

EXPERIMENTS ON THE RELATION OF THE COW TO MILK-DIPHTHERIA.

(Plate II.: Seven Figures.)

BY GEORGE DEAN, M.A., M.B.,

Bacteriologist, Serum Department, Jenner Institute of Preventive Medicine,

AND CHARLES TODD, M.D., D.P.H.,

*Assistant Bacteriologist, Serum Department, Jenner Institute of Preventive
Medicine.*

THE present communication relates to an outbreak of diphtheria where suspicion was directed to the milk supply and where there existed a pathological condition of the udder of the cows from which the milk was obtained.

There has been in Great Britain for many years a prevailing impression among sanitarians that milk is capable of acting as a vehicle of infection in diphtheria. Indeed there is a mass of evidence connecting certain epidemics with the milk supply.

Before the discovery of the specific organism of diphtheria, Mr Power had pointed out in his Report to the Local Government Board in 1878 the connection between the disease and the milk supply in an epidemic investigated by him in North London. He was led by a process of exclusion to the surmise that actual cow conditions capable of affecting directly or indirectly the milk might have brought about the results observed.

A number of more recent epidemics which were carefully investigated,

Devonport, 1882,—Dr Parsons ⁽¹⁾,

Hendon, 1888,—Mr May ⁽²⁾,

Hendon, 1882,—Mr Power ⁽³⁾,

York Town and Camberley, 1886,—Mr Power ⁽⁴⁾,

have emphasised the connection between milk supply and diphtheria.

Howard ⁽⁵⁾ in America has carefully worked out an epidemic in which milk appeared to be the vehicle of infection. In his paper he points out that Escherich has called attention to the fact that, outside of England, no milk epidemics of diphtheria have been reported.

Though a number of diphtheria-like bacilli have been demonstrated in milk, in very few instances has the presence of a virulent organism been recorded.

Bowhill ⁽⁶⁾, in connection with an outbreak of diphtheria at Cardiff, attributed to infected milk, isolated from the suspected milk, a diphtheria bacillus whose virulence for guinea-pigs was proved by Dr Nuttall.

Eyre ⁽⁷⁾ and Klein ⁽⁸⁾ have also recorded the existence of a genuine virulent diphtheria bacillus in milk.

It has always been a question of doubt as to whether the infective material is derived from the cow herself, or from subsequent contamination of the milk by human or other agencies. As stated above Mr Power was inclined to regard the cow as the source of infection.

In several epidemics lesions have been observed in the udders of cows yielding the suspected milk; notably in the outbreaks at Devonport in 1888 ⁽⁹⁾ and York Town in 1886 ⁽⁴⁾; and Dr Thorne Thorne (1891, p. 192), in reviewing the chief epidemics of "milk-diphtheria" in England, sums up as follows:—

"On each occasion of milk-diphtheria to which I have referred there has been evidence, more or less precise, of some cow-ailment, so far trivial, it is true, as to be ignored by those versed in bovine diseases, but either affecting the physical properties of the milk, or being associated with some vesiculation, and later on with 'chapping' or 'scabbing' of the udder and the teats."

In those conditions of the udder which have occurred apart from artificial infection, so far as we are aware, the *Bacillus diphtheriæ* has not hitherto been demonstrated.

Klein ⁽⁸⁾, following Power's observations, attacked the question from the experimental standpoint. He inoculated recently calved cows with cultivations of *B. diphtheriæ* of human origin. He found that these cows "became attacked by a definite illness, having, as one of its manifestations, a peculiar acute eruptive infection of the udder; and that from the milk of an animal made ill in this way the diphtheria bacillus could be isolated by cultivation." There was present also in the animals disease of the lungs, liver and kidneys. All these phenomena he attributed to one cause, viz., the inoculation with the diph-

theria bacillus. The eruption on the udder was not invariably present, nor could the bacilli be always demonstrated in the milk. In a later communication ⁽¹⁰⁾ he pointed out that in two outbreaks of diphtheria, one near Croydon, the other near Bishop's Stortford, lesions occurred in the udders of the suspected cows having the same characters as those produced by experimental inoculation. He did not claim, however, to have demonstrated bacteriologically either the diphtheritic character of the lesions or the presence of the bacillus in the milk. Loeffler ⁽¹¹⁾ criticised Klein's results and held that they required further confirmation. Abbott ⁽¹²⁾ attempted to reproduce in two cows the results obtained by Klein. He succeeded in demonstrating the bacilli at the site of inoculation, but failed to obtain cultivations both from the local lesions and from the milk. In his cows there was no eruption on the udder, nor did he find visceral lesions. Klein ⁽¹³⁾ replied to Abbott's criticism and attempted to show the reason why the results differed from his own. Ritter ⁽¹⁴⁾ also attempting to repeat Klein's experiments failed to show that the diphtheria bacilli passed into the milk.

History of Outbreak.

Our attention was drawn to the present outbreak by Mr Sidney Villar, F.R.C.V.S., who had, in the course of his practice, seen two cows suffering from "cow-pox" and who had heard of the existence of sore throats amongst the consumers of the milk.

The two cows were the property of a gentleman, the milk being used chiefly for his family and servants; any surplus being sold to one individual in the district, who distributed it to a few customers.

The milk from the cows was supplied to Mr F.'s family, consisting of Mr and Mrs F., a baby, a nurse, and two other servants in the house. Of these, Mr F. did not drink milk; the baby and nurse drank the milk only after sterilisation.

Of the members of the household drinking unsterilised milk, Mrs F. had typical diphtheria, first observed on Dec. 11th; one of the two servants had a suspicious sore throat and was seen by the family medical practitioner; but as both servants left the house on Mrs F. becoming ill they escaped further observation.

The gardener, who acted as cowman, and lived near the cowhouse, about a quarter of a mile from Mr F.'s house, had a family consisting of a wife and seven children; and a small quantity of the milk went to this household. One of the children, aged four, was removed to the isolation

hospital on December 11th, with a severe attack of diphtheria, necessitating subsequent tracheotomy. Two cases of sore throat among other consumers of the milk were known to Mr F.

On visiting the place on December 15th we found that the two cows were housed in a small cow-house with a low ceiling, and that they had been turned into an adjoining paddock, with two heifers, their own calves of the previous spring. So far as was known there had been no recent contact with other cattle, either directly or through the cowman. Both cows were in calf. The date of the onset of the disease in the cows could not be accurately determined, but the cowman stated that he had observed something wrong with the teats for a week or ten days.

Appearances observed in the Cows on December 15th.

On the udders and teats of both cows there were present papules and ulcers covered by dark brown scabs (see Plate II, Fig. 1). The papules were, on an average, about the size of a pea, and had a markedly indurated base which extended into the subcutaneous tissue. No vesicles were seen at this examination. In the cow which was experimentally infected at a later period and where precautions were taken to avoid their being broken, vesicles were a very marked feature of the condition, as will be seen in Figs. 2 and 3. The majority of the lesions were in the form of ulcers covered with dry brown crusts and were of the size of a sixpence to a shilling. On removing the crust from one of these there was exposed a slightly moist, fairly smooth surface with an elevated, puckered, cicatricial-looking margin. The largest lesion at this stage, situated on the udder itself, measured about 2 inches by 1 inch, had an irregular margin and appeared to have been formed by the running together of several smaller ulcers. The ulcerative process in this case was considerable, and on removing the crust there was exposed a raw, bleeding, irregular cavity with a depth of at least $\frac{1}{4}$ inch. The severity and persistence of the lesions were no doubt due to the constant removal of the crusts either by the hands of the milker or by the animal lying on, and crushing, the udder.

In Cow 1 there was no evidence of mammitis at this stage; there was an abundant secretion of apparently normal milk and the general health appeared to be little, if at all, affected.

In Cow 2, in addition to the surface lesion of teats and udders, there was a distinct mammitis affecting a posterior quarter of the udder. In

this case the milk was scanty, ropy, semipurulent looking, and was slightly tinged with blood. Shortly after removal to the new station this cow cast her calf.

Bacteriological Examination of Material from Cows.

Cultivations in broth, agar, and solidified blood-serum were made from the ulcers after removal of the crusts, and at the same time samples of milk were taken with the usual precautions, and later, these samples were investigated bacteriologically.

The blood-serum cultivations from the ulcers, in the case of both animals, showed a considerable number of colonies indistinguishable from those of the Klebs-Loeffler bacillus. These were subplated on serum and thus pure cultures were obtained.

The milk of both cows was centrifugalised and blood-serum tubes were inoculated from the deposit. In the cultures from both cases a few diphtheria-like bacilli could be demonstrated and by further plating pure cultures were obtained on serum.

In addition to the colonies of the diphtheria bacillus a number of colonies resembling them, but with a yellow tint, were observed and isolated. This bacillus, though morphologically resembling the *B. diphtheriæ*, in twenty-four hours old cultures on solidified blood-serum was non-virulent for guinea-pigs, and further investigation revealed a number of characters differentiating it from the diphtheria bacillus. Numerous *Cocci* and other organisms were observed; *Streptococci* being especially numerous in the milk of the cow suffering from mammitis.

Brushings were made from the teats of thirteen apparently healthy cows at a small dairy farm. In two cases the cultures on solidified blood-serum showed diphtheria-like bacilli, but these on isolation were proved not to be the *B. diphtheriæ*.

Bacteriological Examination of Brushing from Mrs F's throat.

This gave almost a pure culture of diphtheria-like bacilli which were similarly isolated in pure culture.

Examination of the Bacilli.

Morphologically the bacilli from the cows, from the milk, and from the patient's throat were found to be typical Klebs-Loeffler bacilli of medium size. The virulence of the three races was tested as shown in the following tables of experiments:—

Testing Bacillus from lesion in Cow 1.

A 24 hours' culture of the bacillus in alkaline broth was used :—

Guinea-pig No.	Weight of animal in g.	Dose of culture, etc. injected	Result
1	260	2 c.c. culture	Dead in 48 hrs.
2	278	4 c.c. culture	" " "
3	250	4 c.c. culture + 1 c.c. diph. antitoxin	Lived. Very slight local reaction
4	255	5 c.c. diph. antitoxin and 24 hrs. later 2 c.c. culture	Lived. Very slight local reaction
5	268	5 c.c. diph. antitoxin and 24 hrs. later 4 c.c. culture	Lived. Very slight local reaction

The diphtheria antitoxin employed contained 300 units per c.c. (Ehrlich).

Testing Bacillus from milk of Cow 1. (A 24 hours' culture etc. as before.)

1	258	2 c.c. culture	Dead in 48 hrs.
2	258	4 c.c. culture	" " "
3	258	4 c.c. culture + 1 c.c. diph. antitoxin	Lived. Very slight local reaction
4	248	5 c.c. diph. antitoxin and 24 hrs. later 2 c.c. culture	Lived. Very slight local reaction
5	250	5 c.c. diph. antitoxin and 24 hrs. later 4 c.c. culture	Lived. Very slight local reaction

Testing Bacillus from Mrs F's throat. (A 24 hours' culture etc. as before.)

1	255	2 c.c. culture	Dead in 48 hrs.
2	258	4 c.c. culture	" " "
3	250	4 c.c. culture + 1 c.c. diph. antitoxin	Lived. Very slight local reaction
4	255	5 c.c. diph. antitoxin and 24 hrs. later 2 c.c. culture	Lived. Very slight local reaction
5	265	5 c.c. diph. antitoxin and 24 hrs. later 4 c.c. culture	Lived. Very slight local reaction

These experiments show that the three races of bacilli isolated from the cows, from the milk, and from the patient's throat, were virulent for guinea-pigs, and that diphtheria antitoxin given simultaneously or twenty-four hours before inoculation completely protected the experimental animals.

In addition to the tests mentioned, the object of which was to show that the organisms present were genuine *B. diphtheriæ*, a series of tests was carried out to ascertain the toxigenic power of the three races. As is well known the diphtheria bacillus from different sources varies greatly in this property, and without laying too great stress on the

matter we thought it might be an indication as to whether the three races in question were of common origin.

The three races, after being grown for two passages, on the usual alkaline broth employed in the preparation of diphtheria toxin, gave a good surface growth.

Twenty-seven test-tubes, each containing 10 c.c. of the same alkaline broth, were divided into three sets and each set was inoculated with one of the three races of bacilli. These test-tubes were placed in one incubator at 36° C. for ten days. The nine test-tubes of each series were then mixed and filtered through a Berkefeld filter. Our object in using a large number of test-tubes was to reduce the risk of accidental variations of toxicity. The toxins thus obtained were tested as follows:

Testing Toxin from Bacillus from lesion of Cow 1.

Guinea-pig No.	Weight in g.	Dose of toxin	Weight on successive days etc.					
			1	2	3	4	5	
1	255	1.0 c.c.	245*	dead				
2	270	0.5	250*	dead				
3	262	0.2	246*	dead				
4	250	0.1	250*	225*	dead			
5	260	0.05	254*	245*	235*	225*	225*	dead

Testing Toxin from Bacillus from Milk from Cow 1.

1	275	1.0	255*	dead				
2	258	0.5	250*	dead				
3	278	0.2	265*	dead				
4	270	0.1	255*	252*	245*	245*	245*	dead
5	255	0.05	240*	235*	225*	225*	225*	230

Testing Toxin from Bacillus from Mrs F.'s throat.

1	258	1.0 c.c.	dead					
2	265	0.5	dead					
3	250	0.2	dead					
4	265	0.1	235*	dead				
5	265	0.05	250*	240*	240*	230*	225*	

Note: * = large local swelling.

The toxins from the three races, therefore, show a remarkable correspondence in toxicity.

The virulence of toxin-free cultures, obtained by growing the bacilli

24 hours in broth containing 1% glucose, was tested on guinea-pigs of 250 grammes weight with the following result:

Bacillus from Cow 1	killed at 0.2 c.c.
„ „ milk of Cow 1	„	0.2 „
„ „ throat of Mrs F.	„	0.1 „

A sample of blood taken from Cow 1 on January 23rd, and tested for diphtheria antitoxin, was found not to contain $\frac{1}{2}$ unit per c.c.

The Disease in the Cows.

With the view of further investigating the condition in the cow the two cows were removed on December 19th to an isolated cowhouse in the vicinity of the laboratory, and several miles from the place in which they had contracted the disease.

We obtained a young cow which had just had her first calf, which had been removed immediately after its birth. The cow was placed, on December 22nd, in a stall in the same shed in which the two affected cows were kept; but separated by a partition, and having a separate entrance. A new attendant, who had no connection with the outbreak and who had no other cattle in his charge, was instructed to milk the healthy cow after the affected animals. At first, from a misunderstanding, he carefully washed his hands after milking the diseased animals. On our attention being drawn to this the hand-washing between the milkings was stopped.

On the 31st December, the evening temperature of the new cow (Cow 3) rose to 103° F. and two days later there appeared on one of the teats a vesicle with clear contents, a representation of which is shown on Plate II, Figs. 2 and 3. A vesicle had evidently been already ruptured on the same teat. Cultivations from the fluid of the unruptured vesicle gave no diphtheria bacilli. Next day, at the base of the same teat, there could be felt and seen a papule the size of a pea. Two days later there were present on the teats and udder three lesions covered by crusts. Cultures from these again failed to demonstrate the presence of *B. diphtheriæ*.

Fresh crops of papules passing through the same stages occurred for about a fortnight, and a few of the crusts were still present nearly two months after the onset.

In this cow the process was not so severe as in the case of the first two animals in which the condition persisted somewhat longer.

This experiment demonstrates the contagious nature of the condition in the cow. In the fresh lesions, vesicles, and ulcers, we were unable to demonstrate the presence of the diphtheria bacillus. With the view of still further investigating the point as to whether this was a purely diphtheritic condition and not a diphtheria implanted on another process, we performed the following experiments on two calves:—

Calf 1. On January 6th the abdomen of a calf about six weeks old was shaved and scarified, and the scarification inoculated with crusts from the experimentally infected cow No. 3.

Calf 2. On the same day a second calf of about the same age which had forty-eight hours previously received subcutaneously 10,000 units of diphtheria antitoxin, was similarly shaved, scarified, and infected with the same material. Six days later the calves both showed a distinct eruption on the shaved area, consisting of about half-a-dozen flattened papules, almost petechial looking, with a diameter of about 3 mm., which later increased. This stage rapidly disappeared, giving place to small brown crusts, on removal of which there were seen shallow, smooth, moist ulcers, with slightly indurated edges. These scabs fell off in about a week and there was left a slight cicatrisation which could be felt about a month after the inoculation.

Although, as is shown in the figures from the two calves (see Plate II, Figs. 6 and 7), a slight difference was observable in the two cases, this was trivial, and was probably due to the difference in the texture and pigmentation of the skin. Cultivations were made from the crusts and subjacent areas in both cases on the sixth day after the onset of the eruption, but in neither case could diphtheria bacilli be demonstrated. These experiments appear to demonstrate:—

- (1) That the calf was capable of being infected with the disease existing in the cow.
- (2) That diphtheria antitoxin did not protect against this infection.
- (3) That the diphtheria bacillus could not be found in the lesions in the calves.

These results obtained both from the cows and from the calves point to the conclusion that there was present a specific contagious eruptive condition apart from the diphtheritic infection.

Experiments on Vaccination.

As this condition in many of its features had a resemblance to natural vaccinia in the cow, we thought it desirable to carry out some experiments with the view of elucidating the point:—

Calves 1 and 2, which had already been successfully infected with the disease of the cow's udder, as previously described, were vaccinated on the buttock with Dr Chaumier's Lymph fourteen days later. In both cases a typical vaccinia resulted.

The same tube of vaccine lymph was used on several cases in the human subject, and gave rise to a severe but typical vaccinia. The inoculation of Cow 3 with vaccine lymph six weeks after the onset of the eruption gave rise to a typical vaccinia. Cows 1 and 2 were not vaccinated because as they were multiparae they might have already been the subjects of a naturally occurring vaccinia and have thus acquired a certain degree of immunity. The fact that inoculation with vaccine lymph gave rise to so typical a vaccination in the calves within a fortnight of infection with the material in question, and in the cow within six weeks of the naturally occurring infection, seems to almost preclude the possibility of vaccinia in the case of the original condition in the cows.

The converse experiment was performed on two calves, one of which was vaccinated with Chaumier's Lymph, the other kept as a control. An attempt was made about a fortnight later to infect these calves with the crusts from the cow's udder, but in both cases the experiment failed. This failure was no doubt due to the fact that, owing to a difficulty in obtaining calves, we were not able to carry out the experiment till a very late period in the condition of the cow, when the crusts were no longer capable of giving rise to the disease.

Crusts from the earlier stages of the disease in the cow, which had been preserved in 50% glycerine for six weeks, were also used, but these also failed both in the vaccinated and unvaccinated animals.

Summary.

In this outbreak of diphtheria certain individuals suffered from diphtheria, and others from sore throat probably diphtheritic. These individuals obtained their milk supply from two cows. Members of one household who did not drink milk, or who used it only after sterilisation, escaped infection. The cows yielding the milk were found to be

suffering from an eruptive disease of the udder, and both from the lesions and from the milk cultures of virulent diphtheria bacilli were isolated. The pathological condition in the cows preceded, by a short interval, the onset of the disease in the patients. Having regard to Power's epidemiological investigations, and to Klein's experimental work, this observation was of the greatest interest and naturally suggested the hypothesis that the lesions were due, primarily, to a specific diphtheritic infection of the cow. Further investigations weakened this view, for it must be noted:—

(1) That in a cow intentionally submitted to infection from the diseased cows and in which there occurred the eruptive condition of the udder, neither in the vesicular nor in the ulcerative stage of the disease could diphtheria bacilli be demonstrated.

(2) That in calves infected with the eruptive disease no diphtheria bacilli could be demonstrated.

(3) That in a calf, in spite of the fact that it had received 10,000 units of diphtheria antitoxin, the vesicular eruption was experimentally produced. The last is probably the strongest point in support of the dual nature of the condition in the cows.

It is conceivable that pathological lesions in the cow such as those described, if infected with the diphtheria bacillus, might form a suitable nidus for its growth and permit of the infection of large quantities of milk over a considerable period. Though we have as yet no evidence on the subject it is possible that a profound change in the virulence of the diphtheria bacillus for the human subject might be effected by such passage through the cow¹.

The disgusting habit the milkers in this part of England have, of spitting on their hands before milking, would easily account for the infection of the lesions, even in the absence of obvious diphtheria in the cowman; knowing, as we now do, that apparently healthy individuals are not uncommonly the hosts of the diphtheria bacillus.

The experiments made with the view of ascertaining whether the eruptive condition was genuine cowpox, are opposed to that view; for two calves, and one cow successfully infected with the eruptive condition and subsequently vaccinated with vaccine lymph, developed a typical vaccinia.

The somewhat limited time and material available have prevented

¹ A series of reports on a similar vesicular condition of the udder of the cow, investigated in relation to scarlatina, will be found in the Report of Prof. Brown, of the Agricultural Department of the Privy Council Office, in 1888.



FIG. 1.

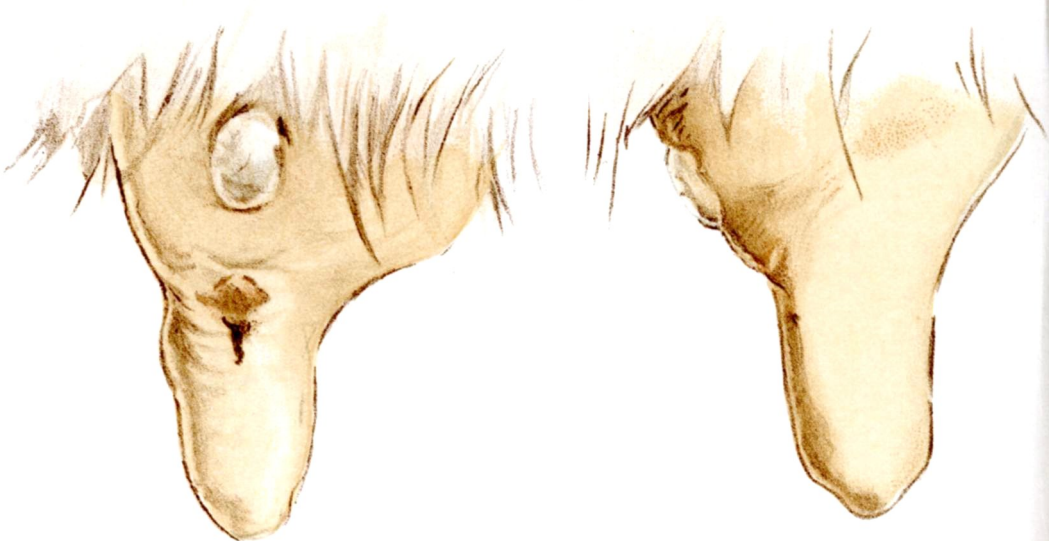


FIG. 3.



FIG. 4.



FIG. 5.



FIG. 6.



FIG. 7.

us from investigating the matter so fully as we should have wished, or on a scale proportionate to its interest and practical importance. We hope at some future time to go further into the matter.

Our best thanks are due to Mr Sidney Villar, F.R.C.V.S., for bringing the outbreak to our notice, and for his help in the veterinary side of the question; and to Dr Carson Smyth for his kindness in allowing us facilities for taking cultures from the throats of his patients.

DESCRIPTION OF PLATE.

PLATE II.

- FIG. 1. Cow 1. Appearance of lesions when first observed on the udder.
- FIGS. 2, 3, 4 and 5. Showing the condition in Cow 3 which had been intentionally exposed to infection.
- FIGS. 2 and 3. Infected teat with intact and ruptured vesicles on the day of their appearance.
- FIG. 4. Ditto 2 days later.
- FIG. 5. Ditto 11 days later.
- FIG. 6. Calf 1. Ten days after inoculation with material from Cow 3. This calf had received no antitoxin. Figure about one-half natural size.
- FIG. 7. Calf 2. Ten days after inoculation with material from Cow 3. This calf had received 10000 units (Ehrlich) of Diphtheria antitoxin 48 hours before inoculation. Figure about one-half natural size.

REFERENCES.

- (1) PARSONS. Local Government Board Reports, 1883.—(2) THORNE THORNE. The Milroy Lectures, 1891, p. 162.—(3) POWER. Local Government Board Reports, 1883.—(4) POWER. Local Government Board Reports, 1887.—(5) HOWARD. *American Journal of Med. Sciences*, Dec. 1897, p. 629.—(6) BOWHILL. *Veterinary Record*, 8 April, 1899, No. 561.—(7) EYRE. *Brit. Med. Journal*, 1899, Part II., p. 586.—(8) KLEIN. *Journ. of Hygiene*, 1901, vol. I., p. 85.—(9) KLEIN. Local Gov. Reports, 1889.—(10) KLEIN. Local Gov. Reports, 1890. (11) LOEFFLER. Internat. Med. Congress, Berlin, 1890.—(12) ABBOTT. *Journal of Pathology and Bacteriology*, 1894, vol. II., p. 35.—(13) KLEIN. *Journal of Pathology and Bacteriology*, vol. II., p. 428.—(14) RITTER. *Ref. Centralblatt f. Bakteriologie*, vol. XIX., 1896, p. 662.