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EBOLA VIRUS: A COMPARISON, AT ULTRASTRUCTURAL LEVEL, OF THE BEHAVIOUR OF THE SUDAN AND ZAIRE STRAINS IN MONKEYS

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Summary.—Histopathological and electron microscopical examination of human liver specimens collected during the Ebola haemorrhagic fever outbreaks in Zaire and Sudan indicated that Zairean strains of the virus produced more extensive lesions. Experimental infection of rhesus monkeys with Zairean and Sudanese strains of Ebola virus produced similar changes to those found in man. In Zairean strain infections large numbers of virus particles were found in the liver, lung and spleen accompanied by extensive necrosis in the spleen. In Sudan strain infections particles were found only in the liver and in greatly reduced numbers. The main distinction lay in the high proportion of aberrant particles found with the Sudanese strain. The possibility of these being defective particles is discussed.

IN 1976 an outbreak of severe and often fatal haemorrhagic fever occurred in Southern Sudan followed almost immediately by a similar outbreak 600 miles away in Zaire. The causative agents were isolated and named Ebola virus (Johnson *et al.*, 1977; Bowen *et al.*, 1977; Pattyn *et al.*, 1977). There was some evidence at the time suggesting that the Zaire outbreak produced a much heavier mortality than that which took place in the Sudan (WHO, 1978).

Ebola virus was found to be morphologically identical to the Marburg agent (Siegert *et al.*, 1967) but antigenically distinct (Johnson *et al.*, 1977). To date no antigenic differences have been detected between the Ebola strains isolated from patients during the outbreaks in both Zaire and Sudan.

Liver specimens from confirmed cases of Ebola virus infection in Zaire all showed fatty change and necrosis of hepatocytes and Kupffer cells but very little inflammatory reaction (Murphy, 1978). Large intracytoplasmic eosinophilic inclusion bodies were found in hepatocytes in two of these livers and Councilman-like bodies were also seen in areas of necrosis. By electron microscopy the inclusions were all found to be full of tubular structures identical to the internal component of Ebola virus particles. Virus particles were visible in extracellular spaces. In contrast, liver specimens collected *post mortem* from fatal infections in the Sudan exhibited very little necrosis but there was some necrosis in spleen and kidney (WHO, 1978). There were, however, considerable numbers of virus particles in Sudan liver specimens when examined by electron microscopy (Ellis *et al.*, 1978). Virus particles were only produced in the liver though some were occasionally identified in the capillaries of other organs. Within the liver the particles were found in extracellar groups, usually associated with recent collagen deposition, and the groups contained a high proportion of aberrant forms, often up to 50% of the particles being without either coat or core. Branching of the virions and torus forms was common. There were few inclusion bodies and no large arrays of precursor material, such as had been described for the Marburg agent (Peters, Muller and Slenczka, 1971).

The striking differences in the histo-

pathological findings in Zairean and Sudanese patients and the markedly different expression of virulence between Zairean and Sudanese strains of Ebola virus both in the field and in laboratory animals (E. T. W. Bowen, unpublished) prompted us to compare monkeys infected with these two virus strains. In this study we compare the effect of the Sudan and Zaire strains of Ebola virus in the organs of infected monkeys and offer some suggestions to account for the marked differences noted.

MATERIALS AND METHODS

Animals and inoculation.—Four young adult rhesus monkeys (*Macaca mulatta*) of either sex were used. For inoculation of virus and sampling procedures the monkeys were anaesthetized by i.m. injection of ketamine hydrochloride (Vetalar®, Parke Davis). Daily rectal temperatures were recorded and heparinized blood samples collected by femoral venepuncture. Monkeys were killed at various stages of infection by i.v. injection of pentobarbitone sodium.

Virus inoculation.—The source of the Zairean strain of Ebola virus was a human acute-phase blood (E-718) sent to us by Professor S. Pattyn, Antwerp. The Sudanese strain source was an acute-phase human blood (Boneface) collected by one of us during the Sudanese outbreak.

Both virus strains were passaged i.p. in guinea-pigs. The virus inoculation was a suspension of guinea-pig liver taken during the late febrile stage of the disease in the third guinea-pig passage as a 10% suspension in phosphate-buffered saline, pH 7·2, containing 0·75% bovine serum albumen (Armour Fraction V) plus 200 u penicillin and streptomycin. Monkeys were inoculated i.p. with 0·4 ml of virus suspension and the dose of virus calculated by parallel i.p. titrations in guinea-pigs and expressed as guinea-pig infectious units (GPIU)/ml. Monkeys received approximately 10^3 GPIU.

Materials for electron microscopy studies.—Both monkeys infected with the Zaire strain were killed on the sixth day and samples of liver, lung and spleen were removed for electron microscopy. One monkey infected with the Sudan strain was killed on Day 12 and samples of its liver, lung, spleen, heart, brain and kidney were taken for examination.

Electron microscopy procedures.—Tissue specimens were immediately placed in 3% glutaraldehyde in 0·1M cacodylate buffer at pH 7·4. They were cut into 1 mm cubes in this fixative in which they remained for 3 days. They were then washed in 0·2M sucrose/0·1M cacodylate buffer with 4 changes over 24 h and post-fixed in 1% osmium tetroxide in cacodylate buffer. After washing in the sucrose/cacodylate buffer and later distilled water for 2 h, the specimens were dehydrated in graded methanols, with a stay of 30 min in 3% uranyl acetate in 30% methanol. Embedding was in Araldite *via* propylene oxide. Sections were cut on an automatic Huxley Cambridge Ultramicrotome, lead stained and viewed with an A.E.I. 801 electron microscope.

RESULTS

Clinical observations

Sudan strain (Boneface).—The clinical course of the disease followed a pattern similar to that exhibited in monkeys infected with the Zairean strain (see below). Monkeys developed fever, anorexia, weight loss, diarrhoea and skin rashes. However, although the course of infection with the Boneface strain appeared to be just as severe, the monkeys survived the infection. Pyrexia occurred on the third day reaching a peak on Days 7 and 8 and persisted in one monkey until Day 14. One monkey was killed on Day 12 but the other monkey survived and remained normal thereafter.

Zairean strain (E 718).—Monkeys became febrile on the third day after infection with temperatures ranging from 40·2°. Pyrexia persisted until the terminal stage of the infection when the temperature became subnormal. By the fourth day the monkeys were quiet and listless; they did not eat or drink and sat huddled in their cages and responded only slowly to provocation. Diarrhoea and weight loss were noticeable features. A maculo-papular skin rash involving the face, limbs and trunk appeared between the fourth and fifth days. The rash faded on Day 6 when both monkeys were moribund.

Electron microscopy

Sudan strain.—One monkey killed on Day 12 had virus particles only in the liver. The particles were very few and difficult to find, but included a high proportion of aberrant forms (Figs 1 and 2). No virions were found in the kidney, spleen, heart, lung or brain.

A few inclusion bodies (which might

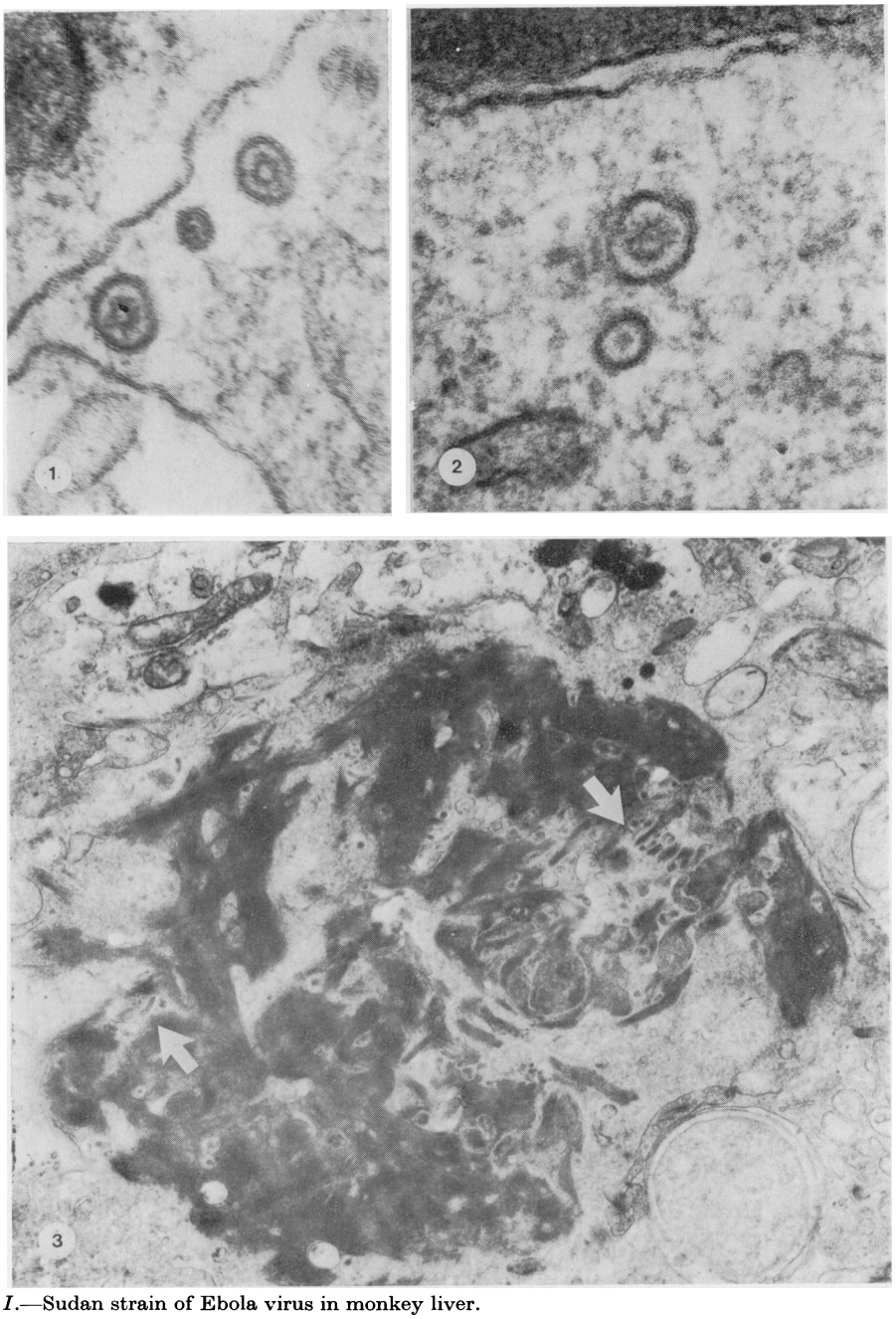


Plate I.—Sudan strain of Ebola virus in monkey liver.

FIG. 1.—Transverse section through an aberrant particle (without a coat) lying between two normal virions. ×114,500.

FIG. 2.—Two virions, the lower of which consists of coat only, without an RNA core. ×114,000.

FIG. 3.—A dense inclusion body with particles (some arrowed) around its periphery. ×114,000.

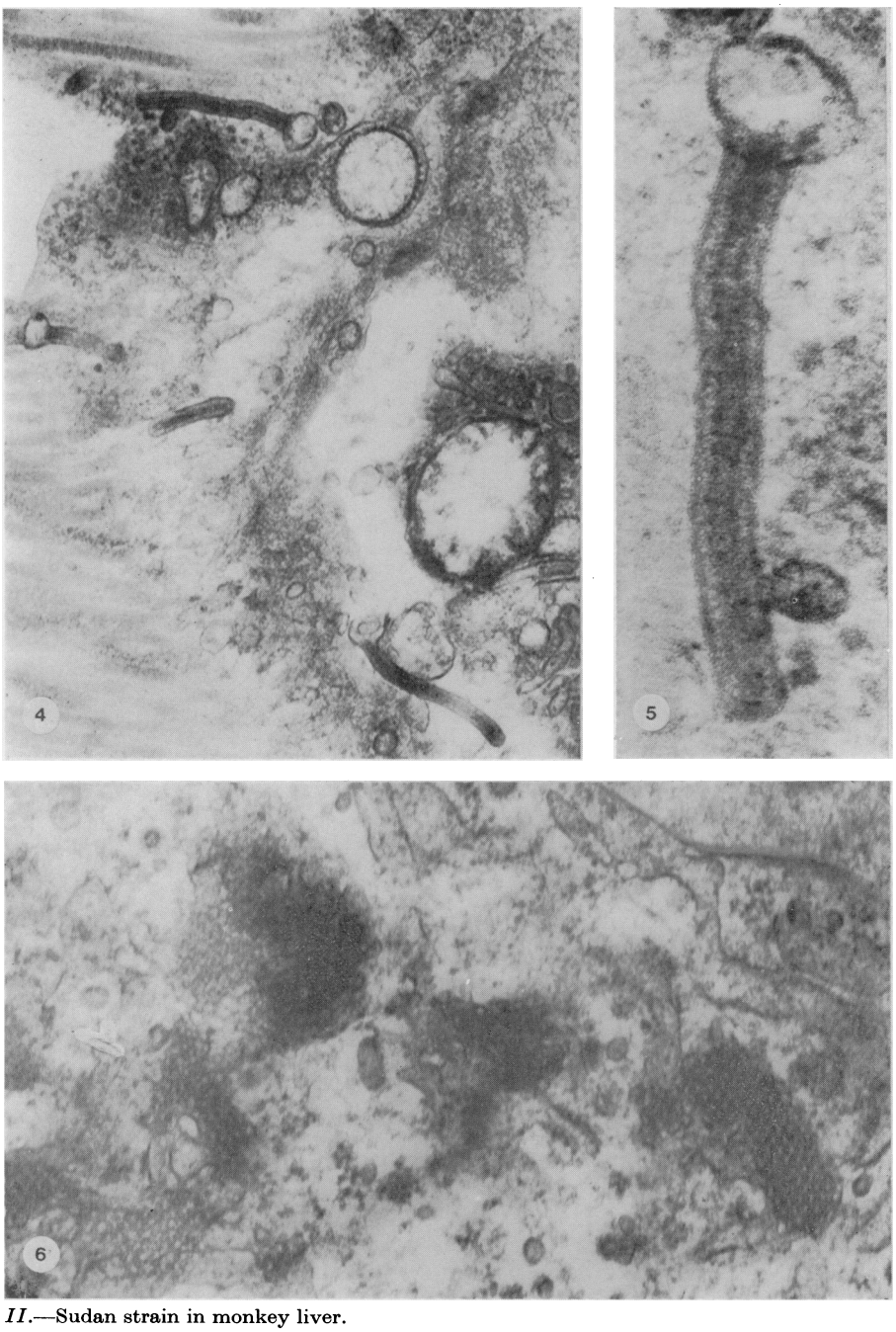
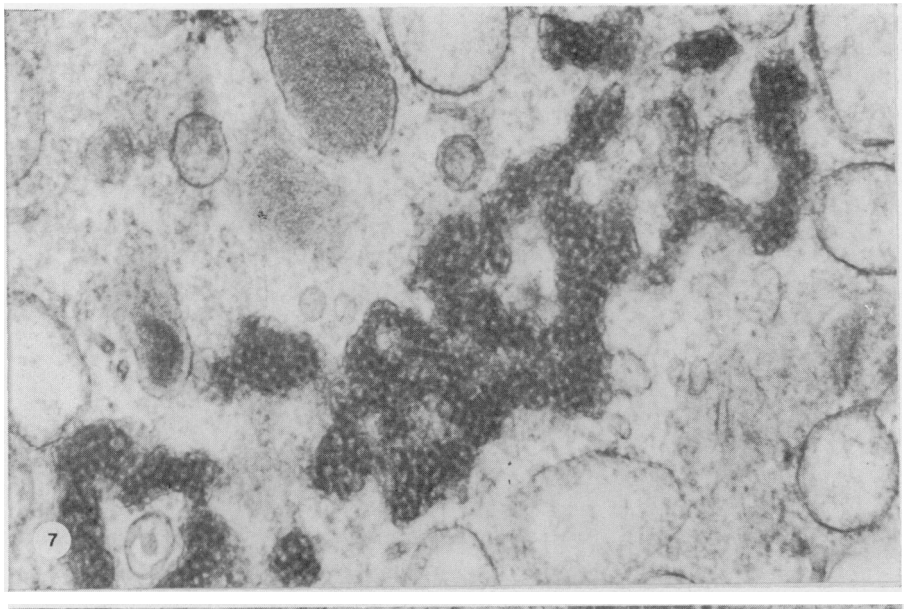


Plate II.—Sudan strain in monkey liver.

FIG. 4.—Particles, three of which are "torus" form precursors, among newly formed collagen fibres. × 28,600.

FIG. 5.—An enlargement of the particle at the top of Fig. 4, showing budding, presumably a method of branch formation. × 112,600.

FIG. 6.—Arrays of viral precursor material found in the hepatocytes of infected monkeys. × 56,300.



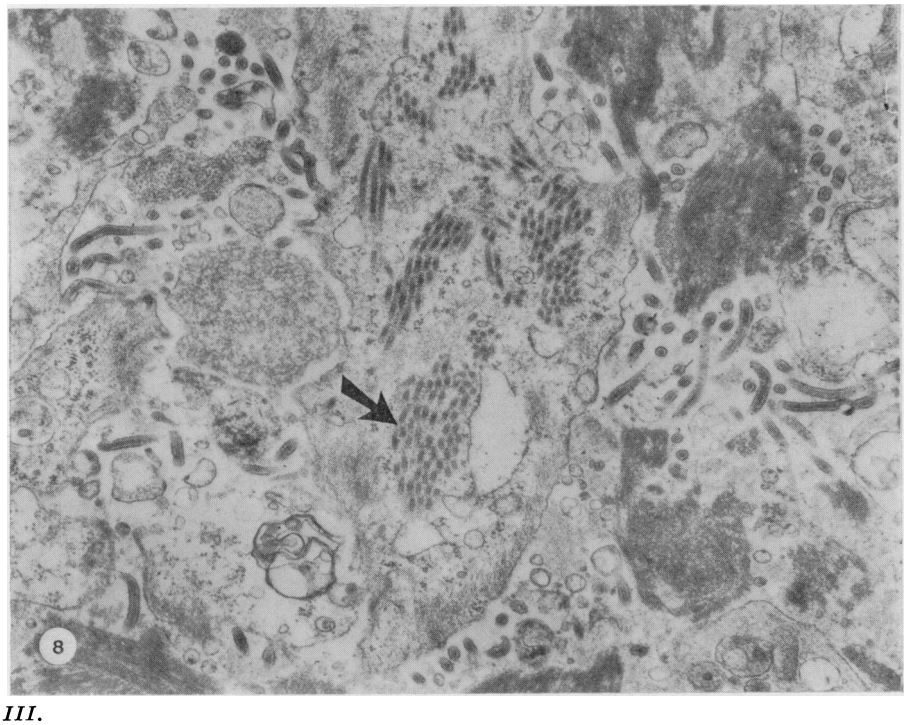


Plate III.

FIG. 7.—Sudan strain in monkey kidney. The osmiophilic material, found in many of the cells in the renal cortex, looks not unlike a protein array, though no clear geometric patterns were ever seen. ×44,300,

FIG. 8.—The Zaire strain of Ebola virus in monkey spleen. Note the large number of particles, with aggregations of RNA cores (arrowed) in the centre. No aberrant particles can be seen. ×17,700.

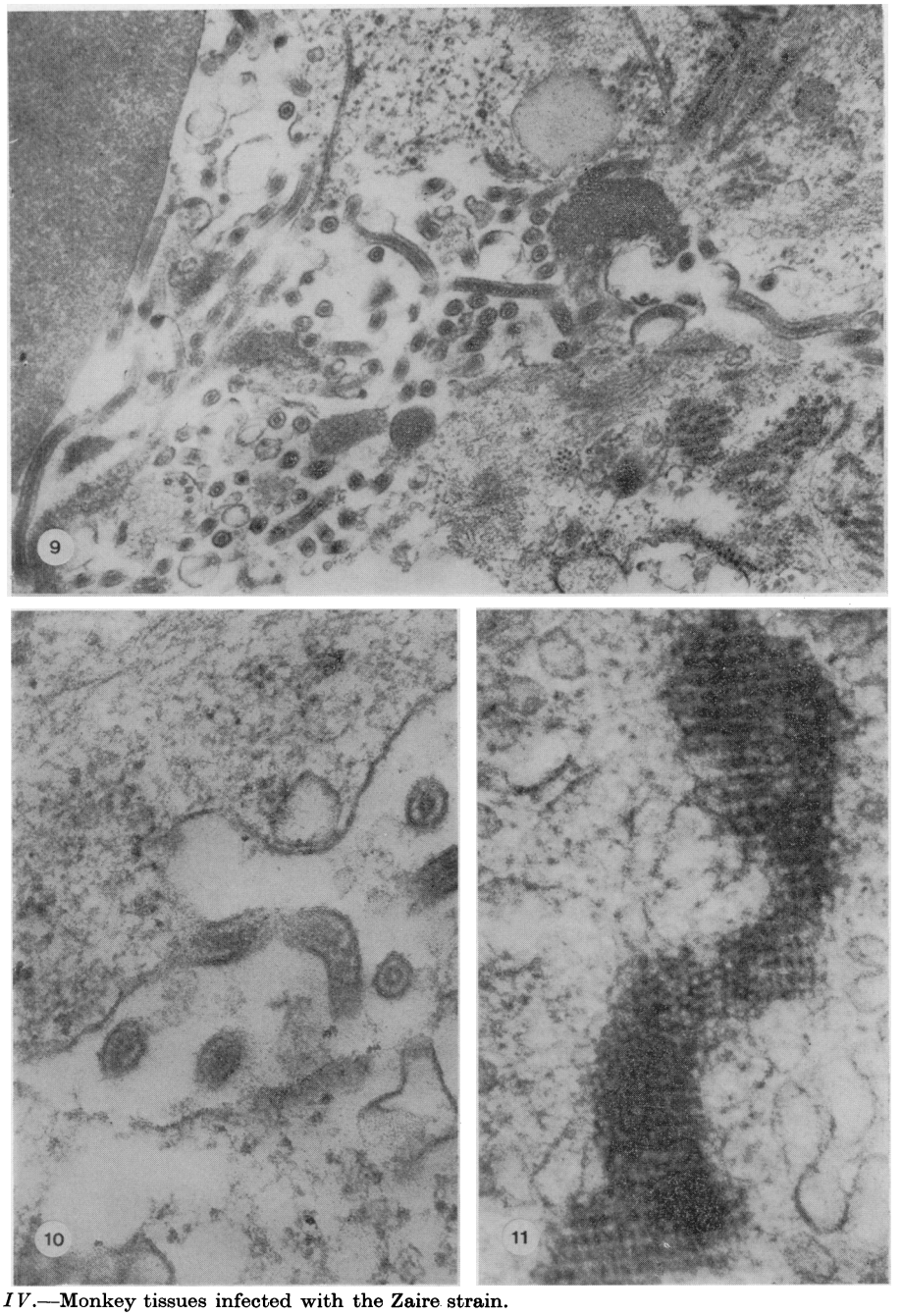
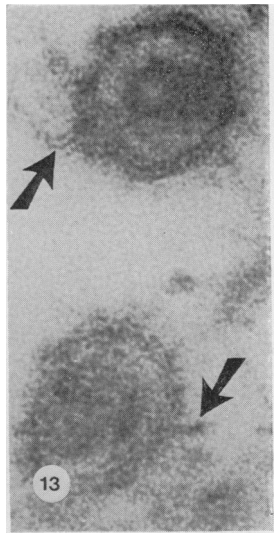


Plate IV.—Monkey tissues infected with the Zaire strain.

FIG. 9.—Monkey spleen. As in Fig. 8, the spleen structure is unrecognizable. Mature particles and cores are plentiful, with few, if any aberrant particles visible. × 28,800.

FIG. 10.—A virus particle budding into extracellular space in an infected spleen. × 72,000.

FIG. 11.—An array of viral precursor proteins in an infected monkey lung (*cf.* Fig. 6). × 72,000.



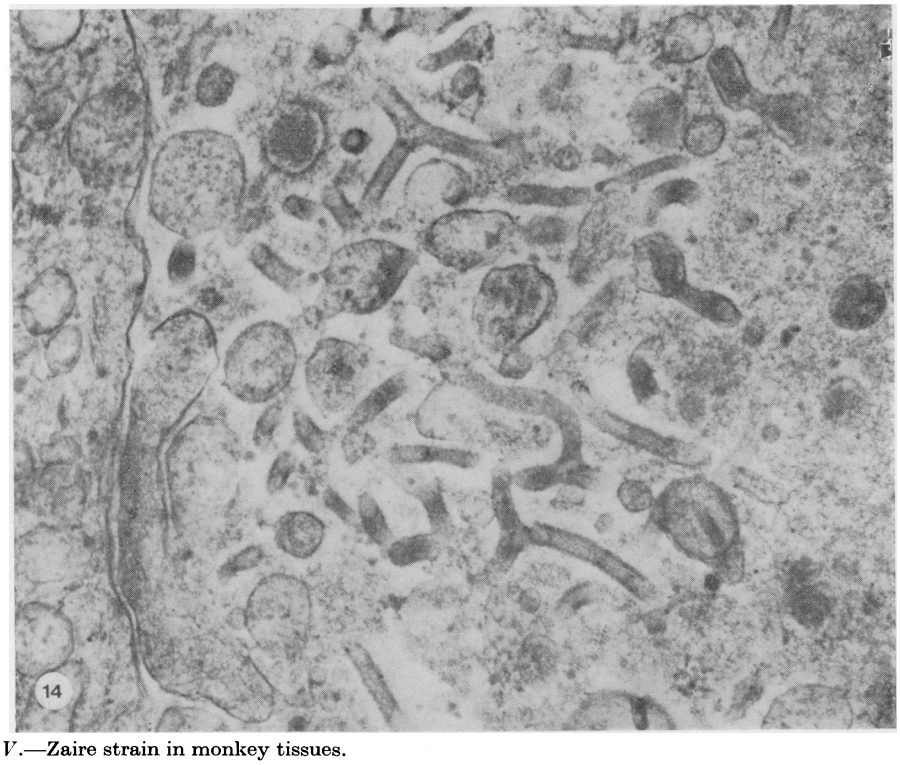


Plate V.—Zaire strain in monkey tissues.

FIG. 12.—Monkey lung. A large group of particles lying among collagen fibres. × 17,800.
FIG. 13.—Cross section of two virions in monkey liver showing evidence of spikes (arrowed). × 285,000.
FIG. 14.—A large group of branched particles in monkey liver. × 44,600.

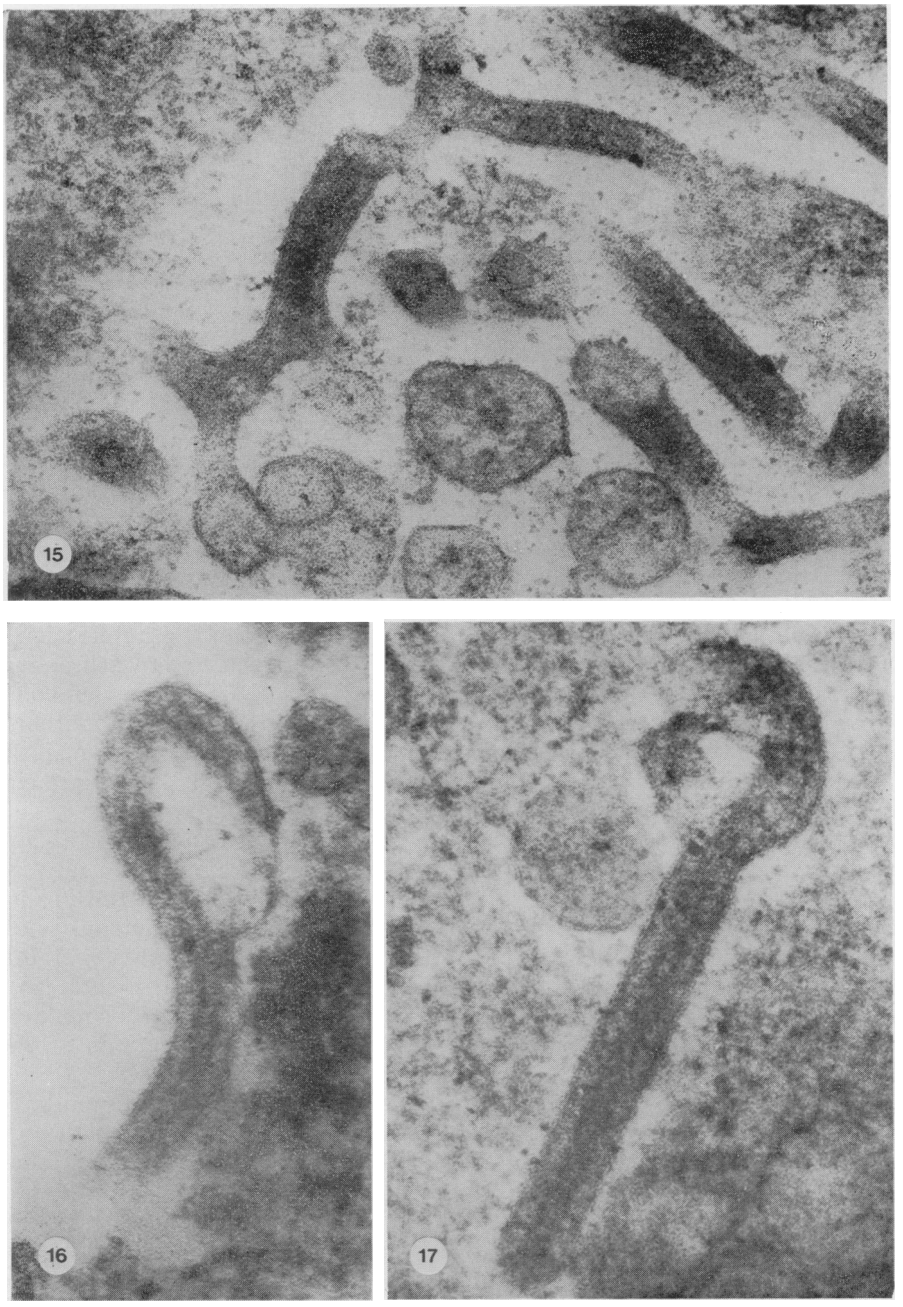


Plate VI.—Zaire strain of Ebola virus.

FIG. 15.—A particle in monkey liver with five branches. × 89,000.
FIG. 16.—Longitudinal section of a particle in monkey spleen, showing the RNA core curling up to make a "torus" form. × 112,400.
FIG. 17.—A later stage in "torus" formation, the ring form nearly complete. × 112,400.

have been large enough to have been seen by light microscopy) contained some particles around their periphery (Fig. 3). Most liver particles were seen near newly formed collagen (Fig. 4). Branching and torus formation was seen (Fig. 5). Some aggregations of possible precursor material (Fig. 6) were also found in the liver. While no particles were found in organs other than the liver, some kidney cells occasionally contained electron-dense material (Fig. 7) similar in appearance to that seen in the liver in Fig. 6.

Zaire strain.—Monkeys infected with this strain were killed *in extremis* on Day 6. Postmortem specimens of the liver, lung and spleen all contained great numbers of virions (kidney material was not available for this study). The spleen appeared to be the worst affected, being totally disorganized, with few cells remaining recognizable (Figs 8 and 9). Large numbers of arrays of cores (Figs 8 and 9) were found in many areas where active budding (Fig. 10) through cellular membranes was apparent. Owing to the extensive destruction of the spleen, it was difficult to decide whether any of the mature particles were actually within the host cells. Large amounts of precursor material (Fig. 8), possible membrane stores, and branching particles were seen within the spleen.

The lungs also contained large numbers of virions (Fig. 12) and arrays of precursor material (Fig. 11), the former usually associated with extracellular collagen deposits.

The liver appeared to contain fewer particles than the spleen and still retained large areas of recognizable hepatocytes. Branching of the particles was very extensive (Figs 14 and 15). Torus formation (Figs 16 and 17) appeared to follow the same pattern as in the Sudan strain. An indication of the "spikes" is seen in Fig. 13.

In all these three organs it was extremely rare to find a single aberrant particle, in spite of the very large number of virions that it was possible to scan with this very heavily infected material.

DISCUSSION

Although many of the details of the Ebola virus outbreaks in the Sudan and Zaire are likely to remain obscure, it has been established that the first reported victims were from Nzara and later in Maridi in the Sudan: that the disease was first thought to be yellow fever and that i.m. inoculations together with inadequate isolation and nursing probably led to parenteral transmission in the early days (K. M. Johnson and Babiker el Tahir, unpublished) in both Zaire and Sudan. Such a sequence could possibly have led to an increase in virulence and mortality in the subsequent outbreak in Zaire.

The different course of the disease and resulting pathology in man (Johnson *et al.*, 1977; Murphy, 1978; WHO, 1978) reported from Sudan and Zaire is paralleled by our findings in the monkey. The apparent limitation of particle replication in the host liver in the Sudan strain infections (Ellis *et al.*, 1978) and the contrasting widespread involvement of other organs such as the spleen and lungs with the Zaire strain (Murphy, 1978) are similar in man and monkey.

Infections with the two strains produce particles that differ markedly in both quantity and in quality. Both in man and monkey particles are not only limited to the liver in the Sudan strain infections (see above) but are found in greatly reduced numbers there, compared with those found with infections of the Zaire strain.

The main distinction of quality between the strains lies in the very high proportion of aberrant particles found in the Sudan strain, where up to 50% may be found to contain either only coats or cores (Ellis *et al.*, 1978). Aberrant particles are extremely difficult to find in Zaire strain infections, in spite of the very large numbers of virions present. The findings with the Zaire strain are very similar to those found in monkeys infected with Marburg virus (Murphy *et al.*, 1971).

It is tempting to equilibrate the lower virulence of the Sudan strain in both man

and monkey with both the low numbers of particles found in victims and the high proportion of these presenting as defective particles.

Finally, although no virions were seen in organs other than the liver during Sudan strain infections, inclusion bodies were found in the monkey kidney (Fig. 7). These did not appear to be the same as the viral precursor material arrays seen in the liver, for example. The possibility arises that they might represent immune complexes, and further work is needed to resolve this problem.

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