THE BACTERIOLOGY OF VACCINIA AND VARIOLA.

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ALTHOUGH it is now just a hundred years since the process of vaccination was first introduced by Jenner as a prophylactic against subsequent invasion of the system by small-pox, it is a somewhat remarkable fact that until quite recently our knowledge of the intimate pathology of both vaccinia and variola has been extremely scanty.

There has been, indeed, no lack of workers in the field; the extreme interest of the subject generally, and particularly the controversy which almost from Jenner's day has raged around the question as to the true relationship of these affections to one another, having been sufficient to attract a great number of observers into this field of research.

MICRO-ORGANISMS IN VACCINE.

The first impulse was given to the work when Chauveau¹ and Burdon Sanderson² demonstrated almost concurrently by means of filtration and deposit experiments that lymph when freed from its contained particles and inoculated on a living animal no longer causes the appearance of vaccinia; while, on the other hand, the precipitate or deposit when employed in similar fashion is capable of producing the disease. In consequence, numerous bacteriologists have since then devoted themselves to the search in vaccine lymph for a micro-organism to which the special and peculiar effect resulting on the inoculation of such lymph is due.

Among the earlier observers who, though they cannot be considered to have succeeded in their search for an organism specific to either vaccinia or variola have yet, by a vast amount of patient research, done much to clear the ground for their successors may be mentioned Keber, Burdon Sanderson, Cohn, Quist, Buist, Carmichael, and Pfeiffer, the names being set out in accordance with the dates of publication of the results of their work.

The first account of the discovery of micro-organisms in vaccine and small-pox lymph was that given by Keber,³ of Dantzig, who evidently regarded the bodies found by him as being the carriers, if not the actual generators, of the virulent principle of these diseases. Within the next two years the occurrence of similar bodies in vaccine lymph was described by Burdon Sanderson and Klebs. In 1872 a most important paper was published by Cohn,⁴ of Breslau, in which he treated the morphological aspects of the subject with much completeness. His observations, which related to both vaccine and variolous lymph, have, as regards the lymph obtained from mature vesicles at least, received entire corroboration from all subsequent workers, with the exception that while he apparently believed the micro-organisms found by him to be of one species only, and to which accordingly he gave the name *micrococcus vacciniæ* or *variolaë*, as the case might be, later observers have shown that organisms of more than one species are usually to be found in any given specimen of lymph.

Cohn calls attention to the fact that in perfectly fresh vaccine lymph the "corpuscles" for the most part occur singly, others being joined together in pairs in a form resembling the figure 8, and he states that after the preparation has been kept for a time, the numbers of "these double cells increase, 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 16, 32, or more corpuscles." He also notes a point of importance in connection with the opacity which is apt to occur in stored lymph, namely, that "in capillary glass tubes the multiplication of colonies sometimes lasts a

long time so that they acquire considerable size, and present themselves as flocculi."

A short communication by Weigert,⁵ dealing with the microscopical appearance met with in the skin at the site of small-pox pustules, was published just previously to the appearance of Cohn's paper. Weigert found that the lymphatic vessels of the cutis were plugged with granular masses of what appeared to be micrococci, provided that the preparations were obtained from persons who had died at a comparatively early period of the disease. When death happened at a later period the micrococcus masses could not be distinguished owing apparently to the abundant corpuscular infiltration of the tissue, which was always noticeable after a certain stage of the disease.

CULTURE OF MICRO-ORGANISMS: QUIST'S EXPERIMENTS.

About ten years later Quist⁶ published a series of experiments dealing with the possibility of cultivating outside the animal body the micro-organisms present in vaccine lymph. He obtained the best results with a culture fluid composed of equal parts of blood serum, glycerine, and distilled water, which he rendered alkaline by the addition of $\frac{1}{10}$ 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.

Quist 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 into the skin of animals in some cases gave rise to a typical eruption of vaccinia.

He does not, however, appear to have been successful with subcultures. Still he showed, as also did Müller, that the specific contagium of vaccinia could exist for a time at any rate in a fluid composed in part of dilute glycerine, and it was probably in consequence of their work that this substance has come to be much used as an addition to vaccine lymph when it is required to be stored.

CARMICHAEL'S EXPERIMENTS.

A certain small measure of success appears also to have attended inoculation experiments in the hands of Carmichael,⁷ 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 consider were the product of one species of micrococcus only. As was doubtless the case in Quist's experiments, the few instances in which true vaccine vesicles resulted at the site of his inoculations must in all probability have been due to the presence, in the material employed, of a small proportion of the lymph originally used for the seeding of his culture medium.

BUIST'S EXPERIMENTS.

Probably the most extensive and laborious series of experiments dealing with the isolation of the various micro-organisms found in vaccine and in variolous lymph are those detailed by Buist,⁸ of Edinburgh, in a work published by him in 1886.

His experiments resulted in the separation of three different species of micrococci, which, when grown on the ordinary nutrient media, gave rise to cultivations of a white, yellow, or orange colour. All these three, Buist appears to have considered to be essential constituents of vaccine lymph, as is evidenced by the fact that he speaks of them as white, yellow, and orange vaccine respectively.

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.

¹ *Comptes Rendus*, lxvi, 1868.

² Intimate Pathology of Contagion: 13th Report of the Medical Officer to the Privy Council.

³ *Virchow's Archiv*, xlii, 1868.

⁴ *Loc. cit.*, lv, 1872.

⁵ *Centralb. f. Bakt.*, 1871, p. 609.

⁶ *Berlin. klin. Woch.*, No. 52, 1883.

⁷ *Trans. Philosophical Society of Glasgow*, 1887.

⁸ *Vaccinia and Variola*, 1886.

PFEIFFER'S RESEARCHES.

It was reserved for Pfeiffer⁹ to show that the various micro-organisms isolated by his predecessors, although constantly to be found in lymph, were identical with certain named species with which he was familiar as also occurring in various tissues and body fluids under circumstances which had no relation either to vaccinia or variola. In consequence, none of these organisms could be regarded as concerned in the specific action of the lymph.

CROOKSHANK'S LIST OF BACTERIA.

Crookshank¹⁰ in his evidence before the Royal Commission on Vaccination, and also in a paper communicated to the International Congress of Hygiene in 1891, states that he has succeeded in isolating from vaccine lymph by the method of plate cultivations an immense number of bacteria, of which he sets out a detailed list.

All of these he recognises as well-known saprophytic bacterial forms, many of which have been shown to be concerned in the processes of suppuration, and none of which, he says, can be regarded as the contagium, seeing that no one of them is constantly present in either human or calf lymph vaccine. As regards the latter statement, I find myself entirely in agreement with him, but the evidence of his portentous list of the bacterial contents of vaccine loses whatever weight might otherwise have attached to it as proving the danger likely to be incurred in the operation of vaccination, for the reason that no statement is made as to whether any, and if so what, precautions had been taken with regard to the collection of the lymph, neither is any attempt made to distinguish between those bacteria which are commonly to be found and those whose presence is exceptional.

THREE SPECIES OF MICRO-ORGANISMS.

As the result of my own work,¹¹ it would appear, however, that there are at least three species of micro-organisms—namely, *Staphylococcus albus epidermis*, *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 vaccine lymph examined; of these the *Staphylococcus albus epidermis* is usually to be found in the upper layers of healthy skin.

Of more importance is the fact that in certain cases, though rarely, I have been able to satisfy myself as to the presence of the *streptococcus pyogenes*, and moreover it is on record in certainly one instance at least that the *streptococcus of erysipelas* has also been isolated from a specimen of vaccine lymph. That untoward results should occasionally in certain cases follow the additional inoculation during the act of vaccination of one or other of the various extraneous organisms which may be present in the lymph employed is hardly to be wondered at; indeed it would appear rather a matter for congratulation that the evil results following the present somewhat empirical practice of vaccination have been in the past so few in proportion to the vast numbers that have undergone the operation.

THE VARIOLA BACILLUS.

In this connection, however, attention may be called to the fact that recently Klein¹² and myself¹³ have found it possible by the use of certain staining methods to demonstrate both in vaccine and in variolous lymph the presence of a small (spore-bearing?) bacillus, sometimes in considerable numbers.

This bacillus, which has also been found by Kent and myself¹⁴ in sections of skin passing through the site of a vaccine vesicle obtained from the calf, is to be found most readily in early lymph, that is, lymph taken on the fourth or fifth day (seventy-two to ninety-six hours) in the calf, and in the human vaccine (or variolous) vesicle about the fifth day of eruption, while later lymph, such as that obtained at the ordinary period for purposes of vaccination, contains but a

few at the most.¹⁵ This fact, which not unlikely has relation to spore formation by the bacilli at about the time of maturity of the vesicle, probably accounts for their presence having been overlooked by previous workers in this field. If proper precautions as to time and method be taken in the collection of lymph, it is usually found that although these bacilli are present, often in extraordinary numbers, other bacteria are conspicuous by their absence.

The fact that these apparently identical bacilli are to be found both in vaccine and in variolous lymph of about the fifth day of eruption tends to support the hypothesis that they in reality constitute the active contagium of the diseases in question, and the further fact that it has not been found possible to cultivate them in any of the artificial media ordinarily employed renders it practically certain that they are not merely "extraneous" saprophytic organisms.

Although not capable of growth on gelatine, agar, etc., the writer has succeeded in obtaining cultivations of the variola bacillus¹⁶ by employing for the purpose the hen's egg, inoculated with an emulsion of small-pox crusts in normal saline solution, and incubated for about a month at the body temperature. With material obtained from such an egg a series of calves have been inoculated, and with lymph of the third remove from the egg numbers of children have been successfully vaccinated.

Without resorting to cultivation experiments, it is possible to prepare with ease and certainty from vaccine lymph a material containing what must be regarded as a pure culture of the organism peculiar to vaccinia,¹⁷ and which, therefore, is of special value for purposes of vaccination.

GLYCERINATED CALF LYMPH.

As already stated, it has been abundantly demonstrated that vaccine lymph, as ordinarily obtained, gives rise, when inoculated into different nutrient media, of which plate cultivations are subsequently made, to growths of various "extraneous" saprophytic organisms. If, however, previous to making a plate cultivation the lymph has been intimately mixed with equal parts of a sterilised 50 per cent. solution of chemically pure glycerine in water and subsequently kept protected from the air and light in hermetically sealed capillary tubes, for a period of from a few days to a couple of months, it will be found that all "extraneous" organisms have now been killed out and no growth occurs in gelatine or agar plates inoculated with the contents of such tubes.¹⁸

In glycerinated calf lymph properly produced we have therefore a preparation which, while even more active as vaccine than the original lymph, can, to the best of my belief, be produced absolutely free from the "extraneous" organisms which at one time or another have been isolated from fresh or stored lymph, with the possible solitary exception when the lymph is obtained from the calf, of bacillus subtilis, the common hay bacillus, which, however, possesses no pathogenic properties. This statement, if it rested on my own authority alone, might no doubt be received with hesitation; but, seeing that since I first published the experiments on which it is based, ample corroboration has been afforded by the fact that other observers, including Chambon, Ménard, and Straus¹⁹ in France, Leoni²⁰ in Italy, and Klein²¹ in England, have arrived at similar results, there can be, I think, no longer any question about the matter.

Such glycerinated lymph, moreover, would appear to offer the most favourable opportunity for the further study of the specific organism of vaccinia and variola, as possible fallacies induced by the presence of "extraneous" organisms of any kind would thus be obviated.

SUPPOSED PSOROSPERMS IN LYMPH.

In consequence, no doubt, of the apparent impossibility which existed until recently of isolating any bacterium from

¹⁵ Besser (*Centralbl. f. Bakt.*, May 4th, 1893) has described a small bacillus as found by him in the papules of a case of small-pox at the fifth day, which can be stained by all the ordinary aniline dyes. He succeeded in growing the microbe, which is aerobic, on agar and broth kept at the body temperature, but not upon gelatine, serum, or potatoes.

¹⁶ BRITISH MEDICAL JOURNAL, January 7th, 1896.

¹⁷ *Trans. Internat. Congress of Hygiene*, 1891, vol. ii., and *Proc. of Royal Soc.*, 1893.

¹⁸ *Journ. of Pathology*, May, 1894.

¹⁹ *Gazette des Hôpitaux*, December 15th, 1892.

²⁰ *Revue d'Hygiène*, August 20th, 1894.

²¹ Private communication.

⁹ *Zeitschrift f. Hygiene*, iii, 2, p. 189.

¹⁰ *Trans. Internat. Congress of Hygiene*, 1891, vol. ii.

¹¹ *Loc. cit.*

¹² Report of Medical Officer to the Local Government Board for 1892-93 (1894).

¹³ BRITISH MEDICAL JOURNAL, September 22nd, 1894.

¹⁴ *Loc. cit.*

vaccine lymph which could be regarded as specific to vaccinia, several observers have sought to prove that organisms of a somewhat higher order than bacteria might be concerned in the production of this malady and also of variola. In this connection it may be mentioned that bodies believed to be of the nature of psorosperms or sporozoa have been described by Pfeiffer,²² Guarnieri,²³ Monti,²⁴ Van der Loeff, Doehle,²⁵ and Sicheer²⁶ on the Continent, and by Ruffer²⁷ and Jackson Clarke²⁸ in this country, as capable of demonstration in and among the epithelial cells of an inoculated area.

Ruffer and Plimmer describe the parasite in question as a small round body, which sometimes 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, though it has not been found enclosed in the latter structure. These observers state that they have found the same organisms in sections of skin from small-pox patients and in pustules of the larynx and trachea.

Pfeiffer describes similar bodies as to be found, not only in the epithelial cells of the vaccine vesicle, but equally in all other vesicular eruptions of man and the lower animals. Pfeiffer, Guarnieri, Ruffer, and Plimmer all assert that these parasites exhibit very slow amœboid movements.

There can, of course, be no doubt as to the occurrence of the appearances described, although one 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 cell enclosures described by Guarnieri, Monti, and Ruffer are said by them to differ essentially in their staining reaction and in their appearance from those of Pfeiffer, Van der Loeff, and others, but also the fact that the most recent experimental work in this direction has been carried out on the cornea of the rabbit—an animal which there is reason for believing to be insusceptible to vaccinia. It may be added also that no such bodies have been satisfactorily demonstrated in vaccine lymph itself. It is therefore 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.

THE VIRUS OF SMALL-POX AND VACCINIA.

On the other hand there appears good reason for believing that the bacillus demonstrated by Klein and myself in reality constitutes the specific virus of small-pox and vaccinia respectively.

It is consequently of interest to note that the operation of vaccination is probably one of the earliest instances on record of the employment of a bacterial inoculation, although of course its true nature was not recognised at the time of its introduction.

A SMALL-POX ANTITOXIN.

Inspired by the investigations of Tizzoni and Cantani on tetanus, early in the course of my work on the pathology of vaccinia and variola, my attention was directed to the question of the production, if possible, of an antitoxic serum,²⁹ which might be of use in the treatment of small-pox, it being well known that vaccination is of practically no avail when once the disease has become developed.

The results of my first experiments in this direction, which were carried out on the monkey, were not very encouraging however, as owing to the fact that at that time I had not succeeded in isolating what I now believe to be the specific organisms of variola and vaccinia, I was only able to employ for my injections the blood serum of a monkey which had been vaccinated on several occasions.

Since my work was carried out a number of other observers have experimented in a similar direction, the results recorded by them being, however, of a somewhat conflicting nature. Thus Kramer and Boyce³⁰ were unable to produce any im-

munity even with large doses of serum from vaccinated calves, and recently Beumer and Peiper³¹ have arrived at a similar conclusion. On the other hand Kenyon,³² and also Hlava and Houl,³³ claim to have obtained successful results, but as far as can be judged from their publications, such claim would appear to rest on somewhat slender foundation, although Hlava and Houl state that in one instance the injection of their serum to the extent of 0.6 to 1.0 c.cm. per kilo of body-weight entirely prevented the action of vaccine lymph inoculated four days later. They have not put on record any experiment with variola as yet.

The fullest and most recent contribution to this subject is a long paper by Bèclère, Chambon, and Ménard.³⁴ The conclusion at which the authors arrive is that the serum of a vaccinated heifer, gathered after the drying of the pustules, immunised to a certain extent against the effects of subsequent vaccination, but the diagrams with which the paper is illustrated show that the antagonism is by no means complete.

It would seem, therefore, that in default of having at command considerable quantities of a pure cultivation of the specific organism concerned, better results might probably be obtained by employing colts instead of calves for production of the serum, since, as Chauveau has shown, inoculation of colts not infrequently gives rise to a generalised eruption, the effect of which on the system is likely to be more potent than when the reaction, as is the case in calves, is limited to the area of inoculation. The writer hopes shortly to be in a position to publish the results of experiments carried out on the lines thus indicated.

If, as appears not improbable, further investigations should be attended by a greater measure of success than has at present been attained, we may hope before long to be in possession of an effectual method for the treatment of those cases in which the system has already become invaded by small-pox, which would form a fitting complement to the Jennerian process of preventive vaccination.

ANIMAL VACCINATION.¹

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INTRODUCTORY.

It is no uncommon error which leads people to suppose that the recent general recourse to the employment of vaccine taken direct from the calf (animal vaccine), as a

³¹ *Berl. klin. Woch.*, August 26th, 1895.

³² *Philadelphia Medical News*, February 2nd, 1895.

³³ *Wein klin. Rundschau*, October 6th and 13th, 1895.

³⁴ *Annales de l'Institut Pasteur*, January, 1896.

¹ By "animal vaccination" is meant in this paper the vaccination of human beings with animal (bovine) vaccine—that is, with vaccine from genuine cow-pox, and by "animal vaccine" is meant vaccine obtained from bovines (the calf). The nomenclature of vaccinia is very defective and misleading. Unfortunately, too, matters have been made still worse of late years by the employment of the terms "vaccine" and "vaccinate" to indicate the agent and the operation of protective inoculation against other diseases than small-pox. This was done by Pasteur with the generous and laudable object of keeping Jenner's great discovery for ever before men's minds. The result is none the less perplexing. Jenner employed the term "vaccine" as specifically referring to the cow (*Lat. vacca—a cow*). Nowadays it is high science to speak of the vaccine of diphtheria, or cholera, etc., when cow-pox and cows are in no ways concerned. The expression "pustule" as applied to the eruption of vaccinia is also entirely inaccurate and misleading, but no more so than the equally common term "vesicle." The specific Jennerian product of vaccination, from which vaccine is taken for vaccinating, is not a pustule nor does it contain pus, either in the human being or in animals. When it has ceased to be specific in character, and is undergoing some form of degeneration, it may or it may not become purulent. To call the "pock" a "pustule" suggests the untruth that pus is used for vaccinating. Neither is the Jennerian vaccine-pock a true "vesicle," though the vaccine may be partially contained in component vesicles; the pock of sheep-pox is a true unicellular vesicle. The term "lymph," too, is quite irrational as applied to the liquid exuded from the pock. It is not "lymph," and does not come from the lymphatics. It is serum mixed with a proportion—greater or less under varying circumstances—of the specific products of the morbid action going on in the successfully inoculated (or spontaneous?) pock. In the existing chaos of nomenclature "vaccination" is thoughtlessly used to signify both the operation of vaccinating and the disease which results from it. I would suggest that "pock" is the most suitable word for the local sore; that "vaccinia" (=cow-pox) should be employed to indicate the disease; and that "vaccine" should be used instead of the very inappropriate term "lymph" to indicate the specific virus of the Jennerian pock.

²² *Die Protozoen als Krankheitserreger*, Jena, 1890.

²³ *Centralb. f. Bakt.*, August 25th, 1894.

²⁴ *Loc. cit.*

²⁵ *Centralb. f. Bakt.*, Band xii, No. 25.

²⁶ *Münch. med. Woch.*, August 20th, 1895.

²⁷ *BRITISH MEDICAL JOURNAL*, June 30th, 1894.

²⁸ *Med. Press and Circular*, July 25th, 1894.

²⁹ *Trans. Epidemiological Society*, vol. xii, 1893.

³⁰ *BRITISH MEDICAL JOURNAL*, 1893, vol. ii, p. 989.

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