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## A COMPARISON OF THE INCLUSION BODIES OF FOWL-POX AND MOLLUSCUM CONTAGIOSUM *

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With the demonstration of the infectious nature of the inclusion bodies of fowl-pox [1] it has seemed possible that inclusion bodies of other filtrable virus diseases might be used in similar experiments. Among these diseases is molluscum contagiosum, which closely resembles fowl-pox with regard to the large size and the distinctness of its intracytoplasmic inclusions. Furthermore, in a recent study of the subject, Goodpasture and King [2] have confirmed the observations of Lipschütz [3] and da Rocha Lima [4] that the inclusions of molluscum contain myriads of extremely minute round bodies — another respect in which molluscum and fowl-pox inclusions are similar. This observation, together with the fact that the molluscum bodies are of such large size, encouraged us in the hope that these bodies might be freed by tryptic digestion for purposes of inoculation, as were the fowl-pox inclusions. Accordingly, a series of experiments was undertaken, the results of which will be reported briefly. Since the gross and microscopic characteristics of molluscum contagiosum have been adequately described in textbooks and numerous papers on the subject, the molluscum body, which is the most characteristic feature of the lesion, may be considered without further description of the infected tissue.

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## The Effect of Trypsin on the Molluscum Bodies

When a piece of tissue from a molluscum lesion is placed in 1 per cent trypsin, the shell of epithelium about each molluscum body digests away, freeing the body (Fig. 1). As may be seen, the bodies thus freed by digestion are oval or round in outline and exhibit a finely granular structure. In addition to this fine granulation, a sort of trabeculation of the bodies can be distinguished especially clearly in a preparation which has been teased out in saline (Fig. 2). These trabeculae were described by White and Robey [5] in 1902, the entire structure being aptly compared to a honeycomb. The authors described the formation of the trabeculae from the cytoplasm of the affected cell. That this explanation is true and that the trabeculae are actually a portion of the epithelial cell is indicated by their reaction to trypsin. A period of thirty minutes in the digesting solution causes the trabeculae to dissolve and allows the various compartments to disintegrate (Figs. 3 and 4). The molluscum bodies still retain their outline and consist of a jelly-like matrix in which the appearance of granulation is conspicuous. The matrix material, after digestion of the trabeculae, is extremely sticky and strings out like a piece of dough when it is touched with a glass or steel point. Active Brownian motion of granules may be seen in the gelatinous compartments, and the granules are easily expressed and many of them become free while still vibrating rapidly.

In their reaction to trypsin the molluscum bodies differ markedly from the firm, compact inclusion bodies obtained by the digestion of a fowl-pox lesion. The fowl-pox inclusions are protected from the action of trypsin, presumably by their lipoid content, and can be digested completely free from adventitious cellular material without interfering with their homogeneity. In order to get molluscum bodies similarly free from cellular material, the removal of their cytoplasmic trabeculae is necessary. With this removal the molluscum bodies are found to be composed of sticky masses and are rendered quite unfit for any sort of manipulation as units. Because of this stickiness, our attempted duplication of the inoculation experiments which were successfully performed in the case of fowl-pox had to be abandoned. Recently a technique has been evolved for inoculating minute portions of the fowl-pox inclusions.[6] This latter technique has not yet been attempted with molluscum, but we feel that it would be practicable, and preferable to attempts to inoculate the entire inclusion bodies.

The gelatinous matrix of the molluscum bodies shows a much greater resistance to the digestive action of trypsin than is shown by the trabeculae. Furthermore, in any fresh preparation, whether the bodies are suspended in trypsin, normal saline or distilled water, the matrix exhibits the markedly granular appearance which has already been mentioned. This appearance is caused by the presence of innumerable Lipschütz granules — minute coccoid structures 0.25 of a micron in diameter within each inclusion. The granules were discovered by Lipschütz in smear preparations stained with Loeffler's flagella stain. He also observed them within the molluscum bodies in fresh preparations. At the present time the Lipschütz granules are recognized by many observers to be as characteristic of the molluscum lesion as are the molluscum bodies themselves, for, wherever the latter are found, there the Lipschütz granules can also be demonstrated. The granules are resistant to the action of trypsin to an even greater degree than the matrix material which surrounds them. In our experience, periods of five hours digestion have made no demonstrable change in their morphology and in all probability they would resist tryptic digestion for much longer periods.

## The Effect of Distilled Water

One of the striking characteristics of the inclusion bodies of fowl-pox is the marked swelling which they exhibit when placed in distilled water. It has been pointed out that this swelling continues until the original volume of the inclusions is increased two or three times.[6] Molluscum bodies, on the other hand, show little or no swelling under the influence of distilled water. The matrix of the molluscum body is probably completely permeable, since it promotes no swelling, while the lipoid material of the fowl-pox inclusion acts as a semipermeable membrane, permitting a marked imbibition of water.

Within the fowl-pox inclusions which have become swollen in distilled water may be seen myriads of minute bodies dancing about in rapid Brownian motion. These bodies, called Borrel bodies after their discoverer, are an exact counterpart of the Lipschütz granules of molluscum. In either disease the minute structures are coccoid in shape, 0.25 of a micron in diameter and react in similar fashion to special stains [7] (Figs. 5 and 6). As may be seen they appear either singly, in diploid or chain form, or in masses. Lipschütz stated that the minute granules are readily differentiated from any impurity or precipitate which might settle on the slide, and, with regard to a carefully made preparation, we can completely corroborate this statement. Moreover, the great uniformity in the size and shape of the granules in the two diseases militates against the argument of those who contend that such granules are mere fragments of chromatin or keratohyaline material.

The most effective method of obtaining the minute elements well dispersed is, in the case of fowl-pox, to allow the digested inclusions to become swollen in a pool of distilled water on a slide and then allow the water to dry.[6] The relatively great tension of the thin film of water abruptly tears apart the lipoid material of the inclusion at the moment the water dries from its surface, leaving the Borrel bodies nicely dispersed (Fig. 6).

The inclusion bodies of molluscum not only show no imbibition when they are placed in distilled water, but, possibly because of their failure to swell in water, fail completely to be broken up when they are allowed to dry out of distilled water. The best preparations of the Lipschütz granules are obtained by making direct smears from the molluscum lesion, or by grinding the lesion, as will be described later.

## Effect of Trituration

A study of the literature indicates that there is a difference in the readiness with which the fowl-pox and molluscum viruses may be passed through filters. Marx and Sticker,[8] and later Juliusberg [9] were successful in filtering fowl-pox virus through Berkefeld filters. Burnet [10] was able only occasionally to obtain passage through a Berkefeld filter and in no case through a Chamberland filter. Juliusberg,[11] on the other hand, obtained molluscum virus after passage through a Chamberland filter, and Wile and Kingery,[12] apparently without difficulty, passed active molluscum virus through a Berkefeld filter. It would seem that the molluscum virus filters somewhat more readily than does the fowl-pox virus.

Since the common mode of preparing virus material for filtration is that of thorough grinding in a mortar, we were interested to observe the different microscopic pictures presented by the smears of ground material in the cases of molluscum and fowl-pox respectively. After grinding bits of molluscum tissue in saline in a mortar for periods of five minutes, smears of the resulting material show the Lipschütz granules to be well dispersed (Fig. 5). After a similar treatment of fowl-pox material the smears show irregular masses of débris with very few discrete, definite Borrel bodies. In the case of fowl-pox, grinding the diseased tissue evidently fails to liberate the Borrel bodies completely, while in molluscum the liberation and dispersion of the Lipschütz granules is practically complete. This difference in the reaction of the inclusion bodies of the two diseases to grinding may again be due to the lipoidal element in the inclusions of fowl-pox.

## Comment

Whether or not the readiness with which the Lipschütz granules are dispersed upon grinding the molluscum tissue is of importance in explaining the apparently greater filtrability of this virus cannot be stated definitely at this time. In the case of fowl-pox, evidence has been presented which would associate the Borrel bodies with the actual virus of the disease.[6] While no such evidence is at present available in the case of molluscum contagiosum, still the similarity of the Lipschütz granules to the Borrel bodies in size, shape and position within the inclusion points to the probability that the minute elements in the two diseases represent the viruses concerned. If such is the case, the ready dispersion of the Lipschütz granules upon grinding and the great difficulty met with in attempting to disperse the Borrel bodies afford an explanation of the relatively greater filtrability of molluscum.

Though the great similarity of Borrel bodies to Lipschütz granules has been pointed out, there is no intention in so doing of indicating a common etiology of the two diseases. The reverse has been demonstrated by numerous observers, and, in our own hands, the inoculation of fowl-pox on man and of molluscum on chickens has in no case given any suggestion of a positive result. Moreover, repeated intravenous injections of molluscum virus into chickens has failed, in our experience, to induce any immunity to subsequent infection with fowl-pox virus. That fowl-pox, particularly, is a highly specific disease is indicated by the fact that a single passage of the closely related pigeon-pox through fowls causes the virus, in most instances, to lose its virulence for pigeons while still producing the typical fowl-pox lesion in chickens. If strains of the virus are specific for such closely related forms as fowls and pigeons, one would not expect the infectious agent of fowl-pox to cause disease in man. Several attempts to infect monkeys (*M. rhesus*) and other laboratory animals with molluscum virus have in our hands proved unsuccessful.

## SUMMARY

1. The inclusion bodies of molluscum contagiosum may be freed from surrounding cellular material by tryptic digestion.

2. Unlike fowl-pox inclusions, the molluscum bodies are found to be sticky and gelatinous after they have been digested. Because of this characteristic they cannot be manipulated readily with the Chambers microdissection apparatus.

3. The gelatinous matrix of the molluscum bodies has a markedly granular appearance due to the presence within it of myriads of Lipschütz granules — minute coccoid structures 0.25 micron in diameter. These granules are identical in size, shape and staining reactions with the Borrel bodies of fowl-pox. They are resistant to the action of trypsin.

4. The inclusion bodies of molluscum, on being placed in distilled water, show little or no swelling. Under similar conditions the fowl-pox inclusions swell markedly, due, probably, to their lipoid material acting as a semipermeable membrane.

5. Trituration of the molluscum inclusions readily breaks them up into the component Lipschütz granules. Fowl-pox inclusions, similarly treated, fail to break up so readily into Borrel bodies. This difference in the reaction of molluscum and fowl-pox to trituration may afford an explanation for the relatively greater filtrability of the former.

6. Fowl-pox and molluscum contagiosum are apparently specific for fowls and man respectively, cross-inoculation experiments having proved unsuccessful. Attempts to transfer molluscum to monkeys and other laboratory animals were also unsuccessful.

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## DESCRIPTION OF PLATE

### PLATE 1

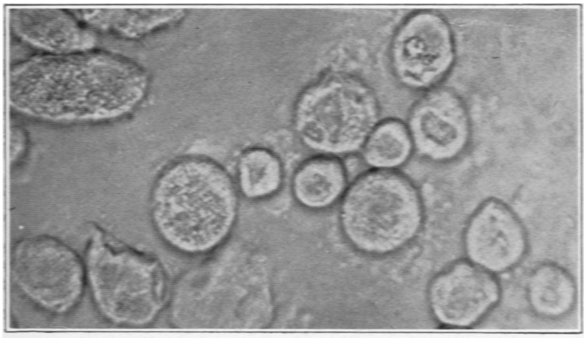
FIG. 1. Inclusion bodies of molluscum contagiosum (molluscum bodies) freed from cells by tryptic digestion. × 550.

FIG. 2. Molluscum body in normal saline, teased free from surrounding cells. Note trabeculation within the inclusion. × 1450.

FIGS. 3 and 4. Molluscum bodies in trypsin with beginning digestion of trabeculae. Note finely granular appeaiance of matrix material. × 1450.

FIG. 5. Lipschütz granules of molluscum contagiosum. Morosow's stain. Photomicrograph taken with white light. × 1860.

FIG. 6. Borrel bodies of fowl-pox. Morosow's stain. Photomicrograph taken with white light. × 1860.



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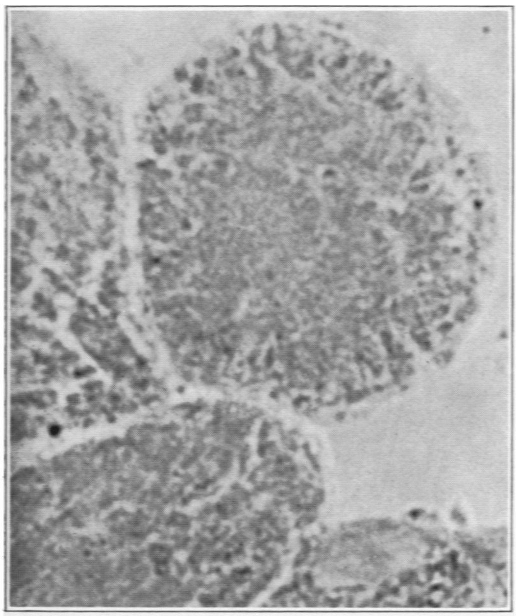

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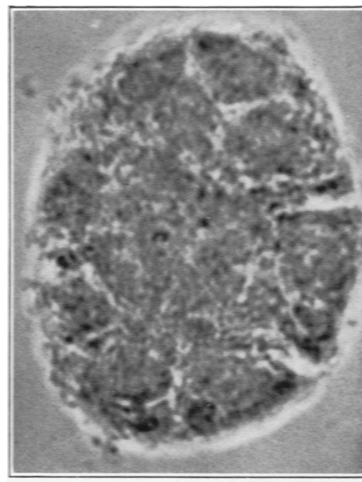

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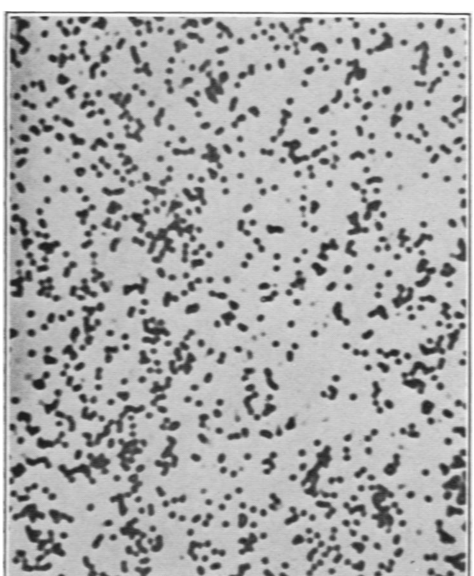

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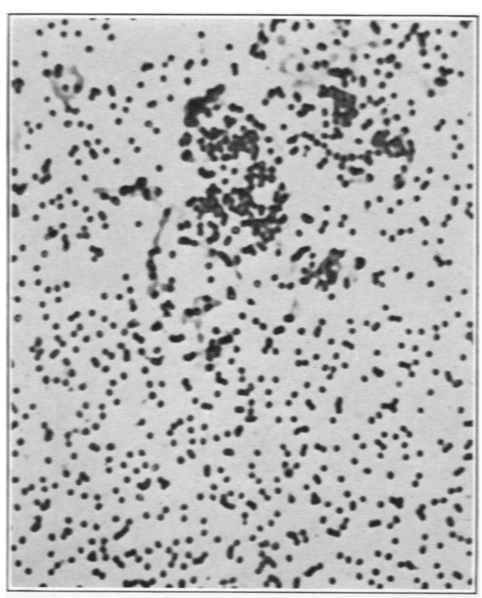

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