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**AUTHORITY**
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DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

Stomatitis papulosa is a relatively mild, contagious disease of bovines, first documented in 1936 by Osterseg and Buna(1). These authors speculated that the causative agent was a filterable virus. Schroaf, Fraub and Beller (2) in 1937 observed changes in the mucosal lining of the mouth of experimental cattle being held for investigations on Trichomonas disease. These changes appeared to be traceable to a same virus. In their principal investigations, they determined, among other things, that the filterable agent was strongly host- and organ-specific. The induced infection appeared only in the mucosal lining of the mouth of cattle. In some cases, the virus could be detected in the blood. The appearance of papules on the feet was not observed. The histological picture of the mucosal lining of the mouth in the area of the papules showed a widening of the epithelial coat around the area of the papules. Ballooning degeneration of the cells of the stratum spinosum and exaggeration of the cells of the granular layer were noticed. The authors found multi-shaped acidophilic inclusion bodies in the ballooning cells. They were located either in the plasma or in the hollow spaces formed by atrophy of the protoplasm, and they often resembled a granular structure. On the basis of the estimated volume of viral particles, from an intermediate filtration, the kinds of cell inclusions, and the histological changes in the epithelium of the mouth, the authors discussed the possibility that the etiological agent is closely related to the pox virus group.
The problem of whether or not this was a pseudo-hoof and mouth disease occurring in cattle in South Africa, identical to Stomatits papulosa, and reported on by Rebekker, Du Torr, and Quinlan (3), by De Kock, Du Torr, and Neitz (4) and by De Kock (5) was not clear until now.

Since Stomatitis papulosa is of less economic importance, it was given little consideration until now. Only recently, since it was noticed frequently in connection with epidemic outbreaks that produced severe economic losses in America, in Morocco, and in northern and central Germany, has more interest been given to it. Wagenor (6) succeeded with cases occurring in northern Germany in identifying them as that disease which had been described in American as X-sickness or hyperkeratosis. The question concerns poisoning by application of chlornaphthalene-containing industrial products, among which wood preservatives and tar- and asphalt-containing products play a role. The last report concerning such a case came in 1954-55 from central Germany where ropes prepared with such materials were the cause of heavy damage among cattle herds. In all of the reports concerning the cases of poisoning, changes in the oral cavity characteristics of Stomatitis papulosa were mentioned. Olsen and Palionős (7) expressed the opinion that a virus-like agent such as that associated with Stomatitis papulosa is involved. In their work, they showed a high microscope picture of the epidermis of an experimentally infected animal that shows characteristics of Stomatitis papulosa and gives the impression that inclusion bodies also are present. Through administration of chlornaphthalene,
the authors were able to make experimental animals more susceptible to
infection. From cases observed in central Germany, Heinig (3) ascer-
tained that the cause agent of the stomatitis was filterable through
a Seitz filter, and that it could be transmitted from normal cattle
independently of chloraphthalamine administration. Heinig (3), Beer (9),
as well as Dedie and Mitra (10) described the acidophilic bodies
seen by Scheuf, Traub and Sellier. Putterske (11) found that, on account
of the reduction in resistance of the animals, the papulous changes show
a strong tendency to spread and only a slight tendency to heal. Also,
in these instances following a less mild case with proliferating papule
formation, he could usually demonstrate acidophilic inclusion bodies.

With regards to chloraphthalamine poisoning, it has been observed
that the virus of Stomatitis papillosa obviously appears much more
frequently than was previously assumed. The breakdown of resistance
might be the reason for the appearance of clinical manifestations.

Early in 1957, Stomatitis papillosa appeared enzootically in cattle
herds in Badens-Wurttembergs, particularly in Handerstainen. These
cases provided the opportunity to investigate electron microscopically
the papulous changes in the mucous membranes of the mouth using ultra-
thin sections and in spot preparations.

MATERIALS AND METHODS

Production of Material: The virus were obtained from a spontaneous
infection of 6-8 month old cattle in a stable in Goppingen, in which
all the animals there were involved. Calves were infected experimentally

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for the study of ultra-thin sections of disease-infected tissues.

From the original material, a portion of papillous mucous membrane, a 20% emulsion in phosphate buffered isotonic saline (pH 7.4) was prepared. Infection occurred on the gingiva of the upper and lower jaw as a result of streak inoculations with supernatant obtained by centrifugation of this material. On the second day, a reddening could be detected; on the third day, fully developed papules had appeared on the inoculation streaks. At this time, a small strip of papulous membrane was incised from the live animal.

Fixing and Embedding: 1 mm pieces of the material were immediately placed in 5% phosphate-buffered formalin solution, pH 7.4. The next day, the specimens were transferred through multiple fresh saline solutions for two hours, left over night in 70% ethanol, then placed for three hours in a solution of 1% phosphotungstic acid and 70% ethanol. They were then dehydrated as usual and embedded in butylnethyl acrylate. The ultrathin sections were prepared with a Sjostrand microtome and collected in 20% alcohol.

Spot Preparations: The preparation of spot preparations followed the method described by herzberg and Kleinschmidt (12). A fresh vertical section was lightly spotted on a coated screen. After a quick washing with water followed by air drying, it was coated with platinum-rhodium at a 21.8° angle.

Sections and spot preparations were examined with the Siemans Elmiscope No. 1. For the preparation of photomicrographs, Kranz-Contrast Ortho plates were used.

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DISCUSSION OF PICTURES

The first three pictures are light microscope photographs of paraffin sections (hematoxylin-Eosin stain). Picture No. 1 is of the epithelial mucosa of the mouth of a healthy calf. The second picture is of a section through the same area, and it that the tissue is from a calf infected with Stomatitis papillae. In this picture, balloononed cells of the stratum spinosum are seen along with inclusion bodies. Picture No. 3 is an enlargement of the same area.

In Picture 4, an electron microscope photograph of an epithelial cell from papulous tissue, magnified 5000 times, the same changes can be seen as in Pictures 2 and 3. The cell boundaries look like arched lines, whose sharp apexes are part of the intercellular bridges. Above and on the left side of the cell, it looks to be discontinuous. Underneath, the cohesion to the neighboring cell is dissolved, and there is a broad intracellular gap. In part of the remainder of the enclosed cytoplasm in a clear empty space lies the shrunken nucleus with two round (slightly) thick structures in its center that are possibly swollen nuclear bodies. In the same space, adjacent to the nucleus, lies a structure that has somewhat the same radius as the nucleus and which undoubtedly is identical to the eosinophilic neoplasms referred to in the light microscope preparations as included bodies. Small, irregularly formed islands, which seem to consist of the same material, are seen to the right of the nucleus. On the inside of the inclusion bodies as well as on their borders lie ovoid-shaped particles about 200 nm large. A large number of ovoid particles are bound into a group with an irregular arrangement and somewhat withdrawn. In the enlargements...
it can be seen immediately that a smaller number of round particles lie within the inclusion bodies, and that these are surrounded by a membrane and have the same size as the void particles. In Picture No. 6, two inclusion bodies with the same arrangement can be seen. On the left and lying underneath, are present only narrow round particles, which contained in a cavity with the void particles. In the interior of the right side lie two inclusion bodies with irregularly formed, centered condensations whose structures appear to be coarse at this magnification. Many of these were found in inclusion bodies. Pictures 7 and 8 show portions of inclusion bodies magnified 60,000 times. The focal condensations appear in both pictures. They show up distinctly from the surrounding loose and foamy material which makes up the great proportion of the inclusion bodies. Numerous small, but also extended foci of this type were also observed. In the enlarged picture, an irregular net-like structure can be seen which has a coarse granular appearance through the thick junctions of the network. In the rounded portions, where these junctions intersect, such as in the center, numerous, round bud-like projections are found. They are enclosed by a membrane that is discontinuous where it meets with the edge of the thickened center. These bud-like projections, about 200 μ large, and rounded, are found inside the foamy materials of the inclusion bodies. They are distinguished from the foamy material in this way only - they are bound on one side with the thickened, granular focal condensation. In Picture No. 9, it can be seen on the left side that this union can also consist of only a narrow, filamentous bridge.
The previously mentioned round and ovoid particles, which could be viral particles or, very possibly, stages of development of viral particles, form a very characteristic film layer in relation to the inclusion bodies.

Inside the fairly thick film layer of inclusion bodies, only the round particles are found. These belong to another characteristic particles of this kind. They are usually smaller than the body with a diameter of about 140 m, and are surrounded by a membrane which seems to be linked in approximately 30-40 close double-walled chains. The entire structure has a diameter of about 207 m. The space between the inner body and the membrane is full of a material that has the same thickness as a structure similar to that of the foamy material of the inclusion bodies. In many particles, fine filaments can be seen radiating from the inner body to the membrane. The slightly ovoid structure of the particle in Picture 9b is probably due to the pressure of the cutting tool. The intersice between the membrane and the inner body is often wider on one side (Pictures 9c and 10), and sometimes the membrane is not completely closed (Picture 10). Whether the form is due to the use of cutting tools or is naturally present is a question for further research.

Likewise, within the foamy material of the inclusion bodies, membranes are also formed in which the inner body is missing and which seems to include only some of the material of the surrounding environment (pictures 9d and 11).
The ovoid particles have some distinguishing features. They are 190 to 240 μm long and 50 to 120 μm wide. They lie only in the foamy material of the inclusion bodies. The impression is given that the material was displaced by them or pulled itself away from them. Where they are found in the inclusion bodies, they are surrounded by open spaces (Pictures 4-6). The ovoid particles appear mainly on the edge of the inclusion bodies, individually or in large groups in the free spaces within the cytoplasm and isolated within the degenerated cytoplasm. In relatively thinner sections and with stronger magnifications, it can be seen that not all of the ovoid particles have the same inner structure. There appears to be a flowing transition between the different forms. The particles in Picture 9b have a relatively simple structure. The small part at the bottom left seems to be cut transversely to its longitudinal axis. It is surrounded by a 4-8 μm membrane-like which is attached from the inside out a layer of somewhat similar width and much less density. Left of it and at the right at two places, both of these layers are interrupted by disintegration. Starting on the inside and going out, progressively wider layers of 3, 5, to 7 μm in width are found which surround an oblong, slightly thicker body. Both of the particles lying in the middle of the picture and having been cut along their longitudinal axis, show a similar structure. Also, their various layers lie bound together around a long inner body. In many of the ovoid particles, the edge appears slightly corrugated or it appears as indentations in many cases. Picture 9c shows a particle exhibiting a number of striated bands of different widths which appear to transverse the particle. Investigations are under way to determine whether or not these are the result of successive disintegrations, such as decomposition.
Here and there in this material occur thickenings which have a net-like structure with wide points of junction and seem to separate the inclusion bodies of the round viral particles. Between the inclusion bodies and the membrane lies a layer displaying a structure similar to the foam-like material of the surroundings. Although distinctions exist, there is the question of similar development between this system and that observed with fowl pox by Morgan and co-workers (13) who studied cross-sections from infected chorioallantoic membranes. The material labeled by these authors as "viroplasma" had a loose, foam-like composition like that of Stomatitis papulosa. The latter authors noted with Vaccinia and fowl pox viral particles a double membrane and a less thickened inner portion lying near the edge of the cytoplasm as well as extracellularly. These forms were considered by them to be a later developmental stage of viruses. Sennhake and coworkers (14) have made similar observations in their investigations of the virus of rabbit fibrosis, and Bauer and Constantin (15) were able to determine in systematic studies on infected tissue cultures that the premature forms, which turned into inclusion bodies after only six hours, are themselves put together from viroplasma and are surrounded by a simple membrane. After 8 days, in comparison, they found fibrosis viral particles that possessed a double membrane and showed a clearing in the center. It has been assumed that the free particles of Stomatitis papulosa represent a later stage of development.
In unfixed wet spot preparations that were prepared directly from infected tissue, only the free oval forms were found. These particles were generally 20 µm larger than those usually found—a difference which may explain the different results with original material. While the canary pox virus prepared with these methods by Herzberg and Kleinschmidt (12) had a square shape, these particles retained their oval shape. This points out that the virus of Stomatitis papulosa is not of the square virus form.

The observations presented were on thin sections of fully developed papules have shown that the electron microscopy of the Stomatitis papulosa virus appear in different forms, from which it must be assured that the question concerns successive stages of the structure of virus particles. The evidence for the correctness of these assumptions will have to be produced through additional investigations of disease altered tissue at different stages of the disease.

**SUMMARY**

The included bodies, demonstrated in the case of bovine Stomatitis papulosa were examined in the electron microscope in ultra-thin sections.

The material of which they mainly consist is loose and foamylike and contains focal consolidations.

Included within this foamylike material, round particles are found with a dense interior body, a surrounding membrane and a less dense layer, filling the interspace. These particles have a diameter of about 207 µm. The existence of incomplete particles of this kind offers the
conclusion due to their special form and situation, that they are build from material of the included bodies. The author presume, that these particles are virus particles. Other ovular particles, which are approximately 215 μ long and 105 μ wide, are considered as virus particles in a different (letter?) stage of development. They are not bedded into the material of the included bodies, but lie freely in great numbers in its hollow space, on its outer margin, within the intercellular gaps and single ones also within the cytoplasm.

These particles were also found in steamed dead-preparations. Contrarily to the virus of canary-smalpox, which is of square structure when demonstrated according to this method, these particles kept their ovular form, thereby underlining the presumption, that the virus of Stomatitis papulosa must not be counted among the square types of virus.

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