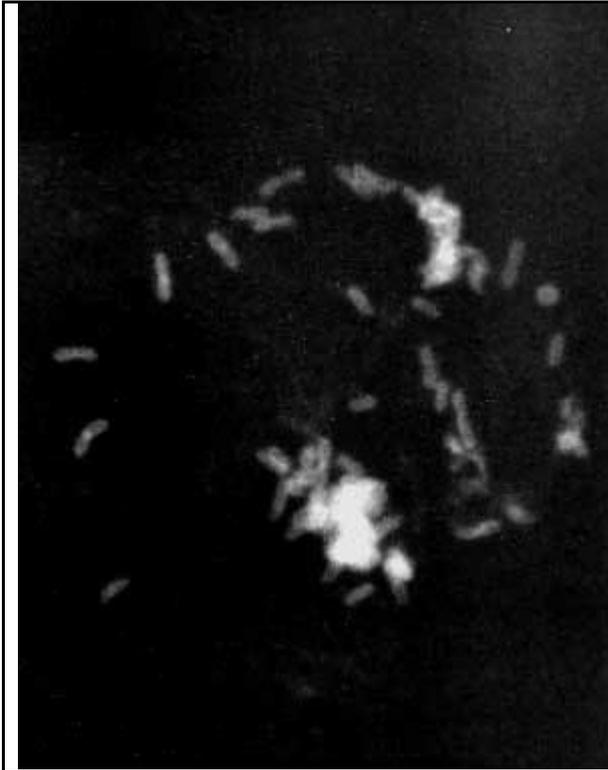
Recovery was complete.

Fig. 1 :

R. rickettsii in skin section of a pink macule taken on about the fourth day of R.M.S.F.

Preparation by Immune Fluorescense (I.F.) x 900.

Prompt Confirmation of Rocky Mountain Spotted Fever; Identification of Rickettsiae in Skin Tissues.

The physician may mistake the clues of headaches, fever, myalgia and rash of tick-borne spotted fever for measles, other viral illness or meningococcemia. These errors, which can lead to delay in treatment, are evidenced by mortality rates of about 7 percent in patients with Rocky Mountain spotted fever in the United States.

Available confirmatory laboratory aids for rickettsial diseases are the Weil-Felix (W-F), complement fixation (C.F.), rickettsial microagglutination (M.A.), and indirect fluorescent antibody (I.F.A.) reactions or the more tedious isolation of causative rickettsia in animals or tissue culture. Regardless of the serologic test chosen, results are available late in illness when serious or irreversible vascular changes or death may have occurred.

Recent attempts to provide earlier diagnostic help include identification of in either monocytes (4) or tissues (5) of experimentally infected animals. Rickettsiae were identified in stained culture monocytes of infected monkeys and by direct or indirect immunofluorescence of tissues of infected animals.

We have successfully identified *R. rickettsii* in skin specimens obtained by biopsy from patients with R.M.S.F. taken during the early and later stages of illness (6).

Five patients with characteristic manifestations of R.M.S.F. have been studied. A small section of skin from a fresh pink macule of a 4 year old girl was taken between the third and fourth days of illness. The specimen was placed in saline, transported on ice and processed promptly. Utilizing the indirect immune fluorescence (I.F.) technique, rickettsiae were identified within about four hours laboratory work time. The procedure was performed by reacting high titered antiserum conjugated with fluorescent isothiocyanate which was placed on properly sectioned skin specimens and examined by dark field microscopy. Rickettsiae have an identifiable morphology and characteristic staining property.

Shown in figure 1 is the I.F. preparation of the rickettsia visualized in the patient, who was shown to have R.M.S.F. by all other laboratory tests.

Rickettsiae were also visualized in other patients whose macular skin lesions were examined later in the first week of illness and in a severely ill, comatose girl on about the eighth day of R.M.S.F. Rickettsiae similar to those shown in figure 1 were visualized although fewer organisms were present.

A more simplified technique of using a punch skin biopsy or needle aspiration of a macule or purpuric lesion should be adaptable to other rickettsial diseases

and certain infections associated with a rash. A recent report has described paraffin fixation of tissue, such as kidney, and identification of rickettsia by I.F.(7) . This technique should be useful for retrospective study of preserved tissues.

There is a significant lag-time between the clinical suspicion of R.M.S.F. and other rickettsia] diseases (when treatment should be instituted) and laboratory confirmation. Specific antibiotic treatment given when the exanthem appears, or not later than about the sixth day of illness, is usually followed by prompt and full recovery.

When therapy is delayed until the second week of illness, extensive vascular and tissue changes may have occurred which makes recovery more difficult, delayed and occasionally followed by death. Management for these patients must include prompt specific chemotherapy and enlightened supportive care which have been described elsewhere. Recovery may occur even at the late stage, although it may be delayed and associated with residuae.

Physicians are not acquainted with rickettsial diseases because of their sporadic occurrence and during the early stages of illness, the presence of fever, headache, malaise and myalgia is non-specific. Many infectious diseases are similar in manifestations at this stage and with the appearance of a rash such infections as measles, meningococcemia and others become suspect. A method of confirmation of one of the serious rickettsioses (R.M.S.F.) by I.F. identification of rickettsiae in early and late skin lesions is now available. In selected patients, this confirmatory test is positive before the results of standard serologic procedures are available.

Certain unsolved problems in pathophysiology and clinical management awaiting solution are (2) :

Unsolved Problems

Pathophysiologic: It is unknown whether the increase in capillary permeability and tissue hemorrhages results from direct effect of rickettsiae in the vascular endothelium, by the toxin, by a combination of each or an immunopathologic reaction. Serial studies of skin lesions utilizing I.F. and other techniques would help clarify this puzzle and help determine whether corticosteroids abate this abnormality.

Increase in capillary permeability with leakage of fluid, electrolytes and albumin accounts for the mild hypovolemia and increase in extra-cellular space. It is unknown whether there is intracellular expansion. Isotopic techniques might help settle this point.

Many features of disseminated intravascular coagulopathy are present in severely ill patients although the mechanism is unclear. Heparin does not appear to be needed therapeutically. Studies of the coagulation and complement systems in primates infected with R. rickettsii intravenously showed that hyperfibrinogenemia, thrombocytopenia, prolonged prothrombin and activated partial thromboplastin times with increased serum fibrin/fibrinogen degradation products occurred with the onset of fever and rickettsiemia. Reactions were greater in fatally ill monkeys. Decreases in amounts of complement fractions C2, C3 occurred in monkeys which developed peripheral gangrenous ecchymoses. Apparently, the hemostatic disturbances in fulminant infection is a direct effect of the infectious vasculitis (8).

Answers to some of these riddles might provide better guides for supportive care including clarification of the indication for use of corticosteroids. Steroids reduce the toxic manifestations and shorten the febrile course but whether they exert a fundamental beneficial influence is unknown.

Microbial Persistence and Recrudescence. Brill-Zinsser disease (recurrent epidemic typhus fever) is an excellent example of microbial persistence in the human host. It is likely but unrecognized that such recurrences occur in R.M.S.F. Q fever, murine and scrub typhus. It would be possible to follow patients convalescent from these diseases when they undergo stress such as during other illnesses or surgical procedures. A suspicious complicating mild febrile illness might be identified by serologic methods and in the instance of recurrence of R.M.S.F. a prompt high titer rise of 7-S antibody using the C.F. or M.A. technique would be confirmatory.

There is ample evidence of persistence of pathogenic *R. prowazeki, R. rickettsii* and *R. tsutsugamushi* in convalescence which have been isolated from lymph node tissue months to years after recovery from the initial illness. Conceivably, a rickettsicidal antibiotic other than chloramphenicol or tetracycline, which are rickettsiostatic, would eradicate such persisting rickettsiae. This is a minor problem.

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DISCUSSION

M. Isaacson : You had one very severe case of generalized Schwarzman-like reaction in one of your Spotted Fever cases and we noted that the patient had been treated with steroids early on. Do you feel this may have in fact precipitated or aggravated the condition ?

T.E. Woodward : We do not think there is any connection. The only time we used steroids was in a very severely ill patient and the reason we used them is aimed at the brain oedema more than anything else. But in a number of patients, the diffuse haemorrhages occur without steroids, I don't think there is any connection. in a severely iZl patient, I would not hesitate one minute to give steroid on a short time basis. I don't think there is any evidence that one has influenced mortality, there is not enough information, but I am convinced that there is no deleterious sideeffect.

M. Isaacson : Why was chloramphenicol used in preference to tetracyclin ?

T.E. Woodward : No good reason, either one is perfectly acceptable. Tetracyclin can be given in lower dosage, but I have no real argument or point either way. We just happen to use chloramphenicol, we are well aware of what has been reported, we think that the side-effect is about 1140.000 but I quite agree with you tetracyclin is just as good.

A. Silberstein : Do you rely on the central venous pressure to determine the fluid input ? T.E. Woodward : None of the patients shown had central venous pressure measurements. I think careful monitoring, looking at skin turgor, and measuring pulse blood pressure are very useful.

KOREAN HEMORRHAGIC FEVER

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INTRODUCTION

Epidemic hemorrhagic fever with renal syndrome was recognized for the first time in 1951 among United Nation troops (1). Since that time it has been known as Korean hemorrhagic fever (KHF) and has remained endemic near the Demilitarized Zone (DMZ) between North and South Korea. In recent years the disease has invaded the southern parts of the Korean peninsula and 100 to 800 hospitalized cases are clinically diagnosed each year (Table 1).

KHF is an acute infectious febrile, often fatal, otherwise self-limited, illness of viral etiology characterized by severe toxemia, widespread capillary damage, hemorrhagic phenomena and renal insufficiency (2).

Similar diseases to KHF as shown in Figure 1, have been described by Japanese from Manchuria (3,4,5), from the Soviet Union (6,7), from Scandinavia (8,9,10), from several countries in Eastern Europe (11) and recently from Japan (12).

In early 1940's Japanese and Russians reproduced hemorrhagic fever by injection of urine and blood of the patients into monkeys (4) and volunteers. The injection of a suspension of *Trombicula* mite obtained from *Apodemus agrarius* into human caused hemorrhagic fever, and mites have been suspected as its reservoir(13). Many attempts have been made to isolate the causative agent of KHF and clinically similar disease.

In 1976 Lee and Lee (14) succeeded in demonstrating an antigen in the lungs of the striped field mouse, *Apodemus agrarius coreae*, which gave immunofluorescent reaction with sera from patients convalescent from KHF and named it as Korea antigen. In 1977 Lee and Tamura 15 reported that epidemic hemorrhagic fever in Japan is related serologically to KHF agent, if not identical. Very recently, Lee et al. (16) have reported that this antigen is the etiologic agent of KHF for the first time and convalescent sera from hemorrhagic nephrosonephritis in the Soviet Union were positive for antibodies.

This presentation is the review of KHF, recent progress of KHF research and serologic relationships between KHF agent and other hemorrhagic fevers of viral and unknown etiology.

EPIDEMIOLOGY

KHF has not been described previously in Korea before 1951, but some workers consider that its epidemicity and pathogenesis suggest the possibility of endemic disease before 1950 and it may had been missed because of lack of knowledge and its rare occurrences in rural areas due to special ecology.

After the Korean War, the disease was designated as endemic in the area of DMZ and since then has gradually spread southwesterly, Fig. 2. A conspicuous increase in the number of the civilian cases was observed in 1970's. However, most cases are still reported near the area of the DMZ where Korean soldiers are stationed. However, considering its epidemiological changing status of KHF for the past 25 years, the imported germ theory in which it originates possibly from North-Eastern part of Asia in the period of Korean War, could not be excluded (17). While a few cases usually occur throughout the year, the seasonal pattern of outbreaks marks in two peaks, small one is in June and large peak in October through December, Fig. 3. The occurrence of more than one case in the same house is extremely rare. Nor has been any information to implicate food, water, clothing in the transmission of KHF. The disease appeared to affect most frequently the age group of 21-50 years although disease cases occur in both sexes, the disease is significantly more frequent in males than in females with the ratio of two to one (18). Main victims are farmers and soldiers stationing in the field. Small numbers of patients have been reported from the surrounding towns recently. The distribution of immunofluorescent antibodies to KHF agent is 1% in city residents, 3.8% in farmers of endemic areas and 1.1% in the soldiers residing in the endemic areas. The antibodies are present some 14 years after the recovery from the disease and there is no case of reinfection yet (16).

The reservoir of KHF in the endemic areas in Korea is Apodemus agrarius coreae. There are about 7 species of field rodents in the endemic areas of KHF but only Apodemus species contained KHF agent (14,16). Three patients had occurred among the personells who dealt with naturally and experimentally infected Apodemus agrarius coreae with KHF agent in our laboratory.

The mode of transmission of the agent from infected Apodemus to normal Apodemus and to man are not clarified yet. As regards arthropod vectors, Asanuma (13) and Traub et al. (19) suggested that Trombiculla pallida was the species most closely fitted to the seasonal incidence of KHF.
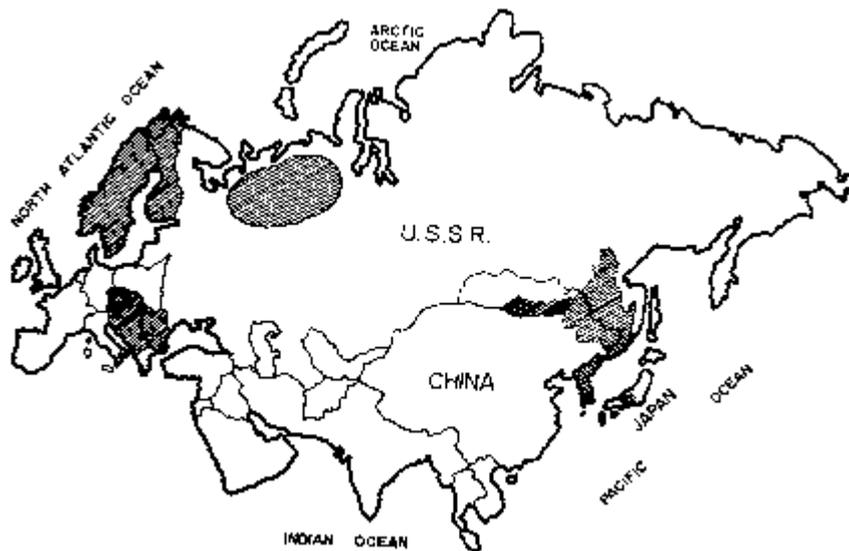

Fig. 1 Map showing endemic areas of hemorrhagic fever with renal syndrome

Japan	Epidemic Hem. Fever	Bulgaria	Epidemic Hem. Fever
Korea	Korean Hem. Fever	Hungaria	Epidemic Hem. Fever
China	Epidemic Hem. Fever	Czechoslovakia	Epidemic Hem. Fever
Inner Mongolia	Mongolian Hem. Fever	Finland	Nephropatia Epidemica
USSR	Hem. Nephrosonephritis	Sweden	Endemic Benign Nephropatia
Yugoslavia	Epidemic Nephritis		

TABLE 1

HOSPITALIZED CASES OF KOREAN HEMORRHAGIC FEVER PATIENTS

Year	US Forces	ROK army	Korean civilian	Total
1951	827			827
1952	833			833
1953	455			455
1954	307		19	326
1955	20			20
1956	28	26		54
1957	13	21		34
1958	15	20		35
1959	79	47		126
1960	10	185		195
1961	27	341		368
1962	29	311		340
1963	11	257		268
1964	22	205	18	245
1965	99	110	2	211
1966	36	82	11	129
1967	31	86	13	130
1968	28	102	13	143
1969	9	134	8	151
1970	13	221	85	319
1971	2	358	311	671
1972	0	203	186	389
1973	0	237	241	478
1974	0	251	170	421
1975	1	370	466	837
1976	4	304	521	829
1977	7	212	288	507
Total	2,906	4,083	2,352	9,341
Fatality	5.00%	6.50%	8.00%	6.50%

ETIOLOGIC AGENT

In early 1940's Japanese and Russians successfully reproduced hemorrhagic fever by injection of urine and sera of the patients in the acute stage into monkeys (4) and volunteers (6,7). Filtered sera of the patients were also produced clinical symptoms, so this disease has been suspected as being of viral origin. Many attempts have been made to isolate the causative agent of KHF and clinically similar diseases. A Russian report of cultivation of a virus in cell cultures from patients with hemorrhagic nephrosonephritis (20) has not been confirmed.

In 1976 Lee and Lee succeeded in demonstrating an antigen in the lungs and kidneys of the Apodemus agrarius collected in the endemic foci, which gave specific immunofluorescent reaction with convalescent sera from KHF patients (14) and named it as Korea antigen. Very recently, Lee et al. have demonstrated that this antigen is the etiologic agent of KHF and is produced by a replicating microbe (16). It passes 0.1 micro millipore filter and antibiotics are ineffective. Under the electron microscope round virus-like particles of about 50 nm in diameter can be observed in crystalline array at the cytoplasm of infected pulmonary epithelia of Apodemus as shown in Fig. 4 (21).

All attempts to establish the KHF agent in hosts other than Apodemus agrarius have been unsuccessful. Various species of laboratory animals as well as more than 20 types of cell cultures all failed to show specific immunofluorescent antibody staining after inoculation of the agent. Apodemus agrarius coreae infected either naturally or experimentally have never showed the clinical symptoms (16). When KHF agent is inoculated the agent begins to appear at lungs 10 days later. After then it can

be identified at kidneys, liver and submaxillary glands. The most amount of agent can be detected toward 20 days, and then start to decline gradually. However, it was still able to be detected after day-60. The agent was serially propagated in Apodemus agrarius as shown in Table 2 and study on characteristics of the agent is in progress.

Immunofluorescent antibody responses to KHF agent after subcutaneous inoculation into rabbits were demonstrated and, the antibodies started to appear at 7 days, reached maximum at 14 days and declined slowly by 60 days.
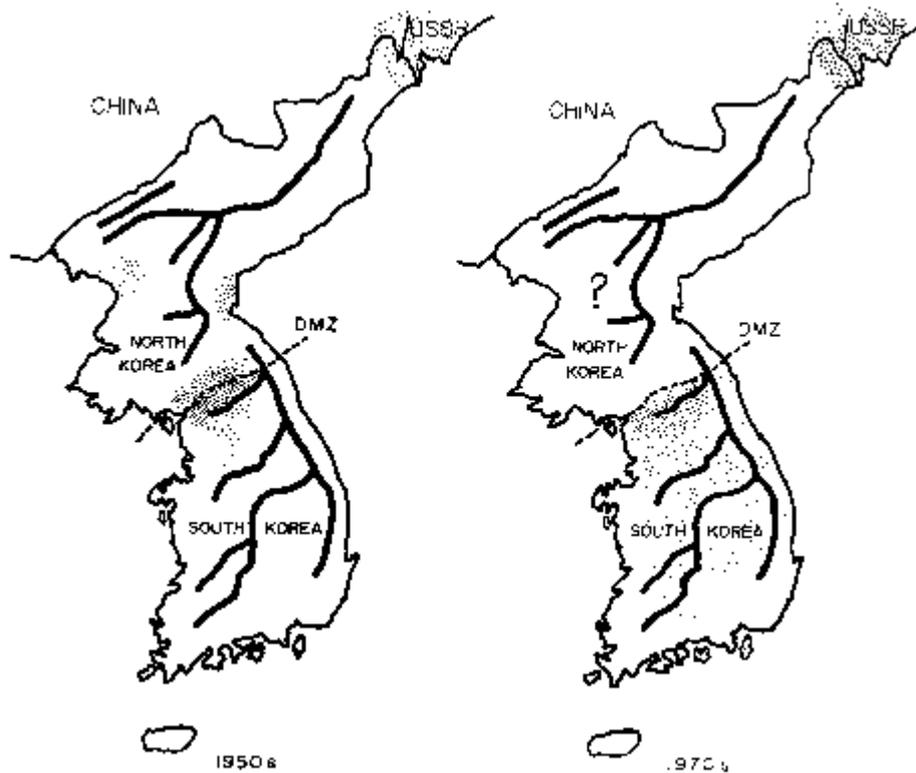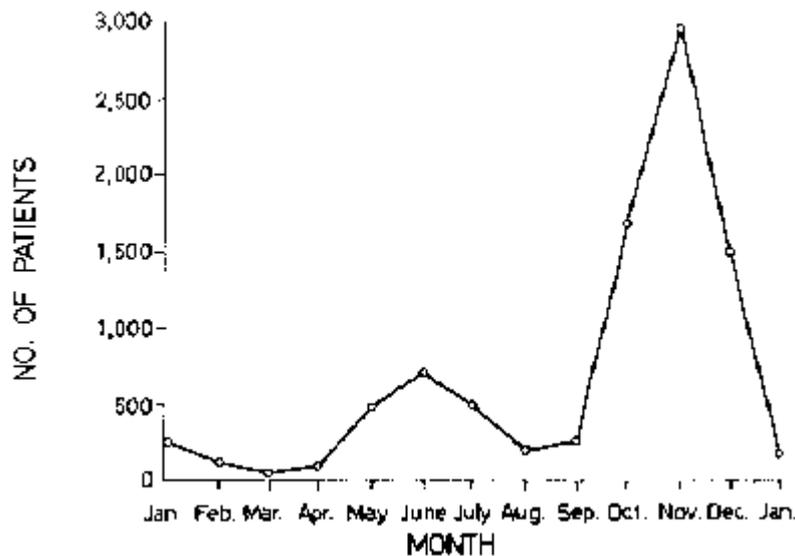

Fig. 2 : Distribution of KHF cases in Korea

Fig.3 Cumulative prevalence of Korean Hemorrhagic Fever from 1951 to 1977.

HUMAN INFECTION

Incubation period is usually 13-22 days, varying from 9 to 42 days. The disease is an acute disease characterized by sudden onset with fever of 3 to 6 days with chills, conjunctival injection, prostration, anorexia, vomiting, hemorrhagic manifestations which begin about the third day, proteinuria about

the fourth day, and hypotension about the fifth, renal disorder for several weeks. The widespread abnormalities of blood vessels, chiefly arterioles and capillaries have been considered as main and initial defect which lead to impairment of function in a number of organs. The resulting clinical, laboratory and functional features are very diverse and there is considerable variation among patients, not only in the incidence of various manifestations of the disease but also in the severity of the illness (2). Moreover the symptoms and signs of the disease are in no way specific and in the early phases diagnosis may be difficult, particularly in respect to differentiation from other febrile illness such as infections of the upper and lower respiratory tract. Approximately 70% of cases show mild course and 30% have several complications, such as shock, bleeding, renal failure, electrolyte imbalance, pulmonary edema, and secondary bacterial infections.

The first immunoglobulin of KHF patient is IgM. This is followed by the appearance and increase of IgG At the same time the immunofluorescent and neutralizing antibodies are produced. They start to appear right after onset of the disease and their levels reach the peak at the time lapse of around 2 weeks. Thereafter they slowly decline over a certain period but (14 years after the experience the antibody continues to be produced at low level. The specific antibody has been identified from the serum of the KHF patients showing not only typical severe clinical symptoms but also mild and subclinical cases (16).

DIAGNOSIS

Due to the lack of specific tests for the etiologic agent of KHF till 1976, clinical manifestation with acute renal failure and laboratory findings such as proteinuria, leukocytosis, thrombocytopenia, and elevated blood urea nitrogen and epidemiologic findings assist in establishing the diagnosis (2,23) and in the fatal case is confirmed by the characteristic lesions (24,25).

In 1976 Lee and Lee (14) demonstrated specific antigen and antibodies of KHF for the first time, and since that time serologic diagnosis of KHF patients has been routinely employed in their laboratory. Specific serologic diagnosis of KHF can be made by demonstrating the increase of specific immunofluorescent antibodies against KHF agent with the sera collected 2 times at the interval of 1 week during course of illness.

Isolation of the agent is difficult but may be recovered from serum taken in the acute stage of the infection (16).

Infected Apodemus agrarius with KHF agent can be recognized by observing the pulmonary and renal tissues by means of FA technique (14,16). Whether it has been infected or not in the past can be realized by detecting specific antibodies from the serum.

RELATIONSHIP BETWEEN KHF AND OTHER VIRAL HEMORRHAGIC FEVERS

In 1962 Gajdusek (11) described in detail natural endemic foci of hemorrhagic fever with renal syndrome and Southern Soviet hemorrhagic fever that occurring from many parts of the world and, possibility of their close relation for the first time. However, there has been no laboratory diagnostic method to study the serologic relationship of similar hemorrhagic fevers because of lack of knowledge on the etiologic agent of the disease.

Very recently, Lee et al. reported that antisera of Lassa, Machupo, lymphocytic choriomeningitis, Pichinde and Tacaribe of the arenavirus group, Marburg and Ebola were negative to KHF agent by IFA technique. In contrast, close serologic relationships between KHF and hemorrhagic fever with renal syndrome in the Soviet Union (16) and epidemic hemorrhagic fever in Japan (15) were established, shown in Table 3.

Furthermore, relationship between KHF agent and other hemorrhagic fevers of unknown etiology that occurring in Scandinavia (9,10) and in Eastern Europe (11) remains to be solved.

TABLE 2

PROPAGATION AND NEUTRALIZATION OF KHF AGENT IN APODEMUS AGRARIUS COREAE

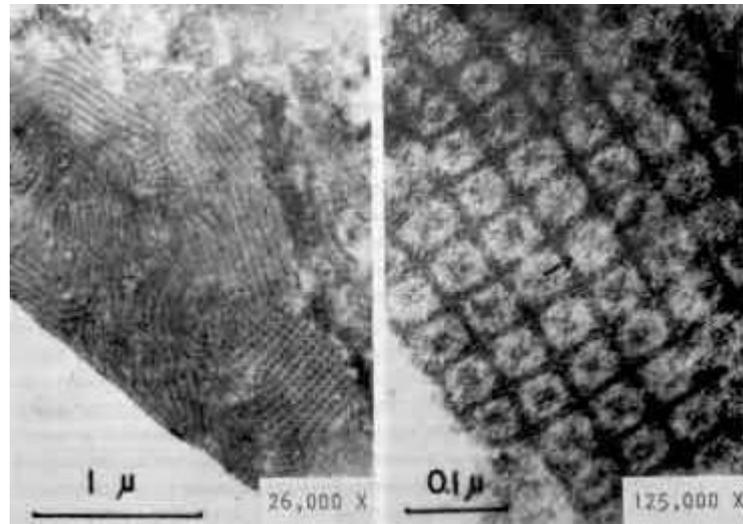| Passage number	Total days in Apodemus	Cumulative log of dilution of original inoculum	Infectivity for Apodemus	Clinical symptom	Titration Apodemus in ID50 /0.1 ml	Neutralization with immune serum ID50/0.1ml in Apodemus
1	20	1.0	10/16	0/16		
4	91	4.0	7/28	0/28		
8	171	8.0	12/12	0/12	$10^{5.3}$	$< 10^{1.0}$
11	231	21.0	21/21	0/21	$10^{7.2}$	
16	324	38.0	15/15	0/15	$10^{6.7}$	$< 10^{1.0}$

Fig. 4 : Electron micrographs of KHF agent in lung tissues of *Apodemus agrarius coreae*
A.   Virus-like particles showing clustering in pulmonary epithelium.
B.   Virus-like particles in crystalline array, round and about 50 nm in diameter.

TABLE 3

SEROLOGIC RELATIONSHIP BETWEEN KHF AGENT AND OTHER VIRAL HEMORRHAGIC FEVERS

Antigen	Name of antiserum	Immuno-fluorescent antibody test
Lung tissue of infected Apodemus with KHF agent76/118, 6th passage	Anti-Pichinde monkey serum	-
	Anti-Tacaribe monkey serum	-
	Anti-Machupo human serum	-
	Anti-Lassa human serum	-
	Anti-LCM guinea pig serum	-
	Anti-Ebola human serum	-
	Anti-Marburg human serum	-
	Convalescent sera of KHF	+
	Convalescent sera of hemorrhagic fever with renal syndrome - Soviet Union	+
	Convalescent sera of epidemic hemorrhagic fever – Japan	+

SUMMARY

Hemorrhagic fevers with renal syndrome are being reported from many parts of the world. It has been reviewed and discussed on KHF and similar diseases which occur all over the Asian and European Continents.

The number of KHF patients in not only soldiers but also civilians tend to increase every year. So it is urgent need to take some measures to prevent this disease. Recently the etiological agent of KHF was isolated, the natural reservoir was demonstrated as Apodemus agrarius coreae and the serological diagnosis also comes to be available, by means of immunofluorescent antibody technique.

The etiological agent did not react with the antisera of arenaviruses but did with convalescent sera of Japanese epidemic hemorrhagic fever and Soviet hemorrhagic nephrosonephritis patients, showing close serological relationship. The relationship between KHF and the similar diseases of unknown etiology occurring in Scandinavia and Eurasia remains to be answered.

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DISCUSSION

S.R. Pattyn : Has this material been inoculated on mycoplasma medium ?

Ho Wang Lee : It does not grow. Antibiotics are without effect on this agent.

NEPHROPATHIA EPIDEMICA

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INTRODUCTION

Nephropathia epidemica (NE) is a clinical entity with a close relationship to the hemorrhagic fever with renal syndrome. The disease has been described by Myhrman (2) and Zetterholm (2) in Sweden. In Finland the first and also largest outbreak occurred during World War II, when during three months over 1000 German and 60 Finnish soldiers in a small area in northern Finland near the Russian border fell ill (3,4). The total number of notified cases of NE in Finland after the war is about 1000 (5,6), in Sweden 400 (7,8) and 60 in Norway (9,10). The seven cases described by Hansen (11) from Denmark as "atypical acute nephritis" may also be regarded as NE.

CLINICAL FEATURES

The clinical picture of NE (for detailed description see Lähdevirta (5)) is similar much less severe than that of the hemorrhagic fever with renal syndrome. The hemorrhagic manifestations are scanty, the hematuria is usually microscopic and lasts only for 2 to 6 days. The mortality is less than 1/200.

The disease has an acute onset. Without prodromal signs the fever rises within a few hours over 39ºC. Headache, nausea, vomiting, somnolence and other neurological symptoms (in a few cases meningismus) follow thereafter. On the 3rd to 6th day the renal involvement develops manifesting as backache, abdominal pain, tenderness over the kidneys, proteinuria (mostly 1 to 1.5 g/1) and oliguria. In most cases microscopical hematuria (5 to 10 erythrocytes per field), leucocyturia and azotemia is seen, as well. The number of blood platelets is often between 50.000 and 150.000 sometimes only 20.000 per mm³. The oliguria (rarely anuria) phase of duration is followed by one week's polyuria and hypostenuria. The decreased concentration capacity of kidneys persists for several weeks. With the onset of the polyuric phase the patients' condition shows surprisingly rapid improvement. The mean duration of the hospitalization is 17 days. The persistent sequalae slight if any. NE seems to be a multisystemic infection and evidence for carditis, hepatitis and meningoencephalitis has also been obtained sometimes. We have found rheumatoid factor activity to be transiently present in the serum of NE patients during the early phase of the disease (Fig. 1). The timing of the activity suggests that it may, like in certain other acute infections (12), be an indication of temporary presence of immune complexes in circulation.
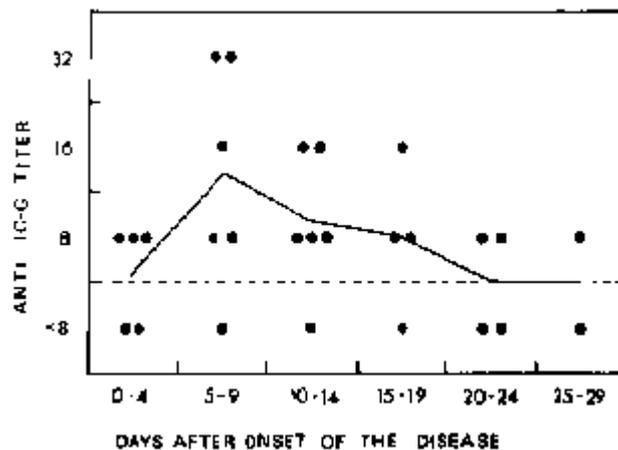

Fig. 1. Occurence of anti IgG globulins in NE patients. Samples from 14 patients have been plotted according to the time of onset of the disease. The mean titers are indicated by the line. In a control group (30 blood donors) two had a titer of 8, the others were negative.
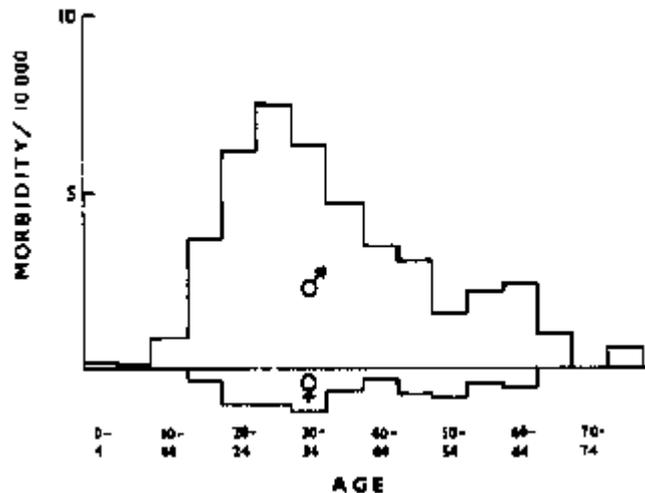
Fig.2. The age and sex distribution of NE patients in Finland (in 1966-1973) (According to Lähdevirta and Elo, 1975)

HISTOPATHOLOGY

According to Kuhlbäck et al. (13) and Lähdevirta (5) the renal histopathological picture of NE is an acute, tubular and interstitial hemorrhagic nephritis with a mild glomerulitis with the interstitial extravasation being the most prominent finding. These findings have been recently confirmed by electron microscopy (14) and by immunohistology. The latter work also suggests that immune complex deposition in kidneys may have a role in the pathogenesis of NE. A direct vascular damage by the plausible agent has not been demonstrated. The renal affection is transitory and morphological sequalae are very slight (16).

EPIDEMIOLOGY

Population distribution. The epidemiology of NE has special features. The great majority of patients (over 80%) are men, mostly at the age of 15 to 35 years (Fig.2). Females and children contract the disease less often and when ill show in average milder symptoms. Perhaps the infection is many times totally subclinical among them. The incidence of NE among the rural population is four times greater than in cities. During the epidemic among the German troops in northern Finland in 1942 the morbidity in a front line combat unit was 48% while no cases were seen in the army staff.

Geographical distribution. NE has a pronounced regional occurrence. Fig. 3 is the map of the home residents of the patients and Fig. 4 shows the morbidity in different parishes in Finland. The morbidity is highest (maximum 1.3%) in certain parishes located in the Lake Finland.

Seasonal distribution. NE has a pronounced early winter incidence. Well over half of the infections occur during November-January (Fig. 5). Most of the cases appear some weeks after the cooling of the weather on autumn.

Annual distribution. For the first time NE was encountered in Finland 1942, then in 1957. After that a periodicity of 3 to 4 years has been noted. The cases have been most numerous during the winters 1965/66, 1969/70, 1972/73 and 1976/77. The numbers of hospitalized patients in the last mentioned winters were 177 and 104.

ETIOLOGY

Several investigations have suggested that rodents are the natural reservoir of hemorrhagic fever with renal syndrome (see e.g. Smorodintsev et al. (17), Casals et al.(18), Trencséni and Keleti (19)) Lee (20) has presented in the preceding paper that the etiological agent of Korean hemorrhagic fever has been found in the field mouse (*Apodemus agrarius coreae*). In Finland (and also in Sweden (8)), the highest NE incidences seem to coincide with the years of a high prevalence of voles (Fig.6). Some other rodents have the same periodicity as the voles and so it is possible that other species also may harbour the agent in nature. However, *A. agrarius* occurs only in the southeastern corner of Finland (not at all in Scandinavia) and *A. flavicollis* in the southern and central parts of Fennoscandia. Among the voles the bank vole (*Cletrionomis glareolus*) seems to be the best candidate. It has spread over the whole of Fennoscandia (excluding northern Lapland). It seems, like NE, to avoid regions of open

fields, being a dweller of forests and bush vegetation. In the autumn, when the temperature drops, bank voles often move into barns getting into contact with each other and with men. This may well be the expansion phase of disease during which the infection spills over the human beings. Presumably later in the winter the gathered voles have passed through the infection and the disease dies out, - human cases are few in the late winter. It is possible that the mites maintain the agent at that time (21,19). The sporadic cases during the summer are for the most part urban dwellers spending their vacation in summer cottages or camping military troops close to microfoci in nature.

More direct evidence for a role of the bank vole has also been obtained. Two persons at the Department of Virology contracted NE one month after dissecting voles collected from the endemic area. There also occurred an unusual family outbreak in the heart of the city of Helsinki. The son of the family had a terrarium with bank voles collected from the endemic area. Five out of the six members of the family contracted NE one after another within two weeks (22).
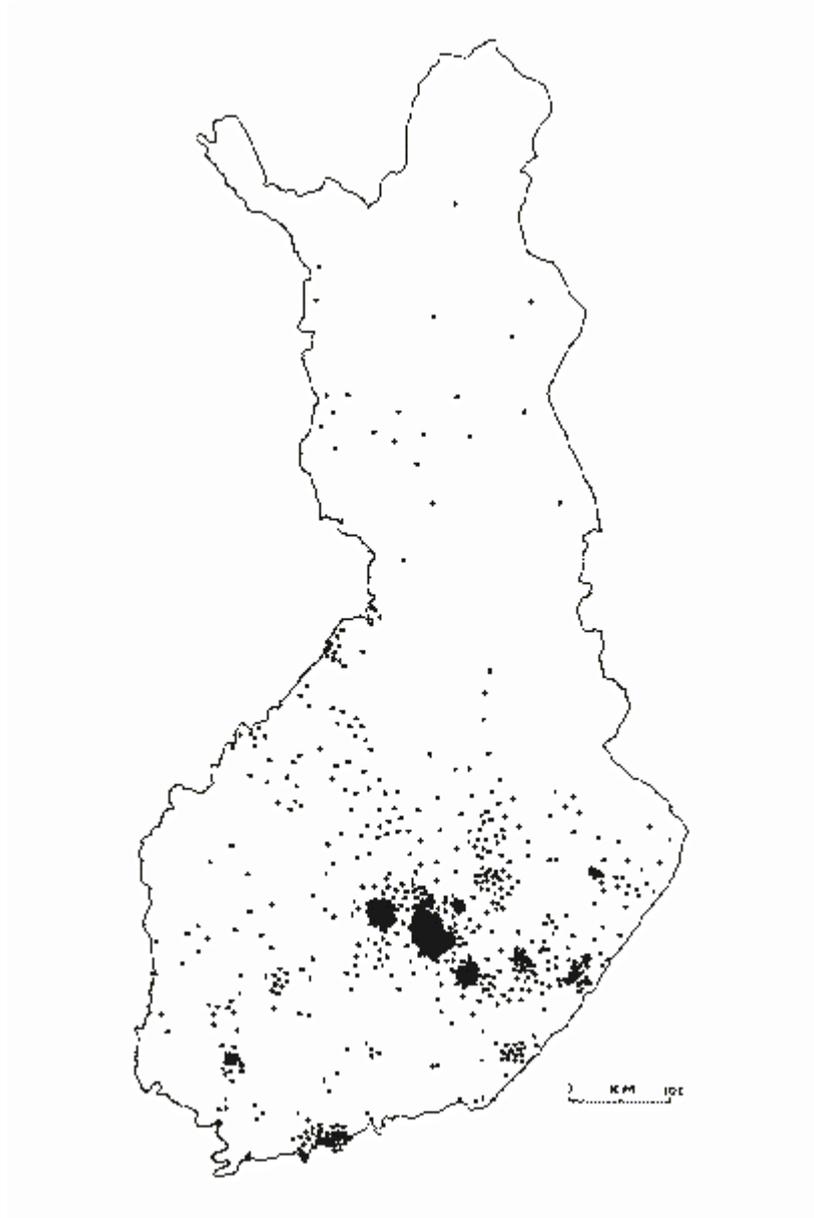

Fig.3 The geographical distribution of house residencies (= the sites of contraction ?) of the NE patients in Finland (June 1966 – May 1977)
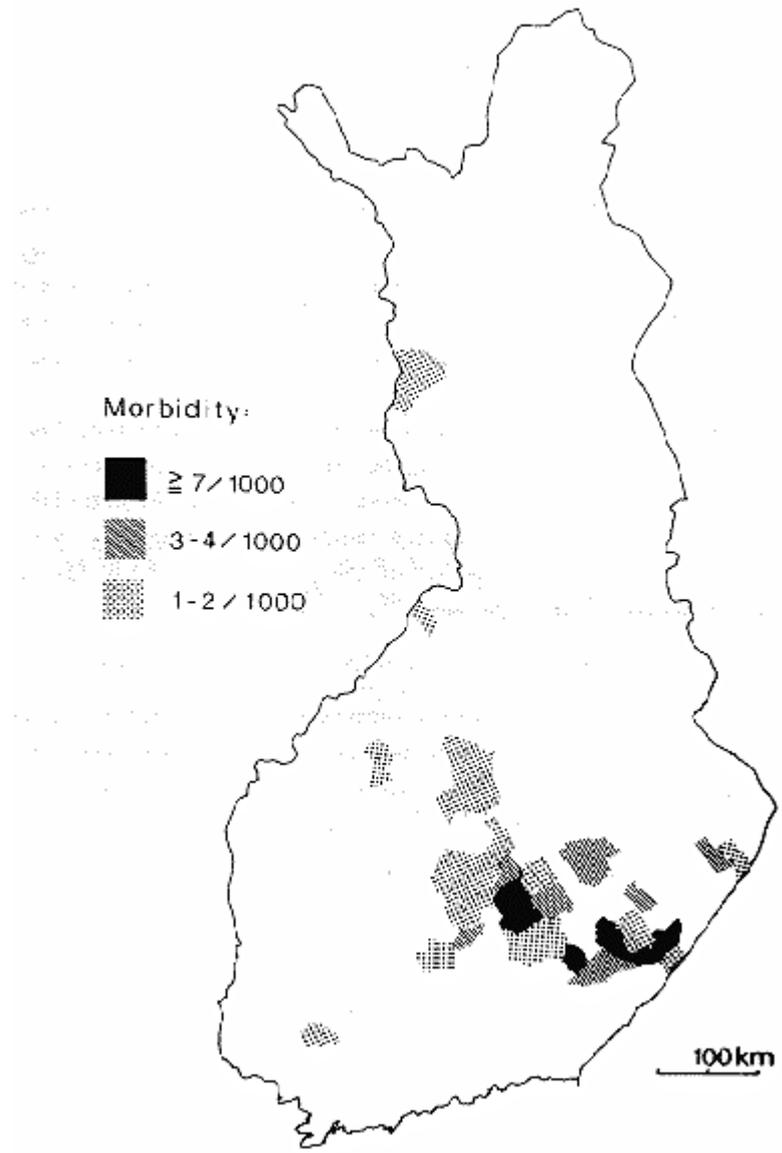
Fig. 4. The morbidity to NE in different parishes in Finland (in 1966 – 1973). (According to Lahdevirta and Elo 1975)
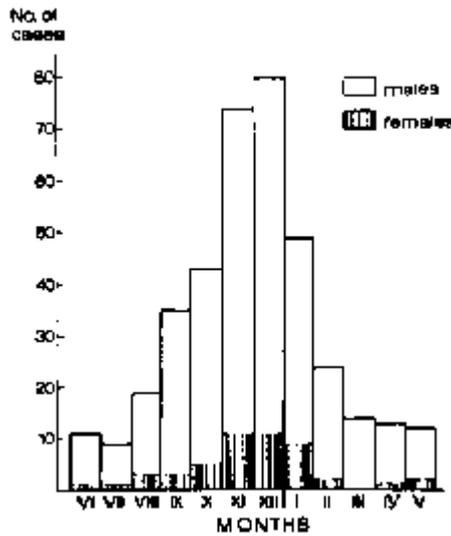
Fig. 5. The seasonal distribution of NE cases in Finland (in 1966-1973).
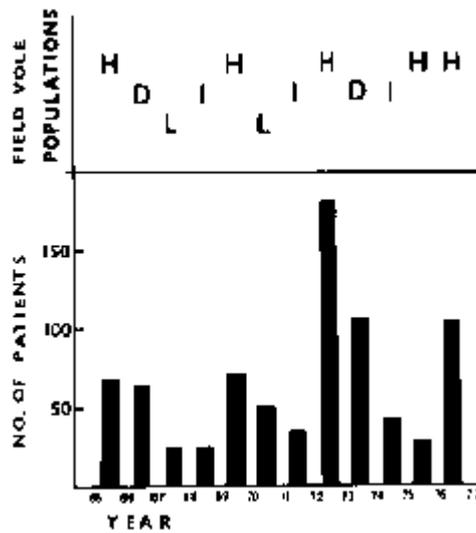(According to Lahdevirta and Elo 1975)

Fig. 6. Annual occurrence of NE cases in Finland as compared to the population density of voles
(symbols: H=high, D=decreasing, I=increasing, L=low density of vole populations, according to
Myllymaki (24) and Myllymaki et al. (25))

ISOLATION ATTEMPTS

The agent of NE is unknown. Some preliminary reports on a successful isolation have been published (e.g. Hoorn et al. (23) in Sweden) but they have been left without confirmation. In Finland the isolation attempts were started in 1964. The efforts were enforced during 1976/77 and concentrated in two different approaches :

1) to search for NE patients at the earliest possible phase of the disease,

2) to collect rodents from the farms where recent cases of the disease have occurred.

Samples from the patients. The samples collected for the isolation attempts are: serum, white cells (both crude buffy coat fraction and lymphocyte cultures), urine (sediment and a concentrate prepared by initial untrafiltration followed by ultracentrifugation), renal biopsy, cerebrospinal fluid, throat swab and faeces. The samples were collected within the first days of the disease (renal biopsies, however,

more than a week after onset). Some samples from the cases of the family outbreak (see above) were obtained even before the disease.

Samples from the rodents. The material includes the pets of the above mentioned family (four bank voles), and both voles (*Clethrionomys glareolus* and *Microtus agrestis*) and mice (*Apodemus flavicollis*) from the endemic area. Blood, urine and different organs (kidney, lung, spleen, liver, heart, salivary gland with regional lymphnodes) were used as source material for isolation attempts.

Isolation methods. All the samples of patients and rodents were inoculated both into experimental animals and cell cultures. The animals were: newborn and weaned laboratory mice, guinea pigs, bank, field and grey-side (*Cl. rufocanus*) voles.

The inoculation route was ip, for the mice also ic (and intranasally sometimes). The primary cell cultures were made from the organs of the inoculated animals at the time they fell ill or some weeks to six months after the inoculation if they kept well.

Besides the experimental animals several cell lines (listed in table 1) were used for isolation attempts.

TABLE I

CELLS USED IN ISOLATION ATTEMPTS

(Origin of established lines is given in parentesis. The other cell types represent adherent primary or early passage cell cultures from the tissues).

Main types:		Additional types:		
Human amnion		Human glia		
Human embryonic skin		Field vole kidney		
Green monkey kidney		Bank vole kidney		
Mouse macrophages		McCoy, X-ray irradiated (Mouse)		
Mouse embryo, total		Lymphoid line (Bovine)		
Mouse embryo viscera		Lymphoid line (Porcine)		
Chick embryo				
		Types used in passages:		
HeLa (Human)		Human embryonic kidney		
Vero (Green monkey)		PK 15 (Porcine) HAK (Hamster)		
Kidney line DK (Dog)		Pt K2 (*Potorous tridactylis*)		
Salivary gland SVG line (Dog)		Singh's *Aedes albopictus* (Mosquito)		
RK 13 (Rabbit)				
BHK21/WI-2 (Hamster)				

Each sample was immediately inoculated in approximately ten different cell cultures, all of the cell types were not constantly available. Three to four blind passages were done.

Attempts to demonstrate the presence of the NE agent primarily carried out by immuno-fluorescence. Cell smears or sections of frozen organs were fixed with acetone (15 min 4ºC) and stained by convalescent sera from NE patients using either direct or indirect method. Additional methods for detecting a possible agent were : 1) electron microscopy, 2) immune electron microscopy using convalescent sera, 3) normal immune adherence using human convalescent serum, guinea pig complement and red cells, 4) immune diffusion with human convalescent sera,

5) interference in cell cultures using Semliki Forest virus as the challenge, and 6) labelling the cultures with radioactive adenine followed by sucrose gradient centrifugation of the supernatants.

In addition primary cell cultures have been prepared from the organs of the voles from endemic area. These cell cultures have been studied as the above mentioned cultures.

Isolation results. In two instances the possible presence of a NE antigen in the initial samples has been detected by immunofluorescence. A buffy coat sample from the member of the "NE-family" taken one week before the onset of the disease showed a clear granular cytoplasmic fluorescence in 10% of all the white blood cells (Fig. 7). At the day of onset of the disease about 1% of the cells were positive in two observed patients. Samples taken after the onset were negative. The unspecified tagging of immunoglobulins cannot be totally excluded. However, when mononuclear leucocytes from

one of the immunofluorescence-positive buffy coats were cultured for 5 days *in vitro* and then inoculated into newly prepared cultures of mouse peritoneal macrophages, an atypical CPE was noted after six days. A similar CPE was reproduced in eight sequential passages by transferring supernatant of the infected cultures onto fresh macrophages. These primary findings were confirmed by reisolating twice the cytopathogenic agent in macrophage cultures. Infectivity titres in these passages remained relatively low, only up to 10^4 per ml. Infected macrophage supernatant also induced CPE in bank vole kidney cells and in kangaroo rat kidney cell line (Pt K2). A partial neutralization of infectivity by two logs in the macrophage system could be demonstrated by using unheated convalescent serum from one of the patients while the respective preinfection serum showed no effect on the CPE. Attempts to show antigens of the agent in the cells by direct or indirect immunofluorescence have not yielded convincing results so far.

Another positive finding of the immunofluorescence was noted in primary cell culture made from the organs of a bank vole of the endemic area. There was a weak but clear cytoplasmic fluorescence in a small fraction of the cells by indirect method. Also here the possibility of an unspecific staining cannot be totally excluded. It is worth mentioning, however, that the two laboratory infections described above most likely were contracted when these primary cultures were prepared.

CONCLUDING REMARKS

Nephropathia epidemica (NE) is less severe than the related disease in the Far East, the hemorrhagic fever with renal syndrome. Especially the hemorrhagic manifestations are in the former disease scanty. The number of hospitalized patients is 20 to 200 per year in Finland, the respective numbers in the Scandinavian countries are smaller. Because the etiologic agent of NE is unknown, the number of undiagnosed, atypical or subclinical infections cannot be estimated.

The incubation time has been supposed to be about three weeks. The onset with high fever is acute without prodromal signs. The clinical picture is dominated by the renal affection : proteinuria, oliguria (later polyuria), microscopic hematuria and azotemia. The interstitial extravasation is the most prominent finding in the renal biopsies. The disease is, however, a multi-systemic infection. Evidence of carditis, hepatitis and meningoencephalitis has been noted rather often. The increased activity of the rheumatoid factor has been found during the early phase of NE. Sequelae of death are very rare. Reinfections have not been observed.

The greatest majority of patients are young rural men who fall ill in the early winter. The periodicity of NE coincides with the fluctuations of the population densities of voles in Finland. Our isolation studies have concentrated on the voles from the NE endemic area and on material derived from a family outbreak apparently caused by pet voles

The results, while still preliminary, suggest that non-cellular patient samples (urine, plasma, etc) yielded negative results but that cellular patient samples (buffy coat) and cellular samples from infected bank voles may yield positive findings.
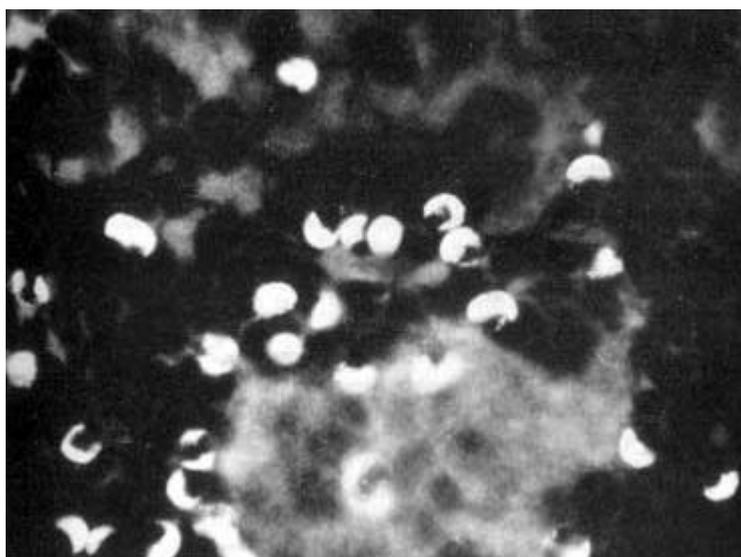

Fig. 7 The cytoplasmatic fluorescence (direct method) seen in the buffy coat cells of a sample taken one week before the onset of NE.

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DISCUSSION

J A. Bryan : Is it your feeling that in your family outbreak all became ill because of contact with the rodents or do you think it was person to person transmission ?

C.H. von Bornsdorff : The incubation period is too short for a person to person transmission. Person to person transmission has never been observed. Rodent-man contact is always responsible.

J.G. Breman : Did you look for antigen in the voles responsible for this household outbreak ?

C.H. von Bornsdorff : We did, but I think too late. We did not investigate them until March and that was too late. Furthermore, cultures were made and we did not make frozen sections.

J. Casals : We learned from our trips in the Soviet Union that they have two varieties of the disease : the one in the Far-East which is certainly the same as the one in Korea, associated with Apodemus agrarius whereas in the European part of the Soviet Union. particularly on the middle Volga and further south, the disease is clinically milder with low mortality somewhat similar to what you described in your country and in Sweden. The latter is associated with Clethrionomys glareolus. There is no evidence as far as we know, whether those two agents are the same or related. But the

disease is really very different, the mortality in the Far East of the Soviet Union is from 5 to 20% whereas in the European region it is no more than 1 or 2%.

SECTION III : PUBLIC HEALTH ASPECTS

1. SURVEILLANCE IN ENDEMIC COUNTRIES

EBOLA HAEMORRHAGIC FEVER : A PUBLIC HEALTH PROBLEM

P. BRÈS

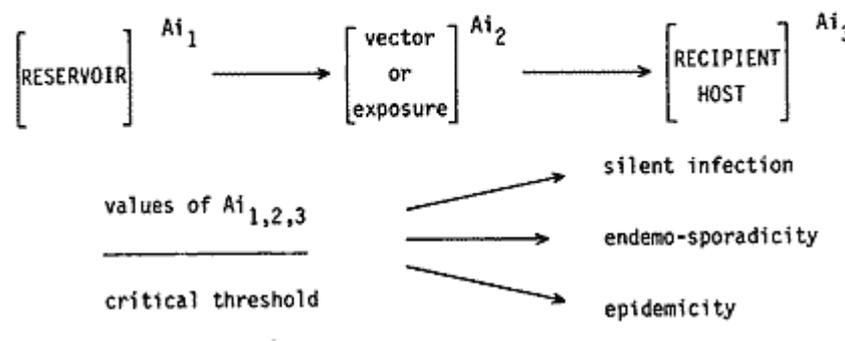World Health Organization, Virus Diseases, 1211 Geneva 27, Switzerland.

The sudden appearance of a "new" communicable disease, such as Ebola haemorrhagic fever which manifested itself in two outbreaks in 1976, was a challenge for those who tried to understand its nature and to carry out appropriate measures under the pressure of current events. Four questions were raised at that time : (1) what was the agent; (2) how did it appear; (3) why were the outbreaks so extensive and (4) what should be done ?

1. <u>What was the agent</u> ? The first agent which came to mind to explain the haemorrhagic syndrome with diarrhoea was Salmonella typhi but the haemocultures were negative. Yellow fever virus was the second possibility since Sudan and Zaire are located in the epidemic zone and the way an epidemic of yellow fever is propagated by Aedes aegypti may to some extent evoke a pattern of man-toman transmission. However, the deaths of three missionaries who had been vaccinated invalidated this hypothesis. We had then to envisage viruses which had already caused haemorrhagic diseases in Africa: Lassa, Marburg, and Crimean-Congo haemorrhagic fever viruses. Rift valley fever virus was not taken into account at that time but has since shown itself to be a possibility even outside its traditional focus although its clinical-pattern is more often like dengue than a haemorrhagic fever. The high attack rate in hospital staff clearly indicated that specimens ought to be sent to high security laboratories. In collecting samples it was recalled that the viraemia of such viruses as Lassa virus may be prolonged and the rise of antibodies may be postponed up to two weeks after onset of the disease. In addition, we now know that electron-microscopy is indispensable to detect a new virus for which the existing batteries of reference antigens and antisera will not give any positive response. The first samples from the Sudan and Zaire were received on 5 October 1976. Ten days later it was found that the agent which had caused the two outbreaks was a new virus morphologically similar to Marburg virus but antigenically distinct. This discovery was simultaneously announced on 15 October by the three laboratories which had participated in the isolation of the virus (1). On 16 October, two epidemiological teams were sent to the Sudan and Zaire, to reinforce consultants already operating in both countries, within the framework of the WHO Emergency Aid Scheme against viral diseases. The outbreaks being exceptional by the high risk of man-to-man transmission the teams had to take appropriate measures to halt the spread of the epidemic to the entire affected countries and to other countries.

2. <u>Why a new virus</u> ? Is the number of viruses in the world finite ? Influenza virus shows that recombinants may occur frequently in natural conditions. The plasticity of arboviruses is well known. However, certain viruses such as measles virus, although very widespread, show only a single type. Did Ebola virus exist before September 1976 or was it a new agent ? We have the same problem with two new Enteroviruses 70 and 71. Hopefully, retrospective serological surveys will provide an answer to this question.

3. <u>Why extensive outbreaks</u> ? Was the introduction of Ebola virus by a single person, or a single animal, in a community all that was needed to start an outbreak ? If so, why did it not occur earlier ? There is some evidence that a single carrier can start an outbreak of influenza, measles, viral hepatitis and several other diseases. Is a single person infected with yellow fever virus, or a single infected mosquito able to start an epidemic ? On the contrary, is it necessary to have simultaneously a certain number "X" of abortive cycles of transmission for one of them to be more successful than the others ? If so, epidemiologists could develop a mathematical model of silent infections with amplication indices (Ai) of each element of the chain of transmission, as in figure 1, and fix a threshold above which such abortive, silent cycles are successful in cre ating an outbreak. This is in fact analogous to what Gorgas in 1908 called the "critical number" below which the population of Aedes aegypti is unable to transmit yellow fever. Seroepidemiological surveys outside the two foci, or in children born after 1976 within the foci, will tell us whether silent infections are common with Ebola virus and in which geographical zones they occur. Epidemiological situations are so often unforeseeable that it will probably be impossible to establish with confidence the above-

mentioned threshold for Ebola virus. It is perhaps more realistic to say that some exceptional epidemiological condition, such as nosocomial transmission, can on rare occa sions enable a silent virus to manifest itself. The way the Ebola virus outbreaks started in the Sudan and Zaire in 1976 can be compared to the way the outbreak started in Germany in 1967. Another hypothesis would be a change in the virulence of the virus.

4. <u>What to do in the face of such outbreaks</u> ? In September 1976, the lack of previous experience with such sudden outbreaks and of such magnitude inevitably meant a good deal of improvisation. Responsible persons had to find a way between insufficient and excessive measures. Political implications often interfered with epidemiological decisions. Communications and transport raised many problems. Logistics were quite a challenge in organizing the mobilization of personnel case finding expeditions, the collection of specimens, their shipment and investigation, the isolation of patients, the procurement of supportive treatment, the preparation of immune plasma, its testing, and the protection of hospital personnel. This last problem was perhaps the least foreseen and the most important.

FIGURE 1
THE AMPLIFICATION INDEX OF TRANSMISSIBLE INFECTIONS

$$\begin{bmatrix} \text{RESERVOIR} \end{bmatrix}^{Ai_1} \longrightarrow \begin{bmatrix} \text{vector} \\ \text{or} \\ \text{exposure} \end{bmatrix}^{Ai_2} \longrightarrow \begin{bmatrix} \text{RECIPIENT} \\ \text{HOST} \end{bmatrix}^{Ai_3}$$

$$\frac{\text{values of } Ai_{1,2,3}}{\text{critical threshold}}$$

→ silent infection

→ endemo-sporadicity

→ epidemicity

CONCLUSIONS

The Ebola virus outbreaks in 1976 created an exceptional situation which the two affected countries, Sudan and Zaire, met so admirably by mobilizing all possible resources. The amplitude of this public health problem called for unprecedented international cooperation. The experience thus acquired will be most valuable should a "new" virus again appear and create such a dramatic situation.

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SURVEILLANCE OF HAEMORRHAGIC FEVER IN ENDEMIC AREAS : SUDAN

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INTRODUCTION

The epidemic occurred in Western Equatoria Province of the Southern Sudan.

Official news of the outbreak reached the Regional Ministry of Health in Juba on the 15th of September 1976. Immediate measures were initiated and help was asked from the Central Ministry of Health, Khartoum, and the World Health Organization.

The Director of the Epidemiology Department there, Dr. Ali Idris, was asked to proceed to the area to investigate the outbreak, help in the diagnosis of the disease and the implementation of control measures. He left Khartoum for Juba on the 3rd of October and was in Maridi the next day.

I would like to mention briefly some of the activities, which have played an important role in the control and prevent further spread of the disease.

1. Compulsory and prompt notification

Information about the disease was distributed to all health points in the Southern Region and also throughout the country. The disease was made notifiable. Health points in the area were instructed to report any suspected cases to the epidemic centre in Maridi and if possible to H/Qs of the Regional Ministry of Health in Juba. All available means of communication and transport were to be utilized for this purpose. From the Regional Ministry of Health the information was passed to the Central Ministry of Health in Khartoum and both Ministries collaborated and organized control activities. The information was also regularly conveyed to the concerned international agencies, mainly the W.H.O.

2. Detection of cases

Teams were formed mainly of students and headed by sanitary overseers or public health officers. These were asked to search the area, house by house, and detect cases in order to organize their prompt removal to the quarantine.

Special teams were formed to inspect the area along the borders to detect those moving in or out of the area. The health workers in all the health points in the area were asked to visit all the houses in their areas for case detection and contact tracing, and to report this information to the epidemic centre.

3. Contact tracing and follow-up

Some teams were instructed to trace the contacts. These were put under surveillance for two weeks, during which they were visited regularly by the surveillance teams.

4. Closure of the area

The affected areas were closed from the other Provinces, and the borders with the neighbouring countries were closed. Movement inside the Southern Provinces was limited and communication between the Southern Region and the rest of the country restricted, except for special circumstances.

5. Complete registration

The information system between the affected districts and the Regional H/Qs of the Ministry of Health in Juba, and between the latter and the Central Ministry of Health, Khartoum, was organized.

Special forms were provided for the registration of cases, suspected cases, and contacts. These forms contained all the necessary information.

6. Health education

This was widely used. All the different types of media were utilized. These included the newspapers, radio and T.V., mobile teams and village chiefs. People were asked not to panic, to report cases to the nearest health points and take the necessary precautions.

CONSTRAINTS

1. Transport

This was the major constraint. The lack of cars and the acute shortage of fuel in the area and in the Southern Region were real obstacles in the way of carrying out many activities. Many of the surveillance teams were asked to roam about in the area on foot. There were difficulties in transporting personnel, supplies and equipment from Khartoum to Juba and from there to the affected areas. A few planes were provided for short periods by the Sudanese Air Force, UNDP and some friendly countries.

2. Communications

Radio communications were deficient and postal services were almost nonexistent especially after closure of the area. Use was made of the available radio communication sets belonging to some voluntary organizations.

3. Runaways

The running away of the nursing and auxiliary staff of the hospitals in the affected districts, and the similar attempts by the patients and their relatives and contacts constituted one of the main problems. It took quite an effort to persuade these people to return to the hospital.

SURVEILLANCE OF HAEMORRHAGIC FEVER IN ENDEMIC AREAS : KENYA

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Whilst Kenya is not known to be an endemic area for Ebola virus infection, its geographical proximity and the considerable volume of air traffic between Nairobi and Juba raised the possibility of virus introduction. Following notification of the outbreak in the Southern Sudan, action taken by Kenya, included the restriction of air movements, follow up of passengers arriving in the preceding two weeks and the institution of national surveillance; this consisted of alerting health services throughout the country and especially at borders and points of entry. A circular was sent to all health facilities outlining the clinical features, method of diagnosis and the required action in the event of a suspect case.

Fundamental problems in carrying out surveillance in predominantly rural populations were discussed. Particular importance must be attached to the provision of protective equipment for staff investigating possible cases and the methods of collection and transportation must be designed to ensure maximum safety whilst providing suitable material for diagnostic purposes. It was suggested that centres should be established which were able to investigate a wide range of viral diseases under maximum security and that countries should be kept informed of centres capable of carrying out this work. Suitable collection and transport equipment needs to be available locally or at least regionally to permit the prompt investigation of possible outbreaks.

THE SURVEILLANCE OF VIRAL HAEMORRHAGIC FEVER IN ZAIRE

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An outbreak of viral haemorrhagic fever occurred in Zaire in the Equateur Province, in September 1976.

The first case was recorded in Yambuku Mission hospital. More than 40 villages, located within a radius of 50 km from Yambuku were involved. A first epidemiological investigation was done in Yambuku by the local health authorities of the Equateur Province on September 15, whereas the central governsent two teams of physicians respectively on September 23 and October 4.

As a microbiologist, I visited the area together with Dr. Omombo, epidemiologist, on September 23.

Our mission was expected to last six days but we shortened it to 24 hours in order to speed the diagnostic procedure since the situation was very serious. We carried out some manipulations without special precautions, except the traditional hygiene of handwashing with soap and disinfection with iodinated alcohol after examination of patients or manipulation of fatal cases.

We collected :
- three liver autopsy specimens from persons who died a few hours before we arrived,
- five blood cultures from febrile patients,
- blood samples for routine serological reactions.

The liver specimens were fixed in formalin and sent to the Institut Pasteur in Dakar, which is one of the WHO Reference Centers for yellow fever. The blood cultures remained negative. The results of the Widal tests were difficult to interpret because the patients live in an area endemic for salmonellosis. Some weeks later, a junior pathologist of Zaire suggested the diagnosis of yellow fever.

Moreover, in order to precise the nature of the disease and to encourage the Mission members, it was decided to evacuate to Kinshasa the nurse missionary who became ill on September 23, and a priest who had travelled with the index case of the epidemic to Kota-Koli, two weeks before.

We travelled with our patients and samples by Land Rover to Bumba, and flew back 24 hours later to Kinshasa via Kisangani.

From N'djili airport the two patients took a taxi to a clinic in downtown Kinshasa.

The nurse's serum was sent to the Institute of Tropical Medicine in Antwerpen, where a new virus was isolated. Unfortunately, the nurse died on October 6. Her companion fell ill on October 8, and died on October 14. A ZaTrian nurse who cared for these two patients fell ill on October 13 and died on October 20. The priest was never infected and did not develop antibodies.

Haemorrhagic fevers are endemic in Africa. For some of them the mode of transmission is well known.

Yellow fever is transmitted from monkey to man by Aedes mosquitoes. Therefore mosquito control measures are partially effective in preventing and stopping the spread of epidemic yellow fever. Vaccination gives a longlasting protection.

In Lassa fever, the virus reservoir is the multimammate rat Mastomys natalensis. In the absence of vaccination against the disease, control measures based on a good knowledge of the ecology of these rats have been effective in the prevention of the spread of the disease.

In the accidental infections by Marburg fever the virus source was the vervet monkey Cercopithecus aethiops imported from Uganda for scientific purposes. In the natural infections, as in the Johannesburg outbreak in 1978, neither the reservoir nor the vector are known.

At present, the source of Ebola virus is also unknown.

Lassa, Marburg and Ebola viruses can be transmitted from man to man. These infections are very dangerous, particularly in Africa, where people live in such a promiscuity that person to person transmission is easily achieved. Surveillance of numerous primary and secondary contacts is difficult.

Therefore, in Zaire, before the nature of the disease was known, we decided

1: - to stop the circulation of patients and families between villages; soldiers guarded the main routes,

2: - to bury the corpses as quickly as possible after death in order to avoid prolonged contact with other members of the family. Because of the high risk of contamination among nurses and other hospital workers in Yambuku, we recommended :

1° - to wear gowns, masks and gloves,

2° - to avoid contact with blood, urine and other body fluids of patients.

The Department of Public Health sent relevant clinical and epidemiological information to most Zaïrian hospitals. Early in the epidemic, the signs and symptoms of the disease are similar to those of malaria or salmonellosis, the prime sign for alarm must be the high mortality rate among patients.

In Zaire, the surveillance of haemorrhagic fevers is presently included in the national public health program. A medical doctor is now present in most rural hospitals and is responsible for the surveillance of the surrounding villages. For the future, we hope to maintain contact with the doctors in the rural areas for permanent surveillance of fevers of unknown etiology.

The mobile teams of FONAMES and FOMETRO distributed all over the country, can also give information to the National Public Health authorities, if they suspect viral haemorrhagic fever. Most of the rural hospitals belong to the Missionaries and have radio contact twice a day with urban hospitals.

Since the outbreak in Yambuku, two micro-epidemics have been notified, but these were false alarms.

Nevertheless, we think that the possibility for new epidemics is real. It would be useful to have a stock of immune plasma in Zaire. Moreover, laboratory facilities for rapid diagnosis of the disease are essential. In the absence of these in Zaire, we depend on collaborative investigations with high security laboratories in foreign countries.

DISCUSSION

S.R. Pattyn : False alarms were mentioned and I would like to ask what are the simplest clinical criteria to exclude haemorrhagic fever.

P. Piot : I don't think there is any single clinical symptom or sign which can exclude the diagnosis of Ebola virus or another haemorrhagic fever infection. Even if the patients are not bleeding, you cannot exclude any of the haemorrhagic feoers. There are only some signs or symptoms which are statistically valuable but not appLicable to individual patients.

O.W. Prozesky : We had two false alarms, this is due to informal surveillance we had organized in South Africa. We have arranged that in such cases the corpses be put in two plastic bags immediately and that a limited autopsy be performed and liver be obtained through the plastic. The liver is to be sent for investigation both in fixed and unfixed forms. In the first case the diagnosis of any Onyalai was made and the second was a case of tick-bite fever.

D.P. Francis : When called into these areas, I don't think there is going to be one isolated case. The individual case of haemorrhagic fever is no doubt very difficult to diagnose. The key at the larger outbreaks are clusters of human to human transmission most of which are in the hospital. The action from then on will be the same, that is to isolate the primary contacts and to get away from the patients.

A. Fabiyi : I am very interested in the five sera Dr. Smith told us about, from haemorragic patients. What were the sera tested against ? The reason why I am asking this is that we have shown that dengue virus is active in Africa, so that dengue shook syndrome could occur, although that would depend on the age of the patients involved. It would be nice to know what antigens were used in the testing of these sera ?

D. Smith : The five cases that I referred to were tested only against Ebola and Marburg. These were sporadic cases that had occurred while we were in the Sudan. It is from an area with very sporadic, usually family cases, three or five at a time, over the last six or seven years. Dr. Metselaar previously had investigated this area with Dr. Casals, they screened for substantially more possibilities.

J. Casals : I don't recall the exact antigens that were used for testing your sera but I believe there were included Chikungunya, Sindbis, West Nile, Banzi, Ntaya and Lassa. Those arboviruses were used because we depend on their cross-reactivity to pick any possible antibodies. Unfortunately, as far as I recall, the tests were not diagnostic. I want to add a comment. As a collaborating Center with WHO, we receive sera from all over the world. We can handle very easily arboviruses because we have the antigens. Sera coming from Africa will have to be tested for particular antigens like Lassa or Congo-Crimean viruses. As long as Drs. Johnson and Webb send us the antigens we can test for these. Marburg and Ebola however are entirely out of our hands since we cannot handle these antigens. Two other antigens pose problems as we are not allowed to handle them because of

interdiction by the Department of Agriculture. These are Rift Valley Fever and Nairobi Sheep Disease. I don't know where the supply of antigens will come from. Finally, I want to ask Dr. Smith whether in his experience he has seen recently or within the last five years, many human cases of Rift Valley Fever in Kenya.

D. Smith : There has been a fairly substantial outbreak in a very remote part of Northern Kenya, part of the Rift Valley. Like all those things we got to know about it after it had happened. There were possibly between 500 and 700 acute cases of a short febrile illness with perhaps 50 or 75 deaths. A very small proportion had haemorrhagic features. We only found 8 or 9 people who were reputedly convalescent, whom we bled and we have the serum now in Nairobi. You highlighted some of the problems. It is a little difficult now, with this wide array of possibilities, to know quite what to do with these sera.

P. Brès : We should do what we did before in similar circumstances : we send these sera to Dr. Casals and, if necessary, he sends the sera further to other places. It takes time, but it is the only thing we can do.

M. Isaäcson : Rift Valley Fever diagnosis is a routine procedure in our National Institute of Virology and we would like to offer the services of that laboratory for Rift Valley diagnosis.

J.G. Breman : I think Dr. Pattyn's question was very critical as to what exactly do you do practically. I think Dr. Prozesky answered that in part. It is very important for everyone to have a clear idea as to what specimens to take because you really can't make the diagnosis clinically nor can you consider every febrile illness as a possible haemorrhagic fever in a rural hospital. Our recommendation was to do as Dr. Prozesky recommended actually, but not even taking fresh tissue as we considered it so lethal, but to put it in formalin. Dr. Muyembe raised an interesting point in that he got a report from Dakar, one of the best places in the world, saying that later on, this was diagnosed as yellow fever and Dr. Murphy showed us Councilman-like bodies which were very impressive. Is indeed the histopathologic picture very much like yellow fever ? We can't really put all these slides under electron microscopy immediately, certainly not in Dakar, Khartoum or Kinshasa. The second question is for Dr. Brès and Dr. Muyembe regarding staff available and specimens that have come in. I think after these epidemics, certainly in Zaire, we have a well-trained corps of national epidemiologists and qualified personnel. Dr. Miatudilla told me he has been designated to do the investigations in the future. I want to ask Dr. Muyembe if, since last year, any pathologic specimens have come in and what has been found.

O.W. Prozesky : we decided on liver because all these haemorrhagic virus diseases reflect in the liver. I don't think that the specimen need tell you whether it is yellow fever, Marburg, Ebola, Rift Valley, you do not have a chance really to diagnose that on histology, which you mostly do, but you can do electron microscopy on that same liver. We do negative staining on the liver juice expressed from it, to visualize the virus. In my limited experience the histology of yellow fever, Marburg, Ebola, is so similar that a specific diagnosis is impossible on one specimen. But a group diagnosis is possible and that is what is needed at that stage.

A.W. Woodruff : In Africa, 90% of fevers will be malaria, to take the blood film under secure conditions wearing gloves with more caution than perhaps has been exercised in most places in the past, remains the central and most important examination. Then comes the clinical decision : is the condition consistent with the presence of malaria, is it wholly explained by the presence of malaria parasites ? Here I think the clinical training of those who go to Africa comes into very forcibly. Physicians should be trained in the recognition of the most likely clinical syndromes that they are going to be confronted with and in particular malaria.

F.A. Murphy : Wouldn't it be practicable if only one specimen would be collected ? One of the problems is that when everything has to be divided and some o'f it is fixed and some is not. frozen material and fixed material cannot be shipped in the same box and everything multiplies. So I think there are great advantages in sending only one piece of liver in the frozen state, this provides material on which a lot of things can be done. it offers the advantage of ultimately arriving at an etiologic diagnosis rather than the frustration of an array of presumptives and not confirmatory hard evidence.

K.M. Johnson : I think I'll have to disagree with my colleague. He is right in terms of the single specimen and what kind it ought to be to obtain the maximum information. My own concern at the moment, in view of the probable sporadic occurrence of these diseases, would be to find the simplest, cheapest way to get any kind of specimen conveniently to some place where something could be learned. If I had to do it, I think I would return to the suggestion and the methods fundamentally used so beautifully for so long in the tropics by -the Rockefeller Foundation and that

is ; put a piece of Liver in a fixative and mail it. You are quite right that it may not be able always to show exactly which virus is involved, but we could even begin to fill in a geographic map of Africa. Latin America, Asia, in terms of the distribution of these kinds of diseases. We would then be in a much better position for future investigations and to really focus on places where we know we want something frozen from, but I understand this means a lot of work to the pathologist.

C.E. Gordon Smith : I think we are building up a beautiful sort of castle in the air. My experience of collecting things from people in tropical countries is that they are not, on the whole, very keen on people rushing around cutting pieces out of them. It is extraordinarily difficult getting post-mortem examinations of even the simplest sort in many countries. The situation you referred to was that of a service working in a highly disciplined situation in South America which would probably never be acceptable in Africa. I would like to hear from the Africans about the realism of the suggestions that have been made, remembering that the situation is quite different during a Large epidemic where everyone is frightened to death and really very draconian measures are possible. But we are talking about the first cases in a place where there never had been any previously.

K.S. Daoud : I agree with Dr. Gordon Smith. The people do not allow any post-mortem especially if it is a first case of disease occurring in that region. Secondly, I don't think it is advisable to take a liver biopsy during life when the liver is very friable and bleeding.

T. Muyembe : Autopsies are avoided because of lack of protective material. Furthermore, autopsies are not allowed. We did not receive any material for diagnosis since the end of 1976.

J.G. Breman : Dr. Gordon Smith's cement is very pertinent, I have had some experience in getting specimens from another area of Africa for cholera, smallpox and yellow fever. We developed a special simple kit with basic material and distributed this in one country of 5.000.000 people, having meetings with 15 of the regional doctors responsible for public health activities. This was after the big epidemics of yellow fever in 1965 and 1971. From '71 to '75 not one specimen had come in to the regional health organization. After this kit was distributed 18 liver specimens were obtained in one year and several series of serum specimens specifically from people with jaundice. The liver specimens were not taken with the menacing-looking viscerotome, but with a thin liver biopsy needle which does not require a major incision. This was generally accepted and the people were motivated. Of course the results were communicated properly to the doctors and we were surprised to see so many specimens.

J. Casals : We assume here that febrile illness that presents haemorrhages in the skin is necessarily Ebola, Marburg or Yellow fever but there are many other such febrile illnesses in Africa and elsewhere and a liver specimen is not going to help much in making a diagnosis. What about dengue haemorrhagic fever ? What about Chikungunya, which in the surveys that we conducted in Nigeria and now in Liberia resembles very much a mild form of Lassa ? Evidently the first thing that one has to do to have a rapid diagnosis is to find the antigen, but this is often missed entirely and we have to depend on serological diagnosis. The serum can be tested in many different laboratories. Dr. Isaäcson can do the Rift Valley fever, perhaps in Kenya they can do the Nairobi Sheep Disease, we ourselves and C.D.C. can do other viruses but are specimens to be sent in five different directions ? What I would like to see is that it could be done all under one roof and be given an answer.

P. Brès : I think the best method is to do what has been done before that is to make contact with the Virus Diseases Unit in Geneva and according to the problem which may be different from one place to the other, you will presumably get some advice where to send the material to. I think that is the best thing I can propose now.

D.P. Francis : We did out first two post-mortems in the Sudan approximately four and seven hours after death. we put the specimens in Ziquid nitrogen within half an hour but no virus was obtained. Therefore I would return to Dr.Johnson's suggestion and mail fix tissues together with sera, as suggested by Dr. Casals.

2. LABORATORY AND FIELD EQUIPMENT

LABORATORY AND FIELD SAFETY EQUIPMENT FOR THE MANIPULATION OF HIGHLY INFECTIOUS AGENTS

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Hazard group 4 viruses, such as Ebola, Marburg, and Lassa, cause serious disease in man, with high mortality, for which there is neither specific cure nor prevention. These viruses, although basically natural zoonotic agents, can be transmitted directly from person to person. Those persons at highest risk appear to be workers engaged in the diagnosis of the disease and the care of patients. Such work often must be done in circumstances where elaborate systems for patient isolation, personnel protection, and disposal of infectious wastes are not available. Evaluation of the specific biohazards involved is a prerequisite for any discussion of specific measures designed to prevent infection among persons exposed to these viruses.

CONTAGIOUSNESS OF EBOLA, MARBURG, AND LASSA VIRUSES

Although little is yet known concerning the contagiousness of Ebola, Marburg, and Lassa viruses, two simple facts have been elucidated and serve as a framework for practical action. The first is that large amounts of virus are present in the blood and in many of the tissues of persons infected with any one of these three viruses. The second is that person-to-person transmission of these viruses by means other than parenteral contact is not common. As documented elsewhere in this symposium, person-to-person transmission was only 5-13% for Ebola virus in Zaire and Sudan. Although precise data are not available for Marburg and Lassa viruses, the fact that these agents have not become epidemic or pandemic in the absence of zoonotic transmission means that they are not, in fact, highly contagious. Were any of them to spread by droplets as do the influenza viruses, for example, our world would surely and literally be a very difficult one in which to live today. This is not to say that we can ignore the droplet route of transmission. Circumstantial evidences of such secondary infection are on record, but almost no experimental work has been done on this general problem.

In summary, I believe that the major hazard in dealing with patients suspected of having hazard group 4 infection is that of sub-integumentary exposure

to blood, secretions, and excretions. Infectious aerosols are a less likely risk. Approaches to these problems will be discussed in terms of primary and secondary containment.

PRIMARY CONTAINMENT

Basic personnel protection can be achieved through scrupulous use of disposable gowns, caps, shoe covers, and gloves. The object is to prevent liquids or semisolids from contaminating the skin, which may be broken. In addition to these garments, either full-face respirators equipped with High Efficiency Particulate Air (HEPA) filters or a combination of goggles and nose-mouth respirators similarly filtered should be used. Although such equipment is not comfortable to wear, particularly in hot, humid environments, and although it is unequivocally frightening to patients, their families, and people in their communities, it serves three critical purposes: it

1) protects the face and mucous membranes from direct contamination,
2) protects the respiratory tract against infectious droplets, and
3) prevents inadvertent autocontamination by touching the face, eyes, and other parts of the body with the hands.

Several details in the use of this basic equipment deserve mention. Gloves should be rinsed in disinfectant while being worn whenever they are grossly contaminated; they should also be rinsed in disinfectant before they are removed. The order in which clothing is removed is also important. The proper sequence is (1) shoe covers, (2) cap, (3) respirator-goggles (which should be stored in containers, such as plastic bags, and reused by a single individual), (4) gown, and (5) gloves.

The equipment and procedures outlined above should be standard for personnel working with patients and for those processing potentially infectious specimens in a field laboratory. Laboratory manipulations create further hazards; for example, a tube breaking in a centrifuge or aerosols generated during pipetting or trituration. Two additional, forms of protection are feasible under field conditions. The first is use of materials other than glass wherever possible. The second is use of a collapsible, flexible-film plastic isolator to provide a negative pressure, HEPA-exhaust-filtered environment for

such work. Isolators which will contain small centrifuges and provide work space for two people are available in the United States for less than 1,500 dollars. Surface decontamination of the isolator and of materials removed from it is achieved by mopping and spraying 2% sodium hypochlorite or 5% peracetic acid solutions. These isolators were used successfully in 1963-64 in San Joaquin, Bolivia, during an epidemic of 700 cases of Machupo virus infection and again in 1976, at Kinshasa and Yambuku during the Ebola virus outbreak. Although electricity is required, the amount needed for such an isolator adds little to that required for other equipment, such as microscopes, lights, and a refrigerator or freezer.

An unavoidable hazard is that posed by the hypodermic needle. Its use should be minimized. Special needles with an antigravity flow valve and evacuated rubber-stoppered tubes should be used for obtaining blood specimens. Needles should be placed in disinfectant solution before being disposed of. Whenever syringes are needed, they should have a mechanism for locking the needle. For specimen collection, disposable equipment is always preferable to reusables.

SECONDARY CONTAINMENT

Isolation

Patients should be isolated from direct and indirect contact with other patients, visitors, and others working in close proximity. A separate facility, however humble, is the best solution. Access to this separate facility must be restricted to the minimum number of properly protected persons needed to provide clinical care. The same principles apply to a field laboratory. Where airflow from such an isolation facility cannot be completely controlled (the most common situation), distance and a favorable location in terms of prevailing winds from the nearest human dwellings are factors to consider and use to optimum advantage.

DECONTAMINATION OF REUSABLES

Primary soaking of instruments in 2-5% sodium hypochlorite is recommended. Beware of commercially available liquid concentrates, which may contain much less of the degradable active ingredients than shown on the label. Look for dry hypochlorite, which is sold for use in swimming pools. If kept dry, its strength will be as advertised. After instruments have been decontaminated in hypochlorite solution, they should be boiled for 20 minutes or autoclaved at 10 pounds pressure for 15 minutes (home pressure cookers will work) before they are washed, wrapped, and sterilized for reuse. Decontaminate with hypochlorite, then boil utensils patients use. Boil all cloth items which must be reused. Do these things in a special location away from the facilities used for other patients. Oil drums make excellent containers for such work.

DISPOSAL OF DISPOSABLES

The first rule for disposing of disposables is to contain such materials. Plastic bags, usually doubled, are the most convenient means of achieving this. Definitive disposal thereafter is a matter of burn and/or bury. Oil drums also make good emergency incinerators. Site them carefully and pay attention to the wind. Items to be buried should be buried deeply. This rule applies especially to cadavres. If plastic bags of sufficient size are not available, cloth strips soaked in phenol or formaldehyde can be used to wrap the body, and similarly soaked sheets can serve as an outer shroud.

TRANSPORT OF SPECIMENS

Principles for safe shipment of highly infectious specimens and specific methods for adhering to them are set forth in Packaging Note 695 of the Industrial Air Transport Association Tariff on Restricted Articles. Briefly stated, these principles call for a tightly sealed primary container, a sealed secondary container which has enough material, such as vermiculite or cotton, inside to absorb the specimen contents should the primary container leak or break, and a shipping container capable of resisting shocks generated by a free fall from 30 feet.

Whenever possible, primary containers should be of temperature-resistant plastic. Suggestions for labelling, documenting, and notifying addresses of packages containing such specimens are also described in the June 3, 1977, issue of the World Health Organization's Weekly Epidemiology Record.

OTHER CONSIDERATIONS

Assuming that future disease outbreaks are caused by agents not significantly more contagious than Ebola, Marburg, or Lassa viruses, the materials and techniques outlined here should suffice to prevent infection in medical personnel and their contacts. I personally see no valid reason for quarantining such

persons in the absence of an overt accident. There is as yet no evidence to suggest that persons incubating these diseases are highly infectious. In any event, it is important that one member of any team charged with investigation and management of an outbreak be responsible for all aspects of biosafety. That responsibility must be accompanied by unequivocal authority for obtaining compliance with the procedures to be followed.

Another question is whether individuals continually exposed to the viruses under consideration should receive passive antibodies to the virus. This, of course, presumes that the etiology of the outbreak has been established.

Detailed discussion of this subject is outside the scope of this paper. A cardinal rule, however, is that the use of such antibodies is not a substitute for adherence to appropriate safety procedures.

MAXIMUM CONTAINMENT LABORATORIES (MCL)

This subject will not be addressed in detail. It is evident, however, that the technology of modern virology exposes laboratory personnel to quantities of hazard group 4 agents which -are rarely or never encountered in nature. No manipulation of such viruses is risk free. Thus maximum primary and secondary environmental protection is mandatory. To protect the outside environment, all air must be specifically filtered and/or incinerated before it is exhausted. Similarly, liquid and solid wastes must be effectively decontaminated by heat or chemical means. Personnel must wear special clothes and take showers before leaving the laboratory.

Primary containment usually consists of a connected line of stainless steel, gas-tight cabinets, operated at an atmospheric pressure significantly negative to that in the laboratory. Double-doored autoclaves and chemical dunk tanks are employed to pass materials into and out of the cabinet line. Detailed requirements for such laboratories in the United States, United Kingdom, and Canada are set out in the Federal Register, Volume 41, N° 131 of July 7, 1977, a handbook entitled Control of Laboratory Use in the United Kingdom of Pathogens Very Dangerous to Humans, issued by the Department of Health and Social Security, and the Medical Research Council Guidelines for the Handling of Recombinant DNA Molecules and Animal Viruses and Cells, respectively.

By now, it should be clear that the costs of constructing and maintaining such laboratories are quite large. They are even greater when one realizes that the worker unit output in the sealed cabinets is both quantitatively and qualitatively less than in ordinary microbiological laboratories. At present, MCL are functioning at the Center for Disease Control (CDC), Atlanta, the U.S. Army Medical Research Institute for Infectious Diseases, Frederick, Md., and the Microbiological Research Establishment, Porten, Down, England.

In an effort to address these problems, CDC has recently completed a new maximum containment laboratory in which the steel cabinet line is replaced by a plastic one-piece suit supplied with air from a source remote from the laboratory. Suits are chemically decontaminated in a special shower at the end of each work period. The cost saving is significant, and easy access is afforded to a much greater variety of equipment. A film depicting this laboratory is now available as a 16-mm sound movie or a 3/4-inch videotape from the national Medical Audiovisual Center, Atlanta, Georgia 30333, U.S.A.

SUMMARY

Both primary and secondary protection against interhuman transmission of highly hazardous agents such as Ebola, Marburg, and Lassa viruses can be achieved under the most difficult field conditions. The major risk is inadvertent exposure to infectious blood, secretions, and excretions. Disposable clothing, respirators, sodium hypochlorite, heat, and disposal by deep burial, together with physical isolation of patients and field laboratories, are the principal tools required. The critical materials should be stockpiled in several centers for immediate availability.

A single individual should be given responsibility and authority for managing the safety program. The guidelines make it possible to replace irrational fear with a positive approach to containing such disease outbreaks without a public health catastrophe. Central to this thesis, however, is the concept that individuals can be found who believe that outbreaks can be contained. Biosafety in the hands of the morbidly anxious nonvolunteer is an invitation to disaster.

Long-term work with these viruses requires maximum containment laboratories (MCL) based on safety concepts so expensive that only three such facilities are currently operational. The use of self-contained plastic suits promises to reduce costs and permit a wide range of technology in the MCL of the future.

DISCUSSION

G.A. Eddy : In general I agree with the concept of a suit Laboratory and we have this on a limited scale at Fort Detrick. It certainly is a lot easier to work in a suit than it is in the class 3 system. We have also experience with Laboratory accidents but all of them occurred in association with the class 3 system. Regarding the handling of fluid waste. I think perhaps one way of handling that is to forget about autoclaving it. At least the viruses that we are working with don't have to be autoclaved in order to inactivate them. A reasonable alternative is the use of a high temperature short-time milk sterilizer. That technology has been very well worked up, they have all sorts of careful controls which provide recirculation in the event the temperature is not sufficiently high. That kind of apparatus is available at fairly reasonable cost. Dr. Johnson, what do you think about allowing a single person to work by himself in a suited system? Would you permit one person to work by himself without anyone else in the Laboratory ?

K.M. Johnson : The answer is no. Of course the complication is double overtime for personnel in the weekends, if animals have to be fed. it is almost impossible for a single person to enter into such a suit, it is almost a two-men operation and our manual does call for at least two people at all times in the suit operation. We will continue to use our present cabinet laboratory far primary diagnosis on unknown materials, The reason is that, while we think that we can find chemicals that will clean our suits from known agents., that might not be true for everything in the world. Therefore our new laboratory will be primarily dedicated to investigation and reagent production for known agents all of which can be tested for chemical inactivation.

I.W. Pinkerton : By what means and how frequently would you attempt to decontaminate the laboratory room and would this put it out of action during any period of time ?

K.M. Johnson : It is planned to decontaminate the Laboratory rooms, both the suit Laboratory and the other one, with vaporized formalin. Eventually it is going to rattle all the equipment, think of ultracentrifuges. In some cases we may make a small compromise by protecting some of the equipment with plastic bags. Provided the animal section can be sealed off, the suit laboratory can be used the next day. The reason is that it has its own air supply

and residual formalin is no problem. For the cabinet Laboratory, it will take about two or three days for all the fumes to completely clear out.

T. Muyembe : What kind of equipment do you recommend for Laboratories in Africa? As you know, we don't have much resources and therefore we are looking for cheaper but adequate long-lasting equipment.

K.M. Johnson I certainly recognize, and we all must, that the infrastructural talents, money and systems that have to be available and have to be continuously maintained to keep one of these laboratories going are not available everywhere and they are not available, not only in Africa but in certain other places either. At the moment, I don't really think that I have an answer that I am willing to propose as such. On the other hand, if you are careful and you have carefully defined what the Limits of the work are going to be, it may well turn out that flexible plastic equipment such as you have seen or could see in the laboratory of Dr. Pattyn and Dr. Van der Groen here in Antwerp, may turn out to be acceptable and useful. What they have is a prototype, I should emphasize, and there has not been any significant amount of work done with class 4 agents in this type of equipment, except in the collapsable plastic isolators we used in the field years ago in Bolivia and more recently in Zaire for doing very simple manoeuvres. in neither of these situations, however, were we attempting to cultivate and grow viruses up to high concentrations. Whether the plastic approach will be feasible and up to what limit and what kinds of procedures for viral work, I don't think I can say. However in theory, I do not see any reason why not. Remember the history of the germ-free animal field, it was originally all stainless steel and heavy glass and eventually was converted into flexible films and it works. I think this could work as well. There are a number of specific problems that need to be addressed and tested thoroughly before one could say yes, I recommend it. I sympathize, I think it is something that deserves real attention because there is a major need, in the countries where these diseases are endemic, to be able to approach them.

R.E. Shope : Dr. Johnson, would you be willing to comment on your experience with the use of immune individuals in field laboratories in an area where you don't have a class 4 facility ?

K.M. Johnson : It's a beautiful solution to the primary containment problem, but it doesn't free you from having to be concerned about secondary containment. For example, this is exactly how we did all our work for ten years in the Panama Canal Zone, on a top floor laboratory which we adapted for secondary control at a very low price. Only people who were immune to Machupo virus were allowed to enter the Lab. All of them had acquired their immunity naturally in the course of field

duty. We never had any break-throughs nor did we ever document booster responses in the staff. The main difficulty was of course that there were very few people in the world with such antibody. But you cannot assemble a group of people of limited education, train them and direct them from outside to do this work.

C.E. Gordon Smith : I think we are all looking forward with great interest to the assessment of your suit laboratory because it will overcome a number of problems we have all faced in the past. One important factor working in a cabinet is arm and hand fatigue that increases the risk factor. To reduce that would be very significant. I wonder if you could at this stage give an idea about the relative costs, capital and running of a suit laboratory as opposed to a cabinet laboratory.

K.M. Johnson : In many ways, the largest cost is in the system of secondary containment. Therefore the first decision is whether there will be autoclaving, boiling, or pasteurizing, or if it is going to be a dry laboratory. I would suggest that for the primary containment system, the initial capital cost is ten times less expensive than a cabinet laboratory. For maintenance, I think expenses are also lower. A further advantage is the basic flexibility in a suit laboratory so that it can be used for other purposes at a lower containment level if programme and future dictates. This is impossible for the cabinet laboratory.

R.A. Coutinho : Do you think that it is advisable that there would be a lot of laboratories that work on these viruses ? When something goes wrong with the laboratory, who is responsible ? Is it the company, the laboratory worker, the university, who is it ?

K.M. Johnson : That is going to depend very much indeed on all of those individuals and all those organizations that you listed as candidates to be responsible and I think it is going to vary from country to country. I don't think that whatever is done the person who scientifically is in charge of running the laboratory is ever going to escape being the primary victim. The commercial companies attempting to be of help in this field will probably do an honest job in trying to test the integrity of filters and all of their constructions, but it will be the user and purchaser of such equipment to have it constantly under his control. All this is the biggest reason why we still know so little about some of these viral diseases.

C.E. Gordon Smith : In setting out safety codes and instructions it is essential that it is clearly defined who is responsible for what, and that adequate responsibility is laid on the lowest members of the team because if they are not too responsible then the load for those higher up in the system is intolerable.

3. HOSPITALISATION

HOSPITALISATION OF PATIENTS SUSPECTED OF HIGHLY INFECTIOUS DISEASE

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Prior to 1945 air travel was a luxury restricted to a few rich people and most travellers from the tropics came by sea to Western Europe or North America. The time taken for the voyage exceeded the incubation period of the major infectious diseases, such as smallpox, so afforded an effective period of quarantine. The speed of modern aircraft and the vast increase in the number of passengers have completely removed these protective barriers and have exposed Europe and North America to much greater risk from exotic diseases prevalent in remote parts of the tropics. Some idea of the size of the problem may be gained from the statistics of passengers using the major London airport at Heathrow, where a trickle of 1,400 passengers a day in 1950 had become a torrent of 100,000 a day in 1976. In 1976 more than 2,000 people arrived from Africa daily at the three airports serving London and well over 700 of these came directly from Tropical Africa where viral haemorrhagic fevers are endemic. This change in travelling pattern has been reflected in admissions to infectious diseases and tropical medicine units, where the problem of the feverish traveller has become all too common. The recognition of Lassa fever and the introduction of a few cases into London caused alarm and gave impetus to the provision of safe hospital accommodation for such patients. My own Department was made responsible for dealing with such admissions from a large area of southern England with a population of approximately 11 million.

It is common for travellers, who have recently arrived from Africa, to develop feverish illnesses and fall under suspicion because of the wide publicity given to Lassa fever, 'Green-monkey' disease and other newsworthy tropical infections. Needless to say the vast majority of these patients have minor respiratory tract infections or malaria and very few fall into the category of viral haemorrhagic fever. It is obviously impracticable and unnecessary to take stringent precautions with all these patients. Yet there is always the lurking possibility that some may be dangerous. Unfortunately there are no distinctive features in the early stages of viral haemorrhagic fevers to enable a firm diagnosis to be made on clinical grounds so judgement has to be made on epidemiological evidence.

We divide patients with unexplained fever, who have arrived from tropical Africa within the previous three weeks, into three categories and admit them to hospital accordingly :

1. Those who have come from major cities in Africa, where the risk of viral haemorrhagic fever is negligible, are admitted to standard isolation rooms with routine barrier nursing.
2. Patients from small towns in tropical Africa are regarded with more suspicion and are admitted to a high-security room with filtered negativepressure ventilation and separate facilities, where they are kept under observation while malaria is being excluded. If parasites are not found and pyrexia continues such patients are transferred to maximum security.
3. The last and potentially the most dangerous group of patients are those who have been living or working in rural areas, medical and nursing staff from country hospitals, contacts of known cases and laboratory workers handling dangerous material. Patients in this group are admitted directly into a Trexler negative-pressure isolator.

I do not propose to discuss in any detail our standard isolation facilities. Suffice it to say epidemiological studies have shown that they afford a high degree of protection against spread of routine infections. Our high-security room provides effective protection for the community but the staff come into direct contact with the patient and rely upon protective clothing as a defence against these dangerous infections for which there is at present no form of active immunization. The hazards to staff from such patients can be greatly reduced by using the Trexler isolator system (1,2). With this system the attendants are separated physically from the patient by a barrier plastic film. Air filters attached to the isolator prevent contamination of the ventilation system and a negative pressure is maintained within the envelopes to prevent egress of airborne particles if the isolator is accidentally punctured. Over the past three to four years we have nursed a considerable number of patients in the Trexler isolator and in the past 18 months we have on average admitted a patient every six weeks. The patients have been mainly young adults but have included a child of 11 years. We have had no experience of nursing very young children or elderly patients in the isolator. Patients are generally aware of their

predicament and accept the restrictions as a necessary precaution. The majority are removed within a few days though an occasional patient may spend a prolonged period within the isolator, especially while waiting for the results of clearance specimens. There have been no problems with temperature or humidity providing the isolator is not exposed to direct sunlight. Noise levels have been acceptable and have not interfered with sleep or listening to radio or television. The convalescent period can be trying because the space available for exercise is very restricted. Much can be achieved to alleviate boredom by reading, listening to the radio, watching television and maintaining a link with the outside world through a telephone.

Initially the nursing staff expressed some misgivings about looking after patients in the isolator but they gained confidence when it had been demonstrated that the techniques were practicable and patients could be effectively nursed within the isolator. Staff now appreciate the protection afforded by the system and prefer to nurse potentially dangerous patients in this manner. A team of 12 trained nurses is necessary to provide effective cover and constant training is essential to ensure proficiency. This can be achieved by weekly training sessions and by partnering a novice with an experienced nurse. The basic skills are usually acquired within two days.

I would not like to give the impression that a negative-pressure isolator by itself is the answer to safe hospitalisation of potentially dangerous patients or that an isolator can be set up in any ward. Ideally the isolator should be sited within a large room in a separate high-security unit to ensure safety in case there should be a major failure of equipment or faulty technique in disposing of contaminated waste. Moreover, there is always the remote possibility that the patient may have to be removed from the isolator in the event of a major emergency, such as fire. There should be direct access for admission of the patient to the isolator and suitable changing and shower rooms for the staff. The patient's room should be fitted with storage racks for supplies, which are replenished daily as necessary. The high-security area should be fully equipped for the disposal of infected material by incineration or sterilization and have facilities for entertainment during convalescence.

Before admission of a patient the isolator is inspected carefully to make sure there are no defects in the plastic envelopes or rubber gloves and the other equipment in the high-security area is tested. The isolator is stocked with sufficient supplies to last for 24 hours. Thereafter fresh supplies are introduced as required and all waste material removed in sealed bags for incineration or sterilization. The patient is usually brought in by special ambulance and placed directly into the isolator, in which case the ambulance crew and the admitting doctor wear full protective clothing. A patient arriving from abroad or from a distant part of England may be transferred in a transit isolator, which can be docked with the main isolator rather in the manner of a space craft, and the patient transferred directly into the main isolator without exposing the staff.

All routine nursing and medical procedures can be carried out with minimal interference by the physical barrier though it is not practicable at present to undertake artificial ventilation or haemodialysis. Blood and other samples can be taken within the isolator without exposing the staff to risk. The samples are removed in sealed plastic bags, welded into a second plastic bag and then packed in a metal box for despatch by special messenger to the appropriate laboratory. Used needles are placed in screw-topped hard plastic containers before removal and incineration.

When it is decided that a patient is not suffering from a dangerous infection the contents of the isolator are dealt with according to routine barrier nursing procedures. The envelopes are subsequently sprayed with a 1% hypochlorite solution and washed thoroughly before re-use. If it is confirmed that the patient has a dangerous infection all the contents of the isolator are removed for incineration or sterilization. The interior of the isolator and the adjacent highsecurity area are fumigated with formaldehyde. The envelopes are later detached from their frames and destroyed by incineration. The room is then refumigated. Should a patient die from a dangerous infection the body can be removed from the isolator into a large plastic bag which can be sealed and separated without contaminating the environment. The body in the bag can then be sealed in a coffin for cremation or burial.

Samples for investigation of dangerous pathogens are dispatched directly to the Microbiological Research Establishment at Porton Down where there are facilities for the safe processing of such material. Problems have arisen with other investigations for we have lacked adequate facilities for complex haematological or biochemical tests. In this age of specialization the laboratories with the necessary microbiological expertise have been deficient in other skills. This is now being rectified and the Public Health Laboratory Service has established a few centres where some of the simpler tests can be undertaken and blood films treated to render them safe for reference to malaria experts. In my view it will be necessary for more complex haematological and biochemical tests to be carried out by appropriate experts using safety cabinets under the supervision of trained microbiologists.

Another problem which has caused us a great deal of trouble is the vexed question of quarantine or surveillance of staff looking after these patients. In the case of the patient with Ebola infection the staff

agreed to go into voluntary quarantine because of the evil reputation of the disease in Africa, the uncertainties about its mode of spread and the lack of scientific evidence about the relatively new techniques being used to contain the infection. Most of the nursing staff were married and the quarantine period of three weeks from last exposure caused a great deal of disruption of family life and some hardship. As a result many were subsequently reluctant to undertake this type of work, not because they had any qualms about the risks of infection but because they were not prepared to submit to quarantine. When you consider that they would be faced with the prospect approximately every six weeks I do not think that their attitude is unreasonable. Fortunately antibody studies after the Ebola episode 3 showed no evidence of infection amongst the 29 staff involved and laboratory investigations using bacteriophage have demonstrated that the isolator system is effective in containing small virus particles. As a result it has been accepted that less rigid measures will be sufficient.

The community physician is primarily responsible for the control of infection including hospital infection. Nevertheless, it has been agreed that we would be responsible for implementing surveillance of hospital staff and of keeping the community physician informed. We keep a register of all staff working in the high-security section and they are kept under daily surveillance for 21 days from last exposure or until the provisional diagnosis of viral haemorrhagic fever has been discounted. No restrictions are placed on the individual nurse or doctor apart from a daily recording of temperature. Should an individual be off-duty or away from home, he or she must telephone the Hospital each day to report the temperature. In the event of a mishap closer surveillance would be instituted. We have found this system to be acceptable to staff and to work well in practice. As far as contacts elsewhere are concerned, the responsibility for surveillance rests with the local community physician who visits twice daily to record the temperature and the appearance of the throat. Should a contact become ill, he is admitted for observation, either into the high-security room or into a Trexler isolator according to the assessed degree of risk.

Over the past four years, we have been gradually acquiring knowledge and experience in dealing with these complex problems. Obviously it is impossible to ensure absolute safety even at the expense of unacceptable curtailment of individual freedom. The measures I have outlined have proved effective in practice though they will no doubt need to be modified in the light of further experience.

ACKNOWLEDGEMENT

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DISCUSSION

K.M. Johnson : Dr. Emond, could you give us some more details on the criteria you do use in making a decision to discharge a patient from the isolator. I'm thinking here particularly of a person for example, who does not bleed and yet you are unable to make a specific diagnosis, how long would such a person have to stay there in terms of negativity on an antibody basis against all the potential bad agents ?

R.T.D. Emond : We find that for a considerable number of patients, the matter resolves itself. The temperature settles down within 48 hours, we keep them in the isolator for 48 hours and then take them out because the matter has resolved clinically. Or we may find malaria parasites in the blood film, treat them and when the temperature settles down, the patient is taken out. The most troublesome of course are the cases where the temperature does not settle down and where we are unable to find malarial parasites and where none of the other tests prove to be positive. In these circumstances, we have to wait until we obtain clearance from the Microbiological Research Establishment of Porton and with Lassa this takes about a week, with Marburg it my be two to three weeks or even more, it depends very much on the individual patient and the judgement has to be nude on clinical and also on microbiological grounds.

O.W. Prozesky : We have been confronted with the problem of those hematology and chemical pathology investigations. There are two ways out of it, one is to put a clinical pathologist in a laboratory attached to the unit, but this is costly since a special set of equipment is needed. The other one is to have these people acquainted with the procedures and work under the guidance of those familiar with the problems, but then the people on duty have to be doubled. So I don't think

there is a really good answer. A practical contribution I can make is that the Coulter counter can be easily decontaminated with sodium hypochlorite.

R.T.D. Emond : Our problem in England is that our laboratories are so mechanized and our Laboratory technicians so specialized that no one nowadays has a general knowledge of pathology that existed ten, fifteen years ago, only the older technicians have the necessary skills to combine microbiology and biochemistry.

T.E. Woodward : Just a word of warning. I have seen patients with plague, typhoid, and typhus with malaria parasites in their blood, it is a little perplexing at times.

R.T.D. Emond : That is why I said that we wait after finding malaria parasites until the temperature responds to treatment. We had one patient transferred from the Hospital for Tropical Diseases found to have malaria parasites. He was treated, the parasites disappeared but the fever didn't. So he was transferred for further observation under conditions of security.

D.P. Francis : I don't want to disagree with anything that is done in the Western World to isolate these patients, I think that the bed isolator approach is very reasonable but I don't think that the people in the developing world should feel that they all have to have a hundred bed isolators. we have no evidence of any airborne transmission of this disease. Only very very close contacts, not even superficial contacts of these highly infected fatal cases, get infected. I think that they can be reasonably well dealt with on the open ward with simple barrier nursing.

J. Casals : What does one propose to do in this scheme of isolation of patients with Ebola, Marburg or Lassa, who may be excreting or secreting viruses for weeks after the clinical recovery? It is very difficult to consider to incarcerate the person for a month or two. How is one to decide when a negative constitutes a negative, do you have to take three sanples and find them all negative before you release him?

R.T.D. Emond : Last year we had a man who had Lassa fever who was over the acute stage by the time we saw him, nevertheless, he was shedding virus in his urine and he did so for 42 days from the onset of illness. He was isolated not in a bed isolator but in a high security room. We allowed him to go out for walks on the grounds of the hospital still carrying his virus because he was a sensible fellow. Finally, we obtained three sets of negative specimens taken at weekly intervals, this took a month or so. The pressure of course comes on to us from the Public Health people who feel very unhappy about someone walking around the community knowing to be shedding virus, they are probably not unhappy about someone walking around the community not knowing to be shedding virus .

THE PLANNING OF A MODERN ISOLATION UNIT IN THE NETHERLANDS

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The Netherlands - just like a number of other European countries - do not have the possibility to isolate, to treat and to nurse adequately patients with or suspected of a highly communicable disease against which full protection by (e.g.) vaccination is not yet available. That is the reason why the big quarantine station "The Heyplaat" in Rotterdam is to be considered as obsolete: still valuable for smallpox but of no use for Lassa Fever or Marburg Virus Disease. That situation has led to setting up a working-group in 1975 for studying the question how to realize in the near future the isolation of patients with a very contagious disease in Holland. Such an isolation should not only protect (para-) medical personnel from infection by the patient but should also allow for optimum treatment and nursing.

After ample discussions the working-group advised the construction of a small isolation unit for 4 patients, situated in the centre of the country not far away from the National Institute of Public Health in Bilthoven. In a small country like Holland one unit in the centre is considered to be sufficient. There is a great chance that the unit will be built on the site of the Hospital "De Lichtenberg" in Amersfoort.

The development of the Trexler Plastic Bed Isolator by Vickers Ltd., appeared to be of crucial importance. It gave the impetus necessary to speed up the discussions about the requirements for the isolation-unit. The discussions in the working-group have not yet been concluded. We hope to arrive at a final digestion of the available information after studying the reports of two delegations of the working-group which have just returned from a visit to the United Kingdom and as well of the condensation of the knowledge gathered at this meeting in Antwerp. The perspective of the isolation-unit to be built seems to be a sound one. The general draught of it has already been drawn. Quite a few requirements, however, have still to be formulated. However immature our plans still may be, for those contemplating to have also modern facilities in their respective countries it may be worthwhile taking note of our endeavours in this respect. Therefore a brief outline of the unit we intend to have is given:

The isolation unit shall contain four patients at most. They will be treated in Plastic Bed Isolators in one ward where for extra security a lower air pressure than its surroundings should be maintained as well. The ward should have an exhaust-ventilator with a H.E.P.A. or "Absolute" filter. The entry to the ward will be through air-locks.

A separate room - connected with the ward - is intended for the convalescent who is considered not to be directly contagious any more but who is still regarded as a potential risk (e.g. semen still containing the pathogen).

Another small room that will also be connected with the ward will serve as laboratory where tests can be performed on pre-sterilized material of the patients.

The medical and paramedical personnel in the unit shall not be isolated.

The unit shall have a recreation room, bedrooms, bathrooms, toilets, etc., for the personnel.

An incinerator and an autoclave shall be installed between the ward and one of the adjacent rooms.

Although the unit will be connected to the hospital by means of a corridor, a small pantry outside the nursing area for preparing coffee, tea, etc., for patients and personnel is considered desirable.

A micro-wave oven (magnetron oven) should also be installed.

The unit should have a basement where the airconditioning equipment could be installed.

Ample technical facilities shall be made available for the patients' care, e.g. connection with the CCU, call and alarm system, mechanical ventilation, oxygen supply, air conditioning regulation, etc.

An ambulance-garage - part of the unit - shall be appropriate for loading and unloading of isolators and for disinfecting the ambulance. Annex to the garage there shall be washing and changing facilities for the ambulance personnel.

Provision shall be made for emergency current in case the main current fails.

The total area of the unit will probably be about 400 sq.metres, i.e. 100 sq.metres per patient.

The question of double-usage of the unit is still under consideration. Double-usage may hamper the rapid clearance of the unit in case a patient with or suspected of a highly communicable disease must

be isolated. On the other hand, double-usage may be very important for the continuous training of (para-) medical personnel in the unit.

At the moment we have one Plastic Bed Isolator in the Harbour Hospital in Rotterdam and a Plastic Aircraft Transit Isolator at Schiphol Airport.

4. TRANSPORT AND INTERNATIONAL SURVEILLANCE

EBOLA VIRUS DISEASE (EVD) SURVEILLANCE AND MEDICAL EVACUATION OF INTERNATIONAL MEDICAL COMMISSION MEMBERS IN ZAIRE

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4. French Army Medical Services, Marseilles, France.

INTRODUCTION

The International Medical Commission (IMC) which was formed by the Minister of Health of Zaire initially consisted of about a dozen members from various parts of the world. Subsequent recruiting in Zaire and elsewhere to establish the many teams required for field surveillance and other duties brought the total to approximately 50 members, mostly of the medical, nursing and paramedical professions but also administrative personnel and mechanics. The latter were required for maintenance of the Commission's fleet of vehicles, radio communication, power supply installation, equipment stores, etc.

The IMC's function was to investigate the cause, clinical manifestations and epidemiology of Ebola Virus Disease (EVD) and to advise and assist the Ministry on measures of control.

Exposure to infection by its members in the field, in the hospitals and in IMC laboratories was expected to occur and, at the time of the IMC's inception, there was every reason to believe that at least some members would develop clinical disease. It was necessary to establish contingency plans to ensure that optimum medical care facilities would be made available. The Yambuku mission hospital had ceased functioning as a health care centre due to the death of almost all of its personnel and the IMC had to provide some of the most basic emergency services such as minor surgery and obstetrics.

An improvised intensive care isolation unit was established to receive suspect EVD cases from the local population but it was recognized by the IMC that it would not be possible to give patients all the benefits of modern medical science under the locally prevailing conditions. The hospital is situated in the Zaire River basin where the vegetation consists of dense equatorial rain forest cover. Roads are non-existant and only a dirt-track requiring 4-wheel drive vehicles gives access to Yambuku, epicentre of the outbreak. All supplies had to be airfreighted by military aircraft, and helicopters were made available to the IMC as commercial air services had ceased its scheduled flights to the epidemic area which had been placed under strict quarantine.

From time to time the generators providing electric power broke down and could have caused critical interruptions of patient monitoring and therapeutic equipment.

Furthermore, the need to care for critically ill friends and colleagues under prevailing sub-optimal conditions would impose a serious additional workload and could be expected to have adverse psychological effects.

Taking cognizance of all these uncertainties and difficulties the IMC decided against nursing its members locally, even with the assistance of fully equipped intensive care teams which could be brought in from elsewhere. It was therefore agreed that IMC members suspected of having contracted EVD would be evacuated.

As evacuation could have serious financial and other repercussions a strict surveillance of IMC members was instituted from the start and criteria for evacuation were established.

Medical surveillance of IMC members. All IMC members were issued with personal medical record forms as reproduced in Fig. 1 and individual thermometers were supplied. Malaria, being hyperendemic in the area would be high on the list of differential diagnosis in cases with PUO (pyrexia of undetermined origin) and adequate prophylactic treatment was therefore prescribed. Only boiled water and bottled beverages were used for drinking purposes and meals were cooked at the mission in order to minimize the incidence of typhoid and other intestinal infections. Typhoid in particular poses problems in the differential diagnosis of EVD.

IMC members were also expected to record the dates on which actual or potential exposure to Ebola virus occurred. This information would be required when deciding whether a PUO was likely to be due to Ebola virus or to some other agent.

Fig. 1   Medical surveillance record used for members of the International Medical Commission.

Criteria for Medical Evacuation of patients suspected of having EVD.

While there was clearly a need for evacuating patients without undue delay it was equally important for patients to be carefully evaluated in order to avoid unnecessary implementation of the evacuation procedures which would be very costly and require international team work.

Major criteria, all of which had to be fulfilled, were

1. Pyrexial illness with one or more other symptoms of EVD of 48 hours duration.
2. A definite history of actual or potential exposure to Ebola virus within 21 days prior to onset of illness.
3. No other demonstrable cause.
4. Failure to respond to anti-malaria treatment.

Evacuation of such patients was to be implemented 48 hours after onset of illness.

Selection of the receiving patient-care centre. Three criteria were used in the selection of the hospital which was to receive IMC members with suspected EVD.

1. Transit time, as determined by distance.
2. Agreement by the hospital and the national health authorities of the country concerned to accept any number of IMC members for isolation and treatment.

3. Prior experience in treating cases of this kind .

Final arrangements were made with the South African Government as Johannesburg is at a reasonably short distance from Kinshasa and travelling time, in theory, should be minimal. The South African Government offered all possible assistance when advised of the position.

Means of transport.

Both minimum and maximum transit times were calculated for the journey from Yambuku to Johannesburg (fig.2).

Minimum.

Yambuku - Bumba by military helicopter	1/4 hour
Bumba - Kinshasa by chartered commercial airliner	3 hours
Kinshasa - Johannesburg by chartered commercial airliner	3 1/2 hours

Inclusive of transfer between the above means of transport it was expected that 8 hours would be the minimum delay between departure from Yambuku and arrival at the Johannesburg Isolation Hospital.

Maximum.

Yambuku Bumba by Landrover	4 1/2 hours
Overnight delay (airstrip at Bumba not equipped for night flying)	12 hours
Bumba Kinshasa by chartered commercial airliner	3 hours
Delay for USAAF aircraft to arrive in Kinshasa from Europe	8 hours
Kinshasa Johannesburg by air	3 1/2 hours
Inclusive of transfers the maximum delay was calculated at	32-34 hours.

It was our opinion that transport of a patient should not be undertaken beyond the quarantined epidemic region unless some form of isolation could be provided to prevent spread. Arrangements had therefore been made to obtain an aircraft negative pressure transit isolator. This was designed to fit into most aircraft used by commercial airlines. An alternate arrangement was the use of a US NASA isolation capsule. Its size necessitated also the provision of an aircraft by the USAAF large enough to accommodate the capsule as no aircraft currently in use by commercial aircraft could load this on board through existing access ports.

In the absence of either means of patient isolation it was envisaged that low flying, thus eliminating the need for pressurization, could safeguard aircrew from droplet infection.

Contingency plans were made accordingly by the three governments concerned in anticipation of EVD occurring among IMC members.

Implementation of contingency plans. On the evening of Friday 26 November a male US Corps volunteer aged 27 years complained of fatigue, cold shivers, headache and backache. His temperature was 37,1°C and it was decided to review the situation the next morning. The following day his temperature had risen to 37,6°C and he complained of abdominal pain, nausea, mild diarrhoea and urinary frequency. A full medical examination, using protective clothing, was carried out but nothing of significance was found. Microscopic examination of a urine sample showed no abnormalities, the WBC was 4680 with normal differential count, platelet count was 143 000 and no blood parasites were detected.

The clinical picture was compatible with the early stages of EVD, but also with that of some other infections. The patient was put on therapeutic doses of chloroquin and cotrimoxazole.

The patient's Ebola-virus exposure record was reviewed. He had been engaged in taking blood samples in the villages but none of the people he had bled had been shown to be active or convalescent cases of EVD. He had also assisted in the virology laboratory in Yambuku where he had handled and microscopically examined, but not prepared, slides with ultraviolet irradiated Ebola virus. It was shown that a recently arrived batch of this antigen still contained live virus in spite of UV treatment. The patient therefore had a potentially positive recent exposure history.

Fig. 2 Map of Africa showing the various localities involved in the 1976 outbreak of Ebola virus disease in Sudan and Zaire. Medical evacuation of an IMC member suspected of having EVD took place from Yambuku to Johannesburg via Bumba and Kinshasa.

The IMC logistics base in Kinshasa was alerted to the possibility of EVD in a staff member and was requested to alert others concerned without however implementing evacuation procedures. In accordance with the contingency plans it was decided to wait a further 36 hours in order to exclude other conditions for which the patient was being treated. That evening his temperature reached 38,3°C with a relative bradycardia of 84 beats/minute. Blood pressure was normal. He was isolated in his room and lock-up toilet and shower facilities were made available for his exclusive use. No visitors were allowed and all medical and nursing care were supplied by two IMC physicians.

On 28 November the patient remained mildly pyrexial and his gastro-intestinal symptoms and urinary frequency had ceased. His WBC had decreased to 4250 with a marked absolute and relative increase of monocytes to 24% of the total WBC.

The patient was moved to the improvised isolation intensive care unit in the hospital. He had proteinuria, a constant finding in all the EVD patients. The logistics base was requested to implement the evacuation programme to the point of full stand-by and readiness for take-off. The aircraft transit isolator had just arrived in Kinshasa. As the patient seemed clinically somewhat improved and the 48-hour period would terminate at nightfall the final decision whether to evacuate the patient was deferred till 6 am the following morning. That morning (29 November) the patient was still pyrexial and he had developed severe generalized backache. His back muscles were tender to palpation, he was nauseous and his pharynx was injected. The IMC logistics vase was requested to inform the governments concerned that evacuation would take place with immediate effect. By implication this was an indication for the administration of convalescent serum. Two units were heated in a 58°C waterbath for 1 hour to inactivate residual Ebola virus. It was then centrifuged and re-transferred to a new bag to eliminate large coagulated masses which had formed during heating. A power failure occurred during centrifugation. This also interrupted radio communications and departure was of necessity delayed.

Contrary to expectations a helicopter was not available and a landrover was equipped to install the patient as comfortably as possible. He was dressed in disposable surgical clothes and accompanied by the two attending physicians one of whom acted as driver. Both wore disposable protective clothing including masks. A spare landrover driven by another IMC member was stocked with drugs and equipment to deal with medical emergencies. The forest tract was in poor shape and the patient in severe pain in spite of analgesia. The drive to Bumba therefore took all of the anticipated 4 1/2 hours.

Temperature of the patient was 38,7ºC. It had been decided to administer the convalescent plasma after boarding the aircraft in Bumba where conditions would be more comfortable. The aircraft was scheduled to have left Kinshasa early in the morning and to arrive in Bumba before the patient. This was not the case and air transport did not arrive until the following morning. The landrover with the patient remained on the airstrip for some time while the IMC member in the spare landrover left to enquire about the fate of the aircraft. Such was the fear of the local populace that parking facilities in the shade of the control tower were denied to the IMC party. To protect the public the patient wore a half-face respirator with filter cartridges. He was sedated with analgesics and Valium and an intravenous catheter was inserted in the left arm. A catheter was used to ensure a troublefree intravenous pathway until arrival in Johannesburg in case of potential medical emergencies occurring during the rest of the journey which was already becoming protracted.

The first unit of convalescent plasma was infused in the landrover, very slowly at first and with syringes containing adrenalin and solucortef at hand. This was followed by slow infusion of 5% dextrose saline. The second unit of plasma was administered that evening after accommodation was obtained in the Bumba guest house with another IMC member.

An Air Zaire 'Fokker Friendship' arrived at dawn together with the aircraft transit isolator (Fig. 3). Appropriate decontamination of linen and utensils used by the patient was carried out at the guest house. He was still pyrexial and complained of severe backache and nausea. Wearing a clean disposable full length gown and half-face respirator the patient was transferred into the isolator without touching its external surfaces. The isolator was sealed after the air extraction mechanism was switched on. This was a prototype apparatus and difficulty was experienced in anchoring the bottle of intravenous fluid in an elevated position. An improvised anchor was devised. During take-off, and subsequent landings and take-offs, constant attention was required to the intravenous apparatus as the infusion rate fluctuated markedly. The sides of the plastic canopy tended to draw in unduly due to excessive suction which we did not know how to regulate. These faults apart, the patient was very comfortable and no problems were experienced in attending to his needs. Kinshasa was reached at 13h00 and a further disappointment awaited the evacuation team when it was learnt that no local aircraft was available for the next leg to Johannesburg. A USAAF C141 'Starlifter' had therefore already left Madrid for Kinshasa where it was expected to arrive that evening at 1900 hours. The next six hours were spent by the patient in the isolator on the back of a truck parked in a remote hangar. It was excessively hot and humid, the isolator started fogging up, the patient's temperature rose to 39ºC and he was in a great deal of pain and discomfort. He was heavily dosed with analgesics and Valium and a battery operated fan was procured. His stock of bottled mineral water was used for a sponge-down in an attempt to prevent a further rise in temperature. These measures were successful in making his condition much more comfortable. During his period in the hangar he started oozing blood from the intravenous puncture site. The USAAF transport aircraft arrived on schedule and took off for Johannesburg at 20h30 by which time the patient was drowsy. Turbulence was expected on the direct route and it was feared that this might precipitate vomiting and problems with the intravenous apparatus. The latter had become important in view of the possibility of bleeding becoming more severe and requiring supportive measures.

A detour was therefore made over the Atlantic Ocean, prolonging the flight by several hours but resulting in a very smooth journey. At arrival in Pretoria the isolator transport to the Johannesburg isolation hospital was handled by the South African Air Force. On arrival at the hospital, i.e. early on day 6 of illness the patient was noted to have a florid morbilliform rash which lasted two days. His platelet count had dropped to 105 000 and his WBC to 4000. His temperature returned to normal on day 6 and he made an uneventful recovery, nursed in the negative pressure containment bed isolator. Details of handling this patient in the bed isolator have been reported elsewhere (1,2). During convalescence his WBC rose to a normal value of 7700 and platelets to 300 000. Other clotting factors as well as liver function tests gave normal values from day 6 onwards. Specimens of urine, blood and throatswabs taken daily from day 2 of illness failed to yield virus, nor could antibody to known viruses or other pathogens be demonstrated.

Fig. 3. The Air Zaire 'Fokker Friendship', one of the IMC's landrovers with the patient, and the negative pressure isolator parked on the Bumba airstrip. The two escorting physicians are shown, one attending to the patient in full protective clothing. The isolator has been placed with its entry port facing the Landrover in readiness for the patient's transfer.

DISCUSSION

A great deal was learnt from this experience. The following are worth noting:

1. The problems posed by a single patient with a PUO and an uncertain exposure history to a lethal virus. The mild oozing of blood on day 5 and the appearance of the characteristic morbilliform rash on day 6 left no doubt in our minds at this stage that this was a case of Ebola virus infection. Subsequent negative laboratory findings were unexpected. Exhaustive investigations in the USA and in South Africa have so far failed to identify the etiological agent in this case. The possibility that the disease was caused by an as yet unknown agent cannot be excluded. A confusing factor was presented by a history of a bite by a half-wild pet civet cat just prior to the patient having joined the IMC, i.e. about 2 weeks before onset of illness. Exposure to rabies could therefore not be excluded but as the patient had been given a full course of duck embryo vaccine (a routine vaccination for Peace Corps volunteers) rabies was not considered a likely diagnosis. It is possible however that the civet cat was the source of another pathogenic agent which may have been the cause of the patient's illness. It is rather curious that a civet cat also featured in the history of the index case of the 1975 South African Marburg virus disease outbreak, although no aetiological association could be established. Treatment was continued with human diploid cell vaccine. Similar problems in deciding whether a patient with PUO from endemic or potentially endemic areas of Lassa fever, Marburg virus disease etc., should be dealt with as an international public health hazard are repeatedly experienced in various parts of the world (3,4,5).

2. The usefulness of a transport isolation system. The aircraft negative pressure transit isolator prototype was tested under the worst possible circumstances and found to be easy to handle. The need for several improvements or modifications were communicated to the manufacturers and these have since been incorporated in subsequent models. No problems we encountered at any stage with the power supply. Spare batteries were available and used while used set was recharged during our delay in Kinshasa.

3. Although a transport isolator was included in the contingency plans, its availability was uncertain at the time the patient became ill. It arrived unexpectedly and had to be assembled in Kinshasa in somewhat of a hurry. Likewise, the South African authorities were faced with the welcome but unexpected arrival of a containment bed isolator, on loan from the CDC, about 12 hours before arrival of the patient. No assembly or operating instructions accompanied the consignment, but the concerted efforts of several organizations succeeded in having it operational by the time the patient arrived. The isolator was designed to operated

on 110 Volt, 60 Hz current but local current is 220-250 Volt, 50 Hz. An adjustment was therefore made by means of a large rhestat, but as the cycles could not be changed this resulted in noisy operation which was troublesome to the patient, Of necessity it should be anticipated that international co-operation may repeatedly result in the movement of bed isolators to areas with different power supplies. Our experience showed that this need not be a handicap since, apart from low-level noise, the unit operated without any problems.

4.   As a result of the uncertainty about the means of isolation to be used during the patient's transport the South Africans were faced with the need to prepare for the arrival of the NASA space capsule, weighing approximately 3 tons and of large dimensions. An 80 ton low-loader tank transporter was made available by the S.A. Defence Force for the transport of. the capsule to the hospital site. Facilities were also at hand for the transport of smaller isolators with unknown voltage requirements.

5.   Although the landrovers and isolator were stocked with every available item and drug that might be needed during an emergency, a file for opening vials had been forgotten and its lack caused some uncomfortable moments when this became evident in Bumba. It is therefore strongly recommended that detailed checklists of equipment and drugs are prepared in advance and kept with the transit isolator. It is also highly advisable to stock up the isolator itself with all routine necessities prior to receipt of the patient as it is time consuming to pass materials into the isolator once the patient is sealed inside.

SUMMARY

Contingency plans were drawn up in the event of an IMC member in Zaire becoming ill with suspected Ebola virus disease (EVD). These provided for the following:
1.   Continuous medical surveillance of IMC members.
2.   Criteria for medical evacuation of IMC members suspected of having AHF.
3.   Selection of the receiving patient-care center.
4.   Means of transport, and isolation of the patient during transport.

The contingency plans were implemented when an IMC member developed a PUO conforming to all our criteria for medical evacuation. Cognizance was taken of the need for avoiding undue delay in getting a patient to an appropriate treatment facility while taking care to evaluate sick staff members as thoroughly as possible in order to avoid unnecessary evacuations.

Unanticipated problems occurred and prolonged the transport time beyond the 34 hours calculated maximum. A negative pressure prototype aircraft transit isolator was used and, apart from minor design faults, this was found to be extremely useful and easy to operate. No major problems were encountered in the administration of nursing and medical care through the plastic module which was put to the test under the most adverse conditions in the heat and humidity of equatorial Africa. A number of minor modifications recommended to improve overall efficiency and patient comfort have been incorporated by the manufacturers in subsequent models.

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INTERNATIONAL SURVEILLANCE AND TRANSPORT OF EBOLA VIRUS DISEASE AND OTHER HAEMORRHAGIC FEVERS: THE UK EXPERIENCE

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INTERNATIONAL SURVEILLANCE: MEASURES TAKEN IN THE UK AT THE TIME OF THE EBOLA DISEASE OUTBREAK IN SUDAN AND ZAIRE 1976

The control of outbreak of any specific disease should be based on an agreed national policy. This must be based on:

   a.   the epidemiology of that disease;
   b.   the organization of government services in that country.

In the UK the control of notifiable infections disease is mainly the responsibility of local government. The doctor responsible is the medical officer for environmental health for each district.

Certain diseases are made notifiable by statutory provisions of the public health acts and regulations by central government to facilitate the monitoring and surveillance. In 1976 the headings, Lassa Fever, Rabies, Marburg Disease and Viral Haemorrhagic Fevers were added to the list.

In the context of the control of infectious disease the DHSS publishes from time to time memoranda incorporating advice for the guidance of the professions on methods of control of such diseases, eg. memoranda have been produced on the control of smallpox, Lassa fever etc.

When the outbreak of Ebola disease in the Sudan and Zaire came to our attention in the UK in mid-September 1976, very little was known about the epidemiology of the disease and in the circumstances it was decided to apply with some minor modifications the same methods of control that had been drawn up for Lassa fever. Fortunately, the memorandum on Lassa fever, giving guidelines on dealing with a case or suspect case of Lassa fever had just been published and circulated to all registered doctors in the UK who were asked to refer to it if needed.

The control measures rested on:

   a.   early identification of known or suspected cases based on the probability that patients were not infected before onset of symptoms;
   b.   their isolation, using only the special facilities designated for the purpose and special transport and designated laboratory facilities;
   c.   surveillance of contacts ensuring cases were identified before there was secondary spread. The limits of the designated infected region were based on WHO reports in October of an outbreak of a viral haemorrhagic fever resembling Marburg disease; initially South Sudan and North Zaire, later South Sudan and the whole of Zaire.

The Microbiological Research Establishment (MRE) Porton was one of the three laboratories which played an important role in international surveillance providing facilities for examination of specimens from world wide sources.

From mid-October all persons coming from the infected area to the UK via ports or air ports were placed under surveillance. There was no restriction on movements of persons regarded as contacts. Port health units were advised by the Department of Health and Social Security 5DHSS) about isolation in a designated hospital for anyone with pyrexia, in consultation with the Medical Officer for Environmental Health (MOEH) and an expert in infectious diseases.

For those without temperature 21 days surveillance was instituted. Any person changing district was notified to the new district and change of country notified to the Embassy of that country, or to the Scottish Office, Welsh Office, Northern Ireland or Eire.

This exercise mainly involved aeroplanes. If a plane arrived at a UK port from an infected area all passengers' names and destination addresses were taken by the Port Health Authority and passed to Central Government (DHSS Communicable Disease Division) who in turn passed each to the appropriate district containing the destination address. The local MOEH was responsible for clinical checking for 21 days. Transit passengers had name, destination, address and, if known, flight number passed to Central Government (DHSS International Health Division) and thence to the health authorities of the country to which they were travelling.

Where indirect flights were involved, eg. from the affected area via a European City, passengers were asked by means of prominent notices in the airport to inform immigration on arrival of their recent stay or travel through infected areas. These people were then referred to the Port Health Unit.

In addition a Press notice was issued by DHSS at the beginning of the outbreak informing the public of the situation and the measures being taken. A central operations room was set up at DHSS for inquiries.

There were loopholes in the system. Certainly a number of passengers failed to notify the immigration officers of recent visits to affected areas and did not reach port health, some coming via other European Capitals also passed through unnotified.

There were inaccuracies in addresses given, eg the Grosvenor Hotel, London was insufficient to distinguish between 3 or 4 hotels. Others did not have a destination address or could not give an exact account of movements within the next day or two. Use of existing medical surveillance machinery was useful but meant that other tasks had to be abandoned. Some areas, especially London areas receiving most of the visitors, were stretched to the limit of their manpower. Had the inflow of traffic from the infected areas been any heavier or had the outbreak lasted longer, there would have been difficulties in maintaining the surveillance scheme.

There was, however, much unofficial co-operation from persons returning from the area and from medical officers of firms with personnel working in the area.

MOsEH and Port Medical Officers were kept informed of the situation in a series of letters sent from the Chief Medical Officer of the Department of Health on such matters as:

i. symptoms of the original Marburg disease;

ii. the present status of the outbreaks;

iii. limit of infected areas;

iv. port and airport control measures etc.

In all some 400 passengers passing through ports of entry into the UK were placed under surveillance for periods of up to 3 weeks, between mid-October and mid-December. None of these were considered as suspect cases although two were feverish. There were no severe clinical conditions discovered in the course of the exercise and no admissions to designated units for isolation.

In general, there was considerable co-operation from the public, local authorities and also those firms employing people in affected areas.

In the event of any future similar infectious outbreaks similar policy would be adopted with modification of reporting and surveillance measures and possibly distribution of manpower, exact policy depending on the nature of the disease involved and what was known about its epidemiology. It is hoped that familiarity with a disease or development of a vaccine would probably allow less stringent vigilance.

TRANSPORT: TRANSIT ISOLATOR FOR AREA MEDICAL EVACUATION OF PATIENTS

Recently several highly infectious diseases have come to the fore which were previously unknown: Lassa fever, Ebola disease, etc. Situations may arise where UK nationals suffering from a highly infectious disease may require transport from abroad to the UK.

Epidemiologically, it is undesirable to move patients from an infected to a non-infected area as this creates the potential risk of introducing and spreading a non-indigenous disease into a new country. It is therefore of prime importance that efforts be made for local facilities to be adequate for treatment of such patients: buildings, equipment and manpower and possibly the setting up of plasma banks in endemic areas for treatment on the spot.

Humanitarian or other considerations may give cause for transfer of a patient from an endemic to a non-endemic area. The problem is then how to do this in the safest way. To this end a transit isolator for use in aeromedical evacuation has been developed in the UK along the lines of the static isolator which has already been produced for use in hospitals.

The UK has proceeded with formation of an aeromedical evacuation team for transport of British subjects with highly infectious diseases, if necessary. The Royal Air Force has agreed to establish and maintain such a team. Having had an aeromedical unit for many years they have the experience and requisite expertise and capabilities to transport ill persons by air.

The team, drawn from this unit, has received training at Coppetts Wood Hospital in London and is continuing with inflight training using a transit isolator developed at the Microbiological Research Establishment, Porton Down.

The present plan is, should a case arise, it would be referred to one or more UK medical experts, if possible in contact with the medical consultant looking after the patient and in conjunction with the RAF doctor involved, and they would decide whether evacuation was medically advisable. An advance

party may be necessary to look at the feasibility of evacuation and set up transport and communication links. Many other problems such as type of protective clothing to be used, hospital facilities to be used in the UK, procedure for dealing with patients' relations, permission for over flying countries en route to the patients etc. are being worked out.

SURVEILLANCE AND TRANSPORT OF PATIENTS WITH SUSPECT VIRAL HEMORRHAGIC FEVERS THE UNITED STATES EXPERIENCE

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INTRODUCTION

Marburg virus caused illness and death in laboratory workers and animal handlers in Marburg (1) and Frankfurt (2), Germany and Belgrade (3), Yugoslavia in 1967 and was responsible for a small outbreak of disease in South Africa (4) in 1975. Lassa virus has been responsible for both epidemic and endemic disease in Sierra Leone, Liberia, and Nigeria (5), and retrospective serologic data have indicated Lassa activity in the Ivory Coast, Mali, and the Central African Republic-Zaire area (6). In the summer and fall of 1976, Ebola virus caused considerable morbidity and from 50-90% mortality in isolated outbreaks in southern Sudan (7) and northern Zaire (8,9). In addition, other viral hemorrhagic fevers have been identified in West (10) and South (11) Africa, South America (12-14), and Asia . Most of these viruses can be transmitted from person to person through either direct or indirect means.

In many countries where these diseases are found there are foreign nationals living and working in rural areas side by side with the indigenous population in settings such as hospitals, schools, and farms. The United States has citizens serving in areas where direct contact with persons harboring these agents is possible and periodically these persons return home for a variety of purposes such as furlough, change of assignment, or medical care. Moreover, since jet age travel has transformed our world into a global community, persons anywhere in the world can and do travel thousands of miles from one place to another within a mere 24 hours. Furthermore, this air travel takes place under conditions where large numbers of people are clustered for extended periods of time in confining environments and are subject to direct contact with an even much larger number of persons in airports. Therefore, the potential for spread of viral hemorrhagic fevers beyond their endemic foci into other areas of the world is very real.

IMPORTATIONS INTO THE UNITED STATES

Whether or not actual importations of hemorrhagic fever into non-affected countries can occur is no longer subject to question but definitely a fact because importations, both recognized and unrecognized, have already occurred in several countries of the world. At least two such importations have occurred in the United States.

The first known importation occurred on March 3-4, 1969 when an ill 52-yearold missionary nurse stationed in Jos, Nigeria was air evacuated from Lagos, Nigeria to New York City in the first class section of a commercial jet airliner accompanied by another missionary nurse and a physician. This patient had nursed a colleague who had died during the previous month with Lassa fever--a then undefined disease--and had assisted at her autopsy. At the time of air evacuation to the United States (the 16th day of her illness), the patient was quite ill with nausea and vomiting and was in shock. The entire first class cabin was utilized for transport aboard the airplane, and the seats in the left section were removed to accommodate the patient, her stretcher (specially designed to bolt to the floor of the aircraft) and equipment for intravenous fluid administration. A heavy curtain was hung across that section to permit the nurse and physician private access to the patient and to minimize exposure to the airline crew and passengers. After arrival in New York, the patient was transferred by ambulance without isolation precautions to a hospital and placed in isolation with full mask, glove, and gown precautions for all attendants. The account of her hospital course and followup have been published elsewhere (16). The physician and nurse who accompanied the patient on the flight did not develop serologic evidence of Lassa fever infection. No serologic survey or follow-up of the flight crew or passengers was undertaken, but it is presumed that no secondary cases occurred among this group (personal communication: J. Lyle Conrad, M.D.).

The second known importation occurred in February, 1976, when a 43-year-old white female American Peace Corps worker from Sierra Leone accompanied by her husband and a Peace Corps nurse returned to the U.S. with an undiagnosed illness which was later recognized as Lassa fever. This incident was studied because of the fear that secondary spread of infection to contacts of this patient might occur (17). Since the exact etiology of the patient's illness was not known prior to her hospitalization in the United States, precautions to minimize transmission of disease were not taken and therefore individuals who shared flights with her from Freetown, Sierra Leone, to London, and from London to Washington, D.C. as well as ground contacts at London's Heathrow Airport and in Washington, D.C. were considered to be at potential risk of infection.

A total of 552 contacts were identified as having had potential exposure to the patient either in Sierra Leone, on flights to and from London, or in Washington, D.C. Responsibility for the 115 contacts aboard the plane from Freetown to London as well as those at Heathrow Airport was assumed by the British government, since the airline company was British and most passengers were enroute to British destinations. No Americans other than the patient and her escorts were aboard that flight. British health officials sent press release notifications to airline passengers and requested that they report any fever or other symptoms of illness to the Quarantine Department by telephone. Of 65 persons (61 who deplaned in Great Britain, 4 who were contacts at London Heathrow Airport), 41 either called in or were subsequently contacted. Fifty-four passengers continued to destinations other than Britain or the United States. Appropriate embassies were notified by the British health officials in these instances. In Japan, contacts were hospitalized for observation during the surveillance period, but specific measures, if any, that were taken in the other countries are not known to us. Thirty contacts in Mobai and Freetown were identified and kept under surveillance by the Sierra Leone government. Responsibility for persons aboard the trans-atlantic flight was assumed by the United States. In the United States after the patient was in hospital isolation and appropriate specimens for diagnosis obtained, State Health Department epidemiologists were contacted by telephone and telegram to inform them of the imported Lassa fever case and to outline a plan for management. Next, in cooperation with the District of Columbia Department of Community Health and Hospital Administration, contacts in the District were identified by retracing the steps of the patient, her husband, and the nurse practitioner from the time of their arrival until admission to the hospital three days later. Each contact was given a printed bulletin briefly explaining the nature of the disease including the early symptoms and informing them of the potential risk of contracting the disease. A surveillance period of 21 days was established to include the longest known incubation period of Lassa fever. In the District, two types of contacts were defined: those at high risk because of face-to-face exposure to the patient and those at presumed low risk because of only casual exposure. The patient's husband and nurse were considered to be high-risk contacts. High-risk contacts were instructed to measure their oral temperature twice a day and report to the Health Department the actual reading plus any symptoms. Low-risk contacts were instructed to report each day any fever or other symptoms of illness. Activities of contacts were not restricted.

In addition to the District of Columbia, 21 states were involved in the surveillance of airline passenger contacts. State Epidemiologists or another designated person in each involved state were contacted to enlist cooperation and request that surveillance data be reported by telephone to CDC. Telephone reporting was conducted daily until all the passengers were located at which time reporting was reduced to twice weekly.

Interstate and international travel of contacts was monitored. As new states became involved in the surveillance system, appropriate state health authorities were alerted. Nineteen persons traveled internationally while under surveillance. High-risk individuals were requested not to leave the country. Low-risk contacts were permitted to leave but appropriate foreign health officials were notified in advance when such travel was agreed upon.

In the District of Columbia, 33 high-risk and 139 low-risk contacts were identified. No fever or clinical illness compatible with Lassa fever developed in any of these contacts within the 21-day surveillance period. Of the 233 passengers and crew aboard the transatlantic flight, one passenger did become ill with a compatible viral syndrome that included fever, cough, and myalgia. He was hospitalized in isolation and his initial leukocyte count was 3,700 WBC. Blood, urine, and throat swab specimens were negative for Lassa fever virus. Blood, urine, and throat swabs were also obtained from the patient's husband and nurse and were negative for virus isolation. Serum specimens from these persons did not contain Lassa antibody.

One month after surveillance ended, a serologic survey of high-risk contacts in Washington, D.C. was carried out, and specimens were analyzed for antibody to Lassa fever by the indirect fluorescent antibody technique. All were negative for antibody. So, in essence, no secondary cases of infection

were detected among either high- or low-risk contacts of this patient both in the United States and abroad.

GENERAL CONCEPTS

Before moving on to a discussion of the specifics of managing a suspect case of surveillance of contacts, let us consider a few general principles of surveillance and prevention, some of which were contained in a report to the Director, Pan American Health Organization by a working group on international surveillance of air travelers (18).

Transmissibility. Thus far, experience with these diseases seems to indicate that transmission occurs mainly through direct personal contact with infected individuals, probably by way of inadvertent percutaneous, oral or mucous membrane inoculation of infected material. Transmission by way of respiratory droplets or aerosols probably also can occur but presumably is a less frequent means of transmission. Additional factors of importance to transmission may possibly relate to the length of time that an individual has been ill and whether virus can still be demonstrated in the pharynx or only in the blood or in urine. Isolation precautions should as completely as possible reflect the virologic and epidemiologic observations. It may be that not all patients with Lassa fever serve as "disseminators" of infection, and it seems useful to keep this in mind as we proceed. Clearly, it will be much easier to make decisions regarding management of contacts and the extent to which isolation precautions should be practised for both patients and contacts when more complete information is available on a larger number of observations documenting duration of virus presence in pharyngeal secretions, blood, and urine.

Local-National Cooperation. So long as people travel internationally to and from endemic areas, diseases such as Marburg, Lassa, and Ebola will continue to be imported occasionally in spite of whatever precautions are taken. When such importations occur, it is unlikely that they will be discovered on arrival at ports of entry and, therefore, recognition will most likely depend upon the existence of an adequate national surveillance network. This suggests that resources to strengthen and improve surveillance efforts should be directed toward state and local health departments and not toward bolstering surveillance at airports. The development of a plan for surveillance and trans port of patients with suspect viral hemorrhagic fevers requires the cooperation of public and private sectors and specifically depends upon a close working relationship between local, state and federal government resources.

International Responsibility. When considering a viral illness such as Marburg, Lassa, or Ebola in the differential diagnosis of an ill traveler, great emphasis must be placed upon whether there has been recent travel in a known endemic or epidemic area. In order for this to continue to be useful for identifying suspect cases, countries must report the existence of highly suspect or confirmed disease activity of an endemic or epidemic nature to the World Health Organization for dissemination to other countries. The performance of adequate surveillance includes not only collecting information but also in sharing that information with those who need to know. Disease control programs are adversely affected by late.or inaccurate international reporting of suspected or confirmed disease outbreaks.

Liaison with Air Transport Organizations. In developing a plan of management, it is best to formulate general national guidelines which can then be adapted to local situations. The plan should involve liaison with the air transport industry as well as organizations both governmental and non-governmental that deal with air travelers. This type of liaison is extremely useful to health authorities which can then make known their specific needs for information such as passenger lists and addresses, seating arrangements, etc. Moreover, periodic meetings with those involved at each level are important to keep all attuned to changing situations such as geographic variation in disease activity, methods for transport and isolation, immunization requirements, specimen handling, and management of ill passengers. Occasionally, the health authority in each country in conjunction with air carriers and others involved should undertake practice drills in managing the transport and quarantine of a suspect case and in locating flight or other contacts so that in the unlikely event of a traveler becoming ill with a hemorrhagic fever-like disease on or after arrival, the health authorities will have had experience in dealing with the problem.

Transport and Isolation. Finally, patients known or highly suspected to have viral hemorrhagic fever and considered to be potentially infectious should not be transported on commercial planes with other passengers. Patients with such diseases should receive the best possible medical care with the best available isolation techniques. If a decision is made to air evacuate such persons, appropriate isolation and/or barrier nursing techniques and facilities should be utilized.

MANAGEMENT OF SUSPECT IMPORTED CASES AND SURVEILLANCE OF CONTACTS

The plan used for management of the Lassa fever case imported to the U.S. in November 1975 was based on an adaptation of the smallpox emergency protocol developed by the Center for Disease Control (CDC) entitled "Comprehensive Action in a Smallpox Emergency". The key elements of this plan are management of suspect cases and surveillance of contacts. (Table 1).

TABLE 1

SURVEILLANCE AND TRANSPORT OF PATIENTS WITH SUSPECT VIRAL HEMORRHAGIC FEVERS MANAGEMENT AND SURVEILLANCE OF CONTACTS

A. Notify State Health Departments.
B. Identify and characterize contacts (high-risk, low-risk, etc.).
C. Notify and inform contacts.
D. Begin surveillance.
 1. Check for symptoms and/or fever
 2. Quarantine
E. End surveillance.

Management of Suspect Cases. Early recognition and detection of suspect cases is of crucial importance to reduce the opportunity for secondary transmission of infection. Physicians and public health workers need to be aware that history of recent travel in an endemic area is an important point to pursue with suspect cases since the early clinical manifestations of illness are generally nonspecific in nature. As soon as a suspect index case is identified, the patient is transported to and placed in a suitable isolation or quarantine facility, specimens are collected, appropriately packaged, and sent to CDC or another qualified laboratory for confirmation of the diagnosis, and state and federal public health authorities are notified. Transport to the isolation facility can either be accomplished using an ambulance and transport isolation equipment or, as an alternative, protective clothing for the patient and accompanying personnel could be used. Isolation of the patient may be accomplished in a conventional hospital setting using advanced barrier nursing techniques or, if available, the patient can be placed in a hospital bed isolator. The patient in Washington D.C. was quarantined in a private room suitable for respiratory isolation and all persons entering the room wore complete protective clothing and special biologic filter masks. Clothing, linens, urine, and feces were treated with sodium hypochlorite. Blood smears for differential WBC counts were fixed with glutaraldehyde and blood collected for cell counts and clinical chemistries was treated with 2% acetic acid or heated at 60°C for one hour. After discharge, the patient's room was disinfected with 0.6% sodium hypochlorite, her mattress was incinerated and her jewelry and personal belongings sterilized with ethylene oxide (17). A suspect case should remain in isolation/quarantine until the diagnosis is confirmed or effectively ruled out. If confirmed, isolation/quarantine should be continued until throat swabs, blood, and urine are negative for viral isolation on at least 2 successive occasions at least 2 days apart.

Surveillance of Contacts. Immediately after identification of a suspect case, state and federal public health authorities must proceed to identify contacts and, depending upon the epidemiologic circumstances, (e.g., whether or not there has been actual face-to-face exposure, exposure to the patient's blood or specimens, etc.) separate them into various risk groups (high, low, etc.). Contacts are then notified and informed of the nature of the problem and the reasons for placing them under surveillance. In the Washington, D.C. situation, both high and low-risk contacts were checked daily for symptoms compatible with the disease (cough, pharyngitis, myalgia, vomiting, diarrhea, abdominal pain, chest pain, headache and abnormal bleeding) and in addition, high-risk contacts had their temperatures checked daily. Surveillance in this situation was continued to include the longest known incubation period for Lassa--21 days (17). This decision to monitor both high- and low-risk contacts daily required considerable time and effort. Therefore, alternatives to this plan need to be considered.

Since individuals at high risk are more likely to become ill than those at low risk, an alternative method could be to limit surveillance to the high-risk group until the diagnosis is confirmed (at which time surveillance of low-risk contacts would also begin) or to limit surveillance solely to high-risk contacts and not place contacts at presumed lesser risk under surveillance at all unless an individual in the high-risk group becomes ill. These alternatives offer the advantage of efficient utilization of the often limited resources that would be required for nationwide surveillance and concentrates them on those at greatest risk. Another option would be to consider restricting the activity of some or all of the highrisk individuals during the entire surveillance period. This would permit greater control and minimize the opportunity for exposure of others. This option would be particularly useful to apply to a

subpopulation of the high-risk group such as medical personnel, spouses, or close associates of the index case since transmission of disease through second, third, and fourth generations is almost uniquely associated with exposures in a medical or nursing care setting or intimate contact among household members.

The next consideration is when to quarantine cortacts and whether there are occasions when quarantine of asymptomatic and healthy contacts should be undertaken. A reasonable position would seem to be to use quarantine only when a contact develops compatible symptoms. In the Washington, D.C. episode, contacts with fever and/or compatible symptoms would have been quarantined. Furthermore, these investigators planned to quarantine all other high-risk contacts if one of their number developed confirmed disease and all high- and low-risk contacts if a low-risk individual developed confirmed disease. Secondary contacts of any primary contact with confirmed disease would also have been placed under surveillance.

Several alternatives with regard to the method of quarantine for contacts who become ill can be considered. One approach would be to have the individual quarantined at home, but this poses a perhaps unwarranted risk to other members of the patient's household and makes regular observations difficult. Quarantine in a hospital facility would obviate the problems of observation and undue exposure for household contacts but presupposes that a hospital would agree to accept such a suspect case and raises quite valid questions about who would bear the cost involved. Other possibilities for quarantine might include hotel or motel rooms or specially-designed quarters such as the mobile quarantine facility which was used to quarantine U.S. astronauts after their return from the moon. Duration of the quarantine period for an ill contact should depend upon confirmation of diagnosis. If specimens collected subsequent to quarantine confirm the diagnosis, the patient and all contacts should be handled as outlined above (see Suspect Case). Ill contacts may be released from quarantine after specimens for viral isolation and serology are consistently negative.

Each of these quarantine alternatives solves some problems but raises others, and none offers the complete solution to a rather difficult and complex problem. An ideal situation would be to have facilities available at selected locations around the U.S. to which ill contacts could be transported so that quarantine, isolation and/or hospitalization, and treatment could be provided. Facilities which theoretically could presently serve or be renovated to serve this purpose exist throughout the country, but considerable work must be done to assure their availability and to equip and staff them. Success in this rather massive project requires planning, discussion, and close cooperation between local, state, and federal health agencies, the Department of Defense, and the health care delivery industry. Action is underway to initiate these activities. In addition, the CDC is moving forward with plans to develop facilities to quarantine/isolate and if necessary provide medical care for a patient with viral hemorrhagic fever. A quarantine facility is being developed on CDC grounds to accommodate individuals with suspect disease or accidental laboratory exposure. Small isolators for ambulance transport and large ones for hospital use and patient care have been purchased. Negotiations are underway with a local hospital to provide space and train personnel for delivering medical care and treatment to an individual in a hospital bed isolator. Specialists in infectious diseases and internal medicine will be identified to direct the medical care of such a patient. Finally, CDC is preparing visual and other training materials for future use in instructing others to develop, operate, and maintain such equipment.

CONCLUSION

Because of the nature and extent of international travel today, it is possible that importations of diseases like Marburg, Lassa and Ebola will take place. It is unreasonable to expect that such importations will be discovered at ports of entry; it is much more likely that state or local health department surveillance activities will bring them to light. Countries should develop comprehensive national programs for the diagnosis, management and surveillance of such patients and their personal contacts. Such a program, originally developed for use in a smallpox emergency situation, was first adapted for use following importation of an individual ill with Lassa fever and has subsequently been revised. No instance of secondary transmission of viral hemorrhagic fever following importation into the U.S. has thus far been documented. Efforts continue to refine and improve a comprehensive plan of action for management of suspect imported cases of viral hemorrhagic fever. Essential issues which remain to be solved include providing for a safe and efficient means of transport of individuals to specifically identified, equipped, and staffed locations around the country for the purpose of quarantine and possible treatment. The U.S. experience in surveillance and transport of patients with suspect viral hemorrhagic fevers has been quite limited. Actions taken and plans for management thus far devised have been tentative and based solely on the best available judgment as to the general application of principles of epidemiology and hospital isolation; this has had to be the approach because of a lack of

adequate knowledge regarding the pathophysiology, epidemiology, and mechanisms of transmission of these diseases. Therefore, our approach to the future management of these problems must remain dynamic and adaptable to new developments in the epidemiology, control and prevention of these diseases.

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DISCUSSION

G.A. Eddy : What laws exist in respect to restricting quarantining and in fact incarcerating patients ? It is quite conceivable that someone who is highly suspected of Lassa fever might not wish to reside in that plastic bag. What do you do with such a patient ?

J.A. Bryan : As far as the U.S. goes, there are no national regulations to handle such a situation and it is very likely that that kind of event is going to occur. Some States, do have legislation that would

permit them to quarantine or isolate individuals although it is usually phrased in a very vague kind of language.

L.M. Roots : The same holds in the U.K. under the Public Health Acts, if a person is a danger to the public, action can be taken.

H. Bijkerk : in the Netherlands we decided to include Lassa and other haemorrhagic fevers in the so-called "Group of notifiable diseases" meaning that they should be notified already on suspicion. This gives the right to the Health Authority in agreement with other experts to put some body in isolation.

P. Brès : I would like to go back to the survey of the different febrile syndrome to be considered in the differential diagnosis. I think that we concentrated very much during these three days on viruses but there are numerous tropical diseases which have to be taken into consideration. I would like to mention malaria that can be confused with encephalitis, meningococcemia, hepatitis, borreliosis, leptospirosis, and more rare diseases such as infection by Streptobacillus moniliformis.

J. McCormick : Speaking about import of HF in the Western world, some groups of people are at high risk for such transport. They are some of the volunteers agency people and missionaries who, unlike the tourists, are going in the areas where these endemic diseases exist. The experience over the last five years in Sierra Leone shows that another Peace Corps volunteer was transferred to the United States with Lassa fever about two years before the one that Dr. Bryan mentioned. Only it was discovered serologically about a year later after she was transported into the country. We established in Sierra Leone a liaison with all of the volunteers and some of the missionary groups, to have blood from them and put their serum away. We instructed them to come to us if ill, so that we can evaluate them. Recently we treated such a person who thought he had Lassa fever for he had been working in an endemic area. It turns out he my not have it, although we have not finished all the virus isolation work. There are probably other areas in Africa where some of these groups work, which are endemic for some of these diseases and it would be worth the time to try to establish liaison and perhaps even bleed them when they go, so that one has the serum already in store and also to instruct them on what they should do in their particular area when someone gets sick enough that he thinks he might have one of these diseases.

D.P. Francis : We can use for these diseases the same logic used in the smallpox eradication programme. For smallpox, it was quickly seen one should spent the money where the disease was and not at one's borders and thus save a lot of money in the long run. If we look at the amount of work being done at this moment to understand the disease especially in the field, it's minimal and the only effort to my knowledge is T. McCormick's on Lassa. It seems to me that with the amount of money spent in some individual laboratories, this kind of effort could be supported for a long time. Thirdly, with all respect to our home countries, I would like, in the now of the teams in the field, to thank South Africa for being the only country that really would welcome us if we were sick.

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