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SMUFD D/A ltr, 17 Feb 1972

TRANSLATION NO. 3372

DATE July 1968

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

ELECTRON OPTICAL INVESTIGATIONS ON PARAVACCINIA VIRUS (V. PIRQUET)

(Following is the translation of an article by T. Nasemann and E. Bauer, Dermatology Clinic and Physical Institute of the University of Munich, published in the German language periodical Klin. Wochen. 35, pages 62-67 (1957). Translation performed by Constance L. Lust.)

In 1892 Danve and Larue reported concerning an anomolous reaction during vaccination against pox. They designated it according to the morphologic picture as "Vaccine rouge" (cherry red pimples in the area of the vaccination.) The viruses isolated from these red pimples behaved biologically different than Variola vaccine virus. Because of this v. Pirquet designated the agents of the "red vaccination reaction" as Paravaccinia. In 1919 Lipschuetz (1919, 1923) gave the elementary bodies of paravaccinia the name Strongyloplasma paravaccine.

Studies by Nasemann and Deubner (1953) and Marchionini and Nasemann (1955) indicated that the "Vaccine rouge" and the actual Melkerknoten (sensu strictiori in the sense of Berger 1955) are caused by the same paravaccinia virus. Clinical and experimental details about the identity of the two illnesses and the etiology of the disease, can be found in the above references as well as those of Kaiser (1952), Katzenellenbogen (1952), Sonck and Penttinen (1954), Bosse (1956), and Nasemann (1956).

Clinical Observation

Jenner at the end of the 18th century already differentiated between the real (actual) Cowpox and the so-called "Fake pox" (Euteropox). The demarcation of the original Cowpox from Variola vera and the present vaccinia virus derives from the studies of Downies (1939,50,53). False pox on the udder of cows are the source of infection for paravaccinia. Cows infected with euteropox as a rule show no general symptoms; the animals have no pain during milking, milk production is normal and appetite remains healthy. Contamination of stables with euteropox may lead to recurrent illnesses for weeks or months; animals may be ill several times. After recovering from the infection only minimal (or none) immunity remains. During milking the paravaccinia virus enters the host via epithelial defects of the skin and leads to specific changes of the epidermis and Corium (hyperkeratosis, degeneration of Rate-cells, eosinophilic intranuclear and intracytoplasmic inclusion bodies, lymph and leucocytotic infiltration of differing degree). Histological studies showed some change in the granuloma teleangiectaticum. Clinicially and histologically these corresponds well to each other for both diseases. Complaints arise only when a secondary bacterial occurrs. The nodes due to paravaccinia disappear in several weeks. The appearance of vaccine rouge after vaccination may be explained by using calf lymph which had been unintentionally contaminated by material of euteropox. (This is rare in Germany; was

very common in Balkan countries). In patients with nodes due to paravaccinia a protective immunization with variola vaccine is positive (takes). Sonck and Pentitinen (1954) reportedly transferred the paravaccinia germ from human to human, despite the presence of vaccine immunity. Berger (1955) later successfully revaccinated previously vaccinated cows.

Biological Characteristics of the Paravaccinia

In stained streak-preparations (Victoria blue, Carbolfuchsion) the elementary-bodies of paravaccinia cannot be differentiated from those of variola vaccinia. Lipschuetz already pointed this out. However, biologically there are clear differences. Contrary to vaccinia virus, paravaccinia cannot be transferred to rabbit cornea nor on to the chorioallantoic membrane of incubated chick eggs. This varying behavior lead to a separation of terminology from the morphologically similar variola vaccinia. The present electron-optic studies were designed to test how far (to what extent) the paravaccinia and variola vaccinia agree in their micromorphology.

Methods and Materials

We have examined fresh, excized material from eight paravaccine infected nodes of udder with virologic methods. During these studies we could regularly demonstrate the virus elementary body (ELK) light optically in streak preparations (also see Nasemann and Deubner 1953). We could not transfer virus dispite high virus content onto corneas or eggs. After vaccinating skin (persinal test) of the upper thigh a cherry-red pimple appeared (similar to vaccine rouge). We only examined two nodes histologically. The above mentioned alterations were found. In serum of patients no vaccinia antibodies (KBR) were found. Since we could find no host cell to grow the virus (except from bovine) eg. egg, mouse cells, rabbit cell line, we could produce no specific paravaccinia antigen. An infected herd of cows near Munich could as yet not be determined. For these reasons we could use only the infected excized tissue (infected human skin) for the electron microscopic studies. Perhaps this lack can be alleviated with aid of tissue culture studies. Such studies are now in preparation.

The nodes of three patients were studied with the electron microscope. Small tissue particles were either pressed carefully onto the object carrier (Zapan treated platinum net) or were indirectly picked up on glass object carriers before drying. In this way the preparations are not very clean since tissue constituents are also picked up. Dispite this we obtained numerous pictures of paravaccinia virus which we could evaluate. We used an AEG Zeiss electronmicroscope. A portion of the preparation was treated obliquely with palladium.

The enzymatic experiments were done according to Peters and coworkers. (Peters and Nasemann 1952,53, Peters and Stoeckenius (54 a,b,c), Stoeckenius and Peters (1955), Nasemann and Huber 1955). For fixation Chabaud-mixture was used 15 min. A part was treated with pepsin (0.02% solution of crystelline Worthington Pepsin, pH 2.0, 37°C 2 hrs; another part was subjected

to stepwise degradation with pepsin and DNAase (pepsin 2 hrs., then 0.1% solution of crystalline DNAase pH 6.0, 37°, 30 min; pepsin 2 hrs). Every time the solution was changed the preparation was washed two times with new distilled water.

The quantitation of length and width of paravaccinia ELK was done on the "clearest" pictures of the "clearest" preparation. We could only measure 20 ELK clearly. The size determination should only be used as a preliminary result. This result await growing this virus in passages in cultures.

Results

Like the (ELK) elementary particle of variola the paravaccinia possess parallelepiped (Ashler) form. They are prima vista not distinguishable. Paravaccinia also belongs morphologically in the pox group. Length 296 mμ (range 165-360 mμ), width 190 (165-230). The elementary particles (ELK) lie unregularly distributed between detritus masses (see figure 1). This cell material can be largely illiminated by treatment with pepsin (figure 5). Occasionally intact skin lamellae were in the preparations. ELK was found between them also. This may be observed in figure 2. In figure 3 the parrallelepiped form of ELK is well recorded. The corners of the form are rounded off. This may be seen even better in figure 4.

Similar to the other viruses with this structure (Variola etc) paravaccinia may be partially degraded with pepsin. This results in membrane-like forms, which surround a central core (not attached) of variable size; also completely empty membranes and a few intact ELK (see figure 5).

If the enzymetic degradation occurs stepwise, pepsin 2 hr. - DNAase 30 min. - pepsin 2 hr., then the central body is also dissolved. Then only empty membranes are found inside which may be found very small, dense bodies (see figure 6). No difference in behaviour to pepsin and DNAase existed between the variola and paravaccinia.

Discussion

Paravaccinia virus is a little more slender than variola and somewhat smaller than Molluscum contagiosum virus. Length ELK of vaccinia 240-380 mμ, width 170-270 (Peters and Nasemann 1952a). Median length of M. contagiosum virus 316 mμ ± 17%, width 245 ± 18% (Peters 1954a). Pepsin attacks paravaccinia ELK as it typically does pox viruses. By treating pepsin and DNAase in combination the DNA containing core is also degraded. The micromorphology of paravacinnia and the other pox viruses is very similar in all ways. They vary clinically and microbiologically. One causes epidermal lesions and cannot be transferred to lab animals or eggs. Paravaccinia causes changes in the epidermis as well as in the Corium; it infects bovines and humans. Humans and a number of animals are susceptible to variola vera and vaccinia virus. Skin and other

tissues are affected. Human and animal pox viruses may be grown in egg cultures. In table 1 the difference between variola -and paravaccinia are summarized. It should be noted that paravaccinia virus is only the pathogen for disease in the sense that Berger (1955) described and is not itself identical to the alterations of cowpox or of the so called vaccinia knots. If vaccinia or cowpox cause an infection, egg culture of excized material is positive and in the patients serum a rise of antibody is found.

Summary

Paravaccinia virus (v. Pirquet) belongs to pox type. By electron microscopy the parallelepiped form was demonstrated; length 296 mμ and width 190 mμ. Pepsin and DNAase attack EL in the same way as the other pox viruses. Microbiologically there are some differences between variola and paravaccinia virus (table 1).

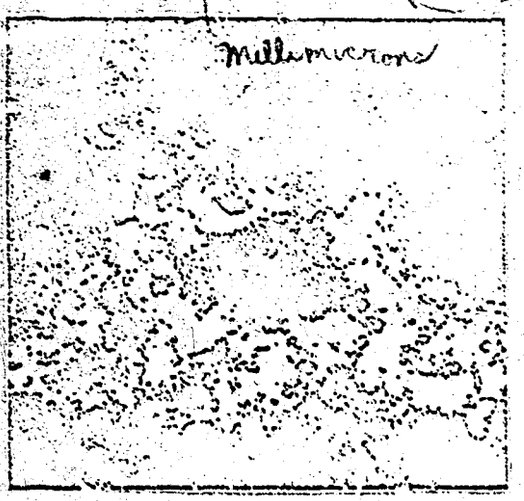

Figure 1

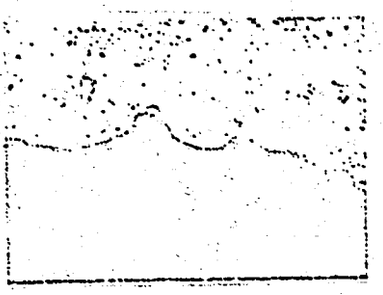

Figure 2

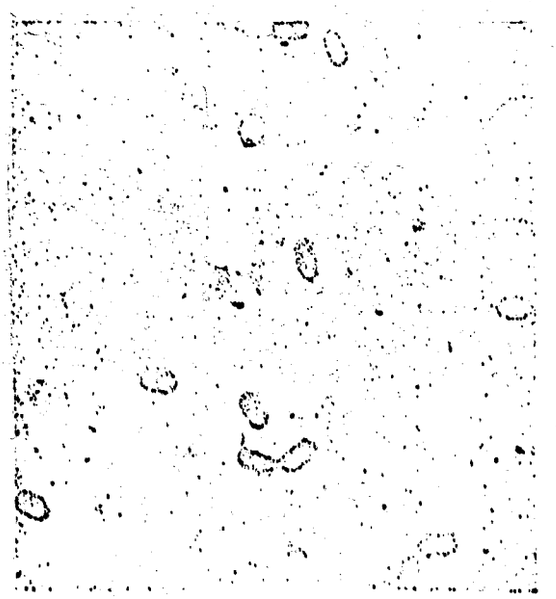

Figure 3

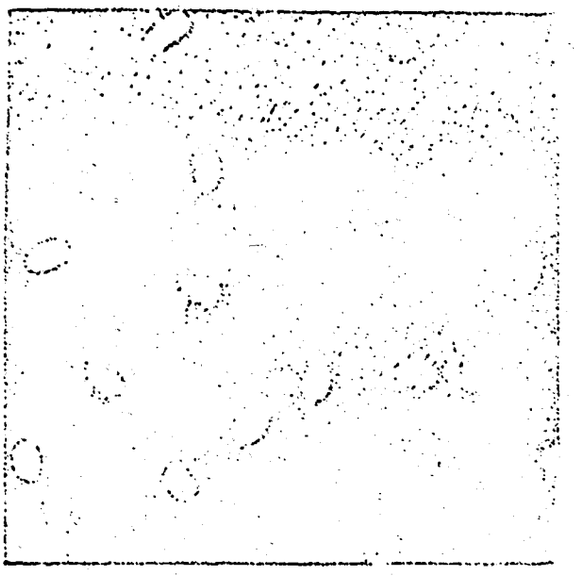

Figure 4

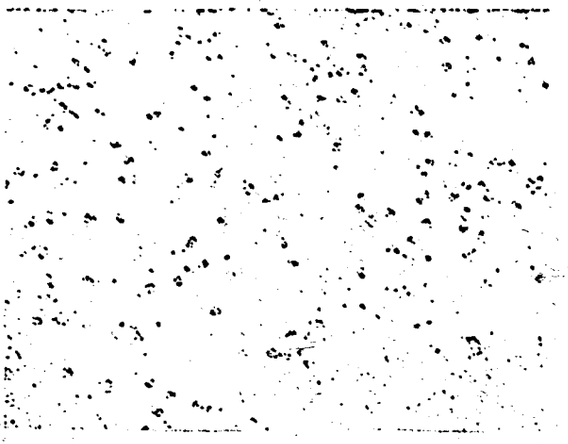

Figure 5

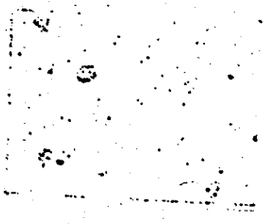

Figure 6

Table 1 - Differences between Variola and paravaccinia

	Variola	Paravaccinia
Paulscher comm. trial	+	-
egg culture	+	-
human to human transfer	+	+
C.F.+	+	-
light optic ELK	+	+
Electron optic	parallelepipet of ELD	same
histology	intracytoplasmic	intranuclear and intracytoplamic

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