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Sir Shirley Murphy
with the Author's Compliments

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W. J. SIMPSON, C.M.G., M.D., F.R.C.P.

Professor of Hygiene, King's College, and Lecturer on Hygiene, London School of Tropical Medicine

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OBSERVATIONS ON THE ETIOLOGY OF VACCINIA AND ON THE CULTIVATION OF THE MICROBE OF VARIOLA.

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In an article dealing with the "Etiology of Vaccinia and Variola," Dr. Klein gives, in the Medical Officer's Report to the Local Government Board for 1892-93, published in 1894, an account and illustrations of the bacillus he found in the early stages of vaccine lymph taken from a calf, and of a similar bacillus in variolous lymph. He was unable to cultivate either.

Studying the same subject from a different point of view, I had, since my arrival in India in 1886, been searching for cow-pox among cattle, with the object, if possible, of cultivating the vaccinia micro-organism from them. I found, however, that the cow-pox I was searching for turned out to be in India a severe affection, and I gradually came to the conclusion that many of the cases were small-pox. In fact the cattle plague in Bengal is called by the Indians gotee and bassunto, two names to denote small-pox. The Indian Cattle Plague Commission of 1871, of which Colonel Kenneth McLeod, I.M.S., was Secretary, came to the conclusion that the Indian cattle plague, which included gotee or matah, was allied to, if not the same as that which used to prevail in England. My experience leads me to the conclusion that the term gotee or matah includes a number of diseases, of which small-pox is one, just as the term rinderpest may include a number of diseases difficult to distinguish from one another. There is a gotee with an eruption and one without an eruption. I am fairly certain that some of the epidemics that go under the name of cattle plague or rinderpest in Bengal are small-pox. Anyone who makes a *post-mortem* examination of a virulent case of small-pox in the human subject and then a *post-mortem* of a case of cattle plague or rinderpest, as seen in Calcutta in a buffalo or cow, cannot fail to be struck by the similarity in the naked-eye appearances of the lesions which have been produced, and their seat. Dr. Duncan Stewart, of Calcutta, as early as the "forties" of last century, suspected a relationship between the two diseases. Veterinary Colonel Hallen, the President of the Indian Cattle Plague Commission of 1871, held a similar opinion.

When the cattle plague prevailed in England and on the Continent in the eighteenth century, it was held by many, especially by M. Vicq D'Azyr and by Dr. Layard, who wrote on the subject in 1756, and again in 1780, "that it was an eruptive fever of the variolous kind." There is also the incident of the vesicle on Mr. Handcock's hand, a veterinary inspector at Uxbridge, in London, who accidentally inoculated himself in 1865 when engaged on the *post-mortem* of a bullock, which had died of cattle plague. The vesicle was seen by Mr. Rayner, Professor Spooner, Dr. Quain and Dr. Murchison, who recognized in it the character of the vaccine vesicle. It was also seen by Mr. Ceely, who stated that it corresponded with some of the cases of casual cow-pox in the milkers.

To return, however, to Bengal. I had in mind the experiments of Macpherson at Murshedabad, who, in 1832, succeeded in obtaining vaccine by inoculating children with the crusts obtained on the twelfth day from an animal suffering from gotee. He tested the vaccination by inoculating two of the children with small-pox virus, but without producing small-pox. There were also the experiences of Mr. Furnell and Mr. Brown in Bengal, who performed similar experiments with animals suffering from gotee, with the result that not vaccine, but small-pox, was produced in the children.

During several years' search among gotee animals I had isolated a diplobacillus from some of them, but the opportunity did not allow of me testing them until 1894, when I came across a buffalo suffering from gotee and with the most profuse eruption on head, neck, body and teats that I have ever seen. The animal a little before its death was shown to several medical men who were asked their opinion as to the nature of the disease. Their reply was unanimous that if it had not been a buffalo they would have said the disease it was suffering from was small-pox. With the assistance of Mr. Haffkine, C.I.E., a diplo-bacterium similar to some of the others previously obtained was isolated. Cultures consisted of rather small diplo-bacteria, which sometimes had the appearance of diplococci, and occasionally a thick diplo-bacillus. This is the type of the culture.

Circumstances did not permit me to test the cultures of this new microbe until some seven to eight weeks after its isolation, but in November, 1894, I inoculated two calves with different aged sub-cultures. For convenience of reference the two calves will be called Calf A and Calf B, and they are classed in Series A.

Series A.—First Set.

In Calf A four out of the fourteen inoculations became vesicular between the fifth and sixth day. Lymph from these vesicles was transferred to another calf, and in this none out of the twenty-one developed, but a secondary vesicle appeared on the ninth day and became a very fine vesicle on the tenth day. The lymph from this vesicle of the tenth day was transferred to a third calf by seven punctures. Vesicles developed at the seat of each puncture, having on the seventh day the appearance of ordinary vaccine vesicles of the sixth day. With this lymph a child was inoculated on the arm and a calf on the abdomen and both showed good vesicles on the sixth day. The vesicles on the calf and child were declared to be vaccine vesicles by the medical men, native and European, interested in the proceedings, among

whom were my assistants, Drs. Ram Chunder Mitter, J. Dutta and S. B. Ghose; also the late Colonel Alexander Crombie, C.B., I.M.S., and Colonel W. G. King, C.I.E., I.M.S., now retired from India and living in England.

From the child other children were inoculated, and each presented well-developed vaccine vesicles. The lymph was transferred from these to other children, and so through a series. The children were later vaccinated with ordinary vaccine lymph, but did not take.

Series A.—Second Set.

Calf B was inoculated in forty places with cultures, and on the sixth day many of the punctures presented a similar appearance to that usually found on the fourth day in ordinary vaccination, but later they receded and dried up. On the eleventh day two well-developed vesicles appeared, and on the twelfth day a third vesicle. The lymph of the two first was transferred to calf B^1 by seventeen punctures, all of which presented good vesicles on the fifth and sixth day and a secondary vesicle appeared on the seventh day. Plate I, fig. 1.

Lymph from these vesicles was transferred to a calf and child. Both showed good vesicles, but on the seventh day a crop of secondary vesicles appeared on the calf and a measly eruption on the child which faded on the ninth day. From this child a series of children were vaccinated, all showing good vesicles and with no eruption. Plate I, fig. 2, shows vesicles on one of the children. The children were later vaccinated and all resisted.

This double set of experiments, expanded in various ways such as re-transferring the lymph from the children on to calves, again from calves to children, and from children to children, lasted from November, 1894, to February, 1895.

Intermediary Set of Series A.

There were some intermediary sets in which fresh cultures were used on calves and mostly raised secondary vesicles, but they were not carried beyond the calves and on to children, because of the early appearance of the Indian rinderpest symptoms among them. I do not propose for the present to deal with this subject, or with the many experiments made to immunize cattle against this disease. I shall confine my remarks to the effects of the cultivated diplobacterium when inoculated on the skin of calves—viz., the production either of vaccine vesicles at the seat of inoculation maturing on the tenth day or eleventh, or the appearance of secondary vesicles on the tenth or eleventh day, and to the observation that lymph taken at an earlier period from vesicles at the site of the inoculation acts in a different way.

During the months of December, January and part of February, the cultures from the buffalo were sub-cultured once a week and a new series of experiments (Series B) was begun with the microbe in February, 1895.

Series B.

In order to eliminate the rinderpest factor as much as possible, I selected another place for the experiments, and isolated the animals for a period of ten days. This being done, three calves, C, D, and E, were inoculated the same day; C with cultures derived from the lung of the buffalo, D with cultures from the spleen, and E with cultures from the liver. C took ill on the seventh day, and died on the tenth of Indian rinderpest; nothing but a secondary papule appeared on the eighth day.

Series B—First Set.

In D, a vesicular rash appeared on the left side of its abdomen on the sixth day, seven secondary nodules or papules appeared on the thighs and scrotum on the seventh day, also a small blister patch on the upper gum. The temperature rose to 105° F. on the eighth day, to 106.6° F. on the ninth day, and the animal had loose motions, yet it continued to feed well. One of the papules became vesicular on the tenth day and four new papules, two under the armpits and a crop on the buttocks, appeared. The temperature was 106.8° F. and there was a generally measly rash on the thighs and abdomen. On the eleventh day two other papules became vesicular, but the vesicles were small. From the three secondary vesicles lymph was transferred to another calf which presented four vesicles on the sixth day, the lymph of which was transferred on the seventh day to two children (A and B) and a calf D^1. Plates I and IV, fig. 3, show vesicles on child A. All gave excellent vaccine vesicles, and from these a third series of calves and children was vaccinated. The children were later vaccinated with ordinary vaccine lymph and all resisted. D calf continued ill until the sixteenth day and then gradually recovered.

Series B.—Second Set.

Calf E had three primary vesicles on the seventh day, but not characteristic. A calf inoculated with their lymph gave small, but unsatisfactory, vesicles. The temperature of calf E rose to 104° F., and there was a vesicular or herpetic rash on the abdomen and thighs. On the eighth day two secondary papules appeared on abdomen, one of which became a vesicle on the tenth day. This vesicle was well raised and developed on the eleventh day, and its lymph was transferred to another calf, E^2, which developed excellent vesicles on the fifth day, from which was started a fourth series of successful vaccinations in calves (Plates I and IV, fig. 4, show vesicles on calf E^2) and children. Plates I and IV, fig. 5, show vesicles on one of the children. Later the children were vaccinated with vaccine lymph and all resisted. The temperature of calf E continued high until the eighteenth day, but beyond this there were no signs of illness. It was vaccinated three weeks later, but did not take.

In the second and third sets of Series B the lymph was transferred from calf to calf and from calves to children, and again from children back to calves.

Series C.

Calf F was inoculated in March with a culture of the microbe kept in hydrogen for thirty-nine days.

PLATE I.

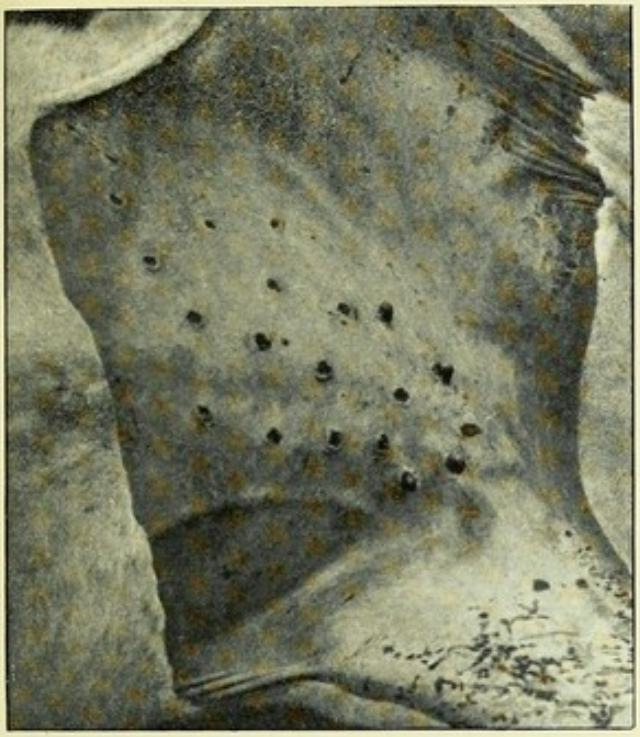

FIG. 1.—Calf B^1 inoculated with eleventh day lymph from vesicles on calf B in 2nd set of series A.

FIG. 2. — Ibrahim, showing vaccine vesicles produced with lymph from a child three removes from calf B^1 in 2nd set of series A.

FIG. 3 — Mungur child A, vaccinated in 1st set of series B.

FIG. 4.—Vesicles of ninth day on calf E^2 inoculated from calf E, which was inoculated February 9, 1895, with a culture 24 hours old of R.P., *i.e.* from microbe isolated from buffalo on September 14, 1894. 2nd set, series B.

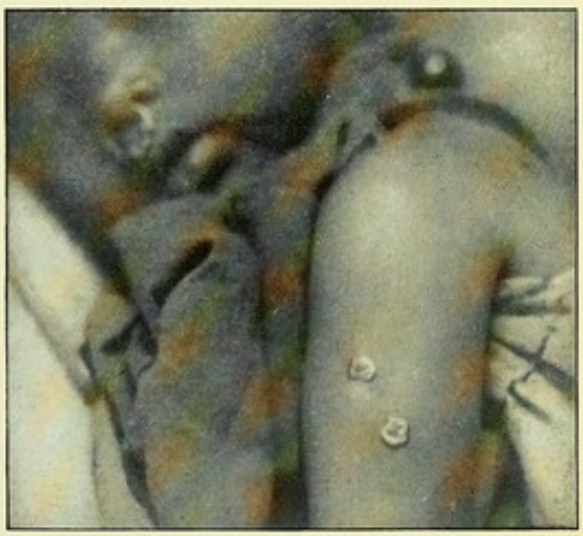

FIG. 5.—Punia. One of the children vaccinated from calf E^2. 2nd set, series B.

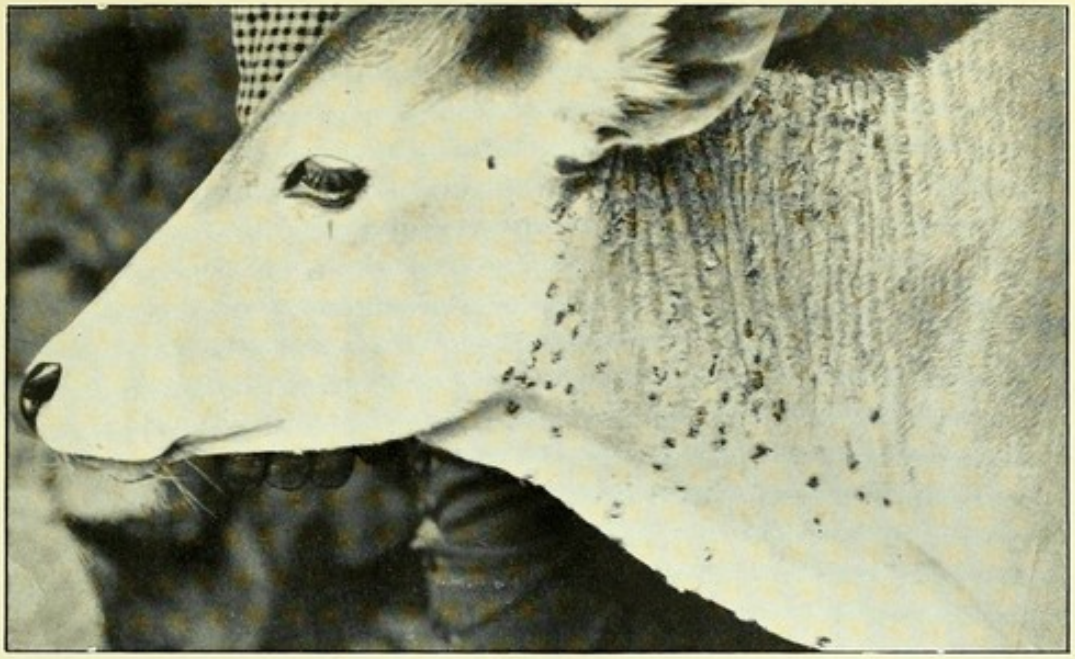

FIG. 6.—Calf F, with eruption left side of neck. Series C.

FIG. 7.—Calf F, with eruption on throat and dewlap. Series C.

PLATE III.

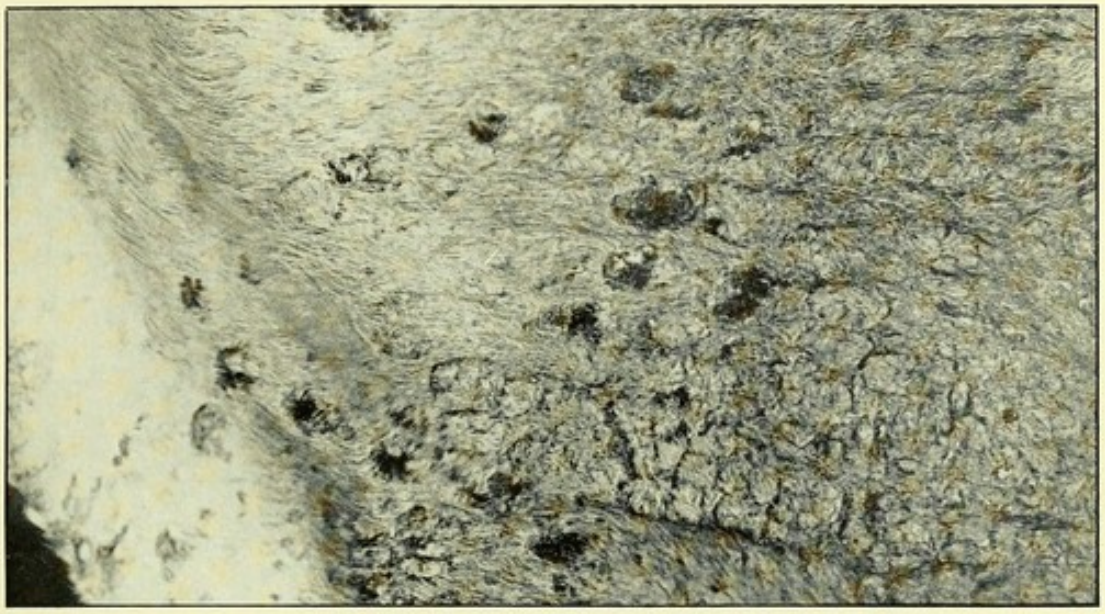

FIG. 8.—Calf F, with eruption on portion of right side and neck. Series C.

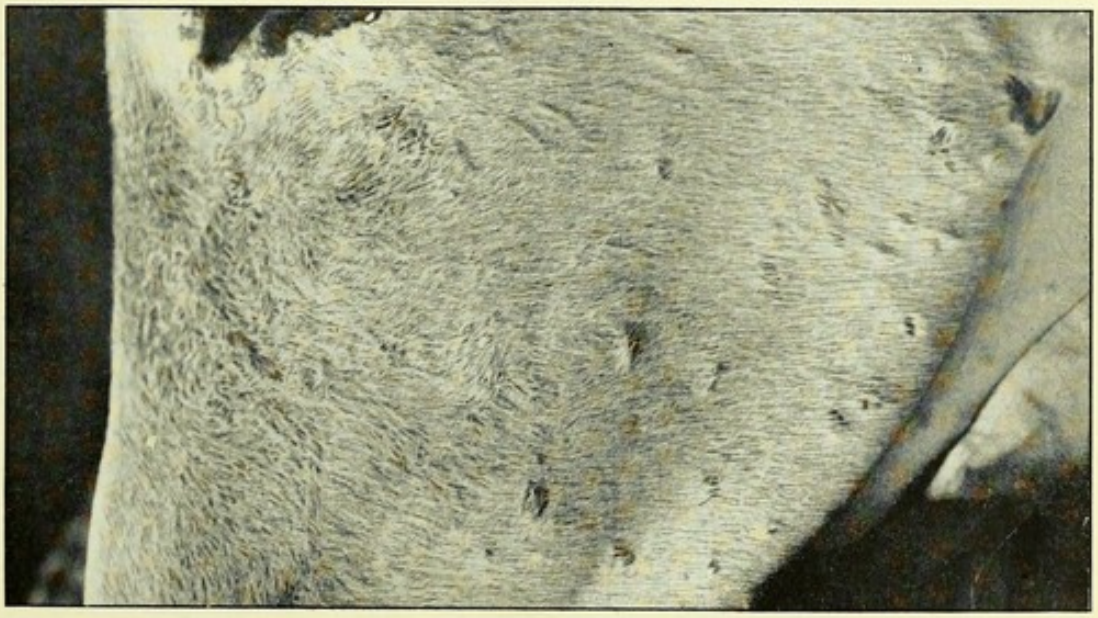

FIG. 9.—Calf F, with eruption on right side of face. Series C.

PLATE IV. To illustrate "The Etiology of Vaccinia." By Professor W. J. Simpson.

Fig. 4, 2nd Set, Series B.—Calf E2, 118 hours, Feb. 24, 1895. From Calf E, inoculated Feb. 9, with Y culture, R.P.

Fig. 4, 2nd Set, Series B.—Calf E2, 193 hours, Feb. 27, 1895.

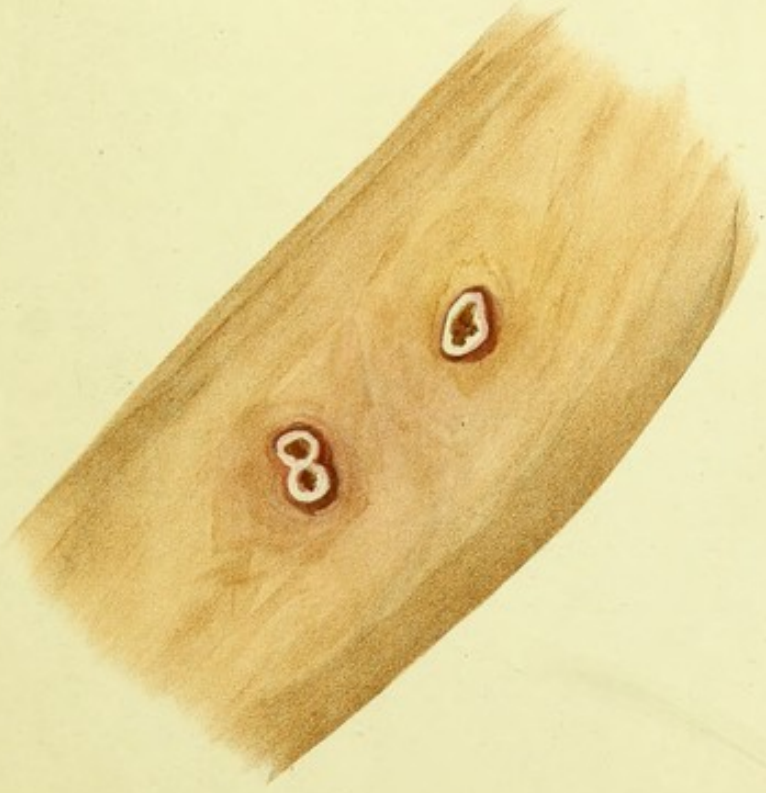

Fig. 3, 1st Set, Series B.—Mungur child A. Left Arm, vaccinated Feb. 25, from Calf D¹, 144 hours. Drawn March 3.

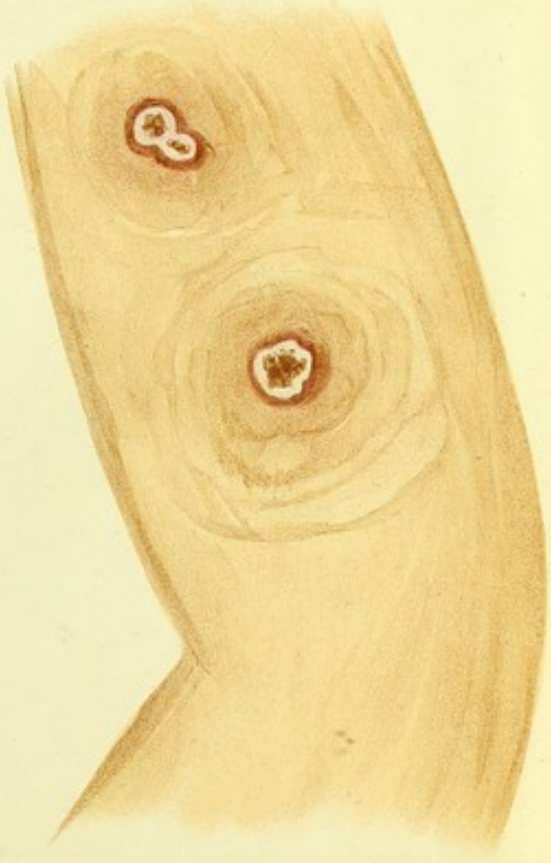

Fig. 5, 2nd Set, Series B.—Child Punia. Vaccinated from Calf E2, on Feb. 23. Drawn Feb. 28, 1895, 3.30, 118 hours.

On the eighth day four conical vesicles appeared at the seat of punctures. They were not umbilicated, but they contained clear lymph, which was transferred to two children, who eight days afterwards showed no signs of having taken, and were, therefore, vaccinated with ordinary lymph. Three days after, one child showed an eruption of twelve papules, which became vesicular on arms, legs and body, and the other child thirty papules, which also became vesicular; both had fever. The results were similar to those following inoculation with variolous lymph of a mild type, such as was familiar to Sutton in the eighteenth century. The local vaccination with ordinary lymph failed in both cases.

Calf F remained well, and was vaccinated on the fourteenth day. It was then vaccinated with ordinary lymph, and was not examined until the fourth day of the vaccination when this was found to have failed, but the animal had on it a profuse eruption. There were crusts over the left eyebrow and under the right eyelid, also over different parts of the face, and on the throat and dewlap; also over the ears there were vesicles and pustules very similar to what is seen in cases of small-pox. There were also pustules on the rump. Plates II and III, figs. 6, 7, 8 and 9, show calf F with eruption on different parts of body. The temperature was 103.2° F. Next day it was 104° F., and there were loose motions. After this the temperature dropped to normal, and the animal, with the exception of its eruption, appeared quite well. A calf inoculated with the crusts and with the serum and lymph squeezed out of the crusts, had a rise of temperature on the sixth day, an eruption on the neck on the seventh day, slimy motions, sore and ulcerated mouth and great weakness on the ninth day and died on the tenth.

Later in the year, during the rains, I began another series of experiments with the cultures, but with a very curious result, possibly due to some contamination that I was unable to discover. A spurious vesicle was produced on the calves, and this vesicle passed through the several stages of papule vesicle crust and scar. It had at some stages a resemblance to a vaccine vesicle, but its final appearance was flat and more like a blister. It could be transferred from calf to calf but not to children, and it did not render the calves immune to vaccination with ordinary lymph, as was the case with the other series.

It may be of interest to note here that it is difficult to maintain effective vaccination from calf to calf or from calf to child in Calcutta during the rains, and that natural small-pox in the human being seems to lose much of its infective power at this season of the year.

The results of these observations bring the subject to a more advanced stage than that reached by the experiments made by me in the years 1884 and 1885, when Medical Officer of Health for Aberdeen and when, owing to the experiments of Chauveau and Klein, the trend of opinion was that it was impossible to produce vaccine by inserting the virus of human small-pox into the cow: that if any vesicle was produced it was a small-pox vesicle, and that the successes of previous experimenters had been due to accidental contamination with vaccine. My experiments then demonstrated that it was possible to produce vaccine by the passage of human small-pox virus through the cow, and that the older experimenters were correct in their observations. I did not publish the results at the time for I wished to supplement them on a larger scale. The fact, however, that such successful experiments had been made, and 1,200 children vaccinated from this source was placed before the Royal Commission on Vaccination by Dr. Cory, in November, 1889, at the instance of the medical department of the Local Government Board, over which Dr. (afterwards Sir) George Buchanan, K.C.B., presided, and I further added to that information when at the request of the Commission I sent to it an account of my successful experiments in the same direction carried out in India in 1892, and which appeared in their sixth report in 1897. In the meantime Colonel W. G. King, C.I.E., had published his classical experiments done in Madras in 1891, and for which he got into much trouble owing to the view held by the medical department, that only small-pox could be produced, and that it was impossible to produce vaccine. Since then, Copeman, Klein and Hime, in England, have also proved its possibility.

The observations recorded in this paper help, I think, among other things, to show that both sets of opponents were correct in their observations, though differing in the interpretation of the results, and that the explanation of the difference lay in the maturity of the vesicle produced at the site of inoculation, or in the production of secondary vesicles. Seventeen years is a long period to elapse between the making of certain observations and their publication, but I was always in hopes of having further opportunities of pursuing the subject. For instance, quite apart from natural small-pox in cattle, giving rise to a disease included under the general term of cattle plague or rinderpest, and which under certain circumstances gives rise to vaccinia in men, the animal has yet to be found that exalts the virus and conveys it to man in the form of epidemic variola. It was because of this line of research and thought that I realized at once the significance of disease in the rat when plague first appeared in Calcutta in 1896. I think, however, it is time these observations should be made known beyond a few friends in order that others, if opportunity arises, may advance this very important subject. Unable in England to follow up this line of research, I recognize that as regards myself, so far as this aspect is concerned, the chapter is closed. But I have not entirely relinquished the study of vaccinia, and I hope in due course to deal with the problem from a different aspect, aided by the experience already gained.

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