should not have been referred to in connection with Conclusion III that no method of administration of chloroform is free from danger, for at least a tentative order of merit of methods, and the cases upon which such tentative order of merit is based. The order would have come out thus:

—	Total Number of Cases.	Cases of Serious Danger or Death. (By + B ₆ .)	Rate per Minute at which CHCl ₃ was used.
"Junker" ...	411	0	5.58 minims.
"Open Method" ...	326	0	Not given.
Lint ...	3,793	23	8.25
Skinner or mask...	2,375	26	8.86
Towel ...	4,441	65	10.6

And from the data given in Table LXVI on page 95, the rate at which the anæsthetic was used would have come out as in the right-hand column above.

I hold no brief for any method; my opinions to-day are those which I have deliberately formulated in the BRITISH MEDICAL JOURNAL upon two previous occasions—in introduction of the discussion on Anæsthetics at Montreal in 1897 with reference to the relative power of ether and chloroform; and in a communication to the Society of Anæsthetists in the following year with reference to the paramount necessity of dosage in the administration of chloroform. The principal conclusion of the first communication was that chloroform is seven times as powerful as ether. The principal conclusion of the second communication was that successful chloroform anæsthesia requires the regular respiration of air in which the chloroform vapour is maintained between the limits of 1 and 2 per 100.

The opinion expressed by the Committee that no method of administration is free from danger is probably correct, but I am convinced that the dangers of anæsthesia, as shown by the annual death-rate returned by the Registrar-General as occurring under anæsthetics, can and will be considerably diminished.

But clinical inquiry, as exemplified in the ninth and final report of the Anæsthetics Committee of the British Medical Association, has said its say after ten years of careful deliberation, and it is evident that further clinical inquiry on these lines can serve no useful purpose.

What has the ordinary administrator of anæsthetics learned from this clinical inquiry? That chloroform is more dangerous than ether (Concl. I), but that as regards methods of administration (III and IV), rate of use (VI), methods of restoration (VII), clinical evidence has not warranted any conclusion. That special circumstances may render the use of some anæsthetic or combination of anæsthetics both safer and easier than that of ether or nitrous oxide (II), but that no indications are derivable from the present inquiry as to the nature of the anæsthetic or combination of anæsthetics that it may be advisable to employ (XXXIV) with the exception of the A.C.E. mixture, which holds an intermediate position between chloroform and ether (XXXI) but is more dangerous to males than females (VIII, XIII). And the most important clinical conclusion is the last (XXXV), that by far the most important factor in the safe administration of anæsthetics is the experience of the administrator, and that in many cases the anæsthetisation is of such importance and gravity that it is absolutely essential that an anæsthetist of large experience should conduct the administration.

This last, and evidently most desirable, condition is not always easy to fulfil, and we must above all look to increased diffusion of precise knowledge for diminution of the death-rate by chloroform. For knowledge to be diffused it must first be acquired. To this end certain definite steps should be taken:

1. An experimental examination of the statement that by proper application of a volumetric method anæsthesia can be certainly effected of any required degree.
2. An experimental comparison of the relative power of various anæsthetics.

3. The determination of the best method of quantitative estimation of anæsthetics in the various fluids and tissues of the body.

4. A careful redetermination of the statements made by Snow, Gréhant, Paul Bert, and Dubois with reference to the percentage of chloroform required for various degrees of anæsthesia, and the percentage of chloroform in the fluids and tissues of animals variously anæsthetised.

A PRELIMINARY NOTE ON THE ETIOLOGICAL AGENT IN VACCINIA AND VARIOLA.

By DR. M. FUNCK

Chef du Laboratoire de Bactériologie de l'Université de Bruxelles;
Director of the Institut Sérothérapique.

[Translated from the French MS.]

THE scientific history of vaccinia dates from 1798, the year in which the memorable discovery of Jenner was published. Since then the practice of vaccination has been fairly rapidly extended to most civilised countries; but, in spite of the continuous researches of a great number of experimenters, we have not yet attained to any certain knowledge of the etiological agent of the affection.

A series of investigations carried on for two years enables me to give here for the first time an experimental demonstration of the cause of vaccinia and of variola. These two diseases are caused by a similar micro-organism, a protozoon of large size, easily observed under a small microscopical magnification.

A detailed report of my experiments with explanatory figures will appear very shortly, but I desire permission to give a summary of work the importance of which will not be overlooked, work which may have a considerable influence on the study of the etiology of a whole group of dreaded diseases—the eruptive fevers.

I propose to prove experimentally the following facts:

1. Vaccinia is not a microbial disease.
2. It is caused by a protozoon easily found in all vaccine pustules and in all active vaccine.
3. The inoculation of this protozoon in a sterile emulsion reproduces in susceptible animals all the classical symptoms of vaccinia.
4. This inoculation renders the animals refractory to subsequent inoculation with vaccine.
5. The variolous pustule contains a protozoon morphologically similar to that in the vaccine.
6. It follows from these experiments that variola and vaccinia are two identical affections, that vaccinia is nothing more than an attenuated form of variola, and that consequently the immunity to small-pox which vaccination confers does not form an exception to the general laws of specific immunity.

I. *Vaccinia is not a Microbic Disease.*

I have compared the vaccines issued by the principal European vaccine institutes. It has long been known that freshly-prepared vaccine, that is to say, the glycerine emulsion of the contents of the vaccinal pustule, contains a very large number of bacteria. It could hardly be otherwise, considering that the vaccine lymph is made by scraping the pustules, and evidently that operation cannot be conducted antiseptically. The fact that vaccine undergoes "autopurification" is acknowledged by most bacteriologists, and we know that in three months most of the microbes originally contained in the lymph have disappeared. I have examined from this point of view 20 specimens of vaccine from different sources, and after being kept in sealed tubes in the dark for three months all were sterile, although they still produced absolutely characteristic pustules. I have proved that the contents of the pustules produced by the inoculation of this purified lymph are themselves sterile, and present the same morphological appearance as the fluid of the ordinary pustules of vaccinia.

It follows, therefore, that vaccine freed from microbes (aërobic and anaërobic) produces the specific pustules.

II. *The Etiological Cause of Vaccinia is a Protozoon.*

When a microscopical preparation of vaccine is made in the ordinary way, that is to say, if the vaccine is spread out on a cover glass fixed after desiccation and stained in cold or hot solutions, it is found that the preparation contains a large

number of ovoid vacuoles which are absolutely clear and transparent and do not take up aniline stains. The microscopical examination of fresh vaccine spread out on an object slide, and examined at once under a magnification of 500 or 600 diameters, shows that the clear spaces of the preparation fixed by heat correspond with very characteristic morphological elements in the vaccine liquid, the existence of which has already been recorded by several observers, and notably by L. Pfeiffer, of Weimar. The merit of having called attention to these corpuscles (in 1887) belongs incontestably to Pfeiffer.

As a rule, these elements occur under three very different forms:

1. In the first place there is a refracting form of a brilliant green colour and perfectly round, measuring from 2 to 10 μ , showing on the warm stage at 37° C. slow but well-marked movements.

2. In the second place ovoid cells are to be seen more or less elongated with a nucleus placed laterally, and containing in the protoplasm masses of a pretty uniformly round shape consisting of small brilliantly green spheres, analogous to the elements above mentioned, and each measuring 1 μ to 3 μ in diameter; these are epidermic cells infected by the protozoon.

3. Lastly a form very frequently met with in all vaccines is represented by raspberry-like bodies (true morulas), sometimes rounded, measuring 25 μ in diameter, and with or without a double contour; sometimes oval, and measuring from 30 to 35 μ in length by 20 to 25 μ in breadth. These elements are cysts (sporoblasts) full of spores, in which the nucleus appears under the form of a voluminous clear spot, sometimes central, sometimes lateral; sometimes they appear pyriform, rounded at one end and pointed at the other. In the contents of the pustules they are frequently to be found in more or less elongated tubular masses formed by the agglomeration of 20 to 40 elements.

As has been said above, these three forms, which represent the different stages of the evolution of the same protozoon, exist in all vaccines and in all vaccinal pustules. The last form—encysted spores—is that most often seen in old vaccines, while in the fresh matter from the pustules it is the first form which is principally found floating amid pus cells.

It does not come within the scope of this short summary of experiments to discuss the distinctive characters of the protozoa in order to class the organism with which we are concerned in a definite zoological group. I shall take up this question in my complete work. As this protozoon was first seen and studied by L. Pfeiffer, I propose to give it provisionally a name which shall recall the researches of this German investigator. As he has placed it among the sporozoa, I shall call the etiological agent of vaccinia "*sporidium vaccinale*." The descriptions of this protozoon found in certain publications are generally inexact. Up to the present time reliance has been placed principally on the examination of pathological sections or on the analysis of the corneal reactions in rabbits which give uncertain results. In sections especially, in spite of precautions taken to fix the parasite, it becomes unrecognisable, and, in order to study it, it is absolutely necessary to adopt the following method.

The sporidium can only be studied conveniently in fresh preparations in emulsions made with bouillon or physiological salt solution. The examination must be made in a hanging drop in a warm moist chamber. For this purpose a perfectly clean cover glass is taken, in the centre of which a drop of bouillon is placed. A trace of glycerinated vaccine (average proportion 1-5) is added to this drop by means of a platinum needle. The liquids are mixed by moving the needle in the drop of bouillon; a cover glass is turned over on to a slide with a cell previously prepared with vaseline, and in half an hour it is examined under the microscope. It is then found that the protozoa of the vaccine have fixed themselves on the under surface of the cover slip. The examination is best made with objective DD, and the compensated ocular 8 of Zeiss. The lower surface of the drop, that is to say the leucocytes and cellular debris, are first got into focus, and the fine adjustment is then moved a few divisions until the protozoa come into view. It is now possible to observe the micro-organisms without any difficulty, and without the other elements of the vaccine showing themselves

in the field of the microscope. An examination made after the preparation has been for several hours in the incubator at 37° C. is still more typical. I shall consider elsewhere the attempts to cultivate this protozoon.

III. *Inoculation of the Sporidium Vaccinale in Sterile Emulsion reproduces in Susceptible Animals all the Symptoms of Vaccinia.*

Since we are concerned with a protozoon there can be no question of cultivation in the bacteriological sense of the word. In spite of the probability of the existence of a protozoon in vaccinia, it was absolutely necessary to obtain a cultivation or a sterile emulsion which would reproduce the disease in animals in order to prove its specificity. The inoculation of vaccine, sterile from a bacteriological point of view, and capable of reproducing the disease, could not be considered as sufficient proof. I have therefore sought to give by a simple experimental arrangement a decisive demonstration of the etiological rôle of the sporidium.

With this object in view I took advantage of the large size of the cysts filled with spores to fish them out with a platinum wire on to discs of agar, and to make them into an emulsion with a sterile liquid. If under these conditions I succeeded in reproducing the disease, it could no longer be doubted that I really had before me the protozoon sought for.

Technique of Inoculation.—For this purpose I spread out on the surface of a disc of ordinary agar some vaccine which was either sterile or contained only a few microbes. After twenty-four hours in the incubator I placed the disc under the microscope (obj. AA. Oc. 2), and with a platinum spatula 0.1 mm. wide I proceeded to fish for the sporoblasts, which with a little practice are very easily recognised. The spatula is made by flattening with a hammer a platinum wire of 0.1 mm. mounted in a glass rod. The spores are made into an emulsion with a drop of bouillon, and a calf inoculated. About the sixth day, when the experiment is properly conducted, the characteristic pustules are observed.

IV. *Infection by the Sporidium Vaccinale Renders Animals Refractory to Subsequent Inoculation with Vaccine.*

When calves are inoculated with fresh emulsions of the protozoon they are found to have become refractory to subsequent inoculations with vaccine. It is no longer possible to obtain the slightest local inflammatory reaction, which shows with certainty that the animal has really undergone vaccinal inoculation by the penetration through the epidermis of the specific cysts. I shall deal in an early publication with the details of these experiments.

V. *The Variolous Pustule contains a Protozoon similar to the Sporidium Vaccinale.*

I have had an opportunity of studying the contents of the vesico-pustules in several cases of typical confluent small-pox. The elements to be found in the vesicles of variola are in all respects similar to those in the vaccine pustules. These forms will be described in my forthcoming work.

Conclusion.

The conclusion to be drawn at the present time from my experiments is that the etiological agent of vaccinia and of variola is identical, a conclusion already rendered probable by the well-known experiment of the transmission of variola to the calf, and of its transformation into vaccinia after three or four passages through that animal (Haccius and Hime).

To return to the history of the question, may I in conclusion be permitted to recall that Keber of Dantzig in 1868 described in vaccine certain corpuscles which he looked upon as the cause of the disease. Protozoa were mentioned in 1881 by Renaut of Lyons, and in 1886 by Van der Loeff of Amsterdam, but as a matter of fact we owe the earliest precise ideas as to the presence of protozoa in vaccine pustules to the remarkable work of L. Pfeiffer in the years 1887-1891. It has been by taking advantage of these elements that I have been able to demonstrate in vaccinia and in variola the constant presence of a parasitic element which is probably a sporozoon (Pfeiffer), and that I have been able to furnish for the first time the experimental proof of its specificity.