OBSERVATIONS ON THE BACTERIOLOGY OF AN EPIDEMIC OF DIPHTHERIA IN A SCHOOL,

WITH SPECIAL REFERENCE TO THE VIRULENCE OF THE ORGANISMS WHICH WERE ISOLATED FROM THE CASES.

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IN November 1906 an opportunity occurred to investigate an epidemic of diphtheria occurring at the Duke of York's School, Chelsea. This is a military school of 551 boys, between the ages of 9 and 14 years, and 40 students, or older lads, 17 to 19 years of age. A certain number of both boys and students were affected.

History of the outbreak.

The epidemic of sore throats began about the 26th of August 1906, immediately after the boys had returned from camp at the seaside. Throughout September, October, and the first half of November, cases of sore throat occurred daily with occasional intervals of a day or two. Almost all of the cases presented the clinical features of follicular tonsillitis.

The earliest case which was clinically considered to resemble diphtheria occurred on 20th September, in a boy who had at the time, in addition to sore throat, a free nasal discharge which continued with an interval for at least two months; his nose and throat yielded in November the Bacillus diphtheriae.

Usually there was a sudden onset with vomiting and a temperature of 101° — 103° F. The illness only lasted four or five days, after which the patient, as a rule, appeared well. In no case was there a rash. No cases of peritonsillar abscess were observed. Altogether 183 boys and students had sore throats.

Albumen was found in the urine of two of the boys who were removed to the Metropolitan Asylums' Board Hospital. But although the urine of many of the patients who remained in the school was examined, in none was albumen found. Paralysis of the fauces was observed in only two cases, and in one of these accommodation was also affected. The general course of the disease was in every case mild, and recovery was uneventful, with the exception given above. All those affected recovered completely.

The epidemic was dealt with in the following manner by Lieut.-Col. Fayrer, the Medical Officer to the Duke of York's School: between 17th and 22nd November 1906, the whole school received a dose of diphtheria antitoxin, 109 boys in whose throats diphtheria bacilli had been found received 1000 units, and the rest 500 units: in addition all those with *B. diphtheriae* were either sent to hospital or isolated.

After the prophylactic injection until the middle of January, only five cases of sore throat occurred, and in none of these was the diphtheria bacillus found. But in one boy in apparent health, a patch of membrane was found on the fauces on 12th December, from which diphtheria bacilli were obtained. The epidemic ended suddenly on the adoption of the plan of isolation of carriers and prophylactic injection.

I first had the opportunity of examining swabs taken from the boys' throats on the 3rd November, and after that I examined all cases of sore throat for *B. diphtheriae*. Swabs taken from the fauces were inoculated the same day on solidified blood serum, and after the tubes had been incubated at 37° C. for about 20 hours, they were examined for *B. diphtheriae*. Its presence or absence was determined by examining films stained by Loeffler's methylene blue.

In order to determine with greater certainty the nature of such strains as were isolated, in addition to observing morphological and cultural characters, experiments with regard to acid production from various carbohydrates, virulence for guinea-pigs, and agglutination were made.

Results of Examinations of Swabs from Throats.

Forty boys were examined whilst actually suffering from sore throat between the 3rd and 18th of November, and of these $15 (= 37 \, {}^{\circ}/_{\circ})$ yielded *B. diphtheriae*.

On 14th November, Dr Boycott, Dr Marshall and myself, took swabs from the fauces of all the boys in the school who were not in hospital, Journ. of Hyg. VIII 4 and on 19th November 33 students were examined in the same way. On these two occasions 537 boys and students were examined and a positive diagnosis of *B. diphtheriae* was made in 118 cases (= 21 °/_o), all of whom, with one exception, appeared to be in good health. In this case a patch of membrane was found on the fauces at the time when the swab was taken. In addition $182 (= 43^{\circ}/_{\circ})$ were found to harbour *B. pseudodiphtheriae* (Hofmann). No attempt was made to count the number of those yielding Hofmann's bacillus in addition to *B. diphtheriae*. The above 182 cases with Hofmann's bacillus were found amongst those not giving *B. diphtheriae*, and the prevalence of Hofmann's bacillus is therefore best indicated by reckoning these cases as a percentage of the 419 cases in which *B. diphtheriae* was not found. Calculated in this way the proportion with Hofmann's bacillus was $43^{\circ}/_{0}$.

If we now take the whole school of 591 boys and lads into consideration (including the 40 students of 17—19 years), and the whole time from the beginning of the epidemic in the last days of August 1906 to the middle of January 1907, the following facts emerge:

Total number of boys and students in school	= 591,
Total number who had sore throats	$=183 \text{ or } 31^{\circ}/_{\circ}$,
Total number found with B. diphtheriae	$= 136 \text{ or } 23^{\circ}/_{0}.$

Incidence of B. diphtheriae.

Of 40 boys with sore throat when examined	15 or 37 % h	ad B. (diphtheriae.
Of 183 boys who at some time had sore throats	55 or 30 %/0	"	,,
Of 591 boys (<i>i.e.</i> whole school)	136 or 23 %/0	,,	**
Of 408 boys who did not have sore throats	81 or 20 %	,,	,,

Very few examinations were made before the 3rd November.

The boys who suffered from sore throats and those who were proved to harbour *B. diphtheriae* were by no means identical, for although 136 yielded *B. diphtheriae*, only 55 of these had had sore throat.

Characters of the Strains isolated.

Twenty strains of bacilli morphologically resembling *B. diphtheriae* isolated from cases taken haphazard were further examined. Four of these strains were from patients who had clinical symptoms when examined (3 cases of sore throat and one of nasal discharge), the rest were from boys who had no symptoms when examined, but 3 of whom had had sore throats about four weeks previous to the examination.

One of these 20 strains (D 73) morphologically and culturally some-

what resembled *B. coryzae segmentosus*, but a dose of 2 c.c. of a 48-hour broth culture killed a guinea-pig in $4\frac{1}{4}$ days. In addition 4 other diphtheroid strains were isolated; 2 of Hofmann's bacillus (A 12, D 39), and 2 organisms resembling *B. coryzae segmentosus* (G 17, G 36). Certain other strains of diphtheria and diphtheroid bacilli obtained from other sources were subjected to the same tests for the sake of comparison (see footnote to Table I).

Morphology.

As described by other writers, films made from 20-hour cultures on solidified serum and stained by Loeffler's blue showed many variations in the length and structure of the bacillus. All the strains showed beading or segmentation and characteristic grouping.

Three of the strains (B 28, C 49, and Y 11), which were most typical of the long form of the *B. diphtheriae*, were amongst the nonvirulent strains. Neisser's stain yielded uncertain results; for instance virulent strains of *B. diphtheriae* (Y 57, St. 28) showed no polar bodies, but diphtheroids (D. 39, Waite) which certainly were not true *B. diphtheriae* had well marked polar bodies.

B. coryzae segmentosus in a film from a 20-hour culture usually appeared as a very short diplococcus-like bacillus, but after 48 hours or more had grown into a long and much segmented form, sometimes with swollen ends. After a few days the beaded forms of the bacillus when stained with Loeffler's blue often closely resembled a chain of streptococci.

None of the 7 strains of non-virulent *B. diphtheriae* isolated in this epidemic resembled those races of diphtheroids described by Gordon (1902) or Graham-Smith (1904) as being related to but not identical with the *B. diphtheriae*.

Acid Production from Sugars.

Liquid media containing $1^{\circ}/_{\circ}$ of glucose, maltose, galactose, laevulose, lactose, cane sugar and mannite were used in these tests. A series of tests was made with a medium consisting of $25^{\circ}/_{\circ}$ beef broth and 75 $^{\circ}/_{\circ}$ peptone water and litmus. The results are given in Table I. Acid was produced by the strains of *B. diphtheriae* in the presence of all these substances except cane sugar and mannite.

A more complete series was carried out with litmus-peptone-water $(2^{\circ})_{\circ}$ Witte's peptone in tap water) as the basis of the medium. This

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$Hiss'$ medium with 1 $^{\circ}/_{\circ}$ carbohydrates.	Kuapp B. diph- theriae +	ours. + = acid after 48 hours. VV = fair virulence. V = low virulence. N = non-virulent. app (1904) and Graham-Smith (1906) are included in this Table. Sources of B. diphtheriae and diphtheroid bacilli examined.	The toxigenic strain No. 8 of Park and Williams. Virulent strains of <i>B. diphtheriae</i> from Dr Blumenthal of Moscow; the bacilli are of very long form and ofte branched. A bacillus from ear discharge in scarlet fever; thick opaque, whitish growth on agar; stained nearly uniformly b methylene blue. From discharge from the middle ear. It resembled 'Ble.' macroscopically; when stained by methylene blue is showe
Hiss' medium v	Graham-Smith B. diph- theriae $+$ $+$ $+$ \mp ? $+$ $ +$ $+$ $+$ B. zerosis $+$ $ +$ $+$ $+$ $ -$ B. coryzae segmentosus $+$ $ +$ $ +$ $ -$ Certain other diph- theroids $+$ $+$ $+$ $ +$ $+$ $ -$	+ S=slightly acid after 48 hours. + = acid after 48 hours. VV=fair virulence. V=low virulence. The results recorded by Knapp (1904) and Graham-Smith (1906) are included in this Table. Sources of B. diphtheriae and diphtheroid bacilli examined.	 P. W. The toxigenic strain No. 8 of Park and Williams. Bl. 2) Virulent strains of B. diphtheriae from Dr Blur (3) Bl. 4) branched. (4) Ble. A bacillus from ear discharge in scarlet fever; tl methylene blue. (5) By. From discharge from the middle ear. It resemble

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 - It resembled 'Ble.' macroscopically; when stained by methylene blue it showed From discharge from the middle ear. regular transverse bands. Бy.
- From the vaginal discharge of a child. It grew feebly in very small colonies on serum and agar. Uniformly barred when stained by methylene blue. ₿ (9)
 - Diphtheroids of the type of B. coryzae segmentosus from catarrhal noses. 18 (2) 21 (1) 6

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series (Table II) gave uniform results which were different from the previous series (Table I) in that acid was never produced from lactose. In the tubes containing cane sugar and mannite, acid was never produced in either series. The growth was good in all the tubes.

It was thought that the muscle sugar in the beef broth might account for the acidity in the lactose tubes of the first series, although no acid had appeared in the presence of cane sugar or mannite. Accordingly 12 strains were tested again in three different media:

- (1) Weak litmus broth as above, without lactose.
- (2) Weak litmus broth as above, with $1 \frac{0}{0}$ lactose.
- (3) Peptone water with $1^{\circ}/_{\circ}$ lactose.

The result is shown in Table III.

Bacillus		Weak broth,	no lactose	Weak broth	, 1 % lactose	Peptone water
diphtheriae	Virulence	2 days	7 days	2 days	7 days	1 ⁰ / ₀ lactose 7 days
St. 28	vv	$+ \mathbf{S}$	S	+	+ +	_
A 67	vv	$+\mathbf{S}$	$+ \mathbf{S}$	+	+ +	-
E 25	vv	+	÷	+	++	-
D 3	vv	-			-	-
Y 3	vv	+ S	+	+	+ +	_
Y 35	v	$+ \mathbf{S}$	+ S	+	+ +	-
Y 57	v	$+ \mathbf{S}$	+ S	+	+ +	-
C 49	N	$+ \mathbf{S}$	-	+	$+\mathbf{S}$	-
St. 17	N	$+ \mathbf{S}$	-	+	+ +	-
P. W.	vv		-	8	VS	-
Diphtheroi	ids from otor	rhoea :				
Ble.	V	-	~	+	++	-
By.	N		-	S	-	-
	$\mathbf{V} = \mathbf{I}$	irulent. ow virulence. lightly acid.		+ = acid. + + = very aci - = neutral		

TABLE III.

The supposition was not found to be justified, for as before, the peptone water with $1 \, {}^{0}/{}_{0}$ lactose remained uniformly neutral; the weak broth without lactose was usually slightly acid after one or two days but later became less acid or alkaline; the weak broth with $1 \, {}^{0}/{}_{0}$ lactose soon became acid and later a strong acid reaction developed in it.

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Virulence.

The virulence of each strain was tested by injecting subcutaneously into three guinea-pigs (about 250 grammes) doses of 0.1 c.c., 2.0 c.c., and 2.5 c.c. respectively of a 48-hour culture in alkaline broth, 0.1 c.c. of antitoxin having been previously added to the 2.5 c.c. dose. The animal which received antitoxin along with the culture remained well in every case. The results are given in Tables IV and V below.

	Dose of a 48 h in alkalir			Occurrence of			hrs.cult ne broth		Occurrence
Strains	2 ^{.0} c.c.	0·1 c.c.	Organism	sore throat	Strains	2.0 c.c.	0 [.] 1 c.c.	Organism	of sore throat
St. 28	†17 hrs.	†36 hrs.	Bac. d.		E 6		-	Bac. d.	
A 67	+36 ,,	†36 "	,,	\mathbf{P} ·	St. 33	-	-	,,	
St. 29	†36 "	†36 "	,, .		St. 22		-	,,	
E 25	†12 "	†84 "	,,		B 28		-	,,	
D 3	†60 ,,	†6 days	,,		¥ 11T		-	,,	Р
G 50	†8 4 ,,	† 8 ,,	,,	i	C 49	~	-	"	
Y3 ·	†20 "	†10 ,,	,,	E	St. 17	~	-	,,	
A 37	†19 "	~	,,		A 12	-		н	
Y 35	† 36 ,,	~	,,	Е	D 39	-	-	\mathbf{H}	
Y 57	†36 <i>,</i> ,	~	,,	Е	G 17	-		B. C. S.	
G 61	†36 "	~	,,	Р	G 36	-		B. C. S.	
E 71	1 36 ,,	~	,,	Р					
D 73	$4\frac{1}{2}$ days		,,						
	Bac. d. =	Bacillus d	liphtheriae	2.			$\mathbf{P} = \mathbf{I}$	oreviously	' .
	H =	,, J	seudo-dipl	htheriae (H	ofmann).		† = d	leath.	
	B. C. S. =	,, c	oryzae seg	mentosus.			~ =li	ved.	
	E =	at time of	f examinat	ion.					

TABLE IV. Virulence for the guinea-pig of the isolated strains.

E and P in the 5th column refer to the occurrence of sore throat in the patient from whom the strain was isolated.

TABLE	V.	Proportion	of	Virulent	and	Non-virulent	Strains.
		Willed to days					

	Killed	n dose or			
	0 ⁻¹ c.c. within	2 ^{.0} c.c. within	Glucose reaction	Morphology	Percentage
4 Strains	$4 \mathrm{days}$	48 hours	Aeid	Bac. d.)	35
3,,	10 "	4 days	,	,, ∫	
6,,	~	$4\frac{1}{2}$,,	,,	,,	30
7,,	-	~	••	,,	35
20					

Bac. d. = Bacillus diphtheriae.

Of the 4 strains regarded on other grounds as *B. pseudo*diphtheriae (Hofmann) and *B. coryzae segmentosus*, none caused death.

Of the 20 strains from the school resembling *B. diphtheriae* morphologically, only four killed within 4 days, when a dose of 0.1 c.c. was

injected; one in 8 days and one other in 10 days; and six strains killed in a dose of 20 c.c. leaving seven strains $(35^{\circ}/_{\circ})$ quite devoid of killing power.

If the 20 strains isolated can be taken as a fair sample of the rest, then $7 \, {}^{0}/_{0}$ of the boys in the school harboured non-virulent *B. diph*-theriae.

In order to make sure that the defective virulence was not due merely to the sample of broth used or to a weak, but not entirely absent, power of forming toxin, 4 non-virulent strains were grown for 10 days in a special alkaline broth which contained $2^{\circ}/_{\circ}$ peptone and which had been found very efficient for making toxin. The same doses as before were injected into guinea-pigs, but in no case did death result.

Agglutination.

To obtain further evidence which should decide the question as to the relationship of the virulent and non-virulent strains of diphtheria bacilli, I applied an agglutination test. For this test I employed the serum of a horse which had been immunised some time before, with the view to testing the value of anti-microbial serum.

In the process of immunisation one race of *B. diphtheriae* was employed, viz. the toxigenic bacillus No. 8 of Park and Williams. At the outset of the immunisation intravenous injections of killed cultures of the bacillus were employed, later the living bacilli were used.

The agglutination results (Table VI) were not uniform for the virulent strains, but the majority of these, 10 in number (including the strains from outside sources), gave positive results. One gave a slight, one a doubtful result, and one was negative; three were not tested.

Of the non-virulent diphtheroids, the two strains of *B. pseudo-diph-theriae* (Hofmann) and the two strains 'Ble.' and 'By.' from ear discharges gave negative results.

Two non-virulent diphtheroids (W and 18 (2)) gave doubtful results; one (21 (1)) gave a negative result.

Of six non-virulent diphtheria bacilli from this epidemic, four gave slight positive, and two negative results.

The agglutination tests indicate differences in the races of virulent diphtheria bacilli, and also a divergence of the non-virulent strains from the majority of virulent strains.

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Normal horse serum 1/50 / 1/100 1/50 / 1/100 + + + + + + + + + + + + + + + + + + +	1 1 1 1 1 1 1 1 1 1 1
+ + + + + + + + + + + + + + + + + + +	+ + + + + § +
+ + + + 20 + + + + + + + + + + + + + + +	iphtheri + + + + +
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+ + + = complete sedimentation. - = no sedimentation.

TABLE VI. Agglutination Macroscopic.

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The Occurrence of Bacillus fusiformis and Spirochaetes.

Another interesting feature of the epidemic was the frequent occurrence in the cases of sore throat of the *B. fusiformis* and of spirochaetes. These organisms were seen in smears made direct from the throat. They were found chiefly in cases in which *B. diphtheriae* was also present.

Of 45 cases of sore throat examined 20 showed *B. fusiformis* and 14 also yielded spirochaetes, both of which were often present in very large numbers in smears made at once from swabs taken from the fauces. Of the 16 cases of clinical diphtheria in which *B. diphtheriae* was found, 11 had also *B. fusiformis* and 10 spirochaetes as well as *B. fusiformis*. If the swabs had been taken a few hours or allowed to dry before the smears were made, *B. fusiformis* and spirochaetes were seldom found, and if found, were present in small numbers.

The frequent occurrence of *B. fusiformis* and spirochaetes on the fauces of patients suffering from diphtheria has been described by Priestley (1906) and Leiner (1906). Leiner considers the coexistence of *B. diphtheriae*, fusiform bacilli and spirochaetes to be quite common, and says that they are found associated mainly in two distinct groups of clinical diphtheria: the one, of very severe—the so-called septic type—the other of milder character with slight general symptoms.

Remarks.

The large number $(21 \, {}^{0}/_{0})$ of boys in the school who were found to harbour *B. diphtheriae* in the fauces, and the large proportion of the strains of bacillus which proved to be either non-virulent or of low degree of pathogenicity for guinea-pigs, is remarkable. Non-virulent strains were estimated to be present in 7 ${}^{0}/_{0}$ of all the boys. The presence of bacilli in the throats of 4 to $10 \, {}^{0}/_{0}$ of apparently healthy children, in connection with outbreaks of diphtheria, has been recorded by Graham-Smith, Thomas, Ustvedt, Pennington, and others.

Graham-Smith (1904) during an epidemic of diphtheria at Cambridge found diphtheria bacilli in the fauces of $3\cdot9\,^{0}/_{0}$ of the children who were contacts (in $2\cdot6\,^{0}/_{0}$ virulent and in $1\cdot3\,^{0}/_{0}$ non-virulent strains) and taking the notified cases and contacts together, in $8\cdot2\,^{0}/_{0}$ (in $6\cdot4\,^{0}/_{0}$ virulent, in $1\cdot8\,^{0}/_{0}$ non-virulent) of the children examined.

Thomas (1905) found from the examination of those children in London schools, who without obvious symptoms of diphtheria were suspected of spreading the disease, that $6.7-7.3 \, {}^{0}/_{0}$ harboured diphtheria bacilli.

Ustvedt (1906) found that in infected schools in towns, bacilli morphologically indistinguishable from diphtheria bacilli, were obtained from $4.5 \, 0/_0$ of the children. In an uninfected country district, none could be found amongst the school children; they were however present in the fauces of $14.21 \, 0/_0$ of those in close contact with patients.

Pennington (1907) examined the fauces of a large number of apparently healthy school children in Philadelphia. He obtained bacilli resembling *B. diphtheriae* from about $10^{0}/_{0}$ of the children and of the strains isolated $35-50^{0}/_{0}$ showed no virulence for guinea-pigs, and $14^{0}/_{0}$ killed guinea-pigs fairly promptly, whereas $30^{0}/_{0}$ he considers showed some pathogenicity, but were of attenuated virulence for guinea-pigs.

Where the observations have been made from cases distributed over a town or district, no relation has been established between the severity of an attack of diphtheria and the virulence of the bacilli for guinea-pigs (Smith and Walker (1896), and Richmond and Salter (1898)).

Cobbett (1901) and Graham-Smith in an epidemic in which cases of very different degrees of severity occurred, found that the strains isolated were of a singularly uniform degree of virulence. They obtained no evidence in favour of the possibility of attenuation of the bacillus.

There is more likelihood, however, that an epidemic within a small area, as in the present case, may have originated from a single strain of bacillus. The low degree of virulence of most of the strains which were pathogenic for guinea-pigs and the high percentage of non-virulent strains associated with a large number of cases of a very mild character can be best interpreted by the assumption that the outbreak in this school was due to an attenuated strain of the *B. diphtheriae*.

The agglutination test which I have used has yielded variable results with virulent and non-virulent strains, but on the whole has tended to accentuate the difference between the virulent and the non-virulent. Other observers have used agglutinating sera for examining strains of *B. diphtheriae* (Lubowski (1900) and Gordon (1901-2)).

Summary and Conclusions.

(1) A localised epidemic of diphtheria of a clinically mild type (both as regards local and general symptoms) was associated with the prevalence in the fauces of patients and contacts of strains of *B. diphtheriae* of low pathogenicity for animals; $35 \,^{\circ}/_{\circ}$ of these strains possessed moderate virulence for guinea-pigs, $30 \,^{\circ}/_{\circ}$ low virulence and $35 \,^{\circ}/_{\circ}$ were non-virulent.

(2) Prolonged nasal discharge after moderately severe diphtheria was in one case associated with a strain of *B. diphtheriae* which was non-virulent for guinea-pigs.

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(3) Acid production in various carbohydrate media proved a valuable means of differentiating diphtheria-like bacilli, but the exact composition of the medium is of importance. Peptone water appears to be especially suitable for the basis as being least liable to variation.

(4) The agglutination test gave a fairly uniform result with most of the strains of virulent *B. diphtheriae*, but three virulent strains did not give a decided positive reaction to this test. Of the non-virulent strains half gave a slight, the remainder a negative result.

Bacillus pseudo-diphtheriae (Hofmann) and certain acid producing diphtheroids gave no reaction.

(5) B. fusiformis and spirochaetes occurred in large numbers on the fauces in this epidemic of diphtheria, in association with the B. diphtheriae.

(6) After all the boys had received prophylactic injections of antitoxin and after the carriers had been isolated the epidemic promptly ceased.

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REFERENCES.

COBBETT, L. (x. 1901). Observations on the recurrence of Diphtheria in Cambridge in the spring of 1901. Journ. of Hygiene, I. p. 485.

- GORDON, M. H. (1902). Report on Bacillus diphtheriae and micro-organisms liable to be confounded therewith. 31st Annual Report of Local Government Board, 1901-2, p. 418.
- GRAHAM-SMITH, G. S. (1904). A study of the Virulence of the Diphtheria bacilli isolated from 113 persons, and of 11 species of Diphtheria-like organisms, together with the measures taken to check an outbreak of Diphtheria at Cambridge, 1903. Journ. of Hygiene, IV. p. 99.
- ----- (1906). The action of Diphtheria and Diphtheria-like bacilli on various sugars and carbohydrates. *Journ. of Hygiene*, vi. p. 286.
- KNAPP, A. (1904). The differentiation of *Bacillus diphtheriae*, B. xerosis, and B. pseudo-diphtheriae by fermentation tests in Serum-water media of Hiss. Journ. of Med. Research, XII. p. 475.
- LEINER, K. (1906). Ueber anaeroben Bakterien bei Diphtherie. Centralb. f. Bacteriol. Abt. I. Orig. XLIII. p. 7.

- LUBOWSKI, R. (1900). Ueber einen atoxischen u. avirulenten Diphtheriestamm und über die Agglutination der Diphtheriebacillen. Zeitschr. f. Hygiene, XXXV. p. 87.
- PENNINGTON, M. E. (1907). The Virulence of Diphtheria organisms in the throats of well school children and Diphtheria convalescents. Journ. of Infect. Dis. IV. p. 36.
- PRIESTLEY, J. (1906). History of a Diphtheria outbreak complicated with Vincent's Angina at the Lambeth Parochial Schools, West Norwood, May to July 1905. *Practitioner*, LXXVII. p. 372.
- RICHMOND, B. A. and SALTER, A. (1898). The Ætiological significance of the Diphtheria bacillus and its variants. *Guy's Hospital Reports*, LIII. p. 55.
- SMITH, T. and WALKER, E. L. (1896). Comparative Study of 42 cultures of Diphtheria bacilli and four cultures of Pseudo-diphtheria bacilli from different localities in Massachusetts. 28th Ann. Rep. of the State Board of Health of Mass., p. 649.
- THOMAS (1905). Referred to by Kerr. Rep. of Medical Officer (Education) to the London County Council for the year ended 31 March 1905, p. 26.
- USTVEDT, Y. (1906). Die Diphtherieprophylaxe und die Bedeutung der gesunden Bacillenträger für die Verbreitung der Krankheit. Zeitschr. f. Hygiene u. Infektionskr. LIV. p. 147.