

A CONTRIBUTION TO THE ETIOLOGY OF DIPHTHERIA.*

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THE microbe, which was first described by Klebs (at the Wiesbaden congress in 1883), then isolated and grown in artificial cultures by Löffler (Mitth. aus dem K. Gesundheitsamte, vol. ii.) from human diphtheritic membrane, was shown by this observer to act virulently on various animals. The Klebs-Löffler bacillus—by which name the diphtheria microbe is known—is the one with which also Roux and Yersin (Annales de l'Institut Pasteur, ii., 1888, No. 12) obtained positive results on guinea-pigs.

In the reports of the Medical Officer of the Local Government Board for 1888-89 and 1889-90, I have shown that there occur in diphtheritic membranes two species of bacilli, very similar in morphological respects, and also in cultures on serum and on agar, but differing from one another in this, that one species, Klebs-Löffler bacillus No. 1, is not constant in diphtheritic membranes, does not grow on solid gelatin at 19-20° C., and does not act pathogenically on animals; the other species, Klebs-Löffler bacillus No. 2, is constant in diphtheritic membranes, in fact is present even in the deeper layers of the membranes in great masses and almost in pure culture, acts very virulently on animals, and grows well on gelatin at 19°-20° C. Löffler, and after him other observers (Flügge, "Die Mikroorganismen," 1886), considered it as a character of the diphtheria bacillus that it does not grow on gelatin below 22° C., but this character, though true of the Klebs-Löffler species No. 1, does not appertain to the diphtheria bacillus species No. 2. In fact, there is no difficulty in obtaining pure cultures of this bacillus on gelatin if a particle of diphtheritic membrane be taken and well shaken in two or three successive lots of sterile salt solution, and from the last lot plate cultivations on gelatin are made. In this way I have obtained the diphtheria bacillus in great numbers of colonies and in pure culture. Zarniko (Centralbl. f. Bakteriologie u. Parasit., vol. vi., 1889, p. 154) and Escherich (*ibid.*, vol. vii., 1890, p. 8) both state that the diphtheria bacillus does grow on gelatin below 20° C.

This bacillus diphtheriæ acts very virulently on guinea-pigs on subcutaneous inoculation; at the seat of the injection a tumour is produced, which in its pathology and in microscopic sections completely resembles the diphtheritic tissue of the human. In human diphtheria the diphtheria bacillus is present only in the diphtheritic mem-

brane, but neither in the blood nor in the diseased viscera; the same holds good for the experimental guinea-pigs. In subcutaneous inoculation with artificial culture, though it causes in these animals acute disease and death—the lungs, intestine, and kidney are greatly congested—the diphtheria bacillus remains limited to the seat of inoculation. It was for these reasons that Löffler concluded that in diphtheria the diphtheritic membrane alone is the seat of the multiplication of the diphtheria bacillus, and that here a chemical poison is produced, which absorbed into the system causes the general diseased condition and eventually death. Roux and Yersin have then separated from artificial broth cultures the bacilli and the chemical products, and, by the injection of these latter alone into guinea-pigs, have produced a general effect. I have in this year's report to the Medical Officer of the Local Government Board (1889-90) shown that in these experiments of injection of cultures into guinea-pigs, an active multiplication of the diphtheria bacilli at the seat of inoculation can be demonstrated by culture experiments; from the local diphtheritic tumour and the nearest lymph glands the diphtheria bacilli can be obtained in pure culture on gelatin.

On various occasions during the last three years information has reached me by health officers (Dr. Downes, Mr. Shirley Murphy, Dr. Thursfield) as to a curious relation existing between a mysterious cat disease and human diphtheria in this manner, that a cat or cats were taken ill with a pulmonary disease, and while ill were nursed by children, and then these latter sickened with well-marked diphtheria. Or children were taken ill with diphtheria, and either at the same time or afterwards the cat or cats sickened. The disease in the cat was described as an acute lung trouble; the animals were quiet, did not feed, and seemed not to be able to swallow; in some cases they recovered, in others they became emaciated, while the lung trouble increased, and ultimately they died. In one instance—in the north of London, in the spring, 1889—this cat malady, occurring in a house where diphtheria soon afterwards appeared amongst the children, was of a widespread nature; a veterinary surgeon—Mr. Daniel—informed me that at that time he had several patients amongst cats affected with the disease, consisting in an acute catarrhal affection, chiefly of the respiratory passages. He furnished me with two such animals: one that after an illness of several weeks had died, another that was sent to me in a highly emaciated state, affected with severe broncho-pneumonia; this animal was paralysed on the hind limbs. In both instances the post-mortem examination showed severe lung disease, broncho-pneumonia, and large white kidneys due to fatty degeneration of the entire cortex. A similar condition is met with in the human subject in diphtheria. Further, I received from Dr. Thursfield, of Shrewsbury, the body of a

* Paper read before the Royal Society by Dr. E. Klein, F.R.S., on May 22nd. This research was undertaken for the Medical Department of the Local Government Board, and was communicated to the Royal Society with the permission of the Medical Officer.

cat that had died after a few days' illness from pneumonia in a house in which children were ill with diphtheria; another cat in the same house that became next ill with the same lung trouble also succumbed. The post-mortem examination of the animal that I received showed severe broncho-pneumonia and large white kidneys, the entire cortex being in a state of fatty degeneration.

Subcutaneous inoculations of cats were carried out with particles of fresh human diphtheritic membranes and with cultures of the diphtheria bacillus (Report of the Medical Officer of the Local Government Board, 1889-90); hereby a local diphtheritic tumour was produced at the seat of inoculation, and a general visceral disease; in the cases in which death followed after a few days the lungs were found much congested; when death followed after one or more weeks, the lungs showed broncho-pneumonia and the kidneys were enlarged and white, the cortex being in a state of fatty degeneration; if the disease in the animals lasted beyond five to seven days, both kidneys were found uniformly white in the cortex; if of shorter duration, the fatty degeneration was sometimes only in patches. Although in these experiments the bacillus diphtheriæ was recoverable by cultivation from the diphtheritic tumour at the seat of inoculation, there were no bacilli found in the lungs, heart's blood, or kidney, and the conclusion is justified that, just as in the human diphtheria and in the diphtheria produced by subcutaneous inoculation in the guinea-pig, so also in these experimental cats the visceral disease must be a result of the action of a chemical poison produced by the diphtheria bacillus at the seat of inoculation.

From this it is seen that the similarity between the artificial disease and the natural disease in the cat is very great, and the question that presents itself is, In what manner does the animal receive or give the diphtheritic contagium in the natural disease? The natural disease in the cat is in its symptoms and pathology a lung disease, and it is reasonable to suppose from analogy that the lung is the organ in which the diphtheritic process in the cat has its seat. The microscopic examination of the diseased lung of cats that died from the natural disease bears this out, the membrane lining the bronchi in the diseased portions of the lobules presenting appearances which in microscopic character coincide with the appearances in the mucous membrane of the human fauces, pharynx, or larynx in diphtheria. But the correctness of the above supposition, that diphtheria has its seat in the lung of the cat naturally diseased, was proved by direct experiment. Broth culture of the bacillus diphtheriæ was introduced into the cavity of the normal trachea without injuring the mucous membrane. The animals became ill with acute pneumonia, and on post-mortem, two to seven days after, there was found extensive pneumonia and fatty degeneration

of the kidney. The bronchi, infundibula, and air-cells of the inflamed lobules were found occluded by, and filled with, exudation, which under the microscope bears a striking resemblance to human diphtheritic membranes, and in the muco-purulent exudation in the large bronchi and trachea the diphtheria bacilli were present in large numbers.

During the last ten or twelve years certain epidemics of diphtheria have occurred which were traced to milk, but the manner in which that milk had become contaminated with the diphtheritic virus could not be demonstrated, although the evidence as to the milk not having been directly polluted from a human diphtheria case was very strong. The epidemic of diphtheria that prevailed in the north of London in 1878, investigated by Mr. Power for the Local Government Board, then the epidemic that occurred in October, 1886, at York Town and Camberley, the epidemic in Enfield at the beginning of 1888, and in Barking towards the autumn of 1888, were epidemics of this character. Mr. Power, in his report to the Local Government Board on the York Town and Camberley outbreak, states (p. 13) that a veterinary surgeon had certified that the cows from whom the infected milk was derived were all in good health, but that two of the cows showed "chaps" on their teats, and he adds that even two or three weeks after the epidemic had come to an end—the use of milk having been in the meanwhile discontinued—he saw at the farm one cow which had suffered chapped teats. At Enfield a veterinary inspector had also certified that the cows were in good health; but at Barking the veterinary inspector found sores and crusts on the udder and teats of the cows.

I have made experiments at the Brown Institution on milch cows with the diphtheria bacillus, which appear to me to throw a good deal of light on the above outbreaks of diphtheria.

Two milch cows* were inoculated with a broth culture of the diphtheria bacillus derived from human diphtheria. In each case a Pravaz syringe was injected into the subcutaneous and muscular tissue of the left shoulder. On the second and third days there was already noticed a soft but tender swelling in the muscle and the subcutaneous tissue of the left shoulder; this swelling increased from day to day, and reached its maximum about the end of the week; then it gradually became smaller but firm. The temperature of both animals was raised on the second and third day, on which days they left off feeding, but after this became apparently normal. Both animals exhibited a slight cough, beginning with the eighth to tenth day, and this gradually increased. One animal left off feeding and ruminating on the twelfth day, "fell in" considerably, and died in the night from the fourteenth to the fifteenth day; the other animal on the

* The cows had been kept under observation previous to the experiment for ten days, and were in all respects perfectly normal.

twenty-third to twenty-fourth day left off taking food, "fell in" very much, and was very ill: it was killed on the twenty-fifth day.

In both animals, beginning with the fifth day, there appeared on the skin of the udder, less on the teats, red raised papules, which in a day changed into vesicles, surrounded by a rim of injected skin; the contents of the vesicles was a clear lymph, the skin underneath was much indurated and felt like a nodule; next day the contents of the vesicles had become purulent, *i.e.*, the vesicle had changed into a pustule; in another day the pustule dried into a brownish-black crust, with a sore underneath; this crust became thicker and larger for a couple of days, then became loose, and soon fell off, a dry healing sore remaining underneath. The whole period of the eruption of papules, leading to vesicles, then to pustules, and then to black crusts, which, when falling off, left a dry healing sore behind, occupied from five to seven days. The eruption did not appear in one crop: new papules and vesicles came up on the udder of one cow almost daily between the fifth and eleventh day after inoculation, in the other cow between the sixth and tenth day; the total number of vesicles in the former cow amounted to about twenty-four on the udder, four on the teats; in the latter they were all on the udder, and amounted to eight in all. The size of the vesicles and pustules differed: some were not more than $\frac{1}{8}$ th of an inch, others larger, up to $\frac{1}{2}$ to $\frac{3}{4}$ of an inch in diameter; they had all a rounded outline, some showed a dark centre. From one of the above cows on the fifth day milk was received from a healthy teat, having previously thoroughly disinfected the outside of the teat and the milker's hand; from this milk cultivations were made, and it was found that thirty-two colonies of the diphtheria bacillus without any contamination were obtained from one cubic centimetre of the milk.

Unlike in the human, in the guinea-pig and in the cat the diphtheria bacillus passed from the seat of inoculation into the system of the cow; this was proved by the demonstration of the diphtheria bacillus in the milk. But also in the eruption on the udder, the presence of the diphtheria bacillus was demonstrated by microscopic specimens and particularly by experiment. With matter taken from the eruption—vesicles and pustules—of the udder, two calves were inoculated into the skin of the groin; here the same eruption made its appearance: red papules, rapidly becoming vesicular, then pustular, and then became covered with brown-black crusts, which two or three days after became loose and left a dry healing sore behind. More than that, the calves that showed this eruption after inoculation became affected with severe broncho-pneumonia and with fatty degeneration of the cortex of the kidney. In the two cows above-mentioned, on post-mortem examination, both lungs were found highly congested, cedematous, some

lobules almost solid with broncho-pneumonia in the upper lobes and the upper portion of the middle or lower lobe respectively; the pleural lymphatics were filled with serum and blood. Hæmorrhages in the pericardium and lymph glands, and necrotic patches were present in the liver. At the seat of inoculation there was in both cases a firm tumour consisting in necrotic diphtheritic change of the muscular and subcutaneous tissue. In this diphtheritic tumour continuous masses of the diphtheria bacillus were present; their gradual growth into and destruction of the muscular fibres could be traced very clearly.

It appears then from these observations that a definite disease can be produced in the cow by the diphtheria bacillus, consisting of a diphtheritic tumour at the seat of inoculation with copious multiplication of the diphtheria bacillus, a severe pneumonia, and necrotic change in the liver; the contagious nature of the vesicular eruption on the udder and excretion of the diphtheria bacillus in the milk prove that in the cow the bacillus is absorbed as such into the system.

From the diphtheritic tumour, by cultivation, pure cultures of the diphtheria bacillus were obtained; a small part removed from the tumour with the point of a platinum wire, and rubbed over the surface of nutrient gelatin or nutrient agar, yielded innumerable colonies of the diphtheria bacillus without any contamination. In cultural characters in plate, streak, and stab cultures and in cover-glass specimens of such cultures, this cow diphtheria bacillus coincided completely with the human diphtheria bacillus, but in sections through the diphtheritic tumour of the cow a remarkable difference was noticed between it and the bacillus from the cultures; inasmuch as in the tissue of the tumour the masses of the microbe, both in the necrotic parts, as also where growing into and destroying the muscular fibres, were made up of filaments, granular threads, some of which possessed terminal oval or flask-shaped swellings. But that it was really the diphtheria bacillus was proved by culture experiments and by cover-glass specimens. In the latter, the transitional forms between typical diphtheria bacillus and long filaments with terminal knob-like swellings, with spherical or oblong granules interspersed here and there in the threads, could be easily ascertained. In the large number of cultivations that were made of the fresh tumour in both cows, the colonies obtained were all of one and the same kind, *viz.*, those of the diphtheria bacillus; no contamination was present in any of the cultivations.

Appendix, May 20th.—At the beginning of the month of April two cats died at the Brown Institution, after having been ill for several days, with symptoms like those of natural cat diphtheria. Between the beginning of April and the beginning of May, 14 cats became similarly affected, some more severely than others, and some died with the

characteristic morbid change. This epidemic, as it may be called, commenced with the illness of the first two cats about the end of March; and the question arises as to how the disease originated in these two animals. No cats had been ill in their shed, and the two affected ones were healthy when received at the institution some weeks before. But during the latter half of March there were in the stables of the institution two milch cows ill with diphtheria induced by inoculation with the human diphtheria bacillus—in fact, the two cows already referred to. The diphtheria bacillus was found in the milk drawn from one of these animals on the fifth day after inoculation, and orders were given to the attendant that the milk of both cows was to be thrown away. This order was not obeyed, for part of the milk was given to the two cats above mentioned, and they sickened as described within a day or two afterwards. It ought to be mentioned that the man in attendance on the cows had also charge of the cats, but, in view of the fact that he was himself free from the disease, the possibility of his having conveyed it from the cows to the cats may be disregarded.

STATISTICAL RESULTS OF THE PASTEUR INSTITUTION.

In a recently published pamphlet* M. Perdrix has issued a concisely tabulated collection of the statistics (corrected up to date) which have appeared at intervals in the *Annales de l'Institut* since January, 1887. Systematic inoculations were commenced at the end of 1885, and as this pamphlet deals with the cases since January, 1886, it is in fact an outline history of the new treatment for rabies to the end of 1889. During these four years, 7,893 cases† from all parts of the world have been inoculated. The fatal cases were 53, equal to a death-rate of '67 per cent. Such a low death-rate may be considered eminently satisfactory, the more so as the number of deaths in each year has steadily diminished, and the low rate is not, therefore, due to any chance succession of favourable cases. M. Perdrix attributes this diminution in part to the experience gained in each year as to the method of inoculation best suited to each case, and in part to patients presenting themselves at much shorter intervals after the injury has been inflicted by the rabid animal.

In recording the cases they are divided into three groups:

* Les Vaccinations Antirabiques de l'Institut Pasteur, Résultats Statistiques (1887, 1888, 1889). M. L. Perdrix, pp. 23. (Charavre et Fils, Sceaux, 1890.)

† 26 cases dying "under treatment" (including in this period, the fortnight immediately following the last inoculation) are excluded, as having already sickened with rabies before presenting themselves at the Institute. If these are included, the total cases number 7,919, and the deaths 79 (equal to a 1 per cent. death-rate).

A. Cases where the animal wounding the patient was proved to be rabid, either by experiment in the laboratory, or by the occurrence of rabies in other animals wounded by the first animal.

B. Cases where the rabies was demonstrated by a post-mortem examination of the animal inflicting the injury.

C. Cases where the rabies was only suspected.

The subjoined table shows for each group the number of cases, deaths, and death-rates:

Year.	GROUP A.			GROUP B.			GROUP C.			Total.		
	Cases treated.	Deaths.	Death-rate %	Cases treated.	Deaths.	Death-rate %	Cases treated.	Deaths.	Death-rate %	Cases treated.	Deaths.	Death-rate %
1886	231	3	1'3	1,926	19	'99	514	3	'58	2,671	25	'94
1887	357	2	'56	1,156	10	'86	257	1	'39	1,770	13	'73
1888	402	6	1'49	972	2	'21	248	1	'40	1,622	9	'55
1889	346	2	'58	1,187	2	'17	297	2	'67	1,830	6	'33
Totals	1,336	13	'97	5,241	33	'63	1,316	7	'53	7,893	53	'67

This table should be read with the next, which subdivides all the cases occurring during the four years:

Site of Wounds.	GROUPS A and B.			GROUP C.			Totals.		
	Cases treated.	Deaths.	Death-rate %	Cases treated.	Deaths.	Death-rate %	Cases treated.	Deaths.	Death-rate %
Face and head ...	593	14	2'36	79	1	1'27	672	15	2'23
Hands.....	3,768	26	'69	619	3	'48	4,387	29	'66
Trunk and Limbs...	2,216	6	'27	618	3	'48	2,834	9	'32
Totals.....	6,577	46	'70	1,316	7	'53	7,893	53	'67

Remembering that the duration of incubation is considered to be on an average 10 to 15 days, and that when the head or face is wounded, the incubation is much shortened, these statistics emphasise the need of early treatment of those wounded in these parts. Unfortunately no information is given in this pamphlet as to the interval elapsing between the injury and the first attendance at the Institute. Of the 26 cases already referred to as excluded, 12 died after head wounds, and 9 after wounds about the hands. The frequency of wounds in the hands is accounted for by the efforts of self-defence, and the fact that the hands are uncovered. A slight amount of protection appears to be afforded by the clothes, due, probably, to a partial cleansing