ON THE RELATIONSHIP OF THE PSEUDO-DIPHTHERIA TO THE DIPHTHERIA BACILLUS.

By G. F. PETRIE, M.D.

Assistant-Bacteriologist, Serum Department, Lister Institute of Preventive Medicine.

The extreme diversity of opinion as to the identity of the Klebs-Loeffler bacillus and the bacillus of Hofmann, and the importance of the subject from the public health standpoint, make it desirable to bring forward any facts which may assist in arriving at a solution of the problem.

For some years after the two organisms were recognised their morphological and cultural characteristics served as a basis for upholding or denying any essential differences between them. In recent years, however, it has followed as a natural outcome of the study of immunity that the methods used for investigating the problems of immunity have been applied in this particular instance, since it is possible to bring about a specific reaction even in the case of closely related bodies. The more important researches carried out on these lines may be briefly mentioned.

Spronck (1896) injected large doses of pseudo-diphtheria cultures subcutaneously into guinea-pigs, and found that the local reaction produced was not influenced by the subsequent injection of diphtheria antitoxin. Glücksmann (1897) ascertained that immunisation of animals with cultures of the Hofmann bacillus did not confer any protection against diphtheria bacilli injected later. Lambotte (1902) prepared a serum which he found contained a "substance sensibilisatrice" for pseudo-diphtheria bacilli. The results of testing this serum on different strains led him to believe that these were very closely related, if not identical organisms—the specific reactions being similar in each case. He found also that there was a certain amount of fixation of this

https://doi.org/10.1017/S0022172400002412 Published online by Cambridge University Press

"substance sensibilisatrice" by Klebs-Loeffler bacilli. It may be remarked that Lambotte employed the Bordet-Gengou test for the fixation of the sensitising substance.

Several observers have attempted to solve the question by means of agglutination experiments but with conflicting results. Lesieur (1901) concludes from his experiments that pseudo-bacilli do not behave otherwise than true diphtheria bacilli towards a specific serum *in vitro*. Gordon's (1903) results showed considerable variations, even in the case of different strains of diphtheria bacilli; depending on whether the bacilli used for obtaining the agglutinating serum, and those used for the tests, were recently isolated or not. Lubowski (1900) immunised a goat with an avirulent diphtheria strain. The serum agglutinated virulent diphtheria bacilli and the avirulent organism but not Hofmann's bacillus.

The experiments about to be described were performed with the purpose of determining whether substances are present in pseudo-diphtheria filtrates which, when inoculated into animals in large amounts, lead to the production of an antitoxic serum for diphtheria toxin.

The work which has been done in this direction hitherto is inconsiderable. With regard to the production of a toxin, the non-pathogenicity of Hofmann's bacillus, *i.e.* the absence of a toxin producing acute symptoms, remains in the opinion of the majority of observers its distinguishing feature. It may be noted, however, that Ruediger (1903) has described pseudo-diphtheria organisms obtained from the throats of scarlet fever patients, which proved virulent to guinea-pigs. Antidiphtheria serum did not give any protection against these bacilli. Hamilton (1904) has isolated similar organisms from the throat in various diseased conditions and on one occasion from a normal throat. On the other hand, Graham-Smith (1904) states that while investigating an outbreak of diphtheria at Cambridge he failed to meet with organisms corresponding to the type described by Ruediger. Lesieur alleges that certain strains of pseudo-diphtheria bacilli, although non-virulent for guinea-pigs in ordinary doses, can nevertheless cause fatal paralyses similar to those due to diphtheria toxin provided that large doses of recently isolated cultures be used, or smaller doses of bacilli rendered artificially active by a method devised by himself. He evidently appears to consider that the paralysing substance is a very similar body, if it is not indeed identical with the toxone in diphtheria toxin. Several years ago Salter (1899) in a communication which is

frequently quoted described some interesting experiments, apparently showing that in filtrates of the Hofmann bacillus substances exist which are identical with the toxoids present in diphtheria toxin. Cobbett (1903) states that he has repeated these experiments without being able to confirm them. Hewlett (1904) also has been unable to obtain corresponding results.

I have carried out experiments in two directions in order to test this point:—

(1) by adding varying quantities of pseudo-diphtheria filtrates to toxin-antitoxin mixtures, and

(2) by immunising horses with large quantities of the filtrates and examining the serum afterwards for antitoxin.

The cultures used comprised 11 races. No. 1 was isolated from a throat-swab which was forwarded for diagnosis. It had been sub-cultivated on serum during a long period, at least 2 years. The remaining strains were recently isolated from swabs:—

1. From nose of patient suffering from throat diphtheria.
2. Throat of same patient.
3. Diphtheritic throat.
4. Throat of a case of scarlet fever with a deposit on the tonsils diphtheritic in appearance.
5. Throat of a case of suspected diphtheria.

6.
7.
8.
9.
10. were isolated from the throats and noses of boys from a school in which an outbreak of diphtheria occurred; the number of cases in which the Klebs-Loeffler bacilli were found was comparatively small.

All these strains had the following characters. They stained by Gram's, but not by Neisser's method. They gave an alkaline reaction to litmus when grown in glucose broth. Preparations from cultures grown for four days in alkaline broth at 36° C. when stained by Loeffler's methylene blue showed uniformly and deeply stained short rods arranged in a parallel manner. Involution forms were rarely seen even in cultures several weeks old. 5 c.c. of bouillon cultures grown for three days at 36° C., beyond slight local reactions at the site of injection, gave rise to no effects in guinea-pigs.

A large volume of culture was obtained by growing No. 1 bacillus in Erlenmeyer's flasks, each containing 200 c.c. of alkaline broth prepared in the usual manner for the production of diphtheria toxin. Another

quantity was grown in Martin's pig-stomach bouillon at 36° C. for ten days. The cultures developed a thick, opaque, slightly wrinkled membrane, of a greyish-white colour on the surface, exactly resembling that seen in diphtheria cultures; the broth was, however, somewhat turbid. The cultures were filtered through a Pasteur-Chamberland filter after growing for 10 days.

A mixed filtrate of the other 10 races was prepared by growing each separately after several sub-cultures in alkaline broth tubes. One lot was allowed to grow for nine days, the remaining flasks being filtered after 15 days' growth. Most of the cultures formed good membranes on the surface of the medium, and all had the peculiar, slightly offensive odour characteristic of diphtheria cultures.

The following Tables set forth the experiments carried out with a view to ascertaining whether toxoids were present in the filtrates. The filtrate was added first to the unit of antitoxin, the mixture being then placed in the incubator for fifteen minutes before adding the toxin. In this way any toxoid which might be present had an opportunity of combining with the antitoxin, thus preventing a corresponding amount of toxin from entering into combination.

All the mixtures were made up to the same bulk with tap-water. The toxin used was a test-toxin employed for testing antitoxic sera, the L+ dose being 0·14 and the Lo dose being 0·08 c.c.

It will be noted that in Table I the results are somewhat irregular in the case where 0·66 c.c. of toxin was given. This can doubtless be explained by the circumstance that the amount of toxin approximates so closely to the L+ dose. In the control experiment with 0·66 c.c. toxin the animal died on the 8th day, but in tests with the same amount of toxin carried out for another purpose the guinea-pig died in one case on the 4th day, and yet another on the 5th day. Differences in the resistance of different animals have also to be taken into account. With regard to the other tests in this table, although in one or two instances the animals which received the pseudo-diphtheria filtrates suffered a greater loss of weight than the controls, it is obvious that the difference comes within the limits of the errors of experiment. In Table II amounts of toxin more nearly approaching the L+ dose were selected. The results of this experiment show clearly that no evidence is forthcoming of there being any difference in the amount of free toxin present in the mixtures.

The dose of toxin given in the experiments set forth in Tables III and IV was such that toxone effects became manifest in 3 or 4 weeks.

Table I.

Testing Toxin – Antitoxin Mixtures + Pseudo-diphtheria Filtrate of No. 1 Bacillus, showing result of addition of the Filtrate to amounts of Toxin varying from a nearly L+ to a nearly L° dose

Guinea-pig	Weight grams	Test	Weight of Guinea-pig on successive days after injection							
1	250	1 I.E. +0·66 c.c. T_5	255 t	240 vl	218 vl	210 vl	200 l	193 vl	195 vl	dead 22nd day
2	258	,, + ,, +1 c.c. Ps. F.	248 l	235 vl	220 vl	dead				
3	255	,, + ,, + ,,	245 m	220 vl	205 vl	185 vl	170 vl	173 vl	170 vl	dead 9th day
4	250	,, + ,, + ,,	225 l	220 vl	200 vl	dead				
5	255	,, + ,, + ,,	258 m	255 vl	253 vl	230 vl	dead			
6	255	,, + ,, +0·5 c.c. Ps. F.	230 vl	215 vl	210 vl	198 vl	205 vl	205 vl	185 vl	recovered
7	255	,, + ,, +0·25 c.c. ,,	258 m	235 vl	240 vl	225 l	215 vl	215 vl	215 vl	dead 22nd day
8	250	,, +0·64 c.c. T_5	258 s	250 vl	240 vl	230 vl	230 l	215 vl	205 vl	dead
9	255	,, + ,, +0·5 Ps. F.	255 m	260 l	250 vl	235 l	240 l	240 l	225 l	
10	250	,, + ,, +0·25 c.c. Ps. F.	238 m	230 l	228 vl	225 m	235 l	235 l	225 l	dead 22nd day
11	248	,, +0·62 c.c. T_5	265 t	242 l	230 vl	225 l	220 l	225 vl	235 vl	
12	255	,, + ,, +0·5 c.c. Ps. F.	240 l	230 vl	220 vl	220 l	215 l	215 l	205 l	
13	250	,, + ,, +0·25 c.c. ,,	240 m	235 m	238 l	245 s	240 s	230 s	220 s	
14	250	,, +0·60 c.c. T_5	240 t	240 s	225 l	215 l	220 l	215 m	215 m	
15	250	,, + ,, +0·5 c.c. Ps. F.	240 m	225 l	218 l	215 m	220 s	210 m	200 m	
16	250	,, + ,, +0·25 c.c. ,,	235 s	225 m	235 l	235 m	235 m	230 s	220 s	
17	255	,, +0·56 c.c. T_5	245 n	240 s	225 l	220 l	220 m	220 t	215 m	
18	250	,, + ,, +1 c.c. Ps. F.	215 m	200 l	195 vl	195 l	200 m	200 m	195 s	
19	245	,, + ,, +0·5 c.c. ,,	230 s	220 l	220 m	225 s	225 s	220 t	220 s	
20	245	,, +0·52 c.c. T_5	245 n	230 t	228 s	215 m	215 m	215 t	215 t	dead 11th day
21	250	,, + ,, +1 c.c. Ps. F.	245 s	228 m	235 s	235 s	225 t	220 t	223 n	
22	250	,, + ,, +0·5 c.c. ,,	240 t	230 s	225 t	228 t	225 t	220 t	228 t	
23	260	,, +0·50 c.c. T_5	258 n	255 t	265 t	245 t	260 t	255 t	255 n	
24	248	,, + ,, +1 c.c. Ps. F.	240 m	230 m	225 t	235 t	230 t	220 t	215 t	
25	255	,, + ,, +0·5 c.c. ,,	275 t	250 n	235 t	235 t	240 n	240 n	255 n	

Note:—1 I. E. = 1 unit of Antitoxin (Ehrlich). T. = Toxin. Ps. F. = Pseudodiphtheria Filtrate.

The Toxin was diluted 1 : 5 with water for convenience of testing; the amount of Toxin represents amounts of this dilution in this and the following Tables.

The letters "n," "t," "s," "m," "l," and "vl," refer to the size of the local reaction, and indicate respectively that there was no local reaction; a trace of reaction; and a small; medium; large; and very large reaction.

TABLE II.

Testing Toxin – Antitoxin Mixtures + Pseudo-diphtheria Filtrate (10 *races grown separately and Filtrates mixed*), *showing result of addition of the latter to amounts of Diphtheria Toxin approaching the L + dose.*

Guinea-pig	Weight grams	Test	Weight of Guinea-pig on successive days after injection							
1	275	1 I. E. + 0·69 c.c. T_5	260 l	250 vl	230 vl	230 vl	—	dead	—	—
2	260	,, + ,,	260 m	255 l	230 l	225 vl	dead	—	—	—
3	255	,, + 0·67 c.c. T_5	260 m	250 l	230 l	210 l	dead	—	—	—
4	250	,, + ,,	240 m	235 vl	210 vl	215 vl	—	180 vl	dead	—
5	265	,, + 0·69 c.c. T_5 + 2·5 c.c. Ps. F. 9 days	255 l	255 vl	225 vl	225 vl	—	dead	—	—
6	260	,, + ,, + ,, ,, 15 days	265 m	270 vl	250 vl	250 vl	—	215 vl	dead	—
7	255	,, + 0·67 c.c. T_5 + 2·5 c.c. Ps. F. 9 days	250 m	250 vl	255 vl	250 vl	—	250 vl	250 vl	265 vl
			Paralysis pronounced on 28th day; dying on 33rd day; dead next day.							
8	250	,, + ,, + ,, ,, 15 days	230 m	215 vl	190 vl	180 vl	—	dead	—	—

TABLE III.

Testing Toxin – Antitoxin Mixtures + Pseudo-diphtheria Filtrate (mixed Filtrates of 10 races), showing effect of addition of latter to amounts of Toxin representing paralysing dose.

Guinea-pig	Weight grams	Test	Result
1	275	1 I.E. + 0·65 c.c. T_5	Died with paralytic symptoms on the 28th day.
2	265	,, + ,, ,,	Marked paralysis on 30th day, but gradually recovered thereafter.
3	270	1 I.E. + 0·65 c.c. T_5 + 2·5 c.c. Ps. F.	Marked paralysis on 23rd day; died next day.
4	260	,, + ,, + ,, ,,	Paralysis on the 23rd day; died on 28th day.
5	250	,, + ,, + ,, ,,	Died on 23rd day.

TABLE IV.

Repetition of Experiment given in Table III.

Guinea-pig	Weight grams	Test	Result
1	260	1 I.E. + 0·65 c.c. T_5	Died on 8th day. P.M. Toxaemia.
2	260	,, + ,,	Showed commencing paralysis on 16th day, which became very pronounced on 21st day; died on 24th day.
3	260	,, + ,,	Showed marked paralysis on 20th day; died on 21st day.
4	250	,, + ,, + 2·5 c.c. Ps. F.	,, ,, ,, ,, 24th day; ,, 28th day.
5	250	,, + ,, + ,, ,,	,, ,, ,, ,, 22nd day; ,, 24th day.
6	250	,, + ,, + ,, ,,	Died on the 11th day.

The results are not conclusive of any marked difference in the paralytic effects produced.

Taken as a whole these experiments may be interpreted as indicating that no toxoids were present in the filtrates of the pseudo-diphtheria bacilli examined.

Immunisation with Filtrates of the Pseudo-Diphtheria Bacilli.

Another method of testing the point was tried. If toxoids be really present as a product of metabolism of the Hofmann bacillus it might be expected that immunisation of a suitable animal with their products ought to lead to the production of an antitoxin in the animal's serum. The validity of this contention will be discussed later.

The process of immunisation was carried out in the following way:

A horse "A" which had not been previously immunised, received doses of pseudo-diphtheria filtrate of No. 1 bacillus, the procedure being exactly the same as in a diphtheria immunisation. Beginning with 3 c.c. it received as a final dose 5 weeks later 1 litre; 3,200 c.c. being given altogether. Before the injections were begun the serum of the horse was tested for normal antitoxin. It was found that it did not contain 1/3 unit per c.c. Ten days after the final injection it was again tested and again was found not to contain 1/3 unit.

A month or two later this horse received 1/10 c.c. of a diphtheria toxin, the M.L.D. of which for guinea-pigs of 250 grammes was 1/100 c.c. No further injections were given until 10 days later. A sample was taken on this day and the serum was again tested. It was found to contain 1 unit per c.c. At the end of the ordinary diphtheria immunisation the antitoxic value of the serum was low (below 100 units per c.c.) and in subsequent immunisations never rose above 400 units per c.c. It may be noted that the morning after a dose of 450 c.c. of the filtrate had been given the horse showed a considerable local reaction.

A second horse "B" was treated in a similar manner. He received 2150 c.c. of the mixed filtrates (10 races grown for 15 days). Two days after the last injection of 1 litre, a litre of the 9 day filtrate was given. The period of immunisation extended over 20 days. Ten days later a sample was taken. Before the injections were commenced the serum was tested and was found to contain no normal antitoxin, *i.e.* not 1/4 unit per c.c. The serum of the sample taken after the immunisation also did not contain 1/4 unit per c.c. The horse was then

given 1/100 c.c. of a diphtheria toxin whose M.L.D. for guinea-pigs was 1/400 c.c. After an interval of 10 days, blood was withdrawn, and the serum again found to have less than a 1/4 unit per c.c. Four days later 1/20 c.c. was given intramuscularly. It was intended to obtain blood for the purposes of a test at the end of 10 days, but unfortunately the horse died suddenly; the cause of death apparently having no connection with the previous treatment.

On the evening after 150 c.c. of the filtrate was administered the horse had a temperature of 104·4° Fahr. and a small swelling the size of the fist over the site of injection. After 300 c.c. he had a local reaction with an oedematous swelling in the brisket and stiffness in walking. The dose of 1 litre of the 15 days' filtrate caused a moderate local swelling accompanied by oedema in the brisket. The symptoms, however, were not nearly so severe as those usually observed during a diphtheria immunisation.

A third horse "C" was inoculated with 650 c.c. of the 15 days' filtrate given by rapid stages, and as a final dose, 1,100 c.c. of the nine days' filtrate. The period of immunisation lasted only nine days. The serum before beginning the treatment did not contain 1/4 unit, and ten days after the last dose was given, again did not have an antitoxic value of 1/4 unit per c.c. The horse was then injected on successive days with 1/100, 1/50, 1/10, 1/2, 1·25 and 3 c.c. of a toxin whose M.L.D. for guinea-pigs was 1/300 c.c. The blood was sampled after an interval of ten days, and the serum was found to contain 1 unit per c.c., but not five units. In the evening after 150 c.c. of the pseudo-diphtheria filtrate had been given the horse had a moderate local reaction. He felt the last dose (1,100 c.c.) considerably, fed badly for a few days, and had a large local reaction.

It is evident from these experiments that the filtrates employed were not capable of producing an antitoxin to diphtheria toxin. Before the conclusion is drawn that no toxoids were present in the filtrates the question must be faced as to whether toxoids alone can give rise to an antitoxin. At the Thirteenth International Congress of Medicine at Paris in 1900 Ehrlich made several observations bearing upon this point which leave no doubt that he believed that they are able to do so. While referring to his own views on the constitution of diphtheria toxin he stated that it was possible to provoke a production of an antibody not only by utilising toxins but also toxoids. As a result of later investigations in conjunction with Morgenroth on the production of isolysins Ehrlich (1900) found that before such an antibody could be

obtained very large quantities of blood must be injected; the reason being that in the blood of the same species elements corresponding to toxophore groups are absent. He considers that what he calls an "ictus immunisatorius" is essential. Wassermann at the International Congress of Hygiene at Brussels in 1903 discussed the matter at some length. His conception of the mechanism of antibody-production may be briefly stated thus:—He asserts that he has never succeeded with quite non-toxic "toxins," *i.e.*, toxins with haptophore groups only, in obtaining a really high antitoxic serum. In order to bring about an "Abstossung" of the receptors a second factor besides the combination of the haptophore groups to the cells is necessary, viz., the stimulus (Reiz) to the cell which is the function of the toxophore groups. He points out, however, that the possibility of immunising with toxoids is beyond question, and gives as an example the basal immunity conferred on guinea-pigs and mice against tetanus by using non-toxic modifications: the only possible method indeed of producing an immunity in these animals against diphtheria and tetanus toxins. Bruck (1904) in a recent paper describes experiments which he thinks strengthens the position taken up by Wassermann. He obtained two tetanus toxins, one practically non-toxic for mice, and the other feebly toxic. He proved the presence of toxoids in these toxins by finding that they were still able to neutralise antitoxin. He then proceeded to immunise rabbits, giving doses up to 1 c.c. of each. In the case where the non-toxic substance was used no antitoxin was produced, while in the other case, a small amount of antitoxin was found in the serum of the rabbit. The objections can, I think, be reasonably adduced that the total quantities of the toxins given were very small,—Ehrlich's isolysin experiments may be recalled in this connection,—and that the rabbit is not perhaps a very suitable animal for the production of an antitoxin. Bruck believes that a slight stimulus is necessary in addition to the action of the haptophore groups for the production of an antibody.

Von Behring's (1904) views on this subject are founded on a very large practical experience. In immunising horses in order to obtain tetanus antitoxin he now uses toxins whose "direct toxic value" has almost, if not completely disappeared, but which have retained their "indirect toxic value," *i.e.*, their power of neutralising antitoxin. He refers to a tetanus toxin which, although quite non-toxic for mice, can be used for immunising horses without the slightest risk, and which in a short time confers upon them a high degree of immunity with a considerable production of antitoxin. Before he recognised this fact he had

empirically adopted the method of adding trichloride of iodine to his toxins in order to bring about a similar result.

The weight of von Behring's authority on this point, together with the fact that Wassermann does not deny that a small amount of antitoxin can be produced by toxoids alone, is confirmatory of the belief that no toxoids were present in the pseudo-diphtheria filtrates used in the immunisation experiments described above. This view gains support from the circumstance that after a small amount of diphtheria toxin was given the antitoxic values of the serum of the horses "A" and "C" were not above the average. It might have been supposed, otherwise, that the receptors produced in abundance by toxoids but remaining attached to the cells would have been set free in the serum when a stimulus provided by even a small dose of toxin was supplied.

In conclusion it may be stated that the two sets of experiments carried out combine to justify the opinion:

(1) that no substances capable of neutralising diphtheria antitoxin are present in filtrates of pseudo-diphtheria bacilli;

(2) that the results of the immunisation of horses with large quantities of the filtrates make it apparent that they do not contain substances capable of stimulating the production of an antitoxin to diphtheria toxin.

It is scarcely necessary to add that if this be the case the differences between the two organisms are accentuated, thereby diminishing the probability that they stand in a close relation to each other. The whole question has a certain interest clinically, since it shows that in cases where Hofmann's bacillus is associated with the Klebs-Loeffler bacillus no toxoids are elaborated by the former which might do harm by combining with antitoxin administered therapeutically.

My thanks are due to Dr Fletcher, Ham Green Hospital, Bristol, and to Dr A. T. MacConkey, for supplying me with the material from which the cultures were isolated. I am also indebted to Dr George Dean for practical suggestions which have been of value in carrying out the experiments.

REFERENCES.

BEHRING (1904). *Aetiologie und aetiologische Therapie des Tetanus*, Berlin, p. 8.

BRUCK, CARL (1904). Experimentelle Beiträge zur Theorie der Immunität. *Zeitschr. f. Hyg.*, Bd XLVI., p. 176.

COBBETT (1903). Discussion on "The Nature of the so-called Pseudo-diphtheria (Hofmann) Bacillus and its significance (if any) in the bacteriological examination for Diphtheria." *Journal of State Medicine*, Vol. XI., p. 609 (Liverpool Congress of Royal Institute of Public Health, 1903).

EHRLICH (1900). *Report of 13th International Congress of Medicine at Paris* (Section of Bacteriology and Parasitology), p. 28.

EHRLICH AND MORGENROTH (1900). Ueber Haemolysine. *Berliner klinische Wochenschr.*, p. 453.

GLÜCKSMANN (1897). Ueber die bakteriologische Diagnose der Diphtherie. *Zeitschr. f. Hyg.*, Bd XXVI., p. 417.

GORDON, M. H. (1903). Supplement. Report on Bac. diphtheriae and microorganisms liable to be confounded therewith. *31st Annual Report of Local Government Board*, 1901, 1902, p. 418.

GRAHAM-SMITH (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. *Journal of Hygiene*, Vol. IV., p. 258.

HAMILTON, ALICE (1904). The question of virulence among the so-called Pseudo-diphtheria Bacilli. *Journal of Infectious Diseases*, Vol. I., p. 690.

HEWLETT, R. T. (9 July, 1904). Supplement to the *British Medical Journal*, p. 16.

LAMBOTTE (1902). Les sensibilisatrices des Bacilles diphtéritiques et Pseudo-diphtéritiques. *Centralbl. f. Bact.*, Bd XXX., p. 817.

LESIEUR (1901). De l'Agglutination des Bacilles dits "Pseudo-diphtéritiques" par le sérum Anti-diphtéritique, *Compt. rend. de la Société de Biologie*, T. 53, p. 819, and Les Bacilles dits "Pseudo-diphtéritiques," Paris, 1902.

LUBOWSKI, R. (1900). Ueber einen atavischen und avirulenten Diphtheriestamm und über die Agglutination des Diphtheriebacillus. *Zeitschr. f. Hyg.*, Bd XXXV., p. 87.

RUEDIGER (1903). *Trans. Chicago Path. Soc.* (cited by Hamilton, 1904).

SALTER (1899). The Pathogenicity of the Pseudo-diphtheria bacillus and its relation to the Klebs-Loeffler organism. *Trans. Jenner (Lister) Institute of Preventive Medicine (London)*, 2nd series, p. 113.

SPRONCK (1896). Ueber die vermeintlichen "schwachvirulenten Diphtheriebacillen" des Conjunctivalsackes und die Differenzirung derselben von dem echten Diphtheriebacillus mittels des Behring'schen Heilserums. *Deutsche med. Wochenschrift*, p. 571.

WASSERMANN (1904). Entstehung und Wirkungsweise der aktiven Stoffe im Immunserum. (Internationaler Kongress für Hygiene in Brüssel, 1903.) Ref. *Centralbl. f. Bacteriologie, Referate*, Bd 35, p. 17.