

# ON THE INFECTIVE AGENTS OF TRACHOMA AND VACCINIA

(STUDY BY ELECTRON MICROSCOPY)

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Based on former investigations we proposed the assumption that the inclusion bodies and elementary corpuscles in virus diseases are coagulated protein particles, and that disseminated outside and inside these bodies are the genuine viruses, which are invisible with the optic microscope (Sugita 1939 and 1940). This assumption has since been confirmed by further studies, which have shown that the agents of both the trachoma and vaccinia, though not resolved by optic microscopes, could be made visible by electron microscopy. These results were presented in an address to the Ophthalmological Society of Nagoya in the summer of 1943 (Sugita, 1946).

## I. EXPERIMENTS

*A. Experiments on the Extraction of the Trachoma Virus.* From human eyelids with typical trachoma, the conjunctivas together with the tarsus were excised. Each flap of conjunctiva-tarsus was then cut into pieces with the scissor and ground in a mortar for about 20 minutes: this was made into an emulsion by addition of about 4 ccm of sterile water. The emulsion was kept upright in an ice-box for about 2-3 hours, and the clear supernatant was transferred to a centrifuge tube and centrifuged at 2 000 r.p.m. for about 10-20 minutes. The clear supernatant in the tube was then centrifuged in an angle ultracentrifuge at 10 000 r.p.m. for five minutes. The viruses are thereby contained in the residue, as is known, and the supernatant was discarded. Next 5 ccm of sterile water was added to the residue. Ultracentrifugation was repeated two to three times under the same conditions (at 10 000 r.p.m. for five minutes). At the end of this the supernatant liquid, *i.e.* the medium, usually has lost its protein reaction. After the liquid was discarded, the residue was suspended once more in sterile water and centrifuged at 3 000 r.p.m. for about 2 minutes, in order to remove bacteria etc., which might have mingled. The supernatant was then decanted into an ultracentrifuge tube and centrifuged at 10 000 r.p.m. for five minutes. The final sediment, obtained at the bottom of the tube and containing the purified viruses, was subjected to electron microscopic investigations.

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In these experiments the following must be borne in mind, viz. since every filterable virus is inclined to have its virulence diminished by washing with water, the duration of contact with water must be as short as possible, when examining virulence in detail. For the same reason, before the conjunctival flaps are resected, the conjunctival sacs must be cleaned only with sterile water, and not with sublimate solution or other disinfectants. Further, instruments must be disinfected by boiling before use and kept scrupulously clean.

*B. Experiments on the Extraction of the Vaccinia Virus.* Used as material in these experiments were the rabbit testes, into which vaccine were injected 4-5 days before use. After enucleation, they were treated similar to the trachomatous material, leaving finally the sediment for electron microscopy. In this case, however, washing with water and ultracentrifugation was repeated 5-6 times, because of the additional proteins that are harder to remove than in case of the trachoma virus.

In addition, in a series of experiments the removed sediment, before examination by electron microscopy, was suspended in a 1/4 dilution of saturated sodium citrate solution for about 30 minutes before ultracentrifugation at 10 000 r.p.m. for 5 minutes.

## II. RESULTS OF EXPERIMENTS

The sediment taken from trachomatous material showed in electron microphotographs many round granules, the diameters of which were under 170 millimicron (*cf.* fig. 1 and 2). Regarding the shape, most were simply grain-shaped, but some looked as if they were accompanied by capsule-like attachments (*cf.* fig. 2).

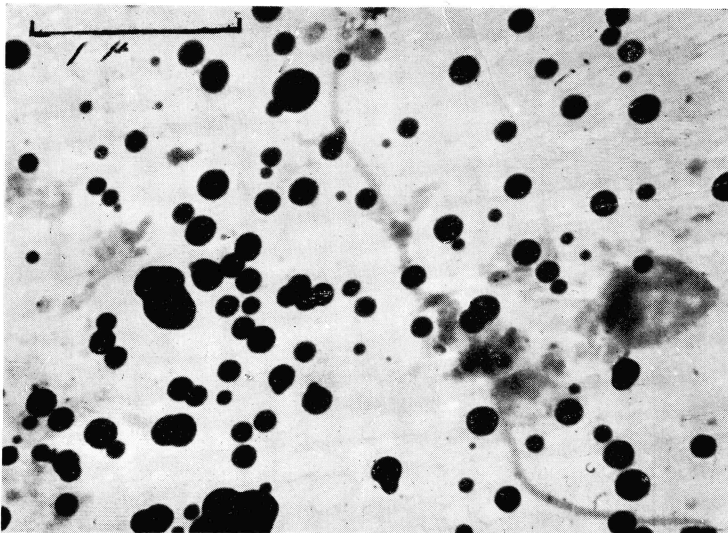


Fig. 1. Trachoma virus  $1 \times 24\,000$ . (130-170  $m\mu$ )

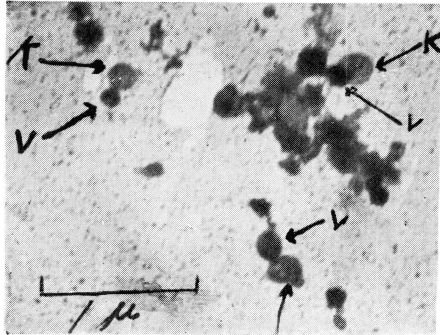


Fig. 2. Trachoma virus(V) with capsule-like attachments(K).  $1 \times 21\,000$

The sediment taken from vaccinia material, showed in photographs many round granules (*cf.* fig. 3 and 4), but their diameters were slightly greater than those of the former, up to about 180 millimicron. Some of the granules were similarly accompanied by capsule-like attachments (*cf.* fig. 4). This state, however, was considerably changed by treatment with sodium citrate, and the density of these attachments was reduced by treatment so strikingly, that they surrounded the granules merely as thin membranes.

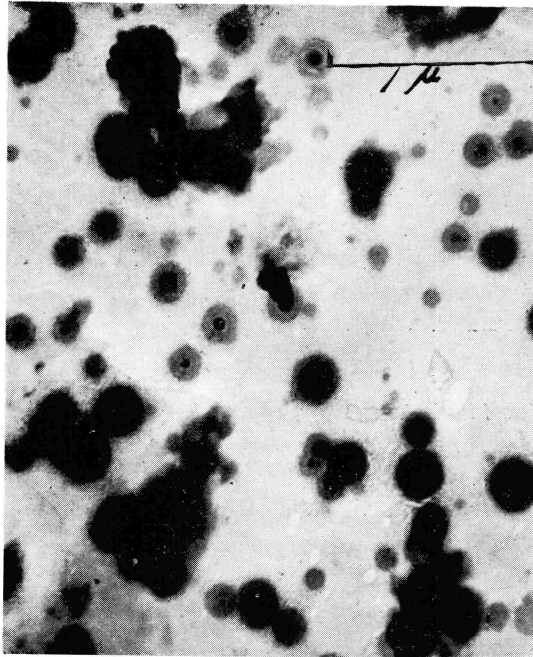


Fig. 3. Vaccinia virus  $1 \times 28\,000$  (140-180  $m\mu$ )

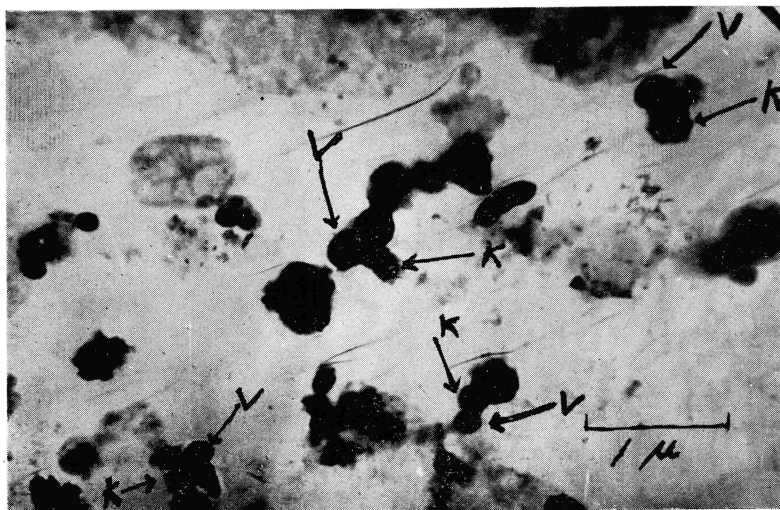


Fig. 4. Vaccinia virus(V) with capsule-like attachments(K).  $1 \times 18000$

### III. EXPERIMENTS ON THE VIRULENCE OF THE SEDIMENTS

The sediments, obtained from both trachomatous and vaccinal material by methods mentioned above and examined by electron microscopy, were examined for virulence, using man and animal, as follows.

A. The sediment from the trachomatous material could evoke typical trachoma, if smeared on the conjunctivas of blind human eyes. Inflammatory signs occurred after an incubation period of 7 to 10 days, and there developed gradually trachoma follicles and eventually all the symptoms of trachoma, in general the same course as reported by former authors, in cases of artificial inoculation.

B. The sediment taken from the vaccinal material was inoculated into the corneas of living rabbits. After 24 to 36 hours the infected parts of the cornea were removed by scratching and stained with Giemsa's stain. Microscopic examination showed Guarnieri bodies in the corneal epithelial cells, revealing infectivity of the inoculated substance.

### IV. CRITICISM

A. *The granules in electron microphotographs are nothing but the images of the viruses.* The sediment obtained as above is a mass of purified and concentrated agents, and can be taken to be so on the basis of physico-chemical investigations of former authors. As infectivity of the sediment was demonstrated in human and animal experiments and as no other formed particles were found in the photographs, these granules must be considered the virus bodies.

B. *Size of the virus particle.* The diameters of trachoma virus particles were under 170 millimicron and those of vaccinia virus under 180 millimicron, and both showed rather wide variations, a fact which is not surprising in viruses of animals.

C. *On the capsule-like attachments.* It is worthy of note that some viruses of both trachoma and vaccinia had capsule-like attachments. Some particles were surrounded by them, while others looked as if they had crawled out from them; however, the intimate relation existing between both could not be overlooked. The capsules could be easily distinguished from the viruses because of their considerably lower density. When treated with sodium citrate, the capsules diminished in size so markedly as to surrounded the virus particles merely as thin girdles. This fact shows that by this treatment a greater part of the proteins were removed from the capsules with sodium citrate, and consequently that the capsules mainly consist of proteins. As to how these capsules come into existence, we believe that tissue proteins or products of the virus adhere to viruses and cause a condition similar to a protective colloid. To explain the origin of these proteins conclusively, further studies are necessary, which should make sufficiently clear, whether they are decomposed proteins in the focus or products of the virus.

#### V. SUMMARY

A. The trachoma virus, removed from human trachomatous conjunctiva and tarsus by ultracentrifugation, appeared in electron microphotographs as round granules under 170 millimicron in diameter.

B. The vaccinia virus removed from inoculated rabbit testes and treated similarly, were visible in the photographs likewise as round granules under 180 millimicron in diameter.

C. The viruses, of both trachoma and vaccinia, often showed capsule-like attachments, which consist mainly of proteins and supposedly form a condition similar to a protective colloid for the virus particles.

#### REFERENCES

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