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The viruses of cowpox and vaccinia.

by K. Berger.

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Current concepts of smallpox vacination are based esentially on the ancient observation that persons infected accidentally or on purpose with cowpox were subsequently protected against human pox. Thus immunity was originally conferred by direct transmission of cowpox virus from the diseased animal to man. Later the procurement of infectious material was made independent of accidental cases of cowpox and the virus was transferred from person to person according to Jenner's instructions. However, sustained human passages induce undesirable properties in the virus, leading to abatement in the vaccine's efficacy in the twenties and thirties of the last century. This situation was corrected by identification of cows with original cowpox, which furnished new vaccines, and by regeneration of the humanized lymph through retro-vaccination, i.e., through interpolation of one or more bovine passages. In the second half of the last century, vaccination with humanized lymph was replaced by immunization with animal lymph, and current smallpox vaccinations are conducted exclusively with material of animal origin. In view of possible degeneration, the virus is not always continued through the same host species, such as cattle, but is exposed from time to time to passages through another host, e.g., man or rabbit.

As the preceding summary indicates, contemporary "vaccination" is based on cowpox (variola vaccina) or the cowpox virus, and the designation "vaccinia virus," the customary synonym for the immunizing virus, still points to that correlation. The situation has been complicated, however by attempts at various immunological institutes to obtain new strains of vaccinia virus not only from newly identified cases of cowpox, but also by experimental mutations of variola virus by means of several animal passages. Reports on such successful mutations (which are not lacking in contradictions, however) have been released by Voigt, Paschen, Groth and others. Oddly enough no attempt has been made until recently to establish whether the vaccinia virus used for immunization still has the properties of original cowpox virus. Aside from occasional findings of certain fluctuations in virulence, the recent literature as a rule makes no distinction between the concepts of "vaccinia virus" and

"cowpox virus." Even nowadays many physicians designate the customary smallpox vaccine (containing vaccinia virus) as "cowpox" vaccine, evidently in the belief that cowpox virus and vaccinia virus are identical. The studies of Downie and coworkers, who were the first to subject the two viruses to thorough differentiation, have shown in recent years that this is not the case. These researchers demonstrated that cowpox virus induces lesions in egg cultures and on rabbit and guinea pig skins which are distinctly different from those caused by vaccinia virus. Without going into details of this investigation, it may be said that the foci developing on the chorioallantoic membrane of the incubated hen's egg and on the skin of rabbits and guinea pigs after infection with cowpox are characterized by a pronounced hemorrhagic nature, while no such tendency is noted in analogous lesions due to infection with vaccinia virus. On the other hand, necrotic lesions appear more rapidly and more distinctly upon infection with vaccinia. Another difference noted by Downie involved the dissimilar appearance of inclusion bodies in cells of the chorioallantoic membrane and rabbit and guinea pig skins infected with cowpox and vaccinia virus. Whereas infections with cowpox virus produced large, compact inclusion bodies without distinct inner structures in stained tissue sections, the enclosed material developing after infections with vaccinia virus frequently was distributed irregularly over varying cytoplasmic areas in the form of granular masses. Another noteworthy observation concerned the occurrence of inclusion bodies in many mesodermal cells (in addition to epithelial cells) in the case of cowpox infections, while these inclusion bodies were relatively rare in mesodermal cells upon infection with vaccinia. Finally, Downie and coworkers found antigenic differences between cowpox and vaccinia virus in direct and indirect complement fixation reactions as well as in neutralization tests using immune sera in cross-absorption.

Other authors have confirmed Downie's findings in principle and have even developed parts of them further. The studies of Herrlich and Mayr deserve special attention in this connection. These investigators proposed a method for the differentiation of different animal pox viruses based on dissimilar pathological lesions in the chicken egg. Echoing Downie, they listed the pronounced hemorrhagic nature of the foci as a characteristic of cowpox lesions on the chorioallantoic membrane, together with the delay in central cellular disintegration when compared to vaccinal foci. They noted further, upon inoculation of the allantoic cavity, that cowpox virus asserts itself very poorly on the entoderm of the chorioallantoic membrane, while infections with vaccinia virus establish themselves as readily as they do on the ectoderm. After infection with vaccinia virus, Herrlich and Mayr found the inclusion bodies to be relatively numerous, small and granulated; after cowpox infections they were less numerous, large, compact and homogeneous. Gispen also noted these differences in inclusion bodies recently. According to his observations, the cytoplasmic inclusions in the case of cowpox are frequently so large that the cell nucleus is compressed against the cell wall in a crescent shape, while these pronounced deformations of the

nucleus occur rarely in vaccinal infections. The same author established unequivocal antigenic differences between the two viruses by precipitation in agar gel. Verlinde also reported on dissimilarities in the morphological appearance of the chorioallantoic membrane.

When the studies dealing with differentiation of cowpox and vaccinia virus are considered in their entirety, it becomes apparent that practically all important investigations in this area date from the recent past, a time in which virology was developed to its current apex by introduction of new working methods. This is odd in the sense that modern virological methods (egg culture, etc.) were not even necessary for the demonstration of difference between the two viruses. As the studies of Downie and coworkers as well as of Herrlich and Mayr have shown, the disparity between cowpox and vaccinia virus is indeed quite conspicuous in egg cultures, but is hardly less impressive on the skin of rabbits and guinea pigs infected by the old method.

We noted additional marked differences in the pathogenetic effect of the two viruses upon instillation in the rabbit cornea. Since these differences have attracted little attention in the past (although the corneal test after Guarnieri and Paul has been employed diagnostically for many decades), a description is given below of the pathological changes in the rabbit cornea after infection with cowpox virus and vaccinia virus.

The technique. Following anesthesia with 2% cocaine solution, the rabbit cornea was scarified in lattice form with a lancet. The virus material was then carefully rubbed into the cornea with a glass pestle. The infectious material consisted of cutaneous efflorescences obtained from rabbits or cows after infection with cowpox and vaccinia virus, or of infected chorioallantoic membranes. The material was homogenized with physiological saline and diluted to such an extent that only isolated foci of epitheliosis developed on the infected rabbit cornea. After intervals of varying length, these foci were visualized macroscopically on the live rabbit by saturation with fluorescein solution or on the enucleated eyeball by fixation in sublimed alcohol. In addition, histological sections were prepared of fixed corneas and stained with hemalum-safranine for demonstration of Guarnieri bodies. The virus strains available for comparative studies included 3 strains of vaccinia virus used by the Viennese Vaccine Procurement Institute in the preparation of smallpox vaccines and 3 strains of cowpox virus furnished by R. Bieling, Vienna, A.W. Downie, Liverpool, and A. Herrlich, Munich.

The macroscopic lesions appearing on the rabbit cornea as a consequence of infections with pox group viruses are due to swelling and proliferation of epithelial cells and concomitant formation of small epithelial elevations on the corneal surface with subsequent formation of ulcers in their center due to epithelial desquamation. Our studies

show differences between the pathological changes after cowpox infections and those after vaccinia infections to the extent that the development of foci and, especially, of desquamation processes is more rapid and intensive in vaccinal infections. To aid visualization of these differences, we have apposed developmental stages of both infections in Fig. 1.

Usually the difference becomes apparent from the third day post infectionem. Up to that term the foci may still be quite similar, although one at times gets the impression even then that the development of cowpox foci is slower. It may happen that a rabbit cornea treated in vivo with fluorescein solution reveals no macroscopically visible lesions 48 hours after infection with cowpox virus, because epithelial desquamation has not yet set in, whereas a vaccinal infection as a rule has produced epithelial defects by this time, at least in the form of minute points. In other cases, cowpox foci may show beginning epithelial desquamation already on the second day, and no distinct difference vis-a-vis vaccinal foci exists at this time. However, in its further development, disintegration is much slower in cowpox foci. Consequently our material showed cowpox foci after 3 days with a total diameter of 1-2 mm and epithelial defects of about 1/3 mm, while vaccinal foci measured 2-3½ mm in total diameter and 1 mm in epithelial defect. After 5 days the total diameter of cowpox foci was 2½-3 mm maximally and the diameter of the central crater measured 1 mm maximally, while vaccinal foci revealed diameters of 4-5 mm in toto and 3 mm across the crater.

When the macroscopic lesions of the rabbit cornea due to infection with cowpox virus are considered in their total development, they recall infections with the virus of variola vera, as described in detail by Paul. Compared to the vaccinal infection, variola also shows a distinctly slower development of lesions in the corneal epithelium and, particularly, a less stormy course. At the current stage of our knowledge, we would classify the vehemence of reactive processes evoked in the rabbit cornea by cowpox infections as being nearly in the middle between variola and vaccinia infections. It seems also that cowpox virus originating with diseased cattle brings about a slower development of the foci of epitheliosis than virus subjected to several egg and rabbit passages.

Aside from the macroscopic aspect, cowpox foci of the rabbit cornea may also show microscopic differences when compared to vaccinal lesions. In certain stages, especially in preparations of the 4th-5th day after infection, some cases reveal Guarnieri's inclusion bodies of a size never associated with vaccinal infections. The histological appearance is so striking at times that Kaiser and Gherardini, among others, have devoted a separate publication to this aspect, in which they point out that previous examinations of thousands of preparations had never shown a similar wealth and size of Guarnieri bodies. As shown in Fig. 2, such preparations contain epithelial regions in which every cell is occupied

by inclusion bodies which not only attain the size of the cell nucleus, but frequently surpass it. The nucleus is then forced to the cell's periphery and appears as a narrow, sickle-shaped structure.

The indicated histological changes are most pronounced at the lesions' center, where tissual disintegration is imminent or already in progress. This circumstance is based on the fact that the pathological process in the cornea proceeds from the point of infection toward the periphery, and for this reason the most recent foci are found at the periphery and the oldest at the center. Consequently the periphery contains the youngest and smallest inclusion bodies, whereas the center shows the oldest and largest ones. Exceptionally large inclusion bodies may be expected in places where central necrosis is particularly slow, allowing sufficient time for maximal development of inclusion bodies. Apparently this requirement does not exist in vaccinia infections due to early inception of desquamation. Judging from past experience, inclusion bodies of truly impressive proportions do not always develop in cowpox infections, but appear primarily in cases where the infectious material is obtained directly from cattle infected with cowpox (as in the case described by Kaiser and Gherardini or in Fig. 2). After passages through other hosts the ability of cowpox virus to develop unusually large inclusion bodies is eventually lost, just as the pathological process in the cornea apparently is less protracted than after infection with original bovine material. It is quite possible, of course, that delayed epithelial desquamation is not the only factor that permits the larger form of inclusion body to develop, but that other influences are involved, perhaps an inherent specific ability of cowpox virus to produce larger inclusion bodies than vaccinia virus under certain conditions.

We attempted a compilation of pathogenetic differences between the viruses of cowpox and vaccinia, found by means of Paul's corneal test, because these dissimilarities had attracted little attention in the past, and because they may be of interest as a supplement to existing data. Another difference, which should be mentioned briefly for the sake of completeness, involves the variation in the morbid course in cattle after infection with cowpox or vaccinia. Without going into details, we want to point out that cowpox in cattle has an incubation stage of 3-8 days and cutaneous efflorescence requires 7-12 days to mature, whereas cattle inoculated with vaccinia virus show cutaneous manifestations after 2 days and fully mature efflorescences after 5-6 days, as known to every producer of vaccine. These descriptions can be found in the veterinary literature (Hutyra and Marek, Wirth and Diernhofer, Zurukzoglu and others).

Illustrations

Fig. 1 a-c. Lesions of the rabbit cornea a. 3, b. 4 and c. 5 days after infection with cowpox or vaccinia virus (magnified about 3 times).

Fig. 2. Guarnieri's inclusion bodies in the epithelium of a rabbit cornea 5 days after infection with cowpox virus f. the efflorescence of a calf infected with cowpox. (Hemalum-safranine stain, magnified 800 X). a. inclusion body, b. cell nucleus.