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ELECTRON MICROSCOPY AND TISSUE CULTURE AS AIDS FOR DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS OF VIRAL DERMATOSIS

(Following is the translation of an article by Theodor Nasemann and Carl Georg Schirren, University of Munich, Munich, Germany, published in the German language periodical Hautarzt 14, pages 447-451, (1963). Translation performed by Constance L. Lust.)

Herpes simplex, Eczema, Zoster and Variola viruses appear frequently and are well known morphologically, characterized diseases of skin. However, frequently special strains with new characteristics of these infectious dermatoses appear, which require differential diagnosis for which a well equiped, modern lab and modern methods are required. For this reason electron microscopy and tissue culture may be used as aids in clinical medicine.

Patient 1, M.A., female, born 26/5/43, student was referred to us because of suspicion of small pox; both sides of throat, red pimples prevelant; blisters the size of peas (see figures 1 and 2)

Mucous membrane of mouth slightly red in throat, temperature 37.5°C (rectal).

Subjective diagnosis: Painful areas and generally tense, otherwise feels fine. Medical history of importance; patient had Variola as a child; no contact with newly vaccinated subjects was demonstrable. Her father had returned five days before from a trip to southern France. (Incubation time for Variola virus it too short). Patient was vaccinated as prescribed at age 1 and 12.

Differential diagnosos: The following diseases were considered on differential diagnosis; Zoster duplex, atypical Variola, Variolois, Vaccinia inoculata, Herpes simplex, Pyodermia.

Measures taken immediately to confirm diagnosis: The physician who referred the case indicated small pox. Therefore, this had to be proved or disproved first. If it was indeed small pox the public health authorities would have to be notified. We isolated the patient in a room and right away took a smear-speciman from one of the blisters. The sample was strained according to Morosow and studied under oil-immulsion. There were an unusually large number of virus elementary particles. However, even an experienced investigator cannot distinguish whether the small dots are viruses of the pox group, or are elements of the Zoster-Variella virus group. It was definitely not Herpes simplex. The Herpes simplex elementary particules cannot be seen with the light microscope. (see Naseman 1962)

A number of clinical observations spoke against the "pox" nature of

the virus. 1) low temperature 2) general well being despite hemorrlogic lesions. Variolois could occur with no symptoms and could cause small lesions. For these reasons a decision had to be sought. The only rapid method available was electron microscopy.

We used a simple direct preparation of the blister contents of the patient and looked as those under the electron microscope. The results were available in two hours. We found round-oval bodies, with hard center cores, with a diameter of 150-165 mu (see figures 3a and b). The diagnosis became obvious with this. No parallelpiped (freestone) viruses were observed in the contents of the blisters. Therefore it was not Varilois or small pox. The virus particles could be clearly differentiated from the Herpes simplex virus. Instead they were more in size and shape like the Zoster-Variolla virus, whose morphology was well known since it was described by H. Ruska (1943). See also Nasemann (1961).

Based on the localization of lesions on the throat and no the diagnosis was Zoster duplex.

Results from virus lab:

1) Inoculation of Hela cell cultures - no cytopathic effect.
2) Inoculation of Allontoic membranes of egg embryoes - negative even after further passages.
3) Complement fixation reactions with Vaccinia antigen negative on 7/5/62, and during convelescence, especially no rise in titer (no Vaccinia inoculata).
4) The contents of the blisters was sterile of bacteria;for this reason those pyodermatoses caused by Staphylococci were ruled out. Nasemann and Bandmann (1965).

These results confirmed the above diagnosis as Zoster. This virus does not replicate in Hela cells or chick embryoes, as opposed to Herpes simplex. The virus can grow in certain human tissue cultures; eg. skin and muscle of human embryoes and monkey kidney (Nasemann 1961).

Summary: In a 19 year old girl a "Zoster" duplex virus infection was confirmed in throat etc. This was aided by electron microscopic examinations; later the viral diagnostic lab also confirmed this.

Patient 2: A.A., male, born 18/2/22, Laborer
Since 5/7/62 (3 days) large blisters (6-7 to a group) about the size of coins appeared inside the foreskin (See fig. 4,5). The blisters are filled (leukocyte migration) and are placed in a red background. The area of lesions is very painful, but patient feels well otherwise.

At first a "Zoster" infection was suspected. From a differential diagnosis viewpoint, Herpes simplex (Herpes genitalis et glutaealis) was considered.

Lab results:

Chick enbryo culture: On the allantoic membrane of several embryoes which had been injected, small white growth appeared after 3-4 days.

Hela cell cultures: Several Hela cell cultures were inoculated with a suspension from the allantoic membranes. After two days a definite CPE was seen. Enucleation was seen with hematoxylin-eosin stained cultures. This could also be seen in ultra this sections of hela cells in the electron microscope.

On this basis a clear diagnosis of Herpes simplex was made.

Multicentric Herpes simplex (Herpes simplex glutaealis et genitalis).

Summary (Epicrises) In a 40 year old man with blisters on the foreskin of the penis multicentric H. simplex was determined in the fluid content of the blisters. This strain of H. simplex from this patient proved to be strongly encephalitis in rabbits (early encephalitis and rapid Exitis letelis of the animals).

Patient 3: P.S., female, born 2/13/19, housewife

14/2/62, very weak, skin and visible mucous membranes pale, temp. 39°, pulse 100/min, no stiff neck. On both sides lymph nodes swollen; more on the left. Patient complained of severe headache and ear ache, left ear shell very swollen, very red in color, A 3 cm. wide band of pussy material is seen behind the auricula (see figure 6). The retroauricular lymph nodes very swollen. Behind right ear shell is a red spot the size of a coin. This is also seen around the anus.

Medical history: For 7 years the patient has been suffering from a chronic ear canal eczema, especially on the left side. Also a chronic eczema behind the ears. The patient has a history of oily hair, dandruff and inflammation in the anal region (occassionally Pruritas ani). On 15/1/62 the one-year old child of the patient was vaccinated against small pox. On 7/2/62 -more than 3 weeks after the child's vaccination- at the time of the scraping off of the vaccine- the patient's eczema became markedly worse. Two days later -5 days before admision to the clinic- lymph nodes in the throat became swollen and severe headaches and ear aches ensued; finally also the patient became febrile rapidly.

From differential diagnosis the following illness were considered: Eczema vaccinatum, Eczema herpeticum, "pyodermic seborrhoic" Eczema, Diphtheria of skin on ear eczema, "Zoster" oticus.

Measures taken immediately to confirm diagnosis. Contents of the blisters was streaked onto slides and stained according to Pasihen and Morosow. Under oil immersion many elementary virus particles became visible. This observation excluded Eczema herpeticatum as well as a banal Pyodermosis. Most probable therefore was a diagnosis of Eczema vaccinatum.

Course of illness and later lab results:

BSG 20/48, Wa. R. negative. Within 3 days an infection with vaccinia virus was confirmed with the following methods.

1) Egg Embryo culture: All inoculated allantoic membranes showed typical, yellow-white pock-shaped colonies. Streak plates of membranes stained according to Morosow allowed identification of large numbers of elementary particles.

2) Hela cell cultures: CPE was seen clearly within two days of inoculation (see figure 7). After three days samples were stained with methyl blue-eosin (Mann). Screening of these preparations under oil immersion showed that in large numbers of hela cells the cytoplasm contained eosinophilic inclusion bodies (see figure 8, arrows point to inclusions).

3) Bacteriological analysis revealed Staph albus and Staph aureus; no (therefore no Diphtheria).

With this the diagnosis was cleared. The disease was a secondary pyodermic Eczema vaccinatum on the base of a "Seborrhoe".

Course of illness; fever rose to 39.8°C; therefore 500 mg Reverin was infused intravenously daily for 7 days; also the first week 5 ml. gamma globulin was administered, and for 14 days, 3 times daily two tablets of Spenitol. The patient was released after 14 days.

It should be mentioned that the isolated Vaccinia virus could be transferred to rabbits. We injected the tongue of the rabbit per and subcutaneously with allontoic suspension from the egg embryo infected with the patients strain of vaccinia virus. After three days it was obvious it was Glossitis papulosa, which was first described by Michelson for humans (see figures 9 and 10). A large part of the tongue surface was affected. Gottron (1929) first showed that Glossitis papulosa (Michelson) was due to a caccinia virus infection. We succeded, in the experiment described earlier, to transfer this freshly isolated vaccinia strain to rabbits which then exhibited a similar illness.

Epicrisis: In a 32 year old woman a diagnosis of secondary pyodermia Eczema vaccinatum was made. The vaccinia virus was isolated from eggs and tissue culture and could be transferred to rabbits. Via this trans-inoculation Glossitis papulosa, known for humans, occurred (Vaccinia-Glossitis for more details also see Schuermann 1958). Two facts should be noted on the Eczema vaccinatum; 1) no Neurodermitis constitutionalis but a seborrhic Eczema, 2) The vaccinia infection occurred relatively late (Mother was infected only after child was vaccinated). The second point bears special watching. It shows how persons with eczema are endangered by vaccinations to others in their surroundings. The secondary infection with Staphylococci was quickly controlled with antibiotics (Reverin).

Summary and Conclusion: In three chosen illnesses the methods of differential diagnosis were thoroughly used; namely electron microscopy and tissue culture. ~~We succeeded in transferring~~ the vaccinia virus to rabbits who then showed the disease picutre of Glossitis papulosa first described by Michelson for humans and ~~that was~~ etiologically described by Gottron. The danger of vaccinia virus infection to those with, as well as without, eczema ~~was~~ pointed out.

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Abb. 1. Atypischer Zoster duplex im Cervicalbereich, linke Halsseite

Figure 1

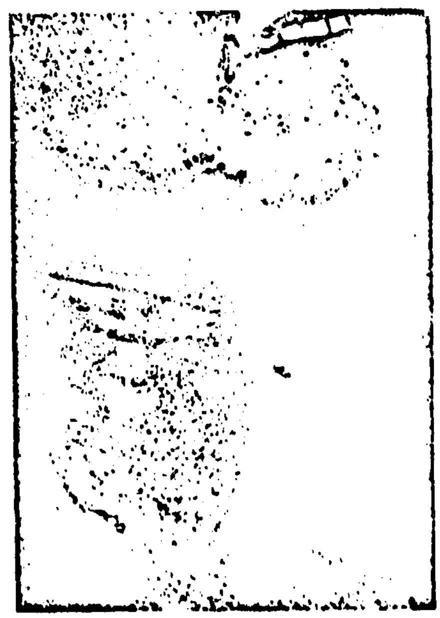

Abb. 2. Wie Abb. 1, rechte Halsseite

Figure 2

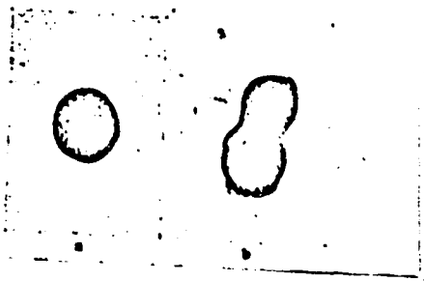

Figure 3

Abb. 4. Herpes genitalis

Figure 4

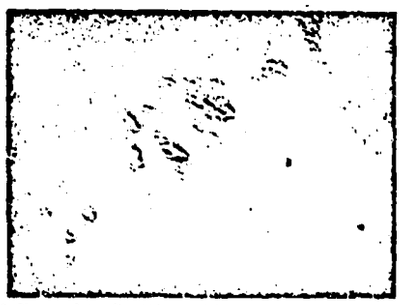

Abb. 5. Herpes glutealis. Abb. 4 und 5 stammen vom gleichen Patienten (multizentrischer Herpes simplex)

Figure 5

Abb. 6. Eczema vaccinatum auf dem Boden einer seborrhoischen Dermatose, sekundär pyodermisiert

Figure 6

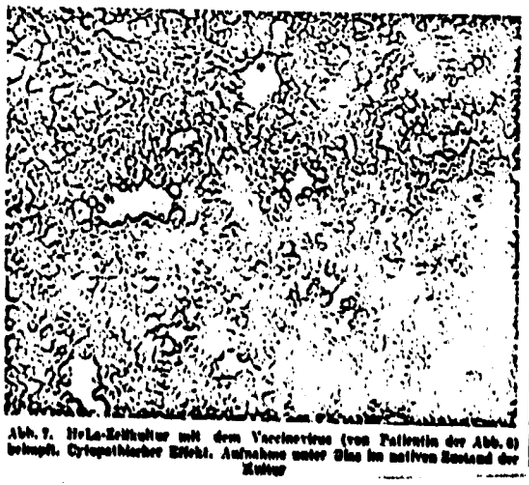

Abb. 7. HeLa-Zellkultur mit dem Vaccinevirus (von Patientin der Abb. 6) beimpft. Cytopathischer Effekt. Aufnahme unter Glas im nativen Zustand der Kultur

Figure 7

Figure 8

Abb. 8. Nach MANN gefärbte HeLa-Zellen (aus Kultur der Abb. 7) mit cytoplasmatischen Guarnierischen Einschlußkörpern des Vaccinevirus (s. die Pfeile). Ölimmersion

Figure 9

Abb. 9. Vaccine-Glossitis des Kaninchens, Infektion mit dem Stamm der Patkutin der Abb. 6, Bild der Glossitis papulosa acuta von MICHELSON

Figure 10

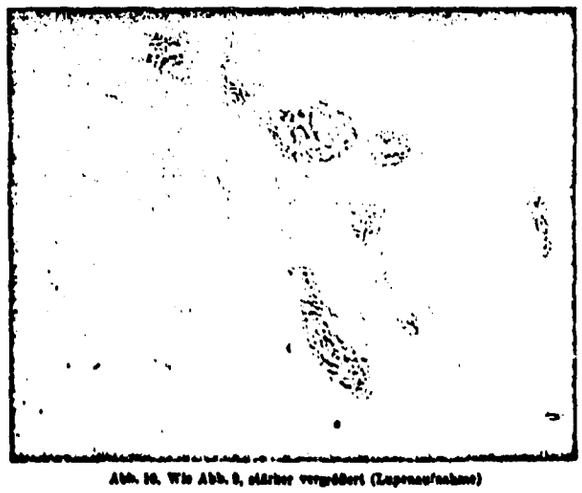

Abb. 10. Wie Abb. 9, stärker vergrößert (Lupenaufnahme)