THE PERMANENCE OF THE SEROLOGICAL PARATYPHOID B TYPES, WITH OBSERVATIONS ON THE NON-SPECIFICITY OF AGGLUTINATION WITH "ROUGH" VARIANTS.

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(With 1 Figure.)

CONTENTS.

1. Introduction.

In a previous paper (Schütze, 1920), a serological classification of strains giving the Salmonella cultural reactions was described. By segregating those showing agglutinatory relationship to one another, two groups were arrived at—the *P. enteritidis* Gärtner and the *B. paratyphosus* B. This latter includes types of diverse origin and pathogenicity, distinguishable in the laboratory by the absorption test; it embraces on the one hand the type labelled "Schottmüller," the well-known virus of paratyphoid B fever, on the other, with perhaps the least agglutinatory affinity to it of any in the group, the more recently described "Hirschfeld" type (the *B. paratyphosus* C of that author). Between these extremes, with varying degrees of agglutinatory relationship to the "Schottmüller" type, come organisms associated for the most part with food poisoning and animal epidemics, for example, the "Mutton" type and the "Hog Cholera" type. In no case can agglutination alone definitely decide to which type a particular strain belongs; only by absorption can certainty be obtained.

The degree of permanence possessed by serological bacterial types, in particular those demarcated by the finer distinctions of the absorption test, is not known.

Observations made upon the constancy of type manifested by a large number of strains during several years of laboratory cultivation are recorded

here, the species being *B. paratyphosus* B with its numerous absorption types. The results are of importance as indicative of the extent to which prototypes maintain their reliability as such.

2. "SUBSTRAIN" VARIANTS.

Criticism has been levelled at the subdivision of the paratyphoid B group by the absorption test on the ground that by choosing a different member of a serological type as prototype and working with serum derived from it, one arrives at a different arrangement of the strains within the types. But this does not appear to be the case, if notice be taken of the existence of what I have called "substrains." A substrain is one which contains less effective agglutinatory antigen than another of the same type. A substrain will agglutinate with and absorb the agglutinins from the serum of a superstrain of the same type more or less badly according to the extent to which it is deficient in effective agglutinatory antigen, but the superstrain always agglutinates to titre limit with and absorbs the agglutinins from the serum prepared from a substrain of the same type.

The most striking demonstration of a substrain is afforded by "Piper 1," a culture received from Capt. Fletcher and isolated by him from the urine of a paratyphoid case in 1917. Normal "Schottmüller" organisms were obtained simultaneously from the faeces. Recognised as atypical, the culture was sent to me for identification. On plating, the culture yielded two types of colonies, both giving the Salmonella sugar reactions; while one showed typical morphology, the other was of the kind now called "rough." With this latter, as it was a self agglutinator, little could be done. The former, "Piper 1," was found to agglutinate very poorly with and absorb not at all the Schottmüller serum

Table I.

Serum	Organism agglutinated	Titre						
"Tidy" serum:								
Unabsorbed	"Tidy"	$\frac{1}{12800}$						
,,	"Piper 1"	$\frac{1}{400}$						
Absorbed with "Piper 1" (1 slope)	"Tidy"	$\frac{1}{12800}$						
"Piper 1" serum:		$\frac{1}{100}$	$\frac{1}{200}$	$\frac{1}{400}$	$\frac{1}{800}$	$\frac{1}{1600}$	$\frac{1}{3200}$	Control
Unabsorbed	"Piper 1"	+ + +	+ + +	+ +	+ +	+	–	–
,,	"Tidy"	+ + +	+ + +	+ + +	+ +	+	–	–
Absorbed with "Piper 1" ($\frac{1}{10}$ slope)	"Piper 1"	–	–	–	–	–	–	–
,, ,, ($\frac{1}{20}$,,)	,,	tr	–	–	–	–	–	–
,, ,, ($\frac{1}{40}$,,)	,,	+ +	+	–	–	–	–	–
,, "Tidy" ($\frac{1}{10}$,,)		–	–	–	–	–	–	–
,, ,, ($\frac{1}{20}$,,)		+	–	–	–	–	–	–
,, ,, ($\frac{1}{40}$,,)		+ +	+ +	+	–	–	–	–

Absorption took place by adding the amounts of culture indicated to 1 c.c. of serum, diluted in the case of "Tidy" serum to $\frac{1}{100}$ and in the case of "Piper 1" serum to $\frac{1}{50}$, and incubating for 1 hour.

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"Tidy" (see Table I). It could not on absorptive or even agglutinatory grounds be regarded as of that type. As it conformed no better to any of the other paratyphoid B types, it was decided to carry out the so-called "mirror" test, *i.e.* to prepare a serum from the strain in question and absorb it with the various type strains. The rabbit yielded a serum with titre no higher than $\frac{1}{1600}$ and further inoculation failed to raise it. It will be seen from Table I that this lowness of titre was not due to any inagglutinability on the part of "Piper 1" itself; there was no better agglutination with the typical Schottmüller strain "Tidy." It will be seen too that absorption of the specific agglutinins is performed equally well by both "Piper 1" and "Tidy."

It would seem as if the antigen mosaic were defective in the case of "Piper 1," only a very small portion of that contained in a typical strain like "Tidy" being present or at any rate capable of engaging in the processes of agglutination, absorption and agglutinogenesis. The effective portion has, however, its counterpart in the more complete mosaic of the typical "Schottmüller" strain. "Piper 1" was therefore called a substrain of the "Schottmüller" type.

But further absorptions of "Piper 1" serum with the other paratyphoid B types proved that the classification of "Piper 1" was not so simple, for of the ten absorption types two besides "Schottmüller" were capable of absorbing the specific agglutinins from "Piper 1" serum, viz. the "Mutton" and the "Stanley" types.

Here, then, were three paratyphoid B types closely related agglutinatorily yet quite distinct absorptively, all of which completely absorb "Piper 1" serum. "Piper 1" is therefore a substrain to all three equally. The agglutinatory antigen in "Piper 1" is apparently so limited in amount that it merely represents that antigen or a portion of that antigen which, in their more complex mosaics, "Schottmüller," "Mutton" and "Stanley" types have in common and by virtue of which they display their agglutinatory relationship. "Piper 1" is, as it were, a common denominator of the three types in question.

Five other strains, resembling "Piper 1" in all respects, have been encountered, but none of them with a history indicating that it was isolated as such; one indeed ("Shanks") when received in 1916 was absorptively a normal "Schottmüller," when retested in 1919 it was seen to have degenerated into a substrain similar to "Piper 1." Two others, "Lister" and "Edinburgh," obtained from Prof. Stenhouse Williams and one old laboratory culture, "Wassermann," had no history, but presumably they had not been regarded as coinciding with paratyphoid B "Schottmüller," for they all three had "Suipestifer" prefixed to their names. The fifth is described later. What clinical significance is betokened by the fact of an organism proving to be a substrain, it is impossible to say. It may be that some of the cultures isolated from time to time and termed inagglutinable paratyphoids are members of this antigenically very depleted group.

Not all substrains differ from the typical so markedly as these six. Simply

by making single cell cultures from a normal "Schottmüller," it was possible to separate out strains (Tidy A and Tidy B) that were in slight and varying degrees substrains to the original culture (Tidy).

Table II shows a series of absorptions demonstrating incompleteness when substrain acts on superserum and completeness when matters are reversed.

Table II.

Serum	Organism agglutinated	Titre $\frac{1}{200}$	$\frac{1}{400}$	$\frac{1}{800}$	$\frac{1}{1600}$	$\frac{1}{3200}$	$\frac{1}{6400}$	$\frac{1}{12800}$	Control
"Tidy" serum:									
Unabsorbed	"Tidy"	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	+ +	−
Absorbed with "Tidy"	,,	−	−	−	−	−	−	−	−
,, "Tidy A"	,,	+ +	+ +	+ +	+ +	tr	−	−	−
,, "Tidy B"	,,	+ +	+ +	+ +	+ +	tr	−	−	−
'Tidy A" serum:									
Unabsorbed	"Tidy A"	+ + +	+ + +	+ + +	+ + +	+ +	+	−	−
Absorbed with "Tidy"	,,	−	−	−	−	−	−	−	−
,, "Tidy A"	,,	−	−	−	−	−	−	−	−
,, "Tidy B"	,,	+ +	+ +	+	tr	−	−	−	−
'Tidy B" serum:									
Unabsorbed	"Tidy B"	+ + +	+ + +	+ + +	+ + +	+ + +	+	−	−
Absorbed with "Tidy"	,,	−	−	−	−	−	−	−	−
,, "Tidy A"	,,	−	−	−	−	−	−	−	−
,, "Tidy B"	,,	−	−	−	−	−	−	−	−

Only the strain "Tidy" with complete antigenic mosaic can absorb from all three sera. The first substrain "Tidy A" can do so from its own and the serum beneath it in the scale, but fails to absorb from its superserum "Tidy," while the second substrain "Tidy B" cannot do so from either of its supersera "Tidy" or "Tidy A." The incompleteness of absorption is not so marked in these cases as it is where "Piper 1" is concerned nor is their antigen so reduced that the heterologous types "Mutton" and "Stanley" contain their counterparts and are thus capable of absorbing their sera. As both "Tidy A" and "Tidy B" can absorb "Piper 1" sera, though the reverse does not occur, the relationship which the strains and types bear to one another may be illustrated as in Fig. 1 (p. 334).

Another instance of prolonged cultivation producing a substrain is afforded by the single cell culture "Tidy A" which, together with the single cell culture "Tidy B," was, as a test of the permanence of their substrain characters, daily subcultured from broth to broth. At the end of about two months "Tidy A" no longer belonged to substrain I but, like the five other cultures already mentioned, to substrain III; it was also found to contain the "rough" variant. "Tidy B" subjected to about half that amount of subculturing had, however, undergone no change.

It has been observed that small changes of position in the scale of antigenic activity, as indicated by the absorption test, often occur. Only twice has a big alteration been recorded, in both cases a descent in the scale; "Shanks" with infrequent agar subculturing during the course of three years and the single cell culture "Tidy A" after 71 broth subculturings had both lost all

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effective agglutinatory antigen beyond what is found in members of substrain III.

With most variations from the normal, diagnosis is easily accomplished by the mirror test. When a culture has been debased to as low a level as that of substrain III in the diagram, it is not possible to allocate the culture to one particular type; *e.g.* "Piper 1" may equally well be a degraded strain of any one of the three types, "Schottmüller," "Mutton" or "Stanley." Another feature common to substrains is the difficulty experienced in making with them agglutinating sera the titres of which in any way equal those easily arrived at with full normal strains.

After intravenous inoculation with heat-killed saline emulsions (doses of 500 million, 2000 million and 6000 million with six to seven days interval)

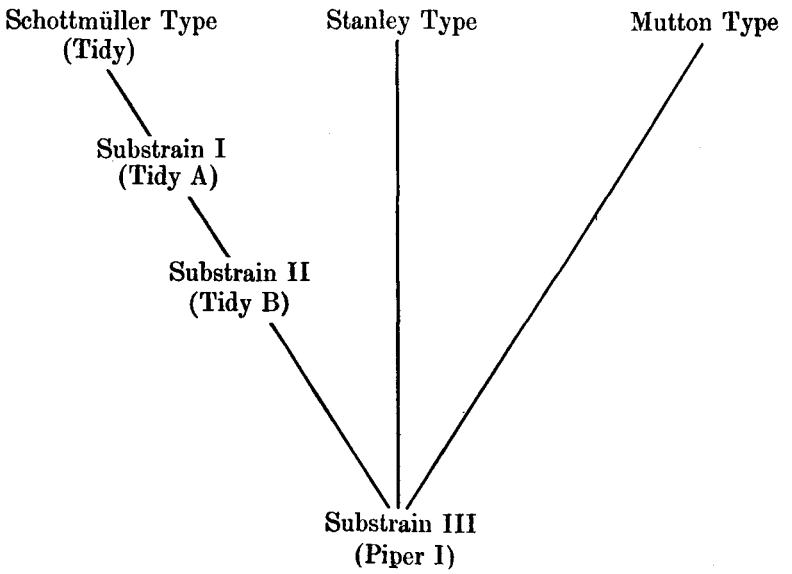

Fig. 1.

rabbits when they receive superstrains yield titres of $\frac{1}{6000}$ to $\frac{1}{12000}$, whereas when inoculated with substrains, they show titres of round about $\frac{1}{1600}$ and further and larger inoculations have little or no effect in raising the potency of the sera. The substrains are thus obviously less effective from an agglutinogenic point of view also.

In the estimation of the agglutinin content of a serum, such markedly substrain cultures as "Piper 1" are likely to make difficulties. In titrating a superstrain serum with a substrain emulsion, for example, one would naturally have to allow for its poor agglutinability, but if it is a serum prepared from a substrain similar to the one being measured, the substrain will register the titre to the full and the employment of any factor arrived at in work done with a superserum would lead one to erroneous results.

When the existence of these substrains is taken into account, the choosing of a different member of the same type as a prototype does not lead to an altered classification.

During the course of this work, it has been seen that even those strains whose serology was registered so long as six years ago, still maintain their places in the same absorptive types. Serologically no alteration except in "altitude" within the type has been recorded after laboratory cultivation extending over that period.

3. "ROUGH" VARIANTS.

There is one other variation that can and does take place during the conservation of cultures, and that is the one recently investigated by Arkwright (1921). The variant has been termed by him a "rough" and it differs from the parent form mainly in the morphology of its colonies, in its stability in saline emulsion, in its appearance in broth culture and in its serological character.

The variation from the normal in the case of the agar grown colonies may be anything from a mere occasional indentation in the edge of an otherwise normal colony to so marked a roughening and flattening of the surface that the growth resembles that of a spore-bearer on potato. In broth culture every stage is seen between a variant that sediments so completely as to leave an absolutely clear supernatant and one that shows but the slightest trace of abnormal precipitation at the bottom of a normally turbid broth culture.

Variations in colonial form and in saline stability do not go hand in hand. The variant giving the roughest colony is not necessarily the most saline unstable and vice versa.

4. NON-SPECIFIC AGGLUTINATION BETWEEN "ROUGH" ALIEN STRAINS.

The other variation already referred to, is a serological one. To a greater or less extent both agglutinatory and absorptive relationship to the original culture is lost and, judged on these grounds, the variant could in some cases be regarded as a new type. The degree of alteration in serological character varies independently of the amount of colonial "roughness" and saline stability possessed by the variant. Although little or no agglutination may take place with a rough strain and its homologous smooth serum, the variant is not inagglutinable, it will respond well to a serum that has been prepared from a rough strain. And, what is very remarkable, is that the rough strains possess the power of agglutinating to a considerable extent with the sera of quite alien species when those sera have been made from rough strains. There exists a serological cosmopolitanism among rough cultures. Thus, for example, rough variants of "Gärtner," paratyphoid A and typhoid strains will agglutinate, sometimes to titre limit, with rough sera of the paratyphoid B group, while the smooth prototypes from which they have been derived, remain quite unaffected. Table III gives the titres of several smooth and rough strains for three rough alien sera.

Table III.

Smooth and rough organisms of species alien to the agglutinating sera		Rough paratyphoid B sera		
		"Schottmüller" type Titre = $\frac{1}{3200}$	"Hirschfeld" type Titre = $\frac{1}{3200}$	"Hog Cholera" type Titre = $\frac{1}{6400}$
Gärtner, "D. H. Bainbridge"	Smooth	$<\frac{1}{100}$	$<\frac{1}{100}$	$<\frac{1}{100}$
„ „	Rough	$\frac{1}{1600}$	$\frac{1}{3200}$	$\frac{1}{6400}$
Paratyphoid A, "S. O."	Smooth	$<\frac{1}{100}$	$<\frac{1}{100}$	$<\frac{1}{100}$
„ „	Rough	$\frac{1}{3200}$	$\frac{1}{3200}$	$\frac{1}{3200}$
Shiga, "550"	Smooth	$<\frac{1}{100}$	$<\frac{1}{100}$	$<\frac{1}{100}$
„ „	Rough	$\frac{1}{1600}$	$\frac{1}{1600}$	$\frac{1}{1600}$
Typhoid, "Howard"	Smooth	$<\frac{1}{100}$	$<\frac{1}{100}$	$<\frac{1}{100}$
„ "Guy"	Rough	$\frac{1}{1600}$	$\frac{1}{400}$	$\frac{1}{800}$

It is seen that while the rough strains disclose an affinity for rough alien sera, the smooth strains fail to do so. That it was not a question of agglutination by serum *per se* whether immune or normal, was demonstrated by controls in which the rough strains remained unaffected. Indeed, for these rough strains serum has in certain concentrations an anti-agglutinatory effect and this may be the reason for the frequency with which inhibition zones are met with when agglutinating rough strains.

It would seem then, as if there were genuine affinity between rough strains as such, but the relationship is not closer than that implied by the agglutination test. By absorption even the more closely related of the heterologous rough strains can be differentiated and the homologous ones identified, just as is possible with smooth strains.

5. The serological diagnosis of "rough" strains.

In Table V are recorded absorptions carried out with a rough "Hog Cholera" serum. "Arkansas," like the strain labelled "Swine Fever," which, similar in every respect, was obtained from the Royal Veterinary College, is an old laboratory culture, and both have gone rough in the course of years of cultivation. To compare the identity of these cultures with more recently isolated and still smooth strains such as the "Hog Cholera XII" of Tenbroeck was impossible with the organisms in their respective states of roughness and smoothness. Table IV indicates what lack of reciprocity there is.

As it is apparently impossible to reconvert a rough into a smooth, Tenbroeck's culture was rendered rough by inoculating from broth to broth; after 7 days at 37° C. the second broth in the series showed signs of abnormal sedimentation and agar plating yielded the rough variant. Absorption of the

rough "Arkansas" serum was then quantitatively carried out with this rough "Hog Cholera" variant as well as with "Arkansas" itself and a rough variant

Table IV.

Serum	Organism agglutinated	Titre								Control
		$\frac{1}{200}$	$\frac{1}{400}$	$\frac{1}{800}$	$\frac{1}{1600}$	$\frac{1}{3200}$	$\frac{1}{6400}$	$\frac{1}{12800}$		
"Arkansas" (rough) serum: Unabsorbed	"Arkansas" (rough)	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	+		–
"	"Hog Cholera XII" (smooth)	+ +	+ +	+ +	+ +	+	–	–		–
Absorbed with "Hog Cholera XII" (smooth)	"Arkansas" (rough)	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	+		–

Table V.

Serum	Organism agglutinated	Titre								Control
		$\frac{1}{200}$	$\frac{1}{400}$	$\frac{1}{800}$	$\frac{1}{1600}$	$\frac{1}{3200}$	$\frac{1}{6400}$	$\frac{1}{12800}$		
"Arkansas" (rough) serum: Unabsorbed	"Arkansas" (rough)	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	+		–
Absorbed with "Arkansas," rough (½ slope)	"	–	–	–	–	–	–	–		–
" " (¼ slope)	"	tr	–	–	–	–	–	–		–
" " (⅛ slope)	"	+ +	+ +	+	–	–	–	–		–
" "Hog Cholera XII," rough variant (½ slope)	"	–	–	–	–	–	–	–		–
" " (¼ slope)	"	+	–	–	–	–	–	–		–
" " (⅛ slope)	"	+ +	+ +	tr	–	–	–	–		–
" "Hirschfeld," rough variant (1 slope)	"	+ +	+ +	+ +	+ +	+ +	+ +	+		–

of the so closely related "Hirschfeld" (paratyphoid C) type which, indeed, Tenbroeck (1920) considers to be serologically identical with "Hog Cholera."

The rough "Hog Cholera" variant absorbs as well as the homologous

strain and it is therefore justifiable to regard "Arkansas" as a rough variant of the "Hog Cholera" type. The rough "Hirschfeld" variant, on the other hand, discloses a relationship to, but no identity with "Arkansas." And as the mirror test proved that it was not a substrain, a similar close relationship but lack of identity has been demonstrated between the rough variants of the "Hog Cholera" and "Hirschfeld" types, just as had previously been shown to exist between their smooth forms.

Many of the old standard laboratory cultures are found to have become rough during their years of conservation. Though a cultural diagnosis remains possible, their serological characters are obscure unless one takes into account the fact that they are variants from the normal. They are to be investigated, not so much by agglutination, which it has been seen, may be misleading, but by absorption, a test which apparently remains specific. The diagnosis of the rough strain "Arkansas," as here described, indicates in what manner the absorption test is to be performed. Given an unknown "rough" culture, one would prepare from it an agglutinating serum and then proceed to establish which of the type strains (in the form of their rough variants, of course) displayed affinity by completely absorbing the specific agglutinins from the serum.

6. The diagnosis of "rough" strains by their growth inhibitions.

There is one other method that may help in the typing of such rough strains and that is the investigation of their growth inhibitions. Attention has recently been recalled to this phenomenon in a paper by McLeod and Govenlock (1921). But here the old Eijkman (1904) procedure of direct inoculation of one strain upon another has been employed. By growing an organism in gelatine at 37° C. for 24 hours, cooling the culture to solidification and inoculating the sloped surface with a loopful of broth culture, one can determine what inhibitions to the growth of other organisms have been established. After one or two days at 22° C. if the inoculated bacillus is not inhibited, a roughening shows up along the track of the loop contrasting with the smooth surface of the gelatine and gradually developing into a definite line of growth.

A comparison of the mutual inhibitions and resistances of smooth and rough variants of the "Hirschfeld" and "Hog Cholera" types with those of the two rough strains "Arkansas" and "Swine Fever" gave the following results. Both "Hirschfeld" variants were capable of inhibiting the growth of both "Hog Cholera" variants as well as that of the two strains "Arkansas" and "Swine Fever," whose diagnosis is in question. On the other hand, neither the "Hog Cholera" variants, nor "Arkansas," nor "Swine Fever," though inhibition between themselves was complete, could inhibit either of the "Hirschfeld" variants. In this test also "Arkansas" and "Swine Fever" agree with the "Hog Cholera" rather than the "Hirschfeld" type. How far this method may be trusted to differentiate closely related serological types remains to be seen. Particularly will it be of convenience, if in this respect

substrains behave like the superstrains of their type and there is thus no necessity to prepare a special serum for the unknown strain in order to perform the mirror test. Laboratories would be relieved, too, of the need of preserving sera corresponding to the various types of this very large paratyphoid B group.

7. THE SEROLOGICAL PARATYPHOID B TYPES.

To the nine of these types, described in a previous paper, has subsequently been added a tenth, the "Abortus Equinus" type. This organism, the frequent cause of abortion in mares (Murray, 1919) is a Salmonella with marked agglutinatory affinity to "Schottmüller" and its allied types, but absorptively quite distinct. The four most commonly encountered types remain: (1) "Schottmüller" (paratyphoid B fever), (2) "Mutton" (food poisoning cases and animal epidemics—it has been recovered from the rabbit, guinea-pig, mouse, calf, duck, parrot, pig, skunk, etc.), (3) "Hirschfeld" (paratyphoid C fever), (4) "Hog Cholera" (associated with the disease of that name); the remaining six have occurred sporadically in man and animals, in food poisonings and continued fevers.

8. CROSS-IMMUNITY WITHIN THE PARATYPHOID B GROUP.

The various types possess to a considerable degree the power of cross-immunisation as witnessed by the following Table VI. The rabbits were all immunised in the same fashion with 24 hours' living broth cultures of the various types—first inoculation 0·01 c.c. subcutaneously, second 0·01 c.c. and third 0·1 c.c. intravenously. After a lapse of 14 days the animals all received varying amounts of a 24 hours' broth culture of Tenbroeck's "Hog Cholera XII," an organism which is so lethal for rabbits that it kills when as few as

Table VI.

Rabbits immunised with living cultures of:	Immunity tested with living "Hog Cholera XII"					
	1 million × M.L.D.		100,000 × M.L.D.		10 × M.L.D.	
	Survivals	Deaths	Survivals	Deaths	Survivals	Deaths
Paratyphosus B, "Schottmüller"	1*	0	0	6	0	3
„ "Hirschfeld"	1	0	2	0	1	0
„ "Mutton"	1	0	1	0	1	0
„ "Reading"	—	—	1	1	1	0
„ "Newport"	1*	0	—	—	—	—
„ "Stanley"	—	—	0	1	—	—
B. enteritidis, "Gärtner"	—	—	—	—	1	0
B. coli, "Escherich"	—	—	—	—	0	1
Streptococcus faecalis	—	—	—	—	0	1
Paratyphosus B, "Hog Cholera"†	—	—	—	—	0	2

* These two survivors were retested after a lapse of a year and still showed immunity to 10 and 100,000 × M.L.D. respectively.

† Immunisation necessarily carried out with killed vaccine. A third rabbit succumbed to a single M.L.D.

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50 to 100 single organisms are injected subcutaneously. The only exceptions to this scheme were the three "Hog Cholera" rabbits, which, because of the virulence of that type, were given the same doses killed by heating to 55° C. for half an hour, and the Streptococcus rabbit, which, being an animal immunised for other purposes, had received nine inoculations of killed streptococci (3000 million–15,000 million) followed by two of living streptococci, each 1000 million, all intravenous.

The results confirm Tenbroeck's statement (1918) that while the "Mutton" type (his "Swine Typhus" or animal paratyphoid B) protects against inoculation with "Hog Cholera," the "Schottmüller" type fails to do so. As far as the number of rabbits employed allows of a conclusion, other types besides "Mutton," including *B. enteritidis* Gärtner, also protect, but as has also been shown by Pratt Johnson in work published elsewhere, those rabbits which are immunised with *killed* cultures of the homologous "Hog Cholera" type, remain unprotected. In the reading of such results it must always be borne in mind that an apparently normal rabbit may have survived spontaneous infection by, say, the "Mutton" type and thus have acquired an unsuspected immunity against "Hog Cholera" inoculation, an immunity not only unsuspected but undemonstrable by serum examination, for the "Mutton" agglutination titre may have disappeared by the time the serum is tested. Both "Mutton" type paratyphoid B and *B. enteritidis* Gärtner are frequent invaders of animal houses and it is possible for the results of such immunity work to be confused.

9. *B. enteritidis* Gärtner and *B. paratyphosus* B agglutinations.

As statements are made from time to time that these two organisms possess unstable serological characters and that a strain may at one time show those of a normal "Gärtner," and at another those of a normal "Mutton" or some intermediate stage between the two, it may be worth while noting here that not only have the laboratory conserved cultures of these two species constantly maintained their original characters, but in a number of isolations made during the investigation of spontaneously occurring epidemics in the animal house, no organism which could in any way be regarded as intermediate between the two, has been encountered. From animals dying spontaneously or in the course of experimentation (diet deficiencies or tubercle inoculations), some 116 "Gärtner" and 21 "Mutton" strains were isolated—111 of the former were recovered from rats and mice, 14 of the latter from guinea-pigs—and in every case the nature of the organism was unequivocally declared without any indication of a confusing serological cross-relationship.

SUMMARY.

1. Among a large number of strains belonging to the various absorption types of the paratyphoid B group and kept under observation in laboratory culture over a number of years, constancy of type has been demonstrated.

2. Only two alterations in the serological nature of certain cultures have been noted; they were due (*a*) to the development of so-called "rough" variants, (*b*) to the degeneration of strains into antigenically less effective "substrains."

Though both are variations within the limits of the absorption type, the serological character of the affected strains may be so obscured that a greater variation than has actually taken place may be ascribed to them, unless the precautions indicated are observed.

3. When "rough" strains are in question, agglutination results are to be mistrusted, for a marked cosmopolitanism in respect of this test has been seen to exist among such alien species as *B. enteritidis* Gärtner, *B. paratyphosus* A and *B. paratyphosus* B.

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