STUDIES ON A PARATYPHOID INFECTION IN GUINEA PIGS.

II. Factors Involved in the Transition from Epidemic to Endemic Phase.

By THEOBALD SMITH, M.D., and JOHN B. NELSON, Ph.D.

(From the Department of Animal Pathology of The Rockefeller Institute for Medical Research, Princeton, N. J.)

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The active stage of the epidemic of guinea pig paratyphoid (1), occurring in the summer months of 1924, was followed by a long protracted endemic period studied up to June, 1926. During this period the percentage mortality showed considerable fluctuation from month to month. It was low when compared with the rate during the active stage except during the hot months of 1925 when there was a slight increase in the fatal cases. In the hope that a study of all cases of paratyphoid occurring subsequent to the early epidemic period might offer some explanation of the drop from the epidemic to the low endemic stage, attention was focused in particular upon the dams and their litters.

It has already been stated that during the epidemic period the mortality among the sows was high. During the later period and up to the present, June, 1926, only 4 sows died of the disease. During the endemic period all sows whose young showed infection with *B. paratyphi* were taken from the breeding house with the surviving young and placed together in a large screened box for observation. These sows and the remainder of the litter were killed at intervals and cultures made from spleen, uterus, gall bladder, and cecum. The blood serum was tested for specific agglutinins towards the epidemic strain. In all, 35 sows with at least one fatal case of paratyphoid in each litter were segregated during the period of observation.

On the basis of the postmortem findings the sows could be divided into three groups. The first group comprised the fatal cases. Death

TABLE I.

Bacteriological Data of Isolated Guinea Pigs and of Their Unweaned Young Which Had Succumbed to Paratyphoid Infection.

No. of guinea pig	Age at death in mos. k. = killed d. = died	Data of sow*						Data of young				
		Spleen	Uterus	Cecum	Gall bladder	Fetal spleen	Serum titer	No. in litter	Deaths	Date of death	Age in days	Spleen culture
										1924		
1	30 (k. Mar. 3, 1925)	+					1:320	3	1	Oct. 16	21	+
2	11 (k. Mar. 25, 1925)	−	−	−	−		1:160	3	1	" 24	6	+
3	25 (k. Mar. 25, 1925)	+	−	−	−		1:80	3	1	" 27	10	+
4	28 (k. Apr. 7, 1925)	−	+	−	−		1:160	4	1	Nov. 1	5	
										1925		
5	12 (k. Apr. 22, 1925)	−	−**	−	−	−	1:320	4	1	Jan. 24	7	+
6	10 (k. Mar. 19, 1925)	−	−**	−	−	1. + 2. − 3. −	1:80	4	1	Apr. 13	3	+
7	11 (k. Apr. 18, 1925)	−	−	−	−		1:80	4	1	" 13	3	+
8	15 (k. Apr. 21, 1925)	−	−	−	−		<1:10	4	1	" 16	3	+
9	25 (k. Apr. 18, 1925)	+	−	−	−		1:40	4	3	" 16	8	+
										" 16	8	+
										" 16	8	+
10	8 (d. Apr. 20, 1925)	+	+	+	+			5	3	Apr. 18		+
										" 18		+
										" 20		+
11	27 (k. Apr. 29, 1925)	−	−	−	−		1:160	2	1	" 25	10	+
12	12 (d. June 13, 1925)	+	+					4	2	June 12	8	+
										" 12	8	+
13	16 (k. Oct. 2, 1925)	−	−	−	−		1:40	3	2	Sept. 17	12	+
										" 17	12	+
14	17 (k. Oct. 2, 1925)	−	−	−	−		1:320	3	1	" 18	8	+
15	17 (k. Oct. 2, 1925)	−	−	−	−		1:20	4	1	" 19	11	+
16	16 (k. Oct. 13, 1925)	+	−**	−	−	1. − 2. −	1:80	4	3	" 29	21	+
										Oct. 8	30	+
										" 12	34	+

* + = positive cultures; − = negative cultures.

** Culture included placenta.

TABLE I—*Concluded.*

No. of guinea pig	Age at death in mos. k. = killed d. = died	Data of sow*						Data of young				
		Spleen	Uterus	Cecum	Gall bladder	Fetal spleen	Serum titer	No. in litter	Deaths	Date of death	Age in days	Spleen culture
										1925		
17	7	−	−	−	−		1:1,280	2	2	Oct. 5	11	+
	(k. Oct. 19, 1925)									" 5	11	+
										1926		
18	21	+	+	+	+			5	4	Feb. 19	3	+
	(d. Mar. 1, 1926)									" 19	3	+
										" 23	7	+
										" 23	7	+
19	20	+	+	+	+			5	4	" 23	7	+
	(d. Feb. 27, 1926)									" 25	9	+
										" 25	9	+
										" 25	10	+
20	32	−	−	−	−		1:320	3	1	" 16	6	+
	(k. Mar. 8, 1926)											

occurred some time after parturition. The sows all showed typical focal lesions in the spleen together with other manifestations of paratyphoid. *B. paratyphi* was obtained in cultures from the spleen and feces, and in two of the cases from the uterus and gall bladder. The blood serum, tested for agglutinins in only one instance, showed no agglutination in a dilution of 1:10. With these cases the majority of the young in the litter died.

The second group included the majority of surviving cases, *i.e.* sows which had recovered from a slight attack of paratyphoid or had been carriers with localization of *B. paratyphi* in the spleen. Within the litters only part of the young succumbed to paratyphoid. The sows of this group showed serum agglutinins for the epidemic strain of *B. paratyphi* in dilutions ranging from 1:20 to 1:1,280. The organism was recovered from the spleen in only two instances. Cultures from the feces and gall bladder were uniformly negative. In one case *B. paratyphi* was obtained from the uterus and in one case from a fetal spleen. It sometimes happened that the sow had been

rebred before removal for the nursing period. The spleen and liver sometimes showed a few foci, with a negative culture, together with other indications of past infection.

The sows of the third group showed no specific agglutinins in a dilution of 1:5. Cultures from the spleen, gall bladder, uterus, and feces were uniformly negative. The spleen and other organs did not show gross changes suggestive of former disease. The sows of the group were regarded as sometime fecal carriers of the organism. The postmortem findings of a selected number of the isolated sows, together with the young of their litters which succumbed to paratyphoid, are given in Table I.

This material affords a basis for certain inferences. (1) The dams, as might have been anticipated, were far more resistant than their offspring. The survivors appeared normal when killed later. (2) In those animals that died, the gross lesions appeared equal in extent and severity to those encountered in the epidemic period. (3) Intrauterine transmission of the infecting agent to the fetus was demonstrated in one case. (4) The bacilli were excreted either by way of the digestive tract, the uterus, or the mammary gland, which in one instance contained a number of abscesses. *B. paratyphi* was regularly isolated from the feces of those sows which succumbed to active infection shortly after parturition. With the sows which showed evidence of inactive infection or of recovery from a past active infection the causal organism was rarely recovered from the feces except when examination was made immediately after segregation. It seemed probable, however, that the majority of the sows were excreting the organism at the time of parturition. If such was the case, it would appear that the carrier state, as evidenced by the presence of *B. paratyphi* in the feces, was of relatively short duration.

To obtain indirect evidence bearing on the persistence of the causal organism in the feces a small series of guinea pigs was fed *B. paratyphi* and the feces subsequently cultured for the organism. The series included one lot of two older stock guinea pigs and one lot of four recently weaned pigs. For comparison a series of eight mice was included. Cultures made from feces collected before feeding were all negative. The animals were fed by pipette on 2 successive days. The guinea pigs received a total volume of 1 cc. of an 18 hour broth

culture of *B. paratyphi*, approximately 800,000,000 organisms. The mice received a total of 0.1 cc. of the same culture, or 80,000,000 organisms. Cultures were made 4 days after the last feeding and then every 7 days until negative. The mice invariably showed a long continued carrier state and were killed before the excretion of the organism had terminated.

The method used in culturing was as follows:

A heavy suspension of fresh feces was made in plain bouillon and incubated at 37°C. for 3 to 5 hours. A small amount of the culture, 0.2 cc., was pipetted into a tube of fermented bouillon containing lead acetate (0.25 cc. of a 1 per cent solution in 5 cc. of medium) and malachite green (0.1 cc. of a 0.1 per cent solution in 5 cc. of medium), and incubated overnight. In the presence of a deep brown or black precipitate and motility, or either, a drop was streaked on the surface of a lead acetate-fermented bouillon agar plate and a thin layer of the same medium superimposed. Deep brown colonies, after 18 hours of incubation, were fished to agar slants and finally tested with a specific antiserum. In the absence of *B. paratyphi*, the lead acetate-malachite green broth generally showed a very scant turbidity, no change in color of the precipitate, and no motility. In the presence of *B. paratyphi* there was always motility, a moderate turbidity, and a browning or blackening of the precipitate.

The guinea pigs were killed shortly after the indicated termination of the fecal carrier state. The mice were killed after a prolonged period of continued excretion. The results of the feeding experiments with the two lots of guinea pigs and with the mice are given in Tables II and III.

Per os administration of *B. paratyphi* to these guinea pigs was not followed by active disease. With the older stock pigs there was an immediate excretion of the organism which continued for a period of approximately 3 weeks. The feces subsequently became negative. The guinea pigs were then killed and autopsied. Lesions were not observed. The organism was recovered from a piece of Peyer's patch of the small intestine in one case and from the spleen in one case. Agglutination tests gave evidence of an immunity response on the part of the host. With the recently weaned guinea pigs there was a delayed and subsequently irregular excretion of *B. paratyphi*. One guinea pig gave but a single culture of the organism from the feces. The fecal excretion of *B. paratyphi* terminated sometime

TABLE II.

Results of Fecal and Postmortem Cultures from Guinea Pigs Fed B. paratyphi.

No. of days after last feeding	Stock guinea pigs		Recently weaned guinea pigs			
	1	2	3	4	5	6
4	Feces +	Feces +	Feces −	Feces −	Feces −	Feces −
11	" +	" +	" +	" −	" +	" +
18	" +	" +	" −	" −	" +	" +
25	" −	" −	" +	" +	" −	" −
27	" −	" −				
28	Killed Spleen + Peyer's patch***+ Serum agglutinins 1:320	Killed Spleen − Peyer's patch + Serum agglutinins 1:640				
32			" −	" −	" −	" −
34			" −	" −	" −	" −
35			Killed Spleen + Peyer's patch + Serum agglutinins 1:40	Killed Spleen + Peyer's patch − Serum agglutinins 1:80	Killed Spleen − Peyer's patch − Serum agglutinins 1:80	Killed Spleen + Peyer's patch − Serum agglutinins 1:20

*** "Peyer's patch" means that a piece of intestinal wall containing a Peyer's patch was cut out and transferred with some fecal material to an agar slant.

TABLE III.

Results of Fecal and Postmortem Cultures from Mice Fed B. paratyphi.

No. of days after last feeding	M 1	M 2	M 3	M 4	M 5	M 6	M 7	M 8
4	Feces +	Feces +	Feces +	Feces +	Feces +	Feces +	Feces +	Feces +
11	" +	Died on 10th day Spleen + Feces +	" +	" +	" +	" +	Died on 7th day Spleen + Feces +	" +
18	" +		" +	" +	" +	" +		" +
25	" +		" +	" +	" +	" +		" +
32	" −		" +	" +	" +	" +		" +
39	" +		" +	" +	" +	" +		" +
46	" +		" +	" +	" +	" +		" +
53	" +		" +	" +	Killed on 49th day Spleen + Feces +	Killed on 49th day Spleen + Feces +		Killed on 49th day Spleen + Feces +
56	Killed Spleen + Feces +		Killed Spleen + Feces +	Killed Spleen + Feces +				

between the 3rd and 4th week. At autopsy the organism was recovered from the intestine in one case and from the spleen in three out of four cases. The immunity response was weak as compared with that of the older guinea pigs.

With mice the feeding of the organism resulted in death in two cases. The survivors showed a persistent fecal excretion of *B. paratyphi.* The animals were killed at the end of the 7th or 8th week after the last feeding and in every case a positive culture was obtained from the spleen and from the feces.

During the endemic period, guinea pigs in other lines of research were scrutinized as far as this was possible A series of 32 stock guinea pigs had each received intraperitoneal injection of material containing *B. abortus.* At varying intervals after injection the animals were killed and the entire spleen cultured. In two cases pure cultures of the epidemic strain of *B. paratyphi* were obtained from the spleen. No gross manifestations of infection were noted in either case. Another series of 10 guinea pigs had received intraperitoneally sublethal doses of a virulent strain of *B. coli.* The animals were killed on the 5th day and the entire spleen cultured in every case. A mixed growth of the injected organism and the epidemic strain of *B. paratyphi* was obtained from one animal. The spleen showed only the exudative membrane occasioned by the *B. coli* strain. *B. paratyphi* was not recovered from the feces.

Several series of stock guinea pigs, selected at random from the benches, were examined during the endemic period for the fecal carriage of *B. paratyphi.* Of 40 animals cultured the organism was recovered from the feces in only one. This guinea pig was one of a series of 12 removed from a bench shortly after the appearance of a fatal case of paratyphoid in it. The fecal culture was positive immediately after segregation. Cultures made 6 and 9 days later were negative. The animal was killed on the 21st day. *B. paratyphi* was obtained in cultures from the spleen but not in cultures from the feces or in cultures inoculated with a piece of a Peyer's patch.

A survey of the possible agencies transmitting the virus from cage to cage and group to group focused attention upon a few gray mice which gain access from time to time to the building from the fields and woods around it. Data already presented clearly showed that

white mice freely discharge the guinea pig strain for some time after having been fed. Early experience of one of the authors with wild mice indicated no appreciable difference of gray and white mice towards hog cholera bacilli. The agency of wild mice was furthermore suggested by the occasional occurrence of a paratyphoid disease among breeding rabbits kept in another wing of the same building. The cases were rare, not more than several in the fall of 1924, a few in 1925, and one in April, 1926. The lesions were characteristic of the less acute type of paratyphoid infection in rabbits, such as necrotic follicles in appendix, necrotic follicles in Peyer's patches, enlarged, firm, congested spleen, and, very rarely, necrotic foci in liver. The isolated bacilli agreed culturally and serologically with the guinea pig strain. Several wild mice examined recently did not carry *B. paratyphi* however.

DISCUSSION.

Spontaneous epidemics among small animals have not been studied in the past with the care warranted by the importance of the phenomenon. Most investigators have perhaps reached the conclusion that the many unknown factors in spontaneous epidemics stand in the way of any trustworthy conclusions to be derived from such study. Hence the experimentally controlled epidemiological investigations of recent years (Topley (4), Amoss (3), Webster (5)). Nevertheless it is the natural epidemics from which must be drawn the problems to be solved. Without such study the problems do not readily present themselves or they may be overshadowed by the artificial conditions of the experiment. In these pages by the natural epidemic is meant the occurrence of disease without the use of cultures artificially administered. In the epidemic reported above the question as to the decline of the epidemic to the endemic level was uppermost. Among the hypotheses to present themselves the following appear to us as involving the major factors.

(*a*) The sudden, acute, highly fatal onset of the infection may be interpreted as an attack upon a number of animals below the normal resisting power of the species which had accumulated during the 6 years of freedom from disease. This accumulation may be due to fortuitous sexual selection going on in the population or to spon-

taneous variation in natural resistance. We may assume that most of the individuals belonging to this category were wiped out in the early weeks of the disease when the breeders were the chief victims. This theory is supported by the fact that no epidemic rise has occurred since the fall of 1924. The occasional appearance of an animal with marked lesions may be due to individually depressing environmental causes, to which the group is subject, as well as to a reappearing, less resistant stock.

(*b*) Another hypothesis assumes an increase in specific immunity towards *B. paratyphi* following the ingestion of minute doses which caused the epidemic to subside towards the endemic level. This theory is not well supported. There should have been found a less abrupt drop in mortality and many more carriers in the exposed guinea pigs during the endemic period. It is not to be denied, however, that the resistance of certain groups may have been raised by the ingestion of minute doses in the early epidemic phase.

(*c*) A third hypothesis assumes a decline in the virulence of the bacillus in passages through successive guinea pigs. Stated differently, this hypothesis assumes that the virus was introduced from a somewhat higher level of virulence, possibly from some other host species, and after several passages was thereby brought into equilibrium with the new host. This hypothesis was tested in several ways. A culture from one of the earliest acute cases was at hand to be tested comparatively with a culture from the later endemic period. The former had been isolated July 14, 1924; the latter March 19, 1925. The possible unreliability of any comparative tests was kept in mind in view of the additional 8 months of artificial cultivation of the early strain. Subcutaneous and intraperitoneal injection of graded dilutions prepared from 18 hour bouillon cultures of the two strains was made in guinea pigs of approximately 350 gm. weight. With the series which received subcutaneous injection a slight difference in virulence between the two cultures was encountered. The older culture showed a virulence approximately four times that of the endemic culture. A dilution range of the bouillon culture of 1:20 to 1:160 was employed. Within that range extensive ulceration at the site of injection resulted with both cultures. Death occurred in approximately 10 days with the guinea pigs which

had received the older culture in a dilution of 1:80. Of the guinea pigs which had received the endemic culture death occurred only following a dilution of 1:20 after a slightly longer period.

The results obtained from the series which had received intraperitoneal injection of the two cultures were less suggestive of a difference in virulence. The intraperitoneal injection of the older culture in a dilution of 1:500 resulted in death in 48 hours. With the endemic culture death did not occur until 8 days after the injection. Dilutions higher than 1:1,000 produced death in 10 to 14 days or more with advanced focal lesions in the spleen and liver. In several later series which had received graded dilutions intraperitoneally no consistent difference in the lethal dose was observed. The guinea pigs employed in the tests were descendents of the original infected population several generations removed from the active period of the epidemic. Individual differences in natural susceptibility might be sufficient to account for fluctuations in the response to graded dilutions.

A second method of comparing the virulence of the bacillus in the epidemic and the endemic period was to study the lesions of cases occurring in these periods. Tissues from 31 animals were examined in fixed and stained sections. This material consisted of breeders, very young and young adult animals, and of cases from the early epidemic and the late endemic period. The material was from both naturally dead and chloroformed animals. A comparative study of this miscellaneous material did not bring to light any histological characters clearly distinguishing the early and subsequent periods. The various changes in spleen, liver, lymph nodes, and intestines were found at all periods and in various ages and a detailed description of these changes is therefore omitted.

A change in virulence has thus not been demonstrated. It must be granted, however, that the methods employed to show such transformation are relatively crude. The change from an epidemic to an endemic level is obviously due to a number of cooperating and mutually interfering factors and all we can state at present is that it actually occurs in all epidemics sooner or later in a stationary population, in spite of the fact that such groups of bacteria as streptococci, pneumococci, and bipolar organisms (hemorrhagic septicemia)

may be raised in virulence by passages through a series of animals. The increase in virulence of the paratyphoid group by passages is not clearly proved by experiment. Moore (2) failed to increase the virulence of hog cholera bacilli by passages through rabbits. Webster (5) failed to show any rise in virulence. Lockhart (6) concludes that the virulence of a single strain of *B. aertrycke* may be significantly increased as the result of animal passage, but in the text he admits that such increase cannot be brought about with any regularity. A prolonged experience of one of us with the hog cholera bacillus since 1885 leads to the conclusion that passages tend downwards rather than upwards in capacity to kill. If experiments in epidemiology are to elucidate natural phenomena, it is to be borne in mind that passages through a series of susceptible animals by inoculation do not imitate nature, for in the spontaneous disease the infectious agent is transmitted through the digestive tract and of what goes on there nothing is known. That the selective action of the host is the same through enteral and parenteral channels may at least be doubted. Furthermore, virulence is regarded by some as the capacity to multiply and kill, whereas in passages the change may be towards greater tenacity of life in the tissues rather than the capacity to multiply and thus destroy. The infection in the population under consideration has now been followed through three summers, the first being that of the original epidemic period. In 1925, the year following the initial outbreak, the mortality was highest during the summer months, reaching a peak in June, with a rate of 4.1 per cent, followed by a decline in July. For 1926, up to September, the mortality was likewise greatest during the summer with a high rate of 6.4 per cent in August. A rough evaluation of the three summer periods may be made by comparing the combined rates for July and August of each year. It was during that period that the majority of deaths occurred at the time of the original epidemic. Taking into consideration the few deaths that occurred in September, 1924, which were included in the original rate computed for that period, the percentage mortality for July and August, 1924, was 16.7 per cent. The rates for a corresponding period of time in 1925 and 1926 were 3.7 per cent and 6.6 per cent, respectively, definitely lower than the rate of the epidemic period.

The infection has therefore been followed long enough to eliminate the various secondary influences such as changes in the character of the food during the four seasons and changes in humidity and temperature. During the occasional excessive heat periods of the summer months deaths among adult guinea pigs have occurred not referable to anything but the heat.

SUMMARY.

Factors bearing on the maintenance of paratyphoid in an endemic state are discussed. There was no evidence of any increase nor any clearly demonstrable proof of a decline in virulence of the causative organism. This persisted within the breeding stock and it is suggested that the sows constituted the chief focus for dissemination of the organism to their young and from these to the population at large. Evidence is presented that the carriage of *B. paratyphi* in the feces was of relatively short duration. Fecal carriage of *B. paratyphi* was commonly associated with a localization of the organism in the spleen. Since it is obvious that some factor or factors must have changed in the transition from epidemic to endemic phase in the presence of younger generations, the hypothesis is tentatively presented that the transition from epidemic to endemic phase is due to a combination of the weeding out of individuals of low natural resistance with a gradual adjustment of the invading organism to the population on a lowered level of virulence.

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