THE BIOLOGICAL CHARACTERS OF A MUCOID VARIANT OF BACILLUS PARATYPHI FROM GUINEA PIGS.

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A mucoid type of *B. paratyphi* was encountered during the course of a spontaneous epidemic of paratyphoid in a previously normal guinea pig population. The active stage of the epidemic occupied a period of 8 weeks in the summer of 1924 and was followed by an endemic stage which has continued with sporadic deaths for 2 years. The normal, smooth type of organism was isolated from the majority of guinea pigs that succumbed to the infection during both stages. Early in the endemic stage, however, two cultures were obtained which differed from the normal in that they were distinctly mucoid and flocculated spontaneously in bouillon. The first culture, No. 1004, was isolated from the spleen of an unweaned, female guinea pig 13 days old. At autopsy the spleen was enlarged, congested, and covered with foci. A mixed growth of the smooth and mucoid types was obtained from the spleen upon plating from the original tissue culture. The second culture, No. 950, was likewise recovered from an unweaned female. The guinea pig died 6 days after birth. At autopsy a slight congestion of the lung was encountered but no typical gross lesions of paratyphoid. A pure growth of the mucoid type was obtained from the spleen.

Mucoid types of the human strains of *B. paratyphi* have been encountered and reported in the literature. Fletcher (1) isolated mucoid varieties of *B. paratyphosus* b from the feces of two chronic carriers and of *B. aertrycke* from the feces during an acute case of meat poisoning. The variants were motile, often small, coccoid bacilli which produced a mucoid substance apparently not as a true capsule and formed large, wet colonies on agar. They were agglutinated in low dilution by an antiserum specific for the normal type. One of the *B. paratyphosus* b variants did, however, absorb the agglutinins from the serum. The other did so imperfectly.

There was no absorption by the *B. aertrycke* variant from an "*aertrycke*" serum. After several daily transplants in peptone water followed by plating the mucoid varieties gave rise to varying numbers of normal colonies. The normal type could likewise be recovered from agar slants of the variant which had been held for several days. The mucoid type was never recovered from the normal, non-mucoid culture.

Thjøtta and Eide (2) isolated a mucoid variant from the urine of a chronic paratyphoid patient. The organism was small, showed few motile forms, and produced a mucoid intercellular substance. It formed large, rounded, wet colonies on agar. Its agglutinability and absorptive capacity were judged to be comparable with those of the normal type, except that the reactions were slower. The mucoid character was regarded as constant although serial plating from single colonies showed at first the production of a small number of normal colonies. The variant was slightly less virulent for mice, upon injection, but the difference was not held to be significant.

Walker (3) found that the prolonged cultivation of *B. paratyphosus* b in bouillon containing specific antiserum gave rise to the production of a mucoid type of the organism. The variant was almost completely non-motile and formed large, umbilicated, moist, waxy colonies on agar. It was agglutinated in low dilution only by *B. paratyphosus* b antiserum. An old agar culture of the variant was transplanted daily in bouillon and reverted to the normal type losing its mucoid nature and becoming motile and agglutinable.

Krumwiede, Cooper, and Provost (4) encountered mucoid variants of *B. paratyphosus* b in the feces of paratyphoid carriers. The mucoid type showed some tendency to produce smooth and rough varieties on cultivation. Absorption tests with the mucoid variant gave anomalous results. It showed little ability to absorb the agglutinins from "rough" and "smooth" antiserums. The low agglutinability and absorptive capacity indicated that the mucoid type differed antigenically from the rough and smooth types of the same strain. It was not believed that the antigenic difference was due solely to the mucoid characteristic.

In Germany considerable attention has been directed towards the appearance of so called *Schleimwall* colonies in cultures as a means of differentiation between *B. paratyphi* b Schottmüller and the meat-poisoning types, *B. enteritidis* Gaertner and Breslau. Freshly isolated cultures of the former when kept at room temperature after 24 hours of incubation at 37°C. were regularly found to form colonies which showed an outer zone or wall radially striated and slimy in nature due to the development of an intercellular material. Such colonies were supposed to be imperfectly developed with the Gaertner strains and absent with the Breslau strains. A critical review of the phenomenon together with a discussion of the rôle of chemical stimuli, such as sodium chloride and increased H ion concentration, in initiating the growth is given by Elkeles (5, 6).

The two mucoid variants under discussion were short, rather plump bacilli. With ordinary methods of staining and in wet preparations

they showed little deviation in size from the smooth type. Sometimes the latter appeared a trifle longer, more distinctly rod-shaped, while the mucoid types more closely approached a coccoid shape. Films made from an 18 hour agar growth fixed with acetic acid and stained with carbolfuchsin showed a faintly stained envelope of indefinite outline surrounding individual cells or pairs of cells. With heavier films the bacteria appeared embedded in a matrix of similarly stained material. With definitely encapsulated bacteria the acetic acid-fuchsin method showed an envelope with distinct boundary. As noted by other workers, the surrounding envelope displayed by the mucoid variants was more in the nature of a viscid intercellular substance than a true capsule. The same method applied to films made from the smooth type showed no enveloping material. Whatever the nature of the envelope it did not appear to affect materially the motility of the organisms. 6 hour bouillon cultures of the mucoid variants showed fine clumps of bacteria together with many free forms. The latter were very actively motile. The clumps also displayed considerable motility. With continued incubation the clumps increased in size and tended to become fixed. Small numbers of free forms were always observed, through 36 hours growth, and these retained their motility.

The mucoid variants gave biochemical reactions identical with those of a smooth type of the epidemic strain. The three cultures fermented dextrose, maltose, levulose, galactose, dulcitol, arabinose, mannitol, and xylose with the production of both acid and gas. Neither acid nor gas was produced from saccharose, lactose, and salicin. Hydrogen sulfide was formed in peptone media but no indole. Gelatin was not liquefied. A strong terminal alkalinity was produced in milk attended by a partial clearing of the medium which presented a slightly yellowish, semitranslucent appearance after 10 days.

The mucoid types differed markedly from the smooth type in the character of growth in bouillon and on agar. The smooth type gave rise to a heavy, even turbidity in bouillon with little tendency towards settling except upon prolonged cultivation. Sometimes a slight scum formed on the surface of the medium but a definite, complete film was never observed. With the mucoid types a spontaneous flocculation followed by sedimentation occurred in bouillon. A 48

hour culture showed a moderate to heavy sediment, a slightly turbid supernatant with floccules in suspension, and generally a distinct surface film.

On agar the smooth type formed transparent, flat, moist but non-mucoid colonies which had a bluish cast and regular border. The colonies reached a size of 4–5 mm. after several days on a thinly seeded plate and tended to become slightly convex showing a deeper colored central zone which gave them a ringed appearance. The mucoid types both formed slightly larger colonies which reached a size of 5–6 mm. after 4 days at room temperature. Sometimes the growth was stringy but more often waxy in nature forming heaped up masses when touched with a needle. The colonies usually became differentiated into a central zone which was either wrinkled with radial striations or granular, and a smooth, outer rim of varying width. At times part or all of the colonies showed only a raised, smooth, central zone. Such colonies when picked into bouillon and plated gave rise to the wrinkled and granular type as frequently as they did to the undifferentiated type of colony.

On agar slants the mucoid types produced a heavy growth which was stringy in nature. On older slants the growth became wrinkled and less definitely viscid. Saline suspensions were formed with difficulty and with the usual salt concentration spontaneous flocculation always resulted. In distilled water and in solutions of reduced salt concentration, 0.4 per cent and less, the suspensions were more stable and showed only a scant sediment upon standing.

The experience of other workers with mucoid variants of the human strains of *B. paratyphi* indicated a tendency towards reversion to the normal type upon culturing. Likewise there appeared to be a tendency for the normal strains, in old culture or under special conditions of culture, to split off mucoid types in varying numbers. Inasmuch as one of the present guinea pig variants was isolated together with the normal type of *B. paratyphi*, it was regarded of interest to determine whether a similar reversion and splitting occurred with the animal strain. The presence of the variant in the guinea pig host might be explained by such a splitting from the normal type of organism.

The two mucoid types were plated on agar from 18 hour bouillon

cultures, a single colony of each type picked into bouillon and again plated after 18 hours. The procedure was repeated through 12 generations, 6 in bouillon and 6 on agar. The bouillon tubes always showed the characteristic growth with sediment, spontaneous flocculation, and surface film. On agar there were produced only waxy or viscid colonies with or without differentiated zones. Daily bouillon cultures of the two types were made through 30 generations and plated at intervals. The results were the same. The characteristic growth was maintained in bouillon and on agar.

The intraperitoneal injection of the mucoid types in low dilution into guinea pigs resulted in death and the recovery of the organism from the visceral organs and the peritoneal exudate. The growth was typically mucoid on agar slants and transplants in broth yielded the usual flocculating growth which on plating produced only waxy or viscid colonies. Similar results were obtained with cultures isolated from rabbits and from mice which had received intraperitoneally fatal doses of the organism.

There was no indication that repeated culturing of the mucoid variants in ordinary media caused a reversion to the normal type. Nor did there appear to be any reversion attendant upon the growth of the variants under parasitic conditions in experimental animals.

Six normal strains of *B. paratyphi*, isolated from guinea pigs which had succumbed to paratyphoid, were similarly carried through 8 successive generations on media, 4 in broth and 4 on agar. The bouillon tubes invariably showed an even turbidity with no flocculation and no surface film. On agar the colonies were all of the same smooth, non-mucoid type. A single strain was carried through 30 generations in bouillon with intermittent plating for colony characteristics. A change occurred in the nature of the growth in bouillon during the series of transfers. The culture developed a surface film and showed a slight tendency towards flocculation with increased sediment. On agar, however, there was no change noted in the nature of the colonies. They were always of the smooth, non-mucoid type.

The same culture was carried through 3 generations in bouillon containing 10 per cent specific antiserum and in 5 per cent peptone water. The transfers were made every 4th day. Plates were poured

from the two kinds of media on the 4th day after the third transfer. In both instances the plates showed two types of colonies in approximately equal numbers, the typical smooth colony and a more opaque type with an irregular, waxy border. None of the colonies were mucoid. The first type produced the usual even turbidity in bouillon. The second type showed spontaneous flocculation in bouillon with sediment but no surface film.

On several occasions cultures were obtained from the spleens of guinea pigs which had received intraperitoneally non-fatal doses of the smooth type of *B. paratyphi*. The spleen, liver, and lymphoid tissue of the intestinal tract gave evidence grossly of some structural change. The blood serum showed a moderate titer of specific agglutinins. The cultures on plating yielded only the smooth type of colony.

There was no indication that the normal, smooth type of *B. paratyphi*, of the present epidemic strain, yielded mucoid variants either with prolonged cultivation in ordinary media or in special media designed to stimulate variation. Growth in the latter media did, however, give rise to a rough type of variant. Moreover the smooth type failed to show any tendency towards variation in the guinea pig under conditions favorable for variation, that is in animals which had acquired some degree of resistance.

The relationship of the two mucoid types to the smooth or normal type of *B. paratyphi* was studied with the aid of the agglutination reaction. Antiserums were prepared by intraperitoneal injection of the three cultures into rabbits. Serums of high titer were obtained. The tests were made in approximately 0.4 per cent salt solution in order to reduce spontaneous flocculation. Readings were made only after 3 hours of incubation at 37°C. With continued incubation a scant flocculation occurred in the controls of the mucoid types. Direct agglutination tests showed no difference between the two types. The limit of agglutination was the same for each culture with the three serums. No difference was noted in the character of flocculation nor in the velocity of the reactions.

Reciprocal absorption tests were employed with the three cultures and their respective serums. The technic of Krumwiede (4) was followed except that absorption was carried out in 0.4 per cent salt

TABLE I.

*Agglutinin Absorption Tests with Normal and Mucoid Type Antiserums.**

Serum	Culture	Direct agglutination	Absorbing culture	Agglutination after absorption	Culture tested after absorption
Normal 922	Normal 922	1:51,200	Normal 922	1:200	Normal 922
				1:200	Mucoid 1004
				1:200	" 950
	Mucoid 1004	1:51,200	Mucoid 1004	1:400	Normal 922
				1:400	Mucoid 1004
				1:400	" 950
	" 950	1:51,200	" 950	1:400	Normal 922
				1:400	Mucoid 1004
				1:400	" 950
Mucoid 1004	Mucoid 1004	1:51,200	Mucoid 1004	1:800	Mucoid 1004
				1:800	" 950
				1:800	Normal 922
	" 950	1:51,200	" 950	1:800	Mucoid 1004
				1:800	" 950
				1:400	Normal 922
	Normal 922	1:51,200	Normal 922	1:400	Mucoid 1004
				1:400	" 950
				1:400	Normal 922
Mucoid 950	Mucoid 950	1:51,200	Mucoid 950	1:400	Mucoid 950
				1:400	" 1004
				1:400	Normal 922
	" 1004	1:51,200	" 1004	1:400	Mucoid 950
				1:400	" 1004
				1:400	Normal 922
	Normal 922	1:51,200	Normal 922	1:400	Mucoid 950
				1:200	" 1004
				1:200	Normal 922

* The absorbing dose was 1:5 and the serum dilution 1:10 throughout.

solution and final readings made after 3 hours of incubation at 37°C. The results of the absorption tests are given in Table I. No outstanding difference in absorptive capacity was displayed by the normal

and mucoid types of *B. paratyphi.* The variants were, however, a little less efficient in the removal of agglutinins from the three serums. The variation in absorptive capacity did not appear sufficiently great to indicate an actual antigenic difference between the two types.

TABLE II.

Preliminary Virulence Test with the Normal and Mucoid Types of B. paratyphi.

Culture	Dilution	Result	Spleen culture
Normal 922	1:2,000	Died 4 days	+
	1:20,000	" 10 "	+
	1:200,000	" 16 "	+
Mucoid 1004	1:2,000	" 10 "	+
	1:20,000	Killed 21 "	−
	1:200,000	" 21 "	+
" 950	1:2,000	Died 10 "	+
	1:20,000	Killed 21 "	−
	1:200,000	" 21 "	+

TABLE III.

Final Virulence Test with the Normal and Mucoid Types of B. paratyphi.

Culture	Dilution	Result	Spleen culture
Normal 922	1:200,000	Died 5 days	+
	1:200,000	" 6 "	+
	1:200,000	" 14 "	+
	1:200,000	Killed 26 "	+
Mucoid 1004	1:200,000	" 26 "	+
	1:200,000	" 26 "	+
	1:200,000	" 26 "	+
	1:200,000	" 26 "	−
" 950	1:200,000	Died 15 "	+
	1:200,000	Killed 26 "	+
	1:200,000	" 26 "	−
	1:200,000	" 26 "	+

A comparative study of the virulence of the normal and mucoid types was made on guinea pigs. As a preliminary graded dilutions

prepared from 18 hour bouillon cultures were injected intraperitoneally into guinea pigs of approximately 350 gm. weight. Little difference was noted in the number of bacteria per cc. with the three cultures. Plate counts gave roughly 600,000,000 bacteria per cc. for each. Dilutions of 1:2,000, 1:20,000, and 1:200,000 in 1 cc. of diluent were injected. One guinea pig only was injected with each dilution. The results of the test are given in Table II.

The preliminary test indicated a considerable difference in virulence between the two types. As a final check four guinea pigs were injected intraperitoneally with 1 cc. amounts of each culture in dilutions of 1:200,000, the highest lethal dose of the normal type. The results of the test are given in Table III.

The final test did not bear out the sharp difference in virulence indicated by the preliminary series. The normal type failed to kill 100 per cent of the test animals in the dilution employed. In addition one of the mucoid types displayed lethal action in that dilution. It seems probable that a difference in virulence does exist in favor of the normal type as the higher, but definite conclusions would be warranted only with a much larger series of animals.

The susceptibility of the mucoid variants to lysis by a filtrate active for the smooth type of *B. paratyphi* was determined. The filtrate employed was obtained from the feces of a mouse infected with paratyphoid through contact with a second mouse which had received *per os* administration of *B. paratyphi*. The method of serial dilution in bouillon was used in determining the activity of the filtrate. Dilutions ranging from 10^{-1} to 10^{-12} were made in 5 cc. amounts of bouillon and 0.05 cc. of an 18 hour bouillon culture added to each tube of the series. Readings were made at the end of 3, 6, and 48 hours incubation at 37°C. The end-point was determined by filtering the three tubes of highest dilution and retesting in series. The filtrate was active for its homologous culture, *i.e.*, the smooth type of *B. paratyphi*, through a dilution of 10^{-10} or 10^{-11}. Complete lysis of the culture never occurred. After 3 hours incubation there was no visible turbidity in dilutions of 10^{-1}, 10^{-2}, and 10^{-3}; from 10^{-4} through 10^{-6} there was a graded turbidity, and from 10^{-7} through 10^{-11} the turbidity was in the same as that of the control. After 6 hours incubation there was a faint turbidity in the first three dilutions which increased

in intensity upon further incubation. Resistant and susceptible bacteria could be isolated from any of the tubes in which the filtrate was active. The filtrate was inactive for the mucoid types. There was no inhibition of growth in any dilution. After 3 hours incubation all the tubes showed a distinct, finely floccular growth identical with that of the control. Further incubation resulted only in increased growth normal in appearance for the mucoid type. Plates poured and streaked at varying intervals of growth showed only normal mucoid colonies.

DISCUSSION.

The guinea pig strain of *B. paratyphi* associated with the epidemic and regarded as the normal type appears to differ from the normal strains of human origin in that it displays a less marked tendency towards variation. Other workers have shown that the human strains commonly give rise to variants of the mucoid and rough types. Variation may occur under normal conditions of growth or it may be stimulated by prolonged cultivation and by rapid serial transfer. Alteration in the chemical nature of the medium by the addition of specific antiserum and by increased sodium chloride, peptone, or H ion concentration may also afford a stimulus to variation. The action of a specific lytic principle may likewise result in variation. The normal guinea pig strain has shown no tendency towards a mucoid variation under normal conditions of growth nor under the stimulus of specific antiserum, increased peptone concentration, or the action of a lytic principle. The latter stimuli do, however, initiate a variation resulting in the appearance of the rough type of variant which flocculates spontaneously in bouillon and is resistant to lysis.

Mucoid variants have not been recovered experimentally from the normal guinea pig strain either under parasitic or saprophytic conditions of growth. They have been recovered, however, from the guinea pig in two cases of spontaneous infection. The mucoid types have been compared with the normal strain as to biological characters. In general they display some difference in character from the mucoid types isolated by other workers from the human strains of *B. paratyphi*. The variants of the latter strains were usually found to be less agglutinable than the normal type and to show a tendency

towards reversion upon cultivation. The variants under discussion display little deviation from the normal type in agglutinability or in antigenic properties. The mucoid character of growth appears constant and no tendency towards reversion is displayed.

Experimental study has failed to demonstrate a stimulus which might account for the appearance of the mucoid types in the guinea pig host. If variation within the guinea pig organism resulting in a splitting from the normal type under the stimulus of resistance were accountable, it would seem that the mucoid types should be more frequently encountered. It is believed, rather, that the two types had a more remote, common ancestry and that the mucoid type as a variant of permanent characters was transmitted to the guinea pig population together with the normal type. Such an explanation likewise fails to account for the infrequency of its occurrence. Differences in virulence might be regarded as an associated factor.

SUMMARY.

The biological characters of two mucoid variants of *B. paratyphi* isolated from guinea pigs have been studied and compared with those of the normal type. The possible origin of the mucoid type is discussed.

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