THE CYTOPATHIC EFFECTS OF VERVET MONKEY VIRUSES

H. MALHERBE and R. HARWIN, *Poliomyelitis Research Foundation,* and M. ULRICH, *South African Institute for Medical Research, Johannesburg*

The introduction of tissue culture methods for the study of poliovirus by Enders, Weller and Robbins in 1949,[1] and the subsequent development of the monolayer cell technique by Youngner,[2] led to the discovery of many viruses. A number of these have been found in uninoculated tissue cultures, and it is helpful to have some knowledge of the viruses likely to be encountered in any particular animal, to avoid ascribing autochthonous agents to materials inoculated into cultures, and to reduce the danger of infection to persons handling cultures or animals.

Extensive use has been made of monkeys for poliomyelitis vaccine production, both as experimental animals for the testing of vaccine and as sources of tissue, especially kidney epithelium, for the cultivation of virus. A number of simian viruses have been isolated from these animals, particularly from rhesus and cynomolgus *Macacus* monkeys from the Far East. A classification of these viruses, based on serological differences and on cytopathic changes observed in unstained tissue cultures, has been developed by Hull and his colleagues,[3-5] each virus having a number following the prefix SV (for simian virus).

In South Africa the vervet monkey *Cercopithecus aethiops pygerythrus* is most commonly used for the production and testing of poliomyelitis vaccine. It has been found convenient to group the viruses recovered from monkeys in this country in numbered categories with the prefix SA (for simian agent), according mainly to the cytopathic changes observed in vervet kidney tissue cultures stained with haematoxylin and eosin. While there is obviously some overlapping between the SV and the SA series, several new viruses are included in the latter group. A more fundamental classification of simian viruses is under consideration, but in view of the greatly extended use of cercopithecus monkeys in several countries during the past few years, it may be of practical value to illustrate the changes produced in tissue cultures by the SA viruses. In previous communications[6-9] some properties of the agents in the first 12 categories have been reported, and this study presents 3 further categories.

METHODS

Monkey Supply and Accommodation

Monkeys are caught by farmers in several areas in the Republic of South Africa, and are railed in small groups direct to the Poliomyelitis Research Foundation in Johannesburg. They are then held in larger groups for varying periods, during which those animals with overt disease are removed. Contact with humans is unavoidable, but is reduced to a minimum, and there has been little evidence of infections acquired from Man. Grouping of monkeys undoubtedly leads to the rapid spread of infection and, while this eventually results in herd immunity, it also produces a high initial mortality and a greater number of latent virus infections.

Virus Isolation

Only a limited study of the respiratory and intestinal tracts of monkeys has been made, but during 8 years kidney tissue cultures have been carefully investigated for the presence of cytopathic viruses. Viruses which do not produce obvious changes in cells have been sought by the haemadsorption technique and by animal inoculation, with negative results.

Materials to be examined were suspended in a balanced salt solution containing antibiotics, and after centrifugation the supernatant fluid was inoculated into monolayer tissue cultures. In the safety testing of vaccine, roller tube or stationary bottle cultures were either inoculated with vaccine or left uninoculated as controls. Cultures were maintained for periods of up to 5 weeks at approximately 37°C. Fluid from any culture suspected of harbouring a simian virus was passaged into coverslip cultures. The use of tissue growing on a coverslip lying free in a roller tube has been invaluable in detecting and identifying viruses. Gross changes in the culture may be observed through the wall of the tube; and subsequent fixation and staining can be carried out with the coverslip in the tube.

Fixation and Staining of Cultures

After withdrawal of the fluid from a culture, fixation with Bouin's solution for one hour, followed by overnight treatment with 70% ethyl alcohol, was employed. As Reissig and Melnick[10] have stressed, the demonstration of inclusions depends largely on the kind of fixative used.

After removal of the fixative, Ehrlich's haematoxylin was applied for 30 - 120 minutes, according to the strength of the particular batch of stain. This was followed by rinses in water and rapid differentiation in 0·25% hydrochloric acid in 70% ethyl alcohol, terminated by immersion in water. After a rinse in 70% ethyl alcohol the culture was then exposed to 1% eosin in alcohol for 2 minutes. Subsequent rinses in alcohol and xylol were followed by mounting of the inverted coverslip on a glass slide by means of a plastic mounting medium.

The figures illustrating this paper were prepared from photomicrographs on Kodachrome film of vervet monkey kidney tissue cultures infected with the SA series of viruses, and were photographed by one of us (M.U.).

RESULTS

Category SA 1

This comprises strains of the most commonly isolated virus, the prototype of which is probably identical with the MK agent described by Rustigian *et al.*,[11] and called simian foamy virus, type 1, by Johnston.[12] The virus has been recovered from mouth swabs and from uninoculated kidney cultures. It is present in small amounts in the kidneys of many monkeys, and if cultures are held for prolonged periods a higher isolation rate results.

Syncytia without inclusions are produced by the mergence of a number of cells whose nuclei migrate towards the centre of the cytoplasmic mass (Fig. 1). Vacuolation or foaming of the cytoplasm may be present in the syncytia (Fig. 1A).

Cultures infected with SA 1 virus can also be readily infected with a number of other viruses which may produce nuclear and cytoplasmic inclusions in the syncytia.

SA 1 virus does not cause haemadsorption or haemagglutination. It is inactivated by ether and chloroform, and is not stabilized by molar magnesium chloride at 50°C.

Category SA 2

With a small proportion of strains recovered from uninoculated kidney cultures and otherwise resembling SA 1, nuclear inclusions are observed in a few of the syncytia (Fig. 2). Further serial passage in tissue culture results in the disappearance of the inclusions after two or three subinoculations. These strains have been placed

in category SA 2, and it is concluded that a second simian agent was present.

Recent experience with neutralization tests on attenuated polioviruses has strengthened this view. The inoculation of serum-virus mixtures into kidney cultures, naturally infected with SA 1 virus, has occasionally resulted in the appearance of numerous large nuclear inclusions in the syncytia; and the omission of antiserum in subsequent passages has led to the unmasking of the poliovirus and the production of its typical cytopathic effect. It is probable that poliovirus, which normally does not cause large nuclear inclusions, may do so under certain circumstances. The presence of well-defined inclusions therefore suggests a masked virus, particularly in view of the fact that the prototype SA 1 virus has been through more than 50 serial passages and has never produced inclusions.

Category SA 3

The sources of strains in this group have included monkey mouth and rectal swabs, and uninoculated kidney cultures. Ragged foci of elongated cells appear in approximately two weeks, and prolonged cultivation does not markedly increase the isolation rate. Numerous eosinophilic inclusions appear in the cytoplasm, coalescing to form a dense body which may occupy the greater part of the cytoplasm (Fig. 3). The production of such inclusions by the group of agents named reoviruses by Sabin[13] was first noted[6] in the SA 3 prototype strain, which is a type 1 reovirus serologically.

Reoviruses are widespread in nature, and are relatively common in monkeys. The SA 3 prototype strain is serologically related to, but not identical with, the agents SV 12 and SV 59.[8] Natural transmission of reovirus within a mouse colony can cause severe disease in suckling mice with a characteristic eosinophilic necrosis of the liver; and the myocardial and other lesions produced in suckling mice by the inoculation of human and simian reoviruses suggest that infections in humans may yet prove to have severe consequences.[8,13]

Category SA 4

Serologically identical strains of this virus have been isolated from uninoculated monkey kidney cultures in the summer months of two years, and the properties of the virus suggest that it is an enterovirus. Features common to other enterovirus infections are noted in the cells (Fig. 4): a large cytoplasmic paranuclear mass displaces the nucleus, which becomes scrolled or crushed. Small eosinophilic bodies may be seen in the nucleus in addition to nucleolar remnants. The virus is resistant to ether and chloroform, and is stabilized at 50°C. by molar magnesium chloride. It is serologically related to, but not identical with, the simian viruses SV 4 and SV 28, both of which produce similar cytopathic changes. Neutralizing antibodies against SA 4 have been present in many of the standard antisera produced in monkeys in the USA against human ECHO viruses, suggesting that infection with related simian viruses may be common there.

Category SA 5

A single strain of this virus was isolated from a rectal swab taken from a sick monkey. The cytopathic effect (Fig. 5) is similar to that of SA 4 virus, but it is not neutralized by antiserum against SA 4, and antibodies against it have not been observed by us in monkey sera from the USA. Other properties of the SA 5 virus also indicate that it is a true enterovirus: it is resistant to ether and chloroform, and it is stabilized at 50°C. by molar magnesium chloride.

Category SA 6

A number of virus strains in this group have been recovered from uninoculated kidney cultures after prolonged incubation, though the growth period is shortened on subculture. Focal lesions are initially produced (Fig. 6). The cells, which stain deep-red, become rounded, and by cytophagocytosis several cells may combine. Usually two eosinophilic inclusions at each end of the nucleus coalesce to form a deeply-staining elongated body.

The inoculation of tissue cultures with suspensions of vervet monkey salivary glands showing histological evidence of infection with the virus of cytomegalic inclusion disease has resulted in cytopathic changes identical with those of the SA 6 strains recovered from kidneys. Serological studies have been limited by the failure to produce suitable antisera in animals, but it is considered probable that the SA 6 strains are salivary gland viruses.

Category SA 7

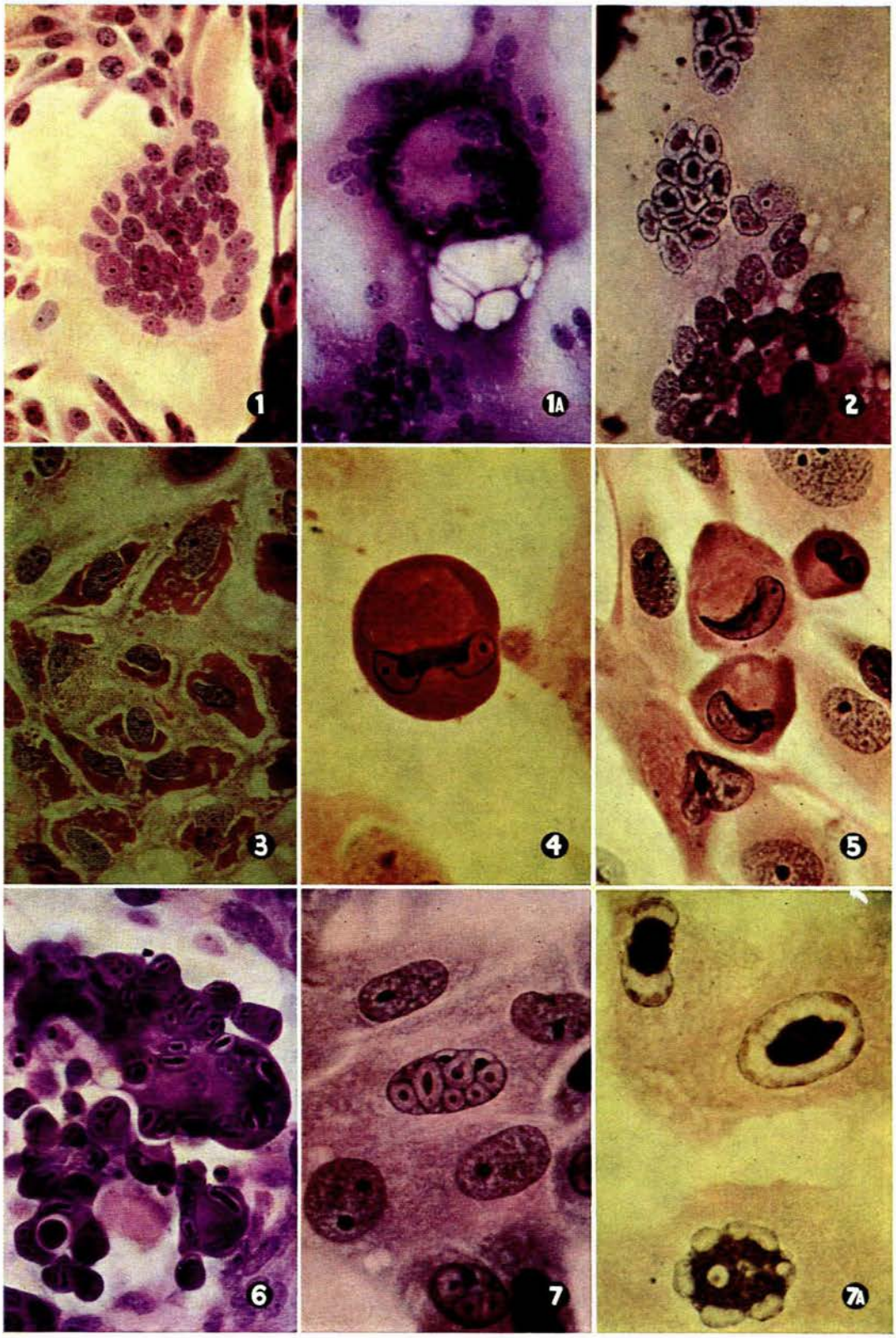
In this group are placed a number of viruses isolated from monkey mouth and rectal swabs, which produce nuclear changes characteristic of adenoviruses. Numerous stages in the development of nuclear inclusions may be seen in the same culture, ranging from early multiple discrete bodies (Fig. 7), through loculated morula-like inclusions, to the final dense, spherical body surrounded by eosinophilic material within the nuclear membrane (Fig. 7A). The prototype strain is not neutralized by antisera against human adenoviruses Nos. 1 - 18, but possesses a complement-fixing antigen common to these. A number of distinct serotypes are placed in this category, and it is likely that some overlapping will be found with the SV series, which includes at least 14 adenovirus serotypes.

Category SA 8

One of the major hazards in the handling of macaque monkeys is the presence of B virus *(Herpesvirus simiae)* which can cause fatal paralysis in man.[14-16] No reports have appeared of the isolation of typical B virus from monkeys in Africa, but two strains of a related virus have

Fig. 1. Effects of SA 1 virus on cells — see text (320x*).
Fig. 1A. Effects of SA 1 virus on cells — see text (320x).
Fig. 2. Effects of SA 2 virus on cells — see text (500x).
Fig. 3. Effects of SA 3 virus on cells — see text (500x).
Fig. 4. Effects of SA 4 virus on cells — see text (1250x).
Fig. 5. Effects of SA 5 virus on cells — see text (800x).
Fig. 6. Effects of SA 6 virus on cells — see text (400x).
Fig. 7. Effects of SA 7 virus on cells — see text (500x).
Fig. 7A. Effects of SA 7 virus on cells — see text (1250x).

* Magnifications all represent sizes before enlargement for printing.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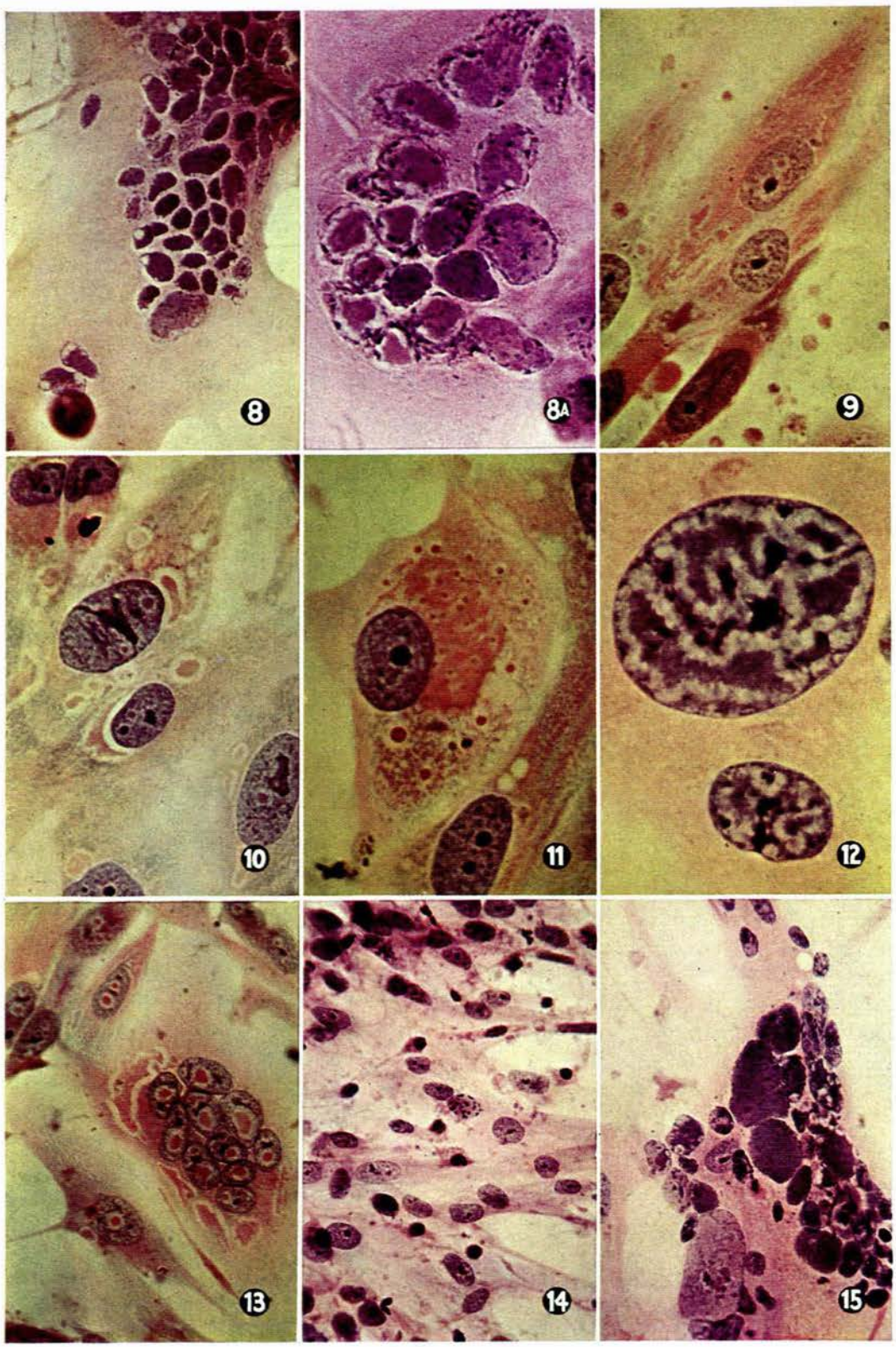
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been recovered by us from the spinal cords of vervet monkeys.[7]

The cytopathic effects of this virus consist of the rapid and widespread appearance of nuclear inclusions followed by syncytium formation (Fig. 8). The inclusions (Fig. 8A) resemble those of *Herpesvirus hominis*, but there is no significant cross-neutralization by antisera prepared with SA 8 virus and *Herpesvirus hominis*. Tests performed in collaboration with Dr. Robert Hull indicate that antiserum to B virus neutralizes SA 8 virus to a significant titre, but that antiserum to SA 8 virus does not significantly neutralize B virus. Inoculation into animals produces lesions similar to, but less severe than, those of B virus, and it is probable that this agent belongs in the *Herpesvirus simiae* group.

Category SA 9

The cytopathic changes consist of small nuclear inclusions and irregular cytoplasmic inclusions (Fig. 9). After a number of serial passages the prototype strain, which was isolated from the mouth of a monkey, continued to produce small nuclear inclusions, but the cytoplasmic inclusions became denser and resembled those of the reoviruses. Unlike the reoviruses, however, SA 9 does not haemagglutinate human O erythrocytes, nor does it haemagglutinate guinea-pig or bovine red cells.

Category SA 10

The prototype strain was recovered from the mouth of a samango monkey, *Cercopithecus mitis*. Cytoplasmic inclusions, rather less dense than those of the reoviruses, are produced (Fig. 10), as well as nuclear inclusions, which tend to be slightly larger than those of SA 9. This virus haemagglutinates human O, guinea-pig and bovine erythrocytes.

Category SA 11

A single strain obtained from the rectum of a healthy monkey has been isolated. The cytoplasm of the infected cell becomes eosinophilic, while characteristic round, loculated inclusions appear in it (Fig. 11). These enlarge to some extent, but always remain discrete. Chromatin stippling is followed by complete degeneration of the nucleus. The virus is not pathogenic in mice.

Category SA 12

One strain only has been isolated, from a kidney tissue culture. Nuclear changes somewhat resembling those of SV 40 are produced, but the inclusions are usually more clearly defined (Fig. 12) and cytoplasmic vacuolation is not a prominent feature. Growth in vervet kidney cultures is slower than for SV 40, and the SA 12 virus is not significantly neutralized by SV 40 antiserum.

Category SA 13

The prototype strain, which was isolated from the mouth of a sick monkey, produces cytopathic changes closely resembling those of measles virus. Syncytia are formed, with prominent nuclear and cytoplasmic inclusions (Fig. 13). Unlike measles virus, however, SA 13 will haemagglutinate human O, guinea-pig, fowl and bovine erythrocytes; and it is not neutralized by measles antiserum.

Category SA 14

One isolation has been made, from a kidney culture. A patchy ragged change occurs in the tissue; the cytoplasm tends to be drawn into long processes; and stippling of the nucleus is frequently followed by coarser clumping of chromatin (Fig. 14). Small eosinophilic dots may occasionally be seen in the nucleus, but definite inclusions have not been noted.

Category SA 15

Only one strain has been isolated, from a kidney culture. Cytopathic changes are slow, consisting of syncytium formation and the production of nuclear inclusions. The latter may resemble those of herpesvirus, but tend to be pleomorphic and bizarre (Fig. 15). This agent does not cause lesions of the skin or central nervous system in rabbits, and is not pathogenic for mice.

CONCLUSION

A satisfactory classification of simian viruses must take into account their structural, physical, chemical and biological properties; but the observation of distinctive cytopathic changes will continue to be essential for the practical recognition of these agents.

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Fig. 8. Effects of SA 8 virus on cells — see text (320x*).
Fig. 8A. Effects of SA 8 virus on cells — see text (800x).
Fig. 9. Effects of SA 9 virus on cells — see text (800x).
Fig. 10. Effects of SA 10 virus on cells — see text (800x).
Fig. 11. Effects of SA 11 virus on cells — see text (1250x).
Fig. 12. Effects of SA 12 virus on cells — see text (1250x).
Fig. 13. Effects of SA 13 virus on cells — see text (500x).
Fig. 14. Effects of SA 14 virus on cells — see text (400x).
Fig. 15. Effects of SA 15 virus on cells — see text (400x).

* Magnifications all represent sizes before enlargement for printing.