THE LESIONS IN RABBITS EXPERIMENTALLY INFECTED BY A VIRUS ENCOUNTERED IN THE ATTEMPTED TRANSMISSION OF VARICELLA.

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Plates 6 to 9.

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During attempts to infect rabbits with the virus of varicella, an active transmissible agent has been encountered which partakes of the characters of the so called filterable viruses. The methods employed, the behavior of the virus in normal and immune rabbits, and the results of attempts to identify the virus have been described in previous papers.[1,2] Early in the work the method employed[1,2] in procuring the virus and the gross and microscopic appearances of the lesions produced by the virus in the testicles, skin, and corneas of rabbits led to the inference that this virus was not improbably the etiological agent of chicken-pox. No proof was forthcoming, however, from immunity studies in rabbits and in man. On the other hand, evidence was obtained that there had been encountered a hitherto unrecognized active transmissible agent which would be of especial interest to investigators working with filterable viruses in rabbits.

The exact source and nature of the virus and its relation to human and rabbit diseases remain to be determined. Nevertheless, the lesions produced in rabbits by the virus and the intracellular changes found in these lesions resemble so closely those seen in varicella[3] and herpes zoster that it seems desirable to describe them without further delay.

[1] Rivers, T. M., and Tillett, W. S., *J. Exp. Med.*, 1923, xxxviii, 673.

[2] Rivers, T. M., and Tillett, W. S., *J. Exp. Med.*, 1924, xxxix, 777.

[3] Tyzzer, E. E., *J. Med. Research*, 1905–06, xiv, 361.

These observations should be of special interest since workers[4-6] have reported that specific lesions occur in the scarified corneas of rabbits inoculated with vesicle fluid from varicella and herpes zoster patients.

Method.

Tissues for examination were removed[7] 3 to 6 days following inoculations with the virus. After many fixatives and stains had been tried, Zenker's fluid with 5 per cent acetic acid, and methylene blue-eosin and Giemsa stains were found entirely satisfactory. When no mention is made of the fixative and stain employed, it will be understood that Zenker's fluid and methylene blue-eosin were used.

Testicles.

Gross Pathology.—The gross pathological changes occurring in the testicles when large amounts of testicular emulsions containing the virus were injected had of necessity to be disregarded. When small quantities of the supernatant fluid from centrifuged testicular emulsions containing active virus were inoculated into the testicles of normal young adult rabbits, significant gross changes also occurred. On the 3rd to 6th day after the inoculation, the testicles became swollen, tense, and tender. Often the scrotum was red and edematous. When the testicles were removed, they were red, swollen, and edematous and the surface was covered with yellowish spots. Upon sectioning the testicles the swollen condition was more evident as the parenchyma bulged out from the restraining capsule. The gross picture was one of acute inflammation.

Microscopic Pathology.—Testicles inoculated with large amounts of the virus showed diffuse lesions involving the whole testicle and consisting of marked degeneration and dilatation of the tubules, engorgement of the blood vessels, and an increase in the cells and fluid in the interstitial tissue (Fig. 1). The cellular infiltration consisted of endothelial leucocytes (Fig. 2), plasma cells, lymphocytes,

[4] Lipschütz, B., *Arch. Dermatol. u. Syph., Orig.*, 1921, cxxxvi, 428.
[5] Bertarelli, E., *Centr. Bakt., 1. Abt., Orig.*, 1909, l, 181.
[6] Gins, H. A., *Z. Hyg. u. Infectionskrankh.*, 1918, lxxxvi, 299.
[7] All operations were performed under ether anesthesia.

and in some instances polymorphonuclear leucocytes. The most characteristic finding was the decided increase in the endothelial leucocytes (Fig. 2), many of which contained two nuclei and nuclear inclusions (Fig. 11). When small amounts of virus were injected into the testicles, discrete lesions were observed in the interstitial tissue with the same cellular changes described above.

Skin.

Lesions Following the Inoculation of Virus on the Scarified Skin.

Gross Pathology.—4 to 6 days after inoculation of the virus on the scarified skin of rabbits discrete lesions appeared in the form of small, superficial, red macules and papules. These persisted for 3 or 4 days, and then disappeared without scar formation. The discrete lesions might be overlooked but when confluent ones occurred along the lines of scarification they were not so likely to be missed. The confluent lesions exhibited more swelling of the tissues and persisted longer than the discrete ones. No definite vesicle formation was observed in any of the lesions.

Microscopic Pathology.—The epidermis was thickened. No definite vesicles were observed. The epidermal cells were swollen and stained a pale pink. The corium was edematous and exhibited a marked cellular infiltration consisting of endothelial leucocytes, plasma cells, lymphocytes, and at times polymorphonuclear leucocytes (Figs. 3 and 4). In some of the endothelial leucocytes nuclear inclusions were seen which resembled those found in testicular tissue.

Lesions Following the Intradermal Inoculation of Virus.

Gross Pathology.—Following the intradermal inoculation of the virus nothing except the effects of trauma was observed for 3 or 4 days. Then the skin at the site of the injection of the virus became red and raised. The reaction at these points increased in size and intensity till the 5th to the 7th day, when the central portion of the inoculated area was often very dark. Extending into the skin around the dark central area was a zone of less intense erythema 2 to 4 cm. in width. The lesions gradually subsided and in a fortnight disappeared without scar formation.

Microscopic Pathology.—The surface of the skin was often covered by a layer of desquamated cells of various kinds. Beneath this layer the epidermis was seen. It was not always intact, however, breaks in its continuity being frequently observed. The epidermal cells were undergoing degeneration, stained poorly, and a few of them contained nuclear inclusions. In the upper part of the corium there was considerable generalized edema with localized collections of fluid suggestive of vesicles (Fig. 5). The cellular infiltration in the corium was marked and consisted of endothelial leucocytes, plasma cells, lymphocytes, and polymorphonuclear leucocytes. The endothelial leucocytes were numerous and many of them contained nuclear inclusions.

Discrete Lesions in the Shaved Skin Following Intratesticular Inoculation of Virus.

Gross Pathology.—In some of the rabbits inoculated only in the testicles areas of skin were shaved. A diffuse or macular erythema which disappeared in 2 or 3 days followed merely this shaving alone. In addition to the lesions thus induced, discrete red macules and papules frequently appeared in the skin 5 to 11 days after the intratesticular inoculations, persisted 3 to 7 days, and disappeared without scar formation. At times a zone of erythema less intense than that of the papules surrounded each lesion. These discrete lesions we have interpreted as the result of a generalized infection with the virus.

Microscopic Pathology.—The epidermis was thickened (Fig. 6). No vesicles were observed. The epidermal cells were swollen and stained poorly. The upper part of the corium was edematous. The cellular infiltration of the corium, consisting largely of endothelial leucocytes and plasma cells, was frequently most marked around small blood vessels (Fig. 6). Many of the endothelial leucocytes were swollen and an occasional one contained nuclear inclusions. Lymphocytes and polymorphonuclear leucocytes, although present at times, were not numerous.

Lesions in Scarified Corneas Inoculated with Virus.

Gross Pathology.—Scarified corneas (cocaine anesthesia was always used) inoculated with virus showed a reaction which was absent

in the controls. The reaction was noticeable in 3 or 4 days, persisted 4 or 5 days, and disappeared rapidly without permanent injury to the eye unless a secondary infection occurred. The corneas were roughened and slightly opaque, especially along the lines of scarification. Increased lacrimation, photophobia, and injection of the blood vessels around the corneas were usually observed.

Microscopic Pathology.—The defects in uninoculated scarified corneas were repaired rapidly with young corneal cells which appeared normal in every respect (Fig. 7). In contrast to this, eyes inoculated with virus exhibited definite lesions in the young corneal cells filling the defects in the corneas produced by scarification. In these lesions very few cells were seen which were not of epithelial origin. Therefore, corneal lesions were most satisfactory for the study of the characteristic intracellular changes which are frequently seen in cells injured by the virus. These cells were swollen and often stained poorly, some had two to four nuclei (Fig. 9), and many had nuclear inclusions (Figs. 8 to 10). Only a few cells were involved in the early lesions. Later when more cells had been injured and the degeneration of these cells had progressed, it was evident that a small vesicle was in process of formation (Fig. 9). Finally, as the cells degenerated more and more, definite vesicles were formed, filled with fluid and the remains of degenerated cells. Around the vesicles many of the cells were in various stages of degeneration and some contained nuclear inclusions (Fig. 10). The early stages of vesicle formation were frequently seen. Well developed vesicles, however, similar to the one shown in Fig. 10 were not observed often. The vesicles were delicate and many of them may have been destroyed in the process of making sections. Only a slight reaction occurred beneath the corneal cells and this usually consisted of a mild infiltration of endothelial leucocytes some of which had nuclear inclusions.

Nuclear Inclusions.

Nuclear inclusions (Figs. 11 and 12) were numerous in endothelial leucocytes in inoculated testicles (Fig. 2) and in corneal cells of inoculated eyes (Figs. 8 and 10). They were less numerous in the endothelial leucocytes of skin lesions and were rarely observed in

epidermal cells. In early lesions these inclusions occurred in the nuclei of otherwise normal looking cells and appeared as small pink dots surrounded by a clear zone, the so called halo. As the lesions became older the inclusions increased in size and were still surrounded by a halo. The uninvolved nuclear material seemed to draw away from the inclusions which finally occupied most of the nuclear space. The remaining uninvolved nuclear material often with the nucleolus was then compressed against the normally thin nuclear membrane and the two together stained a dark blue giving the appearance of an unusually heavy nuclear membrane. In the meantime the cell became swollen and finally death ensued as indicated by the whole cell taking a pink stain. In properly stained sections all the inclusions were pink, in contrast to the blue stain usually taken by nuclear material; the small ones stained homogeneously, the larger ones, however, frequently had the appearance of being an aggregation of the smaller ones.

DISCUSSION.

Local infiltration of the tissues with endothelial leucocytes, swelling of the involved epithelial cells, and the presence of nuclear inclusions in both the endothelial leucocytes and the epithelial cells were the characteristic pathological changes observed in rabbits experimentally inoculated with the virus under investigation. While characteristic of the action of the virus, these pathological changes cannot be considered as unique with it, since similar changes have been found in varicella, herpes zoster, symptomatic herpes, and other diseases resulting from infection with filterable viruses. The point of main interest in the present findings lies in the fact that this well known type of lesion, associated in the general mind with the action of a certain group of viruses, is produced by a hitherto unrecognized entity which partakes of the general characters of the group.

CONCLUSION.

The pathological changes produced by the virus under investigation are similar to those deemed characteristic of a certain well known group of filterable viruses.

EXPLANATION OF PLATES.

PLATE 6.

FIG. 1. Lesion in the testicles of a rabbit, 4th day after intratesticular inoculation, showing the cellular infiltration in the interstitial tissue and the injury to the tubules. × 105.

FIG. 2. Higher magnification of section in Fig. 1, showing nuclear inclusions in endothelial leucocytes. × 1,500.

PLATE 7.

FIG. 3. Normal rabbit skin; the epidermis is thin, the corium not very cellular. × 105.

FIG. 4. Skin 4 days after dermal inoculation; the epidermis is thickened and there is an exudation of serum and cells into the corium. × 105.

FIG. 5. Skin 5 days after intradermal inoculation. There is a layer of desquamated cells on the surface. The epidermis is injured and its continuity is broken in places. Exudation of cells and serum into the corium is very marked. × 120.

FIG. 6. Lesion in the shaved skin of a rabbit inoculated only in the testicles; epidermis thickened, exudation of serum and cells into the corium. The cellular exudation consists principally of endothelial leucocytes and is most marked around small blood vessels. × 105.

PLATE 8.

FIG. 7. Lesion produced in a normal cornea by scarification alone. The eye was removed under ether 4 days after the scarification. × 300.

FIG. 8. Early lesion in an inoculated cornea; degeneration of the corneal cells, some of which contain nuclear inclusions. × 300.

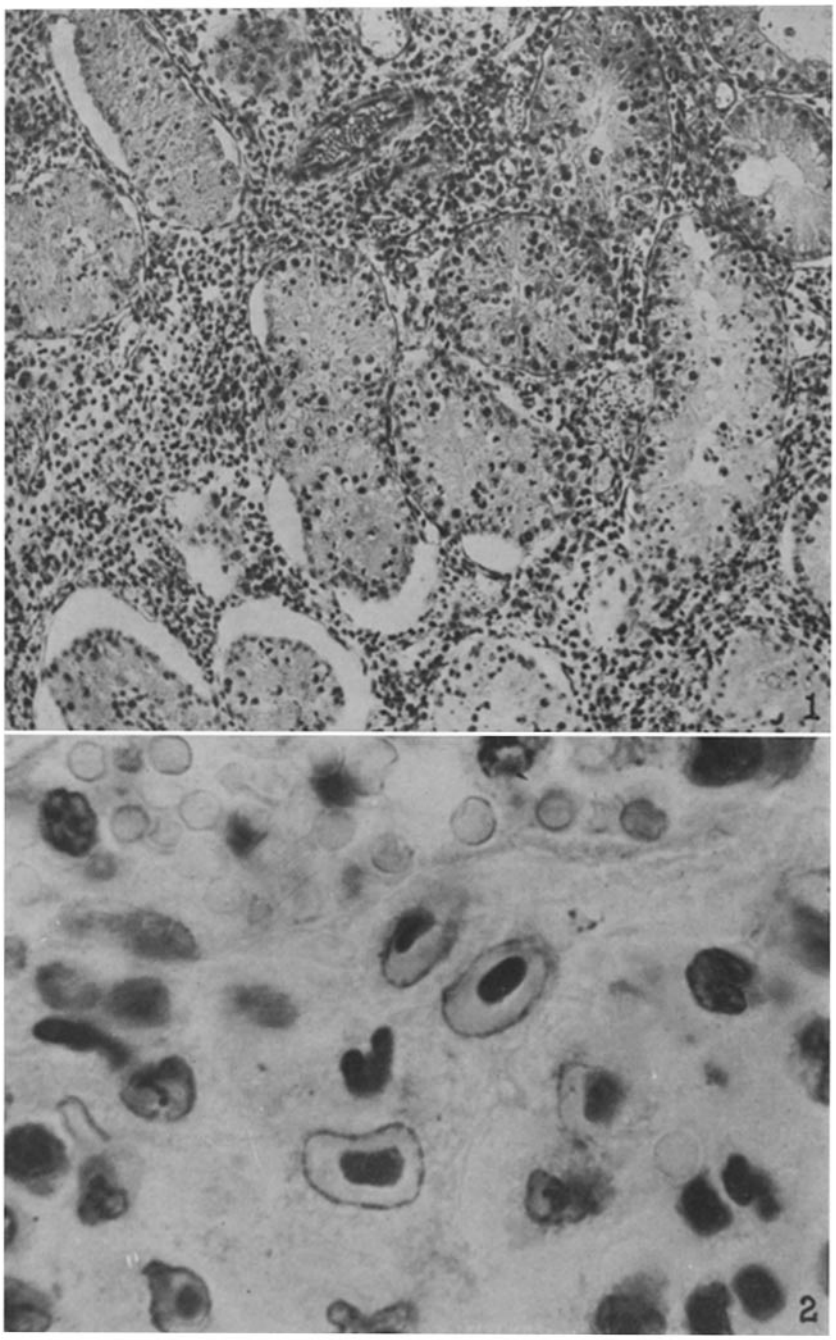
FIG. 9. Early vesicle formation in the cornea of an inoculated eye; degenerating cells with nuclear inclusions, giant cell with four nuclei. × 280.

FIG. 10. Vesicle in the cornea. Many of the cells around the vesicle contain nuclear inclusions. × 300.

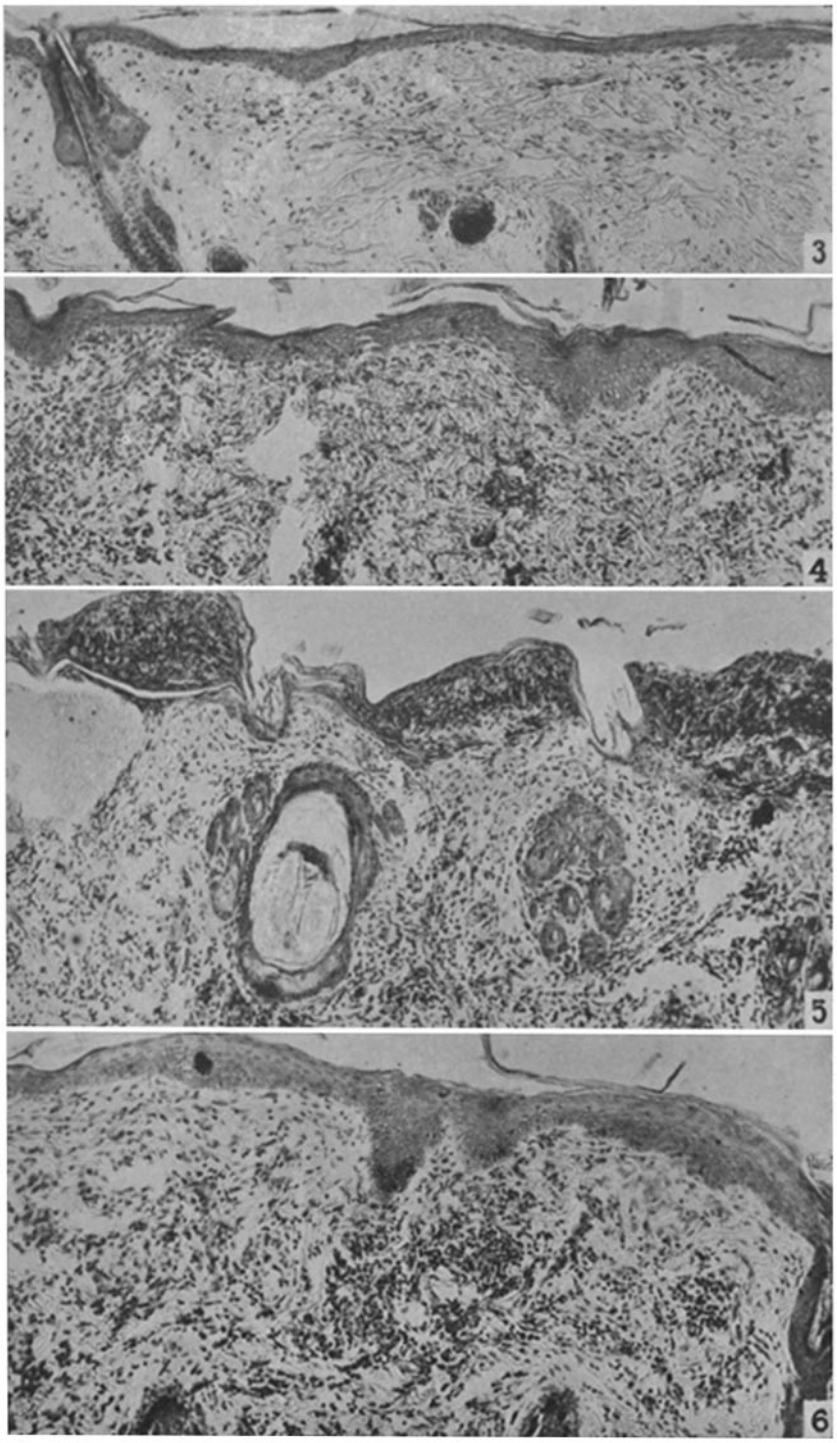
PLATE 9.

FIG. 11. Nuclear inclusions in endothelial leucocytes of an inoculated testicle. One cell has two nuclei both of which have inclusions. Normal cell for comparison. Eosin-methylene blue. × 1,500.

FIG. 12. Nuclear inclusions in corneal cells of an inoculated eye. The small inclusions stain homogeneously, whereas the large inclusions do not always seem to be homogeneous. Each inclusion is surrounded by a halo. Normal cell for comparison. Giemsa. × 1,500.



(Rivers and Tillett: Attempted transmission of varicella.)



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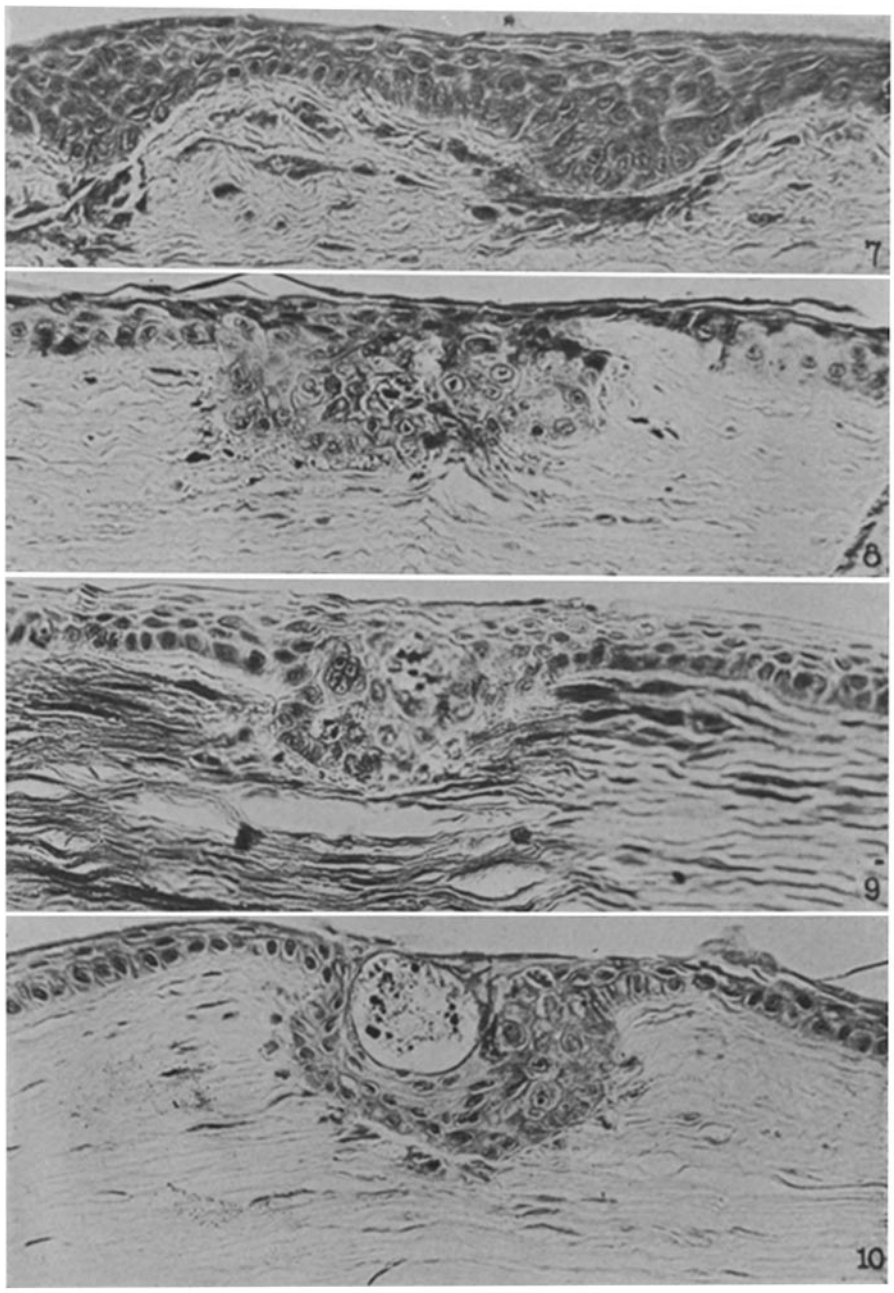

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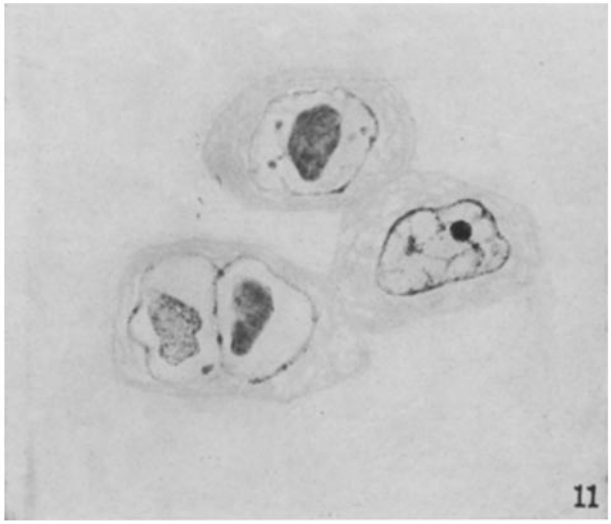

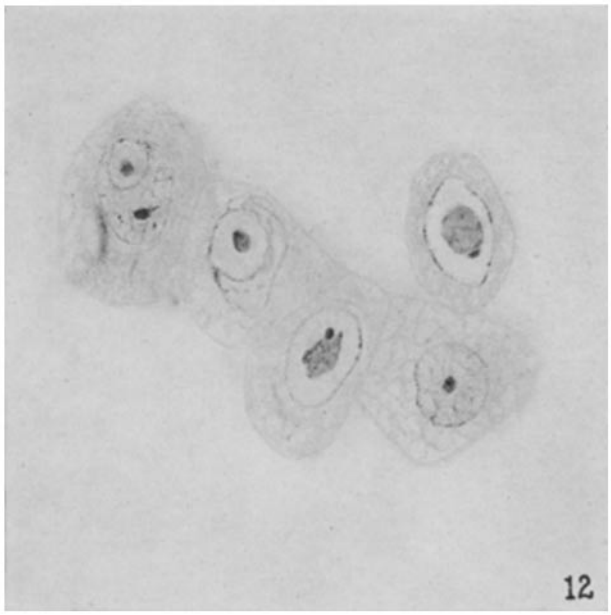

(Rivers and Tillett: Attempted transmission of varicella.)