STUDIES IN BACTERIAL VARIABILITY. THE EXPERIMENTAL PRODUCTION OF A MUCOID FORM OF *B. PARATYPHOSUS* B.

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In the course of experiments which led to the appearance of what I have termed "dys-agglutinable" forms of certain bacteria (Walker, 1922), several non-motile (or only very feebly motile) dys-agglutinable strains of *B. paratyphosus* B were produced at different times. They were all derived from an ordinary "eu-agglutinable" laboratory strain of the bacillus by the method, described by me on previous occasions, of repeated subculture in media containing a proportion of specific agglutinating serum. One of these derived strains presented, in addition to its non-motility and dys-agglutinability, other novel characters of interest, particularly in regard to its growth on the surface of agar. On that medium the appearance of colonies was such as to suggest that the term Mucosus would be appropriate to describe the growth obtained. It at once recalled the description given by W. Fletcher (1920) of a mucoid form of *B. paratyphosus* B obtained by him in two cases from the stools of chronic "carriers."

The original culture from which my mucoid strain was eventually obtained had been an ordinary laboratory strain of *B. paratyphosus* B, in use in the Standards Department, and had shown on plating a mixture of the two common types of colonies described by Arkwright (1921) as "rough" and "smooth."

The dys-agglutinable strain derived from this paratyphoid bacillus by the method already mentioned was obtained in the following manner. A plating on agar, made from the eighth successive subculture in agglutinating serum diluted 1 to 4 with ordinary bouillon, at the end of four weeks from the commencement of this particular experiment, showed two very different types of colony after 24 hours' growth. The one was thin with bluish translucency, and presented the appearance of ordinary paratyphoid colonies. In hanging-drop its bacilli were actively motile. The other was thick, opaque, round, *umbilicated*, yellowish, somewhat waxy and moist looking, and its bacilli were almost, if not entirely, non-motile when examined microscopically.

One of the thick colonies was subcultivated in diluted agglutinating serum (1 to 4), and after 48 hours' incubation it was plated out on agar. The plating

showed colonies which were all of the thick, opaque, yellowish or creamy, moist, waxy-looking type. They were markedly *umbilicated*, the growth being heaped up in a thick ring round the central depression. The diameter of the ring was somewhere about two-thirds of the diameter of the colony, which extended out beyond it to a smooth, round, regular margin. A few of the bacilli showed very feeble motility, the great majority being quite non-motile. A formolised bouillon culture prepared from one of these colonies was highly dys-agglutinable, and gave no more than traces of agglutination in 1 in 50 dilution of standard paratyphoid B serum.

A subculture was again made in the diluted agglutinating serum, and plated three days later. The umbilicated type of colony had now disappeared. All the colonies which grew (about 20) were remarkably slimy both in appearance and consistency. They were semi-fluid looking, large, round, regular, thick, dome-shaped, glistening, white mucoid colonies. One of the colonies was re-plated on sloped agar tubes. In one tube, in which seven colonies were found after 24 hours' growth at 37° C., they were very large (up to $\frac{1}{4}$-inch in diameter), round and thick, white and glistening in appearance, with smooth, even margins and a heaped up surface. The peculiarities of the growth were very striking, and its mucous-like consistency was so marked that at the end of a further period of 24 hours (at room temperature) the colonies had slipped on the surface of the medium in these upright tubes, and "run" half an inch or so downwards, leaving a tail behind them, and presenting an appearance not unlike the gutterings on a candle. In stroke cultures made from these colonies the mucoid character of the growth was equally remarkable, and rapid spread took place from the line of the stroke with thick heaping up along the centre of the growth.

After some days the cultures began to look drier, and seemed to sink down and become flattened. They then presented a peculiarly shiny appearance, with a silvery metallic sheen. But in subculture the extremely mucoid character was regularly re-established for the first two or three days. Few, if any, motile bacilli could be found in hanging-drops made from these cultures. And on testing their agglutinability in formolised bouillon culture they were found to be highly dys-agglutinable, and did not show more than traces of agglutination in 1 in 25 dilution of standard paratyphoid B serum after 2 hours in the water-bath at 55° C. Later on, serum was prepared by inoculating a rabbit intravenously with 1 c.c. of formolised bouillon culture prepared from a mucoid culture which had rested in agar stab for about three months. It agglutinated the "mucoid" organism well, and to high titre (5000), producing the small, compact clumps characteristic of the agglutination of dys-agglutinable cultures. Standard paratyphoid B agglutinable culture was agglutinated only to a titre of 1 in 1000, and standard Aertryck culture only to 1 in 250.

Three months later still, a formolised bouillon culture, freshly prepared from a mucoid culture, which had been preserved on agar slope (sealed up)

during most of that period, was found partially to have regained its agglutinability by standard paratyphoid B serum, and gave its end point trace reading at a dilution of 1 in 250. The reaction was, however, still incomplete, even at 1 in 25.

It is desirable before proceeding further to adduce evidence that the cultures just described, which had undergone a good deal of manipulation, were actually cultures of *B. paratyphosus* B and not accidental contaminations. That this was so was proved by the fact that, after subcultivating the bacillus from the "mucoid" culture, which had rested in agar stab for about three months, through a succession of bouillon cultures it yielded, on plating out on agar, a *proportion* of typical colonies of *B. paratyphosus* B, whose formolised suspensions agglutinated normally with standard agglutinating serum. Subsequently, by continuing the serial subculture in bouillon, the *whole* culture was brought back (in six weeks) to the eu-agglutinable phase. A plating made at this stage showed a mixture of "rough" and "smooth" colonies of the ordinary type, along with *two* colonies which were like smooth colonies, except that they exhibited umbilication.

Bouillon cultures were made from one "rough," one "smooth" and one umbilicated colony. They were formolised and well shaken after 24 hours' growth at 37° C., and were subsequently diluted to standard opacity, and tested with standard paratyphoid B agglutinating serum. The "rough" culture grew as a deposit at the foot of the tube with clear supernatant fluid; it was largely clumped in loose aggregations before it was shaken, and its bacilli were mostly long and distinctly sluggish as regards motility. The other two cultures grew throughout the medium, producing uniform turbidity. Microscopically the "smooth" culture consisted of very short actively motile bacilli with a *very few* small clumps: whereas the culture from the umbilicated colony showed bacilli of average length, some actively motile, others stationary, and some aggregation into clumps of a size intermediate between those seen in the other two cultures. The agglutinability of all three cultures was practically identical, as shown below, where the readings were taken after two hours in the water-bath at 55° C.

Agglutination Test with Standard Paratyphoid B Serum.

Dilution 1 in—	25	50	125	250	500	Control
Culture "rough"	t	t	S	tr	0	0
„ "smooth"	t	t	S	tr	0	0
„ "umbilicated"	t	t –	S –	tr	0	0

The culture, now in a eu-agglutinable phase, gave, as it had done also at the mucoid stage, the reactions proper to *B. paratyphosus* B in lactose, glucose, maltose, milk and Dreyer and Fitzgerald's medium (neutral-red 3 per cent. lactose bouillon).

It is indubitable, therefore, that the mucoid cultures were cultures of *B. paratyphosus* B, and the chief interest of the present observations lies in the fact of this *experimental derivation* from an ordinary paratyphoid bacillus

of a mucoid form of growth, which had apparently all the characters (except active motility) of the mucoid bacillus obtained by W. Fletcher from the chronic paratyphoid B "carriers." Fletcher proved that his mucoid bacillus yielded, after growth in peptone water, colonies of ordinary paratyphoid B. But he was unable ever to "reverse the metamorphosis" by deriving the mucoid phase from an ordinary paratyphoid B colony, even when the latter had itself been thrown off from a mucoid culture.

As regards motility, Fletcher's mucoid bacillus was motile, and presented numerous very active coccoid forms, whereas my mucoid bacillus was at first non-motile. But motility returned during its passage through successive bouillon cultures, on the way to reversion, while it was still mucoid, at about the time that it began to throw off a proportion of ordinary paratyphoid B bacilli, which appeared on plating as typical paratyphoid colonies among the mucoid colonies. At about this stage it was also noted that the mucoid colonies exhibited in some cases a very distinct *nipple-like central elevation* after about two days' growth, as they began to assume a drier character. On replating from a nippled colony a culture which appeared to consist entirely of nipple-forming colonies was obtained, and was carried on through several successive platings.

Fletcher's description (1920) of his mucoid colonies was that they were "dome-shaped, circular in outline, with a regular margin and a mucoid appearance absolutely unlike a paratyphoid colony," and that "when examined by transmitted light they are very translucent, and have something the appearance of frosted glass." Further, he stated that "a fresh culture on agar is thick and slimy like mucus, but after a few days the sliminess disappears...." He found that if part of a 24 hour mucoid colony was spread with the edge of a slide and stained with dilute carbol-fuchsin the individual bacilli do not lie close side by side, but are separated from each other by a matrix in which they are embedded." In all these points my mucoid cultures agreed exactly with his.

The reversal of the difference in relative size between the mucoid bacillus and an ordinary paratyphoid bacillus when dried film staining is replaced by the "relief stain" of Benians (1916), to which Fletcher drew attention, was also noted. In the former case the mucoid bacillus appears the smaller, whereas in the latter its silhouette is much the larger. The difference is apparently due to the presence of enveloping mucoid material in the case of the latter organism which does not stain in films, but makes its presence visible in silhouettes.

Accordingly I conclude that changes in morphological and cultural characters and in agglutinability, similar to those exhibited by the mucoid bacillus found by Fletcher in two "carrier" cases, and proved by him to be paratyphoid B bacilli, *can be produced experimentally*. They were produced by growing an ordinary paratyphoid B bacillus in an environment containing about 25 per cent. of specific immune serum. The presence of this serum

was the only factor in respect of which the conditions of cultivation differed from ordinary laboratory culture of the bacillus. The changes which occurred were, therefore, presumably due to the presence of this factor. Hence it is not an improbable hypothesis that when the same modification—or metamorphosis, as Fletcher called it—occurs during the residence of the bacillus in the human body it has been brought about in a similar manner. That is to say, it may not improbably be due, in part at least, to a reaction of the bacillus to the presence of specific anti-bodies; and may to this extent represent a kind of resistance form. But that this is not the only way in which mucoid forms of intestinal bacteria may arise follows from the observation of Revis (1910) that the prolonged growth of *B. coli* in soil contaminated with human faeces and sterilised, may lead to the appearance, on plating, of large mucoid colonies.

The practical importance of the mucoid form of *B. paratyphosus* B is that in culture it *does not look at all like a paratyphoid.* No one examining platings from stools for the presence of enteric group bacilli would be in the least likely to regard large, heaped up, mucoid colonies as deserving further notice in this connection, unless attention was attracted to them for some special reason. Even then it would, as in Fletcher's study of his mucoid colonies, require a long and careful investigation to establish the true nature of the organism concerned, if its characters had not previously been made familiar. Hence the fact that the existence of mucoid forms of pathogenetic intestinal bacteria has not more frequently been recorded affords no clue to the comparative frequency of their occurrence, and yields no adequate proof of their rarity. It is a truism to say that in work of this kind, and particularly during its routine performance under pressure of time, one is not likely to discover abnormalities for which one is not already on the look out, unless some peculiar circumstance directs attention to them.

The general bearing of the foregoing observations lies in the further evidence that they supply of the extent to which pleomorphism of growth characters may manifest itself. How widely the aspect of the colonies formed on one and the same medium by one and the same bacterium at different times, or even at the same time, may differ is illustrated in the waxy-looking umbilicated colonies, the mucoid colonies, and the nippled colonies of paratyphoid B, as contrasted with the ordinary "smooth" colonies, or these again with the "rough." In the course of this work these *five* distinct types of colony were all, at one or another time, the only type present in particular platings.

SUMMARY.

1. The experimental derivation of a *mucoid* form of *B. paratyphosus* B is described.

2. This form, though at one stage non-motile, agreed closely with the "capsulated mucoid forms" of paratyphoid B, isolated by W. Fletcher from two chronic "carrier" cases.

3. The mucoid paratyphoid B was highly dys-agglutinable, and it would not on serological examination be identified as a paratyphoid B.

4. Its colonies were also entirely unlike colonies of paratyphoid B, being large, slimy, and usually dome-shaped, though at other stages of their metamorphosis they presented either an umbilication, or a nipple-like elevation in the centre.

5. The mucoid bacillus possessed the distinctive sugar reactions of paratyphoid B.

6. On suitable manipulation it reverted to the ordinary form.

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