

THE USE OF THE ELECTRON MICROSCOPE IN DIAGNOSIS OF VARIOLA, VACCINIA, AND VARICELLA

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The clinical differential diagnosis of variola, or generalized vaccinia, and varicella in their early stages is often extremely difficult, and clinically mild cases of variola may be indistinguishable from varicella throughout the course of the disease. Laboratory methods of diagnosis such as flocculation, complement fixation tests, and Paul's rabbit cornea test are only applicable for the diagnosis of variola in the later stages of the disease.

Paschen (1906) described elementary bodies of vaccinia in smear preparations of vesicle fluids for microscopy and later (1919) observed what he believed to be elementary bodies of varicella, which he found to be much smaller in size and of a lower staining affinity than those of vaccinia. Amies (1933) and van Rooyen (1944) confirmed Paschen's findings as to the existence of elementary bodies of varicella in vesicle fluids. Van Rooyen utilized both the difference in the morphology and the frequency of occurrence of the elementary bodies of variola and varicella as a clinical diagnostic method for the differentiation of these diseases in man.

Since the introduction of the electron microscope, this instrument has been employed extensively in morphological studies of viruses, but no studies have yet been published on its application as a diagnostic tool in the differentiation of virus diseases, such as variola and varicella.

During a recent outbreak of smallpox in New York we had the opportunity to collect specimens for electron microscopy of human tissue from clinical cases of variola, generalized vaccinia, and also varicella, and in this paper will be presented electron micrographs derived from these specimens. For comparison are also presented electron micrographs of the virus of vaccinia as prepared from epidermal tissue of a calf and as found in stock calf lymph vaccine for human vaccination against smallpox. The studies were conducted with a RCA electron microscope, type EMU, and the shadow-casting technique of Williams and Wyckoff (1945) has been employed in the production of the metal-shadowed micrographs.

MATERIALS AND METHODS

A specimen presumed to contain elementary bodies of variola was obtained through the courtesy of Drs. Ralph Muckenfuss and George Hirst of the Willard Parker Hospital, New York. This material was recovered from a nonfatal human case on the twentieth day of the disease.

Crusts were collected, ground with silicate, suspended in saline, and centrifuged

at 2,500 rpm for 20 minutes. The elementary bodies were purified by a second centrifugation at 15,000 rpm for 1 hour. The sediment was finally resuspended in 0.9 ml distilled water, thus concentrating the suspension to one-tenth of its original volume. A small drop of the final suspension was then placed on the collodion-covered screens and dried in the usual manner.

We are greatly indebted to Drs. Emmett Holt, Jr., and Robert Ward of the Bellevue Hospital, New York, for two samples from a 10-year-old child who showed multiple open craterform pustules on the vulva 12 days after she had been vaccinated against smallpox. One sample was taken with a cotton swab from the site of the vaccination after the well-formed scab had been removed. The second sample was obtained, also with a swab, from the pustular eruptions in the vulval region. These swabs were washed in solutions of physiological saline and the resulting suspensions were purified by differential centrifugation.

A suspension of vaccinia virus for electron microscopy was kindly provided by Dr. W. Koch of the Biological Division, E. R. Squibb and Sons, New Brunswick. It was recovered from a calf which had been infected intradermally with this agent for routine vaccine production. A small area of the epidermis showing multiple small vesicular lesions was scraped off with a scalpel. The collected material was ground with silicate in a mortar, suspended in saline, and purified by differential centrifugation.

Another suspension of vaccinia virus was prepared from glycerinated calf lymph vaccine as used for human vaccination. This had been held in stock for more than 6 months and was examined for the possible occurrence of morphological changes in virus treated in this manner. The vaccine was suspended in saline and purified as outlined above.

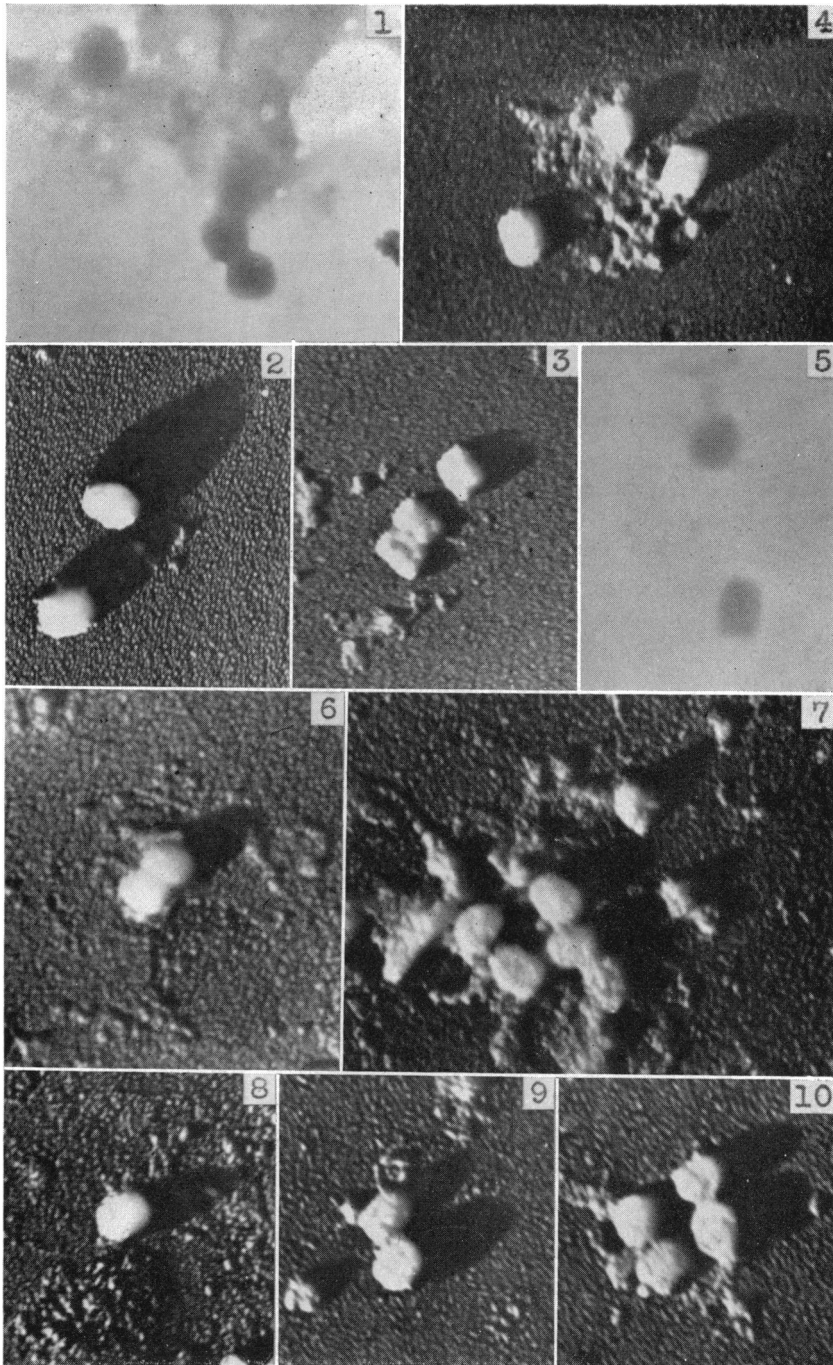
The preparations containing elementary bodies of varicella were obtained from four patients with typical clinical symptoms of chicken pox, one adult case and three children between 2 and 5 years of age. The fluids in these cases were collected with glass capillary tubes from unbroken vesicular skin lesions. The vesicle fluid from the adult patient was taken up with physiological saline and purified by differential centrifugation. Crusts which had formed, in the process of healing, on the pox lesions of this patient were removed, ground with silicate, suspended in saline, and purified as described above.

Only very small amounts of vesicle fluid were available from the children infected with varicella. The differential centrifugation, therefore, had to be abandoned, and the fluids were placed directly on the screens after they had been diluted with distilled water in proportions of approximately 1 in 10.

Nonspecific fluids and crust suspensions were obtained from vesicular epidermal lesions in man caused either by application of tincture cantharides to the surface of the skin or by superficial burns. The specimens were prepared for electron microscopy in a manner similar to that used with the preparations from the adult patient infected with varicella.

RESULTS

Micrographs are presented, in figures 1 to 4, of the suspension containing elementary bodies of variola from the human case of smallpox. It can be seen that



FIGS. 1-4. VARIOLA VIRUS FROM HUMAN CASE OF SMALLPOX

1. Magnification $\times 24,800$.

2-4. Shadowed with 22.1 mg gold at the angle tangent $2/11.82$. $\times 24,800$.

FIGS. 5-7. VACCINIA VIRUS FROM HUMAN CASE WITH SECONDARY VACCINIA

5. Magnification $\times 24,800$.

6-7. Shadowed with 24.5 mg of gold at the angle tangent $2/10.8$. $\times 24,800$.

FIG. 8. VACCINIA VIRUS FROM CALF INFECTED WITH VACCINIA

Shadowed with 24.5 mg of gold at the angle tangent $2/10.8$. $\times 24,800$.

FIGS. 9-10. VACCINIA VIRUS FROM GLYCERINATED SMALLPOX VACCINE

Shadowed with 22.4 mg of gold at the angle tangent $2/9.79$. $\times 24,800$.

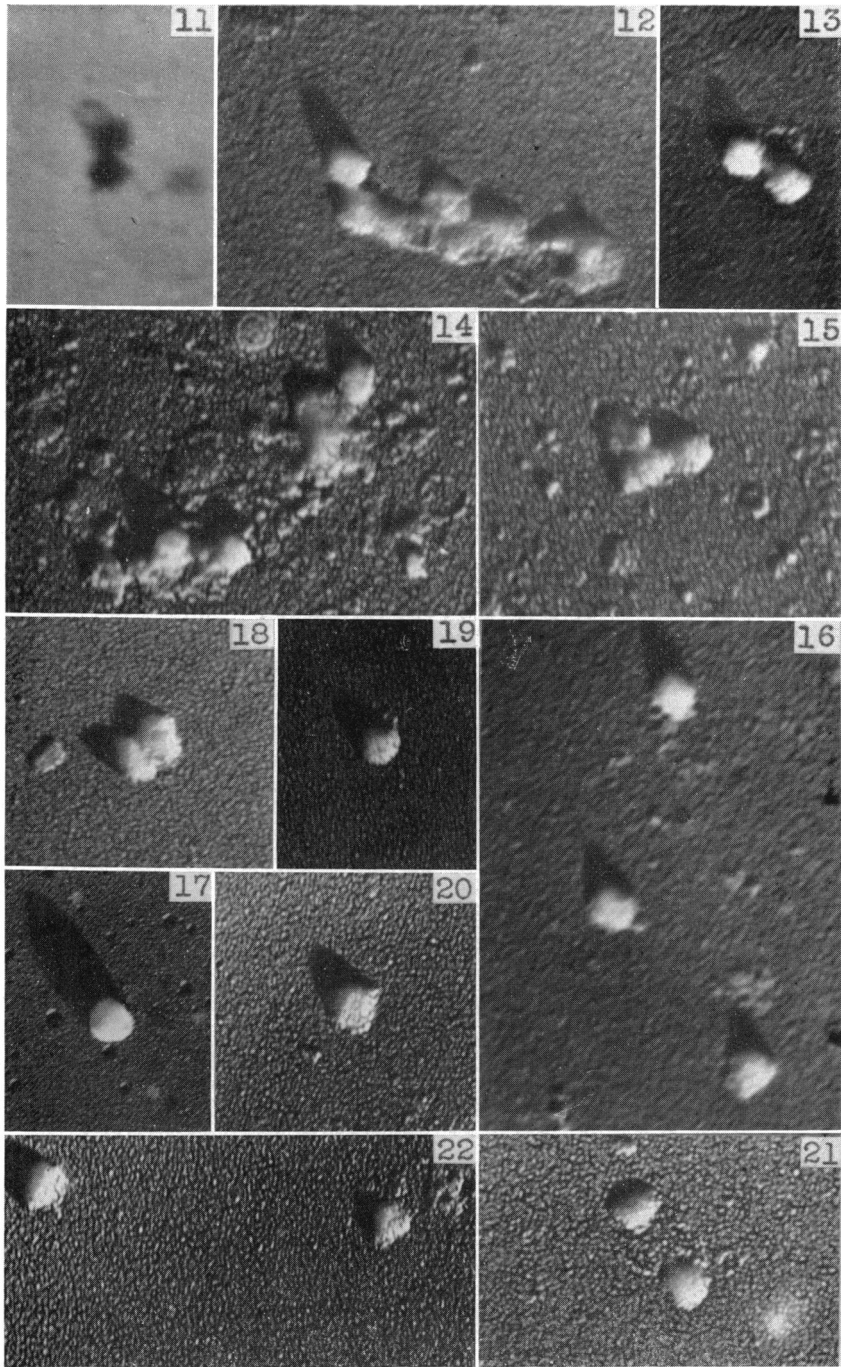
these bodies which were very plentiful have all the morphological characteristics in common with elementary bodies isolated by other workers from chick embryo tissue infected with certain of the pox viruses. The flattened corners of some virus particles and the arrangement in pairs or short chains, in which they are frequently joined to one another at their corners, had been described by Groupé, Oskay, and Rake (1946) and by Groupé and Rake (1947) in vaccinia, canary pox, and fowl pox grown in the chorioallantois of chick embryos, and have been observed in preparations from the variola crusts.

Pictorial data are presented in figures 5 to 7 of suspensions containing elementary bodies of vaccinia that were recovered from the child with a secondary spread of vaccinia. The elementary bodies were found in large numbers in the specimen obtained from the primary lesion. They were morphologically indistinguishable from elementary bodies of vaccinia derived from chick embryo tissues. Similar elementary bodies were observed in the specimen prepared from the secondary (vulval) lesions. Relatively few characteristic particles, however, could be found in this specimen, compared with the number of elementary bodies in the specimen of the primary lesion. This may have been due to the fact that these vesicles were secondarily infected with bacteria as shown by their open pustular nature. A similar observation was made by Amies (1933), who found that elementary bodies always were scanty in stained preparations of varicella fluids if inflammatory cells were coexistent.

Micrographs of elementary bodies of vaccinia obtained from vesicular skin lesions in a calf and micrographs of stock vaccine are presented in figure 8 and figures 9 and 10, respectively. Here again it can be seen that these elementary bodies do not differ in their morphological structure from pox virus particles derived from either human or chick embryo tissues.

Micrographs secured from the human cases of varicella are shown in figures 11 to 19. Figures 11 to 13 and 16 to 19 represent micrographs of elementary bodies of varicella found in vesicle fluids of the various patients. In figures 14 and 15 are pictured micrographs prepared from the varicella crust of the adult patient.

It will be seen that varicella elementary bodies are rather uniform in size and predominantly rectangular in shape. A small number of these are arranged in short chains, in which some are linked to each other at their corners (figure 12). Some bodies appear to be coated with a mucuslike material, and some seem to be connected with stringlike material. Similar characteristics had been observed in elementary bodies of the pox viruses by Sharp *et al.* (1946), by Groupé, Oskay, and Rake (1946), and by Groupé and Rake (1947). Elementary bodies of varicella, however, were not seen as numerous on the screens as those of variola or vaccinia, and they appear to be smaller in size than the latter. Measurements were taken directly from shadowed electron micrographs. The average width of the shadowed elementary bodies of varicella was found to be 210 $m\mu$ (with a range from 197 to 222 $m\mu$) and the average length 238 $m\mu$ (218 to 277 $m\mu$), whereas the average size of elementary bodies of variola or vaccinia, measured under similar conditions, appears to be 244 by 302 $m\mu$.



FIGS. 11-19. VARICELLA VIRUS FROM HUMAN CASES OF CHICKEN POX

11. Magnification $\times 24,800$.

12-13. Shadowed with 21.4 mg of gold at the angle tangent $2/9.79$. $\times 24,800$.

14-15. Shadowed with 23.0 mg of gold at the angle tangent $2/9.79$. $\times 24,800$.

16. Shadowed and magnified as figures 12 to 13.

17. Shadowed with 21.2 mg of gold at the angle tangent $2/9.79$. $\times 24,800$.

18. Shadowed with 21.5 mg of gold at the angle tangent $2/11.82$. $\times 24,800$.

19. Shadowed with 21.2 mg of gold at the angle tangent $2/9.79$. $\times 24,800$.

FIGS. 20-22. PARTICLES FROM NONSPECIFIC LESIONS (SEE TEXT)

Shadowed with 21.8 mg of gold at the angle tangent $2/9.79$. $\times 24,800$.

Electron micrographs of vesicle fluids from the cases with nonspecific skin lesions (caused by tincture cantharides or superficial burns) did not reveal any particles resembling elementary bodies of variola, vaccinia, or varicella. However, a small number of particles (figures 20 to 22) with slight resemblance to elementary bodies of varicella but tending to be flatter were observed in the crust material obtained after irritation with tincture cantharides.

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SUMMARY

Elementary bodies of variola and vaccinia recovered from lesions of smallpox and generalized vaccinia in man can be demonstrated with the electron microscope. The latter resemble elementary bodies of vaccinia obtained from vesicular skin lesions in an infected animal.

Virus particles of variola and vaccinia recovered from human and animal tissues resemble each other closely in their morphological structure. They are also indistinguishable from elementary bodies of these viruses derived from the chorioallantois of the chick embryo.

Elementary bodies can be demonstrated with the electron microscope in vesicle fluid or crusts from cases of varicella. They are predominantly of rectangular shape and morphologically resemble elementary bodies of other pox viruses. They are rather uniform in size but are scantier and smaller than the elementary bodies of variola and vaccinia. No particles resembling elementary bodies of variola, vaccinia, or varicella could be found in vesicular skin lesions caused by either chemical or physical irritation. A small number of particles with slight resemblance to elementary bodies of varicella was observed in crusts of such lesions.

It is suggested that the electron microscope can be used as a tool in the clinical diagnosis of variola, vaccinia, and varicella. If sufficient numbers of bodies can be measured, the electron micrographs might be used in the differential diagnosis between the first two diseases and the last.

During the final assembling of this material for publication a vesicle fluid was made available to us through the courtesy of Dr. Clayton G. Loosli of the Department of Medicine, the University of Chicago. Examination of this preparation showed bodies similar in morphology to those described above for variola, vaccinia, and varicella. The bodies were scanty and gave an average measurement of 209 by 254 m μ . These two facts would have suggested to us that we were dealing with a case of varicella, and it is to be noted that, although there was some doubt in the minds of the clinicians whether the case was generalized vaccinia or varicella (since the rash developed 6 days after vaccination for smallpox), the weight of clinical opinion was strongly in favor of its being varicella.

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