A GROUP OF PARATYPHOID BACILLI FROM ANIMALS CLOSELY RESEMBLING THOSE FOUND IN MAN.

By CARL TENBROECK, M.D.

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

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In a previous paper (1) the writer described a group of paratyphoid bacilli, isolated from animals, that with the usual cultural methods seemed to be the same as paratyphoid B, or *Bacillus schottmülleri*, as Winslow, Kligler, and Rothberg (2) suggest we call the latter organism. Serologically they could be separated from one another by agglutination absorption tests and to a certain extent by the type of clumps formed in immune sera. Five new cultures belonging to this group have recently been studied and a further effort has been made to differentiate them from the human paratyphoids by a method less complicated than agglutination absorption.

Five of the cultures studied came from swine infected with hog-cholera virus, two came from guinea pigs, and one each from a child, a cow, a pigeon, and a mouse. Four cultures of *Bacillus schottmülleri* obtained from Dr. C. Krumwiede were used for comparison. The date of isolation and other facts regarding the above cultures are given in the paper already referred to. In addition, cultures isolated by Dr. Smith in 1918 from five calves have been studied and found to belong to the same group, and still more recently additional cultures of this group have been isolated from swine infected with hog-cholera virus. It is evident that these organisms are quite widespread among our domestic animals but just what relation they have to animal diseases will require much further study. In hog-cholera they may tend to emphasize the intestinal lesions, but cases of this disease occur with extensive intestinal changes from which organisms of this group cannot be isolated.

Cultural Studies.

Sixteen cultures of the animal paratyphoids have been studied culturally and they have all corresponded to one another and to *Bacillus schottmülleri*. The results of the study are given in Table I, which includes the characters of *Bacillus enteritidis* and of the hog-cholera bacillus.

Some of the strains were tested with inosite with unsatisfactory results as the amount of acid produced by the paratyphoids was so small that it seemed possible that it was due to impurities. Other carbohydrates might have been included in the study but they are so

TABLE I.

Cultural Characters of Paratyphoids Studied.

Organisms.	Motility.	Gram stain.	Indole.	Hydrogen sulfide.	Gelatin liquefied.	Dextrose.	Maltose.	Mannitol.	Xylose.	Dulcite.	Arabinose.	Lactose.	Saccharose.	Salicin.	Glycerol.	Raffinose.	Dextrin.
B. schottmülleri (paratyphoid B)	+	−	0	+	0	AG*	AG	AG	AG	AG	AG	0	0	0	0	0	0
B. enteritidis	+	−	0	+	0	"	"	"	"	"	"	0	0	0	0	0	0
Animal paratyphoids	+	−	0	+	0	"	"	"	"	"	"	0	0	0	0	0	0
Hog-cholera bacilli	+	−	0	0	0	"	"	"	"	0	0	0	0	0	Slightly acid.	0	0

* AG indicates acid and gas formation.

expensive that they would be of no practical value in differentiating the two groups. As shown in the table, no cultural differences have been found between *Bacillus enteritidis*, *Bacillus schottmülleri*, and the animal paratyphoids.

In the hope that a difference might be detected between animal and human paratyphoids, by a study of the hydrogen ion concentration, cultures were made in dextrose and xylose broth. Both groups gave a hydrogen ion concentration of pH 5.0 in dextrose broth after 4 days incubation, while in xylose broth the pH varied from 5.6 to 5.2. Some of the animal strains acted more slowly and others apparently utilized more of the carbohydrate than did the human strains. The difference is so slight, however, that it is of no value in classification.

Serological Studies.

When living cultures are used for immunization of rabbits the sera produced will usually agglutinate both the human and animal paratyphoids to the same titer limit. At times, however, one gets a serum that will agglutinate bacilli of the group used in the immunization in higher dilutions than it does those of the other group. This is apparently due to the individual rabbit rather than the bacteria, for the same culture will act differently in different rabbits.

Cultures from both human and animal sources are agglutinated in only the lower dilutions of sera from animals immune to *Bacillus enteritidis* and as the results obtained in the present study do not differ from those previously given they need not be repeated here.

When 24 hour bouillon cultures are used as antigens the clumps formed by bacilli of the same group as the immunizing strain are flocculent and after 2 hours incubation form a mass occupying from one-quarter to one-half the column of liquid. Bacilli of the other group as a rule give very compact clumps which after standing in the refrigerator over night form a thin film on the bottom of the test-tube. This difference in the clumping is striking but unfortunately it does not always hold true. If growth is more abundant than the average, flocculent clumping may occur where a compact type is expected. The amount of dextrose in the bouillon influences the type of clumps formed. Apparently the amount of growth rather than the change in reaction is responsible for the change. As a rule, however, one can differentiate fairly well between the two groups by the type of clumps, but it can only be regarded as a tentative means of differentiation.

When heated or formalinized bouillon cultures or suspensions in salt solution of the growth from agar slants are used as antigens, agglutination will occur in the same dilutions of serum and the clumps are so nearly alike in character that the two groups cannot be differentiated.

When formalinized bouillon cultures are standardized and agglutinated according to Dreyer's method (3) with the readings made after 2 hours incubation at 50°C. in the water bath and 15 minutes at room temperature, the two groups cannot be differentiated by the degree of agglutination or the type of clumps.

Numerous agglutination absorption tests have been made and the results have confirmed those previously reported. The animal strains will absorb from *Bacillus schottmülleri* sera the agglutinin for all the

TABLE II.

Summary of Absorption Experiments with Serum of Rabbit 9, Immunized to Calf-Typhus V by Injection of Living Cultures.

Culture tested.	Titer limit of serum saturated with.					
	Nothing; *i.e.*, control.	*B. schottmülleri* 232.	*B. schottmülleri* 242.	Calf-typhus V.	Swine-typhus I.	Swine-typhus V.
Calf-typhus I	51,200	25,600	25,600	—	—	—
" III	51,200	—	—	—	400	400
" IV	51,200	25,600	25,600	800	—	—
" V	51,200	25,600	25,600	200	400	400
Swine-typhus I	51,200	12,800	12,800	—	200	—
" V	51,200	25,600	25,600	—	—	200
Pigeon-typhus	51,200	25,600	25,600	—	—	—
Mouse-typhus I	51,200	25,600	25,600	—	—	—
Hog-cholera XII	51,200	400	200	—	—	—
" XIII	51,200	400	200	—	—	—
B. schottmülleri 232	51,200	400	200	100	—	—
" " 242	51,200	400	400	—	—	—
" " 225	51,200	200	800	100	—	—

TABLE III.

Absorption of Agglutinins from B. schottmülleri Serum by Calf-Typhus Cultures.

Culture tested.	Titer limit of serum of Rabbit 10, immune to *B. schottmülleri* 232, absorbed with.		
	Nothing; *i.e.*, control.	Calf-typhus V.	Calf-typhus I.
Calf-typhus I	12,800	200	400
" IV	12,800	100	100
" V	12,800	200	400
Swine-typhus I	12,800	200	400
B. schottmülleri 225	25,600	25,600	25,600
" " 232	25,600	25,600	25,600

animal cultures without removing those for the human cultures, and, *vice versa*, *Bacillus schottmülleri* will remove from the sera of animals immune to the animal cultures the agglutinin for the human cultures

and will not remove those for the animal strains. The calf and swine cultures are identical in this respect as is shown in Tables II and III.

While the great majority of the agglutination tests was made with sera of animals immunized by the injection of living bouillon cultures, the possibility that sera produced by the injection of heated cultures might differentiate the two groups has also been considered. The serum of an animal immunized by three injections of a suspension of agar slant growth, killed by heating to 60°C. for 1 hour, agglutinated both groups of bacilli to the same degree. When sera of animals immunized by bacilli heated to 70° for 1 hour were tested, it was found that the bacilli of the immunizing group were agglutinated in a somewhat higher dilution than were those of the other group. The difference was not great enough to make it a valuable means of differentiation. Better results might be obtained by using heated and washed bacilli, but this has not been tried.

Since the type of clumping indicates that the difference in these two groups lies in the flagella, antigens were prepared by shaking suspensions of the bacilli for a short time, centrifugalizing, and using the supernatant fluid for precipitation and complement fixation tests. When the antigen belonged to the same group as the immune serum, the precipitate was more flocculent than when it belonged to the other group, but the amount of precipitation was about the same. When such antigens were used for complement fixation, the inhibition of hemolysis was the same with sera of rabbits immune to either group. Formalinized bouillon cultures used as antigens in complement fixation tests were likewise of no value in differentiating the two groups. These results agree with those of the previous paper where extracts of the bacilli were used as antigens.

The results of the serological tests show that the animal paratyphoids which are usually agglutinated to the titer limit in sera of animals immune to *Bacillus schottmülleri* can best be separated from the latter by agglutination absorption tests. With the methods commonly used in agglutination tests they would be classed as *Bacillus schottmülleri*.

Cross-Immunization Tests.

In the previous paper it was noted that the injection of living cultures of the swine paratyphoids immunized rabbits to a virulent hog-cholera bacillus, whereas rabbits treated in the same way with living cultures of human paratyphoid were not immune. This seemed at that time to indicate that the swine cultures were more closely related to, or possibly were a variety of, the hog-cholera bacillus. These tests have been repeated and the results are given in Table IV.

It will be seen that the calf cultures also immunize rabbits to the hog-cholera bacillus, whereas the human cultures do not. Examination of the records shows that the injection of the animal cultures produces a more severe type of reaction than does the injection of the human cultures. The local lesion is larger and the rise in temperature following a subcutaneous reaction is higher.

Two rabbits were each given a subcutaneous injection of 0.1 cc. of a 24 hour bouillon culture of two strains of human paratyphoid and were chloroformed 1 week later. There was a slight local lesion from which the organisms injected were cultured. The spleen and other organs were normal and cultures from as much as 0.5 cc. of blood, a piece of liver as large as a pea, and stab cultures from the spleen were sterile. Pea-sized bits of spleen from one rabbit showed no organisms, while from the other there was a growth which proved to be due to the organism injected. 1 week after an intravenous injection of 0.1 cc. of 24 hour bouillon cultures, two other rabbits were chloroformed and their spleens were found to be slightly enlarged. Cultures from the spleen of one of these rabbits showed a growth due to the organism injected, whereas the spleen of the other animal failed to show such organism. The blood, liver, and bile of both rabbits were sterile.

These results show that there is very little growth of the human paratyphoid bacilli in rabbits when they are used in the same amounts that will produce a general invasion by the animal cultures. It therefore seems probable that the immunity to the hog-cholera bacillus produced by the animal paratyphoids is due to the fact that they multiply in the body and increase the resistance enough so that the animals are able to withstand the hog-cholera bacilli injected, when the amount of the latter used is about ten times the minimal lethal dose.

TABLE IV.

Test of Power of Various Paratyphoids to Immunize Rabbits to a Virulent Hog-Cholera Bacillus Culture.

Rabbit No.	Inoculated with.	1st injection.	2nd injection.	3rd injection.	Weight Nov. 18.	Result of subcutaneous injection of 0.000001 cc. of 24 hr. bouillon culture of Hog-cholera XII. Rabbit series, Nov. 18, 1919.
		1919	*1919*	*1919*	*gm.*	
1	Swine-typhus II, bouillon culture.	Oct. 4. 0.1 cc. subcutaneously.	Oct. 25. 0.01 cc. intravenously.	—	2,523	Lived.
2	Swine-typhus IV, bouillon culture.	Oct. 4. 0.1 cc. subcutaneously.	Oct. 25. 0.01 cc. intravenously.	—	2,700	"
3	Calf-typhus I, bouillon culture.	Oct. 4. 0.1 cc. subcutaneously.	Oct. 25. 0.01 cc. intravenously.	—	2,164	"
4	Calf-typhus III, bouillon culture.	Oct. 4. 0.1 cc. subcutaneously.	Oct. 25. 0.01 cc. intravenously.	—	2,684	"
5	*B. schottmülleri* 232, bouillon culture.	Oct. 4. 0.1 cc. subcutaneously.	Oct. 25. 0.01 cc. intravenously.	—	2,509	Death in 7 days.
6	*B. schottmülleri*, 242, bouillon culture.	Oct. 4. 0.1 cc. subcutaneously.	Oct. 25. 0.01 cc. intravenously.	—	2,561	" " 10 "
7 Control.	Calf-typhus III, suspension of agar growth, heated 1 hr. at 70°.	Oct. 17. 0.5 cc. subcutaneously.	Oct. 24. 1 cc. subcutaneously.	Nov. 1. 2 cc. subcutaneously.	2,520	" " 9 "
8 Control.	Swine-typhus II, suspension of agar growth, heated 1 hr. at 70°.	Oct. 17. 0.5 cc. subcutaneously.	Oct. 24. 1 cc. subcutaneously.	Nov. 1. 2 cc. subcutaneously.	2,752	" " 6 "
9 Control.	No previous treatment.	—	—	—	2,453	" " 8 "

The intimate relation of these organisms is shown by cross-immunization tests made with mice. These animals were immunized at the same time by subcutaneous injections of 0.0001 cc. followed by intraperitoneal injections of 0.005 cc. of 24 hour bouillon cultures. After they had recovered from the latter injection their immunity towards other cultures was tested by intraperitoneal injections of 0.005 cc. of 24 hour bouillon cultures. The results of the test are given in Table V and show that the animal and human cultures immunize against one another and that the calf culture immunized against the swine and *vice versa*.

TABLE V.

*Test of Cross-Immunity of Mice to Paratyphoids of Other Animals.**

Mice immune to.	Result of intraperitoneal injection of 0.005 cc. of 24 hr. bouillon culture.					
	Calf-typhus V.		*B. schottmülleri* 232.		Swine-typhus V.	
	Lived.	Died.	Lived.	Died.	Lived.	Died.
Calf-typhus V	4	0	4	0	4	0
B. schottmülleri 232	3	1	4	0	4	0
Swine-typhus V	3	0	3	0	3	0
Controls. No previous treatment	0	4	1	3	0	4

* See text for methods of immunization.

DISCUSSION.

The question arises whether we should place these animal paratyphoids, that culturally are the same as *Bacillus schottmülleri*, in a separate group because they fail to absorb the agglutinins from the serum of animals immune to the latter. This failure to absorb agglutinins seems to be a very fundamental difference and these organisms should be regarded as a distinct variety of paratyphoid. No differences either cultural or serological have been detected between the strains derived from swine, calves, and the few strains from the other species that have been studied.

A common name is desirable for this group of organisms and if it is found, upon further study, that these organisms are the same as the

Aertrycke bacillus, isolated by de Nobele (4) from an outbreak of food poisoning, the name of *Bacillus aertryckei* would be appropriate. It is possible that there is one host that harbors these organisms and from it the other animals become infected. If this should prove to be the case, a name indicating this host would be the logical one. On the other hand, the naming of the animal from which a particular culture is isolated is desirable and for the present I propose that they be called typhus with a prefix denoting the animal from which they were isolated. The name typhus has only its long use to commend it, as the organisms are not like *Bacillus typhosus*, nor do they produce a disease that very closely resembles typhoid fever. Another objection is that in the cultures from the smaller animals the word typhus has been used in connection with the paratyphoid disease that is so common. In my experience most of these cultures from mice and guinea pigs belong to the *enteritidis* group and can be separated by their specific agglutination characters, though organisms of the group under consideration also occur. *Bacillus enteritidis* also occurs in the larger animals. I have found it in one pig, Jensen (5), Meyer, Traum, and Roadhouse (6), and others have found it in calves with diarrhea, and Graham, Reynolds, and Hill (7) have found it in an acute disease of horses. In the future it would be well to call these *Bacillus enteritidis* and reserve the use of mouse-typhus, guinea pig-typhus, etc., for the organisms that we have considered in this paper.

It is evident that the group that has been considered in this paper has been encountered before. I have already (1) pointed out that many of the strains of so called *Bacillus suipestifer* probably belong to this group. Bock (8) noted that when he saturated the sera of animals immune to mouse-typhus, *Bacillus suipestifer*, or bacilli obtained from outbreaks of food poisoning with *Bacillus schottmülleri* the agglutinins for the last bacillus were removed while those for the first three organisms were not affected. Sobernheim and Seligmann (9) in studying paratyphoid bacilli noted that three cultures classed as *Bacillus schottmülleri* formed fine clumps in the serum of an animal immune to *Bacillus schottmülleri*. They immunized a rabbit to one of these strains heated to 70°C. and found that the serum agglutinated the three cultures to the same degree while the other *Bacillus schottmülleri* cultures were agglutinated in only the lower dilutions of serum. Especially significant

are the observations of Bainbridge and O'Brien (10). They compared cultures from cases of food poisoning with those from undoubted cases of paratyphoid fever. In *Bacillus schottmülleri* sera the former produced fine clumps and did not absorb the agglutinins for the immunizing strains, whereas the latter formed flocculent clumps and absorbed all the agglutinins from the serum. Their control cultures of *Bacillus suipestifer* acted the same as those from food poisonings, but as these controls were obtained from German laboratories it is probable that they were the same as those I have called swine-typhus. Krumwiede, Valentine, and Kohn (11) separated from *Bacillus schottmülleri* by absorption tests a number of organisms obtained from rodents which probably belong to this group.

As noted above, when suspensions of agar slant growth or killed cultures are used as antigens in agglutination tests the difference between these animal cultures and *Bacillus schottmülleri* could not be detected and it seems possible that the so called paratyphoid B bacilli that Jensen (5), Christiansen (12), and others have associated with diarrhea in calves are the same as the organisms I have called calf-typhus. Many of the paratyphoid B bacilli isolated from food poisonings quite possibly belong to the same group but from the literature one cannot draw any conclusions as the diagnosis has usually been made on the agglutination test without supplementary absorption tests. This subject is important because we want to know what type of infections man gets from the lower animals.

There are several well recognized groups of pathogenic animal paratyphoids besides *enteritidis* and the group considered here. The hog-cholera bacillus, or better, *Bacillus cholerœ suis*, described by Smith (13), differs from the others by being highly pathogenic for rabbits. More recently (1) it has been shown to have distinct cultural differences from *Bacillus schottmülleri*. The Voldagsen bacillus described by Dammann and Stedefeder (14) and the "Ferkel typhus" bacillus described by Glässer (15) seem to be identical. They are differentiated from the other paratyphoids by their failure to act on mannitol and the fact that they produce little or no gas. *Bacillus abortus equi*, first described by Smith and Kilborne (16) and later studied by Meyer and Boerner (17) and others, resembles *Bacillus schottmülleri* culturally, except that it fails to produce hydrogen sulfide, and on agar forms a

dry brittle growth. Serologically Meyer and Boerner, and Murray (18) place it in a group by itself. In its virulence for rabbits Smith and Kilborne pointed out that it resembled a mildly virulent hog-cholera bacillus.

Jordan (19) and Reerstorp (20) have found a variety of so called intermediate paratyphoids in the intestinal tract of normal swine, and from children Lewis (21) and others have obtained paratyphoids which have been classified culturally by Graham-Smith (22). It is difficult to determine just what relation these paratyphoids of the normal digestive tract bear to the established groups. They differ serologically and culturally from the members of these several groups but it is conceivable that under certain conditions they might invade the body and change their characters.

Smith and Reagh (23) discussed the possibility of the host changing the agglutinative characters of organisms and such a possibility should be considered here. After passing from animal to man and becoming adapted to the latter, it is quite possible that organisms might change both their cultural and agglutinative characters. Careful study of food poisoning outbreaks due to eating meat containing these animal paratyphoids might throw some light on this subject. If such a change does occur it would result in much confusion. I have from time to time modified slightly the cultural characters of some of these paratyphoids by passage through animals, but on the whole the cultural and especially the agglutination characters are remarkably constant.

CONCLUSIONS.

1. In addition to the paratyphoid bacilli already named there exists a group which occurs in a variety of animals and which culturally is the same as *Bacillus schottmülleri*. As a rule this group can be separated from the latter by the type of clumps formed when bouillon cultures are used as antigens, while other antigens and complement fixation tests have failed to differentiate it. Agglutination absorption tests sharply separate the animal from the human paratyphoids.

2. No differences have been detected between organisms of this group derived from a number of animals and a common name for them is desirable, but for the present it seems better to call them calf-,

swine-, mouse-, etc., typhus, according to the animal from which they were isolated.

3. Evidence exists in the literature that these organisms have been associated with food infections in man, particularly with what have been called paratyphoid B infections, but this function, as well as the part they play in animal diseases, is a subject for further study.

4. Well defined groups of paratyphoid such as *Bacillus cholerœ suis*, the Voldagsen bacillus, *Bacillus abortus equi*, and *Bacillus enteritidis* are found in animals in addition to the organisms considered in this paper, and every attempt should be made to range newly isolated organisms in one or the other of these well recognized groups.

5. One of the objects in continuing this work was to find a method of differentiating these animal from the human paratyphoids less complicated than agglutination absorption. This object was not realized; the two groups are very similar and agglutination absorption seems to be the only means of classifying them.

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