

THE BIOLOGICAL IDENTITY OF THE FRIEDLÄNDER BACILLUS.

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Although immunological reactions have been applied to the identification of members of the *Bacillus mucosus capsulatus* group, the refractoriness of these organisms to agglutination by immune sera has stood in the way of a satisfactory classification on this basis.

In spite of the success of Landsteiner,¹ Babes,² Klemperer and Scheier,³ and Bertarelli⁴ in agglutinating Friedländer or rhinoscleroma bacilli with immune sera, other investigators (Clairmont,⁵ Sicard,⁶ Defalle,⁷ and Porges⁸) have found these organisms inagglutinable in their native condition, so that recent workers have employed the method of Porges,⁸ which is directed towards dissolving the mucoid capsule or hydrolyzing the protein material which holds the bacilli in an inagglutinable suspension. But this treatment by heating in acid solution has been found by Streit⁹ to lead to an increase in the susceptibility of the bacterial

¹ Landsteiner, K., Ueber die Folgen der Einverleibung sterilisirter Bakterien culturen, *Wien. klin. Woch.*, 1897, x, 443.

² Babes, V., Ueber das Rhinosklerom, in Kolle, W., and von Wassermann, A., *Handbuch der pathogenen Mikroorganismen*, Jena, 1st edition, 1903, iii, 420.

³ Klemperer, F., and Scheier, M., Ueber Identität der Ozaena- und der Rhinosklerombacillen mit Friedländer'schen Bacillen, *Z. klin. Med.*, 1902, xlv, 133.

⁴ Bertarelli, E., Die Kapselbacillen, insbesondere ihre Systematik und die durch sie bedingten immunitären Reaktionen, *Centr. Bakteriol., 1te Abt., Ref.*, 1906, xxxvii, 343.

⁵ Clairmont, P., Differentialdiagnostische Untersuchungen über Kapselbakterien, *Z. Hyg. u. Infektionskrankh.*, 1902, xxxix, 1.

⁶ Sicard, A., *Compt. rend. Soc. biol.*, 1899, i, series 11, 813.

⁷ Defalle, W., Sur le rôle de l'enveloppe des microbes, *Ann. Inst. Pasteur*, 1902, xvi, 602.

⁸ Porges, O., Ueber die Agglutinabilität der Kapselbakterien, *Wien. klin. Woch.*, 1905, xviii, 691.

⁹ Streit, H., Zur Frage der Agglutinierbarkeit von Kapselbacillen, *Centr. Bakte iol., 1te Abt., Orig.*, 1906, xl, 709.

suspension to normal sera and to spontaneous agglutination, as well as to immune sera. For this reason as well as for the variation in the length of treatment required to make different strains agglutinable, the method has not, in the hands of Streit⁹ and Beham,¹⁰ been found suitable for diagnostic purposes.

Cultures made to grow in a dry or capsule-free state, by development at low temperature (110°C.), or on potato agar, or by an artificial selection of the smaller and dryer colonies for several generations, have been found by Streit and Beham respectively to be agglutinated by immune sera. Since these dry cultures have been found by these writers, and by von Eisler and Porges,¹¹ to regain their capsulated mucoid state on animal passage, this simpler method does not appear suited to the recognition of bacilli in their most characteristic condition, as direct from the animal body.

EXPERIMENTAL.

The present study was undertaken as part of an immunological study of the capsulated Gram-negative bacilli. The material consisted of fifty strains, some isolated in this laboratory, and the rest collected from various sources.¹² All the organisms could be placed in the group comprising Friedländer's bacillus, *Bacillus mucosus capsulatus*, etc., and *Bacillus acidi lactici*, *Bacillus aerogenes*, etc.

For comparison of the cultural characteristics the organisms were grown in parallel streaks on dextrose agar in large (15 cm.) plates. In this way it has been possible to distinguish a Friedländer type, with colonies gray, translucent, of fluid, often syrupy consistency, and transparent watery margins, from an *aerogenes* type showing variations but having in common opaque ivory-white colonies, of a more pasty consistency. This cultural distinction is the main difference between the two organisms described by Kruse,¹³ and may be

¹⁰ Beham, L. M., Die agglutinatorischen Eigenschaften der Kapselbacillen und Anwendung der Serumagglutination bei den Trägern von Kapselbacillen, *Centr. Bakteriol., Ite Abt., Orig.*, 1912, lxvi, 110.

¹¹ von Eisler, M., and Porges, O., Ueber die Differenzierung der Kapselbakterien mit Hilfe agglutinierender und präcipitierender Immunsera, *Centr. Bakteriol., Ite Abt., Orig.*, 1906, xlii, 660.

¹² For these I am indebted to Dr. W. Rothberg of the American Museum of Natural History, Dr. J. G. Dwyer of the College of Physicians and Surgeons, Dr. E. G. Stillman of the Hospital of The Rockefeller Institute for Medical Research, and Miss M. Olmstead of the Presbyterian Hospital.

¹³ Kruse, W., in Flüggé, C., Die Microorganismen, Leipsic, 3rd edition, 1896, pt. 2, 185.

regarded as the basis of the classifications of Clairmont⁵ and of Strong.¹⁴

Eleven of our fifty strains belong to the Friedländer type. The cultural difference in our experience has been constant both for young and old cultures, but can be recognized surely only when the different cultures are grown upon the same plate. The fluidity of the Friedländer strains varies somewhat upon different samples of media, and on blood agar the growth is more luxuriant and opaque, but the mucoid characteristic is retained in all cultures and we have not been able to observe distinct moist and dry growing phases. Two further strains show a translucent mucoid growth on dextrose agar, but resemble *Bacillus coli* on plain agar. One of these, No. 12, from the intestine forms gas from lactose; the other, No. 48, from a normal mouth forms acid but no gas on lactose and saccharose, and can be distinguished by this means also from the eleven type strains. It is possible that such variable strains as these have been observed by authors who note a drying out of the cultures on long continued cultivation or a relation between mucoid growth and the water and sugar content of the media (Fürst¹⁵). Other organisms of a more colon-like growth are distinguished culturally without difficulty.

The source of the Friedländer type strains, and their fermentation reactions with dextrose, lactose, and saccharose are given in Table I.

Rabbits have been immunized with killed suspensions of agar growths of Strains 3, 24, and 27. Intravenous inoculations were made every 4 or 5 days. Eleven of the fourteen animals died in the course of the treatment, all but two dying in from 2 to 6 hours after a dose slightly larger than the previous one. At autopsy the findings of distended lungs, petechial hemorrhages of the serous surfaces, and greatly lengthened coagulation time of the blood have pointed to an anaphylactic death.

In the blood of the seven animals examined it has been possible to demonstrate agglutinins for the homologous strain after 19 to 23 days of immunization. For testing agglutination 0.1 cc. of a broth

¹⁴ Strong, L. W., Ueber die Kapselbacillen, *Centr. Bakteriolog., 1te Abt.*, 1899, xxv, 49.

¹⁵ Fürst, T., Untersuchungen über Kapsel- und Hüllenbildungen bei den sogenannten Kapselbakterien *Centr. Bakteriolog., 1te Abt., Orig.*, 1910, lvi, 97.

TABLE I.

Series No.	Source.	Dextrose.	Lactose.	Saccharose.
3	Bronchopneumonia.....	Acid and gas.	No acid or gas.	Acid and gas.
4	Lung.....	" " "	" " " "	" " "
17	Ropy milk.....	" " "	" " " "	" " "
23	Ozena.....	" " "	" " " "	" " "
24	Normal mouth.....	" " "	" " " "	" " "
25	Lobar pneumonia, blood.....	" " "	" " " "	" " "
27	Meningitis.....	" " "	" " " "	" " "
36	Sputum; child with pneumonia.....	" " "	" " " "	" " "
37	Sputum; child with pneumonia.....	" " "	" " " "	" " "
47	Normal mouth.....	" " "	" " " "	" " "
50	" "	" " "	" " " "	" " "

culture has been used, in a total volume of 0.5 cc. of serum dilution. The titer has been low for most sera not exceeding 1:10, but for two of the three animals that survived a longer immunization values of 1:40 to 1:60 were found. The addition of the bacteria to concentrated serum, 1:1 or 1:5 dilution, produces an almost immediate coarse flocculation which soon settles and is compacted into a firm disc in the bottom of the tube. In higher dilutions the flocculation is finer, slower in appearing, does not form so firm a disc, and in the highest dilutions does not produce a clearing of the tube.

This result presents a striking similarity to the agglutination of *Pneumococcus mucosus* by the immune serum recently described by Wadsworth and Kirkbride.^{16, 17}

Quantitative relations between serum and culture are strictly maintained so that 0.1 cc. of serum will cause complete agglutination of the same amount of culture whether it is in a volume of 0.2 or of 1.0 cc. The concentration affects only the speed of the reaction. The stability of Friedländer bacillus emulsions has been noted by Porges, Streit, and Bertarelli, and is so marked that clearing of broth or saline emulsions is impossible even with the highest centrifuge

¹⁶ Wadsworth, A. B., and Kirkbride, M. B., A note on the production of anti-pneumococcus sera, *J. Exp. Med.*, 1917, xxv, 629.

¹⁷ This immune serum was kindly placed at my disposal by the authors.

speed obtainable. This is due, as Porges¹⁸ explains, to the presence of dissolved bacterial protein substance. The soluble material is present in broth cultures, which are found considerably less agglutinable than equally turbid suspensions of bacteria washed in a Berkefeld filter or by centrifugation. The following protocol illustrates this point:

Serum 24-A	1 : 10	1 : 20	1 : 40	1 : 60
Culture 24 (24 hr. broth culture).....	±	—	—	—
Emulsion of washed bacteria from same culture.....	+++	+	±	—

The dissolved protein may be obtained by filtration from broth cultures as soon as turbidity has begun to appear, as early as 4 hours after planting. This substance reacts specifically with immune serum to give a heavy flocculent precipitate so that it is probable that it hinders the outflocking of the bacteria mainly by altering the quantitative relations between serum and substrate.

The viscid fluid obtained by washing out the peritoneal cavity of an infected animal is readily flocculated by immune serum, and capsules can be demonstrated in the agglutinated mass from this, as well as from broth cultures and washed bacterial emulsions. Examined in the hanging drop, the capsules appear in saline solution or normal serum only as halos of refraction, but on the addition of concentrated immune serum come immediately into prominence, swell to half the diameter of a red blood cell, and show a sharp non-refractile periphery. A similar observation was made by Landsteiner,¹ but appears to have been overlooked by later investigators. In weaker sera, agglutination may take place without the occurrence of this change. By the fuchsin-copper sulfate method the swollen capsules take a uniform heavy stain.

These observations do not support a morphological conception of the capsule as an impermeable envelope; they indicate rather that the

¹⁸ Porges, O., Ueber die Beziehungen zwischen Bakterienagglutination und Ausflockungserscheinungen der Kolloide, *Centr. Bakteriol., 1te Abt., Orig.*, 1906, xl, 133.

peculiar properties of the Friedländer bacillus protein determine the nature and the extent of its reaction with immune serum.

The eleven strains belonging to the Friedländer cultural type were agglutinated by immune sera developed against strains from pneumonia, from meningitis, and from a normal mouth. For a given serum, the agglutination titer was approximately the same for all strains. There was no evidence on which to base a subgrouping; unfortunately the supply of high titer serum was exhausted before complete absorption experiments could be applied to this point. The remaining thirty-nine strains representing different cultural types were not agglutinated by any immune serum even in 1:1 dilution.

CONCLUSION.

We conclude therefore that this series of eleven lactose-negative organisms of the Friedländer type, grouped together by Perkins¹⁹ on the basis of fermentation reactions represents a single biological group. It can be distinguished from *Bacillus aerogenes* and other similar bacilli by cultural, fermentative, and serological reactions. There appears to be a close analogy between this group and *Pneumococcus mucosus* in the possession of a fixed cultural type, and the behavior toward immune serum. Both represent apparently a single biological group. Unfortunately no immune sera have been developed against the two strains that grew in moist and dry phases; it is possible that with immune sera for these light could be thrown on the relation suggested by Fitzgerald²⁰ that the capsulated bacilli represent a parasitic development of the *Bacillus coli* group.

I wish to express my indebtedness to Professor Zinsser for his suggestions, and my appreciation of the interest and advice of Professor J. Gardner Hopkins, both of the Department of Bacteriology of the College of Physicians and Surgeons of Columbia University.

¹⁹ Perkins, R. G., *J. Infect. Dis.*, 1904, i, 241.

²⁰ Fitzgerald, J. G., *J. Infect. Dis.*, 1914, xv, 268.