Onderstepoort J. vet. Res. 39(3), 133–138 (1972)

ELECTRON MICROSCOPIC AND SEROLOGICAL STUDIES ON SIMIAN VIRUS S.A. 11 AND THE "RELATED" O AGENT

G. LECATSAS, Veterinary Research Institute, Onderstepoort

ABSTRACT

LECATSAS, G. Electron microscopic studies on Simian virus S.A. 11 and the "related" O agent. *Onderstepoort J. vet. Res.* 39(3), 133–138 (1972).

Electron microscopic and serological studies carried out on Simian virus S.A. 11 and the O agent in MK2 cells have indicated that these viruses differ considerably. While S.A. 11 virus is associated with vesicles of the rough endoplasmic reticulum, with viral inclusion bodies and with membranous elements, O agent shows no such relationships but occurs in vacuoles and in crystalline array in the cytoplasmic matrix. No cross neutralization could be demonstrated.

INTRODUCTION

Viruses recovered from South African *Cercopithecus* monkeys have been studied by Malherbe & Harwin (1957) and Malherbe, Harwin & Ulrich (1963). One of these viruses (S.A. 11, strain H96) was isolated from a rectal swab taken from a healthy vervet monkey in 1958 (Malherbe & Strickland-Cholmley, 1967). O agent, a virus isolated from intestinal washings of cattle and sheep (Malherbe, Strickland-Cholmley & Geyer, 1967), showed eosinophilic cytoplasmic inclusions in vervet monkey kidney cell cultures similar to those of S.A. 11 (Malherbe & Strickland-Cholmley, 1967). Similarities in serology, pH instability, resistance to desoxycholate and certain other characteristics suggested that these viruses were virtually identical (Malherbe & Strickland-Cholmley, 1970). Els & Lacatsas (1972) have shown that the viruses have a similar morphology in negative contrast with a distinctive shell covering the capsid externally. This shell differs morphologically from that shown by the bluetongue type viruses.

This investigation attempts to characterize and compare the viruses in fine cytopathology and serologically by means of a cross neutralization test.

MATERIALS AND METHODS

Virus and cells

S.A. 11 (strain H96) and O agent virus were kindly supplied by Dr. H. H. Malherbe, Poliomyelitis Research Foundation, Johannesburg. The viruses were passaged separately in roller tube cultures of LLC-MK2 cells. Cells were harvested after about 5 days, depending on the stage of cytopathic effect. O agent virus was found to be considerably slower in production of cytopathic effect and was invariably harvested 3 days later.

Cross neutralization test

Rabbits were immunized by a series of five intraperitoneal inoculations of O agent and S.A. 11 infected BHK21 cell cultures given at weekly intervals and their sera were inactivated at 56°C for 30 minutes. The sera were assayed for O agent and S.A. 11 antibodies by mixing undiluted serum with serial ten-fold dilutions of virus. After an incubation period of an hour at 37°C, BHK21 cells were inoculated with serum-virus mixtures in a cross neutralization test. Controls were included using serum from uninoculated rabbits.

Electron microscopy

Ultramicrotomy

The method of preparation of thin sections for electron microscopy described by Lecatsas & Weiss (1968) consists basically of fixation of cells in gluteraldehyde followed by OsO_4, dehydration in graded ethanol dilutions, embedding in Epon and sectioning on a Reichert OmU2 ultramicrotome. Thin sections were double-stained with uranyl acetate and lead citrate and viewed in a Siemens Elmiskop 1A electron microscope normally operated at an accelerating voltage of 80 kV. Particle size was determined from negatives taken at an instrumental magnification of 40 000, using a Nikon Model 6C comparator.

RESULTS

Electron microscopy

Measurement of virus particle diameters in sectioned material yielded a figure of 70 nm for both viruses, which is markedly larger than the figure of 55 nm quoted by Malherbe & Strickland-Cholmley (1967), while the enveloped S.A. 11 particle has a diameter of about 85 nm. Both mature particles show a dense core with a less dense capsid.

S.A. 11 shows a number of fine cytopathic characteristics which are specific for this virus. Fig. 1 shows the occurrence of mature virus progeny particles in the cytoplasmic matrix, where they are associated with membranes of the smooth endoplasmic reticulum (SER). S.A. 11 particles in crystalline array have not been found. The presence of virus particles in vesicles of the rough endoplasmic reticulum (RER) is very common (Fig. 2 and 9). Some of the particles in Fig. 2 are clearly enveloped by a membrane derived from the vesicle in which they lie. Other particles in the same vesicle are not enveloped. Intracytoplasmic and intranuclear membranous elements are characteristic of the fine cytopathic effect caused by S.A. 11 in MK2 cells (Fig. 3 and 6). Myelin type inclusion bodies (Fig. 4) and lysosome-like bodies are commonly found and suggest that breakdown of incoming or progeny particles occurs in these bodies. Viral inclusion bodies associated with "empty" particles are found in the cytoplasm and, in one instance, in the nucleus (Fig. 7 and 6). The close association of viral inclusion bodies and RER vesicles containing virus particles as depicted in Fig. 9 is common and suggests a mechanism for the formation of these virus particles. The mitochondria in S.A. 11-infected cells are commonly found to have a vesicular matrix.

O agent virus differs in a number of respects from S.A. 11. It is rarely found in association with the RER, but occurs frequently in vacuoles, as shown in Fig. 10 and 11. The fine cytopathic effect does not produce membranous elements as is the case with S.A. 11 and viral inclusion bodies, similar to these produced by S.A. 11, have not been found in O agent-infected cells. Progeny O agent virus particles are frequently found in large groups and in crystalline array. Fig. 12 and 13 show

Received 16 August 1972 - Editor

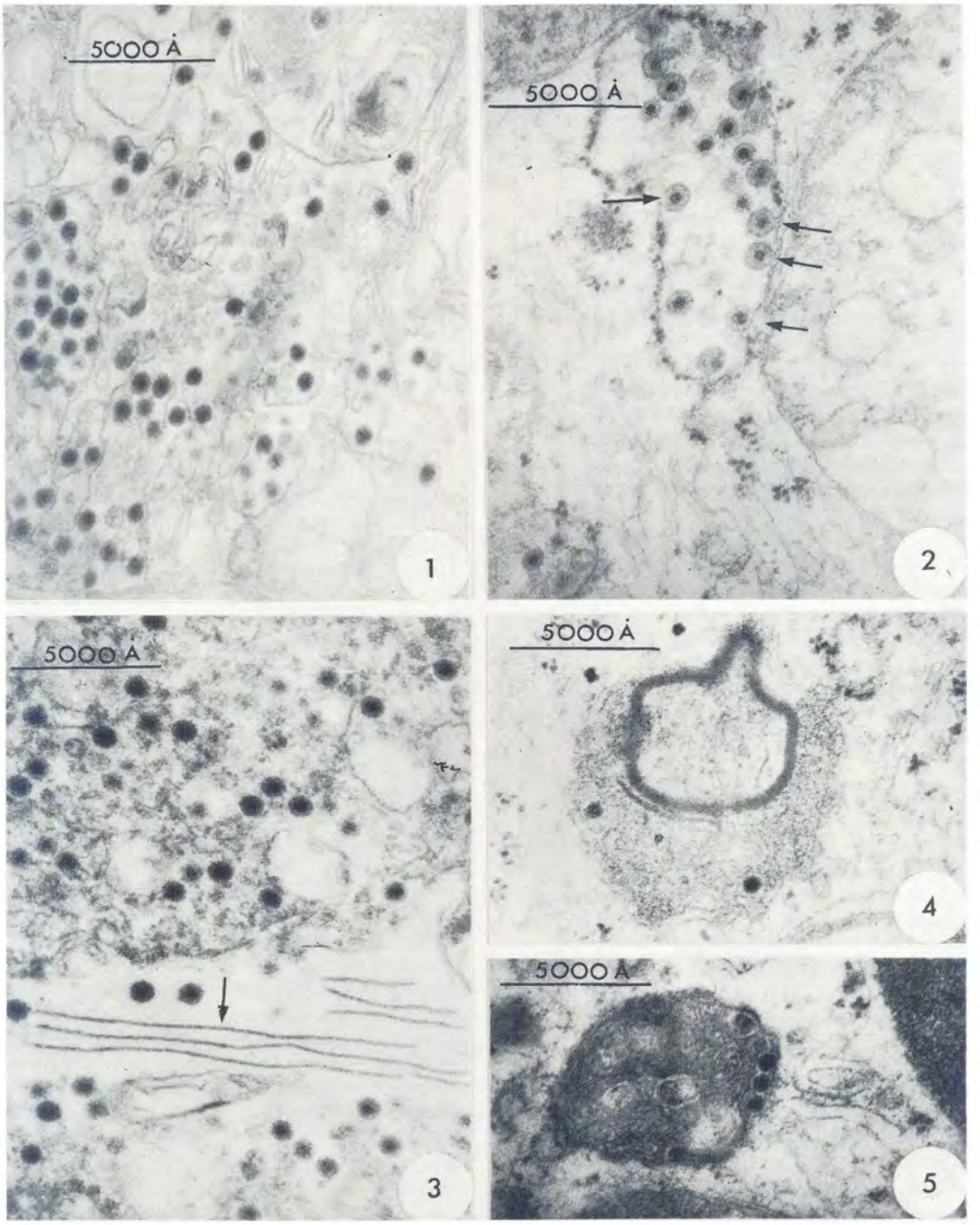

FIG. 1 Mature S.A. 11 virus particles lying in vesicles of the SER

FIG. 2 Mature S.A. 11 virus particles lying in a vesicle of the RER. Enveloped virus particles and some in the process of envelopment (arrows) by the vesicle membrane, are evident. Unenveloped particles are also evident

FIG. 3 Mature S.A. 11 virus particles in the cytoplasmic matrix and membranous elements (arrow) characteristic of the fine cytopathic effect

FIG. 4 Myelin-type inclusion body containing apparently degraded virus particles

FIG. 5 Lysosome-like inclusion body containing viral nucleoids

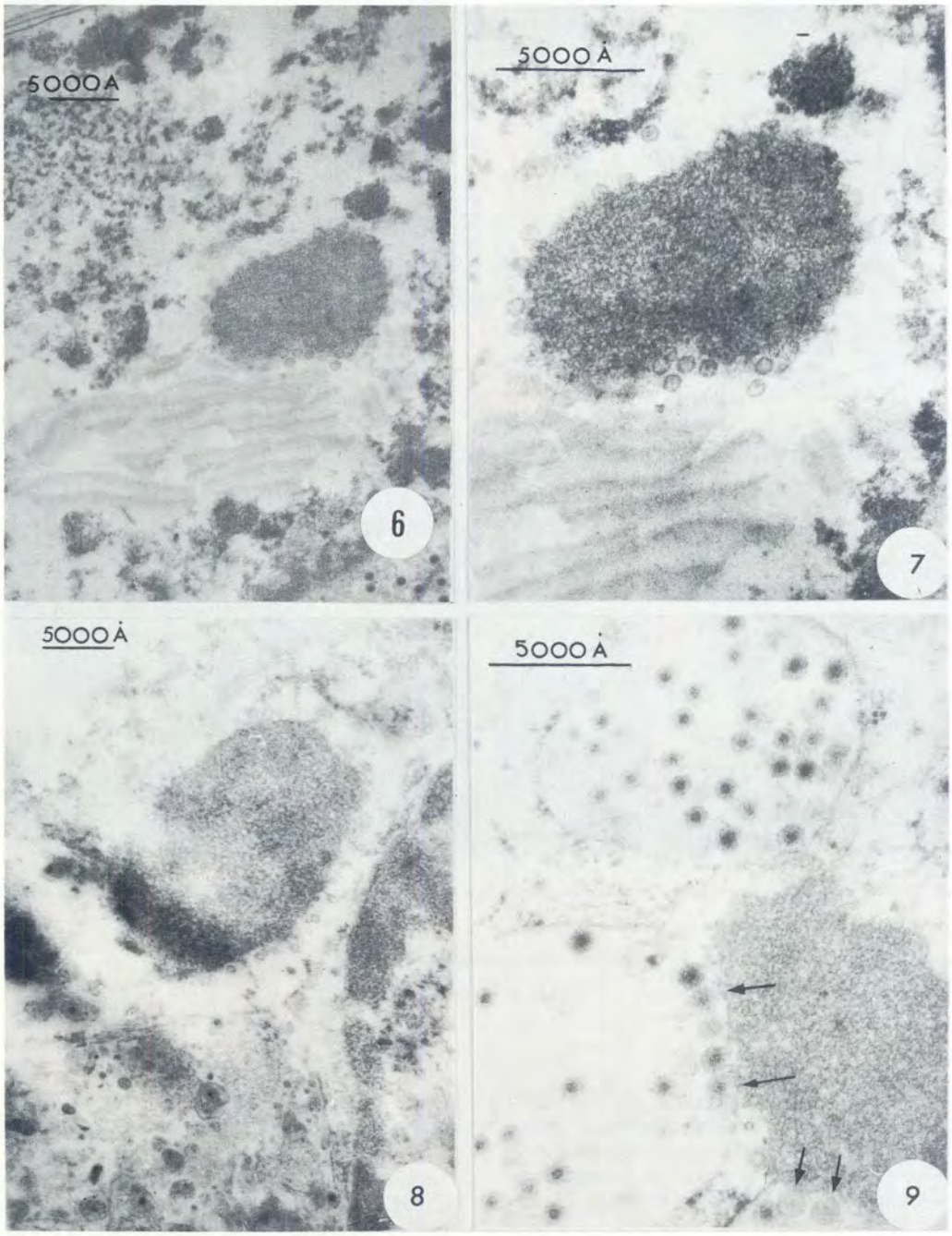

FIG. 6 Viral inclusion body in the nucleoplasm. "Empty" virus-like particles are evident at the periphery of the inclusion body. Isolated virus-like particles are also evident in the cytoplasm and in the nucleoplasm which also contains membranous elements to those demonstrated in Fig. 3

FIG. 7 Enlargement of inclusion body shown in Fig. 6

FIG. 8 Viral inclusion body in the cytoplasm with peripheral virus-like particles. The structure is similar in many respects to that shown in Fig. 6 and 7

FIG. 9 Mature virus particles, enveloped and unenveloped, in vesicles of the RER. The viral inclusion body is closely associated with the lower vesicle and virus particles, apparently arising from its matrix (arrows), appear to enter the vesicle

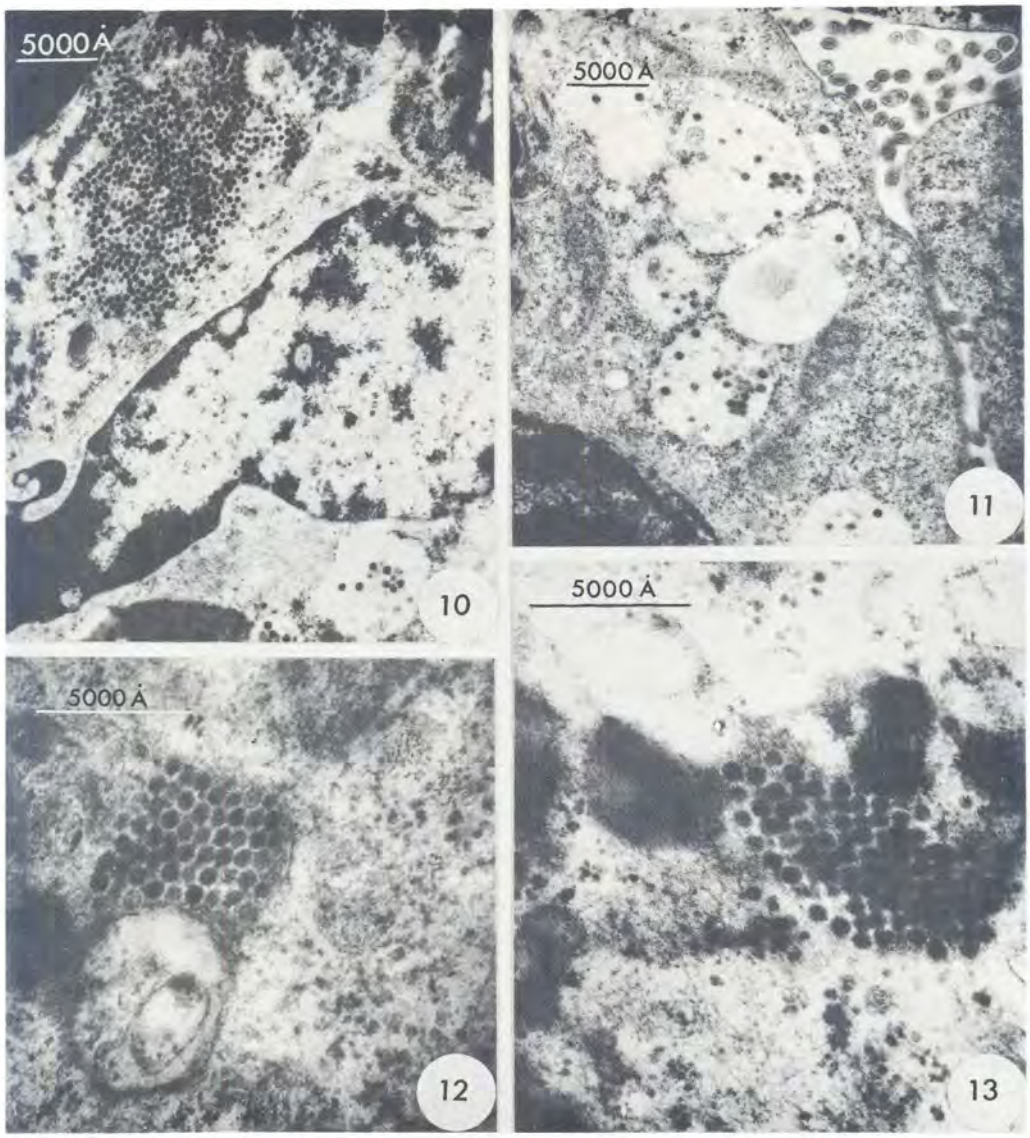

FIG. 10 Mature O agent particles lying in the cytoplasmic matrix in a dense group above and in a vacuole below the nucleus

FIG. 11 Mature O agent virus particles in cytoplasmic vacuoles

FIG. 12 Group of O agent virus particles in crystalline array in cytoplasmic matrix

FIG. 13 O agent virus particles in crystalline array in cytoplasmic matrix

such virus crystals. Enveloped particles, as shown in S.A. 11-infected cells, have not been found in O agent-infected cells. The nucleus in O agent infected cells does not appear to be visibly involved in virus formation. However, nuclear implication in the S.A. 11 fine cytopathology cannot be ruled out.

TABLE 1 Neutralization indices of S.A. 11 and O agent pre-inoculation and post-inoculation sera in relation to control virus. Fixed serum-varying virus technique

	Control virus $TCID_{50}$	S.A. 11 pre-serum	S.A. 11 post-serum	O agent pre-serum	O agent post-serum
S.A. 11 virus . . .	4,5	0	4,0	0	0
O agent virus . . .	3,0	0	0	0	2,0

Cross neutralization test

Table 1 gives the result of the cross neutralization test. No neutralization of O agent by S.A. 11 antibodies occurred, and similarly, S.A. 11 was not neutralized by O agent antiserum. Normal rabbit control sera failed to neutralize either of the viruses. S.A. 11 was neutralized to the extent of about $10^{4,0}TCID_{50}$ by its antiserum, while O agent was neutralized by about $10^{2,0}TCID_{50}$ by its antiserum.

DISCUSSION

The formation of S.A. 11 progeny particles appears to occur in viral inclusion bodies which are closely associated with vesicles of the RER. Mature particles appear to pass into the vesicles on liberation from the inclusion bodies. However, unenveloped progeny particles which appear in the RER vesicles could perhaps enter the vesicles through channels of the ER mem-

branes and are consequently naked. The significance of the viral inclusion bodies in the nucleus is not apparent at this stage. It is also not known whether the nuclear and cytoplasmic viral inclusion bodies are in fact homologous although they appear to be structurally similar if not identical. Similarly, the significance of the membranous elements remains unexplained. However, the latter characteristics can be used as identifying criteria specific for S.A. 11 virus, at least in MK2 cells. The breakdown of incoming virus particles in myelin-type inclusion bodies is plausible and serves again to distinguish S.A. 11 from O agent, which showed few, if any, of these bodies.

On the basis of fine cytopathic effect, O agent can easily be distinguished from S.A. 11 by the absence of membranous elements in both nucleus and cytoplasm, by the predominance of mature progeny particles in vacuoles, the rare association of virus particles with elements of the RER, the absence of viral inclusion bodies, the absence of intracytoplasmic enveloped particles and the appearance of particles in crystalline array. Formation of O agent in the cell appears to involve a different pathway with respect to the assembly of progeny particles. The fact that unenveloped virus particles of both types have similar diameters does not necessarily imply a close relationship between the two viruses.

Contrary to the results reported by Malherbe & Strickland-Cholmley (1967) no cross neutralization could be demonstrated. Electron microscopic studies on BHK21 cells infected with bluetongue or African horsesickness viruses carried out in our laboratory showed that strain differences in each virus could not be distinguished on the basis of fine cytopathic effect. Taken together with the present electron microscopical observations it is strongly suggested that S.A. 11 and O agent are not identical viruses.

ACKNOWLEDGEMENTS

Thanks are due to Dr. A. Theodoridis for facilities in carrying out certain neutralization tests and to Mr. L. M. Pieterse and Miss Joan Hoogenhout for technical assistance.

REFERENCES

ELS, H. J. & LECATSAS, G., 1972. Morphology of Simian virus S.A. 11 and the related O agent. *J. gen. Virol.* (In press).

LECATSAS, G. & WEISS, K. E., 1968. Formation of Wesselsbron virus in BHK21 cells. *Archiv. ges. Virusforsch.*, 27, 332–338.

MALHERBE, H. H. & HARWIN, R., 1957. Seven viruses isolated from the vervet monkey. *Brit. J. exp. Path.*, 38, 539–541.

MALHERBE, H. H., HARWIN, R. & ULRICH, M., 1963. The cytopathic effects of vervet monkey viruses. *S. Afr. med. J.*, 37, 407–411.

MALHERBE, H. H. & STRICKLAND-CHOLMLEY, M., 1967. Simian virus S.A. 11 and the related O agent. *Archiv. ges. Virusforsch.*, 22, 235–245.

MALHERBE, H. H. & STRICKLAND-CHOLMLEY, M., 1970. The viruses of vervet monkeys and baboons in South Africa. *In:* Symposium on Production and Use of Laboratory Animals, CSIR, Pretoria.

MALHERBE, H. H., STRICKLAND-CHOLMLEY, M. & GEYER, S. M., 1967. Viruses in abattoir waste. *In:* Berg, G. (Edit.). *Transmission of Viruses by the water route.* New York: John Wiley and Sons.

Printed in the Republic of South Africa by the Government Printer, Pretoria, by Heer Printing Co. (Pty) Ltd., Pretoria.