THE MILROY LECTURES

ON

THE NATURAL HISTORY OF VACCINIA.

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LECTURE II.

MORPHOLOGY AND CHEMISTRY OF VACCINE LYMPH.

FRESH vaccine lymph, taken before full maturity of the vesicle, is a clear, transparent, limpid fluid, almost colourless in man, and slightly straw-coloured when obtained from the calf; this difference in colour depends on the varying quality of the normal pigment present in the blood plasma. Calf vaccine lymph is also somewhat more viscid than human lymph, and does not flow so readily when the vesicle is punctured, for which reason it is necessary to use compression forceps in the ordinary method of collection of calf lymph. Examined microscopically, vaccine lymph contains a certain amount of epithelial debris. Leucocytes are usually present also, the number depending on the stage at which the lymph is taken; few, or even none, are to be found in the contents of the vesicle when first formed, but at or after the period of maturation they may be so numerous as to render the lymph turbid, or even puriform. A few red blood corpuscles may be noticeable, although most observers will hardly agree with the statement made by Dr. Husband to the Royal Commission on Vaccination that this is invariably the case.

In stained specimens particularly, micro-organisms of one or more varieties can readily be demonstrated, the actual numbers being dependent to a certain extent on the care with which the lymph has been collected.

Vaccination lymph, chemically speaking, consists of the plasma of the blood. When freshly obtained, therefore, it is faintly alkaline in reaction; but it tends to become distinctly acid after a time when stored. In addition to the various salts and proteids normally present in the blood plasma, vaccine lymph contains some substance—possibly of the nature of a toxin or ptomaine—which results from the vital activity of the specific organism peculiar to vaccinia. That this is so may be proved either by filtration of the lymph through porcelain or by exposure of it to a temperature of about 50° C. If, as I have shown, such filtered or sterilised lymph be inoculated on the skin in the usual manner, no obvious effect is produced at the point of inoculation; but it will be found that the animal has been rendered temporarily immune to the effects of subsequent vaccination with lymph of normal potency.

BACTERIOLOGY.

As long ago as the year 1809 Sacco placed on record the fact that in vaccine lymph there might be found certain granules, generally collected together into masses and possessed of the power of automatic movement. The observation appears to have stood alone for more than half a century; but in 1863 Dr. Lionel Beale gave an impetus to the investigation of the morphological constituents of vaccine lymph by his description of the existence in it of transparent or hyaline particles of extreme minuteness. Dr. Beale expressed the opinion that these granules constituted the contagious principle of the disease, but he does not appear to have obtained experimentally any facts in support of his belief. Hallier and Zurn (1867) and Keber (1868) called attention to the presence in vaccine lymph of numerous micrococci, or "grains," as they were termed by the latter observer, who also evidently regarded the bodies found by him as the carriers, if not the actual generators, of the virulent principle.

It is, however, to M. Chauveau (1868) and Professor Burdon Sanderson that we owe the first real step towards the elucidation of the micro-pathology of vaccinia, these observers having demonstrated that vaccine lymph, when freed from

its contained particles and inoculated on a living animal, no longer causes vaccinia, while, on the other hand, the precipitate or deposit, separated from it in one way or another, when employed in similar fashion, remains capable of producing the disease.

Chauveau found that vaccine lymph taken at the stage of maturation of the vesicles contained minute granular bodies, to which he gave the name of *corpuscles noirs*; and not infrequently also much larger bodies resembling the leucocytes of pus. He further found that if the lymph was diluted ten times, and allowed to stand, these leucocytes when present gradually subsided to the bottom of the containing vessel, and could then be removed. When this was done, and the material employed for an experimental vaccination, a local result was still obtained, thus showing that the active principle was contained, not in these leucocytes, but either in the granules or in the fluid portion of the lymph.

In order to determine this point, Chauveau devised a method of diffusion by which he was eventually able to separate the granules from the fluid portion of the lymph. The layer which contained the "granules" in suspension, was found to be active as vaccine, normal vesicles being obtained as the result of its inoculation.

Professor Burdon Sanderson shortly afterwards repeated Chauveau's work, employing certain improvements in the method which seemed likely to render it more accurate, and thus to afford results of greater scientific value. He also made use of another method of his own, consisting in the filtration under pressure of the liquid to be tested through a layer of unglazed porcelain, by which means all solid particles were effectually separated from the fluid containing them. Professor Sanderson fully confirmed the results announced by the French investigator. Further, Chauveau, by largely diluting vaccine lymph with distilled water, and employing the mixture for vaccination, found that the difference between the effect of inoculating extremely diluted lymph and that of inoculating the lymph itself without any addition of water, does not manifest itself, as might be expected, in the size of the individual vesicle or vesicles produced. He found, on the other hand, that the difference consisted entirely in the numerical relation of successes to failure. If successful, inoculation with a liquid containing an extremely small proportion of lymph may communicate the disease as completely as the original lymph itself, a result which is not only perfectly consistent with the fact that contagium is particulate, but which is in itself a necessary consequence of its being so. As bearing on this question, it may be mentioned that Schultz, of Berlin, has quite recently been engaged on an investigation having for its object the determination of the extent to which, in order to save time and expense, glycerinated vaccine lymph could be diluted without losing its efficiency as vaccine. In the course of his experiments, he found that an emulsion containing 1 part only of vaccine pulp in 2,000 parts of glycerine and water was capable of producing locally a normal vesicle in a small proportion of the cases in which it was employed for vaccination.

In 1872 Cohn of Breslau treated the morphological aspects of the subject with much completeness. His observations, which related to both vaccine lymph and variolous lymph, received general corroboration from all subsequent workers. He, however, apparently believed certain micro-organisms found by him to be of one species only, to which accordingly he gave the name *micrococcus vaccinæ* or *variolæ*, as the case might be; whereas later observers have shown that organisms of more than one species are usually to be found in any given specimen of crude lymph.

Cohn called attention to the fact that in perfectly fresh vaccine lymph the "corpuscles" for the most part occur singly, but that others are joined together in pairs in a form resembling the figure 8; and he states that after the lymph has been kept for a time, the number of the "double cells" increases, and soon chains of four begin to be distinguishable. These chains are usually curved or in zigzags; their attachment one to another is evidently very slight, as they can readily be displaced. After a few hours' observation they are seen to be all aggregated into irregular colonies or clumps, each consisting of sixteen, thirty-two, or more corpuscles. He also noted a point of importance in connection with the opacity which is apt to occur in stored lymph—namely, that

[1950]

"in capillary glass tubes the enlargement of colonies sometimes lasts a long time, so that groups of them acquire considerable size, and present themselves as flocculi."

Klebs (1873) isolated from vaccine lymph micrococci united together in the form of tetrads, which he considered as specific, and to which he gave the name of *tetracoccus vaccinæ*. Cornil and Babes (1873) also met with micrococci.

Koch (1882) found micrococci in the vaccine vesicles of a child, and noted that human lymph freshly prepared with glycerine contained what he appears to have regarded as the specific bacteria of vaccine. These developed but slowly even in the incubator, and were met with in fewer numbers in old lymph, even though it still remained active.

Quist (1883) published a series of experiments dealing with the possibility of cultivating outside the animal body the micro-organisms present in vaccine lymph. The culture fluid employed by him was composed of equal parts of blood serum, glycerine, and distilled water, this mixture being rendered alkaline by the addition of $\frac{1}{1000}$ part of carbonate of potash. After sterilising this fluid by exposing it to a temperature of 60° C. for an hour and a half on three successive occasions, it was inoculated with a minute piece of sterilised sponge soaked in clear lymph, or with a piece of vaccine "crust" which had been washed in distilled water and then carefully dried. He found that growth eventually occurred both on and below the surface, the former consisting of minute scales, while the latter gradually settled to the bottom of the vessel as a fine sediment. The scales, forming a scum on the surface of the fluid, he found to be composed of swarms of micrococci, which when inoculated on the skin of calves in some cases gave rise in his opinion to a typical eruption of vaccinia. Although Quist did not attain any great measure of success in the immediate object of his inquiry (the identification of the vaccine organism), still he showed that the specific contagium of vaccinia could exist, for a time at any rate, in a fluid containing a considerable proportion of glycerine. Voigt isolated three species of bacteria by making gelatine plate cultures of vaccine lymph. Of these, one, which alone was constantly to be found, he considered to be specific, and consequently termed it the *vaccinococcus*.

In 1886 Meguin called attention to the fact that the number of micrococci contained in vaccine lymph increased considerably from the second day onwards after the removal of the lymph. During the same year (1886) Dr. Buist succeeded in isolating from vaccine lymph three varieties of micrococci which, when grown on nutrient media, gave rise to colonies of a white, yellow, or orange colour. From specimens of variolous lymph he succeeded in obtaining one organism only, the colour of which was white when grown on solid media. With none of these cultures did he obtain any definite result on the inoculation of calves, monkeys, or human beings.

A certain small measure of success appears to have attended inoculation experiments in the hands of Carmichael (1887), who does not, however, give any detailed description of the morphological characteristics of the growths which he obtained, and which, by the way, although differing from one another in colour, he seems to have considered were the product of one species of micrococcus only. As was doubtless the case in Quist's experiments, in the few in which true vaccine vesicles resulted at the site of his inoculation, success was in all probability due to the presence in the material employed of a small proportion of the lymph originally used for the seeding of his culture medium.

Pfeiffer (1887) recorded the existence in vaccine lymph of micro-organisms of several different forms, including a yeast (named by him *saccharomyces vaccinæ*), two *sarcinæ* (*lutea* and *aurantiaca*), a short bacillus allied to *proteus vulgaris*, but differing from it in not being pathogenic, and certain micrococci, of which one, the *staphylococcus cereus albus* (Passet), was of constant occurrence. Pfeiffer, however, believes that the specific agent of variola and vaccinia is not to be sought for among the bacteria, but rather belongs to the class of sporozoa. This subject will require further notice later on.

Professor Leoni (1890) met with several pyogenic microbes in fresh vaccine lymph, notably the *staphylococcus pyogenes albus*, which was invariably present.

As the result of my own work (1891) it would appear that there are at least three species of micro-organisms—namely,

staphylococcus albus (epidermidis), *S. pyogenes aureus*, and *S. cereus flavus*, corresponding probably to Buist's white, orange, and yellow vaccine respectively, one or more of which are almost universally to be found in every specimen of human or calf vaccine lymph examined. Of these the *S. albus* is usually to be found in the upper layers of healthy skin of unvaccinated persons. In addition I have been able to satisfy myself as to the occasional presence of the *streptococcus pyogenes*. I myself have never detected the *streptococcus* of erysipelas, but Dr. Klein has placed on record the fact that in one instance he isolated this microbe from a specimen of human vaccine lymph, the use of a portion of which was believed to have given rise to the occurrence of the disease.

Professor Crookshank (1891) succeeded in isolating from various specimens of vaccine lymph, by the method of plate cultivation, an immense number of bacteria, including micrococci, bacilli, torulæ, etc. All of these he recognises as well-known saprophytic bacterial forms associated, some of them, with processes of suppuration, but none of which, he says, can be regarded as the contagium of vaccinia, seeing that no single one of them is constantly present in vaccine lymph, human or bovine.

M. Besser (1893) found in the papules of a case of small-pox at the fifth day a small bacillus which he is disposed to look upon as the specific organism of variola. He obtained cultures on various nutrient media, coverslip preparations from which always showed the organisms arranged in palisade-like rows.

The late Professor Straus (1893) found that the seeding of gelatine plates with fresh vaccine material, whether glycerinated or not, gave rise to the appearance of numerous colonies of micrococci, among which the *staphylococcus pyogenes aureus* and *albus* were most frequently met with. When glycerinated lymph which had been kept for a considerable period was employed in similar fashion, the number of colonies was greatly diminished.

M. Anthony (1893), in the course of the examination of a number of different specimens of vaccine lymph, glycerinated or otherwise, finds that four different kinds of micrococci and three species of bacilli are apt to be present. Of these, what he terms from its appearance the porcelain micrococcus is invariably present in fresh vaccine. M. Anthony concludes that vaccine lymph cannot be regarded as above suspicion as long as it is found to contain any microbes other than the porcelain micrococcus. The inoculation of cultures of his porcelain bacillus on heifers afforded only negative results.

Dr. Klein agrees with me in maintaining that none of those microbes which ordinarily can be cultivated from vaccine lymph are "an essential inhabitant in vaccine," or "can have anything to do with its active principle." In the report of the Medical Officer to the Local Government Board for 1892-93 he, however, described a peculiar extremely minute bacillus as occurring in calf lymph, and in variolous lymph of human origin, obtained at an early phase of the disorder, in calf lymph 72 to 96 hours after vaccination, in human variola during the third or fourth day. In both instances the lymph was collected aseptically. Only clear lymph as free as possible from epidermal debris was used for film specimens, which, after heating and treatment with 30 per cent. acetic acid for some minutes, was subjected to prolonged staining in alcoholic gentian violet. Some of the films of calf lymph (collected after removal of the epidermis as a whole) showed an abundance of these minute bacilli, generally massed together, some of the specimens looking indeed like film specimens of an artificial culture. Lymph of early human small-pox vesicles showed the same bacilli, but not so abundantly. Calf lymph of later stages, five or six days old, showed no bacilli, or only a few here and there. In certain of these bacilli Dr. Klein succeeded in demonstrating spore-like bodies which, together with the absence of the bacilli in the lymph of later stages, led him to the conclusion that disappearance of these bacilli from late lymph was possibly the result of their having broken up after the formation of spores, which might alone be present about the time of maturation of the vesicle. Dr. Klein adds that these bacilli are incapable of cultivation in the ordinary culture media, no growth whatever having been obtained by him on solidified blood serum, glycerine agar, ordinary agar, sugar gela-

tine, or ordinary gelatine, although in specimens of the same lymph as was employed for this purpose, and which on transference to the calf produced perfect vaccine vesicles, bacilli were found in abundance.

Dr. Buttersack (1894) described the appearance in vaccine lymph, dried on cover glasses, of a dense network of threads associated with numerous granules which he considered as peculiar to vaccine. These threads he regarded as the growing vegetative part of a vaccine fungus, the granules, in his opinion, representing spores. It appears fairly certain, however, that what he believed to be a mycelium with spores in reality consisted merely of threads and granules of fibrin or of some other albuminous substance.

In this same year (1894) I published a description of numerous minute bacilli sometimes to be found in considerable numbers in the vaccine vesicles both of the calf and human being at an early stage of their development, in this respect apparently confirming the experience of Klein, which was published in the same year and of which mention has already been made. Similar bacilli were also demonstrated by Mr. Kent and myself in sections of skin passing through the site of a vaccine vesicle obtained from the calf.

An account of certain experiments dealing with the possibility of cultivating these micro-organisms, and also a similar one isolated from variolous material, I propose deferring for the present.

Landmann (1895) found that in Germany no less than 80 per cent. of certain children vaccinated successfully presented an excessive inflammatory condition of the arm, sometimes of an erysipelatous or hæmorrhagic character. In searching for the cause of these unfortunately numerous complications Landmann undertook an elaborate study of the lymph distributed from thirteen of the German institutes. As a result of his research he found that the number of germs present in the various specimens of lymph varied from 50 to no less a number than 2,500,000 per c.cm. Among the microbes which he succeeded in isolating were the streptococcus pyogenes, the staphylococcus albus, and staphylococcus aureus.

Two American observers, Dr. Stephen C. Martin and Professor Ernst, recorded (in 1895) the isolation of a micro-organism which they considered to be specific to vaccinia. This was a short thin bacillus, which they found was capable of growth on serum obtained from ox or horse's blood, but not on other media. The growth, which is of a white colour, forms chains, and under certain circumstances, according to these observers, takes on the form of a micrococcus. With a cultivation of this microbe of the fourteenth generation they produced a vaccine vesicle on an infant in 1 attempt out of 10, better success, however, attending their attempts at inoculation of a calf.

M. Le Dantec (1895) found staphylococci in lymph, the reactions of which, he says, shows that they are related to the ordinary pus cocci. These staphylococci differed according to the species of animal from which they were obtained; those isolated from calf lymph liquefying serum, while those obtained from human lymph did not do so. M. Arloing (1896) concluded that the virulent agent of vaccinia must be of the nature of a soluble toxin.

M. Paul (1896) published a voluminous account of his researches on the occurrence and significance of pathogenic micro-organisms associated with vaccine lymph. He lays stress on his observation that the staphylococcus pyogenes aureus is by far the most ubiquitous of the microbes that are found in vaccine lymph, although it is certainly not an essential concomitant. He finds, moreover, that it exhibits both variable resistance and variable virulence. A sample of lymph, for instance, of supposed Jennerian stock, he found to contain this staphylococcus in a phase of much higher virulence and resistance than was a specimen of this same microbe obtained from a sample of calf vaccine lymph. This staphylococcus, he adds may be present without causing suppuration; but the use of a lymph containing it in large quantities should naturally be avoided. According to this observer the number of bacteria present in fresh lymph appears to be independent both of the origin of the stock from which it is derived and of the method of vaccination employed. This, as shown elsewhere, does not accord with my experience.

Quite recently (1897), in conjunction with Dr. Blaxall, I have carried out a further extensive series of investigations on the bacterial flora of calf vaccine, with the outcome that, as the results of former work had led us to anticipate, and as has been repeatedly shown by other observers, calf lymph (or rather vesicle pulp) may contain a large number of micro-organisms which are in no way concerned with its specific activity. Human lymph, as a rule, we find to contain remarkably few microbes, but conceivably this might not be the case were the whole substance of the vesicle removed as is now usual in collecting from the calf. We have shown also, as have other investigators, that lymph, if carelessly collected, may be found to be contaminated with many of the numerous saprophytes common to dust, etc., in addition to containing certain possibly pathogenic microbes.

On the other hand, lymph taken directly from calves vaccinated with vaccine material rendered free from extraneous organisms, as, for instance, glycerinated lymph a month after emulsification, often presents remarkably few colonies of "extraneous" organisms. In one such sample only four colonies appeared on a plate culture of the lymph—one of staphylococcus cereus albus, and three of a light brown yeast.

The fact that in vaccine lymph or in the vesicle pulp micro-organisms are found which, under certain circumstances, are known to be provocative of suppuration, does not in any way imply that the lymph in which they are present is purulent, or that if such infected lymph were employed for vaccination in the human being suppuration would necessarily ensue.

The recent Berlin Commission, indeed, report that of 18 samples of lymph examined, only 5 contained staphylococci which were pathogenic for small animals, and, further, that no harm resulted on the vaccination of children with these particular specimens of lymph. From this circumstance it is evident that the virulence of these micro-organisms for rodents, for instance, cannot be accepted as a measure of their power to affect injuriously the body tissues in the healthy human subject. The streptococcus of pus must be a rare inhabitant of calf lymph, the Berlin Commissioners, indeed, stating that they never once met with it in the course of the examination of 64 samples of lymph. In human lymph it is by no means of so infrequent occurrence.

With reference to the question of the possible presence of the tubercle bacillus in lymph, I am not aware of any authentic record of its having been found even in lymph derived from the calf. But, as I shall presently have occasion to show, we are enabled, by the use of a special method of preparing and testing vaccine lymph, to obviate any possibility of danger to which the presence of the tubercle bacillus or other pathogenic microbes in the original specimen of lymph might conceivably give rise.

In 1894 Dr. Klein and I, as previously mentioned, showed concurrently, though independently, that in specially stained specimens of vaccine lymph taken at a period antecedent to full maturity of the vesicles, minute bacilli can be demonstrated, often in considerable numbers, and in practically pure culture. These bacilli cannot be found, or only with difficulty, in mature lymph, for the reason probably that they have by then given place to spores.

For some time it appeared impossible to obtain further evidence as to the rôle of these bacilli, as they altogether refused to grow on any of the ordinary culture media, and under either aerobic or anaerobic conditions, though this very fact obviously tends to show that they are not of a merely saprophytic nature. In the following year, however, the results of certain experiments appeared to prove that it is possible to obtain a growth of these bacilli in a particular culture medium, and even to carry on such growth in secondary cultivation.

As it seemed to me, the desideratum for the purpose of inducing growth of the supposititious micro-organisms was a medium which had not been artificially sterilised, which *a priori* should afford the necessary pabulum, and which at the same time, whether it was to be considered aerobic or anaerobic, was secure in its circumstances against contamination. Hence the choice appeared to lie between the intraperitoneal fluids of one or another living animal and the hen's egg, which has already been made use of as an alternative culture medium.

As regards intraperitoneal fluid I foresaw difficulty, in that it is apt to contain leucocytes, which might easily vitiate experiment, and accordingly I adopted the hen's egg as a matter of first instance. I had no experience to guide me as to the best method of carrying out the inoculation of the egg, or as to the period of incubation best suited for the object I had in view, and my attempts for a time, therefore, met with but small success. My inoculations were carried out by first cleansing the surface of the egg with corrosive sublimate solution and afterwards with alcohol, and then breaking a small hole in the shell by means of a sterilised needle and forceps. Through the opening thus made I mixed the yolk and albumen of the egg by means of a platinum needle, and then carried out the inoculation by means of a glass capillary pipette, which had recently been drawn out in the blow-pipe flame. For the purpose of such inoculations I employed variegated crusts obtained from the small-pox ships of the Metropolitan Asylums Board. These crusts were rubbed up in a small glass mortar with a minimal quantity of water or sterilised normal saline solution. Of the resulting emulsion a drop or two was then taken up into the glass pipette and the inoculation carried out. Finally, the small hole in the egg was closed up, either by means of a small pledget of sterilised cotton wool soaked in collodion or with melted sealing wax. The eggs inoculated in this manner were then placed on beds of cotton wool, and kept in the incubator at a temperature of 37° C. Here they were left for varying periods of time, a month being the period which was eventually found to be that which was most desirable.

On opening an egg thus treated I found that in its interior was contained a creamy material, which in great part had replaced the ordinary egg contents. This material examined in cover-glass specimens appeared to contain a pure culture of one organism only, namely, a bacillus which morphologically was not to be distinguished from the bacillus which I have previously described as to be found in early vaccine lymph.

My next step was to attempt to grow it in subculture, and to make observation also of the effect of its direct transference by cutaneous inoculation into the calf. As regards attempt at subculture in all media most usually employed for such purpose, including gelatine, sugar gelatine, agar, glycerine agar, sugar agar, and serum, these at this time failed, as subculture of the bacilli contained in vaccine lymph had always failed, when the ordinary culture media are adopted, but with the calf it was different, and as the result of inoculation of egg cultures on this animal I obtained a strain of lymph which, after being passed through a series of calves, was successfully employed for the vaccination of children. The calves were all subsequently vaccinated, with negative results.

More recently, Dr. Blaxall and I have been further investigating the possibility of the cultivation in artificial media of the organism specific to small-pox and vaccinia respectively. Our work received considerable impetus by the discovery that certain culture tubes which had been inoculated with vaccine lymph, but which appeared to remain completely sterile, nevertheless contained a growth of a microbe indistinguishable morphologically from that which I found in egg cultures, and which although invisible to the naked eye, became obvious when cover glass impressions were taken from the surface of the nutrient medium, and afterwards stained and examined with the microscope. Dr. Blaxall subsequently devised a method by means of which pure cultivations of apparently the same organism were obtained on the surface of agar plates, and this not only when vaccine lymph, both of human and bovine origin, was employed, but from variegated lymph as well. From these plates agar tubes and tubes of peptone beef broth were also in turn inoculated. In these also, in the first removes, practically no growth was visible except on careful examination by reflected light, but as generations further removed from the original stock were obtained the growth became more obvious, showing as a haziness in the fluid media, and as a semitranslucent greyish-white line on a sloping surface of agar.

Broth cultures of the ninth and succeeding generations, when employed for the inoculation of calves, afforded in our first series of experiments results which we regarded as most encouraging, seeing that we were enabled to carry on the infection to other calves, and subsequently to children, in all

of which it took the form of apparently typical vaccinia. Some weeks after, the calves were vaccinated with active calf lymph, but without result. A later series of experiments, however, were not attended with success. The reasons for so great a divergence in our results at one and another time are by no means obvious, unless the fact of increased vegetative function shown in the later subcultures of the microbe may have had as a concomitant a lessened degree of pathogenicity. On this point we are at present unable to make any definite statement, but needless to say the investigation is being continued.

Another direction in which our research work has been extended was suggested to us by certain statements in the report of the Berlin Scientific Commission. In that report an account is given of certain experimental inoculations performed by Freyer with the juice of the spleen, liver, inguinal and mesenteric glands, and marrow of previously vaccinated calves. Using such material, vesicles were produced by the employment of almost every organ, which shows in Freyer's opinion that the vaccine germ must be conveyed to them by the circulating blood. This general distribution of that micro-organism takes place, he states, up to three or four weeks after vaccination, but no mention is made by him as to the earliest period at which it was found to occur. The greatest amount of success was obtained by the use of inguinal gland and spleen juice. It is further stated that since calves were successfully vaccinated with this material the postulate of an absolutely bacteria-free efficacious lymph was complied with.

It is of course obvious that where generalised vaccinia occurs as the result of a local vaccination, as, according to Chauveau, is always the case with solipeds, the micro-organism concerned must have been conveyed to a distance from the original point of insertion by means of the circulation. Presumably this may also take place, although perhaps to a more limited extent, in that form of the disease where, as in the case of the vaccination of the human being or the calf, the resulting disorder remains to all appearance strictly localised, a secondary eruption being prevented by the spleen or other organ, such as the liver, being enabled to seize upon and ultimately destroy any of the specific germs that happen to be carried thither.

It seemed to me that if Freyer's results were capable of confirmation, the possibility of vaccinating by means of spleen juice, for instance, must depend on the employment of such material at a period sufficiently late to ensure the active principle having reached the particular organ, and antecedent to that at which it would have been destroyed. I thought, also, that if the most favourable period could be determined, it would be possible then to obtain a material free from bacterial growth other than that on which its potency for vaccination depended—a material, therefore, which would afford exceptionally favourable opportunity for the isolation of the organism specific to vaccinia.

But unfortunately, notwithstanding the categorical statements in the report of the German Commission, we have been unable, as the results of numerous experiments, to obtain similar results, *quâ* vaccination, to those described by Freyer. Neither have we been able to demonstrate the presence of any micro-organism in culture media inoculated from the fresh spleen, liver, or glands obtained at various dates after vaccination or in the interior of fresh or hardened sections of these same organs. We have therefore temporarily abandoned this portion of our inquiry pending the receipt from Freyer of more detailed instructions.

PROTOZOA.

Renault (1881) first called attention to the fact that peculiar bodies which he regarded as parasitic could be demonstrated in the epithelial cells of the vesicles in vaccinia and variola. Van der Loeff, on examining in the warm chamber a hanging drop of clear calf vaccine lymph, discovered "numerous small bodies capable of independent movement." In 1887 L. Pfeiffer described the occurrence in lymph, obtained from cases of vaccinia and variola, of small, unicellular, generally spherical bodies, which he believed to constitute the specific contagium of these diseases. These parasites he found in still greater numbers in the Malpighian layer of the skin, multiplication taking place by division, and also by endo-

genous formation of spores. These statements were in part confirmed immediately afterwards by Rieck. Guarnieri having invariably found, in the epithelial cells of the skin in the vesicular stage of small-pox, small particles half as large as the cell nucleus or even smaller, next attempted (in 1892) the cultivation of similar bodies in the living animal. For this purpose he inoculated the cornea of rabbits and guinea-pigs with vaccine lymph, with the result that these protozoa, as he considered them, appeared in the cells of the epithelium covering the cornea. After an interval of about fifty hours from the time of inoculation the surface of the cornea was scraped and the material examined in a hanging drop of aqueous humour. Guarnieri also employed for examination sections taken through the site of inoculation and stained in various ways. In either case he found numerous parasites within the epithelial cells of the affected area, generally occupying a zone of the protoplasm immediately surrounding the nucleus, but not intruding upon it. In the hanging drop they appeared as little refractile bodies endowed with slight amoeboid power. Examination of sections further showed that they vary much in shape and size, and, further, that they were most numerous in the deep layers of the epithelium, the superficial cells not being involved. To this "parasite" Guarnieri gave the name of *cytocytes vaccinae*. Monti obtained similar results, and both Guarnieri and Monti exhibited, at the International Congress of Medicine at Rome, preparations in which they demonstrated the presence of the parasite.

Ruffer and Plimmer, who in this country had been working at the subject, also attended the Congress, and were thus able to compare their own specimens with those shown by Guarnieri and Monti, thereby establishing, according to Dr. Ruffer, the fact that the protozoa described by all of them were identical in character. Ruffer and Plimmer (1894) described the alleged parasite as a small, round body, which, when treated with appropriate reagents, appears to have a more darkly staining centre. It is, they say, about four times the size of an ordinary staphylococcus, and generally lies in a clear vacuole in the protoplasm of the epithelial cells of the stratum Malpighii, and occasionally indents the nucleus, although they have not found it enclosed in the latter body. These observers state that they have found the same organism in sections of skin from small-pox patients and in small-pox pustules of the larynx and the trachea.

In the same year (1894) L. Pfeiffer not only confirmed the results obtained by Guarnieri, but announced also the discovery in the blood of persons suffering from small-pox and in the blood of children and calves that had been vaccinated, of amoeboid cells, which he regarded as parasites. These he found to be most numerous during the pyrexial stage of the disorder, whether variola or vaccinia. These bodies, which are somewhat larger in the blood of the calf than in that of the child, being in the latter about a quarter the size of an average blood corpuscle, are, Pfeiffer states, provided with pseudopodia and are occasionally flagellated. According to this observer they also contain from one to four nuclei, which are readily stained. They occur free in the blood, not being contained in cells as is the case in the vaccinated cornea; nevertheless, Pfeiffer considers that they represent a similar stage in the life-history of the organism, of which the more mature form is of a cystic nature, to which he gives the name "*monocystis epithelialis*." This cyst he believes to give rise, in due course, to a number of spores. It should be mentioned that he describes similar bodies as occurring in various other vesicular eruptions of man and the lower animals.

Ogata (1895), as the result of careful microscopic examination of stained film preparations of vaccine lymph derived from the human being and the calf, and also of human varicellous lymph, concludes that certain protozoa which he has been able to demonstrate in each case should be classified with the gregarinidae. Further confirmation of Guarnieri's experiments was afforded in 1895 by von Sicherer, Ernst Pfeiffer, and Mr. Jackson Clarke. According to E. Pfeiffer the bodies vary considerably both in size and shape, being sometimes as large as a red blood corpuscle. They resemble the corpuscles also in their affinity for certain staining reagents.

There can, of course, be no doubt as to the occurrence of most of the appearances described, although we may not be

prepared to accept the interpretation put upon them; and in this connection it is well to bear in mind not only that the bodies enclosed in cells, described by Guarnieri, Monti, and Ruffer, are said by them to differ essentially in their staining reaction, and in their appearance from those of the Pfeiffers, van der Loeff, and others, but also that all the more recent experimental work in this direction has been carried out on the cornea of the rabbit and the guinea-pig, both of them animals which there is reason to believe are insusceptible to vaccinia. It is therefore, I think, conceivable that the "parasites" in question may represent merely the result of epithelial irritation caused by the scarification, together with that (of a non-specific nature, however) set up by the vaccine lymph employed.

That such is indeed the case there is now a considerable amount of evidence to show. Thus Ferroni and Massari (1893) expressed the belief that Guarnieri's so-called parasites are in reality derived from the nuclei of the cells, or are due to the immigration of leucocytes. They base their opinion on the results of certain experiments devised by them in which they inoculated the cornea of rabbits and guinea-pigs with croton oil and Indian ink. On subsequent examination of stained sections they were able to demonstrate the presence in the epithelial cells of numbers of small bodies apparently identical with those described by Guarnieri. It should, however, be stated that E. Pfeiffer failed to find them under similar circumstances, although they were invariably present after the inoculation with vaccine lymph.

In the *Annales de l'Institut Pasteur* for April, 1897, appeared a most important paper by M. Paul Salmon. This investigator brings to bear on the subject a thorough knowledge of modern staining reactions, by the light of which he shows that Guarnieri's parasites, while differing in their affinity for certain stains from either the protoplasm, nucleus, or nucleolus of the epithelial cell, correspond in all respects, as judged by such tests, to the nuclei of the migratory leucocytes, the chromatin masses of which rapidly break down in the substance of the invaded cells. The paper is illustrated with drawings showing all stages of the process of invasion of the corneal epithelium by the leucocytes, from that of their first appearance in the underlying tissue to that of their final dissolution, represented by the presence of minute granules of chromatin only, in the protoplasm of the epithelial cells. No one, I think, comparing this plate with the drawings and descriptions of the corneal "parasites" of Guarnieri and others, can fail to be struck with the truth and completeness of Salmon's explanation of the facts previously observed.

Recent researches have tended to confirm the statements of L. Pfeiffer as to the presence also in the blood of variola and vaccinia, especially during the pyrexial stage, of minute, free, granular amoeboid cells. It is, however, doubtful whether it is possible to demonstrate a nucleus. These cells, as already mentioned, are regarded by Pfeiffer as the causative agent of these diseases. This question has been investigated by Dr. Walter Reed (1897), whose attention was directed in the first instance to the microscopical examination of fresh preparations of the blood of vaccinated monkeys and calves, and subsequently to that of vaccinated children and small-pox patients. Of 11 vaccinated animals (9 monkeys and 2 calves) whose blood was examined, Reed found in the blood of 7 (6 monkeys and 1 calf) "granular amoeboid bodies, having a diameter about one-third that of a red blood cell" during the active stage of vaccinia, which on the decline of the local inflammation again disappeared. As showing, however, that these bodies have no causative relationship to vaccinia is the fact that Reed occasionally met with quite similar bodies in the normal blood of healthy children and of monkeys.

As the outcome of the most recent investigations dealing with the question under discussion, it must, I think, be admitted, that although Guarnieri and others, who have described the presence of protozoa in the blood and tissues of man and the lower animals, during certain stages of invasion by variola or vaccinia, are undoubtedly correct in their statements as to the occurrence of the particular bodies described by them, they are altogether mistaken as to the true nature and affinities of these bodies. There would, indeed, appear to be good reason for the belief that these so-called parasites are in fact nothing more nor less than the fragments of

amœboid leucocytes, which had originally invaded the peripheral circulation, and in turn the particular tissue affected, under the influence of chemotaxis.

VARIOLA AND VACCINIA IN THE MONKEY.

Professor Michael Foster suggested to me some years ago that in view of possible deterioration of lymph stocks, after employment through a long series of generations, it would be well, if possible, to devise some practical means of investigating the efficiency of the protection afforded by some of those at present in use against variolous infection.

The inoculation of small-pox on the human subject being now a penal offence, I turned my attention to the monkey tribe, on account of their similarity in many respects to man, although assured on high authority that they were not susceptible to either vaccinia or variola. On putting the matter to the test, however, I found that the inoculation of vaccine and variolous lymph each gave, in my hands, successful results in every instance in which I have tried it on the monkey. Briefly described, the *modus operandi* has been as follows: The upper arm, or occasionally the inner surface of the thigh, is shaved on both sides of the body; the skin is then washed with a solution of some disinfectant, such as corrosive sublimate or carbolic acid, and afterwards with alcohol. Each arm (or thigh) is then scarified in three or four places, and the lymph well rubbed in.

In the first series of experiments I used ether as an anæsthetic, but I afterwards found this quite unnecessary, as the monkey appears by no means to dislike the scratching process, becoming absolutely quiet as soon as the operation is commenced, and remaining with limbs limp and eyes half closed, as if hypnotised, until its termination, when it at once becomes again as lively as ever.

In the case alike of variola and of vaccinia the local result of inoculation attains its acme (*quâ* vesiculation) in the monkey, as in the human being, about the eighth day. The first signs of reaction appear usually on the third day, by which time, if variolous lymph has been used, there is a distinct, though very thin, crust over the site of inoculation. In the case of vaccinia the appearance of crust at this date is less marked. By the fifth day vesiculation in both cases has generally commenced, this becoming more obvious up to the eighth day, though even then, it is much less marked in variolous cases than in those which have been vaccinated, the difference being recognised with the greatest ease. Later the vesicle gives place in either instance to a pustule, by which time there is not infrequently considerable swelling of the skin, and subcutaneous tissue, and of the nearest lymphatic glands; the pustule gradually dries up and a scab is formed, which is more pronounced in the case of vaccination than variolation, and which falls off some time during the third week, if the monkey has not picked it off before. The chief difference noted between the effects resulting from the local inoculation of these two diseases is that in the case of variola there is more or less of a crust from the first; that vesiculation is less marked than in vaccination; that about the ninth to the eleventh day a general eruption may appear, which in some instances, as in a monkey which at the request of Professor Foster was inoculated at Cambridge, covers the whole surface of the body; and that the final scab at the site of inoculation is not so elevated in the variolated as in the vaccinated animal.

In both cases there is usually a rise of body temperature, which is more marked and longer sustained in variola than in vaccinia. After variolation it was noticed in several cases that the monkey suffered from diarrhoea, that its eyes were suffused, and that it was not as active as usual. A peculiar odour was also noticed, quite distinct from the well-known smell of "monkey." In no instance had the experiment a fatal termination.

Having thus proved to my own satisfaction that monkeys are susceptible not only to vaccination but also to small-pox, I next determined to make trial as to the protection against small-pox afforded in the monkey by previous vaccination, and the protection against vaccination afforded it by variolation, and I went on to compare the effect produced by the use of human and of calf vaccine respectively. For this purpose humanised lymph was obtained from Birmingham, where, to the best of my belief,

the same strain has been continuously carried on by means of arm-to-arm vaccination for the past thirty-eight years. The calf lymph used was obtained from the Government Animal Vaccination Station in Lamb's Conduit Street, while small-pox lymph was supplied to me from the Hospital Ships, from Warrington, and from Manchester.

In no one of numerous experiments performed on these lines at intervals of one to five months after the primary inoculation, whether vaccinal or variolous, did anything in the nature of a successful result follow the first or subsequent reinoculations.

From these experiments it would appear that the protective power of lymph obtained from these three different sources, when inoculated on the monkey, is practically identical in all respects.

THE EVOLUTION OF THE DUM-DUM BULLET.

BY SURGEON-MAJOR-GENERAL J. B. HAMILTON. A.M.S.

THERE seems to be so much misunderstanding on the subject of "expanding bullets" as distinguished from "explosive bullets," that perhaps a short description of the new so-called "dum-dum" bullet will be interesting to the profession. I propose briefly to review what I may call the evolution of the present projectile, and show the various steps that led to its adoption.

Everyone is familiar with the old spherical musket bullet, the projectile that prevailed from the invention of gunpowder up to the period of the Crimean war. With it Marlborough, Napoleon, and Wellington fought their battles, and its only variation was the "belted bullet" used by rifle regiments, which was merely the ordinary spherical bullet, with a zone or belt raised round one circumference to enable it to fit into the grooves of the rifle.

Shortly before the Crimean war the conical bullet was introduced. It was shaped like a sugarloaf, "and was a distinct advance on the spherical form, as it gave greater range and penetration." The "Minié" was the first outcome of this new invention, followed by the "Enfield," a muzzle-loading rifle of considerable power throwing a conical bullet, solid in front, with a hollow in the base, which caused the lead to expand and take the grooving of the rifle. So far the bullets were solid, and caused much the same class of wound as the old spherical ball.

When the Prussians adopted the needle gun, with which they so conspicuously defeated the Austrians, the English Government was forced into arming its troops with a breech-loader, and adopted the Snider principle of breech action, which had this great advantage, that it was feasible to convert the Enfields in stock into breechloaders. With the introduction of the Snider changes were made in the bullet; a hollow, filled by a plug of wood or clay, was introduced into the front part of the bullet, and the base was also hollowed and plugged, a diaphragm of solid lead being left in the centre. This was a most deadly projectile, the plug in front expanding the bullet on impact and causing terrible injuries to soft part and bones. The next step was to form a hollow in the front, over which the lead was "drawn," leaving a space filled with air; then came a solid portion of lead, and finally a hollow in the base plugged with clay. This was a more deadly bullet than the previous one, and its "smashing" powers were so great that it was adopted for sporting purposes. On soft-bodied animals, such as tigers and panthers, its effects were wonderful, the biggest tiger often dropping dead to a single shot when well placed. This form of Snider bullet, though called "expanding," was really explosive, its bursting power being probably due to the air it contained being greatly compressed on striking, acting like a small charge of powder exploded by the impact.

This bullet was far more deadly to man and animals than anything that had before been invented, excepting, of course, "explosive" bullets, which I shall now briefly describe. Speaking generally, there were two forms of "explosive bullets" used by sportsmen (I speak in the past tense, as these bullets are now practically abandoned): one the bullet cast with a cavity in the apex, extending about two-thirds of its length, the other a bullet cast in two parts and "swaged" together in a machine. The first form was very simple, the