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POCK DISEASES *

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The aetiological agents of the pock diseases are the so-called elementary bodies, first discovered by Borrel in fowl-pox and sheep-pox (Borrel bodies), and by myself in variola vaccine (Paschen bodies). Morphologically they are identical. We have here to do with minute, coccoid, sharp, round corpuscles, which divide into two dumb-bell shaped parts, and are always to be found in enormous quantities. They pass through the Berkefeld filter V, and can readily be deposited from the filtrate by high-speed centrifugalization—namely, 10,000 revolutions or more per minute.

INCLUSION BODIES: SIZE, STAINING METHODS, AND DISTRIBUTION

I have fixed their size in the coloured preparation at from 0.2 to 0.25 μ . In tests based upon mathematical and physical calculations Bechhold arrived at the value of 0.21 to 0.23 μ , while filtration experiments carried out by Elford and Andrews suggested that vaccinia virus particles have a diameter of 0.125 to 0.175 μ —that is, considerably smaller than those of Bechhold and Schlesinger.

Ledingham judged correctly that the actual diameter of the naked Paschen bodies is smaller than 0.2 μ . The stained bodies which he obtained in pure suspension in formalized saline solution are obviously smaller than they would be in their natural element, where their size is increased by the addition of colouring matter and the presence of serum protein in the lymph.

In 1911 Jacobsthal and I showed that the elementary bodies of infant lymph are specifically agglutinated by immune serum. Ledingham, by an improved method for obtaining pure suspensions of elementary bodies, was able to follow up the agglutination in the hanging drop—the stained clumps consisted entirely of elementary bodies.

Presumably, the Paschen bodies possess a lipid mem-

brane, and are thus very resistant to the process of drying. They cannot be shown with the usual dyes, but only by using hot Löffler mordant, followed by heated acid carbolic or aniline fuchsin, gentian, or methyl-violet. The methods of Fontana-Tribandean, Morosow, and Giemsa also render them visible. A slight trick is, however, first necessary—namely, to minimize the layer of serum surrounding the elementary body. The dry smears are, before staining, placed perpendicularly in saline solution made with distilled water (I prefer Ringer solution) for several minutes. After they have dried they are fixed in methyl-alcohol, and are then ready to be stained.

The Paschen bodies can be regularly demonstrated in large numbers in infant and cow-pox lymph, also in the rabbit virus lymph—especially in the two-day virus, which is almost a pure culture. They are also found in the content of variola pustules—that is, in variola vera, varioloid, and alastrim. The same bodies were found by me in appropriate material from various countries—Germany, Switzerland, England, Portugal, Haiti, Togo—and in the primary, secondary, and early throat lesions in inoculated pox (Togo). When the cornea of the rabbit is vaccinated with variola vaccine material, besides the Guarnieri bodies the Paschen bodies can always be demonstrated when using the "Klatsch" method. When vaccinating with neurolapine (neurovirus) the Paschen bodies are always found in great quantities in the metastatic lesions of the skin and internal organs (lungs, spleen, liver, adrenal glands, testis), in the smears as well as in the sections, when the method of Levaditi is used.

Are they Causative Agents?

In the case of fowl-pox, according to the method of Goodpasture and Woodruff, I believe it would be possible to isolate separate Guarnieri bodies from the pustules of vaccinated infants and to infect with them, if this should be actually desired as a crucial experiment, to prove the nature of the agents concerned. Actually, this proof has already been given by the morphological demonstration of the Paschen body in the tissue culture (Nauck and Paschen) and in the vaccine culture in a medium free from cells (Eagles and Ledingham). In vaccination with cultures originating from an infant lymph they always, in spite of high potency, produced the same type of reaction as the usual calf lymph. On the other hand, the typical reactions of neurolapine, with accompanying necrosis and haemorrhages, were obtained with cultures derived from testis vaccine, kindly sent me three years ago by Dr. Eagles. E. Gildemeister made similar observations with his cultures (as he informed me personally), which also started from testis vaccine. Thus we see that a quality acquired by a virus through organ passage is maintained in artificial cultures *in vitro*. Considering the results I have obtained, I believe myself justified in declaring that the so-called Paschen bodies, discovered by me, are the aetiological agents of the variola vaccine. I owe special thanks to Professor Ledingham for having given credit to the Paschen bodies by the excellent work which he has now been carrying on for many years.

INTERRELATION OF POCK DISEASES

Starting from a common source—the original pock (probably the human pock)—and resulting from the continuous passages through one and the same host (that is, the same species of animal), three different strains have developed. These are: human pox, fowl-pox, and sheep-pox. It is true that in a great number of animals the pock virus is only primarily present, and develops later into ephemeral pock eruptions. As a rule, the illness soon wanes, cannot be carried on in passage, and leads to no epidemics. This process can be experimentally

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repeated. The same species of animal shows only unimportant local reactions after deliberate inoculation; it is, however, immune against a second pock vaccination, and also against cow-pox.

The most marvellous experiment in the realm of medicine—namely, the transformation of the aerially contagious virus of human variola through the cow or horse into the vaccinia phase, which produces only local reactions and is non-contagious—was brought about by Nature herself in primeval times. Presumably cow-pox is as old as pox itself. The theoretical explanation of the transformation of variola into vaccinia met with the greatest difficulties, and it was only due to the united efforts of many scientific investigators that light was brought into this darkness. It is highly probable that the manner in which the virus penetrates into the body, and the route it takes, play a very important part. The pock agent, which is disseminated in the air by the patient when speaking, coughing, or spitting, finds its way into the mucous membrane of the upper respiratory organs of contacts. In the case of throat lesions I have demonstrated the presence of numerous elementary bodies, especially in the early stages. In susceptible individuals the cells surrounding the part where the virus has lodged become irritated and damaged, as is evidenced by proliferation, various kinds of cell degeneration, and coagulative necroses. The process attacks the neighbouring lymph and blood vessels, and leads to thrombophlebitic processes with enormous increase of the virus. Finally, there must develop a septic focus in the course of the incubation period. This lesion must lie in the lungs; otherwise there is no explanation for the sudden enormous inundation of the blood stream, and for the occurrence of metastases in skin and internal organs (liver, bone marrow, adrenal glands, testis, and spleen). Certainly no one has so far seen this primary focus. As an explanation of the process I agree with the excellent work of Helmuth Nathan of Hamburg (published in *Virchow's Archiv*), who succeeded in demonstrating macroscopically a pulmonary venous thrombophlebitis in twenty-eight of thirty-one patients who at necropsy showed multiple abscesses.

If there were no such process it would be impossible to understand the course of variola inculcata. Most scientists deny an increase of the agent in the circulation, but the occurrence of an incubation period before generalization of the virus could not be explained by the agent simply having to wander through the lungs. The sudden inundation of the blood with virus shows that primarily one or more lesions giving enormous multiplication of virus must have been created. Experimental work with neurolapine in the case of rabbits, about which I shall speak later on, entirely supports this hypothesis.

PATHOLOGY

In the case of variola we have, therefore, a typical septic process, whereas in vaccinia the state of affairs is entirely different. Certainly, even with vaccinia the agent circulates regularly in the blood; but there is only a bacteraemia, and we have here a minus variant. This minus variant can be experimentally removed—at least partially. It is done by forcing vaccinia virus to accustom itself to a tissue which is strange to it (as, for instance, the brain or the testis), or to higher temperatures (Armstrong's heat-resistant vaccine virus). Many passages are necessary before the virus is made to multiply, and in later passages enormous increase takes place. Levaditi was able to infect with neurovaccine in dilutions of 1 in 10 millions. This neurovaccine (neurolapine or testilapine) is very similar to variola; Bijl called it the variola of rabbits. The special characteristics of it are the formation of metastases and a

liability to necroses and haemorrhages. It is only since neurolapine has been experimented with that there have been occasional reports of the "spontaneous" appearance of pox in rabbit cages (Levaditi, Armstrong). In consideration of the part (100 per cent.) that the lungs play in metastases with neurolapine, one might perhaps consider the possibility of infection through the respiratory organs.

The experimental study of neurolapine, introduced by Levaditi and his colleagues, has made a breach in the doctrine, set up by Levaditi himself, of the elective affinity of vaccinia—and especially of neurolapine—for cells and tissues of epiblastic origin. In numerous works (Walthard, Winkler, Demme, Gildemeister, Douglas, Smith and Price, Ledingham and Barratt, Bijl and Frenkel, Paschen, etc.) it has been demonstrated that the vaccine virus also affects the mesenchymal tissue. Douglas, Smith, and Price showed, in their work on generalized vaccinia in rabbits, with especial reference to lesions in the internal organs, that almost all the tissues of the body may develop macroscopic lesions, no injury or irritation being necessary. The internal viscera most commonly affected are the lungs, liver, spleen, adrenals, and the organs of reproduction. The mesodermic origin of an organ does not mean a non-susceptibility to vaccinia. According to the scientists mentioned, and also to Sobernheim and Paschen, such so-called affinities are merely the result of acclimatization to a new environment. Given the opportunity, the virus might be able to attack any tissue as easily as calf lymph attacks the epidermal surface. The lesions have a distinctive appearance microscopically and macroscopically, and are unlike any other lesions with which we are conversant. The lesions in the internal organs are always bacteriologically sterile, and contain the virus in sufficient quantities to reproduce the disease. They compare the generalized diseases in the case of rabbits with the alastrim or the uncomplicated variola. In the case of virulent pox all investigators found infections complicated by the presence of pyogenic cocci, chiefly streptococci. The authors suppose that the difference between alastrim and small-pox consists in the former being called forth alone by the pox virus, the latter by the pox virus combined with pyogenic cocci. The comparison is not quite adequate in so far as the neurolapine almost regularly kills the rabbit without the co-operation of bacteria. I believe that animals often die through the absence of adrenaline. The cortex of the adrenals, which are of mesodermal origin, frequently only shows rests of normal tissue.

FEATURES OF EXPERIMENTAL VIRUS INFECTIONS

Ledingham and Barratt, Frenkel and Bijl, Lillie and Armstrong, McIntosh and Scarff, Paschen, and others give exact descriptions of the alterations in tissue in experimental vaccine-neurolapine—for example, neurotesticular lapine. According to Ledingham the neurolapine obtained by testicular or brain passage has not acquired new properties compared with dermovaccine, but rather it has become possessed in an intensified form of properties shared also in a limited degree by strains of cutaneous source. The observed increase in endotheliolytic and endotheliotoxic powers must augment not only the chance of invasion from a skin area, but also the strength of the inundation, and it is presumably on the latter that the production of actual visceral lesions will depend.

In the case of vaccine virus propagated from dermis to dermis, and also by subcutaneous inoculation, a slightly increased tendency is shown at the spot itself, presumably in consequence of the unusual conditions of growth in the mesoderm under anaerobic conditions; it is only possible to maintain them virulent through two passages

at the outside. Presumably this is also the cause of the short decreased immunity after intradermal vaccination of the child, as shown by Kirsch. On the other hand, dermolapine carried on to the epilated superficial part of the skin (ectoderm) of the rabbit develops very quickly and luxuriantly. After two days the vaccinated area is dark red and papular, and contains the agent in enormous quantities, as in pure culture. Neurolapine and testilapine vaccinated in the same manner develops much more slowly; it must first gain the germinal layer suitable to it; it then increases with great rapidity, leading to those serious changes which are so well known—necrosis, widespread haemorrhage, and quickly developing gelatinous oedema.

The lesions in the lung in the case of neurolapine are of special importance as regards the further fate of the animal. The pock virus, which gets into the lungs through the blood, invades the alveolar walls and first of all attacks the cells of the septa. The epithelial cells swell, the endothelial cells proliferating rapidly. Serious vascular changes appear in the lung, the blood vessels being, as a rule, extremely congested, while some are thrombosed. The small veins especially show an inflammatory infiltrate in their walls. Perivascular infiltration and severe necroses are frequent. Proliferation is the most obvious symptom, with a mesenchymal reaction in the shape of a cellular perivascular infiltration (Bijl, Armstrong)—processes which are also found in post-vaccinal encephalitis and in organ metastases. The lesions are always in the neighbourhood of vessels. The site of inoculation is immaterial: the way always leads to the lungs, and from there, after propagation of the virus, to the left heart and the general circulation. The fundamental difference from dermolapine lies in the fact that in the latter the agent simply passes the lung without causing lesions. Where overdoses of dermolapine have been given only a hyperaemia occurs in the lungs. Sometimes after vaccination with neurolapine—also after cutaneous vaccination—typical metastases appear on the cornea (Armstrong, Paschen); I have only observed this once after vaccinating with dermovaccine. When intraperitoneal inoculation was made with material from the later passages of testilapine, Douglas, Smith, and Price found an increased affinity of the virus for the peritoneal cells. The organs of reproduction were attacked with remarkable frequency; the bladder, too, showed numerous lesions.

FORMATION OF INCLUSION BODIES

Great importance has always been attached to the inclusion bodies observed in virus infections. With inclusion bodies that have appeared after vaccine inoculation of the cornea, uniformly similar lesions are not found, as I showed in 1911. Borrel declares in an article which appeared in 1924: "La véritable origine des inclusions semble complexe."

Five different factors participate in the formation of the inclusion bodies:

1. Phagocytosed leucocytes.
2. Extruded nucleoli.
3. Protruding chromatin.

4. A great part are formed through lesions in the plasma with components of plastin and chromatin without inner structure. They are not specific, as they also appear after vaccination with diphtheria, Shiga, and gas-gangrene toxin. With this vaccine it is a case of a varying toxin that exerts a dissociating effect upon the chromatin and plastin parts that are normally united in the plasma.

5. Cap-shaped lesions on the nucleus. With the best colouring and illumination, the minutest bodies are found that presumably correspond to elementary bodies. These are colonies of agents. When making tests with infant, calf, and variola pustules only these inclusions are found.

whilst the inclusions of Group 4 are missing. Most likely, the variability in the conditions of the current plays a decisive part.

According to Goodpasture, Woodruff, and Buddingh vaccinal lesions in the chorio-allantoic membrane of the chick embryo offer a most excellent opportunity for studying Guarnieri and Paschen bodies in their relation to each other. There is no doubt that the Guarnieri bodies consist, at least in part, of colonies or masses of Paschen bodies, a hypothesis which I have long upheld. Vaccinia attacks the cells of the three germinal layers, whilst fowl-pox only attacks ectodermal and (to a less degree) entodermal epithelium. Woodruff and Goodpasture agree with the view held by Rocha Lima, Lipschutz, and myself that the inclusion bodies of fowl-pox consist of elementary bodies enclosed in a lipid capsule. Apart from the difference in the size attained and their time of appearance, the inclusions of fowl-pox, whether they appear in the skin or the cornea of the chick, have, without exception, a lipid component, which is never found in vaccine. So far as this reaction is concerned, therefore, vaccine virus represents a minus variant. Saito sees in this a difference between fowl and mammalian pox agents which is independent of the substrates. The pustule produced by vaccinia on the skin or comb of the chick does not show the histological features of fowl-pox, but those of the vaccinia or variola pustule, without formation of Guarnieri bodies; it contains, however, elementary bodies. In fowl-pox the agent generalizes soon after insertion, and invades all the tissues, but without metastases. At the most the lungs are hyperaemic, but show no lesions. The virus remains active for a long time in the body, and ovum and embryo (my own observations) are liable to be infected.

ROLE OF INSECT VECTORS

Recent experiments have proved that in epidemics of fowl-pox a vector plays an important, if not the chief, part. This transformation of variola to fowl-pox through passage of blood-sucking insects—such as *Culex pipiens*—will most probably have taken place in primeval times, when mankind and animals lived together in the most primitive habitations. Infections of healthy chicks with *Culex pipiens* and *Aedes aegypti* which had sucked the blood of fowls suffering from fowl-pox have been successful. Carl Stuppy has carried on these experiments at the Hamburg Tropical Institute, and has been able to prove that the infective properties of infected gnats were still active at the expiration of eighty-four days. Probably they remain infectious, active virus-carriers all their lives, though this property only starts from a certain date after sucking—possibly the tenth day. That proves that the virus propagates in the body of the gnat. Conditions here are similar to those in the case of yellow fever and dengue, and vaccination with the isolated intestinal tracts and salivary glands of gnats have given positive results. Sections through the stomach of the gnat reveal in the epithelial cells suspicious nodular inclusions beside the nucleus, in which up to the present the Borrel bodies have not been detected. The work is being continued. It should be emphasized that experiments made with gnats were entirely negative in the case of rabbits profusely vaccinated with vaccinia. In sheep-pox we have to do with a very infectious disease, characterized by a nodular eruption, especially in the skin, the lungs, the liver, and the kidneys. Characteristic inclusions are found in the pocks. Haemorrhages from the size of a pin's head to the size of a pea lie under the pleura. The lesions are similar to those of neurolapine. The manner in which the epidemic spreads is not exactly known, but it is quite possible that here, too, it is a question of infection through an insect vector.