The BACTERIOLOGY of VACCINIA, etc.

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THEESIS
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THE
BACTERIOLOGY of VACCINIA and VACCINE LYMPH
with a Study of the
ACCIDENTS WHICH COMPLICATE VACCINATION and the
RELATION of MICRO-ORGANISMS THERETO:
and of the
EFFECT of various CHEMICAL SUBSTANCES on VACCINE LYMPH.
PREFACE.

The experimental work in connection with the subject of this Thesis was carried out during the Winter Session of 1897-98, in the Pathological Laboratory of the University of Edinburgh; and I desire to gratefully acknowledge the kindness of Professor Greenfield for so readily granting me permission to carry on my investigations there, and also to express my thanks for the many valuable suggestions I received from him.

My thanks are also due: especially to Dr Robert Muir, the University Lecturer on Bacteriology, and my teacher of this subject, for his constant help, and for the many valuable practical hints he gave me while carrying out my researches: to Dr Cadell, Dr Husband, Dr Cory, of the National Vaccine Institute, London, Dr J. B. Buist, Drs Chambon and Ménard, Paris, and others, for supplying me with vaccine lymph, and to Dr Harvey Littlejohn for other assistance. I also wish to acknowledge the courtesy and helpfulness of Mr Richard Muir, Laboratory Assistant, University Pathological Laboratory.

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INTRODUCTION.

The subject which I have discussed in the following pages is only a small part of the large and important subject of Vaccination. Although often tempted to do so, I have been careful not even to mention, much less to discuss, any of the many other important and interesting aspects of this question.

The subject of Vaccination is, at the present time for various reasons, attracting much attention; so that its literature, already very great, has increased to enormous proportions during recent years, and much of it is of a popular character. Nor is this to be regretted by the Medical Profession; for this is essentially one of those subjects which call for the educated influence of trained and thinking men, in order that the unthinking (scientifically) may have presented to them in a true light, and in their proper proportions, the true and essential facts of the case, and their interpretation. The profession of Medicine has nothing to lose, but much to gain, scientifically, by the diffusion of "more light" on this important question. The subject discussed in this Thesis is a case in point. That serious, and sometimes fatal, accidents have occurred in connection with the practice of vaccination, no fair-minded man can deny; nor is it even expedient to attempt to conceal these facts, for the more closely they are examined, especially in re-
lation to the very large number of Vaccination operations performed, mainly upon very tender subjects, the more clearly will even the lay mind see, when other general facts are also considered, that the operation of Vaccination is one which offers the minimum of risk with the maximum of benefit. In short, it will be clearly seen, that this small surgical operation, with all its risks and dangers, is infinitely safer than many of the everyday actions of life. That no operation is absolutely safe, is a truth which was impressed upon us, as students, by one of our teachers of Surgery; but, on the other hand, a similar statement may be made regarding any one of the most common daily events. It is probable that many more people suffer serious, or even fatal, injury by the use of tram cars, than by the operation of Vaccination, and yet no one is foolish enough to suggest that their use should be entirely discarded. At the same time, our duty to the public, as well as to ourselves, demands that we should make, not only the operation of Vaccination, but also every other operation, as safe as it is possible for human skill and forethought to make them. Injuries which might have been prevented have occasionally occurred in connection with the practice of Vaccination, and not until this fact has been acknowledged, and the position honestly faced, will a determined effort be made to reduce not only these, but all other Vaccination dangers, to an irreducible minimum. An endeavour has been made in this Thesis to ascertain the nature and
extent of these injuries, not merely because of their scientific interest, but rather that, knowing the danger and its extent, we may be in a position to avoid, if not to abolish, it.

Many investigators in different parts of the world are at work upon the subjects of Vaccinia and Variola. Stimulated, probably, by recent advances in Bacteriology, and our knowledge with regard to the questions which pertain to immunity to disease and the effects of serums thereupon, a large number of workers are engaged upon researches into these aspects of the subjects we have just mentioned. Many things seem to indicate that, perhaps, very soon, we shall be in possession of information regarding the essential nature and cause of Vaccinia and Variola. If this cause is bacterial, and it is possible to obtain, in suitable quantities, the micro-organism of the disease or diseases in a pure condition we shall then, probably, be independent of the use of calf and human lymph, for it will then be possible, it is hoped, to use this pure culture in a solution of pure sterilised glycerine, for Vaccination purposes. The discovery and isolation of the micro-organism will also raise and probably settle, important questions with regard to the true relationship between Vaccinia and Variola.

This discovery will also, in all probability, enable us to obtain a small-pox serum, which will both protect against and abort this disease, especially in its early stage, and thus be similar in its action to the anti-diphtheritic serum we now possess.
The subject I have investigated is here treated in three sections.

In the first section, I have attempted to give an account of the bacteriology of Vaccinia and Vaccine Lymph, based upon the work I carried out in the Pathological Laboratory; and by means of an historical sketch, I have shown the present state of our knowledge of this subject.

In the second section an effort has been made to arrive at a definite conclusion regarding the nature and extent of some of the accidents and injuries which occasionally complicate Vaccination, and to ascertain how the micro-organisms found in vaccine lymph are related thereto.

In the third section a description is given of an experimental research upon the effect of glycerine, formalin and other chemical substances upon vaccine lymph and its "extraneous" micro-organisms. An account is also given here of the work done and the conclusions arrived at, by other investigators in the same field.

A Bibliography of the subject under discussion as an appendix. The figures above the line in the text refer to corresponding numbers in the Bibliography.
SECTION I.

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The BACTERIOLOGY of VACCINIA

and of

VACCINE LYMPH.

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Historical Sketch.

Chauveau and Burdon Sanderson almost simultaneously gave the first impulse to work in this field by demonstrating that vaccine lymph from which all solid particles had been separated, was incapable of producing vaccinia; while, on the other hand, the solid particles, if invaccinated, were capable of producing this result. These investigators separated the solid particles contained in the lymph from the fluid, by means of filtration and deposition. These experiments seem to disprove the theory which has been advanced, that the essential and specific cause of vaccinia was not a living micro-organism, but an enzyme or a ferment, which was capable of so acting upon the living tissues, that it could reproduce itself. This inference obviously rests upon the fact that it has hitherto been found impossible to isolate and cultivate upon the ordinary culture media an organism to which could be attributed the effects of vaccinia. No other evidence of this theory has been given.

Monckton Copeman repeated with modifications the experiments of Chauveau and Burdon Sanderson. Fifty
cubic centimetres of calf lymph were intimately mixed with normal salt solution, and with a mixture of glycerine and distilled water. This diluted lymph was aspirated through a Kitasato's porcelain filter, with the necessary precautions. With the filtrate so obtained, a number of human beings and calves were inoculated, but in no single instance was any result obtained.

This experiment confirmed the results of previous observers; yet Dr Copeman did not regard it as conclusive. Because, as he states, "Sydney Martin has demonstrated in connection with his work on the products resulting in nutrient media, from the vital processes of the anthrax bacillus, that if any of the alkali albumen in his artificial serum has failed to become converted into albumenose, such alkali albumen is separated off during the process of filtration through porcelain, to which the fluid is subjected by him, with the object of removing the bacilli themselves." He therefore concludes that although nearly every one accepts the theory of a "Contagium vivum", yet the method of filtration does not demonstrate with absolute certainty that the essential principle of vaccine virus is particulate. Similar objections can also be urged against deposit experiments.

In 1809, Succo drew attention to the presence in vaccinal lymph of grains joined together in masses, which had the power of spontaneous motion.

Fifty-eight years afterwards Hallier and Zuon des-
cribed "microcognes caudés, coniques," the presence of which he had discovered in vaccine lymph.

Keber of Dantzig, in 1868, published an account of the discovery of micro-organisms in vaccine and small-pox lymph. He evidently regarded these bodies as the carriers, if not the active principle, of the virulent properties of these diseases.

Two years afterwards the presence of similar vesicles in vaccine lymph was discovered by Klebs and Burdon Sanderson.

In 1872, Cohn of Breslau published an important paper in which he treated the morphological aspects of both vaccine and small-pox lymph. He describes only one species of micro-organism, and gave it the name of micrococcus vaccinae or variolae, as the case might be. In perfectly fresh lymph, he says, these "corpuscles" occur singly or joined together in pairs; but that when the lymph has been kept for some time, the number of double cells increases, and soon chains of four, and clumps of sixteen or thirty-two are formed. He also considered that the opacity which is apt to occur in lymph stored in capillary tubes, is due to a multiplication of these cocci.

In 1871, Weigert published a paper dealing with the histology of the vaccine vesicle. He found that the lymphatic vessels of the cutis were plugged with granular masses, (which appeared to be micrococci,) provided that the persons examined died soon after being vaccinated.
Coze and Feltz (1872) also demonstrated the presence of very small bacteria in vaccine lymph, and Klebs (1873) separated from the lymph a tetrad, to which he gave the name tetracoccus variolae, and which he considered the specific organism.

Koch (1883) found micrococci in the vaccinal pustules of a child, and observes that, provided the lymph remains active, the older it is, the fewer bacteria it contains.

Quist published, in 1883, an account of a series of experiments dealing with the cultivation in media of the micro-organisms found in vaccine lymph. He used a culture fluid composed of equal parts of blood serum, glycerine, and distilled water, rendered alkaline by the addition of carbonate of soda. After sterilising this fluid, he inoculated it with a piece of sterilised sponge soaked in clear lymph, or with a piece of vaccine crust, which he had soaked in distilled water. Growths resulted in the form of minute floating scales, which gradually settled at the bottom in the form of a fine sediment. The scales when inoculated on animals seemed to produce typical vaccine vesicles, although he had found that they consisted of colonies of micrococci only. Probably the media absorbed some of the vaccine virus from the sponge, or crust, which being preserved in this glycerinated mixture, was transferred to the vaccinated animal and produced the disease. Subcultures produced no result. It was probably in consequence of Quist’s work, in con-
junction with that of Müller, that glycerine came to be added to stored vaccine lymph.

Voigt in 1885 isolated from vaccine lymph three kinds of organisms by means of gelatine plate cultures:

(1) A small round organism, which may occur alone or in clusters or chains. These organisms give rise to greyish-white round colonies on nutrient gelatine, which they do not liquefy. When heifers were inoculated with this organism they became immune to vaccine; and Voigt seems to have regarded this organism as the essential cause of the latter disease.

(2) A small microbe resembling the one just described, but which did not liquify nutrient gelatine; but it produced pustular eruptions and it conferred immunity to vaccinia.

(3) Large cocci which liquified gelatine, and produced on this medium colonies of a yellowish-green colour.

(4) Small cocci which also liquified gelatine, and gave rise to colonies of a yellowish-grey colour.

Carmichael of Glasgow also produced growths of cocci from vaccine lymph, and although he did not give the morphological characters of the growths he obtained, he seems to have considered that they were the product of one species of micrococcus only, although they differed from one another in colour. He used the cultures so obtained for inoculative experiments, and announced that he obtained true vaccine vesicles. Probably these were caused by a portion of the origin-
al lymph, which he had used in making his cultures. Sub-cultures do not appear to have been made.

Buist\(^{(2)}\) in 1886, published the results of a most extensive and laborious series of experiments. He separated three different species of micrococci, which, when grown on nutrient media, gave rise to growths of a white, yellow or orange colour. These micrococci Buist considered to be the essential cause of vaccinia, and in consequence he named them the white, yellow and orange vaccine. With none of these did he obtain true vaccine vesicles on the inoculation of calves, monkeys or human beings.

Guttmann (1886), Marotta (1886), Megnin (1886), Hlava (1887) and Garré (1887) all reported somewhat similar results.

Pfeiffer\(^{(32)}\) confirmed the above results, and also showed that the organisms which he and others had isolated, and which appear to be constantly found in vaccine lymph, were not different from certain species which were often found in various tissues and fluids of the body, quite apart from vaccination. None of these organisms could, therefore, be regarded as the essential cause of vaccinia.

He concludes that the specific cause of vaccinia and variola belongs not to the bacteria, but to the sporozoa, and that, in the adult, this parasite has a spherical form. He named it the "\(\text{\textit{monocystes epithelialis}}\)."

Cruickshanks\(^{(13)}\) having failed to find a specific or-
ganism in vaccine lymph, or pus, made a systematic examination of the different kinds of bacteria found in different specimens of calf lymph. He gives a very long list of different forms found in the various specimens - in fact a number very much larger than any previously given - but concludes that none of them is peculiar to vaccine lymph; that there was no bacterium constantly present in calf and human lymph, and there was not one which could be regarded as the contagium.

Most of the organisms found were well known saprophytic bacteria, and some of them were identical with bacteria commonly found in suppuration.

Professor Cruickshanks does not state what precautions, if any, were used in collecting the lymph, and as his statement with regard to the number and variety of organisms commonly found in vaccine lymph is at variance with the results of every other investigator in this field, we cannot attach the same amount of importance to them as might otherwise have been done.

Many other observers have worked in this field during recent years both in England and on the Continent, but their results generally are not different from those already described.

Copeman as a result of many examinations of vaccine lymph concluded that one or more of the following three organisms were almost universally to be found in every specimen examined:

Staphlococcus pyogenes aureus,
Staphlococcus cereus flavus,

Staphlococcus albus epidermis.

Copeman adds that these probably correspond to Buist's yellow, orange and white vaccine. In addition to these he has occasionally found an organism which he believes to be the staphlococcus pyogenes. Inoculative experiments were made by him on calves, rabbits and man, but in no case was anything resembling a vaccine vesicle seen. In one case the rabbit died, apparently from septicaemia after inoculation from a mixed growth of these organisms.

In 1894 Copeman and Klein, working independently, reported that they had found it possible, by the use of certain staining methods, to demonstrate both in vaccine and variolous lymph, the presence of a small, apparently spore-bearing bacillus, sometimes in great numbers.

These organisms are \( \cdot 4 \mu \) to \( \cdot 8 \mu \) in length, and one third to a half of this in thickness. They are generally thinner and stain better at the ends than in the middle. They occur in groups of from three to ten in both the lymph and the tissues. In the centre of the protoplasm there is often a clear globule, which is looked on as a spore.

These bacilli are said to be found in lymph taken from the calf at a period antecedent to the full maturity of the vesicles. Later and in mature lymph, they are found only in small numbers, and then with difficulty.
This bacillus has also been found by Kent, working in association with Copeman, in sections of the skin passing through the side of a vaccine vesicle obtained from the calf.

Later still, Copeman has reported that he has succeeded in growing this organism in the following manner: (he had previously discovered that it would not grow on any of the ordinary media.)

The surface of a hen's egg was rendered aseptic by means of corrosive sublimate and alcohol. A hole was made in it by means of a sterilised needle, and the contents mixed. After this the egg was inoculated with small-pox crusts which had been rubbed up in sterile normal salt solution, and finally the small hole was plugged with sterilised cotton wool, or closed with collodion. The eggs were then incubated at a temperature of 37°C for varying periods of time, - a month being the period which was eventually found to be most suitable. At the end of this time the eggs were found to contain a creamy material, which on microscopical examination seemed to be a pure culture of the organism already described. It again refused to grow on the ordinary media.

Four different calves were invaccinated with cultures obtained from the creamy matter contained in the inoculated and incubated eggs. Good vesicles were produced in each case; and from these other calves were vaccinated, and good vesicles again resulted. Children were vaccinated from each of these second calves,
and in each case good vaccine vesicles resulted. In other two cases, the lymph was put through a third calf, from which good vaccine lymph was obtained. This was used to vaccinate a large number of children by Dr Cory, and "all these children showed excellent marks when inspected a week later."

Dr Copeman, however, himself concludes that these results were inconclusive because "these experiments of mine on the bovine animal had to be conducted, at any rate in this preliminary stage, at Lamb's, Conduit Street, and the subjects of them had to be calves that on each occasion of experiment by me, were at the same time vaccinated on some part of their bodies with the calf lymph current at the station." The risks of other contaminations were also great in such a place as the National Vaccine Institute. "So indeed these experiments have to be repeated."

After I had commenced my investigations, Blaxall\(^{(3)}\) published an account of his researches upon the same subject. All his specimens of calf lymph were obtained from the National Animal Establishment. He found the following organisms in the specimens he examined:

\[
\begin{align*}
(I) \quad & \begin{cases} 
\text{Staphlococcus cereus flavus.} \\
\text{Staphlococcus cereus albus.} \\
\text{Large Yeast, orange coloured.}
\end{cases} \\
& \text{Passet.} \\
(II) \quad & \begin{cases} 
\text{Small Yeast, light brown colour.} \\
\text{Small Yeast, pale salmon colour.}
\end{cases} \\
(III) \quad & \text{Staphlococcus pyogenes albus.} \\
(IV) \quad & \text{Staphlococcus pyogenes aureus.}
\end{align*}
\]
(V) Staphlococcus pyogenes citreus.
(VI) Bacillus Mesentericus vulgatus
(VII) Bacillus Subtilis.
(VIII) Moulds and surcinæ.

The first two were always present, the third frequently, and the fourth was much less frequently observed. The others he regarded as accidental contaminations.

Pfeiffer, Monti, Guarnieri, Doehlé, Ruffer and Jackson Clarke have described bodies, which they believe are of the nature of psorosperms or sporozoa, which occur in and among the epithelial cells of an inoculated area.

Ruffer describes this parasite as a small round body, which may have a darkly stained centre. It is about four times the size of an ordinary staphlococcus, and generally lies in a clear vacuole in the protoplasm of the epithelial cells of the stratum Malpighii. The same organism has been described in the skin of smallpox patients.

The authorities mentioned above do not, however, agree in their descriptions of this organism, and especially with regard to its staining reactions. This body has not been demonstrated in vaccine lymph, while, on the other hand, similar bodies have been demonstrated in so many conditions that it is impossible to ascribe any specific significance to them.
A SMALL-POX ANTITOXIN.

During recent years, many efforts have been made to discover and prepare an antitoxin, which might be used in the treatment of small-pox.

The fullest contribution to this subject has been made by Béclère, Chambon and Ménard. They found that the serum of a vaccinated heifer, which had been gathered after drying the pustules, produced immunity to a certain extent, against the effects of subsequent vaccination.

Kenyon and Hlava also claim to have obtained successful results. In one instance they state that the infection of 0.6 to 1.0 cc. per kilo of body weight of their serum, prevented the action of vaccination four days later.

A large number of investigators, such as Copeman, Kramer and Boyce have obtained negative results with a similar serum.
The main object of my own investigations upon this subject was the discovery, isolation and, if possible, the growth on artificial media of the micro-organism, which is almost universally supposed to be the cause of vaccinia. It was necessary, therefore, in the first place, to examine a large number of different specimens of vaccine lymph, in order to ascertain if it were possible to demonstrate the constant presence therein of an organism other than those already described. Previous workers in this field, especially during recent years, have generally used calf lymph in their investigations. It seemed to me, therefore, that it might be advantageous, while not excluding calf lymph from my enquiries, to first examine specimens of human lymph. As compared with calf lymph, it has, for microscopical examination, some advantages, not the least of which are that it is much less viscid, and much clearer, because of its relative freedom from granular debris. To me, however, it presented a still greater advantage. I have been obliged to obtain all my specimens of calf lymph from a distance, and though I believe that most of them were collected and stored under antiseptic precautions, I cannot be absolutely sure of this fact. But it is different with the specimens of human lymph I have used. I have been able to collect most of them myself, and I have been able
to insure that they were collected under most strict antiseptic precautions, in a way which would be difficult to apply to the collection of lymph taken from the calf.

The method I employed was as follows. I thoroughly cleansed the skin surrounding the vesicles, and the vesicles themselves, with a strong (1 in 1000) solution of periodide of mercury. This I did by means of a large soft camel-hair brush. After allowing this solution to remain in contact with the skin and vesicles, for some time, I washed it off, in a few cases, at first, with absolute alcohol; but later I employed distilled sterilised water for this purpose, and I again applied this with a soft camel-hair brush which I had rendered aseptic.

To collect and store the lymph, I used capillary glass tubes, most of which I prepared myself, immediately sealing the ends by heat, when I had drawn out the tube. I occasionally used the ordinary commercial capillary tubes; but these were thoroughly sterilised in the hot air steriliser, and immediately closed by heat; after which they were again subjected to hot air sterilisation.

I carried these tubes in a sterilised case, and when about to be used the ends were broken off with sterilised forceps, and the tubes were passed through a flame before the lymph was collected.

The vesicles were sometimes ruptured by means of the sharp end of these tubes; but more frequently I
found it more expeditious to use for this purpose a small knife, which I had immediately before sterilised in the flame of a spirit lamp. After the lymph was collected, the tubes were immediately sealed by heat.

I collected in all forty-one sets of specimens of human lymph in the manner just described, and eight others were sent to me, and each of these was carefully examined by me, first on the day on which they were collected, and also later, on various dates, according to the number of tubes in each set of specimens. (Each set was collected from a different source.)

I prepared films in the usual way, either by fixing them by means of heat, or with a saturated solution of corrosive sublimate, and afterwards prepared them for staining in the usual manner. Two stains especially, alcoholic gentian violet and thionin blue gave the best results.

By this method I was able to demonstrate in most of the specimens examined, the presence of the ordinary staphlococci, and occasionally also other organisms.

In eight out of the forty-nine specimens I examined, I also demonstrated an organism, apparently similar to the one described by Copeman, Klein and Kent. This organism was very small, apparently about one third the length of the tubercle bacillus; but almost as thick. The ends were somewhat pointed, and they occurred singly or in groups of from three to eight. I made several attempts to cultivate this bacillus on
agar-agar, glycerine-agar, and gelatine, by means of a streak culture in tubes and plates; but without success.

I also used tubes of agar streaked with a film of blood, and others covered with a film of egg albumen, but with no better result.

In this respect I have simply confirmed the results of Copeman, Klein and Kent, who all failed to cultivate the supposed bacillus of vaccinia on artificial media.

It should be noted that the lymph I used was taken from the vesicles on the eighth day in every case, and possibly this explains my failure to detect the above described bacillus more frequently than I have done, if it be a fact, as stated by Copeman, that the bacillus occurs in greatest numbers in the lymph during the fifth or sixth day, or perhaps even earlier, especially in the calf; and that later, spore formation takes place.

I also examined a large number of specimens of calf lymph, but with two or three possible exceptions, I failed to detect any bacillus similar to the one already described, nor could I demonstrate the constant presence in the lymph of any other characteristic organism.
The MORPHOLOGY of VACCINE LYMPH.

Before describing the results of my investigations upon the bacterial growths which are commonly found in vaccine lymph, I may here give a short account of the morphology and composition of calf and human lymph, founded mainly upon my own observations.

If lymph be collected just before the full maturity of the vesicle, it is found to be a clear, perfectly transparent, very slightly viscid fluid. In man it is almost colourless, but that obtained from the calf is slightly yellowish, probably due to a difference in the blood pigment. Calf lymph is also much more viscid than human lymph, and has a strong tendency to coagulation; in fact in almost every specimen of pure calf lymph, examined by me, there was a central thread of coagulum. This I rarely found in human lymph. Microscopically it showed leucocytes, generally in small numbers at this stage, cells, cell debris, epithelial cells and debris, and red blood corpuscles. The latter I found in every specimen examined. In unstained, and especially in stained specimens, microorganisms can, in almost every case, be demonstrated. They occur, as a rule, singly or in pairs; but not unfrequently large masses may be seen in different parts of the field. They are generally free in the fluid; but, in several specimens examined, a number were seen inside cells and leucocytes.
If lymph collected after the maturation of the vesicles be examined, it is generally more opaque, leucocytes, cells, and cell debris are more numerous. There is an increase in the number of cocci, but the blood discs appear to be fewer.

Chemically speaking, vaccine lymph consists of the serum of the blood, or rather of its plasma. When collected it is slightly alkaline in reaction, but it becomes distinctly acid when stored. In addition to the various salts and proteids usually present in the blood-plasma, it is said to contain some substance, probably of the nature of a ptomaine, which results from the activity of the specific organism of the disease. Evidence of this is found in the fact, that if the lymph be filtered through porcelain, or exposed to a temperature of about 50°C, the filtrate or sterilised fluid, if inoculated on the skin, will produce no effect at the point of vaccination; but it will be found that the animal has been rendered immune to subsequent vaccination, produced in the usual way.

The opacity which sometimes occurs in human lymph when it has been stored for some time in capillary glass tubes, has been the subject of much discussion. This opacity is certainly not always caused by coagulation of the lymph, for I have found no coagulum in a number of old tubes in which this opacity was very well marked. Copeman states that this opaque lymph is uncertain in its action as a vaccinal agent. He has also made cultivation experiments with such lymph, and
also simultaneously, for purposes of control, with samples from tubes of comparatively fresh lymph, and he has found that "many more colonies are likely to result in plates established from the old tubes, than from those established from more recently stored lymph." He concludes, therefore, "that the opacity of old stored lymph is, in the main, the outcome of the multiplication in it of aërobic bacteria, the ancestors of which were in the lymph when first collected, although their numbers were then so comparatively small as not to render it in any way turbid."

My own experiments with old stored calf lymph do not warrant me in accepting this conclusion.

I have been able to obtain through the kindness of Drs Husband and Cadell, specimens of old stored lymph, some of which were comparatively clear, while others were very opaque. Plate cultivations were made from each kind of lymph, equal quantities of both being used, and in not a single instance did I find that the plates inoculated with opaque lymph, yielded more colonies than the plates inoculated with comparatively clear lymph; indeed, in several instances the latter produced more numerous bacterial growths than the former.

This opacity does not, as a rule, show itself until the lymph has been stored for a considerable time; while my experiments seem to prove that active bacterial growth in the lymph comes to an end during its third week of storage.
Most public vaccinators know from experience, that at times, apparently quite healthy vaccine pustules will produce a lymph which is very opaque. I have collected specimens of such lymph, and also, at the same time, specimens of beautifully clear lymph; and on making comparative plate cultures, with equal quantities from each, I found that, as a rule, about an equal number of bacterial growths resulted for each kind of lymph. Microscopical examination, however, showed that leucocytes and granular debris were more numerous in the opaque than in the clear lymph. This, I think, suggests the probable correct explanation.

In tubes stored for a long time, the cells and granules may break down into a much greater number of particles, and these for optical reasons may give rise to the opacity.

At my request Dr Husband vaccinated a child with somewhat opaque lymph from a tube which had been stored for four years, with the result that perfect vesicles were produced. Dr Cadell repeated this experiment with lymph which had been stored for three years, and although the vaccination was successful, the vesicles were not quite perfect.
BACTERIAL GROWTHS in HUMAN LYMPH.

In all forty-nine specimens of human lymph were examined, not including fourteen specimens of old human lymph which I also examined, and in most of which I also found organisms.

The lymph was, as a rule, examined on the day of collection, and streak cultures on agar-agar, in stopped tubes were made, and in most cases also, plate cultivations in nutrient gelatine; and the resulting growths were examined three to five days afterwards, and sub-cultures made when necessary.

The following is a list of the organisms which I isolated from the various specimens, arranged in order of frequency: -

(I) Staphlococcus pyogenes albus.
(II) Staphlococcus pyogenes aureus.
(III) Staphlococcus cereus albus.    Staphlococcus cereus flavus.  Passet.
(IV) Staphlococcus pyogenes citreus.
(V) Bacillus subtilis
(VI) A bacillus similar to the last, but which did not liquify nutrient gelatine; probably similar to the bacillus subtilis similans as described by Cruickshanks.

It is unnecessary to give an enumeration of the growths which resulted from the inoculation of each separate specimen. The following list will sufficient-
ly show how they were distributed:

Eight tubes produced growths of the staphlococcus pyogenes albus only.

Twenty-five tubes produced colonies of both the staphlococcus pyogenes albus and aureus. Three of these also showed colonies of the staphlococcus pyogenes cereus, and one in addition produced colonies of the Bacillus subtilis.

From five tubes pure cultures of the staphlococcus pyogenes aureus only were obtained.

In one tube the last mentioned bacillus in the above list was combined with the staphlococcus cereus albus.

In three tubes the staphlococcus pyogenes albus and the staphlococcus cereus were combined, while one of the three contained in addition the staphlococcus cereus flavus.

In seven tubes the staphlococcus cereus albus and flavus were combined.

The fourteen tubes of old human lymph produced very similar results, with two exceptions, where no growth at all resulted. The organisms in the remaining twelve were distributed as follows:

Five contained the staphlococcus albus and aureus, and two of them contained the Bacillus subtilis in addition.

One contained the staphlococcus pyogenes albus only, while another contained only the aureus. Two produced colonies of the staphlococcus pyogenes albus and
and Bacillus subtilis; and in other two the latter bacillus was associated with the staphlococcus cereus albus, while in one case it occurred alone.

The tubes, etc., in the first series were carefully disinfected, while, in the second series, they were, I believe, used as they came from the maker's hands. This fact, I think, will sufficiently explain the almost constant presence of the Bacillus subtilis in the second series.

Two of these tubes of human lymph, which on cultivation produced organisms, had been stored for nine years. Another had been stored for three years, and none of them had been stored for a shorter period than one year.

In a previous part of this paper, I have stated that I, at first, employed heat or saturated mercuric solution to fix the lymph upon the cover slips. I could not obtain very good results by this method. Even the thinnest possible coating of lymph in many cases, made the preparation almost opaque, and therefore most difficult to examine microscopically. This was especially the case when the film was stained with methylene blue, even after repeated washings.

When corrosive sublimate was used, it required very prolonged washing in order to free the film even moderately from the fixing agent, and the result of this was that all the stains used were frequently more or less precipitated.
At a later period of my investigations, while experimenting upon the effects of Formalin on calf lymph and its contained organisms, I discovered that in this substance we had a more satisfactory fixing agent than either of the two I had previously employed. Lymph films on cover slips, if allowed to float, film downwards, upon Formalin either full or half strength, even for a few seconds, are at once satisfactorily fixed. They can then be at once stained with any of the ordinary dyes, without washing the fluid off, although it is better to do so. A most satisfactory preparation is thus easily and quickly obtained, and one in which the objects to be examined are more clearly defined than they are by either of the above mentioned methods.

This method is also equally good for making film preparations of micro-organisms; and I believe that for this and other similar purposes, it will prove most useful in ordinary clinical work. Films may remain on this substance for a long time without being damaged in any way, and it is certainly much more expeditious than the methods just mentioned.
I examined twenty-five specimens of pure unmixed calf lymph, and also sixteen specimens of so-called glycerinated calf lymph, which I received from different calf lymph stations at home and abroad. The majority of the specimens of pure calf lymph were obtained from the National Vaccine Institute, through the kindness of Sir Richard Thorne and Dr Cory. The remainder were obtained from Paris (M. Chambon and Dr St Yves Ménard), Brussels, and from a London commercial firm.

In the National Vaccine Establishment, in Paris, and in most public and private vaccine lymph establishments, antiseptic precautions are taken, first when the calves are inoculated, and secondly when the lymph is collected. After the calf has been fixed for the operation, the part to be vaccinated is shaved, and then carefully washed with soap and hot water, after which it is again washed with an antiseptic, generally corrosive sublimate, which is afterwards removed with hot water. At the end of from 96 to 144 hours, the calf is again strapped to the table, and the parts covered with vesicles are washed with corrosive sublimate, or some other antiseptic, all traces of which are removed by means of hot water, before the vesicles are clamped and punctured and the lymph collected. Care is also taken to sterilise the capillary tubes used.
I give below a list of the micro-organisms which I isolated by the methods already described, from twenty-five specimens of pure calf lymph arranged in order of frequency: —

(I) Staphlococcus pyogenes albus.
(II) Staphlococcus pyogenes aureus.
(III) Large orange coloured yeast.
(IV) Staphlococcus cereus albus.
(V) Small yeast, yellowish in colour.
(VI) Staphlococcus cereus flavus.
(VII) Staphlococcus pyogenes citreus.
(VIII) Bacillus subtilis.
(IX) A mould.
(X) Staphlococcus pyogenes.

These were distributed as follows: —

In four tubes growths of the staphlococcus pyogenes albus only resulted.

In sixteen tubes the two pyogenic cocci (albus and aureus) were found together, combined in one tube with the staphlococcus pyogenes citreus, in other three with a large orange-coloured yeast, and in another with a small yellowish-coloured yeast.

Two produced colonies of the large orange-coloured yeast and colonies of the staphlococcus cereus albus.

Two contained the staphlococcus cereus albus, combined in one case with the staphlococcus cereus flavus and a mould, and in another with the Bacillus subtilis.

One contained only the staphlococcus pyogenes aureus.
Another tube when inoculated on agar produced colonies of the staphlococcus pyogenes and the staph-
lococcus pyogenes albus.
COMMERCIAL GLYCERINATED CALF LYMPH.

The twenty-five specimens of commercial glycerinated calf lymph which I examined, showed, without exception, numerous colonies of micro-organisms, when agar tubes or nutrient gelatine plates were inoculated. The following is a list of the organisms which I isolated by tube or plate cultivations, from each specimen:

Tube I. Staphlococcus pyogenes aureus and albus.
 II. Staphlococcus pyogenes aureus and albus, Staphlococcus percius albus and the bacillus subtilis.
 III. Similar to last.
 IV. Bacillus subtilis sim
 V. Bacillus subtilis.
 VI. Staphlococcus pyogenes aureus and albus, and bacillus subtilis.
 VIII. Staphlococcus pyogenes albus and staphlococcus cereus flavus.
 IX. Similar to last.
 X. Similar to last, with the addition of bacillus subtilis.
 XI. Staphlococcus pyogenes albus and aureus.

Tubes XII to XV. Four tubes received at the same time from one firm. Each contained the staphlococcus pyogenes albus and aureus, and two contained in addition
the bacillus subtilis, while one also contained the staphlococcus cereus flavus.

Tube XVI. Staphlococcus pyogenes albus and aureus with the bacillus subtilis.

" XVII. Staphlococcus pyogenes albus.

" XVIII. Similar to last.

" XIX. Staphlococcus pyogenes albus and aureus and staphlococcus cereus albus.

" XX. Staphlococcus pyogenes aureus and albus.

" XXI. Similar to last.

" XXII. Staphlococcus pyogenes albus.

" XXIII. Staphlococcus pyogenes aureus and albus, and the staphlococcus cereus albus.

" XXIV. Staphlococcus pyogenes albus.

" XXV. Staphlococcus pyogenes aureus and the staphlococcus cereus albus.

With two exceptions every one of these specimens showed very dense growths of the micro-organisms enumerated above.
The NUMBER of MICRO-ORGANISMS in VACCINE LYMPH, and their RATE of GROWTH.

Both in human and calf vaccine lymph the number of organisms present was invariably large. One very small platinum loopful — about one tenth the quantity which would be used in an ordinary vaccination of three or four insertions — produced, on an average, more than one hundred colonies when inoculated on agar or nutrient gelatine, and a plate preparation made.

I found it rather difficult to determine the exact rate of increase, when the lymph was kept stored in the usual capillary tubes; but the results I obtained will permit of the following general statement. At the end of a week, the organisms had increased to six or eight times their original number. During the second week, the increase was very small, and at the end of this period demonstrable increase seemed to cease. The growth in human lymph was greater and more rapid than in calf lymph, and in many cases it appeared to stop at the end of a week or ten days.

The rate of increase also seems to depend upon the size of the capillary tube used, or, in other words, upon the quantity of lymph in one tube, and upon the atmospheric conditions in which it is kept. In a tube larger than usual, the increase is much greater in proportion, and this increase can be demonstrated
for, at least, three weeks; and lymph kept in tubes which are stored in a warm place, show a more rapid increase, than lymph stored under different conditions, but the increase comes more quickly to an end in the former than in the latter case.
The VIRUS of VACCINIA and GLYCERINATED CALF LYMPH.

This description and enumeration of the bacterial growths found in calf and human lymph, brings to light one of the difficulties encountered by the investigator into the essential bacteriology of vaccinia. If an organism is the cause of the disease, as is believed by so many, it must be contained in the lymph, either in a mature or spore condition. It may, therefore, be possible to demonstrate this organism in calf and human lymph, obtained from the vesicles at the proper time, but the large number of other "extraneous" organisms present, may to some extent hinder, or altogether inhibit, its growth when plate cultivations are made. This difficulty presented itself to me at the very commencement of my researches; and was one of the main factors which directed my attention to the effect of glycerine and other substances upon vaccine lymph. In the third part of this paper, I shall give an account of the results of my investigations upon this part of the subject. I may here state, however, generally, that I found in chemically pure and sterile glycerine, combined in certain proportion with sterile distilled water, an efficient means of destroying the organisms which I have described, while, at the same time, the efficiency of the lymph as vaccine material was unimpaired. This result, however,
I did not attain until I had worked at the subject for several months, and other engagements have prevented me from more fully experimenting with this sterile glycerinated lymph. I have, however, failed to obtain any growth by inoculating it upon the ordinary media, including glycerine-agar, and sterile egg albumen.

Following the lines indicated by Copeman, I have attempted to obtain a positive result by the inoculation and incubation of fresh, newly laid hens' eggs. The shell was first thoroughly sterilised by brushing it with water, and then with a strong solution of corrosive sublimate, which was afterwards removed with distilled sterilised water. I examined bacteriologically several eggs prepared in this manner; and in every case I obtained growths of micro-organisms from the mixed contents of the egg.

I also inoculated with sterilised glycerinated lymph, in the manner already described in the historical section of this paper, an egg prepared by this method, and allowed it to incubate at a temperature of 37 °C for eight days. I then inoculated agar tubes and plates, and also glycerine-agar tubes, with the mixed contents of the egg, and after incubation obtained growths of several different organisms, one of which was the staphlococcus pyogenes albus. The results of these experiments seem to indicate, then, that a hen's egg, however carefully cleansed on the outside, is never perfectly sterile, and that, therefore, it is useless as a medium whereby pure cultivations of an or-
ganism may be obtained.

In the future, however, it may be found possible to obtain pure cultures of the micro-organisms of vaccinia, by inoculating the peritoneum or the internal organs of a rabbit, guinea-pig, or other animal, under antiseptic precautions, with a sterile glycerinated preparation of vaccine lymph. With this material the work of the investigator in this field has been very much simplified, and we may reasonably expect that in a very short time new developments will be made in this important practical subject.
SECTION II.

The ACCIDENTS which COMPPLICATE VACCINATION and the RELATION of MICRO-ORGANISMS THERETO.
THE
ACCIDENTS WHICH COMPLICATE
VACCINATION
AND THE
RELATION OF MICRO-ORGANISMS
THERETO.

Introduction.

The Royal Commission which was appointed in May 1889 to enquire into the subject of Vaccination, was directed, inter alia, "to enquire and report as to the objections made to vaccination on the ground of injurious effects alleged to result therefrom, and the nature and extent of any injurious effects which do, in fact, so result."

This, I venture to think, is a matter of very great practical importance, Not only has the efficacy of vaccination as a protection against small-pox been denied; but it has also been asserted that it causes the disease against which it is intended to protect, and it is alleged that the injuries caused by it are so numerous and so terrible, that there is no justification for a practice which is so dangerous to the life and health of the community. A well known writer has
recently said that "a thousand children have been certainly killed by vaccination during the past twenty years, and an unknown, but probably much larger number injured for life."

The medical profession cannot afford to look lightly upon such statements: the issues at stake are too great. It has now been proved beyond the possibility of doubt that such statements have influenced a very large number of people in their attitude towards the subject of vaccination. In England and Wales during the years from 1874 to 1883, both inclusive, an average of 4.73 per cent per year of all the children born during these years, were unaccounted for in the vaccination returns; i.e. after the necessary deductions had been made for children who had died unvaccinated, or had proved insusceptible to vaccination or who had had small-pox. During the years from 1884 to 1893, both inclusive, an average of 9.89 per cent per year were unaccounted for. The figures become still more striking when notice is taken of the yearly increase during the latter decennium. Thus -

<table>
<thead>
<tr>
<th>Year</th>
<th>Per Cent Unvaccinated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1884</td>
<td>5.5</td>
</tr>
<tr>
<td>1885</td>
<td>5.8</td>
</tr>
<tr>
<td>1886</td>
<td>6.4</td>
</tr>
<tr>
<td>1887</td>
<td>7.1</td>
</tr>
<tr>
<td>1888</td>
<td>8.5</td>
</tr>
<tr>
<td>1889</td>
<td>9.9</td>
</tr>
<tr>
<td>1890</td>
<td>11.3</td>
</tr>
<tr>
<td>1891</td>
<td>13.4</td>
</tr>
</tbody>
</table>
1892 - 14.9 per cent unvaccinated.
1893 - 16.1 " "

During this decade a total of 8,951,129 births were registered, and their history with regard to vaccination may be tabulated thus:

Certified as insusceptible to vaccination 17,672
Died unvaccinated . . . . 927,752
Had small-pox . . . . . . 260
Vaccination postponed by medical certificate . . . 118,814
Unvaccinated . . . . . . 767,946
Successfully vaccinated . . 7,118,685

Thus, leaving out of account the 118,814 children whose vaccination was postponed by medical certificate, we find that one tenth of these available children born during the period under consideration were allowed to evade the Vaccination Laws in England and Wales.

These unvaccinated children were not, however, equally distributed over England and Wales. Several large centres of population had been allowed to almost wholly evade these Laws.

In London the percentage of births left unaccounted for rose from 6.5 in 1883 to 16.4 in 1891, or an average for nine years of 9.9 per cent per year.

In Dewsbury, Leicester and Gloucester the percentages of births left unaccounted were as follows:

Dewsbury. 1884 . . 37.6
1882 . . 12.6 1885 . . 47.2
1883 . . 20.0 1886 . . 37.5
In Scotland the proportion of children primarily vaccinated has remained practically the same during the decade 1884 to 1893. During the latter period an average of 4.35 per cent per annum of the total births after the necessary deductions had been made, were unaccounted for in the Vaccination returns. During the decade before this an average of 3.12 per cent per annum were unaccounted for.

These figures bring to light a very serious state of affairs. Year after year an increasingly large number of people is being allowed to evade one of the most, if not the most, important Public Health enact-
ments of the country, with the result that there is at the present moment in the community a very large number of children below the age of fourteen or fifteen who are not protected against an attack of small-pox by vaccination. The risk to themselves is great; but the danger to the community is still more serious.

No one can doubt that this is the result of the opposition to the practice of vaccination which, especially in some parts of the country, has grown rapidly during the past fifteen years, although it has existed to some extent for a much longer period. In the three English towns mentioned above, the anti-vaccinationists have been most active in their propaganda, and their success is only too evident.

The success of these agitators is, I venture to assert, mainly due to this fact, that by means of a large number of cases of injury or so-called injury said to be caused by, or connected, in some way, with, vaccination, they have been enabled to appeal to the emotions and sympathy of that class upon whom is laid the responsibility of obtaining vaccination for their children and who at all times regard with repugnance the infliction of even such a small injury as vaccination causes, upon such tender subjects. Serious injuries and even death have undoubtedly been caused by vaccination, and perhaps more frequently are found in association with it; and although the per-centagge of such injuries and deaths to the total number of vaccinations is very small, yet the gross total is sufficient-
ly alarming when found as a result of, or in connection with such an apparently safe and simple operation. And it should not be forgotten that one case of death or injury in connection with vaccination in any town or district is often sufficient to bring the practice of vaccination to a standstill for a time, and this is especially the case when some active anti-vaccinationist makes it his business to spread broadcast the facts of the case, not unfrequently in a distorted and exaggerated form.

I therefore would lay emphasis on this statement. The argument against vaccination which is founded on the injurious results which are found in connection with it, is the one which appeals with the greatest force to the largest number of people, and the one which has frightened so many from having the operation performed upon their children.

It is therefore most essential on this, perhaps the lowest possible ground from the scientific point of view, that the medical profession should know the etiology of, nature and extent of such injuries, so that means may if possible be taken to prevent, or at least minimize, them.

I wish here to make this the object of my enquiry, and to look at the subject, especially in connection with what I have recorded in a former section, where I have stated the results of my enquiries into the composition and bacteriology of calf and human lymph.
THE METHOD OF ENQUIRY.

Those who oppose vaccination because of its alleged injurious results have followed two lines of attack. In the first place it is asserted that evidence of its injurious results is afforded by an examination of individual cases in which injury or death has followed upon, or resulted from vaccination; and in the second place it is stated that an examination of infantile mortality from certain specified diseases during certain periods will show that vaccination is most harmful in its results.

It will be convenient in this enquiry to follow these two lines; and in the first place I shall examine the statistics of deaths and injuries which are asserted to have occurred in connection with vaccination.

I am indebted to the reports and appendices of the Royal Commission for most of the figures I shall use.

THE MORTALITY FROM, OR IN CONNECTION WITH, VACCINATION.

The number of deaths which result annually from vaccination has been recorded for many years in the returns of the Registrar-General. Until 1880 the number of deaths from erysipelas was separately recorded; but since then all deaths from vac-
cination have been included under the heading "Cow-pox
and other Effects of Vaccination."

The following table shows the number of deaths
from erysipelas from 1859 to 1880:

<table>
<thead>
<tr>
<th>Years</th>
<th>Range</th>
<th>Annual Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>1859 - 67</td>
<td>2 to 13</td>
<td>6.8</td>
</tr>
<tr>
<td>1868 - 71</td>
<td>9 to 24</td>
<td>18.0</td>
</tr>
<tr>
<td>1872 - 80</td>
<td>16 to 39</td>
<td>28.5</td>
</tr>
</tbody>
</table>

In the Final Report of the Vaccination Commission
it is pointed out that from 1859 - 71 the population
of England and Wales from nineteen to twenty-two mil¬
lions, and that the number of children vaccinated in¬
creased very much between 1868 and 1871, owing to the
Vaccination legislation of 1867. The Vaccination
Acts of 1871 also increased considerably the number of
primary vaccinations.

The following remark is made by the Commissioners
on the figures: - "Of course the greater the number
of the vaccinated amongst the children born in any
given period the greater, caeteris paribus, would be
the number of cases of erysipelas after vaccination,
without any necessary connection between the two."

This remark, I venture to assert, is obviously one-sid¬
ed. When these statistics are examined in the light
of present day knowledge, we are compelled to acknow¬
ledge that a certain proportion of these deaths may
be attributed to vaccination as a cause, and that as
the number of vaccinations increased so the number of
deaths rose, probably pari passu. The truth of this
statement, I think, will be found below.
The following table shows the number of deaths classified as due to cow-pox and other effects of vaccination from 1881 to 1895.

<table>
<thead>
<tr>
<th>Year</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>1881</td>
<td>58</td>
</tr>
<tr>
<td>1882</td>
<td>65</td>
</tr>
<tr>
<td>1883</td>
<td>55</td>
</tr>
<tr>
<td>1884</td>
<td>53</td>
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<tr>
<td>1885</td>
<td>52</td>
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<tr>
<td>1886</td>
<td>45</td>
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<td>1887</td>
<td>45</td>
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<td>1888</td>
<td>45</td>
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<tr>
<td>1889</td>
<td>58</td>
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<td>1890</td>
<td>43</td>
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<td>1891</td>
<td>43</td>
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<tr>
<td>1892</td>
<td>58</td>
</tr>
<tr>
<td>1893</td>
<td>59</td>
</tr>
<tr>
<td>1894</td>
<td>50</td>
</tr>
<tr>
<td>1895</td>
<td>56</td>
</tr>
</tbody>
</table>

From 1881 to 1889 inclusive, a total of 476 deaths was certified as due to vaccination, or an average of 53 a year. During the following six years the average has remained nearly the same. There was an average of one death to 14,159 primary vaccinations during the first nine years, the total number of vaccinations being 6,739,902.

From 1886 to 1891 vaccination was certified as the cause of death in 279 cases, and there is good reason to suppose that the proportion of deaths due to primary vaccinations was somewhat higher than during the previous nine years.

In Scotland twenty-two deaths were certified as due to vaccination during the years 1883 to 1890 inclusive, or a proportion of one death to 38,872 primary vaccinations.

During the years from 1888 to 1896, 205 alleged
cases of injury in England and Wales were fully enquired into by officials of the Local Government Board, and 421 additional cases were investigated by the Commission on vaccination. Full particulars of most of these cases have been published in the ninth Appendix to the Final Report of the Commission, and I propose to use this material as the basis of an enquiry into the nature and amount of the injury caused by vaccination, and I shall also endeavour to ascertain how much of this may be prevented, and in this and the following section I shall attempt to indicate, mainly upon the results of my own investigations, how the injuries may be prevented or at least reduced to an unappreciable minimum.

THE NATURE AND EXTENT OF THE INJURIES CAUSED BY, OR FOUND IN CONNECTION WITH, VACCINATION.

It will be convenient, in the first place, to make a detailed study of the 205 cases in which death occurring on or before the 1st November 1888, and the 30th December, 1891, had been alleged or suggested to have been caused or connected with vaccination.

These 205 fatal cases may be classified thus:

I. Inflammatory or septic complications, ... 142
II. Those in which a suspicion of syphilis was raised ... ... ... ... ... ... ... ... 16
III. Complicated by various diseases, . . . 8
IV. Complicated by non-specific skin eruptions 9
V. Those in which it was doubtful what, if any, influence was exercised on the result by vaccination. . . . 30

The last class we may at once set aside as unworthy of detailed consideration, and useless from the point of view of the present inquiry. A careful examination of the published history of each of these thirty cases will fully convince any impartial observer that vaccination had no causal relation to the fatal result nor to the illness which led to it.

Of the remaining 175 cases, the large number of 142 have been classified as "inflammatory". The complications included under this are erysipelas, ulceration, abscess, cellulitis, pyaemia and septicaemia; or in other words, the complications were those which are common to all open wounds and lesions of the skin.

The proportion of septic cases should, I think, be stated as even larger than this, as many of the cases classified under II and III in the table should be included in No. I, as investigation will show that inflammation or sepsis in some form was the true cause of death.

Here, perhaps we should also state, that this proportion of inflammatory to other complications following vaccination, is observed in all the available returns. Drs Barlow and Acland investigated 189 cases of vaccinal complications and found that 94 of them came
under the heading of "inflammatory"; and an examination of the analysis of the fatal diseases connected with vaccination, during the period 1886 to 1891, made by Dr Ogle, shows that erysipelas is credited with almost one-half of the total number of deaths, and if we add to this the class which includes pyaemia, septicaemia, and also a number in which severely inflames arms occurred, we shall find that two thirds of all the deaths in connection with vaccination have been connected with inflammatory or septic causes. The Commissioners on Vaccination in their Final Report say, "An examination of the particulars of the cases of alleged deaths and injury from vaccination, to which our attention has been called during the last six years, shows that the death or injury has been attributed in the great majority of cases to one or other of these diseases (erysipelas, ulceration, abscess, pyaemia and septicaemia), and chiefly to erysipelas."

Inflammatory and septic complications are then, as we should expect, the most common complications of vaccinia, and of these erysipelas is the chief.

**ERYSIPELAS and VACCINATION.**

This term is used, I think, by the majority of medical men to designate an acute infectious inflammation, of a cutaneous or mucous surface, the great characteristic of which is a tendency to spread, mostly by continuity, and which is associated with general
febrile symptoms. Its immediate cause, as was first shown by Fehleisen, is a streptococcus, in all probability identical with the streptococcus pyogenes.

Some writers have included under this term various forms of spreading sub-cutaneous inflammation, and although many of these are probably allied to, if not identical with it, the above description will best serve our purpose.

Its most important predisposing condition is a wound, often indeed a mere abrasion of the skin, such as is made by scarification as in vaccination. Certain people are especially liable to attacks, while, on the other hand, experiments seem to indicate that certain individuals are immune to it.

Of the 142 children whose deaths were alleged to have been caused by vaccination, 113 were attributed directly to erysipelas, arising in connection with the vaccination.

Before we can, however, enter upon an enquiry into the truth of this allegation, two preliminary subjects demand our attention.

**IS ERYSPIELAS IDENTICAL WITH VACCINIA.**

In the first place it is necessary to mention the view propounded by certain writers on this subject. It has been asserted that the erysipelatous virus is inherent in vaccine lymph however obtained,
and that erysipelas is inoculated whenever vaccination is performed. The authority of Jenner and the early vaccinators is claimed for this statement, on the ground that he described "Erysipelas inflammation" as characteristic of the true, as opposed to the spurious, cow-pox.

Jenner doubtless seems to have inclined towards the view that erysipelas and vaccinia were allied to one another, for he gives an instance in which he believed that true cow-pox was conveyed to a herd of cows and communicated to the milkers by "an extensive inflammation of the erysipelatous kind, appearing without any apparent cause upon the upper part of the thigh of a suckling colt." Jenner may indeed have believed that the two diseases were identical; but surely no one will assert that this proves them to be so, and ask us to limit the pathological knowledge of today to statements made in 1799. Jenner probably had more reason for this belief than we have, for, I think, I am justified in saying that the symptoms which accompanied vaccination were more serious in his day than in ours, because of the insanitary and dirty conditions under which it was so often performed, and probably also because the first strains of lymph were more virulent than now.

It is true, as I shall have to show more fully again, that erysipelas sometimes attacks an arm after vaccination, that the specific virus of erysipelas has been introduced by means of vaccination, and that sometimes it is most difficult, or impossible, to define
precisely the boundary line between normal areolous inflammation and erysipelatous inflammation, yet none of these facts, nor all of them taken together, prove the identity of the two diseases.

The following facts, the evidence for some of which I shall give later, sufficiently disproved the contention that the two diseases are identical:

I. Spreading inflammation of the arm from the vaccinal wound is only of very occasional occurrence, and is then characterised merely as a "complication".

II. Erysipelas is caused by a specific micro-organism, and if these are inoculated with the lymph, spreading inflammation of the cutaneous tissues will most probably result. This micro-organism has only occasionally been demonstrated in vaccine lymph.

III. Vaccine lymph can be freed from the pathogenic and saprophytic micro-organisms usually found in it without impairing its efficacy as a vaccine agent.

IV. No one has attempted to prove that the areola which forms round healthy vesicles contains the streptococcus of erysipelas or that it can communicate erysipelas to others.

THE INCUBATION PERIOD OF ERY SIPELAS.

The second preliminary subject which calls for investigation is the length of the incubation period of post-vaccinal erysipelas.

This is very uncertain. Fehleisen, as a curative
measure, inoculated seven patients, the subjects of malignant tumour, with the streptococcus erysipelatus, and found that the incubative period ranged from fifteen to sixty-one hours. The method he employed was to make superficial scarifications, and then to rub in the pure culture.

Ritzmann states that it varies from eight hours to two days; while Pujos considers that it is from one to eight days.

A large number of cases of erysipelas occurring in connection with vaccination, in which the source and time of inoculation were more or less accurately known, have been investigated and published by the Medical Department of the English Local Government Board, for the information of the Vaccination Commission and for other purposes; and an analysis of this material will probably help us to arrive at a working theory regarding the incubation period of, at least, post-vaccinal erysipelas.

We shall, however, probably find it difficult to arrive at an exact determination of this question, because the results of the action of the contagium of erysipelas on any individual organism will depend, as will other contagia, upon not one but many factors. The following factors must all be taken into account in determining this action and its results:

I. The quantity of the contagium.
II. The virulence of the contagium.
III. The state of health of the individual infected.
IV. The idiosyncracy of the individual.

V. Probably also the concurrent introduction of some other contagia, or its presence in the system. This may act in one of two ways. In the first place its growth may be absolutely inhibited, or merely minimised; and in the second place, its growth may be more or less promoted and intensified.

In the following pages, I shall examine groups of cases of erysipelas following vaccination in which there was opportunity for the introduction of the contagium locally either at the time of the vaccination or on the eighth day when the vesicles were opened or when the child was inspected.

I. The first series of cases we select from the 205 already mentioned. From these I select only forty undoubted cases of the disease, and of this number not fewer than twenty developed erysipelas during the first week after vaccination, viz.: -

First day . . . 1
Second day . . . 3
Third to fifth day 4
Sixth day . . . 2
Seventh day . . . 2
Eighth day . . . 8

Eleven of the forty showed symptoms of illness during the second week, i.e. after inspection, viz: -

Ninth day . . . 2
Tenth day ... 2
Eleventh day ... 2
Thirteenth day ... 1
Towards end of the week, or particular day not ascertained ... 4

Of the remaining nine cases, seven developed the disease during the third week, and the other two later.

It is impossible here to discuss each of these cases separately; but a careful examination of the published records of the twenty cases which occurred during the first week after vaccination, forces one to the conclusion that they were in all probability infected on the day of vaccination. No other source of infection seems to have been possible; and the following facts also, I think, confirm this opinion. Among these twenty cases, there were eight in which the co-vaccinees (i.e. those vaccinated with lymph from the same source) suffered, and thirteen in which the subsidiary co-vaccinees (i.e. those vaccinated by the same person at the same time, but not with lymph from the same source) suffered from erysipelas.

The date of infection of the eleven cases in which erysipelas commenced during the second week, cannot, with three exceptions, be determined with any degree of accuracy. One of the cases which commenced on the eleventh day, was probably infected on the day of vaccination. The child lived in healthy surroundings, the vesicles were not interfered with in any way, and were not opened on the day of inspection, and on that occasion
occasion there was no apparent source of other infection. Altogether, the facts of the case convince us that here we have an example of delayed incubation. Two other cases opened on the eighth day, and second and third days after respectively, developed erysipelas, apparently from inoculation on that occasion.

No reliable opinion can be expressed regarding the remaining forty cases in this series.

II. A series of twenty-six cases which was reported from Henstead to the Vaccination Commission (21) is specially important from our present point of view. All of the vaccinations in this series were performed by a Locum Tenens to the Public Vaccinator on four different days and at four different stations. Accidents in the shape of erysipelas, burst vesicles or inflammation occurred in every one of them, and two of the children died in consequence. Almost criminal carelessness was shown by the vaccinator. Many of the vaccinifiers had inflamed arms, the instrument employed was also used for other purposes, and was not cleansed between the operations. After careful enquiry, it was ascertained that the first indications of mischief were observed on the following days - on or after vaccination:

- On the 1st day ... 4 cases.
- On the 2nd day ... 5 "
- On the 3rd day ... 6 "
- On the 4th day ... 2 "
- On the 5th day ... 3 "
On the 8th day ... 2 cases
On the 9th day ... 2 "
On the 10th day ... 1 case.
Uncertain ........ 1 "

Thus it was ascertained that the mischief commenced during the first week in twenty cases, and during the second week in five cases. The arms were inspected on the eighth day, and on that day one of the five which subsequently developed erysipelas had burst or broken vesicles, and in two other cases the vesicles were opened and the lymph used for vaccination purposes. No information is given, unfortunately, regarding the co-vaccinees.

The facts in this series seem to indicate that twenty of the cases, at least, were inoculated at the time of vaccination, and that all of them developed erysipelas on or before the fifth day. I am also almost convinced that the case which presented itself with burst vesicles on the eighth day and which subsequently developed erysipelas had been inoculated with the others on the first day, although we cannot use it to assist us in determining the length of the period of incubation, as it was again exposed to infection on the eighth day. The other two cases in which the vesicles were opened, were also again exposed to infection, and, probably the same should be said of the remaining two in which the vesicles were not opened.

On the other hand, however, it is not improbable
that they were also inoculated on the day when vaccination was performed; but that the number of organisms originally introduced was so small, or the resistance so strong, that the patients were able to withstand the onset of the disease longer than the others; and of course, it may also be argued that more organisms were introduced on the eighth day, especially in the cases in which the vesicles were ruptured or opened.

III. In this series, which occurred in Thingoe, there are very good reasons for believing that the vaccinator was personally infected, and probably also his clothing, and that while in this condition he infected a number of the children which he vaccinated.

Ten hours previous to the date of the first vaccinations, the vaccinator had been dealing with the septic body of a person whom he had just previously attended. On this day he vaccinated nine persons, seven with human, and two with calf, lymph. On the eighth day all appeared to be doing well; but on the ninth, and twelfth days, and during the course of the second week, three of the children were attacked by erysipelas; two of whom died.

These three cases were the only ones of the nine whose vesicles were opened on the eighth day.

On this day he vaccinated eleven children at the same station, and used lymph from the children who subsequently developed erysipelas, as well as from other sources. Of these children one developed erysipelas on the fourth day, two on the eighth day. All the
others did well.

This series, which includes the two sub-series just mentioned, is, I think, very important, for it seems to indicate that the virus of erysipelas may apparently lie, as it were, latent for nine or twelve days.

In the first sub-series three children out of nine vaccinated did badly, and although it was said that on the eighth day "they appeared to be doing well", it was subsequently ascertained, however, that one of them at least had been very unwell during the first week. Lymph was used from each of these three children, on the eighth, when "apparently well"; with the result that abnormal symptoms developed in a certain number of the sub-vaccinees from each case.

I am firmly of opinion that in this sub-series we have an example of the prolonged incubation of erysipelas, and that when they were brought to the vaccination station on the eighth, they were incubating that disease, as lymph taken from them seems to have had the power of inducing the same disease in others. This, I think, is a more reasonable explanation, than that which supposes that the vaccinator had retained his personal infectiveness for eight days, and that he was more liable to affect children on that day than on the day when he was first in contact with septic material.

The evidence of the second sub-series seems clearly to point to the conclusion that the three children
who suffered out of the eleven vaccinated, had been inoculated from the three children of the first sub-series, and that the inflammation commenced in them respectively on the fourth and eighth days.

If we conclude that the fourteenth October was the day of the vaccinator's infectiveness, and that he was free from infection on the twenty-first, we arrive at the incubation period for the six cases as follows:

1 case . four days
2 cases. eight days.
1 case . ninth day.
1 case . twelfth day
1 case . during second week.

The last case mentioned above should probably not be included in this series. The erysipelas was very slightly marked, while the most pronounced symptom was an axillary abscess.

There is another feature of great interest and importance in this series of cases. On the 14th December when the vaccinator was in a condition of personal infectiveness, he vaccinated nine children, only three of whom suffered from erysipelas, while the other six wholly escaped. All must have been equally exposed to infection, and probably all were infected more or less, yet only one third of the whole number suffered from the disease. How did the other two thirds escape?

Again on the 21st eleven children were vaccinated mainly from three children who at the time seem to have
been incubating erysipelas, and who subsequently developed this disease, and yet only three of the sub-vaccinees developed erysipelas. Some of the others, at least, must have been inoculated. How did they escape? Our present knowledge of the conditions which determine the growth and interaction of bacteria and living tissue, and the relation of this to disease, does not enable us to give a definite answer to this question. All we can say at present is, that probably the children who escaped either were inoculated with a smaller number of the specific organism, or that their tissues formed a less suitable nidus for their growth and development, or probably, both causes contributed to the final result.

IV. A fourth series of cases was reported in 1878 by the Plomesgate Union. It relates altogether to twenty-eight vaccinations.

There was in the district at this time a case of epilepsy, with which erysipelas was associated. There were only two medical practitioners in the district, — the Public Vaccinator and his partner, and although the case of epilepsy was mainly under the charge of the latter, the Public Vaccinator also visited it on more than one occasion, and in this way he (or his clothing) became personally infective. The patient died on October 3rd, the day on which the first three of the series of twenty-eight vaccinations were performed with lymph (human) from the National Vaccine Establishment.
It will be instructive to make a detailed study of these cases, as they seem to indicate that a pathological relationship exists between some of the abnormal conditions which are found in association with vaccination, and also, I think, shows that abnormality is not always characterised by a gross lesion; but is also shown by other circumstances, such as delay or imperfection of the vaccine vesicles, tenderness and a tendency to early and ready rupture.

On October 3rd three children were vaccinated by the Public Vaccinator, with the following result:

First Child. (1) In the second week the vesicles were inflamed and discharging; and two months afterwards the scabs had not separated.

Second Child. (2) Only one third of the insertions succeeded, and on the night of the eighth day the arm began to inflame, and the child was restless. Towards the end of the second week erysipelas began on the left side of the head, and spread to the forehead and the side of the face, and this was followed by an abscess over the left scapula.

Third Child. (3) On the eighth day there was a very decided areola with broken vesicles.

On October 10th the vesicles in all three cases were opened and the lymph used for vaccination purposes with the following result:

(1) The lymph from this case was used for two children (a and b).

(a) On the eighth day the arm was much inflamed,
and on the seventieth day severe erysipelas, which subsequently spread largely and proved fatal.

The lymph from this child was used to vaccinate two children on October 17th, both of whom developed erysipelas. In one of them the inflammation began in the course of the first week, and erysipelas was definitely established on the eighth day, and was associated with abscess in the pectoral region. This child recovered. In the other child erysipelas developed on the sixth day, and then rapidly spread and proved fatal.

(b) On the eighth day the arm was inflamed from the elbow upwards, and about the third week an axillary abscess developed.

The lymph from this case was employed in vaccinating four children, with the following results:

(1) No inflammation; but sores formed which were long in healing.

(2) "Places never rooved over rightly, but became wounds that discharged; skinned over and broke out again."

(3) On the second day the arm was discharging, and later was much inflamed.

(4) On the eighth day the vesicles were discharging, and inflamed, and secondary sores formed on the shoulder and arm.

(II) The lymph from this case was used for three children (a, b, c).

(a) In the second week the arm was said to be
much inflamed. No lymph was taken from this patient.

(b) Arm much inflamed in the second week.

Lymph from this case was used to vaccinate one child, in whom erysipelas developed on the third day, and extended to the fingers.

(c) In the second week the arm was much inflamed, and there was purulent discharge from the vesicles. Secondary pustules followed.

Lymph from this patient was used to vaccinate two children, in one of whom the vaccination was quite normal; but in the other there was much inflammation during the first week.

(III) Lymph from this case was used to vaccinate one child, whose arm on the eighth day, was much inflamed, and more so later. (Most probably a case of erysipelas.)

On October 10th, other seven children were vaccinated at the same station with the remainder of the National Vaccine lymph which had been used on October 3rd:

I. Arm inflamed on eighth day, subsequently the inflammation extended, and secondary pustules formed round the vesicles.

II. Much inflammation in the second week, with swelling under axilla.

III. Apparently normal on the eighth day, but subsequently the arm inflamed to the elbow and shoulder, and pustules formed round vesicles.
Two children were vaccinated with lymph taken from this case, in one of whom erysipelas developed on the second day, and ultimately proved fatal. In the other erysipelas developed on the seventh day (the vesicle was reported to have been scratched on this day), and extended to neck and back. The child recovered.

IV. Erysipelas in second week.
V. Course normal.
VI. Course normal.
VII. During the first week the vesicles broke down and discharged, and the following week the arm was much inflamed, and a large ulcer formed.

One child was vaccinated with lymph from this case, and although the vesicles rose prematurely, there was not much inflammation.

It is, I consider, beyond dispute that this series of accidents and deaths, was directly caused by infection conveyed at the time of vaccination. There were two possible sources of infection. The Public Vaccinator had, admittedly, been in attendance upon a case of erysipelas, and when he performed these vaccinations on October 3rd and 10th, he was probably personally infected. On the other hand, however, the lymph received from the National Vaccine Institute may have been taken from a vaccinifer who was potentially, if not actually, erysipelatous, i.e. who was at the time incubating the disease.

It is impossible to decide which of these sources
is to be blamed, or whether both had a share in the result. The important point from our present point of view is that the infection was conveyed at the time of vaccination, and thus it is possible for us to determine approximately the length of the incubation period in the different cases.

In this series of twenty-eight cases, there were twenty-five cases of vaccinal injury, eighteen of which, at least, appear to have been erysipelatous. Of these eighteen cases, eleven developed the disease during the first week, and seven during the second week. The cases occurring during the second week may have been inoculated on the day of inspection, when some of the vesicles were opened; but, in view of the fact that an equal number of the cases which subsequently developed erysipelas were untouched on the eighth day, I think we are justified in concluding that in a certain number of cases the incubation period was prolonged to from ten to twelve days.

The above facts justify the conclusion that although the usual incubation period of erysipelas is from two to three days, yet in some cases, it may be prolonged to from eight to twelve days. The great majority of cases of vaccino-erysipelas occur during the first five days after vaccination; and a large number also manifest themselves on or after the eighth day. Cases occurring at this later period appear to have, as a rule, a shorter period of incubation than those which occur immediately after vaccination.
THE SOURCES OF INFECTION.

In the course of my inquiry into the length of the incubation period of post-vaccinal erysipelas I have indirectly shown that the lymph used or the method of vaccination was the cause of the disease in several cases.

We shall now enquire more systematically into this aspect of the question.

I have already stated that whilst investigating the composition and bacteriology of calf and human lymph, I examined a specimen of calf lymph which showed numerous chains of streptococcus pyogenes. Agar tubes were inoculated with this lymph, and numerous typical colonies of the same bacillus resulted. Dr Monckton Copeman, Dr Klein, and other observers have also, on several occasions, found the so-called streptococcus erysipelas in different specimens of calf and human lymph. I should also add that in one specimen of human lymph examined by myself, and obtained under antiseptic precautions, the microscopical examination showed several apparently typical chains of streptococci; but that no growth of this coccus resulted when agar plates were inoculated with the lymph.

We may therefore conclude that both calf and human lymph collected under ordinary conditions, sometimes contain the specific contagium of erysipelas.

The other possible sources of infection, apart altogether from the circumstances of the children after vaccination, are:
I. The infectiveness of the vaccinator, clothes or person.

II. The infectiveness of the instruments used.

III. The infected condition of the station.

IV. The infective condition of other children or adults present on the day of vaccination.

V. Similar infective conditions on the day of inspection.

VI. The condition of the child's arm.

Among the 205 cases of alleged injury which have formed the main subject of my enquiry, we have examples of infection from each of these sources, some of which I have already mentioned, and also others which are not included in this number.

The evidence justifies us in concluding that 150 of these 205 cases were of a septic or inflammatory nature: and of these 118 were erysipelatous in character. Inflammatory symptoms manifested themselves in most of these cases during the first and second week. The number commencing during each of the weeks succeeding vaccination being distributed as follows:

Commencing in the first week .. 42.
" second " .. 41.
" third " .. 20.
" fourth " .. 13.
" fifth " .. 4.

Undetermined ............. 3.

With one or two exceptions, the cases of erysipelas which began during the first week, appear to be
due to one or other of the causes mentioned above; and about sixteen or more than one third of the number which commenced during the second week, were due to the same causes.

In thirty-two of these cases, the evidence distinctly shows that either the lymph or the vaccinator was at fault. I shall quote two cases in which it was shown by bacteriological examination that the lymph was at fault. Two children (cases XXXI and LXV in the IX Appendix to the Report of the Royal Commission) were vaccinated at different dates by the Public Vaccinator of H - Union from lymph stored in tubes. The lymph apparently had been taken from the arm of a child in whom vaccination had run an abnormal course, and in whom erysipelas had supervened. In the first child, erysipelas commenced on the third day, and by the eighth day it had spread over the vaccinated arm, the chest, side of the head and opposite arm; on the fifteenth day the child died. In the second case the first vaccination did not take; but seven days afterwards it was again vaccinated from the same stock of lymph. By the fourth day erysipelas was well marked, and before the death of the child on the twenty-second day, it had spread over the greater part of the child's body. No cause, other than the lymph, could be ascertained. Two tubes of the lymph used were submitted to Dr Klein for examination: - (1) unopened, and (2) one partially used, it was believed, for vaccinating the second child. Dr Klein reported that
the lymph from the first tube showed on cultivation, colonies of "staphylococcus pyogenes albus liquefaciens". The original lymph produced no inflammation on the ears of rabbits. The colonies from the second tube of lymph "were all streptococcus of erysipelas" and very numerous. Inflammation was produced on a rabbit's ear by inoculation with the original lymph, and with sub-cultures of the micro-organism. Other similar cases might be quoted from the same report, and also from other sources; but the above is sufficient to show, I think, that lymph contaminated with the virus of erysipelas may be expected to show the results of the inoculation of such organism, if used in vaccination.

I do not, however, assert that it will invariably show such a result; for I have already shown, that some children, in a manner unknown to us, sometimes escape this evil consequence.

A series of three cases which occurred in the practice of Mr H - a Public Vaccinator, may be given as illustrations of those cases of invaccinated erysipelas caused by the personal infectiveness of the vaccinator. Two of the children were vaccinated on the 14th November 1889, and the third a week later. In each of these three cases erysipelas developed, from which the children died. No other cause, except the one about to be mentioned, could be assigned. Up to the 14th October Mr H - the Vaccinator, had been attending a case of psoas abscess, with "discharge of such a virulent nature that the nurse suffered severe-
ly from abscesses, undoubtedly caused by it, and he himself ---- contracted several angry phlegmons on his hand". On the morning of the 14th October he was attending this case until 2 a.m., and he commenced his vaccinations at 1 p.m. on the same day. No one can doubt that the infection in these cases was conveyed by the operator. Several other children, vaccinated at the same time, and under similar circumstances, suffered from erysipelas, but subsequently recovered. Other children apparently exposed to the same infection on the same days, wholly escaped.

In treating of the incubation period of erysipelas, I have already drawn attention to other possible sources of infection connected with the methods, or place, of vaccination, such as the condition of the instruments used, the insanitary condition of the station, the unhealthy condition of children or other persons present, and upon these I need not again enlarge. It should be noted, however, that these conditions affected each child not only on the date of primary vaccination; but also, and perhaps more especially, eight days later, when the patient again appeared for examination, and especially if the vesicles are interfered with in any way. A certain proportion of the cases in which erysipelas first appeared during the second week, appear to have been due to the invaccination of septic material at the time of primary vaccination; but a still larger number of them are due, I believe, to renewed exposure to infection on the eighth
day. During the first week after vaccination, most cases of erysipelas develop on the second or third day, and fewest on the sixth day; and during the second week the maximum is reached on the ninth day after vaccination, and the minimum on the fourteenth day. No other general cause, except the second exposure to infection, can be given for this remarkable result. The chief source of danger is, I consider, the interference with the vesicles on the eighth day. In private practice the risk of infection from this operation cannot be great; but it is very different in public practice. The stations are often very insanitary, or at least are rendered so by the patients who frequent them.

In forty-three other cases in which vaccination was followed by erysipelas during the first three weeks there was evidence to show that there were sources of danger apart from the lymph used, or the method or place of vaccination.

It is unnecessary to quote cases illustrative of these sources of danger; an enumeration of some of them will conclusively show that they constitute a very real risk:

I. Exposure to erysipelatous or other septic infection.

II. Improper management, e.g. the application of cream with a feather which had been "picked up in the back yard".

III. Unwholesome or filthy surroundings.

IV. Exposure to infective fevers.
V. Illness or delicacy of vaccinee.
VI. The use of "shields".

Before concluding the consideration of erysipelas in relation to vaccination, it should be pointed out that erysipelas occurring in connection with vaccination is not different from erysipelas arising under other conditions. The records of foundling institutions, especially those of Vienna and St. Petersburg, and other facts, prove that vaccino-erysipelas may be communicated to other persons. Its essential cause is the same as in the case of erysipelas occurring under ordinary conditions.

CONCLUSIONS.
I. Erysipelas is the most frequent and serious complication of vaccination.
II. Its occurrence is comparatively infrequent in proportion to the very large number of vaccinations.
III. Its essential cause, as in ordinary surgical erysipelas, is the streptococcus pyogenes.
IV. This organism has been demonstrated in calf and human lymph in a small proportion of cases. Careful examination of a large number of specimens of ordinary vaccine lymph might, however, possibly prove that it is oftener present than past experiments have shown. The presence of a large number of other microorganisms, micrococci especially, makes it very difficult to detect and isolate the streptococcus pyogenes. If the lymph is only examined microscopically, and not
by cultures also, it will often be overlooked, especially if only a few are present.

V. In a certain number of cases, it has been invaccinated by contaminated lymph; but in a still larger number its invaccination has been due to carelessness on the part of the vaccinator, or through some fault in the method or place of vaccination. In a very large proportion of cases, the vaccinee has been infected by causes extraneous to the lymph, or the methods or place of vaccination.

OTHER SEPTIC COMPLICATIONS OF VACCINATION.

The most serious are ulceration, cellulites, abscess, septicaemia, and gangrene. Certain skin diseases might also be included, such as eczema, and contagious impetigo, as they are, in all probability, due to microbial infection.

It is almost impossible however to consider these separately from erysipelas. They are so frequently found in association with it, or in the same series, that one is tempted to conclude that they arise from a common cause. When we consider, however, that almost every specimen of calf or human lymph, even when collected under strict antiseptic precautions, contains numerous pyogenic cocci, it is most surprising that the cases of septic complications are so very few. Our knowledge of the bacteriology of vaccine lymph
would certainly lead us to expect that ulceration, abscess, pyaemia, etc., would more frequently complicate vaccination than erysipelas does. The streptococcus pyogenes has only occasionally been demonstrated in vaccine lymph, while I found the staphlococcus albus or aureus or both in almost every specimen examined, and frequently in very large numbers. They grow and increase rapidly in the lymph, and therefore we cannot conclude that the lymph in any way renders them less active. Possibly, however, the upper layers of the human skin are more resistant to the ordinary pathogenic cocci than they are to the streptococcus of erysipelas; or of the essential cause of vaccinia is a micro-organism it may have a stronger inhibitive influence upon these organisms than upon the streptococcus when vaccination has been performed.

It is difficult to form a correct estimate of the frequency of the occurrence of these diseases in connection with vaccination by the statistical method. A fatal result more frequently follows an attack of erysipelas in this connection than it does in the case of ulceration, abscess, etc., and, therefore, the rate of mortality from erysipelas is, to a large extent, a criterion by which we can estimate the frequency of this complication. It is different, however, in the case of the accidents under consideration, for in them the rate of mortality is but small in comparison to the frequency of the occurrence of the disease. An examination of the 205 cases of vaccinal injury from
which death resulted, will show that thirty-two of the deaths were caused by one or other of the above mentioned diseases, including one from tetanus. It should also be noted, however, that many of the cases of erysipelas were complicated by ulceration and abscess. But, on the other hand, a very large number of cases have at one time or another been reported of the occurrence of ulceration, abscess, impetigo contagiosa, etc. in connection with vaccination, and in which recovery took place. (By this I mean that the gross total reported during several years was large; and not that it was large in proportion to the number of vaccinations performed.) We are therefore justified in concluding that ulceration, glandular abscess, impetigo contagiosa and other septic diseases do, in a certain fairly large proportion of cases, complicate vaccination, and that in a very much smaller proportion of cases a fatal result occurs from these causes.

To what extent then is the vaccine lymph or the method of vaccination to blame for the occurrence of these injuries? That the lymph is to blame in some cases, and the vaccinator in others is beyond dispute. The facts which I have already adduced when considering the subject of erysipelas sufficiently prove this. But other causes are also at work, and they may shortly be arranged as follows probably in the ordinal of frequency:

I. The insanitary and dirty surroundings of the patients, and the carelessness of the mothers and
others, combined with unwise treatment and the use of unsuitable local applications. Under this we may also include the local prevalence of disease.

II. The unhealthy condition of the child.

III. The vaccine lymph, or the infectiveness of the vaccinator, either in person or instruments.

In illustration of the first set of causes, a few extracts from the cases recorded in Appendix IX to the Report of the Royal Commission may be given. They are recorded in each case under the heading "general conditions".

House "extremely filthy and badly kept" — surrounding — "filthy. There was no privy, no drainage. Slops stagnated in puddles close to the door; alongside a refuse heap, upon which excrement was deposited" — "extremely poor, dirty and squalid", — "child illegitimate" — vaccinated when three days old in a workhouse infirmary, from which it was removed in two weeks" — application of "cold bread poultices and cream to the arm" — child sucked at left breast while the "mother had at the time discharging abscess on her right breast" — "lotions, Fuller's earth, starch, violet powder, and cream in turn applied to the vesicles," — "dirty shield used", — "arm rubbed with a dirty dyed sleeve," — "measles and scarlatina prevalent," — "scarlet fever in same row of houses," — "five other children one of whom was suffering from tubercular disease."

With regard to the second cause, it may be noted
that Balzar, Wheaton, and Dyke Acland have recorded cases of gangrene at the point of vaccination, which occurred after vaccination in syphilitic children.

It is also a well known fact that the great majority of non-erysipelatous septic complications occur in badly nourished, delicate children.

We come now to the consideration of the third cause. It has already been shown how the vaccinator may become the agent, apart from the lymph, in introducing infection at the time of vaccination. Only one other question still remains for consideration; is it possible to cause these septic conditions by means of vaccine lymph? Clinical experience seems most strongly to indicate that it is; and this opinion is, I consider, strongly suggested by bacteriological evidence. As already shown, numerous bacteria are to be found in all specimens of vaccine lymph when taken from the child or calf. Examination of both kinds of lymph shows that the staphlococcus aureus and albus are more frequently found therein than any other micro-organism. And these are just the organisms which, as a rule, are found associated with ordinary septic conditions, similar to those under consideration. It has also been shown that if the staphlococcus pyogenes aureus is injected under the skin of one of the small rodents, an abscess will arise at the seat of inoculation, and general infection may follow. Again, this organism, if rubbed into the human skin will cause a regular crop of cutaneous furuncular abscesses. The
same organism is also found in many skin diseases, especially in those which are accompanied by suppuration; in acute abscess; acute osteomyelitis, and other diseases. The staphlococcus pyogenes albus is found in similar positions, and under similar conditions. It is more frequently found on, or in, the skin, and the staphlococcus epidermidis, as described by Welch, is probably identical with it. The staphlococcus pyogenes albus is, however, considered to be only slightly pyogenic, and its action in producing abscess formation when injected subcutaneously, or of setting up pyaemia when injected into the circulation is considered inferior to that of the staphlococcus pyogenes aureus. Welch considers that this organism is the usual cause of the small abscesses which form around tense skin stitches.

In view then of these facts we are compelled to conclude, that such accidents of vaccination as ulceration, abscess, contagious impetigo, osteomyelitis and the septic intoxications, when occurring in connection with vaccination, although more frequently due to, or connected with, other causes, are in a certain proportion of cases, directly caused by invaccination with the vaccine lymph, of the pyogenic micro-organisms which it contains.

Tetanus has also occurred as a complication of vaccinia. Acland states that it has been known to follow vaccination in South Africa in a series of adult natives, who immediately after being vaccinated,
worked under a tropical sun. One case of this disease is recorded in Appendix IX to the Report of the Royal Commission. It occurred in a female child aged two months, twenty-three days after vaccination. No blame could be attached either to the lymph or to the method of operation; there was, however, a possible source of infection in the neighbourhood of the patient's house.
TUBERCLE and VACCINATION.

The assertion that it is possible to inoculate tuberculosis by vaccination is supported by very little evidence.

M. Degive stated at the Congrès pour l'étude de la Tuberculose, at Paris, in 1889, that it had been proved that the vaccine pustule could transmit tuberculosis. This statement was found to rest upon two series of experiments performed respectively by three Greek physicians and by M. Toussaint.

The three Greek experimentors inoculated two rabbits with lymph taken from a vaccine vesicle from a person suffering from advanced phthisis. In one of the rabbits a tubercle was noticed at the point of inoculation at the end of twelve days, and in five weeks there was generalised tuberculosis.

M. Toussaint inoculated the vulva of a tuberculous cow with lymph taken from a typical vesicle on a healthy child. On the seventh and eighth day he took lymph from this cow, and with it inoculated four rabbits, a pig, a cat and a pigeon. The cat and pigeon, when killed on the sixtieth day, showed no signs of tuberculosis. All of the rabbits were found to be tuberculous; and the pig when killed 142 days after inoculation, was found to have suffered from wide-spread tuberculosis.

These experiments stand alone and are entirely unverified by other investigators. In themselves, too,
they are open to serious objections, as the methods employed were open to several sources of fallacy. No weight can be attached to the first series by the three Greek physicians. No details are given, and the number of experiments performed were far too few whereon to base any reliable conclusion.

Toussaint's experiments are also unreliable. The cow used was tuberculous, and the vaccinations were made on a part most liable to be contaminated by excreta or otherwise.

No reliable evidence, therefore, is forthcoming in support of the thesis that lymph from a tuberculous person or cow can directly convey this disease.

On the other hand, however, a large mass of evidence seems to point most strongly towards an opposite conclusion.

Cases of localised tuberculosis have occurred after tattooing and post-mortem cuts; but it is probable that in these cases the knife penetrated the skin so as to infect the subcutaneous tissues, and other influences were also at work. Cruickshanks says "the possibility of the inoculation of skin wounds (by tubercle) is open to doubt."

Professor Chauveau endeavoured in five cases to produce tuberculosis by means of inoculation through the skin, but in every instance the results were negative.

Josseraud, Lothar Meyer, Straus, Peiper and Acker
have conducted numerous experiments to ascertain whether the lymph taken from persons obviously suffering from tuberculosis contains the tubercle bacillus, or is capable of transmitting tuberculosis.

Josseraud obtained lymph by vaccinating individuals who were undoubtedly suffering from tuberculosis, and then with the lymph thus obtained, rabbits and guinea-pigs were inoculated by injections into the peritoneal cavity, under the skin, and into the anterior chamber of the eye. Forty-seven animals were experimented upon, and the post-mortem examination gave negative results in forty-three cases; one only had enlargement of the glands near the point of inoculation. In no case was conclusive evidence of tuberculosis obtained.

Similar results were obtained by the other observers.

Lothar Meyer (Medical Times, 1885) vaccinated with antiseptic precautions, five patients suffering from advanced tuberculosis; and from these patients lymph was taken from the seventh to the thirteenth day and most carefully examined for tubercle bacilli, and in every case with a negative result.

Dr Schmidt inoculated rabbits and guinea-pigs with tuberculous matter by scarification, in a manner similar to what is followed in vaccination, but in every case had a negative result; while "control"animals which had been inoculated by the similar material by
injections into the peritoneal cavity were found on post-mortem examination to be widely infected.

Poole also performed analogous experiments with a similar result.

The Royal Commission on Vaccination caused enquiries to be made into numerous cases of alleged accidental infection of tuberculosis by vaccination, and in not a single case could it be shown that the vaccination of tubercle had taken place.

Because cows are well known to be most susceptible to tubercular disease, it has been thought that calf lymph would become a potent means of disseminating tuberculosis. A very little consideration will serve to show that this suspicion is entirely unfounded. Vaccine lymph is not obtained from cows, but from calves, and in the majority of cases from very young calves, and it is a well known fact that calves are very little liable to this disease. In Augsburg only one tuberculous calf was found amongst twenty-two thousand two hundred and thirty which passed through the public abattoir. Först also states that only one case of this disease occurred among thirty-four thousand four hundred calves. And even this small danger may be avoided either by not using the lymph until a post-mortem examination of the calf has been made and proved satisfactory, or by the use of Koch's Tuberculin test, which is now regarded as most reliable for diagnostic purposes. Another means of precaution will be point-
ed out later.

We cannot deny the possibility of the invaccination of tubercle, but the risk of this accident is so very slight that we need hardly take it into practical consideration. If vaccination is properly performed, it seems almost impossible that it should become a means of disseminating tuberculosis.
LUPUS and VACCINATION.

Several cases are on record in which it is stated that lupus had affected the seat of vaccination at varying times after vaccination; but in all of them it is most difficult to determine whether this was due to the invaccination of the tubercle bacillus, or whether it was due to infection acquired in another way.

As already mentioned, a localised form of tuberculosis has been produced by the operation of tattooing, and post-mortem room porters have also suffered in a similar way. No fair comparison can, however, be drawn between such cases and vaccination. Tattooing is a very much more serious operation than vaccination. The area covered is usually much greater, and the risk of subcutaneous infection is also more serious. Post-mortem room porters are, on the other hand, constantly exposed to infection, and the hand and fingers upon which the growths occur, are not unfrequently the seat of deep cuts and abrasions.

Jadassohn (quoted by Morris) reported a case in which lupus was directly inoculated. A woman was tattooed on the fore-arm by a man suffering from pulmonary tuberculosis, from which he afterwards died. This man used his saliva to dilute the ink; and typical lupus nodules appeared on the tattooed parts.

One case (IX Appendix, p.242) was reported to the
Royal Commission on vaccination, and investigated by Dr T. Dyke Acland. M. D. was vaccinated in June, 1890, in four places; two of the wounds did not heal for two years, while the other two were not completely healed seven years afterwards. At this date there was on the upper and outer side of the left arm an undoubted zone of lupus which measured $3\frac{1}{4}$ by $3\frac{2}{3}$ inches. There was no history of tuberculosis in the family. The source of the lymph used could not be traced. Dr Acland concludes that the case is one of lupus chiefly affecting the skin, but that there is no evidence to show whether it was invaccinated, or merely an intercurrent disorder attacking a weak spot."

Mr Hutchinson examined several reputed cases of lupus following vaccination, and concluded that there was not sufficient ground for believing that lupus resulted directly from vaccination.

M. Vidal states that numerous attempts to transfer lupus by inoculation from one person to another have failed. This risk must be exceedingly small, for it is difficult to conceive how the essential etiological factor of lupus can gain an entrance into the lymph; certainly only one or two investigators have demonstrated its presence in a few samples, out of a very large number examined. On the other hand, predisposed children after vaccination must be more susceptible to infection than before, for it is a well known fact that scars and lesions often are the start-
ing points of the disease. According to Besnier, "slight injuries, burns, sores, blisters, infantile eczema, are very commonly the immediate cause of lupus." If this be true, it seems remarkable, not that one or two cases of lupus have followed vaccination, but that this result has not occurred in a much larger number of individuals.
LEPROMY AND VACCINATION.

The relation of leprosy to vaccination is not a point of very great practical importance in this country, but in several of our dependencies and in other places it is a subject of great interest and discussion.

The bacillary origin of the disease has not been quite definitely proved according to Koch's postulates. Notwithstanding this, few authorities hesitate to believe at present, that the organism which is almost constantly found in association with it is the true cause of the disease.

Is it then possible to spread this disease by means of vaccination? Not a few observers assert that it is, and they have brought forward cases in support of this view. Before this question has been satisfactorily answered, we must determine;-

(I) If the bacillus leprae has been found in lymph taken from an individual suffering from leprosy.

(II) If it is possible to spread the disease directly by inoculation.

With regard to the first point positive evidence is very scanty. Dr Edward Arning in 1884, vaccinated several lepers in Hawaii, but in no case did the vaccination take. In the following year he again repeated his experiments, but only in three cases did the vaccination take. Two of these were of the an-
aesthetic variety, and the other was tubercular. In the former two cases he failed to detect the bacillus leprae, but both the lymph and crusts from the tubercular case contained it.

Simpson of Calcutta has also described bacilli in lymph taken from lepers. In Almaru ninety-three specimens of vaccine lymph taken directly from lepers were examined by three members of the Indian Leprosy Commission, but only in three cases were bacilli found, and one of these was from lymph taken from a vesicle produced over a nodule, while another was from a vesicle formed on an anaesthetic patch.

Beaven Rake and Buckmaster also examined a large number of similar cases, most of which showed no trace of the bacillus. Although in most cases the bacilli have been found for the most part within the protoplasm of the round cells of the granulation tissue, a few have also been found in the hair follicles, in the glands of the skin, and occasionally in the surface epithelium. These facts point to the possibility of the development of the bacillus leprae in vaccine vesicles formed upon apparently healthy skin.

Opinion varies greatly on the next point; whether it is possible to spread the disease by inoculation.

Experiments on animals have in nearly all cases given a negative result. In some cases the bacillus leprae was found after inoculation in different parts of the body, but the evidence seems to support the view that they spread by diffusion and not by prolifer-
Inoculation of the human subject has been performed a considerable number of times, but the results were inconclusive or negative.

Arning inoculated an apparently healthy convict, and three years afterwards the man developed leprosy. This case, however, although at one time regarded as conclusive, is not so, for it has since been found that several members of his family - a son, nephew, and maternal cousin, have also developed leprosy. Danielssen, Bargilli, Holst, Tilsch, and Profeta have also made similar attempts, but with a negative result.

If we regard leprosy as a bacillary disease, analogy would lead us to expect that it could be inoculated on the human subject; but up to the present time there is no positive evidence in support of this view. None of the experiments which have been made have been performed under proper conditions. For an experiment of this kind to be absolutely conclusive, it should be made upon a subject who could never have been in contact with the disease, and in a country where leprosy is not endemic. And even then inoculation would probably require to be performed by means of deep incisions, and by a method quite different from what is required in vaccination.

It is unnecessary here to enter into the question of the contagiousness of this disease. That it is contagious may be taken as proved, although at present it is difficult to determine under what conditions, and
in what manner it becomes so. In its spread, other factors, such as heredity, bad sanitation, climatic influences, unwholesome and putrid food, etc., are also probably at work.

We come now to the question, is vaccination to be regarded as an etiological factor in the dissemination of leprosy? Bacteriologically we are compelled to regard this as a possibility, as the bacillus leprae has been demonstrated in a few specimens of lymph taken from leprous subjects. Inoculation experiments, however, tend to prove that the invaccination of this disease is not possible.

For many years the invaccination of leprosy has been regarded as a possibility, and many observers in different parts of the world have collected and published cases and statistics bearing on this subject.

In 1867 Sir (then Mr) Erasmus Wilson in the Appendix to the Report on Leprosy of the Royal College of Physicians, London, alluded to the possibility of the invaccination of leprosy.

Dr Gavin Milroy (Report on Leprosy and Yams in the West Indies, 1871) gave a resumé of the answers which he received to the following interrogations from large numbers of West Indian Medical practitioners: - "Have you any reason from personal experience or observation to believe that leprosy has ever been communicated by vaccination? Or do you know of any authenticated instances of such connection?" Not one of
the medical practitioners to whom the questions were addressed, knew personally of any such connection; and in nearly every instance, the answers received were in the direct negative.

Dr Arning observes that "there can be no doubt as regards the synchronousness of the diffusion of leprosy and the introduction of vaccination in the Hawaiian Isles," but he explains that the coincidence may be explained in other ways. He also, however, speaks of a very remarkable local accumulation of fresh leprosy cases which took place in 1871 - 2 at Lahaina, about a year after a universal arm-to-arm vaccination had been carelessly performed - fifty to sixty cases occurring, the place having been up to that time comparatively free from the disease.

Dr Beaven Rake, Superintendent of the Trinidad Leper Asylum, and a member of the Indian Leprosy Commission, alluded to this subject in several of his Reports from 1885 onwards; but concludes "that he is unable to produce any evidence in favour of the possibility of introducing leprosy by vaccination."

The well known case published by Professor Gairdner need only be referred to. The child of a medical practitioner living in one of the West Indian Islands where this disease was endemic, was vaccinated from the arm of a native infant, who subsequently, it was said, developed leprosy. Another white child was vaccinated from the doctor's child, and in after years
both became lepers.

Further enquiry into this case has amply shown how unreliable it is. Dr Gairdner in a subsequent letter says that the native child was "probably not an actual or apparent leper." Again no information is given regarding the time when leprosy appeared in the two white children, and the situation upon which it first developed; and during the time between vaccination and the development of leprosy both children were living in a leprous country, and therefore were exposed to infection.

Dr Swift of Molokai, Montgomery of San Francisco, Daubler of the Cape, Hillis in British Guiana, Chew in Calcutta, and several other observers have published cases in which there was some reason to suppose that leprosy had been invaccinated.

All the cases which have been published are, however, open to serious objection. All the patients reported upon had either come from, or resided in endemic areas, or in some other way had been exposed to infection. They had been in contact with lepers, or had to do with food which might have become contaminated by them.

At the present time we have no reliable positive evidence in support of the statement that vaccination is a means of the dissemination of leprosy; and such positive evidence cannot be obtained until vaccine lymph from undoubted leprous cases, has been used upon subjects who in no way have been exposed to infection.
from this disease.

We must, however, admit that there is a risk in using lymph from native children in endemic areas, and that leprosy in a few rare cases may have been invaccinated.

For the following reasons we believe that this risk is very small: -

(I) Many specimens of lymph from leprous patients have been examined, and only in a very few cases has the bacillus leprae been found.

(II) Calf-lymph is now extensively used in places where this disease is prevalent; or human lymph from non-leprous districts is imported.

(III) In Finland, Norway, Jamaica, and in some parts of India, etc., where leprosy is steadily declining, the practice of vaccination is increasing in greater ratio.

(IV) The large majority of lepers - about eighty per cent in India, - have not been vaccinated at all.
SYPHILIS and VACCINATION.

Of all the diseases said to be caused, or connected with, vaccination, syphilis is naturally regarded with the greatest abhorrence. Until quite recently many authorities regarded the inoculation of syphilis in this way as most improbable. We cannot now hold this opinion, for it has been definitely shown that the invaccination of syphilis is possible, and this is just what we would, a priori, expect; for, although the specific virus has not yet been isolated, analogy seems to justify the view that it is a microbial disease.

The inoculation of syphilis at the time of vaccination may be due to one of the three following causes:

(I) Vaccination by lymph taken from a syphilitic child.

(II) Contamination of the instrument or wound, either at the time of vaccination or subsequently.

(III) Personal infectiveness of the vaccinator.

The experiments\(^{(a)}\) of Dr Cory of the National Vaccine Institute, on himself, not only prove the possibility of the invaccination of syphilis from contaminated human lymph, but also provide valuable information regarding its clinical history.

On four occasions Dr Cory vaccinated himself with lymph from children known to be syphilitic. On the first occasion vaccination was successful, but the
vesicles matured early, and declined after the fifth day. No syphilitic trouble resulted. About two years later, he again vaccinated himself from a syphilitic child, but neither vaccinia nor syphilis resulted. On a third occasion the experiment was repeated with a negative result. On the fourth occasion he was vaccinated in three places from a child, the subject of obvious congenital syphilis. Extreme precautions were taken, especially to avoid the admixture of blood. On the second day the insertions were red, with small areolas, and the arm was quite healed in six days. On the twenty-first day, however, a red papule formed at two of the insertion points. These gradually increased until the thirty-fifth day, when a yellow spot appeared in the centre of one of the papules, and on the next day a scab formed, which in two days was removed, leaving a small ulcer. On this day Sir George Humphrey and Mr Hutchinson saw the arm, and both considered that the lesions were syphilitic. After this the diseased parts were removed, but five days later an enlarged and painless gland was felt in the axilla. On the forty-fifth day the lower wound was indurated, and some sloughing occurred. The pain in the axilla was severe, and the glands were enlarged and tender; and on the fiftieth day there was a distinct feeling of illness. Subsequently rheumatic pains were felt, the cervical glands became painful, a roseolous eruption appeared on various parts of the body. Later, tertiary symptoms manifested themselves, but ultimate-
ly succumbed to treatment.

The history of this case affords, I believe, a
typical history of the sequence of events in invaccin-
ated syphilis; and proves, once for all, that this dis-
ease can be inoculated at the time of vaccination.

The question now presents itself, is this acci-
dent of frequent occurrence in the ordinary practice of
vaccination?

The evidence brought before the Royal Commission
on Vaccination proves that in England the occurrence
of this accident is exceedingly rare.

Of the two hundred and five cases of alleged
deaths from vaccination reported upon by the Medical
Inspectors of the L. G. B., thirteen were asserted to
have been connected with syphilis.

In three of these cases, no syphilitic taint could
be discovered in the parents or in their other children.
Two of the three did not show any of the typical
symptoms of invaccinated syphilis, and the fatal result
was sufficiently accounted for in other ways. The
third case presents more difficulty. After vaccina-
tion four very small vesicles formed, and the arm healed
normally. In four months, however, after vaccina-
tion, the child suffered from a sore mouth, and subse-
quently from a sore cheek, and "general eczema of the
head and face", and after two or three months "diar-
rhoea set in and "thrush"and the child died exhausted.
The mother suffered from sores on the hand, arm and
breast; but unfortunately her later history was not ob-
tained. This may have been a case of invaccinated syphilis; but it is impossible to pass a more decided opinion from the facts given.

In other five of the fifteen cases there was a distinct history of inherited syphilitic taint.

In the remaining six cases no evidence could be produced to support the statement that the children had suffered from invaccinated syphilis. One of them apparently died from septic infection, probably caused by the vaccination; another died from pneumonia, and there was a decided tuberculous taint in two others.

In his evidence before the Commission, Mr H. H. Taylor put in a table containing thirteen English cases of "alleged cases of vaccinal syphilis."

Four of these had been reported upon by Mr Hutchinson, and were undoubtedly cases of vaccino-syphilis; and another case which had occurred in 1871, and had been reported by Mr T. Smith in the Clinical Society's Transactions, was also most probably connected with vaccination. The evidence regarding the other cases was most unreliable and inconclusive.

During the years 1889 - 96, over which the Commission sat, not a single well authenticated case of this disease, except probably the one already mentioned, was brought to light, although the anti-vaccinators seem to have exhausted their ingenuity in this matter. During these years nearly five and a quarter millions of primary vaccinations were performed in the United Kingdom.
Further evidence of the rarity of the disease is afforded by the fact that amongst 30,000 patients at the Hospital for Sick Children, Great Ormond Street, London, Dr Robert Lee has seen only one case of supposed vaccino-syphilis; and at the East London Hospital for Children, Dr Radcliffe Crocker, who during many years has made special inquiries as to this occurrence, has not seen or heard of one such case. If cases of vaccino-syphilis occurred with even moderate frequency we would certainly expect to meet them oftener than is the case in these Institutions.

The question we are considering may also be looked at from a different point of view. During the past twenty years the number of deaths from syphilis among children under one year of age has increased in Great Britain, and it has been asserted that this increase is due to the inoculation of syphilis at the time of vaccination.

A careful study of the records of the mortality from syphilis will show that it is most largely fatal during the first three months of life. Now, in England and Wales this is the period of compulsory vaccination. But, on the other hand, it should not be forgotten, that as a matter of experience it is found that vaccination is delayed as long as possible. If, therefore, the invaccination of syphilis were a thing of frequent occurrence, we would expect an increase of the mortality from this cause to manifest itself, not during the first, but in the second or third quarter.
It is important to note in this connection, that of the 205 cases in which a fatal result occurred between the 1st November, 1888, and the 30th November, 1891, and which were attributed to, or said to be connected with, vaccination, the majority of, at least, 160 of these deaths did not take place until the fourth or later months. Only twenty were recorded as below the age of three months. Whatever be the cause of the increase of syphilis and the mortality therefrom during the early years of life, there is no evidence to show that it is due to vaccination.

From Scotland similar evidence is forthcoming. The age of compulsory vaccination here is six months, so that a comparison has to be made between the mortality from syphilis in the first half and in the second half of the first year of life. During the years 1855 - 1863 which immediately preceded the period of compulsory vaccination, the proportion of deaths from syphilis in every 1000 deaths in Scotland from that disease was, at the age 0 - 6 months, 575. In the period 1864 - 1875, the corresponding proportion was 612, and in the period 1875 - 1887 it was 647. In the same periods, the similar proportion of deaths at the age 6 - 12 months was 109, 118, and 109 respectively. It will thus be seen that while the proportion of deaths at the age 0 - 6 months had increased during the periods 1853 - 1863 and 1876 - 1887, the proportion at the age 6 - 12 months had remained stationary.

In Ireland the practice of vaccination has not
declined during recent years, and yet the mortality of infants from syphilis has largely diminished. During the two years 1864 - 5 the mortality averaged 124, while during the two years 1887 - 8 the average was only 40.

In Leicester, during the period 1883 - 87, the practice of vaccination fell almost totally into disuse. If vaccination were to any serious extent a cause of syphilis, we should expect to find that during these years the mortality from this cause in infants had considerably decreased. This, however, was not the case. For we find that as between the period 1863 - 67, and the period 1883 - 87 an increase in the infantile mortality in England and Wales of 24.7 per cent only, the increase between the same periods in Leicester was no less than 69.3 per cent.

The statistics from these combined sources prove, beyond dispute, that vaccination has not been responsible for even the slightest appreciable increase of the infantile mortality from syphilis.

Can syphilis be communicated by lymph taken from the calf?

Nearly all authorities are agreed on this point, that syphilis is a disease peculiar to man, and it has been shown by Koch that the syphilitic virus is destroyed by passing through an animal. Animals do not suffer from this disease, and all attempts to inoculate them with it have given a negative result. In the
light of present knowledge we are therefore justified in concluding that it is impossible for syphilis to be conveyed by calf lymph, (unless, of course, it is contaminated after being taken from the calf), even though the calf has been vaccinated from a syphilitic child.

In Germany the use of calf lymph is almost universal, and it is interesting to notice that during the years 1889 - 93, when a total of twelve and a quarter millions of vaccinations and revaccinations were performed, not a single case of vaccino-syphilis was recorded.

Conclusions.

(I) It is possible to invaccinate syphilis.
(II) Statistics prove that this accident is extremely rare in the ordinary practice of vaccination.
(III) Calf lymph taken directly from the calf cannot convey the syphilitic virus.
GENERAL CONCLUSION.

In this survey of the more important bacterial diseases which occasionally complicate vaccination it has, I venture to think, been demonstrated, that although many other causes are at work, there is evidence to show that in a certain proportion of cases, of cases, vaccine lymph and the methods employed in vaccination, have been the means whereby certain septic diseases have been invaccinated, and septic injury inflicted.
The Influence of Vaccination Upon the General Infantile Mortality from Certain Diseases.

This question does not fall directly within the scope of our inquiries; but for the sake of completeness, it will be necessary to shortly examine the main facts of the case.

The diseases selected for such a comparison have not always been the same; but latterly the list has included tubes mesenterica, diarrhoea, bronchitis, pyaemia, skin diseases, syphilis, convulsions, cholera, diphtheria, pneumonia, atrophy and debility, whooping-cough, erysipelas and scrofuls. A return was obtained by an order of the House of Commons showing the deaths from these diseases, at three periods, viz., 1847 - 1853, 1864 - 1867, and 1868 - 1875.

The first six of these diseases showed an increasing, the next four a decreasing, mortality; whilst the remaining four showed an irregular mortality. All the diseases taken together showed an increasing mortality. This increase was at once, by many, assigned to vaccination as a cause. But such an opinion is obviously untenable. The evidence available does not warrant us in holding that the increase in certain of these diseases was due to vaccination, any more than it warrants the conclusion, that the decrease in certain others was due to the same cause. During the period 1874 - 1891
there was a large decrease from phthisis, pneumonia, and convulsions; but no one thinks of attributing this to the influence of infantile vaccination.

If vaccinia is the cause of other diseases, and has increased the mortality therefrom, we should expect to find that the general rate of infantile mortality had increased as a whole. On the contrary, however, this rate has been decreasing, as the following table will show. The mortality of infants under one year is stated in the proportion of their deaths to births:

<table>
<thead>
<tr>
<th>Year</th>
<th>Mortality per 1000 births.</th>
</tr>
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<tbody>
<tr>
<td>1838 - 1842</td>
<td>152.</td>
</tr>
<tr>
<td>1847 - 1850</td>
<td>154.</td>
</tr>
<tr>
<td>1851 - 1860</td>
<td>154.</td>
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<tr>
<td>1861 - 1870</td>
<td>154.</td>
</tr>
<tr>
<td>1871 - 1880</td>
<td>149.</td>
</tr>
<tr>
<td>1881 - 1890</td>
<td>142.</td>
</tr>
</tbody>
</table>

That injury occasionally results from vaccination in individual cases has been already proved; but the infantile death rate affords us no means of ascertaining accurately the exact measure of the extent of this injury. If any conclusion is allowable from it, it is this, that the number of such injuries is so very small that it exercises no appreciable influence upon the general rate of infantile mortality.

The following passage from the Final Report of the Royal Commission on Vaccination gives a good epitome of the present state of knowledge on the subject:
"Without encumbering our report with the details relating to pyaemia, bronchitis, diarrhoea, and skin diseases, which are all said to have increased owing to the mischievous influence of vaccination, we may confidently say that there is no evidence to justify the statement. It is, however, worth while pointing out that comparing, as before, the period of 1883 - 87 with the period of 1863 - 67, the increase of deaths under one year of age from diarrhoea and dysentery in Leicester was 4·2 per cent., whereas in England and Wales it was 0·5 per cent. A similar comparison in respect of bronchitis shows the increase in Leicester to be 112·8 per cent., in England and Wales 73·3 per cent. It seems clear that as regards general infantile mortality, Leicester has not gained by its avoidance of vaccination. Whilst in England and Wales the mortality of children under one year of age had, between the periods selected for comparison, decreased 7·5 per cent., in Leicester the decrease was only 2·8 per cent."

"Upon the whole, then, we think that the evidence is overwhelming to show that, in the case of some of the diseases referred to, vaccination cannot have produced any effect upon the mortality to a substantial, we might say an appreciable, extent."

In this connection, a word may be said on the influence of vaccination on latent disease. It is a matter of somewhat common experience that certain diseases occasionally appear after vaccination, and are
by some attributed to it as a cause. Tuberculosis, and congenital syphilis are, perhaps, the most common of these diseases. But it is quite in accordance with every-day experience to find that disease, hitherto quiescent, becomes lighted into activity by some cause which has no real relation to the disease in question. In some of these cases it is difficult to exclude vaccination as a cause; but in the great majority the circumstances and history of the case, when fully gone into, will sufficiently show that vaccination has no causal relation with the disease in question.
SECTION III.

The EFFECTS of GLYCERINE and other SUBSTANCES upon VACCINE LYMPH and the MICRO-ORGANISMS which it CONTAINS.
THE EFFECTS OF GLYCERINE AND OTHER SUBSTANCES UPON VACCINE LYMPH, AND THE MICRO-ORGANISMS WHICH IT CONTAINS.

Introduction.

In the first part of this paper we have shown that numerous bacteria can be grown in various nutrient media from all specimens of ordinary vaccine lymph, all of which are already well known both morphologically and pathologically. To none of them, therefore, can be assigned the role of the actual vaccine virus. Or, as Professor Cruickshanks shortly puts it: "none of these different species of bacteria are peculiar to vaccine lymph; there is no bacterium constantly present in human and calf vaccine, and there is not one which can be regarded as the contagium."

We have also seen that the organisms which most commonly are found in vaccine lymph are similar to those which are usually associated with various pathological conditions, such as ulceration, abscess, pyaemia and erysipelas; in short, the pathological conditions which are most apt to complicate vaccination. That they have been the cause of such complication, in a small proportion of cases, no reasonable person can deny; indeed the wonder is that they have not produced
these evil results in a much larger number of cases, considering the vast number of persons who have undergone the operation of vaccination. In view of this fact, and especially because of the strong feeling against vaccination which is only too prevalent at the present time, a number of investigators have attempted to devise means whereby these "extraneous" organisms may be rendered innocuous, without at the same time affecting the efficacy of the lymph as a vaccinal agent.

If only it had been possible to isolate the contagium of vaccinia, and to carry on without loss of power pure cultures of it in some nutrient medium, from generation to generation, this part of the problem would have been solved; but this result has not yet been attained, and it is therefore necessary, at present, to continue the use of vaccine lymph, and to endeavour to ensure its purity as far as possible.

HISTORICAL SKETCH.

Monckton Copeman was one of the earliest workers in this field. In 1890 he in the first place tried to kill out the "extraneous" organisms of vaccine lymph by exposing it for definite periods, to a temperature considerably above blood heat — a method, which in the hands of Kitasato had proved so successful in the isolation of the bacillus of tetanus. The lymph was exposed to a temperature ranging between 38 C. and 40 C. But the experiments did not prove successful. After exposure for an hour at the lower
temperature, plate cultures were made from the lymph, and in a day or two, a few points of growth made their appearance. The higher temperature, on the other hand, although it stopped the growth of all "extraneous" organisms, appeared to exercise an injurious effect upon the lymph, as far as vesiculation was concerned. A less delicate and more reliable method was therefore desirable.

Some time afterwards Copeman announced that he had devised such a method. It consisted in the addition of glycerine and distilled water in certain proportions to the lymph or lymph pulp.

Müller\(^{(77)}\) in 1866 had shown that lymph might be diluted with three times its bulk of glycerine, and still retain its properties as vaccine matter unimpaired.

Later still Quist\(^{(80)}\) demonstrated the same fact.

In 1867 Landrin used glycerine as a preserving agent with human lymph.

In 1882 the Committee on Revaccination in Milan recommended the use of glycerine mixed with calf lymph, and as a result of this calf lymph was prepared in this way, and was preserved and distributed in goose-quill tubes.

In Germany Pissin and then Pfeiffer employed this method of preservation. In 1884 a Government Commission, inter alia, enquired into this subject.

The knowledge thus obtained has been taken advantage of at many Continental vaccine stations, and by several of the commercial houses which supply calf
lymph. By this method a much larger supply of lymph is available, and it is generally more free from opacity than lymph prepared in the usual way.

With the same end in view, glycerine was used by Dr Stephen Mackenzie at the London Hospital in 1870 - 1871, the mixture being made up immediately before it was required.

I am informed by Dr Cadell that glycerine was used for a similar purpose in Thistle Street Dispensary many years ago. In this case human lymph was mixed with glycerine, and no impairment of its efficiency could be detected. None of these observers seem to have ascertained its effects upon the microorganisms contained in the lymph.

In 1892 M. M. Chambon and St Yves Ménard published a paper dealing with this subject. In this paper, they relate their experience of glycerinated calf lymph, which had been kept for a considerable period in sterilised sealed capillary tubes. They state that lymph which, in its fresh state, had given poor results, produced after fifteen days admixture with glycerine, a passable vesicle; and after forty, fifty and sixty days, a typical one. Plate cultures made (by Straus) from this glycerinated lymph when first mixed gave rise to numerous colonies of various microbes, especially staphlococcus pyogenes albus and aureus; but when it had been stored fifty to sixty days plate cultures therefrom remained absolutely sterile.
I may remark here that I have examined eight specimens of glycerinated calf lymph, guaranteed sterile, which I had received on different occasions from this Paris firm, either directly or through their Edinburgh agents, and in every case, plate cultures produced colonies of various organisms, especially staphylococcus pyogenes aureus and albus.

In a paper read before the Medical Congress at Rome, in 1894, Leoni states that he found that calf lymph as freshly collected contains large numbers of micro-organisms, some of which are capable of exerting pathogenic properties, when inoculated into the system along with the true vaccine virus. But he adds that these organisms disappear completely from, or their number is greatly decreased in, vaccine which had been prepared with glycerine and allowed to stand from one to four months.

Vaillard and Antony (1894) concluded "that in growing older the germs contained in glycerinated pulp diminish considerably in number; but do not completely disappear: even after seven months, the pulp may still contain living germs."

In 1896 a Commission was appointed by the German Government to enquire and report as to the best methods for the collection, preservation, storage, distribution and use of vaccine lymph. It included the following members, Dr Koch, Schmittdmann, Pfeiffer, and Frosch, and the Directors of various Vaccine Institutes. In their Report the Commissioners arrive at the conclusion
that fresh calf lymph contains numerous microbes, the number of which, on the addition of glycerine, diminishes as the age of the mixture increases. According to their investigations the streptococci when mixed with glycerine are destroyed in eleven days, and the diphtheria bacillus in twenty days.

Dr. Schultz, the Director of the Berlin Institute, found that keeping for a few days, after admixture with glycerine, sufficed to destroy most of the bacteria usually present, and that their destruction was more rapid when glycerine was present to the extent of 60 per cent, than when the proportion reached fifty per cent only.

No other investigator has arrived at such a result. According to my own investigations, if the number of bacilli is small, and the amount of glycerine great, the organisms will disappear more rapidly than usual; but the former condition especially does not hold with regard to the majority of specimens of calf or human lymph.

Various other chemical agents have been used to render vaccine lymph free from micro-organisms. Kita-sato has recently published a series of experiments in which he sought to free vaccine lymph from extraneous bacteria by the addition of carbolic acid to the extent of 0.5 per cent to 8.66 per cent. The lymph was thus rendered free from living bacteria; but it was rendered useless as vaccine material.

Other investigators have also experimented with
carbolic acid, salicilic acid, boracic acid, but with no definite result.

Blaxall\(^{1}\) confirms the results of previous investigators with glycerine prepared and used according to Dr Copeman's method.

In the four experiments carried out by him with calf lymph, he found that at the end of four weeks after glycerination no growth resulted from the inoculation of agar plates with the glycerinated lymph. This lymph in three out of the four cases gave positive results when used in vaccination. Ianoline and vaseline were also used by him in a similar manner; but he found that the bacteria in calf lymph, when mixed with either of these substances, tended to become largely increased, as shown by plate cultivation. These two substances have recently been introduced by two officers of the India Medical Service, neither of whom, however, brings forward any bacteriological evidence as to the germicidal action of the substances they advocate.

The practice with regard to the amount of glycerine added varies very much.

Dr Copeman uses a fifty per cent solution of glycerine in distilled water, which has been rendered aseptic by heat. Four parts by weight of this solution are used to one part by weight of vaccine lymph. The capillary tubes which he uses are also carefully sterilised. These tubes are sealed after being charged with a mixture of lymph and glycerine and allowed to stand in the dark for "some weeks". He concludes that
"in glycerinated calf lymph properly produced we have a preparation which, while even more efficient as vaccine than the original lymph, can be rendered entirely free from the 'extraneous' organisms, which, at one time or another, have been isolated from fresh or stor¬
ed lymph, with the possible solitary exception of the bacillus subtilis, which, however, possesses no patho¬
genic properties."

M. M. Chambon and Ménard add without measurement about an equal quantity of glycerine to the lymph or pulp. Everything is most carefully sterilised.

In Brussels a similar method is employed.

In Berlin the amount of glycerine and water used are in the following proportions: -

Epithelial pulp . . . . . . 1 part.
Glycerine . . . . . 7 parts.
Boiling water . . . . . 7 parts.

In Dresden the proportion is as follows: -

Water . . . . . . . . . 3 parts
Glycerine . . . . . . . . 1 part.

Four parts of this mixture are added to one part of vaccinal pulp.

In Cologne the proportions are: -

Pulp . . . . . . . . . . 5 grammes.
Water . . . . . . . . . . 25 grammes.
Glycerine . . . . . . . . 25 grammes.

In Geneva, Dr Haccius used the following propor¬
tions: -
Vesicle pulp .... 1 part.
Glycerine ....... 2 parts.
Water ........... 1 part.

It is interesting to note that while in the London Animal Vaccine Establishment, where glycerinated lymph is not yet in use, a single calf can provide lymph for only 200 to 300 vaccinations, while on the other hand it is no unusual thing abroad to provide from a single calf an amount of glycerinated lymph that will serve from 4000 to 6000 vaccinations. In Berlin one calf can be made to provide sufficient lymph for not less than 15,000 vaccinations.
Experiments upon the Effects of Glycerine and Other Substances upon Vaccine Lymph and the Organisms Contained Therein.

Introduction.

Four main causes combined to direct my attention to this aspect of the subject of vaccination: -

I. The Commissioners on vaccination in their Final Report (1896) say "In connection with this subject, our attention has been drawn to the experiments made by Dr Copeman, as to the effect of the storage of vaccine lymph in glycerine. The conclusions at which he arrives are that the addition of glycerine, whilst it leaves the efficiency of the lymph undiminished, or even increases it, tends to destroy other organisms. If it be the fact that the efficacy of the lymph remains unimpaired, its storage in glycerine would largely diminish the difficulties in the use of calf lymph, which are inseparable from calf-to-arm vaccination. The investigation has not reached a point at which it is possible to pronounce with certainty whether the anticipated results would be obtained. . . . The question is one a further investigation of which is desirable."

II. In a Report to the Local Government Board
(July 1897) on the preparation and storage of glycerinated calf vaccine lymph, Sir Richard Thorne says, "Much remains to be ascertained by careful scientific research in order to learn what are the precise conditions under which glycerinated calf lymph can be prepared and stored so as to secure to the utmost freedom from extraneous, and especially from pathogenic, micro-organisms, whilst at the same time retaining to the utmost the undiminished protective value of the lymph material against small-pox. We learn that in every country visited further research is being made in this direction, and in Germany a special Commission of medical and bacteriological experts have been appointed by the Government to study and report upon the subject."

III. My researches into the essential cause of vaccinia, convinced me of the absolute necessity of, at first, devising a method whereby the extraneous micro-organisms of vaccine lymph might be inhibited, without, at the same time, hindering the development of the specific cause of the disease.

IV. Examination of several specimens of so-called glycerinated calf lymph, made me very incredulous regarding the claims put forth on behalf of glycerine as a germicidal agent; it was easily demonstrated that every sample examined contained large numbers of several different micro-organisms.
I made altogether thirty separate experiments upon this subject. In seventeen of these experiments I added a solution of glycerine in water in one of two proportions to the fresh calf or human lymph, having first determined by plate or sloped agar tube cultivations what organisms they contained. In other five of these experiments I also added a large number of similar or other organisms to the mixture of glycerine, water and lymph. In seven experiments, I used no lymph, but simply added a number of various bacteria to the glycerine solution, and in one case, I added a number of the staphlococcus pyogenes albus to pure distilled water.

In six of the experiments, in addition to the lymph and glycerine solution, I added Formalin in different proportions, and in one case Permanganate of Potash was added.

The glycerine used was Price's "Pure Glycerine", of which the following analysis has been made by Dr Wilson Huke:

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific gravity</td>
<td>1.26</td>
</tr>
<tr>
<td>Water</td>
<td>0.64</td>
</tr>
<tr>
<td>Ash</td>
<td>0.00</td>
</tr>
<tr>
<td>Glycerine</td>
<td>99.36</td>
</tr>
</tbody>
</table>

The glycerine was first thoroughly sterilised in a Koch's steam steriliser, by intermittent sterilisation. Pure distilled water which had been kept at boiling point for a considerable time was then added. In the first fourteen experiments, I used a solution which
contained 75 per cent of glycerine and 25 per cent of water, and in the remainder a solution of glycerine in an equal quantity of water was used. Having carefully mixed the glycerine and water in these proportions, the mixture was again thoroughly sterilised as before. In mixing the vaccine lymph with the glycerine solution care was taken to make, and to keep, all the materials thoroughly sterile; and the capillary glass tubes in which the lymph was stored, after being carefully and intimately mixed with the glycerine solution, were rendered perfectly sterile by dry heat.

The lymph was stored in small boxes which were kept in the dark, at the usual winter temperature of the Pathological Laboratory.

Every capillary tube, when the lymph was required for testing purposes, was first sterilised, before the ends were broken off with sterile forceps. Immediately after the mixture of lymph and glycerine solution had been made, plate or sloped agar tube cultivations were instituted with a definite and constant quantity of the mixture from one of the capillary tubes, and I was thus able to satisfy myself of the presence of micro-organisms, and their probable number. Afterwards, in some cases weekly, and in others fortnightly, plate and agar tube cultivations were made with a similar quantity of the mixture to what I had used on the first occasion when the mixture was made.

In the first series of experiments (I - XIV) I used the stronger solution of glycerine, and added it
in the proportion of two of glycerine solution to one of vaccine lymph. In the second series (XV - XXX) the weaker solution was used, and it was added in the proportion of four of glycerine solution to one of vaccine lymph.

EXPERIMENTS.

I shall now proceed to give a detailed account of the various experiments.

Experiment I.

The stronger solution of glycerine was inoculated with a large quantity of the staphlococcus pyogenes albus. Cultures made from the mixture gave dense growths of this organism. For ten weeks regular cultural examinations were made, with the result that a gradual diminution of the number of organisms was demonstrated. At the end of this period, only nine colonies were produced from the contents of one capillary tube, and seventeen days later, no growths resulted. Three other subsequent examinations showed that the mixture was free from micro-organisms.

Experiment II.

This was made with a specimen of vaccinal pulp from which profuse colonies of staphlococcus pyogenes aureus and albus were produced. It was mixed with the stronger solution of glycerine, to which a large number of the bacillus coli had been added. A sloped agar tube immediately inoculated with the mixture, showed a large number of colonies of all three organ-
isms. Regular examinations were made during the first forty-one days. Then it was found that the bacillus coli had entirely disappeared, and that from one capillary tube only thirty colonies of the two cocci resulted. Nine days later, a similar quantity produced only one colony of staphlococcus pyogenes albus. A week later, no growth occurred; and this result was confirmed on two other occasions when nutrient gelatine plate cultivations were made.

Experiment III.

Calf lymph supplied by Dr. Cory of the National Vaccine Establishment was used. It contained four organisms, and a small light yellow yeast: - the staphlococcus pyogenes aureus and albus, the staphlococcus cereus flavus, and the bacillus subtilis similans.

The stronger solution of glycerine was added to the lymph, and a sloped agar tube was immediately inoculated from the mixture. Colonies of all four organisms and the yeast resulted. In twenty-one days cultural examination showed that the mixture was free from organisms, and two subsequent examinations confirmed this.

This lymph after being glycerinated for six weeks, was once used for vaccination purposes; but only one small vaccine vesicle resulted.
Experiment IV.

A calf lymph which contained the staphlococcus albus and aureus was used. It was mixed with the stronger solution of glycerine, and the inoculation of a sloped agar tube therefrom showed the presence in moderate quantity, of the two micro-cocci already mentioned.

In twenty-three days cultural examination proved that the lymph was free from living micro-organisms; and two subsequent agar plate preparations confirmed this. This lymph was not used for vaccination purposes.

Experiment V.

To pure distilled and sterilised water there was added a large quantity of the staphlococcus pyogenes albus. After four months profuse growths of this organism were obtained, when the mixture was inoculated on the usual media.

Experiment VI.

In this experiment calf lymph already glycerinated was used, after a small quantity more of glycerine had been added to it. It produced, immediately after being mixed, numerous colonies of the staphlococcus pyogenes aureus. After being stored for eighty days it still produced many colonies of the same organism, although they were not so numerous as at first.
Experiment VII.

Calf lymph which contained the staphlococcus pyogenes aureus and albus and the bacillus subtilis was used. It was mixed with the stronger solution of glycerine in the usual proportion, and this mixture when inoculated on agar gave rise to colonies of the above mentioned micro-organisms. Weekly cultural examinations demonstrated a steady decrease in the number of micro-organisms, and on the thirty-third day it was found to be quite free from them in a living condition. Subsequent examination confirmed this.

After six weeks storage, this lymph was used to vaccinate a child. Three insertions were made, and good vesicles resulted.

Experiment VIII.

The stronger solution of glycerine was mixed with a large quantity of the tubercle bacillus. Tubes of sloped glycerine-agar inoculated immediately with this mixture, showed characteristic colonies of this bacillus after eight days incubation. A similar result was obtained two weeks later; but the colonies were not so numerous. The same medium was again inoculated from the mixture after it had been stored for twenty-six days; but no growth was apparent twenty days later. Subsequent inoculations were also negative. When the mixture had been stored for about five weeks, Dr Muir injected a large quantity of the mixture into the peritoneum of a guinea-pig. The animal, which
was very weak and thin when inoculated, died in eight days; but no evidence of tuberculosis could be obtained on post-mortem examination. Another guinea-pig was inoculated by Dr Muir in a similar manner, from the same mixture. This animal died nearly six weeks after inoculation, on the morning of the day on which it had been determined to kill it. Examination again gave a negative result. No trace of tuberculosis could be found.

Experiment IX.

For this experiment, two vaccinal crusts from a child's arm were used. They were thoroughly powdered, and then intimately mixed with the stronger solution of glycerine. An examination of this mixture showed that it contained the staphlococcus pyogenes albus and citreus and the staphlococcus cereus flavus. A month later no colonies were produced by the inoculation of this mixture upon agar. Later examinations also gave similar results.

Experiment X.

Calf lymph which contained the staphlococcus pyogenes albus, was mixed with the stronger solution of glycerine, to which had been added a fairly large number of the typhoid bacillus. Both these organisms were demonstrated by the immediate inoculation of the mixture upon sloped agar tubes. After being stored for thirty days, an agar tube was inoculated with the mixture from a capillary tube, and a plate preparation
made. Only eight colonies of the micro-coccus appeared; but there were numerous colonies of the typhoid bacillus. A month later both the typhoid bacillus and the staphlococcus had lost their vitality; and subsequent examination confirmed this result.

Experiments XI and XII.

These experiments are mainly a repetition of experiments II and X. Two different quantities of the stronger solution of glycerine were inoculated respectively with the bacillus coli and the typhoid bacillus and stored in the usual manner. During the first thirty days both preparations produced colonies of their respective bacilli, when cultural experiments were carried out; but the number of colonies gradually diminished during this time. Later examination failed to demonstrate the presence of the bacillus coli in a living condition; but this result was not attained with the bacillus of typhoid until it had been stored for just over forty days.

Experiment XIII.

To calf lymph which contained the staphlococcus pyogenes aureus and albus there was added a large quantity of the staphlococcus pyogenes citreus, and then it was mixed with the stronger solution of glycerine. Thirty-eight days after, numerous growths resulted from the inoculation of this mixture upon agar, although they were much less profuse than when the mixture was originally made. Two weeks later examination showed
that the mixture still contained micro-organisms; and subsequent examinations during the following two weeks, still gave the same results.

Experiment XIV.

The old calf lymph used in this experiment contained the staphlococcus aureus and albus in very large numbers. It was mixed with the stronger solution of glycerine, and the mixture immediately examined by inoculating agar tubes, when most profuse growths of the two micrococci showed themselves. Forty days afterwards only one growth resulted from the inoculation of a nutrient gelatine plate with the contents of one capillary tubeful of the mixture, and during this period fortnightly examinations demonstrated a gradual diminution in the vitality of the organisms. A week later, no colony appeared when agar was inoculated, and subsequent examination proved that the mixture was sterile.

Experiments XV to XX.

Calf lymph containing one or more of the following organisms was used in this set of experiments: - staphlococcus pyogenes albus and aureus, staphlococcus cereus albus and flavus, bacillus subtilis, and also yeasts. The weaker solution of glycerine was used in the proportion of four parts to each part of lymph. After the glycerine solution and the lymph had been thoroughly mixed, cultural examination demonstrated the presence of a fairly large number of micro-organ-
isms in every specimen. The lymph was stored as usual and weekly examinations were made. Nutrient gelatine plate cultivations demonstrated that the various mixtures were sterile at the end of the following periods:

- Two mixtures, . . . 26 days
- One mixture, . . . 30 days.
- One mixture, . . . 32 days.
- One mixture, . . . 34 days.
- One mixture, . . . 41 days.

The lymph as prepared in these six experiments was used to vaccinate several children, four in public Dispensaries and two in private.

In four cases most typical vaccine vesicles were produced, which ran a normal course. In one case the result was doubtful, and in another the lymph quite failed to produce vesiculation, although a subsequent vaccination of the same child ran its course much more quickly than usual, in fact, it was more like the course of re-vaccination than of vaccination.

Experiments XXI to XXVI.

These experiments are similar in this respect, that in each Formalin: 1 in 10,000 or 1 in 20,000, was added to the mixture of glycerine solution and lymph. In two of them calf lymph was used, in two human lymph, and in the other two micro-organisms were added to the glycerine solution. In each I used the weaker solution of glycerine and added it in the same proportion as in the last series. The specimens of calf and hu-
man lymph contained one or more of the micro-organisms I have already mentioned in the previous set of experiments.

To the two specimens of calf lymph there was added four parts of the weaker glycerine solution, and sufficient Formalin to make the resulting mixtures equal in strength respectively to one in 10,000 and one in 20,000 of the latter substance. In the former case, no growth appeared when agar was inoculated with the mixture on the ninth day of storage, and in the second case, no growth appeared on the thirteenth day. Subsequent inoculations also gave negative results. The lymph from the first set of tubes was used, at periods ranging from the eleventh to the fifteenth day of storage, to vaccinate three children; but it absolutely failed to produce vesiculation. No irritation or discomfort of any kind was produced by it.

To each of the specimens of human lymph glycerine was added in the same proportions, and sufficient Formalin to make the resulting mixtures equal in strength to one in 10,000 in both cases. In one case, inoculation of agar by the mixture, when it had been stored for nine days, produced no result; but in the other case, colonies of micrococci were obtained by inoculating agar tubes after the mixture had been stored for twenty-six days. Four days later, no positive result was obtained. Subsequent cultural experiments proved, in both cases, that the lymph was sterile.

In the two remaining experiments in this series
sufficient Formaline to make the respective mixtures equal in strength to 1 in 10,000, and 1 in 20,000 was added to the weaker glycerine solution, to which had been added a large number of the staphlococcus pyogenes albus. After being stored for fifteen days the one to which had been added the larger proportion of Formalin, produced no result when it was inoculated on agar, while the other set proved to be sterile on the seventeenth day. These results were confirmed by further inoculations of nutrient gelatine plates and agar tubes.

Experiment XXVII.

Calf lymph was mixed with four parts of the weaker solution of glycerine to which had been added a large number of the staphlococcus pyogenes albus and aureus - which also were originally contained in the lymph. After being stored for sixty days, the colonies produced on agar plates were almost as numerous as on the date when the mixture was made.

Experiment XXVIII.

To a quantity of the weaker solution of glycerine there was added a quantity of the ataphlococcus pyogenes albus about equal to what is found in an average specimen of calf lymph, and to this mixture there was added just sufficient Permanganate of Potash to cause a slight colouration. Ten days later agar tubes were inoculated with the mixture, when it was shown that a considerable diminution had taken place in the number
of living micro-organisms, as compared with the condition of the lymph in this respect on the day on which the mixture was made. After the mixture had been stored for thirty-one days, agar plates were inoculated from it, with a negative result.

Experiment XXIX.

In this experiment I used calf lymph which contained numerous chains of streptococcus pyogenes, and a few staphlococci. The weaker glycerine solution was added to it in the usual proportions. Inoculation of this mixture on agar tubes produced many colonies of the streptococcus pyogenes and a few of the staphlococcus pyogenes albus. After the mixture had been stored for twenty-six days, it failed to produce any colonies of the streptococci when agar tubes or plates were inoculated with it, although a few colonies of the staphlococcus made their appearance. A few days later the mixture was perfectly sterile, and three subsequent cultural examinations confirmed this.

Experiment XXX.

A considerable quantity of the streptococcus pyogenes was mixed with a portion of the weaker solution of glycerine which was stored in the usual manner. The usual experiment demonstrated the presence of the streptococcus in the mixture; which was regularly examined every few days by the inoculation of sloped agar tubes. The number of colonies gradually diminished, and an agar plate which was inoculated on the twentieth
day of storage gave a negative result. Further examination proved that the mixture was quite sterile.
EXAMINATION OF EXPERIMENTAL RESULTS.

These experiments fully confirm the claims advanced regarding the effect of a solution of pure sterile glycerine in distilled sterile water, upon the micro-organisms usually found in vaccine lymph. They also serve to indicate some of the conditions and limitations of this method of sterilising vaccine lymph. An examination of the results obtained will make this clear.

Ten fresh samples of calf lymph were glycerinated and stored in the manner already described. In four of these two parts of the 75 per cent solution of glycerine in water was used, to each part of the lymph; and in the others the lymph was mixed with four parts of the fifty per cent solution of glycerine and water. In these ten cases, the glycerinated calf lymph was rendered sterile in from twenty-one to forty-one days, the average being 29.7 days. The exact time required for each separate specimen is stated below, the first four being the mixtures in which the stronger solution of glycerine was used:

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>21</td>
</tr>
<tr>
<td>II</td>
<td>23</td>
</tr>
<tr>
<td>III</td>
<td>33</td>
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<td>VI</td>
<td>26</td>
</tr>
<tr>
<td>VII</td>
<td>30</td>
</tr>
<tr>
<td>VIII</td>
<td>32</td>
</tr>
<tr>
<td>IX</td>
<td>34</td>
</tr>
<tr>
<td>X</td>
<td>41</td>
</tr>
</tbody>
</table>

The stronger solution of glycerine seems to be
better adapted for the purpose under consideration than the weaker one, as, in my experiments, it rendered the lymph sterile in, on an average, twenty-seven days, while the weaker solution required an average of 31.3 days.

Copeman seems to consider that the bacillus subtilis is more resistant to the action of this glycerine solution than the other micro-organisms usually present in vaccine lymph, as he mentions it as a probable exception to the general rule. I have not, however, found any difficulty with this organism. Several of the ten specimens mentioned above contained this bacillus; and in every case the action of the glycerine solution upon it was similar to its action upon the other organisms. Examinations of commercial glycerinated specimens of calf lymph show that this bacillus is more frequently contained therein than in fresh specimens of calf lymph. The probable reason for this may be found in the fact that the lymph in imperfectly sterilised capillary tubes, which form one of the most common habitats of the bacillus subtilis, has had added to it, probably, numerous colonies of this common micro-organism; and, as I shall show later, the addition of any micro-organisms to what is already in the lymph, makes a very considerable difference in the action of the glycerine solution upon the lymph.

When it had been ascertained that the lymph was perfectly sterile, I tested eight of the ten specimens by using it in the vaccination of children, with the
following result: -

Five produced good vesiculation.

Two produced very imperfect vesiculation.

One failed absolutely to produce vaccinia.

This result I consider was not unsatisfactory.

The result obtained was sufficient to prove that it is possible by means of glycerine to free vaccine lymph from its "extraneous" micro-organisms, without, at the same time, impairing its efficiency as a vaccine material.

The specimen of calf lymph which contained the streptococcus pyogenes and the staphlococcus pyogenes albus was rendered sterile in thirty days; but the streptococcus failed to grow after the twenty-sixth day. Experiment XXX showed that this latter micro-coccus when mixed in a solution of glycerine and water failed to grow after twenty days storage. We are therefore justified in concluding that glycerine exercises its full germicidal action upon this less resistant micro-organism in from twenty to thirty days.

The experiments with Formalin and Permanganate of Potassium are interesting; but prove nothing practically, except perhaps that the essential cause of vaccinia is most susceptible to the prolonged action of the usual chemical antiseptics. Other similar experiments seem also to point to this conclusion.

The experiments in which a large number of bacteria was added to newly glycerinated calf lymph, or simply to the glycerine solution, give important indica-
tions regarding the conditions under which vaccine lymph should be prepared and stored. The inference to be drawn from these experiments is that when the lymph contains more than an average number of bacteria, the specific action of glycerine upon it is very much slower and more uncertain. Whenever lymph contains more than its usual number of micro-organisms, the time taken by glycerine to destroy their vitality is so much prolonged that there is some risk of impairing the efficiency of the lymph as vaccine material.

It is important to note also that some of the above experiments seem to prove that glycerine exercises a germicidal action upon the tubercle bacillus, the bacillus coli and the typhoid bacillus. The results obtained in all three cases are very remarkable, and especially is this so with the tubercle bacillus, in view of the well known fact that this micro-organism grows best, especially in sub-cultures, upon an 8 per cent glycerine-agar medium. It is remarkable also that the bacillus coli should prove less resistant to the action of the glycerine solution than the bacillus of typhoid. It will probably be found that glycerine exercises a germicidal action upon many other different bacteria.

CONCLUSIONS.

The practical conclusions to be drawn from these experiments have already been indicated.

They have proved, I think, that in glycerinated
calf (or human) lymph, properly prepared, we have a preparation which, while at least not less efficient than the fresh lymph as vaccine material, is entirely freed from the extraneous micro-organisms which are usually contained in unprepared vaccine lymph. In most cases this result may be obtained by the storage of the lymph in a cool dark place, for from three to five weeks. Bacteriological examinations should, however, always be made in order to ascertain if this result has been obtained.

These experiments also show how important it is that a sepsis or antisepsis should be most rigorously carried out at every stage. Glycerine cannot, as it were, cope with a larger number of bacteria than is found in an average specimen of calf lymph, especially in the short period of time during which the lymph can be allowed to stand. Care should, therefore, be taken to ensure that no additional organisms are added to it at any time.

To ensure this result, in the first place, the calf should be most carefully prepared for the operation. The parts to be vaccinated should be carefully washed with hot water and soap, and a good antiseptic, and this should be again washed off with boiling water that has been allowed to cool. For vaccination purposes, sterile glycerinated lymph should be used, and all the instruments should be surgically clean. During the period between vaccination and the collection of the lymph or pulp, the calf should be kept under the most
favourable hygienic circumstances. Before the lymph is collected, the vaccinated part should be cleansed as before; and great care should be taken to ensure that the vessels into which the lymph is received are perfectly sterile.

Similar precautions should be taken in preparing the lymph. Too much importance cannot be attached to the thorough sterilisation of the glycerine and water, and the capillary tubes in which the mixture is permanently stored should be beyond suspicion.

All these preparations will, however, be futile unless similar precautions are taken by the vaccinator who uses the lymph. In the past, vaccination by many has been carried out in a very rough and ready manner. It cannot too soon be recognised that vaccination is a surgical operation, although a very "minor" one, and that it is liable to many of the risks which accompany other operations. The most dangerous of these risks, as in so many other operations, are those connected with sepsis; and every possible precaution should be taken to make the operation an "antiseptic" one. The vaccinator cannot always control the conditions and surroundings under, and in, which the operation is performed; but he can ensure personal cleanliness, and the cleanliness of the instruments, etc., he uses. Even in Public Vaccination Stations, it should not be difficult to have the child's arm washed with soap and warm water, and also with some antiseptic solution.
The most satisfactory instrument to use is an ordinary fairly large-sized needle. A large number of these can easily be obtained, and a separate one should be used for each child. Such needles can also be easily and thoroughly sterilised by boiling immediately before use.

The care of the arm after the operation is entirely beyond the control of the operator. No satisfactory method of dressing or covering the arm antiseptically has been devised, although this plan has many obvious advantages. Probably the best advice we can give the parents is to cover the arm with a clean soft handkerchief, after the lymph has dried, and not to put a shield over it.

If these precautions were carried out, and a sterile glycerinated calf lymph used, I am convinced that the sometimes serious accidents and complications of vaccination would be even fewer than they are at present. If, however, this result were not obtained, a cause of blame against the medical profession would be removed, and some of the most serious objections to vaccination would be nullified.

I should, however, state in conclusion that I make these claims only for glycerinated calf lymph properly prepared, combined with the other precautions I have mentioned. I cannot include in this category the "glycerinated" calf lymph supplied by the majority of British commercial firms, and the three or four Continental firms which send lymph to this country. As
already stated, I have examined specimens from most of these sources, and in not one single instance did I obtain a sterile glycerinated lymph. Most of the tubes I examined are, bacteriologically, as bad, if not worse, than pure unglycerinated lymph, in fact, if obliged to make a choice between the two, I should much rather use the latter than the former.

If, then, the use of glycerinated calf lymph is to become general, it is necessary that means should be taken to secure that a regular supply of aseptic lymph is available to every Medical Practitioner; and no one can doubt that, when Vaccination is compulsory, it is the duty of the Legislature to make arrangements whereby such a supply may be obtained, not only by Public Vaccinators but also by every Medical Practitioner.
APPENDIX.

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BIBLIOGRAPHY.
BIBLIOGRAPHY.

(1) Acland . . . Cases 202 and 207 in Appendix IX of Royal Commissioners' Report.

(2) " . . . System of Medicine, Albutt. Vol. II.

(3) Acker . . . Die Uebertragbarkeit de Tuberculose durch die Vaccination.


(6) Arning . . . Reports to the President of the Hawaiian Board of Health. I, II and III. 1884, 1885, and 1886.

(7) " . . . Journal of the Leprosy Investigation Committee, No.II.

(8) " . . . International Medical Congress, 1890.

(9) Baltzer . . . La France Médicale. 1890.


(12) " . . . Annales de l'Institut Pasteur, 1896.

(13) Blaxall . . . Report of the Medical Officer to the Local Government Board. 1895 - 96.


(16) " . . . Pathologie der Vaccine. 1875.

(18) Buckmaster. Journal of the Leprosy Investigation Committee. No.IV.

(19) British Medical Journal, 1884.

(20) Buist . . . Vaccinia and Variola. 1886.

(21) Butel . . . La Tuberculose des animaux et la phtisie humaine. Paris 1887.


(26) Chambon et St Yves Ménard. Gazette des Hopitaux. 1892.


(31) Copeman, Monckton. The Bacteriology of Vaccine Lymph. Transactions, Seventh International Congress of Hygiene and Demography, 1891. Vol.II.
(33) " . . . " " " 1896.
(34) " . . . Journal of Pathology and Bacteriology, 1894.
(37) " . . . Proceed. of Royal Society, 1893.
(39) " . . . Bacteriology and Infective Diseases, 1896.
(40) " . . . History and Pathology of Vaccination, 1889.
(41) Creighton. Natural History of Cow-pox and Vaccinal Syphilis, 1887.
(42) Danbler. . . Monatschrifte für Praktische Dermatologie, 1889.
(43) Delobel et Cozette. La Vaccine et la Vaccination, Paris, 1897.
(45) Degive . . Transmission de la tuberculose par la Vaccination.
(47) Eichstedt. Epidémie d'impetigo contagiosa a la
suite de la Vaccination, 1892.


(49) Färst. Die Pathologie der Schutzpocken. Impfung, 1896.


(53) Hillis. Leprosy in British Guiana.

(54) Hlava. Wein Kline. Rundschau, 1895.

(55) Hutchinson. Illustrations of Clinical Surgery Fascic. VI.

(56) Jenner. An inquiry into the causes and effects of the Variolae Vaccinae, 1798.

(57) Josserand. Contribution a l'étude des contaminations vaccinales, 1884.


(61) Klein. Report of the Medical Officer to the Local Government Board, 1884.

(62) " Royal Commission on Vaccination. Appendix IX to Report.

(63) " Micro-organisms and Disease, 1896.


(67) Leclainche et Conte. Epreuve des genisses vacciniferes par la Tuberluline. Congres pour l'étude de la Tuberculose chez l'homme, 1893.

(68) Leoni. . . Revue d'Hygiene, 1894.


(71) McVail. . . Vaccination Vindicated, 1887.


(76) Morris, Malcolm. Diseases of the Skin, 1894.

(77) Montgomery. Occidental Times, 1890.


(79) Muir and Ritchie, Manual of Bacteriology, 1897.

(81) Perron . . De l’impétigo d’origine vaccinale.  


(83) Poole . . Vaccination Eruptions, 1893.


(86) Rake Beaven. Trinidad Leper Asylum Report, 1885, 1886, and 1887.

IX Appendix.

(88) Ruete. . Cultures pures du vaccin de génisse.  
Bull. Med. 1891.


(91) Sanderson, Burdon. Intimate Pathology of Conta-gion.


(93) Schmidt . . Medical Times, 1885.


(95) Straus, Chambon et Ménard. Recherches expérimen-tales sur la vaccine chez le veau.  
Bull. Méd. 1890.

(97) Straus. . . La Tuberculose et son bacille. Paris. 1895.
(98) Swift... . Occidental Times. 1890.
(100) Tebb . . Leprosy and Vaccination. 1893.
(103) Toussaint. Note présenté a l'Académie des Sciences le 8 Aout. 1881.
(104) Vidal. . . Lupus Vaccinal. Annales de Dermatologie et de Syphiligraphie. 1889
(105) Viennois . De la transmission de la syphilis par la Vaccination. Archives générales de Médecine. 1860.
(106) Voigt. . . Deutsche medizenische Presse.
(108) " . . . Vaccination proved useless and dangerous. 1889.