

ON THE PRESENT STATE OF OUR KNOWLEDGE  
AS TO THE ÆTIOLOGY OF VACCINIA.

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[Read in the Section of Pathology, January 10, 1902.]

WHEN I received my present appointment at the Local Government Board, and was entrusted with the supervision and control of the calf lymph supplied by Government to vaccinators throughout the country, my close attention was very naturally called to the question as to the nature of the virus of vaccinia, and its relationship to that of small-pox.

It is impossible for anyone who has familiarised himself with the methods of investigation whereby so many obscure problems in the causation of infectious disease have been cleared up to stand idly by and witness week after week the vaccination of the calf and the regular appearance of the outcrop of pustules, without seeking to learn something as to the cause of the phenomenon. Again, the fact that inoculation with this calf-derived material confers upon the human subject immunity against what at first sight would seem to be quite a different and more deadly malady—the much-dreaded small-pox—is one that runs counter to our latter-day conceptions as to the specificity of the *materies morbi* in each one of the infective diseases.

Furthermore, there was the reflection that the discovery of protection so conferred against a seemingly different malady was merely empirical—the result of a happy chance, and not founded on any deep knowledge of the real conditions. Yet the immunity conferred by vaccination exceeds in certainty

and duration any similar condition produced in the human subject as the result of accurate knowledge during the bacteriological epoch. That the exciting cause must be a micro-organism seems, from analogy, to be quite certain. We know of nothing else that, introduced into the animal economy in infinitesimal quantity, is at the end of a definite period present in incalculably larger amount, and then quickly disappears, leaving behind it a persistent and profound alteration of the system which enables us to approach unscathed one of the deadliest of infections.

That the discovery of the micro-organism must be a matter of much difficulty was evident from the numerous alleged discoveries that have been made from time to time, only to be disproved by others or admitted by the would-be discoverer himself to be premature and unfounded. Still, there was the phenomenon, and there was I acquainted with the methods of research. The cause must be a micro-organism. It must be in the tissue of the vaccinated area. If not cultivable, it may at any rate be demonstrable by histological methods. Then why not look for it? Were it easy to find it would have been found long ago. Hence the search was not to be lightly undertaken. In order to find a passage through this maze, where so many had already lost themselves, it would clearly be necessary to find out how much of the ground had already been explored, what avenues had already been followed up and found to lead nowhere, what untraced paths there still remained which held out fair promise of success.

So much I have now accomplished, and my object to-night is to lay before the Section a brief statement of the present state of the question at issue.

First, why does cow-pox give immunity against small-pox? Because the two diseases are caused by the same virus, which, occurring naturally in man, produces variola, but which, by passage through the system of the bovine animal, undergoes

attenuation, so that when re-inoculated on the human subject the effect is no longer a generalised pustular eruption but a single vesicle limited to the seat of inoculation.

By passage through the calf a thorough change in the character of the virus must take place, for it loses its power of convection through the air, and loses its infectivity save by inoculation.

Many investigators have succeeded in variolating the cow—*i.e.*, in producing on that animal an eruption by inoculation with the contents of variolous pustules. The first of these seems to have been Gassner, so far back as 1807. In 1839 von Thiele repeated the experiment with success. Having obtained material from human small-pox, he inoculated it on the udders of calves, and so obtained a vesicular eruption which he continued from calf to calf for 75 generations. Meanwhile he vaccinated no less than 3,000 individuals from the same strain with typical results.

In England, Ceely, about the middle of last century, demonstrated conclusively the inoculability of human small-pox on the cow, and the transmissibility of the virus from cow to cow, the identity of the eruption so produced with natural vaccinia, and the immunity against small-pox produced by the inoculation of man with variolo-vaccine so produced. Since Ceely's time his results have been abundantly confirmed by the successful variolations of the cow at the hands of Voigt, Fischer, and others.

With the exception of the French school, headed by Chauveau, which holds that the two diseases are absolutely distinct, and fails to give any adequate explanation of their mutually protective power, their identity is now admitted by all competent to form an opinion on the subject.

Whoever discovers the microbic agent in vaccinia will, therefore, have also discovered that of small-pox.

In order to discover the micro-organism one would naturally

examine the tissues which are presumably altered as the result of its presence—the parasite, in the case of small-pox, being deposited apparently from the blood, and in that of vaccinia being directly implanted by human agency. Weigert seems to have been the first, in 1874, to make an accurate examination of the minute anatomy of the skin lesion in small-pox, and a few years afterwards Unna published some valuable works on the same subject.

Quite recently a most careful and complete investigation of the histology of the vaccine vesicle has been made by Dr. Gustav Mann, of the Physiological Laboratory, Oxford, and published by him in the Supplement to the Ninety-eighth Annual Report of the Local Government Board for 1898–99.

This investigation was carried out on carefully fixed material, collected by Dr. Copeman from the vaccinated calf, and representing every stage of the vaccine process, from the first hour to the tenth day. The methods are those of the most advanced histological technique, and the results are set forth in a series of beautiful photographs—some from drawings made carefully to scale, some from the actual preparations. Through the kindness of Dr. Mann, I am in a position to show some of his results by means of lantern slides and preparations, for the loan of which I am greatly indebted to him.

[DEMONSTRATION.]

The search for the specific vaccine organism has gone along two paths—search for a bacterium ; search for a protozoon.

First, as regards bacteria ; absolutely fresh lymph from an aseptically opened vesicle on the arm of a child is bacteriologically sterile ; as collected under ordinary circumstances it contains cocci. Calf lymph always seems to contain cocci. Over 50 different kinds—staphylo-, strepto-, in fact every variety—of cocci have been found in vaccine lymph.

The irritating nature of calf lymph is erroneously ascribed

to these cocci. Lymph very rich in cocci was found by the German Lymph Association to exercise in many cases no irritating effect, and to be devoid of vaccine power. Active lymph causing a vigorous local reaction was often found by them to be sterile.

The cause of the process cannot, therefore, lie in the presence of cultivable cocci, and all attempts to establish these as producers of the malady have failed. The latest attempt is Czapslewski and Vanselow's *Micrococcus quadrigeminus*. The same holds good of the bacilli that have from time to time been described as the morbid agents.

Unsuccessful attempts to demonstrate a bacillary etiology were made by Klein, Copeman and Kent in 1892-94.

The latest is Nakanishi's attempt to make out that the "Xerous" bacillus is the cause of the disease.

*Protozoal Ætiology.*—Bacteria having proved impotent, and Chauveau, Braidwood, and Vacher having shown that lymph freed from its corpuscular elements by filtration or diffusion is deprived also of its vaccine powers, nothing remained but protozoa, and the first author who seems to have recognised amœbæ in vaccine lymph is Van der Loeff, in 1887 (*Monatschr. f. prat. Dermat.*, 1887). In small-pox vesicles he found similar organisms. Independently of Van der Loeff, L. Pfeiffer, the head of the Government Vaccine Department at Weimar, and the founder of an influential school of thought tending to give prominence to the protozoa as producers of disease in man and the lower animals, came forward about the same time (*Korrespondenzbl. d. ärtz. Vereins in Thüringen*, 1887, No. 2) with the discovery of a protozoal parasite which passed its early developmental shape in the epidermal epithelium. He describes it as forming spores within a capsule, and classed it with the sporozoa. He gave it the name of *Monocystis epithelialis*. Its spores were, he considered, present in humanised

and calf lymph, but with our existing optical methods were not distinguishable from cocci.

Whatever be thought of Pfeiffer's organism, he deserves the credit for being the first to make the important step of regarding the vaccine process as an epithelial infection.

Guarnieri, in 1892, made the next step in advance by selecting as the seat of his experimental inoculations the front of the rabbit's cornea, and thereby doing away with the vascular and leucocytic phenomena evoked by the irritation of the parasite, and so powerfully tending to obscure the real nature of the phenomena.

Leucocytes take two or three days to reach the centre of the inoculated cornea, and during that period the intimate features of the vaccine process can be watched in their absolute purity. The inoculation is performed with great ease after a little practice. A fine needle dipped in active sterile lymph is entered obliquely near the centre of the cornea, so as to penetrate to a very slight extent between the epithelial cells. On no account is a deep thrust to be made or the anterior chambers to be perforated. When the operation is properly performed there is no alteration whatever in the appearance of the eye for 24 hours. On careful observation the epithelium is then seen to be very slightly thickened, and this thickening extends for 2 or 3 mm. round the point of inoculation. Even at the end of 50 or 60 hours there is no opacity, but the central elevation is seen to be surrounded with small transparent projections.

After the third day, ulceration, with local turbidity, sets in, and may spread to the lamellar part and perforate into the anterior chamber, causing hypopion. If the animal is not killed the process gradually terminates, leaving a central leucoma.

If the rabbit is killed towards the end of the second day, before either leucocytes or cocci have arrived on the scene,

and the cornea excised and fixed in sublimate, each cell of the deeper layer of corneal epithelium at the point of inoculation is seen to contain a small, round body, lying beside the nucleus, and, roughly, about half the size of it. The body lies in a clear area or vacuole in the protoplasm, and not unfrequently depresses and bulges in the nuclear membrane. Guarnieri considered these "bodies" to be genuine parasites belonging to the protozoa. Shavings of the cornea teased out in aqueous humour and examined on the warm stage revealed the "bodies" performing amoeboid movements. The "bodies" stained well with hæmatoxylin, carmine, and the basic anilines. A nucleus could be made out, and, finally, Guarnieri considered that he had discovered two modes in which these bodies reproduce themselves—one by direct division into two parts, the other by segmentation, so as to form a mass something like a blackberry or morula. The actual division into segments and infection of fresh epithelial cells by the young amoebæ were not observed. Pfeiffer recognised in Guarnieri's sporulating form his own *Monocystis epithelialis*, and the parasitic nature of Guarnieri's bodies was fully admitted by many of those who saw his preparations at the International Medical Congress in Rome in 1894.

Amongst English pathologists, Ruffer, Plimmer and Jackson Clarke expressed their full agreement with Guarnieri's contention. Many others, however, who saw the bodies denied the validity of Guarnieri's interpretation of them, and a great deal of experimental work has since been done, the outcome of which I shall endeavour very briefly to lay before the Section. Some\* took the view that irritation of the cornea with various substances, such as croton oil, osmium, or Indian ink, could produce intracellular "bodies" such as Guarnieri described. Salmon, working in the Pasteur Institute, convinced himself, as the result of careful staining, that the supposed parasite

\* Massari and Ferroni, 1893.

was nothing but the hyperchromatic residue of the nucleus of a broken down, wandering leucocyte. Hückel, in 1898, made a most careful and exhaustive study of the inoculated rabbit's cornea by modern histological methods, and came to the conclusion that the parasitic nature of the bodies was not proven. He looked upon them as a specific degeneration of the cytoplasm of the epithelial cells, brought about by the presence of the as yet undemonstrated parasite. Gustav Mann, whilst unable to satisfy himself as to their origin, is also convinced that these bodies are not parasites. On the other hand, L. Pfeiffer repeated Guarnieri's work, and convinced himself that chemical irritants were unable to produce anything resembling the specific "bodies" in the corneal epithelium. Von Sicherer also confirmed Guarnieri in 1898, but a more important contribution was made by Ernst Pfeiffer in 1895 (*Centralblatt für Bakt.*), who saw from the first to the eighth hour after vaccination the bodies lying *between* the cells of the corneal epithelium. From the eighth to the tenth hour he saw very minute ones penetrating into the epithelial cells, approach the nucleus, become surrounded by a somewhat clear area, and even by a sort of radiation in the encircling cytoplasm. He also saw them producing a depression of the nuclear membrane. He made the further important observation that filtered lymph, or lymph that has lost its power, does not, on corneal inoculation, give rise to the formation of "bodies." Finally, he showed that shavings of the infected epithelium from the rabbit's eye, if introduced into the cornea of other animals, produced similar changes, and that, after several such passages from eye to eye, a calf could be successfully vaccinated from the scrapings of such an eye and the lymph used for vaccinating children.

In 1896, v. Wasielewsky, a pupil of L. Pfeiffer, took up the subject energetically, and undertook to solve the following questions :—



1. Can one keep on indefinitely producing the characteristic changes in the rabbit's cornea by serial inoculations? and

2. Does the specific character of the process as tested on calves and children persist along the whole length of such a series of rabbits' corneas?

Both of these questions he answered—as the result of his patient work—in the affirmative.

He kept on inoculating from rabbit cornea to rabbit cornea, always reproducing the specific “bodies.” In one series he re-inoculated every second or third day, in another every fourth or sixth day, always with the same result. He sometimes inoculated both eyes of the animal, using one for the microscopic examination, the other for re-inoculation. Some times he inoculated the same cornea in two places, and used one for each purpose. Sometimes, as a more crucial test, and with the expertness begotten of long practice, he detached the little kernel of swollen epithelium from the front of the cornea with a sterile lancet, placed it on a sterile slide, and divided it in two parts, one of which he used for re-inoculation, whilst he sought for “bodies” in the other. With rabbit corneas of the sixteenth and also of the twenty-fifth generations calves were successfully vaccinated, and, after the termination of the process, were tested with active calf lymph, and found to be immune. The lymph obtained from the calf vaccinated with the cornea of the twenty-fifth generation was used for vaccinating children with typical results.

The corneal epithelium of three rabbits belonging to the forty-sixth generation was mixed up with glycerine, tubed and taken to the vaccine institute, where it was used three hours later for vaccinating seven children, six insertions being made in each case. The result in a week was :—

- 1 child had no vesicle.
- 2 children had 1 vesicle.
- 1 child had 4 vesicles.

2 children had 3 vesicle .

1 child had 6 vesicles.

The vesicles were perfectly typical.

The remainder of the same epithelium was microscopically examined and found to contain the typical "bodies."

When a specific property in this case—that of producing typical vaccinia—can be transmitted through an indefinite number of generations in certain cells, and when these cells are found to differ from normal cells in a certain respect only—viz., the possession of certain "bodies"—one is certainly justified in holding that the presence of these bodies is ætiologically related to the vaccinal activity of the cells in which they are contained.

My own conviction is that the solution of the question as to the causation of small-pox and vaccinia is indissolubly bound up with the interpretation of the "bodies," whether we look upon them with Hückel as merely the effect of the presence of the (invisible) parasite, or with Guarnieri as the actual protozoan parasite, the producer of an "epithelial infection."

We shall not have to wait long—if I may venture on a prediction—for the settlement of this question. I now conclude by showing some photographs of these "bodies" on the screen.

[DEMONSTRATION.]

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DR. A. N. MONTGOMERY held that we never had a vaccine pustule—the pustule was the result of want of cleanliness. Pure lymph gives vesicles only. He would like to hear from Dr. McWeeney if he had ever found these Guarnieri bodies in lymph supplied to the Cow-pock Institution? Could these bodies be cultivated and vaccination be produced from the cultures?

DR. MCWEENEY declared he was at one with Dr. Montgomery in saying that pure lymph never produces a pustule. He had not as yet commenced a systematic search for the bodies.