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THEIR MANIFESTATIONS AND INTER-RELATIONS IN THE LOWER ANIMALS.

AN EXPERIMENTAL RESEARCH.

By S. MONCKTON COPEMAN, M.A., M.D. Cantab.

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An Experimental Research.

By S. MONCKTON COPEMAN, M.A., M.D.Cantab.;
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(*Read: April 19th*, 1893.)

In the course of certain experiments on the bacteriology of vaccine lymph, an account of which I had the honour of bringing before this Society last year,* I was confronted by an insuperable difficulty (as it then appeared) in the practical testing of certain of the results of my work. Continuous experimentation on children being obviously out of the question, I was naturally led to turn my attention to the discovery, if possible, of some one or more of the lower animals, which by reason of their passing through the various stages of vaccination, and more especially variolation, in a manner comparable with that witnessed in the human subject, might serve me for control experiments.

With this object in view I carefully looked up all the available literature on the subject, but the result of such search, unfortunately, was not encouraging.

In the attempt to give experimental confirmation to the theory of Jenner, who arguing from the prophylactic effects of vaccine against human small-pox, termed cow-pox variola vaccinæ, or small-pox of the cow, numerous observers, including Thiele, McMichael, Sonderland, Ceely, Badcock, Martin and Reiter, and, in more recent times, Voigt, Hime, Haccius, Klein, and others, have inoculated animals of the bovine species with human variolous lymph. In a considerable number of these experiments, however, no local result has apparently been obtained; and even in those cases which have been regarded by their authors as successful, the result of primary transmission has borne

* *Transactions of the International Congress of Hygiene*, London, 1891; and *Transactions of the Epidemiological Society*, 1892.

little resemblance, either in the appearance at the site of inoculation or in the accompanying symptoms, to small-pox as witnessed in the human subject. In point of fact, so wide has been the divergence, that the result obtained has been looked upon by these observers as a transformation rather than an attenuation of variola, and as therefore to be more properly termed variolo-vaccine rather than variola. In other words, such a modification of variola is apparently produced by its transference to bovines that the resulting local affection cannot be distinguished from ordinary cow-pox or vaccinia.*

Although it should be stated that such an interpretation of the results thus obtainable has been strenuously called in question by Chauveau, and by others following in his footsteps, still the fact remains that the total effects obtainable by variolation of these animals bear so little resemblance to human variola, local or general, as to render them useless for the immediate purpose of my own experiments.

In many animals, among which may be mentioned horses, pigs, and dogs, a local effect, passing in greater or less degree through the stages of papule, vesicle, and pustule, can be obtained as the result of inoculation with vaccine lymph. Thus Chauveau, after experiment by subcutaneous and intravascular injection of vaccine lymph, arrived at the conclusion that horse-pox and cow-pox are one and the same disease. In these animals, however, as is found among bovines, the induced vaccinia generally runs a shorter course than in the human subject, while variolation of them rarely or never succeeds.

In addition, I have experimented with vaccinia on rabbits, guinea-pigs, and rats, but as yet with no very definite results.

This being so, I turned my attention to the monkey tribe, on account of their similarity in many respects to man, although assured on high authority that they were not susceptible to either vaccinia or variola. On putting the matter to the test, however, I was agreeably surprised to find that this was not the case, the inoculation of vaccine and of variolous lymph having each of them given, in my hands, successful results in every instance in which I have tried it on the monkey. Briefly described, the *modus operandi* has been as follows: The upper arm, or occasionally the inner surface of the thigh, is shaved on both sides of the body; the skin is then washed with a solution of some disinfectant, such as corrosive sublimate or carbolic acid, and afterwards with alcohol. Each arm (or thigh) is

* To this subject it will be necessary to return in a description of a further stage of my research.

then scarified in three or four places and the lymph well rubbed in.

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

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

In both cases there is usually a rise of body temperature, which is more marked and longer sustained in variola than in vaccinia. After variolation, it was noticed in several instances that the monkey suffered from diarrhœa, that its eyes were suffused, and that it was not as active as usual. A peculiar odour was also noticed, quite distinct from the well-known smell of "monkey".

In no instance had the experiment a fatal termination.

Having thus proved to my own satisfaction that monkeys are susceptible not only to vaccination but also to small-pox, I endeavoured to discover whether any record existed of an epidemic of the latter disease attacking these animals in a state of nature. Mention of such an occurrence, as was pointed out to me, is to be found in the article "Mammalia", in the first edition of Cassell's *Natural History*.

No apparent support having been forthcoming, however, for the statements here made, the account has been omitted, as I was informed by the publishers, from later editions.

Murray, in a pamphlet on Small-pox, Chicken-pox, and Vaccination, published in Melbourne in 1869, also states that "Dr. Jackson has described an epidemic of variola that occurred over New Granada among monkeys". Whether this statement has any greater foundation in fact than the first, I am, however, unable to say.

In his book on Variola and Vaccinia, I find that Buist of Edinburgh describes the effects of variolation of monkeys, and gives plates showing the results he obtained, but he does not appear to have made trial of vaccine lymph in similar fashion.

I next determined to make trial as to the protection against small-pox afforded in the monkey by previous vaccination, and the protection against vaccination afforded it by variolation, and I went on to compare the effect produced by the use of human and of calf vaccine respectively. For this purpose humanized lymph was obtained from Birmingham, where, to the best of my belief, the same strain has been continuously carried on by means of arm-to-arm vaccination for the past thirty-eight years. The calf lymph used was obtained from the Government Animal Vaccination Station in Lamb's Conduit Street, while small-pox lymph was supplied to me from the Hospital Ships, from Warrington, and from Manchester.

The following experiments will serve as illustrations of the system I pursued:—

Experiment 1.—On July 13th, 1892, I vaccinated a male rhæsus monkey in four places on the left arm with calf lymph, on points taken at the A. V. E. on the previous day. On July 20th, the eighth day, the appearance presented was undistinguishable from that of a successful case of human vaccination.

On July 23rd the vesicles had given place to four large scabs raised nearly one-eighth of an inch above the surface of the arm, while the surrounding inflammatory

zone had nearly disappeared. The axillary glands on the same side were considerably enlarged. The arm was drawn on this day by Mr. Lapidge.

By August 9th the scabs had all come off and the monkey was again vaccinated, but this time on the opposite arm in two places, and also on the inside of the right thigh in two places also, with humanized lymph of ascertained activity. The animal was carefully examined day by day up to August 17th, but no sign of the second vaccination having "taken" was observed.

Experiment 2.—On July 19th a large female rhæsus monkey was inoculated by scarification in eight places (four on each arm) with two tubes of somewhat opaque S. P. lymph from the Hospital Ships. It was obtained from a patient *æt.* 19, stated to have been vaccinated in infancy, who was suffering from a semi-confluent attack of the disease. The lymph had been sealed up in carefully sterilized tubes. On July 23rd (the fifth day), the right arm showed papulation and commencing vesiculation. The glands slightly enlarged; left arm not so good. The lymph from which this arm had been done had coagulated in the tube, and very little could be got out. On July 28th a drawing was made by Mr. Lapidge, and on the following day the animal was seen by Dr. Thorne Thorne, Mr. Power, and Dr. Sherrington. On August 18th, a month after inoculation, it was vaccinated in four places on the inner aspect of the right thigh with A. V. E. lymph from calf No. 2809. No result of any kind followed the operation.

Not to weary you with details of the numerous experiments which I have performed on similar lines at intervals of one to five months after the primary inoculation, whether vaccinal or variolous, I may perhaps briefly show, by means of a table,* the general method of experimentation.

1.	V.	with	H. L.	...	...	Rv. with C. L.
2.	V.	„	H. L.	...	...	Rv. „ V. L.
3.	V.	„	H. L.	...	...	Rv. „ H. L.
4.	V.	„	C. L.	...	..	Rv. „ H. L.
5.	V.	„	C. L.	...	...	Rv. „ V. L.
6.	V.	„	C. L.	...	...	Rv. „ C. L.
7.	V.	„	V. L.	...	...	Rv. „ H. L.
8.	V.	„	V. L.	...	...	Rv. „ C. L.
9.	V.	„	V. L.	..	...	Rv. „ V. L.

No. 8 was afterwards again revaccinated on two occasions, first with V. L. and secondly with C. L. In none

* In this table H. L. = Human lymph; C. L. = Calf lymph; V. L. = Variolous lymph; V. = Vaccinated; Rv. = Revaccinated.

of these experiments did anything in the nature of a successful result follow the first or subsequent revaccinations, although in No. 8 the experiments lasted over a period of five months.

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

I desire next to call attention to the fact that, although protection against subsequent vaccination or inoculation is undoubtedly afforded by the effect produced on the system generally, as the result of the sequence of events at the site of operation that I have described, yet that these local manifestations are apparently not essential for the production of some protection.

My attention was first called to this possibility when, a year or two ago, I was experimenting with lymph which had been sterilized by heating at a temperature of 60° C. Such lymph not only gave rise to no bacterial colonies in plate cultivations made from it, but also it produced no local effect whatever when calves were vaccinated with it. On the first occasion that this occurred I thought that by chance the particular calf might have been the subject of an unnatural insusceptibility, especially as nothing resulted from a subsequent vaccination of it a little later with lymph, which from its effect on other calves and on children was obviously of normal potency. But on repeating the experiment on other calves the course of events was invariably similar, so that no doubt remained in my mind that something of the nature of protection had really been afforded by the primary operation in each instance, although nothing in the way of local reaction had been perceptible.

A similar course of events has been observed by Chauveau, Klein, and myself, in the course of variolation experiments on the calf.

By the introduction of vaccine lymph into the subcutaneous tissue of the back of the monkey, the lymph having previously been diluted with normal saline solution in order to obtain a quantity of fluid easier of manipulation, I find that protection is obtained against subsequent vaccination carried out in the usual manner. In one of the two instances in which I have made trial of this method, a local tumour, possibly an abscess, resulted, probably due to irritation from septic organisms or their products in the lymph.

Whether if glycerinated lymph were used the formation

of such a tumour would be prevented in every instance I am not yet in a position to make any dogmatic assertion.

On looking up Chauveau's work, I find that he has pursued the same method with bovines and horses, and that he confirms me in the opinion that a generalized influence over the system affords (for a time at least) immunity from vaccination obtained without production of local result.

I have also found two instances recorded in recent years in England in which "insusceptibility" to vaccination had apparently been conferred on the child during intra-uterine gestation by an attack of small-pox* in the one case, and by revaccination of the mother in the second. If these cases are trustworthy, such protection must have been afforded through the medium of the blood, or rather of the blood plasma, which alone is capable of diffusion between the maternal and fœtal circulations.

With the hope of throwing some light on this problem, and at the same time making trial of the value of what might possibly afford a new method of protection against small-pox, I devised the following experiment, on somewhat similar lines to the well-known work of Tizzoni and Cantani on tetanus. In a monkey which had been successfully inoculated with variola five months previously, and after an interval of three months had again been variolated and also vaccinated without result, the carotid artery was dissected out and tied with antiseptic precautions, after ætherisation of the animal. A glass canula was then inserted into the artery below the ligature, and about 30 c. c. of blood received into two test tubes, each of which contained a few drops of oxalate solution. The blood which was thus prevented from coagulating was then centrifugalized, and rather more than half the total amount of transparent slightly yellow-tinted plasma was obtained, the red corpuscles remaining in a dense mass at the bottom of the tubes. Of this plasma, about 8 c. c. was then carefully inserted into the peritoneal cavity of a second monkey through a minute incision in the linea alba. Two days after the operation both animals appeared perfectly well.

After an interval of fourteen days, on April 7th, 1893, the second monkey was vaccinated on the left arm and also on the inner aspect of the right thigh with calf lymph on points, obtained from the A. V. E.

I had no opportunity of seeing the animal again until the eighth day, owing to compulsory absence from town, but

* See also Report of Vaccination Committee, Epidemiological Society, 1885-86.

it was then evident that complete protection, at any rate, had certainly not been afforded by the operation.

All the scarified places had "taken", although in each instance the result produced was a somewhat abortive one, as was noticeable on comparing this animal with another which had been vaccinated on the same day and with the same lymph, and which therefore served as an excellent "control".

Some effect had therefore apparently been produced on the system of the monkey by the procedure adopted, but at the same time it was obviously but slight, even though the amount of plasma injected was considerable in relation to the size of the animal. It would appear that the use of the plasma of an immune animal is not likely to afford any result of value sufficient to render it desirable that trial should be made of it on the human subject.

It is conceivable that the oxalate used for the prevention of coagulation may have in some way prevented the effect which otherwise might have been produced on the system, but in view of the fact that Dr. Sherrington has found that leucocytes will live for as long a period as fourteen days in such oxalated plasma, this explanation hardly appears feasible.

Vaccinia and Variola in Bovines and Solipeds.

It is desirable now to consider briefly what is the relationship, if any, which exists between variola and vaccinia. To this end, as a preliminary, let us review the various means by which infection of the animal organism can be brought about experimentally in the case, first, of vaccinia, and afterwards of variola, as in this way only can adequate grounds for a judgment be obtained.

Such experimental methods may be tabulated as follows: —

1. Puncture, incision or scarification of the skin.
2. Injection (*a*) into the cellular tissue, beneath the skin or conjunctiva; (*b*) into the lymphatics; (*c*) into the blood-vessels.
3. Infection through the respiratory tract.
4. Infection through the digestive tract.

With regard to vaccinia, the local results of infection of the skin, more particularly in bovines and the human being, are so well known that it would be a waste of time to enter into any description here. It only remains, therefore, to

call attention to the fact that, as Chauveau has pointed out, a general eruption may follow the vaccination of colts, although unknown as a result of the operation in the case of calves. Accessory vesicles are indeed occasionally seen, but they usually arise at the site of accidental wounds caused by shaving, etc. It is to be remembered also that a spontaneous eruption arising independently of auto-inoculation is not unknown in the human being, as witness the case of his own child, recorded by Dr. Longstaff.

Chauveau has also placed on record a large number of experiments in which he injected vaccine lymph subcutaneously, and also into the lymphatics and blood-vessels of colts, and in a few instances heifers also. With heifers he never obtained any appreciable result, but with colts a generalized eruption followed in four out of eleven cases in which injection was made into the lymphatics, and in eleven out of twenty-seven cases in which the blood-vessels were made the channel of infection. A like effect followed insertion of lymph into a pouch made in the subcutaneous tissue in the neck. The generalized eruption produced in these various ways by the injection of vaccine lymph Chauveau denominates as *horse-pox*.

These experiments as yet I have not had the opportunity of repeating.

That generalized vaccinia can be communicated by means of the *digestive tract* there is considerable evidence to prove, more particularly in the human being. A case is recorded in which M. Cazal of Agde, in 1810, administered to a young girl, apparently refractory to vaccination, a pinch of powdered vaccine crusts in soup, in the hope of obtaining a more definite effect. As a result, constitutional symptoms were observed at the end of six days, which were followed by a general eruption of 180 vesicles all of clearly vaccine type.

Dr. Etienne mentions a case in which a child *æt.* 4 sucked the vesicles on its arm, which had been injured nine days after the operation. Dr. Richard also gives an account of a girl *æt.* 8 years, who sucked the vaccine vesicles on the arm of a younger brother. In both instances a generalized eruption of vesicles followed after an interval of about four days.

More recently somewhat similar cases have been placed on record in this country by Drs. Martin, Acland, Colcott Fox, and others.

Experimentally, a like result was obtained by Chauveau in two colts, which he infected by the addition of vaccine lymph to their drink.

Similarly, variolous infection may be obtained by way of the digestive tract, according to Marsillac,* who states that variolous matter spread between slices of bread and butter was given to two children and to a dog, and that by this means the children as well as the dog suffered in consequence from a very mild attack of small-pox. In this connection attention may also be called to an observation of Ceely's, which is, I believe, but little known. He records† an instance in which five out of eight milch cows sickened with cow-pox within twelve or fourteen days of their having been seen to be licking over a quantity of flock from the bed on which a patient had died of confluent small-pox, and which had been spread out in the field for purification. It is of course possible, however, that infection by way of the respiratory tract may have been a factor also in this case.

Being much struck by perusal of this statement, I endeavoured to imitate the occurrence experimentally. For this purpose I blew out the contents of about a dozen capillary tubes of small-pox lymph (many of them less than half full) which I had had by me for some time, into a small quantity of normal saline solution. This was afterwards diluted to about half a pint with normal saline, and after the addition of some small-pox "seeds" from the Hospital Ships, administered by means of a feeding-bottle to a heifer about two months old. The calf remained apparently well, and presented no symptoms of any sort for some days afterwards, but on March 11th (the tenth day) we found on examination that the mammary gland, which was hardly noticeable at the time of operation, was obviously swollen and "knotted". The swelling increased for the next two days until the mass measured four or five inches across, and then commenced to die away without any eruption having appeared. It was suggested to me that it would be well to repeat the experiment on a cow which had recently calved, and in which, therefore, the mammary gland is fully functional, as in the case of Ceely's cows. This, however, I have had no opportunity of doing up to the present. Subsequent vaccination of this calf induced an eruption of a somewhat abortive character. I also experimented on a fox-terrier dog by feeding it with small-pox crusts from the ships. No result followed, but I afterwards learned

* Quoted by Viborg of Copenhagen, in *Med. and Phys. Journal*, vol. viii, p. 272, 1802.

† "Observations on the Variolæ Vaccinæ," *Transactions of Provincial Med. and Surg. Association*, 1842.

that there was some doubt as to whether the package containing the crusts had not been "disinfected" by mistake before leaving the ships.

The only recorded instances of the *intra-venous* injection of variolous lymph of which I know are to be found in a paper by Dr. Klein* in the Reports of the Medical Officer to the Local Government Board. The paper deals mainly with attempts at variolous inoculation of bovines, carried out in 1878, which at the time were considered to be altogether unsuccessful. Among them Dr. Klein mentions two cases in which lymph previously diluted with normal saline was injected into a subcutaneous vein of the ear. The only result noted was some slight suppuration of the tissues of the ear; but it is to be observed that these injections were each done at an interval of eight days after a previous inoculation by scarification of the skin. As I have already shown in the case of vaccine lymph, and as Chauveau has also shown with regard to variolous inoculation, a certain amount of protection, at any rate, would have resulted from the previous operation, even though that also had brought about no obvious effect.

It should be mentioned, however, that Klein was successful in producing a general eruption in the sheep by means of an intra-venous injection of the lymph from a case of sheep-pox.†

Sonderland of Barmen, in 1830, claimed to have caused infection by way of the *respiratory tract*, but his method of experimentation has failed in the hands of later observers.

On turning, however, to the question of infection from skin inoculation, whether by puncture, incision, or scarification, the literature of the subject becomes so vast as to make it extremely difficult to condense the opinions of the numerous observers within reasonable limits. In consequence of the view, which has received much scientific support from the times of Jenner onwards, that cow-pox represents merely a modification of human small-pox brought about by residence in the tissues of the cow, the experiments to which I now have to refer have almost invariably been carried out on bovines.

It is quite impossible here to do more than mention the names of those who have taken up this line of research, except in the case of one or two of those more recent observers who have engaged in the work during the past few years. I append here a list of such names, placed, as

* Report of Medical Officer to Local Government Board, 1879.

† Rep. of Med. Officer to L. G. B., New Series, No. III, 1874.

far as possible, in chronological order. It is a noteworthy fact that every observer mentioned, with the exception of Chauveau and his colleagues of the Lyons Commission, claims to have obtained positive results as regards the production of typical vaccinia after one or more removes, as the result of variolation of the cow :—

Chronological List of Observers who have carried out Variolation Experiments on Bovines.

1807. Viborg, Copenhagen.
„ Gassner, Gunsburg.
1828. McMichael, Egypt.
1830. Sunderland, Barmen.
„ Numann, Utrecht.
1832. MacPhail, Baltimore.
1836. Thiele, Russia.
„ Martin, Allteborough, Mass.
„ MacPherson, India.
1839. Reiter, Munich.
„ Ceely, England.
1840. Badcock, England.
1863-65. Chauveau, Lyons.
1868. Shortt, India.
„ ? John Greene, Birmingham.
1871. Chauveau, Lyons.
1881. Voigt, Hamburg.
1886-90. Fischer, Carlsruhe.
1889. King, Madras.
1890-91. Eternod and Haccius, Geneva.
1892. Simpson in India, and Hime, Klein, and myself in England.

Although there is so great a unanimity of opinion among those who have worked at this subject, it must be confessed that many of the earlier experiments are practically worthless owing to the conditions under which they were carried out. Some of the main objections are based on the frequently concomitant use of vaccinal and of variolous lymph in the same animal, and the want of care as to the cleanliness and freedom from vaccine contamination of lancet and "points" used in the experiments. A somewhat similar objection might also be urged against much of this work, even that of quite recent date, in that, as it is perhaps not surprising, it should have been carried out for the most part in vaccine establishments and by persons engaged in the practice of vaccination. Such criticism, whatever may be the exact value to be attached to it, would, I believe, hold in the case of most of the experiments on the lines indicated which have been done since the date of the Lyons Commission, with the exception of those which have within the twelve months been carried out by Klein and by myself.

Without doubt, however, the largest amount of research work in this direction during recent years is that of Haccius, the Director of the Swiss Vaccinal Institute. In a work entitled *Variolo-Vaccine*,* which has been recently published, and which is profusely illustrated with beautiful

* *Variolo-Vaccine*, par Ch. Haccius, Genève, 1892.

drawings and photographs, he gives an account of no less than eight successful series of experiments, in each of which a strain of variolous lymph, obtained in the first instance from the human being, was carried on from calf to calf through, in some instances, as many as six or seven removes. The general result was that by the second, or at most the third remove the effect produced was altogether undistinguishable from that which we are accustomed to see in a calf which has been successfully vaccinated.

With the lymph of the fifth or sixth remove a number of previously unprotected children were vaccinated with unfailing success. The resulting vesicles could in no way be distinguished from ordinary vaccination vesicles of equal age. It is particularly stated that every precaution was taken, as, for instance, the sterilization of the lancet before each operation, to prevent the accidental transmission of vaccinia.

The single experiment which was published last year by Dr. Hime of Bradford differs from those of Haccius in that the former observer obtained in the calf, which was directly inoculated with variolous lymph, three vesicles having the appearance and running through the usual course of typical vaccination vesicles.

From the brief account of his experiments, which has been published by Simpson, it would appear that his experience has been very similar to that of Hime.

Concerning Dr. Klein's results I, at present, know but little, for the reason that, as his experiments were carried on concurrently with some of mine, I purposely refrained from seeking information on the point. I took this course, feeling that if it should afterwards appear that we had obtained very similar results, such mutual corroboration would be all the more valuable from a scientific point of view.

My own experiments on this point I now propose to describe as succinctly as possible.

Experiment 1.—On August 11th, 1892, I inoculated a cow-calf, about six weeks old (C), in thirty-two incisions and two scarified patches on the abdomen. For this purpose I made use of two capillary tubes of variolous lymph taken from a vaccinated girl *æt.* 16, a patient at the Hospital Ships, who was suffering from a typical attack of discrete small-pox. She had been admitted on July 26th, and the lymph was taken on July 30th, with antiseptic precautions, into tubes which had been previously sterilized.

When the calf was examined on August 13th nothing

was to be seen, except that a slight scab was forming over the scarified patches. Two days later, however (on August 15th), some of the incisions, more particularly the more posterior ones, looked somewhat red and elevated, while in their course were several small points more distinctly elevated than the rest. On the inner aspect of each thigh, quite apart from any of the incisions, was noticed a crop of shotty and apparently slightly vesicular pimples. On the right side also were a few isolated pimples at some distance from the patch. At this stage the calf was photographed and a drawing made by Mr. Lapidge, which I have brought for your inspection.

On August 17th (the eighth day), with the aid of compression forceps, I scraped three of the incisions, removing lymph and crusts together, with which Calf D was inoculated the same day in fifty-two incisions. To finish the history of Calf C, however, I may state that, in the first place, there was never any appreciable rise of temperature or apparent illness; that by August 19th the eruption was commencing to crust over; and, finally, that rather more than a month later (on September 22nd) I vaccinated in twenty-three long incisions with calf-lymph obtained from the A. V. E., but without producing any result whatsoever.

Experiment 2.—On August 17th a cow-calf (D), two months old, was inoculated in fifty-six linear scarifications with lymph and crust obtained from the previously-mentioned incisions of Calf C. Two days later it was obvious that the experiment would be successful, as the lines of incision were all distinctly raised and surrounded by a delicate pink flush.

On August 20th (the fourth day) all the incisions had evidently "taken" well, the areola around each being now about $\frac{1}{8}$ inch wide. On the same day this calf was photographed, and another, Calf E, inoculated from it. It was also drawn in colours by Mr. Lapidge. A month later (on September 22nd) it (D) was vaccinated in seventeen incisions, each about two inches in length, with calf-lymph obtained from the A. V. E. Again the result was absolutely negative.

Experiment 3.—On August 20th, 1892, a small cow-calf (E), seven weeks old, was inoculated in twenty-seven linear incisions from Calf D with both lymph and crusts. In this instance, again, every incision had "taken" on the fourth day, when another drawing was made of the appearances presented. On August 25th some of the "places" were distinctly vesicular along their margins.

For reasons of economy the series was not continued further, and although some tubes of lymph were collected from this calf, I did not feel justified, without further experience, in trying its effect on children. Unfortunately, I had not fully appreciated at this time the possibility of using monkeys for purposes of control.

Experiment 4.—On November 29th, 1892, I inoculated a calf (F) in thirty-seven incisions and one scarified patch with five tubes of variolous lymph obtained from a case of confluent small-pox in an unvaccinated child *æt.* 3 years at Warrington. As no result followed, I inoculated again on the ninth day with a further supply of lymph from Warrington, in the hope of obtaining Ceely's result in the revivification of the former incisions. Again, however, I was doomed to disappointment.

Experiment 5.—A young cow calf (G) was inoculated on December 12th, 1892, in forty-eight linear incisions, with Warrington lymph from a case of confluent small-pox in an unvaccinated man aged twenty-three.

On December 15th a slight blush was visible at the site of all the incisions, and several little "shotty" points were noticeable to the finger when pressed over the skin, particularly on the inner surface of the right thigh. On the evening of the same day I was unfortunately attacked by an illness which confined me to hospital for nearly a month, so that I had no opportunity of observing the further progress of this case, which, apparently, gave every promise of success.

Experiment 6.—The last experiment of this nature which I have performed, and of which the result is shown in a photograph, need not be related in detail, as owing to pressure of other work I was unable to get back to town before what had evidently been minute vesicles on the tract of some of the posterior incisions had practically dried up. As a matter of fact, another calf was inoculated from this one, but did not "take", possibly in part for the reason that it was extremely young, having to be fed on milk entirely, and because it was suffering from a severe attack of diarrhœa at the time.

Thus far, then, I have obtained an undoubtedly successful result in one case only out of four attempts; but I have at any rate been able to satisfy myself that it is possible to variolate the calf; and further, that the result obtained in the first instance becomes greatly modified in the course of successive removes; and again, that animals which have been thus treated are no longer susceptible to vaccination.

I may mention here that, by way of precaution, the scalpels used were invariably first carefully sterilized in a flame; that, after use, the table was thoroughly washed with carbolic acid and hot water, and during the intervals of use kept exposed to the open air.

In none of my experiments, then, did I obtain anything resembling a true vaccine vesicle in the calf first inoculated, as happened in Simpson and Hime's cases. On the other hand, I must say that I have never yet met with the precise appearances described by Chauveau in the Report of the Lyons Commission. How these discrepancies are to be explained it seems impossible to say as yet, but I hope that by making trial under varying conditions I may hereafter be enabled to solve the problem. As far as can be judged from what I have done at present, I find myself more in agreement with Haccius, whose variolo-vaccine lymph, as he calls it, has now been carried through many generations of children without producing any of the ill effects of variolous inoculation.

Those who deny the relationship of small-pox and cow-pox will say that these children have been variolated and not vaccinated. One, of course, admits that they *have* been variolated, in the sense that they have been inoculated with lymph descended from a case of human small-pox, just as was probably the case in the enormous number of children on whom Badcock's lymph was used, but differing from the mild inoculations of Adam Dimsdale and the Suttons in that the resulting disease is no longer contagious. Such procedure is also strictly comparable with those methods of protective inoculation by the use of attenuated virus, which of late years have given such valuable results in the prevention and treatment of various zymotic diseases. If, then, it can be conclusively proved that small-pox lymph, by passage through the system of the calf, can be so attenuated and altered in character as to become deprived of its power of causing a generalized eruption, and, more important still, that by such treatment it loses its contagious nature, while causing at the site of inoculation what is undistinguishable from a typical vaccine vesicle, it is, I think, difficult to find any ground for the assertion that vaccinia is not a modification of, if not identical with, variola, especially as these two diseases exert a mutually protective action against one another. On the other hand, as I have abundantly shown in an earlier section of this paper, vaccinia, when passed through the organism of animals other than the cow, is capable in certain

cases of so far regaining its virulence as to give rise again to a generalized eruption.

I am aware that Crookshank and others assert that this power of mutual protection is no argument, for the reason that there are several diseases which, when inoculated, cause a local effect somewhat similar to that produced by variolation or vaccination, and which exert a so-called temporary antagonism against small-pox.

This question the time at my disposal on the present occasion will, unfortunately, not allow me to discuss, but I must confess that up to the present I have seen nothing adduced in the way of evidence which would lead me to the conviction that these two diseases, which protect against one another, are yet absolutely dissimilar in nature, such a state of things being altogether opposed to what we at present know of the natural history of infectious diseases. In any case such statements should, I think, be received with all reserve, unless supported by an amount of trustworthy experimental evidence, far in excess of any that is at present forthcoming.

Bacteriology of Vaccine Lymph.

I desire now to turn to a branch of my subject which I have had the honour on a previous occasion of bringing, in part, before the notice of this Society. I refer to the study of the bacteriology of vaccine lymph, and to the lessons which may be learnt from such a study, as to the means of storage best adapted for securing sustained purity of the lymph and the unimpaired manifestation for indefinite periods of the action peculiar and essential to its use.

In the volume recently published of the *Transactions* of the Bacteriological Section of the Hygiene Congress, held in London in August 1891, will be found two papers dealing to a greater or less extent with this subject; the first by myself, the second by Professor Crookshank. Although working quite independently, we both arrived at the conclusion, in which we are corroborated by Pfeiffer and many other observers, that although numerous bacteria can be grown in various nutrient media from specimens of vaccine lymph obtained in the ordinary way, yet that to none of these could be assigned the *rôle* of the actual vaccine virus, which as yet remains unidentified. For this reason I had assigned to these bacteria the term "extraneous", signifying thereby that their presence is not in any way

essential to the successful action of the vaccine lymph. In this also Professor Crookshank agrees with me, for he says: "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. To sum up, most of them are well-known saprophytic bacteria, and some are identical with bacteria commonly found in suppuration. Vaccine lymph is a most suitable cultivating medium for micro-organisms, and bacteria invariably get access to the contents of the vaccine vesicle."

A lengthy list is given by Crookshank of the various bacteria which may be isolated from human or calf lymph by the method of plate cultivations, but he does not state whether any and, if so, what precautions had been taken with regard to the collection of the lymph with which he experimented; neither does he attempt to distinguish between those bacteria which are commonly to be found, and those whose presence is exceptional. I have shown, however, that there are three species of micro-organisms, one or other, or all, of which is almost universally to be found in every specimen of vaccine lymph examined, of which one, the staphylococcus albus, is usually to be found in the upper layers of healthy skin. Of more importance is the fact that in certain cases, though rarely, I have been able to satisfy myself as to the presence of the streptococcus pyogenes, and, moreover, it is on record in certainly one instance at least that the streptococcus of erysipelas has also been isolated from a specimen of vaccine lymph.

That untoward results should occasionally, in certain cases, follow the inoculation during the act of vaccination of one or other of the various extraneous organisms which may be present in the lymph employed is not to be wondered at; indeed, I take it that it is rather a matter for wonder that the evil results following the present somewhat empirical practice of vaccination have been, in the past, so few in proportion to the vast numbers that have undergone the operation. Still, in view of the feeling against vaccination which is prevalent at the present time, it is most desirable that we should not, if it be possible to avoid it, run any risk of performing a septic inoculation on a patient under the guise of vaccination.

If only it had been possible to isolate the contagium of vaccinia (or variola), and to carry on, without loss of specificity, pure cultures of it in some nutrient medium from generation to generation, this portion of the problem would

be solved; but, although the number of observers who have turned their attention to this point is legion, and although a claim is every now and again advanced, success cannot as yet be said to have been obtained.

Fortunately, however, we have at our disposal other means of satisfactorily ensuring the purity of vaccine lymph.

Some two years ago, when experimenting on the possibility of isolating the specific contagium of vaccinia and variola, it struck me that the exuberant growth of evidently "extraneous" organisms, which I obtained in all the various nutrient media employed, might be capable of superseding the more important organism for which I was seeking. I therefore turned my attention to the discovery, if possible, of some means of treating vaccine lymph which should inhibit all extraneous organisms without injuring its potency for vaccination.

Another point which directed my research work at that time in the direction I have indicated, was that my attention was called to the opacity which commonly occurs after a longer or shorter period in lymph which has been stored in sealed capillary tubes, and to the deterioration in activity of the lymph which is found to be a concomitant of such opacity.

In former papers I have shown that this opacity of old stored lymph is quite independent of any coagulation in the lymph, as no coagulum was found on breaking many of the tubes in which it was most marked. Further, I have shown that if cultivation experiments are carried out with the contents of tubes which have become opaque, and also simultaneously, for purposes of control, from tubes of comparatively fresh lymph, many more colonies result in the plates poured from inoculations of the old tubes than in those for which more recent lymph is used. I concluded, therefore, that we are apparently justified in considering that the opacity of old stored lymph is, in the main, the outcome of an enormous multiplication of aërobic bacteria, the ancestors of which were present in the lymph when first taken, although their numbers are then so comparatively small as not to render it in any way turbid. These evidently find in the lymph, especially when removed from the body, a suitable medium for their subsequent multiplication, while at the same time it would appear that such growth has the result of gradually inhibiting the specific effect proper to the vaccine virus itself.

In this case also, then, the obvious indication was not

only to prevent such multiplication of extraneous organisms subsequent to storage in the usual manner, but, if possible, to remove them altogether without injury to the actual contagium of vaccinia.

Without detailing the various experiments which were carried out with this object in view, a full account of which I have already published, it is necessary in this place to state briefly the lines on which the work was carried out. In the first place I made trial of the method of fractional heating, which had, in the hands of Kitasato, met with such brilliant success in the isolation of the bacillus of tetanus. Proceeding in this manner, and in every experiment observing the precaution of making control cultures, I presently arrived at a temperature exposure to which is apparently incompatible with the continued existence of those micro-organisms which can ordinarily be grown when vaccine lymph is inoculated into nutrient jelly. This required temperature ranged between 38° C. and 42° C.; but whereas, after exposure for an hour at the lower register, a few points of growth are occasionally seen in plate cultivations after the lapse of a day or so, while the higher temperature sometimes appeared to exert an injurious effect on the lymph, as far as regards the normal vesiculation which should result from its inoculation in the living animal, I sought for some method of readier application and requiring less delicate manipulation. This I at length found in the admixture with the lymph of a definite proportion of 75 per cent. glycerine in distilled and sterilized water prior to storage in capillary tubes, which latter had been sterilized by heat.

Many years ago Müller showed that lymph might be diluted with three times its bulk of such a mixture and still retain its properties unimpaired, a fact which has been taken advantage of at many of the continental vaccine stations, and by more than one purveyor of trade lymph. An argument in favour of the use of lymph diluted in this way with glycerine is found in the fact that such a mixture does not dry up nearly as readily as ordinary lymph, and, therefore, in the hands of operators all but the most expert, affords greater facilities towards the attainment of a uniformly successful series of vaccinations. The fact also that a much larger supply of lymph is thus made available is so obvious that it needs not to be insisted on.

Not only is lymph thus treated efficient as vaccine in the old sense of the word, but, as time goes on, instead of losing its effect on inoculation, its potency actually becomes in-

creased. Experiment shows also that in tubes filled with such diluted lymph opacity does not apparently result. As I have previously stated—an observation, by the way, for which I may fairly claim priority—the glycerine inhibits the growth of, and, after a longer or shorter interval, kills off altogether, those aërobic bacteria which I have termed "extraneous". This effect may be demonstrated by making from tubes of glycerinated lymph a series of plate cultivations at gradually increasing intervals of time, together with control cultivations from tubes of untreated lymph. (Photographs of such plates I show you to-night.)

With the assistance of Dr. Cobbett I have also carried out a series of glycerine experiments on a larger scale, not only with the organisms which can be grown from vaccine lymph, but also with numerous other species of bacteria, both pathogenic and saprophytic in nature. With details of these experiments, which we shall hope to publish shortly, I need not detain you now. I will here merely state that the net outcome of our work appears to show that while some pathogenic organisms remain active in admixtures of broth and glycerine, or even in pure glycerine, for longer or shorter periods, other pathogenic, and, as far as our experiments go, all saprophytic, organisms are killed out in from three or four days to as many weeks.

At the time of writing I have seen, for the first time, a paper (dated September 1892) by Achille Sclavo, Director of the Health Bacteriological Laboratory at Rome, on the Preservation of Viruses in Glycerine. In this paper, which is very short, the author does not do much more than just refer to the use of glycerine as an addition to vaccine lymph, and has obviously not altogether appreciated the importance of the results to be derived from such admixture. Indeed, he appears to mention it merely as an exception to a rule, which he considers fairly general, that many bacteria are preserved rather than destroyed by glycerine. As, however, he confirms in several particulars the results at which we have arrived, it will be well to briefly epitomise the paper in question.

"Glycerine is added nowadays in almost all vaccine institutions to vaccine emulsion, which by this means preserves its virulence for months, and even for years.

"As a proof of this I adduce the following*:—Dr. Krieger, after some experiments with calf lymph, overlooked a bottle of lymph in a press. He found it, and

* "Note sur le Vaccin de Genisse," par M. E. Deschamps, *Revue d'Hygiène*, N. S., p. 654.

used it *eleven* years afterwards, producing normal pustules and passing it through other subjects.

"The virus of rabies, too, remains unaltered in glycerine for a long time (Roux), and is active after six months (Ulfreduzzi).

"Further, many bacteria live well in glycerine, and they may be found preserved in glycerinated vaccine emulsion for various lengths of time. Their number, however, gradually diminishes, so that after some months' preservation the emulsion may retain its specific activity, but contain only a sparse number of living bacteria.

"I have extended my experiments in glycerine preservation to pathogenic bacteria found in the tissues of animals dead of infectious disease, using quite neutral unsterilized glycerine. I put spleens of such animals into 80-100 c.c. of glycerine, and kept them at the temperature of the room.

"Teased portions were subsequently inoculated subcutaneously in living animals. The bacteria used were the diplococcus of Fränkel, the bacillus of fowl cholera, and that of anthrax (? spore form)."

As the result of these experiments, Sclavo arrives at the following conclusions:—

The *diplococcus of Fränkel* preserves its virulence in glycerine for not less than sixty-seven days (also at forty-four and fifty-eight days).

Bacillus of fowl cholera—virulent after seventy-four days in glycerine, but killed by stay of four months.

With *anthrax*, virulence gradually decreased, ceasing in one experiment on the seventh and in another on the ninth day. At earlier periods incubation was prolonged in the living animal, and there was extensive subcutaneous œdema, while the bacteria showed a tendency to form long filaments in the blood, so that it may be that *the anthrax b. underwent a gradually increasing degree of attenuation in the glycerine.*

Of considerably greater importance from my point of view, however, is a paper by Chambon and Ménard which recently appeared.* In it these gentlemen relate their experience of the use of glycerinated vaccinal lymph when kept for a long time in capillary glass tubes previously sterilized and closed by the blow-pipe. The results they obtained with lymph originally good were highly satisfactory, and even lymph, which in its fresh state gave mediocre results, in

* *Gazette des Hôpitaux*, Dec. 15th, 1892.

the course of fifteen days produced a passable vesicle, and, after forty, fifty, or sixty days, a typical one. The improvement seemed due to the gradual extinction of parasitic microbes under the influence of glycerine and time.

Prof. Straus made plate cultures with their glycerinated lymph, which showed that when fresh it gave rise to numerous colonies of various microbes, especially staphylococcus pyogenes aureus and staphylococcus albus, while the glycerine lymph, fifty to sixty days old, remained absolutely sterile, intermediate specimens presenting fewer and fewer microbes as they became older. These experiments were repeated many times, and invariably with similar results.

This evidence, so entirely corroborative of my own work, is all the more important, as it appears certain, from a perusal of the original paper, that the authors were ignorant of the precisely similar results at which I had previously arrived, and which were published nearly twelve months before the appearance of their article.

There can thus, I venture to think, no longer be any doubt as to the superiority of the method of lymph storage which I advocate over the perhaps simpler method which has been so strenuously insisted on in England of late years, and I trust, therefore, that some authoritative pronouncement may before long be made on the matter, as it appears to me, at any rate, to be one which is of the utmost importance in relation to the present wide-spreading objection to vaccination on the score of the possible dangers incidental to the operation.*

No one, of course, denies that vaccination is liable to be complicated with erysipelatous, phagedænic, or pyæmic conditions; but all these latter undoubtedly are specific diseases due to specific causes, and when they arise in connection with cow-pox, whether in the cow or in man, are the results, not of the vaccine virus, but of specific poisons of a different kind, which have either been introduced with the vaccine virus or have inoculated the vaccinal sores at a later period.

It has, nevertheless, been frequently affirmed by some opponents of vaccination that erysipelas is an essential concomitant of vaccinia, and that given certain conditions —as, for instance, a lessened vitality in the child, or insani-

* From experiments which I have carried out, in part since this paper was read, it would appear that lanoline possesses the property of inhibiting the growth of saprophytic organisms in a similar manner to that exhibited by glycerine.

tary surroundings—it will gain the upper hand, causing injury and perhaps death, the ordinary manifestations of the vaccine virus itself either not appearing at all, or becoming aborted. Indeed, some go to the absurd length of considering the areola, which normally appears about the eighth day, as merely an indication of the presence of erysipelas in a more or less nascent condition.

Seeing that the streptococcus of erysipelas grows fairly readily, though it must be confessed somewhat capriciously, in ordinary nutrient media, it is obvious that, supposing no growth of any sort is obtained in a plate cultivation of glycerinated lymph of ascertained activity, the above-mentioned thesis cannot be sustained. In view, however, of the possible occurrence of the streptococcus in vaccine lymph, I thought it desirable to specially study the effect produced by the addition of glycerine to various nutrient media, which have been seeded from a pure cultivation of the streptococcus.

Such a cultivation I obtained through the kindness of Dr. Armand Ruffer. As, however, my experiments have not as yet been sufficiently exhaustive, I must defer details to a subsequent period, merely stating now that, in this case also, glycerine appears to inhibit the development of the streptococcus.

In conclusion, it may be well to briefly summarise the results at which I have arrived:—

1. The organisms to which the specific action of variolous and of vaccine lymph is due as yet are undiscovered.

2. Although this is the case, all the most reliable evidence, experimental and otherwise, is in favour of the organisms of these two diseases being identical in nature; the phenomena of vaccination being most probably due to an attenuation of the virus, caused in a similar manner to that witnessed in chicken cholera and other diseases, by sojourn in the tissues of an animal which is more or less refractory.

3. Monkeys react to inoculations of variola or vaccinia in a similar manner to the human being, and therefore afford a valuable means of controlling the results of experiments on the mutual relationship of these two diseases.

4. It is possible to isolate from vaccine (and also, to a less extent, from variolous) lymph a number of micro-organisms, none of which, however, have anything to do with the specific action of the lymph.

5. These bacteria belong in great part to that class which is concerned in the processes of suppuration, though

others, both pathogenic and non-pathogenic in nature, may be present also.

6. These "extraneous" bacteria may be destroyed if present, and in any case a guarantee of their absence may be afforded by the methods of treatment of the lymph which I have advocated.

7. By my method lymph may be preserved unimpaired for an indefinite period. Indeed, its specific activity, so far from being destroyed, may actually become intensified.

There now only remains the pleasant duty of expressing my sincere thanks to all those gentlemen who have aided me in my research. To Dr. Ricketts and Mr. Colclough, of the Hospital Ships, to Dr. Gornall, of the Warrington Small-pox Hospital, and to Dr. Berry, of the Monsal Fever Hospital, Manchester, I am indebted for supplies of variolous lymph; to Dr. Ruffer for a pure growth of the streptococcus of erysipelas; to Mr. Stanley Kent and Dr. Edmunds for photographs; and to Professor Foster, Mr. Power, Dr. Sherrington, Dr. Acland, and others, for much kindly help and criticism.

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