

THE DIFFERENTIATION OF THE PARATYPHOID-ENTERITIDIS GROUP

VII. IRREGULAR AND VARIABLE STRAINS

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While the majority of the strains of the paratyphoid-enteritidis group that I have had under observation during the past six years have given, whenever tested, uniform cultural and agglutination reactions, others have shown considerable irregularity and variability. Since these observations may throw light on the interrelationships of the subgroups and help to explain the frequent discordant statements in the literature, the history of some of these variable strains is here recorded.

No. 10.—This culture came to me originally (in 1913) from the collection of the American Museum of Natural History where it had been sent from the Hygienic Laboratory of the U. S. Public Health Service in Washington, D. C. It was isolated from human blood in a case of paratyphoid fever and was considered a para B type. When it was first received by me it gave irregular cultural and agglutinative reactions. Sometimes dulcitol was fermented, sometimes not; sometimes milk would remain acid for a week or more and sometimes it would turn alkaline within a few days. In other respects its reactions were inconstant. On Nov. 3, 1913, it failed to agglutinate in a 1:250 dilution with a para B serum (strain 2, titer 1:4,000) while on April 26, 1914, a positive agglutination of 1:1,000 was observed with fresh serum from the same rabbit (strain 2, titer 1:4,000). The culture was plated on plain agar and on endo medium and the colonies were apparently all alike. Twelve colonies were picked from Endo medium to plain agar slants and from these in 24 hours inoculated into litmus milk. Four strains produced alkali (like typical para B) within 4 days, the other 8 at 4 days were more acid than the control. One of the acid cultures (5) began to change about this time and in 7 days was more alkaline than the control. In 14 days one other (10-9) had become alkaline. In 18 days the remaining 6 acid strains had begun to change and were like the control and at the end of 31 days all were more alkaline than the control.

Two of the strains showing early alkalinity (10-8, 10-11) did not ferment dulcitol, but two of the acid strains (10-3, 10-6) produced acid and gas in 3 days (not in 48 hours). The latter in their slow attack of dulcitol as their behavior in milk, resemble the typical paratyphoid strains.

One of the strains producing early alkalinity in milk (10-10) was selected for the stock culture of this organism and transferred in the usual way with the other cultures. After two years it was plated and twelve colonies picked

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from the plate. All were alike in their behavior toward milk (early alkalinity), xylose (acid, but no gas) and galactose (acid, but no gas), thus showing that the strain had bred true and possessed stable characters.

TABLE 1
SHOWING CHARACTERS OF 12 STRAINS PICKED AT RANDOM FROM PLATINGS OF
TEST-TUBE CULTURE

| | Milk | | | | Dulcitol* | | Xylose* | | Sorbitol* | | Galactose* | | Agglutination† | |
|-------|--------|--------|---------|---------|-----------|---------|----------|---------|-----------|---------|------------|---------|----------------|------------|
| | 4 Days | 7 Days | 14 Days | 31 Days | 24 Hours | 10 Days | 24 Hours | 10 Days | 24 Hours | 10 Days | 24 Hours | 10 Days | Para B (12) | Para A (4) |
| 10-1 | a | a | a | alk | 0 | a+ | 0 | 0 | +a | +a | +a | +a | 0 | + |
| 10-2 | a | a | a | alk | 0 | a+ | 0 | 0 | +a | +a | +a | +a | ? | ? |
| 10-3 | a | a | a | alk | 0 | a | 0 | 0 | +a | +a | 0 | +a | 0 | + |
| 10-4 | a | a | a | alk | 0 | a+ | 0 | 0 | +a | +a | +a | +a | 0 | + |
| 10-5 | a | alk | alk | alk | 0 | 0 | a | a | a | a | a | a | + | 0 |
| 10-6 | a | a | a | alk | 0 | a+ | 0 | 0 | +a | +a | +a | +a | 0 | + |
| 10-7 | a | a | a | alk | 0 | a+ | 0 | 0 | +a | +a | +a | +a | 0 | + |
| 10-8 | alk | alk | alk | alk | 0 | 0 | a | a | a | a | a | a | + | 0 |
| 10-9 | a | a | alk | alk | 0 | a | 0 | 0 | +a | +a | +a | +a | 0 | + |
| 10-10 | alk | alk | alk | alk | 0 | 0 | a | a | a | a | a | a | + | 0 |
| 10-11 | alk | alk | alk | alk | 0 | a | a | a | a | a | a | a | + | 0 |
| 10-12 | alk | alk | alk | alk | 0 | 0 | a | a | a | a | a | a | ? | ? |

* Gas production is indicated by the + sign.

† To save space only the final results are given here, although all strains were tested in various dilutions from 1:250 up to the titer limit (paratyphoid B 12 = 1:2,000; paratyphoid A 4 = 1:5,000). Only perfectly definite agglutination affinities are recorded; two strains, 10-2 and 10-12, gave doubtful results. The strain 10-5 showed the most unmistakable relation to the paratyphoid B type, but in no instance was the agglutination reaction as marked as with the homologous strains or with other strains belonging definitely to paratyphoid B or paratyphoid A groups.

No. 66.—This culture was received in October, 1913, from Dr. C. J. Hunt, labeled "B paratyphoid B. Stock strain from University of Pennsylvania, obtained by them from Cushing, Boston." From this notation it was thought possibly to be the strain isolated by Cushing in 1900 from a rib abscess and designated by him "Bacillus O."¹ It cannot, however, be definitely determined that this is the original Cushing strain. Cultural descendants of bacillus O seem to have given aberrant results. At the time when it was first isolated it produced alkali in milk rather slowly (not until after 8 days) and was agglutinated by a "hog cholera" serum. Buxton² a little later noted that bacillus O turns litmus milk faintly blue in 10 days and remarks that this strain is "somewhat erratic." Proescher and Roddy,³ working with what was apparently this same strain some years afterward, state that "the results obtained by Cushing are remarkable in that we find it impossible to repeat them. A complete examination of this bacillus made with the greatest of care, and employing all the known means of identification, described in another part of this work, prove the organism isolated by Cushing, and named 'bacillus O,' to be a typical paratyphoid A bacillus; whereas his result would put it in the paratyphoid B group."

In my hands this culture, No. 66, has given results during a period of six years that place it definitely with the suipestifer group. The milk reaction is of the unmistakable para B suipestifer-enteritidis type. Dulcitol fermentation is either absent or much delayed. Eight out of 30 colonies picked from agar plates to agar slants and after 24 hours' incubation inoculated into dulcitol

¹ Bull. Johns Hopkins Hosp., 1900, 11, p. 156.

² Jour. Med. Research, 1902, 3, p. 201.

³ Archives of Int. Med., 1910, 5, p. 263.

all gave gas and acid in 15 days, but none within 48 hours. The remaining 22 were negative in 15 days. Only one of the 30 had produced gas in arabinose at the end of 15 days. All the agglutination tests have indicated a relationship to the *suipestifer* type. The following example will suffice.

TABLE 2
SERUM PARATYPHOID B 12 ABSORBED WITH *B. SUIPESTIFER* 167

| Strain | 250 | 500 | 1,000 | 2,000 | 5,000 | 10,000 | 20,000 |
|----------------------------|-----|-----|-------|-------|-------|--------|--------|
| 12 before absorption..... | +++ | +++ | +++ | +++ | ++ | ++ | + |
| 12 after absorption..... | ++ | ++ | ++ | + | + | tr | 0 |
| 202 before absorption..... | +++ | ++ | ++ | ++ | + | tr | 0 |
| 202 after absorption..... | ++ | ++ | + | tr | tr | 0 | 0 |
| 66 before absorption..... | +++ | ++ | ++ | + | tr | 0 | 0 |
| 66 after absorption..... | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Other absorption experiments made at different times have given similar results. Whatever the original source and history of this culture, therefore, there is no doubt that it is a *suipestifer* and not a paratyphosus B type. In the absence of a definite pedigree history, however, it is not absolutely certain that No. 66 is a descendent of Cushing's bacillus O isolated in 1900.

No. 47.—This strain, originally isolated in 1911 from the feces of a water-borne case of paratyphoid,⁴ gave constant reactions for some time after it was first received (1913), but in about a year began to show some irregularity in dulcitol fermentation. On plating, two types from apparently similar Endo medium colonies were found, one fermenting dulcitol rapidly in 24 hours, the other not fermenting until after 4-5 days and in some instances not within 15 days. The slow dulcitol fermenters also attacked arabinose tardily or not at all. Agglutinative as well as cultural differences were observed.

TABLE 3
AGGLUTINATIONS OF TWO TYPES OF STRAIN 47 WITH TYPICAL PARATYPHOSUS B (12) SERUM

| | 250 | 500 | 1,000 | 2,000 | 5,000 | 10,000 | 20,000 |
|--|-----|-----|-------|-------|-------|--------|--------|
| 12..... | +++ | ++ | ++ | ++ | ++ | + | 0 |
| 47 ₁ (fails to ferment dulcitol within 15 days)..... | +++ | ++ | ++ | + | tr | 0 | 0 |
| 47 ₂ (ferments dulcitol with gas production within 24 hours)..... | +++ | +++ | +++ | ++ | ++ | + | tr |

The rapid dulcitol fermenters agglutinated with paratyphosus B serum in higher dilutions than the nonfermenters. This suggests a splitting off from strain No. 47 of a type akin to *B. suipestifer*. The rapid dulcitol fermenters bred true and showed stable characters (100 colonies) 4 years later.

Nos. 169 and 175.—These two strains were isolated in 1915 from the lungs of two "virus hogs" at the hog cholera serum plant, Ames, Iowa. Both when first received showed the fermentative and agglutinative characters of what I have called the *B. suipestifer* type.⁴ The change that occurred in No. 169 has been previously described.¹ I have only to add that a few months later No. 175 altered in exactly the same way so that now both cultures possess the characteristics of the paratyphosus B type.

⁴ Jour. Infect. Dis., 1917, 20, p. 457.

No. 179.—When first isolated by Robinson,⁵ this strain was recorded as failing to ferment dulcitol and in giving permanent acidity in litmus milk. Since coming into my hands it has produced acid and gas in dulcitol within 24 hours and has turned litmus milk alkaline in five days. No variation has been noticed in two years.

No. 134.—This is the atypical strain isolated by Dorset in 1899 from the spleen of a pig⁶ dead of acute hog cholera. It was kindly sent to me in May, 1914, by Dr. Dorset with the memorandum that it had "recently been tested out on the various laboratory mediums and found to have the same characteristics described in the enclosed reprint." It has shown no deviation from the original culture since coming into my hands. In addition to the fermentation reactions recorded by Dorset (glucose = acid, no gas, lactose = 0, saccharose = 0), I have found that the other carbohydrate reactions correspond exactly to those of the typical *supestifer*⁴ save that in no case is gas produced (sorbitol, maltose, mannitol, galactose, xylose, rhamnose = acid; salicin = 0). Neither dulcitol nor arabinose is attacked within 15 days. Its agglutinative reactions are typically those of *B. supestifer* as determined by the absorption test.⁴ This organism seems to be essentially similar to the nongas-producing strain of the hog cholera bacillus isolated by Tenbroeck from an old laboratory culture.⁷

No. 205.—This strain was kindly sent me in June, 1916, by Dr. J. G. Cumming labeled "*B. enteritidis* VII. Isolated Dec., 1914, by S. G. from stool of calf during epidemic of diarrhea in the dairy; agglutination 1:10,000. Typical sugar reactions." The organism has been typical in all respects save that arabinose is usually not fermented at all and dulcitol is attacked very slowly; in this respect it differs from all the other *B. enteritidis* strains I have had under observation and bears the same relation culturally to them that *B. supestifer* bears to *B. paratyphoid* B. Its agglutinative reactions, however, show no divergence whatever from the other members of the *B. enteritidis* group.

No. 48.—This culture was originally isolated from feces and was received by me in 1913. It was sent as the A type and its cultural characteristics relate it definitely to this group; it has been so described in an earlier paper.⁴ Xylose is not fermented, dulcitol fermentation is slow and arabinose rapid. It is agglutinated by *B. paratyphosus* A serum in dilution practically to the titer limit. It is, however, also agglutinated with certain *B. paratyphosus* B serum (from three out of four rabbits tested) to the titer limit. This does not seem to be due to an admixture or splitting off of types, such as observed in No. 10, but rather to a mingling of common agglutinating affinities in the same cell. Twenty-five colonies picked from a plating of this culture showed no deviation in their characters.

Nos. 62, 115, 161, 169, 175.—The origin and history of these strains have been given in an earlier article.⁴ The five strains are all of porcine origin and some of these have been carried in laboratory collections under the name of hog cholera bacillus. As I have shown previously, however, they possess the chief characteristics, both cultural and agglutinative, of the *B. paratyphosus* B type. Similar strains from hog cholera cases have since been described by Krumwiede, Kohn and Valentine,⁸ and by Tenbroeck.⁹ This interesting group of organisms, although resembling very closely the *paratyphosus* B type of

⁵ *Ibid.*, 1915, 16, p. 448.

⁶ Eighteenth Ann. Rept., Bureau of Animal Industry, 1901, p. 566.

⁷ *Jour. Exper. Med.*, 1916, 24, p. 213.

⁸ *Jour. Med. Research*, 1918, 33, p. 89.

⁹ *J. Exper. Med.*, 1918, 28, p. 759.

human origin, differs in some respects. I have elsewhere¹⁰ pointed out their different behaviors in lead acetate agar, and Krumwiede, Kohn and Valentine have called attention to some agglutination differences in the strains they studied. Tenbroeck found that in agglutination experiments the type of clumps formed is different, and that when injected into rabbits such strains produce an immunity to the hog cholera bacillus while *B. paratyphosus* does not.

The existence of this subdivision of the *B. paratyphosus* B type, which is of porcine origin and unmistakable relationship to *B. suis*, adds to the complicating difficulties within the group and has probably been responsible for much of the classificatory confusion in which the paratyphoid-enteritidis group has been so long involved.

A particularly large number of variations and irregularities have been reported for the paratyphoid-enteritidis group. Anaerogenic strains comparable to No. 134 have been found belonging both to the *B. paratyphosus* B¹¹ and to the *B. suis* types. Some of these have been isolated from animal bodies, but others like the strain of *B. suis* isolated by Tenbroeck⁷ and that of *B. paratyphosus* B, isolated by Loewenthal and Seligmann¹³ have apparently developed out of gas-producing strains grown in culture mediums in the laboratory. One member of the group, *B. sanguinarum*, which is found not uncommonly in epidemics among barnyard fowls (fowl typhoid), ferments characteristically a number of carbohydrates, but in no instance produces gas.¹⁴ Another avian paratyphoid bacillus, *B. pullorum*, typically produces gas in glucose broth, but anaerogenic strains of this organism have been observed by Rettger and Koser,¹⁴ Smith and Tenbroeck,¹⁴ and Mulsow.¹⁴ Anaerogenic strains themselves sometimes show variation. The strain of *B. pullorum* received from Smith and Tenbroeck by Krumwiede and Kohn¹⁵ resumed or acquired the ability to ferment glucose. As a rule, however, such variation as has been observed consists in the loss of gas-producing power originally present. No gas-producing strain of *B. typhosus* or of *B. sanguinarum* has to my knowledge ever been reported. Acid production in glucose broth is shown without exception by all members of the group.

¹⁰ Jour. Infect. Dis., 1917, 21, p. 571.

¹¹ Oette, E.: Centralbl. f. Bacteriol., I, O., 1913, 68, p. 1; Wagner, G.: Ibid., 1913, 71, p. 25; Ohno, K.: Ibid., 1914-15, 75, p. 288.

¹² Preisz, H.: Cited by Tenbroeck, Jour. Exper. Med., 1916, 24, p. 213; Bock, F.: Arb. a. d. k. Ges., 1906, 24, p. 238; Graber, K.: Cited by Tenbroeck (Footnotes 7 and 9); Bainbridge, F. A.: Jour. Pathol. and Bacteriol., 1909, 13, p. 443.

¹³ Loewenthal and Seligmann: Berl. klin. Wchnschr., 1913, 50, p. 250.

¹⁴ Moore: 12th and 13th Annual Reports, Bur. Animal Ind., U. S. Dept. of Agr., 1895; Rettger: Jour. Med. Research, 1908, 18, p. 227; Rettger and Koser: Ibid., 1917, 35, p. 443; Smith and Tenbroeck: Ibid., 1915, 31, p. 503; Krumwiede and Kohn: Ibid., 1917, 36, p. 509; Mulsow: Jour. Infect. Dis., 1919, 25, p. 135.

¹⁵ Jour. Med. Research, 1917, 36, p. 515.

The fermentation of carbohydrates other than glucose is apparently less profoundly related to fundamental or specific qualities. At all events a very wide range both of variation and of variability exists with respect to the ability to attack certain carbohydrates. In addition to the strains described in this paper a number of similar instances are scattered throughout the literature. While a certain proportion of such cases are doubtless to be attributed to cultural impurity or to mistakes in labeling, there is a residuum that can hardly be explained in this way. Savage¹⁶ notes that one strain ("Hog Cholera Maryland"), which in his tests showed inability to ferment dulcitol, had previously in Morgan's hands produced acid and gas from this carbohydrate. The same strain was received by me in 1902 direct from Prof. Theobald Smith and in my hands has never fermented dulcitol. Dulcitol and arabinose are two carbohydrates that appear to be attacked with considerable irregularity. As shown by the writer in an earlier paper,⁴ some strains of porcine origin attack dulcitol, while others fail to show any acid production within 15 days. Variations in avidity for this carbohydrate were also shown by strains of the paratyphosus A type. Arabinose likewise is attacked tardily by some porcine strains, not at all by others. Changes in the fermentative power of certain strains for this carbohydrate have also been noted.

Mulsow¹⁷ has observed some particularly interesting variants with respect to maltose fermentation among strains of the two avian paratyphoid types *B. pullorum* (ordinarily not fermenting maltose) and *B. sanguinarium* (ordinarily maltose +). Sorbitol is attacked by some strains of *B. sanguinarium* and not by others. The mammalian types of paratyphoid bacilli attack both these carbohydrates with much greater constancy, and relatively few instances of variability have been reported. Grote,¹⁸ however, has recorded the appearance in a stock culture of *B. paratyphosus* of a variant unable to ferment maltose and differing also from the maltose-fermenting stock in the kind of colony formed on Drigalski medium. In the course of about six months the power of fermenting maltose was slowly acquired.

Agglutination reactions in general are subject to a considerable range of variation, and in the paratyphoid-enteritidis group especially are to be accepted only guardedly as criteria of relationship. Perhaps the most striking case of lack of correlation between agglutination

¹⁶ Report of Medical Officer, Local Gov't. Bd., London, 1909, p. 430.

¹⁷ Jour. Infect. Dis., 1919, 25, p. 135.

¹⁸ Centralbl. f. Bakteriöl., I, O., 1913, 70, p. 15.

reactions and cultural characteristics is the close agglutinative relationship of the culturally diverse avian paratyphoid bacilli and *B. typhosus*.¹⁹ Mulsow has shown further that *B. enteritidis* and *B. abortus equinus* also manifest agglutinative affinities to this group. On the other hand, as well known, *B. enteritidis* and *B. paratyphosus* B, while agglutinatively distinct, possess the closest cultural similarity. It is interesting that *B. sanguinarium* and *B. pullorum* agglutinate in about equal degree with *B. typhosus* serum, altho the slow rhamnose fermentation of *B. sanguinarium* would seem to indicate that it is more clearly related to the typhoid bacilli than is *B. pullorum*.

It is hardly possible to catalog all the variations that have been noted, especially with relation to agglutinative properties. Some observers²⁰ have recognized the existence of a "Paratyphosus C" type resembling *B. paratyphosus* B in its cultural characteristics, but differing agglutinatively. I have not been able to secure any cultures of the so-called "C" strains and hence do not know what relation they bear to the types described in the first article of this series. One of the most remarkable changes in agglutinative qualities yet observed has been described by Sobenheim and Seligman.²¹ This consisted in the singular behaviour of certain *B. paratyphosus* B strains which became inagglutinable to *B. paratyphosus* B serum, and at the same time acquired the property of being agglutinated by *B. enteritidis* serum. The serum produced, however, by inoculation of these converted strains agglutinated *B. paratyphosus* B cultures and not *B. enteritidis* strains. I have not observed this change in the cultures I have had under observation for six years.

Considering all the evidence, there seems no escape from the conclusion that variations both in nature and in artificial test-tube cultures are exceedingly common throughout the paratyphoid-enteritidis group. These variations affect agglutination and fermentation characters, as well as less fundamental qualities. At times a special tendency to variability seems to exist within the confines of a test-tube culture, and a number of varieties are split off from the parent stock. This is well illustrated by culture No. 10 described in this paper. The stimuli leading to such outbreaks of variability are at present practically

¹⁹ Smith and Tenbroeck: *Jour. Med. Research*, 1915, 31, p. 503; Rettger and Koser (Footnote 14); Krumwiede and Kohn (Footnotes 14 and 15); Mulsow (Footnote 14).

²⁰ Heinemann, W.: *Centralbl. f. Bakteriol.*, I, O., 1912, 66, p. 211; Mackie, F. P.: *Jour. Roy. Army Med. Corps*, 1919, 33, p. 154.

²¹ *Centralbl. f. Bakteriol., Beiheft*, 1911, p. 50.

unknown. The conditions under which these epidemics of variability occur suggest the possibility of conjugation phenomena rather than the direct action of environment.

As with higher forms of life, the variations do not often, if ever, overstep certain limits. The ability of the paratyphosus-enteritidis subgroup to attack rhamnose as contrasted with the lack of avidity of *B. typhosus* for this carbohydrate, the inability of the *B. paratyphosus* A strains to attack xylose, and the fundamental quality of fermenting glucose possessed by the whole coli-typhoid-paratyphoid group seem rarely subject to variation and are perhaps on this ground to be regarded as more fundamental than agglutinative reactions.