

## SEVEN VIRUSES ISOLATED FROM THE VERVET MONKEY

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THE vervet monkey, *Cercopithecus aethiops pygerythrus*, is the animal most commonly used in South Africa for the laboratory isolation and maintenance of strains of poliomyelitis virus, and for the production of poliomyelitis vaccine. Following the introduction by Enders, Weller and Robbins (1949) of cultures of non-nervous tissue for the growth of this virus, various tissues from the vervet monkey have been used for this purpose, the most suitable tissue for large-scale cultures, and also the most sensitive for the isolation of poliomyelitis and a number of other human viruses, being renal epithelium prepared according to the trypsinization technique of Youngner (1954).

It is known that viruses may accompany tissues cultivated *in vitro*, manifesting themselves after variable periods in the cultures. The first of the group of agents now classified as adenoviruses was recognized through the occurrence of spontaneous degeneration in cultures of human adenoids (Rowe, Huebner, Gilmore, Parrott and Ward, 1953), and workers in the field of poliomyelitis research have isolated simian viruses from cultures of rhesus and cynomolgus kidney tissue (Hull, Minner and Smith, 1956). It is the object of this paper to describe briefly the effects in tissue culture of seven simian viruses which have been encountered since 1954 in the laboratories of the South African Poliomyelitis Research Foundation. Six of these viruses were isolated from uninoculated cultures of renal tissue from the vervet monkey, and one from the faeces.

Until a standardized classification of simian viruses has been adopted, it is proposed to call this particular group the SA viruses; and the following is a brief account of their behaviour in tissue culture.

SA1 (Fig. 1) produces syncytia with aggregates of nuclei of normal appearance, and is possibly identical with the MK virus described by Rustigian, Johnston and Reihart (1955). Vacuolation is frequently absent, and it is not yet known whether this represents a true difference between strains. It is the virus most commonly encountered in kidney cultures, usually appearing 10 to 14 days after preparation of the culture, the period being considerably shortened with passage. Syncytia arise in multiple foci, later coalescing to involve the entire culture which then flakes from the glass.

SA2 (Fig. 2) which has been isolated on several occasions from monkey kidney, produces intranuclear inclusions in syncytia. After several passages the inclusions tend to disappear, leaving syncytia with abnormal chromatin patterns. This agent does not have the characteristics of herpes B virus.

SA3 (Fig. 3) has been recovered frequently from kidney cultures. Cytopathic effects appear in foci about 10 to 14 days after the preparation of cultures, extending to involve the whole tissue over a period of days on initial isolation. The cells,

which show a fine refractile granularity, develop elongated processes in a reticular pattern before they fall off the glass. Staining with haematoxylin and eosin demonstrates large red cytoplasmic inclusions. These commence at multiple points in the cell, increasing in size and fusing to form homogeneous masses which lie around and over the nucleus. Similar inclusions are formed in cultures of HeLa cells and human amnion. This agent, which is clearly of simian origin, is neutralized by an antiserum, kindly supplied by Dr. A. B. Sabin, against the human ECHO 10 virus. Cytoplasmic inclusions identical in appearance with those produced by SA3 are observed in monkey kidney cells infected with ECHO 10 virus.

SA4 (Fig. 4) is more rapid in its cytopathic effect which somewhat resembles that produced by poliomyelitis virus. In preparations stained with haematoxylin and eosin, the characteristic features are margination of chromatin with an eosinophilic even appearance of the central part of the nucleus, followed by the formation of a large pale paranuclear area and lateral displacement of the nucleus which becomes crumpled or scrolled. Two eosinophilic dots are frequently seen in the open ends of the scrolled nucleus. Changes somewhat similar have been described by Reissig, Howes and Melnick (1956) in cells infected with poliomyelitis virus, but pallor of the nucleus followed by scrolling is more often seen in cells infected with SA4. This agent has been isolated on numerous occasions from kidney cultures.

SA5 produces a cytopathic effect similar to that of SA4, but is not neutralized by the serum of monkeys naturally immune to SA4.

SA6 (Fig. 5) has been recovered on three occasions from kidney cultures. Foci of rounded swollen cells appear after 14 days or more, progressing in size only very slowly, and passage does not markedly increase the rate of cell destruction. When stained with haematoxylin and eosin, the cells show lateral displacement and elongation of the nucleus which has, characteristically, either two small inclusions or one long inclusion. Binucleate masses are common, and there is increased phagocytosis of one cell by another. Fig. 5 shows a small focus with typical changes.

SA7 (Fig. 6) was isolated from monkey faeces in the course of a poliomyelitis virus feeding experiment. Growth in kidney epithelium is more rapid than in the case of SA6, and the nuclear inclusions are distinctive. Inclusions are formed in an annular pattern around the nucleolus, and can be seen to be multiple, with concentric structures. These later fuse into a homogeneous dense mass which is round or oval, being less elongated than that produced by SA6.

The majority of cultures in which these agents have been isolated consisted of trypsin-dispersed kidney epithelium grown at 36°–37° either as stationary cultures in Roux bottles or as roller tubes. The nutrient medium usually comprised Hanks' balanced salt solution containing 0.5 per cent lactalbumen

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#### EXPLANATION OF PLATES

FIG. 1.—Syncytium in monkey kidney epithelium produced by SA1 ( $\times 380$ ).

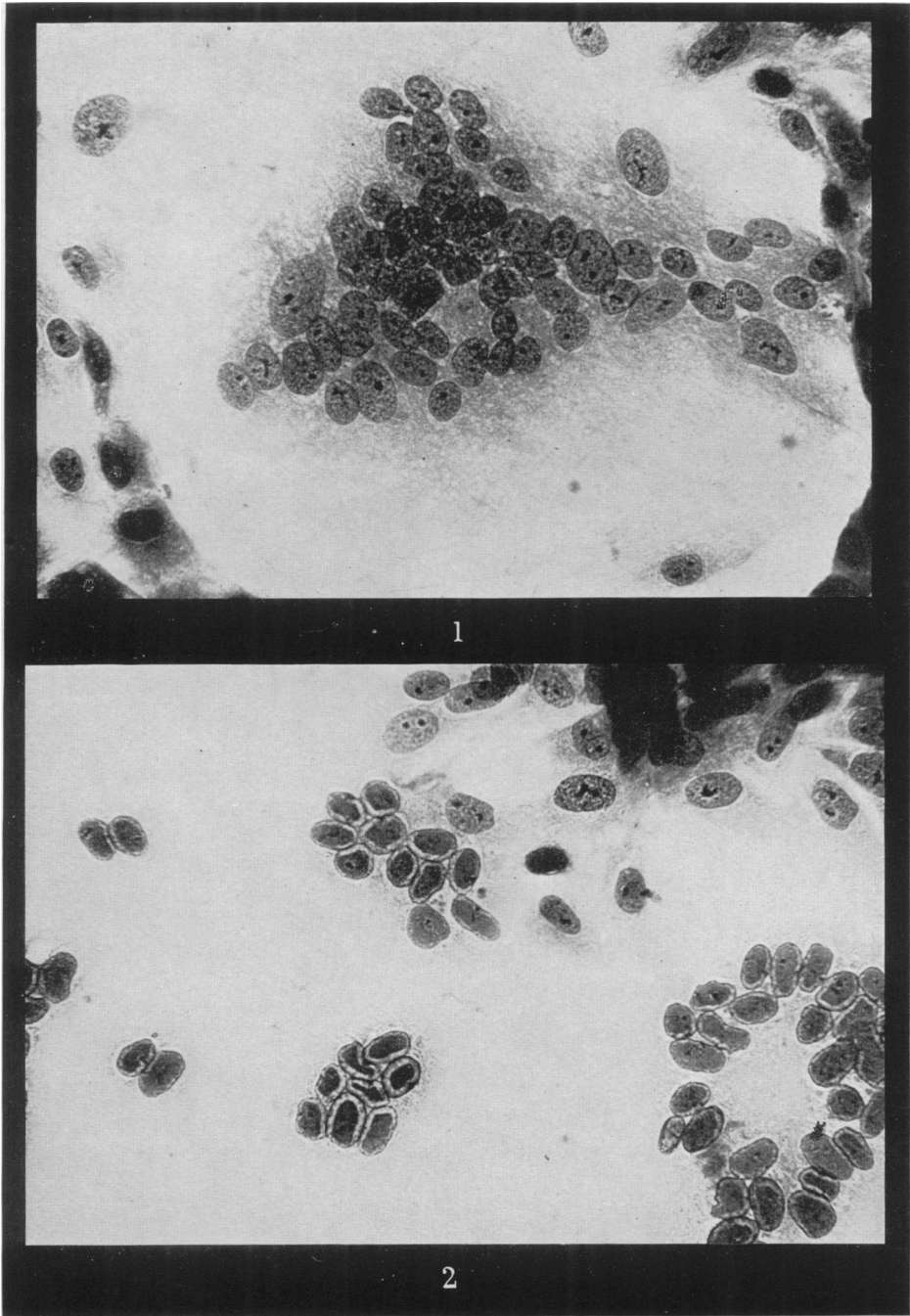
FIG. 2.—Intranuclear inclusions in syncytia produced in monkey kidney epithelium by SA2 ( $\times 380$ ).

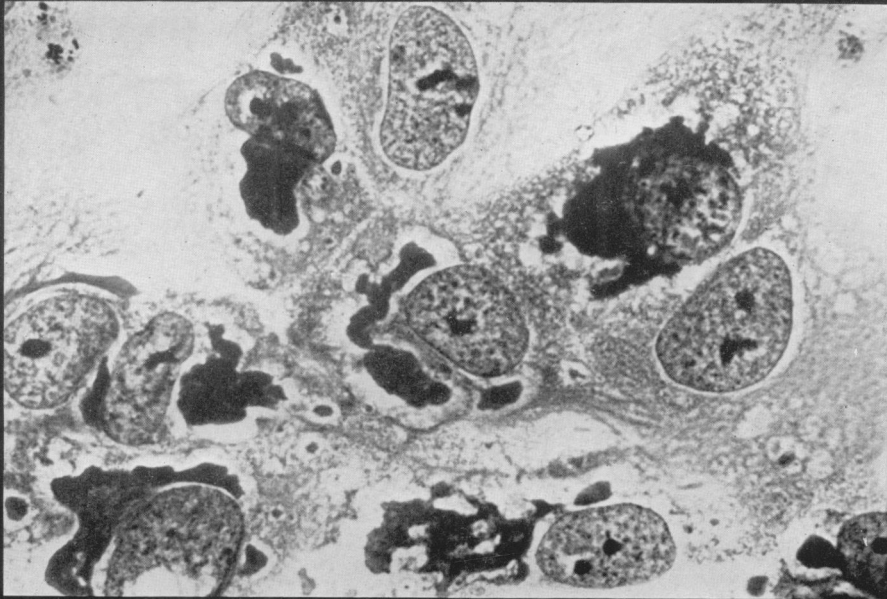
FIG. 3.—Cytoplasmic inclusions produced by SA3 in monkey kidney epithelium ( $\times 960$ ).

FIG. 4.—Monkey kidney epithelial cell infected with SA4 ( $\times 960$ ).

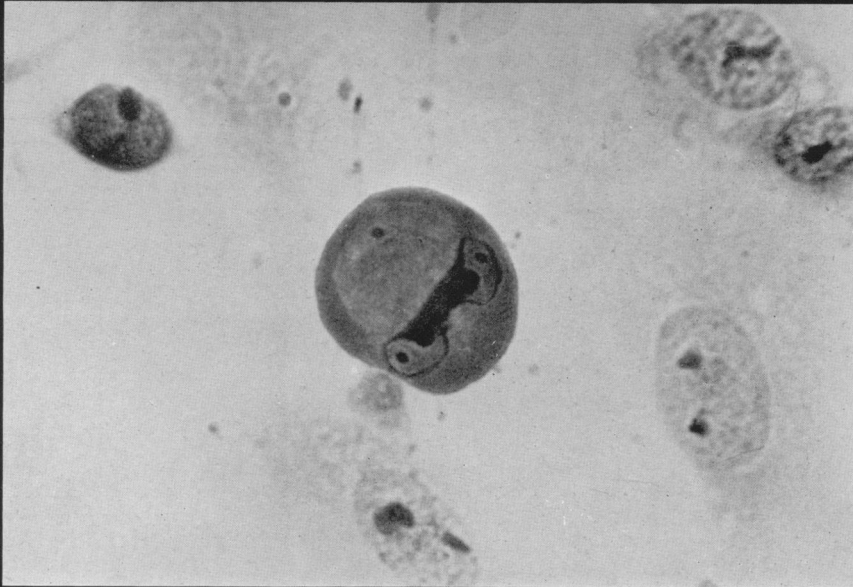
FIG. 5.—Focus of monkey kidney epithelial cells infected with SA6 ( $\times 380$ ).

FIG. 6.—Intranuclear inclusions in a kidney epithelial cell infected with SA7 ( $\times 1200$ ).

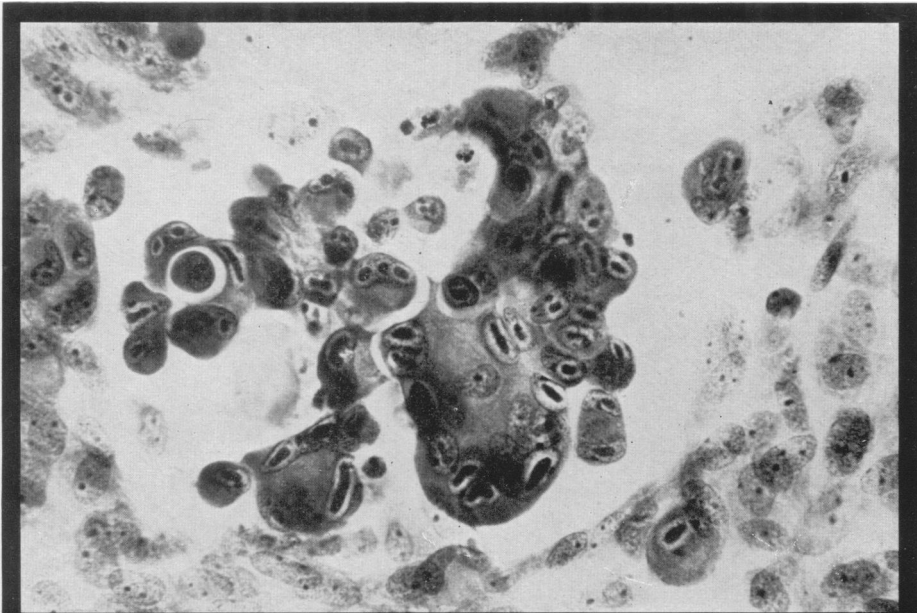




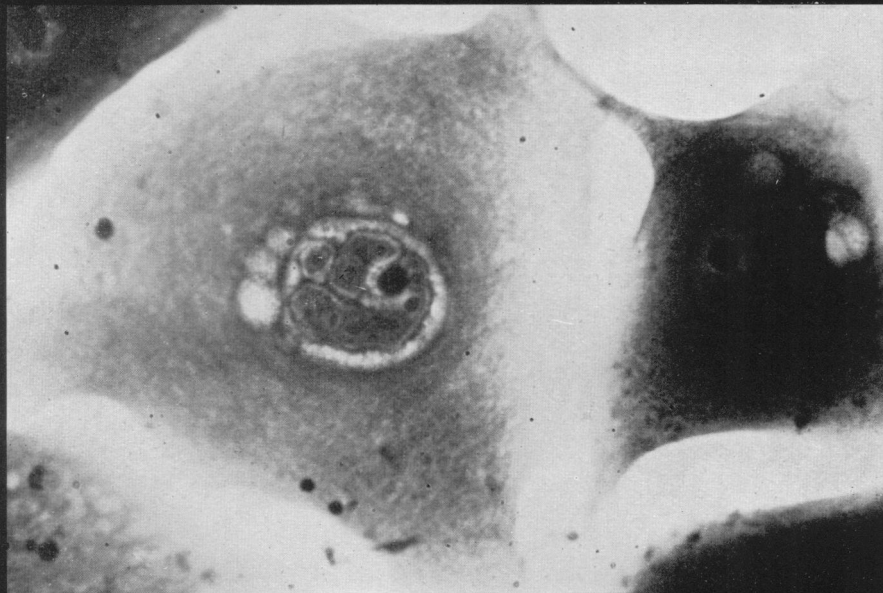
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hydrolysate, with the addition of up to 5 per cent horse serum. The possibility that some of the viruses were derived from either the lactalbumen or the serum has been considered, but is not accepted as likely since their appearance can be clearly seen to follow the use of certain batches of tissue, not batches of serum or lactalbumen. Precautions against contamination during the removal of kidneys make it improbable that the source of virus was not the tissue itself; but it is not possible to ascertain whether virus was present within the cells or in the blood contained in the kidney.

The use, for certain purposes, of small tissue batches consisting of kidneys from one or two monkeys only has led to a marked reduction in the number of isolations of SA3 and SA4, but SA1 continues to be frequently encountered in these small batches. The occurrence of such viruses in tissues used for the isolation of pathogenic agents from human material can be misleading, and an acquaintance with the viral concomitants of any tissue is essential. Inoculation of coverslips lying free in roller tubes, which can be fixed and stained in the tube, provides an additional means of identifying the causes of cellular degeneration, and serves as a useful screening precaution when simian viruses are frequently encountered.

More complete studies of the properties of the SA group of viruses are in progress, but it is felt that a preliminary description of their appearance in tissue culture will be of use, in view of the wide use made of simian tissues for viral cultivation.

#### SUMMARY

The cytopathic effects in tissue culture of seven viruses recovered from the South African vervet monkey are illustrated and briefly described.

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