Psychiatric Institute

STUDIES ON A PARATYPHOID INFECTION IN GUINEA PIGS.

III. A Second Type of Salmonella Naturally Appearing in the Endemic Stage.

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The course of a natural outbreak of paratyphoid disease in a guinea pig population was outlined in a preceding paper.[1] The active stage, which occupied a period of 8 weeks in the summer of 1924, was followed by a long endemic stage characterized by sporadic deaths. A single type of *Bacillus paratyphi* was held to be the agent throughout both stages. Continued investigation showed that a second, serologically different type had come into the population during the summer of 1926. The disease was still endemic at the time. The uniformity of the guinea pig stock had not been altered, meanwhile, by the addition of animals from outside sources. Mention should be made here of a similar occurrence reported by Lynch[2] who described the spontaneous appearance of a second Salmonella type in a mouse population. Further reference to the report will be given in a subsequent paper.

Since the introduction of specific infection into the guinea pig population all animals that died from natural causes have been autopsied. In all cases a bacteriological examination, generally of the spleen alone, has been made. Previous experience had shown that the spleen yielded a positive culture, in the majority of cases, whether the animal was a carrier or in a state of active disease. Cultures that were presumptively identified as belonging to the Salmonella group were tested by direct agglutination with a specific antiserum. From

[1] Nelson, J. B., and Smith, T., *J. Exp. Med.*, 1927, xlv, 353.

[2] Lynch, C. J., *J. Exp. Med.*, 1922, xxxvi, 15.

the onset of the epidemic up to the summer of 1926, the strains isolated from active cases and from carriers had shown sufficiently uniform agglutination reactions to be regarded as individuals of one general type. One serum employed in identification agglutinated its homologous strain through a dilution of 1:51,200. With this serum the strains examined agglutinated either to the titer limit or in the next lowest dilution, 1:25,600. It is realized that conclusions based on the outcome of direct agglutination tests alone may be misleading. The serum in question, however, was tested against a comprehensive series of other members of the Salmonella group and found to be low in group agglutinins. Of the types examined none agglutinated in a dilution higher than 1:800. Even though the isolated strains were not subjected to a more exact analysis by absorption it is believed that the position taken as to their unity is secure.

The first culture of the second type was isolated from a breeding sow, on July 28, 1926. At autopsy the animal showed a congested spleen. Focal lesions were not visible grossly. Peyer's patches of the small intestine were prominent and likewise congested. From the spleen there was obtained a motile bacillus which produced hydrogen sulfide and which failed to ferment either lactose or saccharose. Tested with the stock antiserum it agglutinated only in low dilution, 1:400. The following month three additional spleen cultures were isolated. These gave identical findings. Two of the guinea pigs were unweaned young. Only one showed suggestive lesions, the presence of small white plaques in the cecum and a congested spleen. The third culture was from a young, weaned guinea pig which showed an enlarged spleen together with minute focal lesions.

Animal injection was resorted to as a preliminary measure in the identification of these strains.

1 cc. amounts representing a 1:4,000 dilution of an 18 hour bouillon culture were injected intraperitoneally into 350 gm. guinea pigs. A single animal was employed for each strain. After 14 days the four guinea pigs were chloroformed and autopsied. In each case the postmortem findings were typical of paratyphoid infection. There was, however, a considerable individual variation in the type and extent of the changes produced. All showed enlarged spleens partially covered by an exudative membrane. There were no foci. Two showed purulent fluid in the gall bladder accompanied in one case by scattered liver foci. The

third showed focal lesions alone, and with the fourth the liver was normal. Three showed focal lesions in the lymphoid tissue of the small intestine, while the fourth showed small white plaques in the cecum. Pure cultures of an organism possessing characters identical with those of the injected culture were obtained from the spleen in each case. It seemed evident from the above findings that the four cultures were Salmonella types.

Rabbits ·ere given serial intraperitoneal injections of heated killed suspensions of two strains for the production of antiserums. The two immune serums agglutinated the four cultures equally through a dilution of 1:12,800. A strain of the first type was agglutinated in low dilution only, 1:800. The absorptive capacities of the two immunizing strains as tested by reciprocal agglutinin absorption were identical. The above results in conjunction with the animal tests establish the position of the four cultures as identical strains of a second Salmonella type. From August on sporadic deaths due to one or the other of the two types, provisionally designated *B. paratyphi* Types I and II, have occurred. The relative distribution of the two types within the population will be considered in another paper. It may be said that 87 cases of the second type have been observed and studied during a period of 10 months.

In addition to the different agglutinative relationships of the two types of *B. paratyphi*, there was some indication that their loci of development in the animal host tended to differ. As previously noted,[1] the most constant manifestation encountered in fatal cases of the earlier type was the presence of focal lesions in the spleen together with enlargement and congestion. Focal involvement of the lymphoid tissue of the small intestine was often observed and somewhat less frequently of the liver. With the introduction of the second type of infection the number of cases showing typical focal lesions in the spleen has decreased. Thus, from the autopsy records of 50 consecutive cases of each of the two types, focal lesions were observed 29 times with Type I and 17 times with Type II. When present in the latter type, however, the focal changes showed no difference grossly from those of the earlier form of infection. In the absence of foci, congestion and slight enlargement of the spleen were commonly observed. Focal involvement of the Peyer's patches of the small intestine was also less frequent. In the liver, on the other hand, it

was more often observed than in Type I cases. Lesions in the cecum have likewise been more frequent with the Type II infection. The wall of the cecum was generally congested. At times the congestion was diffuse, involving the entire wall or extensive areas of it. At times it was circumscribed, involving only the lymphoid tissue which stood out as deep red circular patches. In addition, yellowish or yellowish white plaques varying in number and in size were present. Sometimes the plaques were located on the peritoneal surface, sometimes on the mucous surface. At times a diffuse exudate coated the mucous surface giving a dense opaque appearance. Frequently the cecal contents was fluid or semifluid.

Other gross changes noted at times in individual cases included involvement of the cervical and mesenteric lymph nodes with hypertrophy and congestion, a purulent fluid in the gall bladder, and firm adhesions binding the liver or spleen to the peritoneal wall. In several instances an acute peritonitis was encountered. These cases, which were limited to the stock, weaned guinea pigs, were marked by a tenacious exudative membrane on the surface of the liver and spleen, a seromucoid exudate coating the intestinal tract and peritoneum, and a considerable volume of turbid, mucoid fluid in the abdominal cavity.

Involvement of the genital tract was often observed in the case of adult females from the breeding cages. The uterine wall was swollen and diffusely congested. At times a thick, mucopurulent exudate was present in the lumen. At times small exudative plaques were found on the peritoneal surface of the uterus. In several instances an abscessed condition of the mammary gland was encountered. In young female guinea pigs uterine changes aside from a slight congestion were not conspicuous. Involvement of the male genital tract was rarely encountered.

The relationship of the second type of *B. paratyphi* to other members of the Salmonella group was studied by means of direct agglutination and by agglutinin absorption. An antiserum against Strain 1149, the first Type II culture isolated, was employed in the direct agglutination tests. The antigens were fresh, unheated, saline suspensions prepared from 18 hour agar cultures and were standardized to equal opacity. The agglutination limits of Salmonella types and

one strain of *B. typhi* with the Type II antiserum are given in Table I. Of the cultures employed, guinea pig Strains 1149 and 922 were Types II and I, respectively, from the present epidemic. Guinea pig Strain IV was isolated during a Boston epidemic in 1908. As noted in a previous paper,[1] it was agglutinated by a Type I serum in low dilution only. The rabbit strains were isolated during a slight outbreak among the stock rabbits in the fall of 1926. The mouse cultures were Mouse Typhoid I and II from The Rockefeller Institute in

TABLE I.

Agglutination Limits with Type I and Type II Antiserums.

	Antiserum	
	Type I	Type II
Guinea pig paratyphoid 922	1:51,200	1:800
" " " 1149	1:400	1:12,800
" " " IV	1:200	1:12,800
Rabbit " 5	1:51,200	1:400
" " 22	1:400	1:12,800
Mouse " I	1:51,200	1:800
" " II	1:200	1:12,800
Rat " V	1:51,200	1:800
Calf " I	1:400	1:12,800
Swine " IV	1:200	1:12,800
B. paratyphi a Schottmüller	1:200	1:800
" " b Rowland	1:200	1:6,400
" " *aertrycke* 387	1:200	1:12,800
" *enteritidis* Gaertner (Kral)	1:400	1:400
" *choleræ suis* X	1:100	No agglutination at 1:100
" *typhi* X	1:3,200	1:3,200

New York. The Rowland strain of *B. paratyphi* b was obtained through the courtesy of Miss Georgia Cooper of the New York City Department of Health. The other cultures were selected from the departmental collection.

The Type II serum agglutinated a number of the miscellaneous Salmonella cultures to the titer limit. Both of the serums were high in group agglutinins for *B. typhi*, while both were low for *B. enteritidis* and *B. choleræ suis*. The level of group agglutination for the opposite

type was somewhat higher in the case of the Type II serum. In the routine examination of cultures from positive cases of paratyphoid the limit of group agglutination of the Type II serum with the opposite type, however, varied from 1:200 to 1:800. The Salmonella cultures which showed a high agglutination with the Type II serum were selected for further identification by agglutinin absorption In each case the serum in a dilution of 1:25 was absorbed twice with a 1:10 volume of packed and washed cells. The absorption was carried out at 37°C. for 5 hours, followed by approximately 16 hours at ice box temperature. The absorbed serum was finally tested in a two-fold dilution series ranging from 1:50 through 1:25,600. The results

TABLE II.

Agglutination Limits with Type II Serum after Absorption.

Absorbing culture	Agglutination after absorption	
	Absorbing culture	Homologous culture
Guinea pig paratyphoid 1149		1:100
" " " IV	1:200	1:200
Rabbit " 22	1:100	1:100
Mouse " II	1:50	1:50
Calf " I	1:200	1:200
Swine " IV	1:100	1:1,600
B. paratyphi aertrycke	1:100	1:200
" " b Rowland	1:50	1:6,400

of the absorption tests with the Type II serum are given in Table II. Absorption with the homologous strain lowered the agglutinin content of the serum to the same level for all the cultures.

The absorptive capacity of the guinea pig culture, Strain IV; the mouse culture, Type II; the calf culture, Strain I; and the *aertrycke* type of *B. paratyphi* corresponded very closely to that of the homologous culture. By the direct method these cultures were agglutinated in low dilution only by the Type I serum. A close relationship between them and the Type II organism is indicated. The absorptive capacity of *B. paratyphi* b was approximately half that of the homologous culture. The reduction in titer of the serum for the

Type II antigen after absorption with *B. paratyphi* b was probably due to the removal of group and not specific agglutinin. By the direct method the culture was agglutinated in high dilution. The difference in absorptive capacity of the two cultures appears sufficiently marked to establish their non-identity. The Swine IV antigen reduced the titer of the serum approximately 87 per cent as compared

TABLE III.

Agglutination Limits with Mouse II, Calf I, and B. paratyphi aertrycke Serums after Absorption.

Serum	Culture	Direct agglutination	Absorbing culture	Agglutination after absorption	Culture agglutinated
Mouse II	Mouse II	1:51,200	Mouse II	1:100	Mouse II
			" II	1:100	Guinea Pig 1149
	Guinea Pig 1149	1:51,200	Guinea Pig 1149	1:100	Mouse II
			Guinea Pig 1149	1:100	Guinea Pig 1149
Calf I	Calf I	1:51,200	Calf I	<1:50	Calf I
			" I	<1:50	Guinea Pig 1149
	Guinea Pig 1149	1:25,600	Guinea Pig 1149	<1:50	Calf I
			Guinea Pig 1149	<1:50	Guinea Pig 1149
B. paratyphi aertrycke	*B. paratyphi aertrycke*	1:25,600	*B. paratyphi aertrycke*	1:50	*B. paratyphi aertrycke*
			B. paratyphi aertrycke	1:50	Guinea Pig 1149
	Guinea Pig 1149	1:25,600	Guinea Pig 1149	1:50	*B. paratyphi aertrycke*
			Guinea Pig 1149	1:50	Guinea Pig 1149

with the 99 per cent reduction by the homologous antigen. It seems necessary to assume that some specific agglutinin was removed and that the two cultures bear a relationship other than through their group agglutinogen.

In order to establish the identity or close relationship of the cultures which showed nearly equal absorptive capacities reciprocal

tests were made. The Calf I, Mouse II, and *aertrycke* cultures were employed. Rabbits were immunized with the three antigens and agglutinating serums of high titer obtained. The period of immunization was longer than employed in the preparation of the Type II serum and the titer limits of the serums were correspondingly higher. Absorption was carried out, as before, with the homologous culture and with the Type II culture. The results of the absorption tests are given in Table III.

The agglutinative affinities of the second organism as established by reciprocal absorption relate it to the *aertrycke* type of *B. paratyphi*. The identity of the Type II culture, the Mouse II culture, and the Calf I culture is similarly indicated. The identity of the Type II culture, the Guinea Pig IV culture, and the Rabbit 22 culture, while unconfirmed, is suggested. The Swine IV culture appears to be a less closely related organism, but bearing some specific agglutinogen in common with it. The second organism is related to the Type I strains, *B. enteritidis*, *B. paratyphi* a and b only through group agglutinogen. The content of group agglutinogen is low except for the latter organism from which it is differentiated only by agglutinin absorption. It also bears group agglutinogen in common with *B. typhi*. The indicated relationship of the Guinea Pig II, Mouse II, and *B. paratyphi aertrycke* strains is in agreement with the work of Webster[3] and of Edwards and Rettger.[4]

SUMMARY.

The spontaneous appearance of a second paratyphoid infection in a guinea pig population during the endemic stage of an earlier epidemic is reported. A comparative study of the gross pathology of the two infections indicated a difference in the loci of development of the respective organisms in the animal host. The two types were readily differentiated by direct agglutination with specific immune serums. From its agglutinative affinities the second organism was judged to be an *aertrycke* type of *B. paratyphi*.

[3] Webster, L. T., *J. Exp. Med.*, 1922, xxxvi, 97.

[4] Edwards, P. R., and Rettger, L. F., *J. Bact.*, 1927, xiii, 73.