Studies on the Marburg Virus
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With 2 Figures

The events and circumstances of the outbreak of Marburg virus disease in Germany and Yugoslavia during August and September, 1967, have been fully documented by a number of authors in the Deutsche Medizinische Wochenschrift, March 26, 1968, and in other publications [1, 2, 3].

We briefly reported our preliminary findings in 1968 [4], because we felt that it may not have been sufficiently appreciated that the virus could multiply undetected in monkey kidney tissue cultures unless the cells were stained to reveal the virus-induced cytoplasmic inclusions. The present communication summarizes our investigations to date, with the exception of the examination of South African baboons and vervet monkeys for natural infection with Marburg virus, which is the subject of a separate paper [5]. Our objectives have been clearly defined on a practical basis:

1. To establish the identity of the virus isolates made in our laboratory,
2. To assess techniques for the rapid and reliable detection of the virus,
3. To provide additional information of value in the handling of this agent.

Virus Isolations

Our first isolation was made in tissue cultures prepared from the kidney of the vervet monkey Cercopithecus (aethiops) pygerythrus, which had been inoculated with serum from the patient Kliebe, sent in 1967 by Professor R. Siegert for serological investigation. The virus was re-isolated twice from this serum.

Through the courtesy of Professor Siegert and Dr. Gordon Smith, further samples of blood and tissues were obtained; and we recovered (and re-isolated) similar viruses from the bloods of patients Hartz and Flak, and from the liver of a rhesus monkey infected at Porton.

Laboratory Facilities

Although we were accustomed to handling a variety of simian viruses in our laboratory, special facilities for dealing with this particularly dangerous agent had to be improvised. A steel hood for tissue culture and complement fixation work was made, the air being drawn in through open front ports and passed out through filters irradiated by ultraviolet lamps. The tissue culture work involved 2800 cultures, of which more than 1000 contained multiplying virus; and over 2000 stained coverslip cultures were examined.

For studies in animals, we emptied an experimental monkey wing and used rooms at the furthest end. Sealed plastic enclosures for cages were improvised: 2 monkeys in separate cages, and up to 8 small animal cages, could be accommodated, accessible by way of tape-sealed plastic doors. Precautions were taken.
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To prevent cross-infection between monkeys and small animals, and air was constantly under negative pressure in the cages. Evacuated air was drawn first over heated coils, then along three 36-inch ultraviolet lamps in series, and finally into a box irradiated by four 36-inch ultraviolet lamps before being discharged into the air-conditioning exhaust duct.

**Tissue Cultures**

Except for the preparation of bulk virus stocks, roller tube cultures containing free coverslips were maintained at 36–37 °C. Various media appropriate for the different tissues tested were used, with foetal calf serum added. Most of the coverslips were fixed with Bouin’s solution followed by 70% ethyl alcohol, and were stained with haematoxylin and eosin (or eosin and phloxine).

Our first isolation had shown that while an infected sheet of monkey kidney cells might appear intact (Fig. 1), staining of the cells and high-power microscopic examination would reveal eosinophilic cytoplasmic inclusions (Fig. 2).

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**Fig. 1.** Vervet monkey kidney tissue culture 14 days after infection with Marburg virus. Magnified 200 ×

**Fig. 2.** Same culture as shown in Fig. 1, magnification 480 ×