A STUDY OF PARATYPHOID BACILLI ISOLATED FROM CASES OF HOG-CHOLERA.

By CARL TENBROECK, M.D.

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

(Received for publication, September 25, 1918.)

Many swine infected with hog-cholera undoubtedly find their way into our food supply and it would seem that a thorough knowledge of the bacteriology of the disease is of importance from a practical as well as from a theoretical point of view. In the earlier days of the work on this disease attention was chiefly confined to the identification of the hog-cholera and swine-plague bacilli, while since the discovery that a filterable virus is the cause of the condition the bacteriology has to a considerable extent been poorly done or neglected altogether. These so called secondary invaders probably vary with the locality from which the animals are obtained, and also with the virulence and possibly other factors associated with the virus. Accordingly the findings I have to report are a contribution to the bacteriology of the disease rather than a bacteriological study, for only two strains of virus have been used and the majority of the animals came from a single herd. *Bacillus coli*, *pyocyaneus*, *alkaligenes*, several species of anaerobes, and several species of unidentified aerobes have been isolated, but this report will be confined to a description of the paratyphoid bacilli found, because of their economic importance, in that they may be the cause of disease in man, and because they bring up some interesting points in the classification of this group.

HISTORICAL.

The classification of the paratyphoid group is extremely difficult and confusing, for, until recently, there have been no cultural differences between members of the group that could be relied upon, cross-agglutination reactions are common, and animal inoculations have not been made. Dr. Smith (1) stated that the only reliable means of differentiating the hog-cholera bacillus from closely related forms

was by inoculation into rabbits. The former caused a definite disease after subcutaneous injection, resulting in death in from 7 to 12 days with marked changes in the lymphoid tissue, and in freshly isolated cultures with necroses in the liver. The failure to make rabbit inoculations probably accounts for the failure to differentiate the hog-cholera bacillus from paratyphoid β in spite of the statement of Uhlenhuth, Hübener, Xylander, and Bohtz (2) that such inoculations are of no value in differentiating the two.

Dr. Smith noted that the hog-cholera bacillus grew less readily in bouillon than did typhoid or other paratyphoids. Ford (3) suggested that the hog-cholera bacillus could be differentiated from the other members of the group by its failure to ferment arabinose. Jordan (4) and Krumwiede, Kohn, and Valentine (5) have recently shown that the hog-cholera bacillus cultures which they have studied failed to ferment arabinose and in addition that dulcite was fermented little or not at all, and the latter authors have extended the observations of Weiss and Rice (6) on inosite and they show that paratyphoid β is the only member of the group that ferments this carbohydrate. Jordon and Victorson (7) show that the hog-cholera bacillus differs from paratyphoid β in that it fails to form hydrogen sulfide in peptone agar containing lead acetate, whereas the latter and *B. enteritidis* promptly cause a blackening of the medium. A reaction that is somewhat similar is noted by Krumwiede, Kohn, and Valentine (5). They find that in a serum water containing 0.1 per cent dextrose and 1 per cent of Andrade indicator paratyphoid α and the hog-cholera bacillus fail to reduce the fuchsin while paratyphoid β and *enteritidis* give a colorless or at most a faintly pink coagulum at the end of 24 hours incubation.

By means of the agglutination reaction Uhlenhuth, Hübener, Xylander, and Bohtz (2), Savage (8), Jordan (4), and Krumwiede, Kohn, and Valentine (5) agree that paratyphoid α and β and *enteritidis* can be differentiated from one another, but the first authors state that paratyphoid β and hog-cholera bacillus cannot be differentiated by this reaction, while the others say that they can, especially when absorption tests are made.

When one examines the literature of the paratyphoid bacteria, it is found that most workers have considered all members of this group to be hog-cholera bacilli, no matter whether they came from normal or diseased swine. Grabert (9) is often cited as having isolated hog-cholera bacilli from normal swine but the only carbohydrates he used were dextrose, lactose, and saccharose, and in immunizing rabbits two animals survived the injection of 0.01 cc. of a bouillon culture intravenously, which goes a great way towards showing that he did not have typical hog-cholera bacilli. Seiffert (10) compared three strains of what he calls hog-cholera bacilli with paratyphoid β and found that they were the same. It should be noticed, however, that he made no tests for pathogenicity and two of the strains formed acid and gas from arabinose, while the other was not tested, and all of them formed hydrogen sulfide. Not one of the tests used by Uhlenhuth, Hübener, Xylander, and Bohtz would differentiate

the hog-cholera bacilli from other paratyphoids, and as they did not make rabbit inoculations it is impossible to say whether they were working with the true hog-cholera bacillus or with another member of the group. Krumwiede, Kohn, and Valentine (5) seem to think that the hog-cholera bacilli are the only paratyphoids found in swine, for, after showing that paratyphoid β and the hog-cholera bacillus can be separated both culturally and serologically, they conclude that the former is found only in man.

EXPERIMENTAL.

As the cultures to be described came from swine infected with the virus of hog-cholera it seemed wise to repeat the newer cultural tests for the hog-cholera bacillus. Six strains, all of which had been studied by Dr. Smith and pronounced true hog-cholera bacilli, were used and it was found that all six failed to ferment arabinose in 10 days and all but one failed to ferment dulcite in the same time. This one culture which did ferment dulcite failed to do so after it had been passed through a pig, showing how unstable this property is. All the cultures failed to produce a brown color in peptone-lead acetate agar and all gave a decidedly pink color in Krumwiede's dextrose-Andrade-serum water.

The cultures to be described were isolated during the course of some experimental work on hog-cholera in which two strains of virus were used. One of the strains came from Dr. V. A. Moore, while the other was obtained from a spontaneous outbreak in New Jersey. During the work thirty-eight pigs were used, weighing between 20 and 45 pounds. The majority of the animals were raised on the Institute farm where their parents had been isolated from other swine for 3 years. Thirty-three of the pigs were infected by exposure or by the inoculation of bacteria-free material containing the virus, while the remaining five were spontaneous cases obtained from nearby places. Most of the animals were killed in from 7 to 12 days after their temperature began to rise, while some were allowed to die. All showed characteristic lesions of hog-cholera and the sera of many of them were used to infect other pigs.

Cultures were made as a routine from the liver, spleen, and kidney, after searing the surface of the organs, by digging out bits of tissue with flamed forceps and transferring these to agar slants and often-

TABLE I.

Source of Cultures.

Culture No.	From.		History of swine inoculated.			Disposed of.
	Swine No.*	Organ.	Date.	Weight.	Material.	
			1917	*lbs.*		
Swine-typhus I	149	Liver; spleen; kidney.	June 29	18	Fed viscera from spontaneous case of cholera.	July 7. Died. Autopsy 12 hrs. post mortem but animal had been kept cold. Few hemorrhages under capsule of kidney. Fibrinous exudate in cecum. Hemorrhagic pneumonia.
Swine-typhus II	152	Liver; spleen; kidney; lymph node.	July 20		Exposed to spontaneous case.	Aug. 14. Died. Autopsy 5 hrs. post mortem. Typical lesions of hog-cholera.
Swine-typhus III	151	Liver.	Aug. 1	47	Exposed to Swine 152.	Aug. 30. Moribund. Chloroformed and autopsied at once. Typical lesions of hog-cholera.
Swine *enteritidis* I	161	Liver; spleen.	" 21	40	" " fresh spontaneous case of cholera.	Sept. 19. Chronic hog-cholera. Killed. Autopsy shows healing lesions of cholera.
Swine-typhus IV	192	Liver; spleen; kidney.	Dec. 12	21	Inoculated with organ emulsion of rats that had been inoculated with virus 7 days before.	Dec. 19. Sick. Killed. Typical lesions of cholera.
			1918			
Swine typhus V	194	Liver.	Jan. 29	25	Inoculated with filtered emulsion of liver, spleen, and kidney of virus pig.	Feb. 8. Moribund. Chloroformed. Typical lesions of hog-cholera.

* The numbers used belong to a series that was begun many years ago so that they in no way indicate the number of animals used.

times to fermentation tubes containing bouillon. Where growth occurred pure cultures were obtained and from one or more organs of six of the thirty-eight swine bacteria belonging to the paratyphoid group were isolated.

The numbers given to these cultures and a condensed history of the animals from which they were obtained are given in Table I. It is worthy of note that four of the animals were exposed to spontaneous cases of hog-cholera while the other two were purchased from a neighboring farm before inoculation. The animals to which the first four were exposed did not show paratyphoid bacteria in their organs but they must be looked upon as a possible source of the infection. Of a large number of our own animals inoculated with bacteria-free virus not one has shown paratyphoid bacilli in its organs, indicating that they do not carry these organisms in their gastrointestinal tracts.

Cultural Characteristics.

All six cultures resemble one another in that they are motile, Gram-negative rods of about the size of the typhoid bacillus. They grow readily on the ordinary media. On agar they form translucent bluish colonies, while in bouillon they cause a turbidity of about the same density as that caused by the typhoid bacillus. They do not liquefy gelatin or produce indol. They all form acid and gas in fermented bouillon containing 1 per cent dextrose or mannite, while they fail to attack lactose or saccharose. In Durham tubes containing fermented bouillon plus 1 per cent of Andrade indicator and 1 per cent xylose, dulcite, or arabinose they form acid and gas in 24 hours from all three carbohydrates, while neither acid nor gas is produced in salicin bouillon in 10 days. In lead acetate-peptone agar they all produce a brownish color in 24 hours, indicating the formation of hydrogen sulfide. In the medium of Krumwiede made of sterile horse serum one part, sterile distilled water four parts, containing 1 per cent of Andrade indicator and 0.1 per cent dextrose, all six cultures produce in 18 hours a practically colorless coagulum containing gas bubbles. In litmus milk they all give a distinctly alkaline reaction in from 3 to 6 days.

By comparing the cultural reactions of these bacteria with those given for the hog-cholera bacillus it will be seen that there are distinct differences, and from the findings of Jordan and Krumwiede already referred to they would have to be classed as paratyphoid β or *enteritidis*. These two may be differentiated by the fact that the former ferments inosite, but the one test made with the swine cultures was unsatisfactory and no more of the carbohydrate could be obtained.

Pathogenicity.

When injected into the subcutaneous tissues of the rabbit in large numbers these cultures cause a local infection from which the animals recover. The tests are summarized in Table II where it will be seen that 0.1 cc. of a 24 hour bouillon culture failed to kill any of the rabbits.

TABLE II.

Virulence Tested.

Rabbit.		Subcutaneous injection of 0.1 cc. of a 24 hr. bouillon culture of strain.	
No.	Weight.	Culture No.	Effect.
	gm.		
1	2,476	Swine-typhus I	Slight loss in weight with open ulcer at site of inoculation. Recovery.
2	1,572	" I	Slight pyrexia, loss in weight, and local lesion. Recovery.
3	2,505	" II	Slight pyrexia, marked loss in weight, and local lesions. Chloroformed 1 month after inoculation. No lesions found; organs sterile.
4	2,740	" II	Pyrexia, marked loss in weight, and large local lesion. Recovery.
5	2,325	" III	Dead in 6 days. Rabbit septicemia.
6	2,168	" III	Pyrexia, loss in weight, and local lesion. Recovery.
7	2,609	Swine *enteritidis* I	" " " " " " " "
8	2,137	" " I	" " " " " " " "
9	2,500	Swine-typhus IV	Loss in weight and local lesion. Chloroformed 19 days after inoculation. No lesions found and large pieces of liver and spleen were sterile.
10	2,226	" IV	Pyrexia, loss in weight, and local lesion. Recovery.
11	1,167	" V	Amount injected 0.5 cc. Rise in temperature, loss in weight, and large abscess at site of inoculation. Recovery.

In some cases the local lesions were quite extensive and it is probable that had injections been made into the peritoneal cavity death would have resulted. Such injections did not seem necessary as the object was to differentiate these cultures from the hog-cholera bacillus, for, as has been emphasized above, the latter organism always kills the rabbit when injections of 0.1 cc., or even less, of a 24 hour bouillon culture are made into the subcutis. It should also be noted that two of the animals were killed, one 19 and the other 30 days after the injection and that no lesions were found and cultures made by transferring large bits of liver and spleen to bouillon remained sterile, indicating that there had been no general invasion of the body of the animals.

Four mice were fed a bouillon culture of one of these strains (Swine-typhus III). 6 days later one was killed, and though it was apparently well, cultures from the liver and spleen showed a paratyphoid bacillus that was probably the organism ingested. The remaining three animals were not visibly affected by the feeding.

White rats were inoculated with 0.1 cc. of 24 hour bouillon cultures of four of these strains. They showed a slight loss in weight and a local lesion, but they had apparently recovered at the end of 22 days, when they were killed and cultures made from their livers and spleens. Organisms of the paratyphoid group were obtained from two of these rats.

A pig weighing 37 pounds was fed 50 cc. of a 24 hour bouillon culture of Swine-typhus III mixed with ground food, all of which was eaten. The animal showed no effect from the feeding either in its temperature, its appetite, or the consistency of its feces for the next 8 days. It was then inoculated with virulent hog-cholera virus and killed 9 days later when it was very sick. Autopsy showed the typical lesions of hog-cholera. Cultures made from bits of the liver, spleen, and kidney were sterile, showing either that the bacillus was not present in the digestive tract or, if present, it did not have the power to invade the body as does the hog-cholera bacillus when it is fed to a pig and the animal later is inoculated with virus.

The results of the animal inoculations show that the six cultures are not nearly so pathogenic for rabbits as are the hog-cholera bacilli. My experience with paratyphoid β is limited but it seems that these

swine cultures produced larger local lesions than the former does, but this may be due to the fact that the swine cultures have been isolated more recently than the human strains worked with.

Serological Tests.

In preparing animals for immune sera the rabbits used in testing the virulence of the different strains of swine cultures were utilized. After they had recovered from the subcutaneous injection of 0.1 cc. of a 24 hour bouillon culture they were given an intravenous injection of 0.01 cc. of a bouillon culture of the same age. 10 days after the last injection they were bled. This method of treatment gave sera of high agglutination titer but it is possible that such sera differ from those produced by the injection of large numbers of bacteria killed by heat. Uhlenhuth and Hübener (11) state that they get better agglutinating sera for the paratyphoid group by immunizing with killed than with living cultures. Though the organisms are motile they say that the type of agglutination is not characteristic and that all gradations between fluffy and fine clumps may be observed.

When tested against sera produced by the injection of living cultures it was found that one of the strains was sharply marked off by the fact that it failed to be agglutinated by the sera of animals immune to the others and the serum of a rabbit immune to it did not agglutinate the others. The data substantiating these statements are found in Table III.

The serum of this rabbit immune to Swine *enteritidis* I was then tested on other paratyphoids from animals and man and it was shown that the culture is one of *Bacillus enteritidis*, for its serum agglutinates other *enteritidis* cultures as well as the majority of the animal cultures. These consist of two mouse, four rat, three guinea pig, and one dog typhus and two human *enteritidis* cultures. Absorption tests show that these animal and human strains will absorb the agglutinins for the swine as well as for the other strains that are agglutinated by this serum.

The other five strains, which for convenience have been called swine-typhus, appear to be the same serologically as well as culturally. They are agglutinated to the same titer limit and any one of them will absorb the agglutinins for the others.

TABLE III.

Agglutination of Swine Cultures by Sera of Rabbits Immune to Strains That Differ Serologically.

Culture tested.	Limit of agglutination with serum of rabbit.	
	Immune to Swine-typhus III.	Immune to Swine *enteritidis* I.
Swine-typhus I	51,200	100−*
" II	51,200	100−
" III	51,200	80
Swine *enteritidis* I	1,600	51,200
Swine-typhus IV	51,200	100−
" V	12,800+	320
Hog-cholera Arkansas	1,600	80−
" XII	1,600	160−

* Figures followed by a minus sign indicate that there was no agglutination in this the lowest dilution used.

Relation of Swine-Typhus to Hog-Cholera Bacilli.

Though cultural differences have been found between these cultures and those of the hog-cholera bacillus, it is interesting to compare the two serologically.

The agglutination relationship to various hog-cholera bacillus cultures is shown in Table IV, where it will be seen that there is a certain amount of cross-agglutination but the limit of clumping is much lower than in the control strains, and in addition the sediment, after the tubes have been incubated 2 hours and refrigerated over night, is firm and compact, whereas with the homologous strains it is loose and fluffy.

Absorption tests show that cultures of Swine-typhus I and II will take from the anti-hog-cholera bacillus serum the agglutinins for themselves and Swine-typhus V, although the agglutinins for the hog-cholera bacilli are practically unchanged, and that the hog-cholera bacilli will take from the Anti-swine-typhus III serum the agglutinins for themselves without removing those for Swine-typhus III. These facts are shown in Table V.

Thus we see that while there is a certain relationship, as shown by

TABLE IV.

Agglutination Relationship of Hog-Cholera and Swine-Typhus Bacilli.

Culture tested.	Limit of agglutination with serum of rabbit.	
	Immune to Swine-typhus III.	Immune to hog-cholera bacillus.
Swine-typhus I	51,200	12,800×*
" II	51,200	12,800×
" III	51,200	6,400×
" IV	51,200	6,400×
" V	12,800+	1,600×
Hog-cholera bacillus Nebraska	1,600×	
" " Denver	3,200×	
" " Arkansas	3,200×	25,600+
" " X	400×	
" " XI	1,600×	
" " XII	1,600×	12,800

* × indicates that the sediment was firm and compact, +, the highest dilution tested, clumping very marked.

TABLE V.

Absorption of Agglutinins from Hog-Cholera Bacillus and Swine-Typhus Sera by Swine-Typhus and Hog-Cholera Bacilli Respectively.

Serum.			Cultures tested with highest dilution in which agglutination occurred.				
Rabbit No.	Immune to.	Absorbed with.	Swine-typhus II.	Swine-typhus I.	Swine-typhus V.	Hog-cholera Arkansas.	Hog-cholera XII.
12	Hog-cholera bacillus, Arkansas and Nebraska.	Nothing; *i. e.*, control.	12,800	12,800	1,600	25,600+	12,800
12	" "	Swine-typhus I	200	200−	200−	20,000	5,000+
12	" "	" II	200	200	200−	20,000	20,000
12	" "	Hog-cholera XII				500	200−
2	Swine-typhus I	Nothing; *i. e.*, control.		6,400+	6,400+	6,400+	
2	Swine-typhus I	Hog-cholera Arkansas.		6,400+	6,400+	100	

the cross-agglutination between the hog-cholera bacilli and these swine paratyphoids, they can readily be distinguished by the type of clumps formed and by absorption tests.

Relation to Paratyphoid β from Man.

As these strains as far as they have been studied culturally are identical with what Jordan (4) and Krumwiede, Kohn, and Valentine (5) regard as typical paratyphoid β, it is of interest to compare serologically the cultures from man with those from swine. Four paratyphoid β cultures which had been isolated comparatively recently were obtained from Dr. Krumwiede, and when tested it

TABLE VI.

Agglutination of Paratyphoid β Bacilli by Swine-Typhus Sera.

Culture tested.	Limit of agglutination in serum of.			
	Rabbit 13, immune to Swine-typhus I.		Rabbit 6, immune to Swine-typhus III.	
	Limit of agglutination.	Type of sediment.	Limit of agglutination.	Type of sediment.
Swine-typhus I	25,600	Fluffy.		
" III	25,600	"	51,200	Fluffy.
Paratyphoid β 225	6,400	Compact.	1,600	Compact.
" β 232	12,800	"	1,600	"
" β 234	6,400	"	1,600	"
" β 246	12,800	"	800	"

was found that they did not agglutinate to the titer limit with the serum of rabbits immune to the swine-typhus cultures. This is shown in Table VI. The numbers of the paratyphoid cultures are the ones used by Krumwiede, Kohn, and Valentine (5).

Not only do these human cultures fail to agglutinate to the titer limit of the sera but the clumps when first formed are small and granular and after standing in the refrigerator over night the sediment is compact, resembling that formed by non-motile bacteria.

Rabbits were at once immunized against two of the human paratyphoid strains and the swine cultures tested, with the results given in Table VII. It will be seen that the swine cultures fail to agglu-

TABLE VII.

Agglutination of Swine-Typhus Bacilli by Paratyphoid β Sera.

Culture tested.	Limit of agglutination in serum of.			
	Rabbit 14, immune to Paratyphoid β 225.		Rabbit 15, immune to Paratyphoid β 246.	
	Limit of agglutination.	Type of sediment.	Limit of agglutination.	Type of sediment.
Paratyphoid β 225	25,600	Fluffy.	25,600	Fluffy.
" β 246	25,600	"	25,600	"
Swine-typhus I	6,400	Compact.	25,600	Compact.
" II	6,400	"	25,600	"
" III	6,400	"	25,600	"
" IV	6,400	"	25,600	"
" V	3,200	"	6,400	"

TABLE VIII.

Absorption of Agglutinins from Swine-Typhus and Paratyphoid β Sera by Paratyphoid β and Swine-Typhus Bacilli Respectively.

Serum.			Cultures tested with highest dilution in which agglutination occurred.			
Rabbit No.	Immune to.	Absorbed with.	Swine-typhus I.	Swine-typhus III.	Paratyphoid β 232.	Paratyphoid β 246.
13	Swine-typhus I	Nothing; *i. e.*, control.	25,600	25,600	12,800	12,800
13	" I	Paratyphoid β 232	25,600	25,600	200—	200—
13	" I	Paratyphoid β 246	25,600	25,600	200—	200—
			Swine-typhus I.	Swine-typhus III.	Paratyphoid β 225.	Paratyphoid β 246.
14	Paratyphoid β 225	Nothing; *i. e.*, control.	6,400	6,400	25,600	25,600
14	" β 225	Swine-typhus I	200—	200—	12,800	12,800
			Swine-typhus II.	Swine-typhus IV.	Paratyphoid β 225.	Paratyphoid β 246.
15	Paratyphoid β 246	Nothing; *i. e.*, control.	25,600	25,600	25,600	25,600
15	" β 246	Swine-typhus II	200—	200—	6,400	12,800
15	" β 246	" IV	200	200	12,800	12,800

tinate to the titer limit in one serum, while in the other they do agglutinate to the titer limit, but in both sera the sediment is compact, while the sediment formed by the human culture is fluffy, resembling a mass of loose cotton.

Absorption tests, the results of which are given in Table VIII, show that the swine-typhus cultures will not absorb the agglutinins from the human paratyphoid β sera, nor will the paratyphoid β bacilli absorb the agglutinins from the swine-typhus serum.

TABLE IX.

History of Cultures Which Resemble Swine-Typhus Cultures in Their Agglutination.

Culture tested.	History.					Agglutinated by swine-typhus serum, titer 51,200.
	Isolated by.	Date.	Locality.	Source.	Diagnosis.	
Paracolon VI	Dr. N. B. Wherry.	1908	San Francisco, Cal.	Liver of 3 yr. boy.	Status lymphaticus.	25,600
Mouse-typhus I	Dr. Marshal Fabyan.	1911	Mass.	Mouse.	Mouse typhoid.	51,200
Guinea pig-typhus I	Dr. Theobald Smith.	1895	"	Guinea pig.	Pseudotuberculosis.	51,200
Guinea pig-typhus IV	Dr. P. A. Lewis.	1908	"	" "		51,200
Cattle-typhus.	Dr. Mohler.	1904	Washington, D. C. (?).	Cow's brain.	Suspected rabies.	25,600
Pigeon-typhus.	" "	1904	New Jersey.	Heart's blood of pigeon.	Infectious enteritis.	25,600

We have seen that absorption tests show that the hog-cholera bacilli and paratyphoid β differ from these swine-typhus cultures, and the question arises as to whether the last are distinctly different from the paratyphoids that are so widely distributed among various animals. With this question in mind the series of stock cultures collected by Dr. Smith was tested against one of the anti-swine-typhus sera and six cultures were found that agglutinated to the titer limit of the serum and that gave the characteristic fluffy clumps formed by the immunizing strain. It is interesting to note that the cultures from the boy, pigeon, and cow had been sent in as possible

hog-cholera bacilli on account of their unusual virulence for laboratory animals. Culturally they correspond to paratyphoid β or to the swine-typhus cultures and not to the hog-cholera bacillus. The source of these cultures and the date of their isolation are given in Table IX.

It will be seen that the cultures have come from a variety of animals and from widely different localities in the United States. They not only form characteristic clumping in the serum of rabbits immune to the swine-typhus cultures but they absorb the agglutinins for the immunizing culture as is shown in Table X. The

TABLE X.

Absorption of Agglutinins from Swine-Typhus Serum by Paratyphoids from Other Species.

Swine-typhus serum.	
Absorbed with.	Limit of agglutination for immunizing strain.
Nothing; *i. e.*, control	51,200
Paracolon VI	2,500
Mouse-typhus I	2,500
Guinea pig-typhus I	800
" " IV	500
Cow-typhus	400
Pigeon-typhus	3,200

absorption has not been complete in any case but this is probably due to the facts that the serum was of very high titer and not enough bacteria were added. The reduction is, however, great enough to show that an absorption has taken place.

Complement Fixation Experiments.

Another method of studying the relationship of such closely allied groups of organisms is by means of the complement fixation test. Antigens were prepared from two paratyphoid β strains, two swine-typhus strains, and two hog-cholera bacilli cultures as follows:

The growth from one Blake bottle agar slant was suspended in 10 cc. of sterile distilled water and transferred to a sterile bottle

that was tightly closed. The bottles were heated 1 hour in a water bath at 60°C. and then shaken ½ hour, after which they were placed in the refrigerator for 7 days. They were again shaken for ½ hour

TABLE XI.

Complement Fixation Tests Using Swine-Typhus Antigens.

0.05 cc. of serum.		Readings and amounts of antigen.						
Rabbit No.	Immune to.	Swine-typhus I.			Swine-typhus III.			
		0.005 cc.	0.0025 cc.	0.00125 cc.	0.01 cc.	0.005 cc.	0.0025 cc.	0.00125 cc.
13	Swine-typhus I	0	++	++++	0	0	0	++++
16	" III	0	++	C.	0	0	++	++++
15	Paratyphoid β 246	0	++	"	0	0	++	C.
14	" β 225	0	+++	"	0	0	+++	"
12	Hog-cholera Arkansas and Nebraska.	+++	C.	"	C.	C.	C.	"
17	Hog-cholera XII	C.	"	"	"	"	"	"
18	*B. pullorum* III	"	"	"	"	"	"	"
19	Normal.	"	"	"	"	"	"	"

TABLE XII.

Complement Fixation Tests Using Paratyphoid β Antigens.

0.05 cc. of serum.		Readings and amounts of antigen.						
Rabbit No.	Immune to.	Paratyphoid β 225.			Paratyphoid β 246.			
		0.005 cc.	0.0025 cc.	0.00125 cc.	0.01 cc.	0.005 cc.	0.0025 cc.	0.00125 cc.
13	Swine-typhus I	0	0	+++	0	0	0	0
16	" III	0	0	++	0	0	0	0
15	Paratyphoid β 246	0	0	+++	0	0	0	++
14	" β 225	0	0	+++	0	0	0	++
12	Hog-cholera Arkansas and Nebraska.	+++	C.	C.	0	C.	C.	C.
17	Hog-cholera XII	C.	"	"	++++	"	"	"
18	*B. pullorum* III	"	"	"	++	"	"	"
19	Normal.	"	"	"	C.	"	"	"

and placed in the refrigerator for 7 days. At the end of this time 9 cc. of each suspension were added to 1 cc. of 8 per cent sodium chloride solution in a tube and centrifugalized at high speed

for 1 hour. These gave slightly opaque solutions that, on account of their high complement-fixing properties, had to be diluted before they could be used. The hemolytic system used was anti-sheep corpuscle rabbit serum, washed sheep corpuscles, and guinea pig

TABLE XIII.

Complement Fixation Tests Using Hog-Cholera Bacillus Antigens.

0.05 cc. of serum.		Readings and amounts of antigen.							
		Hog-cholera XII.				Hog-cholera Arkansas.			
Rabbit No.	Immune to.	0.01 cc.	0.005 cc.	0.0025 cc.	0.00125 cc.	0.01 cc.	0.005 cc.	0.0025 cc.	0.00125 cc.
13	Swine-typhus I	0	++++	C.	C.	++	C.	C.	C.
16	Swine-typhus III	C.	C.	"	"	+++	"	"	"
15	Paraty-phoid β 246	0	C.	C.	C.	0	++++	C.	C.
14	Paraty-phoid β 225	++++	"	"	"	+++	C.	"	"
12	Hog-cholera Arkansas and Nebraska.	0	+	++++	C.	++	+++	C.	C.
17	Hog-cholera XII	0	0	0	++++	0	++	++++	"
18	*B. pullorum* III	C.	C.	C.	C.	C.	C.	C.	C.
19	Normal.	"	"	"	"	"	"	"	"

complement. The hemolytic amboceptor, complement, and antigens were titrated and all the controls made, but only the actual results of the test are given in Tables XI to XIII. The method used was to decrease the amounts of antigen and use a constant amount of serum. The readings given are those of hemolysis, 0 indicating no

hemolysis or complete fixation of complement, ++ slight, +++ moderate, ++++ almost complete, and C. complete hemolysis.

The results show that the swine-typhus and paratyphoid β antigens act in practically the same manner, causing fixation to occur to about the same degree with sera of animals immune to either group, and causing little or no cross-fixation with sera of rabbits immune to the hog-cholera bacillus. The hog-cholera bacilli antigens are more irregular in their action, for they cause some fixation when used with the sera of rabbits immune to either the swine-typhus or the paratyphoid β cultures, but fixation with hog-cholera sera takes place with higher dilution of the antigens.

In the light of the agglutination tests complement fixation does not give the marked differentiation of the strains that one would expect, but on the contrary, the close interrelation of the members of this group is clearly shown.

Cross-Immunization.

All the above tests show that the five swine-typhus cultures are more closely related to paratyphoid β than they are to the hog-cholera bacillus. It was surprising, therefore, to find that rabbits immune to these swine-typhus cultures were also immune to a virulent hog-cholera bacillus. Animals immune to each of the cultures have been tested and only one, inoculated with Swine-typhus V, died as the result of the injection of hog-cholera bacilli.

The immunity is one of degree only, for while the animals are apparently little affected by the hog-cholera bacilli and live for months, they show upon autopsy a much enlarged spleen, and when large bits of this organ are transferred to bouillon a pure culture of hog-cholera bacillus is obtained. A similar condition in rabbits inoculated with hog-cholera bacilli of low virulence has been described by Dr. Smith (12).

The question naturally arises as to whether paratyphoid β will immunize rabbits to the hog-cholera bacillus, but it will be seen by examining the results given in Table XIV that in two instances the rabbits inoculated with paratyphoid β were not immune, whereas animals treated in the same way with swine-typhus cultures showed

little or no reaction to an inoculation with virulent hog-cholera bacilli. From the results of this experiment it seems that these swine-typhus cultures are more closely related to the hog-cholera bacillus than are the paratyphoid β cultures.

TABLE XIV.

Immunity Tests.

Rabbit No.	Immunization.		Aug. 16, 1918. Subcutaneous injection 1 millionth cc. of 24 hr. bouillon culture of Hog-cholera XII. No. of organisms injected 1,810.	
	Culture used.	Inoculations of bouillon cultures.	Weight.	Result.
		1918	*gm.*	
14	Paratyphoid β 225	July 5. 0.01 cc. subcutaneously. " 22. 0.01 " intravenously.	2,026	Death in 10 days. Typical lesions of hog-cholera bacillus infection.
15	Paratyphoid β 246	" 5. 0.01 " subcutaneously. " 25. 0.01 " intravenously.	1,822	Death in 11 days. Typical findings at autopsy.
13	Swine-typhus I	" 5. 0.01 " subcutaneously. " 25. 0.01 " intravenously.	1,533	Pyrexia, but no loss in weight.
16	Swine-typhus III	" 22. 0.01 " subcutaneously. " 31. 0.01 " intravenously.	1,917	No pyrexia or loss in weight.
20	*B. bronchisepticus.*	" 17. 0.2 " "	1,412	Death in 10 days. Typical lesions.
21	Control.		1,848	Injection one-tenth that given the others. Death in 11 days. Typical lesions.

SUMMARY AND CONCLUSIONS.

During the course of some experimental work on hog-cholera, paratyphoid bacilli were isolated from 16 per cent of the pigs. Culturally these organisms are the same as paratyphoid β isolated from man, while they show several differences from hog-cholera bacilli. In their slight pathogenic effect on rabbits they also differ from the hog-cholera bacillus. In their agglutination in sera produced by the injection of living cultures, one of the cultures, isolated from a chronic case, corresponds to *Bacillus enteritidis*, while the other five are apparently in a class by themselves. They resemble paratyphoid β

more closely than hog-cholera bacilli, but the type of clumps formed and absorption experiments show that they are different from either. Whether these differences are enough to make it necessary to put them into a class by themselves is questionable, but the fact that when injected into rabbits they produce an immunity to the hog-cholera bacillus, while paratyphoid β does not, is additional evidence in favor of such a classification. Complement fixation experiments have been of little value in differentiating the members of this group, but on the contrary show their close relationship. It seems probable that some of the cultures that are described in the literature as hog-cholera bacilli really belong to this group, which would account for much of the confusion that exists in the classification of the interesting, truly pathogenic bacillus that at one time was thought to be the cause of hog-cholera and in the series of animals with which we have worked has not appeared once. Whether the ingestion of pork containing these bacilli would cause disease in man is a question that can only be decided by a more careful bacteriological study of the organisms causing food poisonings and paratyphoid fever.

BIBLIOGRAPHY.

1. Hog cholera: its history, nature, and treatment, *U. S. Dept. Agric., Bureau Animal Industry*, 1889, 69.
2. Uhlenhuth, Hübener, Xylander, and Bohtz, *Arb. k. Gsndhtsamte.*, 1909, xxx, 217.
3. Ford, W. W., *Med. News*, 1905, lxxxvi, 1126.
4. Jordan, E. O., *J. Infect. Dis.*, 1917, xx, 457.
5. Krumwiede, C., Jr., Kohn, L. A., and Valentine, E., *J. Med. Research*, 1918, xxxviii, 89.
6. Weiss, H., and Rice, J. L., *J. Med. Research*, 1916–17, xxxv, 403.
7. Jordan, E. O., and Victorson, R., *J. Infect. Dis.*, 1907, xxi, 554.
8. Savage, W. G., *J. Hyg.*, 1912, xii, 1.
9. Grabert, K., *Z. Infectionskrankh., parasit. Krankh. u. Hyg. Haustiere*, 1917, iii, 218.
10. Seiffert, G., *Z. Hyg. u. Infectionskrankh.*, 1909, lxiii, 273.
11. Uhlenhuth, P., and Hübener, E., in Kolle, W., and von Wassermann, A., Handbuch der pathogenen Mikroorganismen, Jena, 2nd edition, 1913, iii, 1021.
12. Smith, T., *U. S. Dept. Agric., Bureau Animal Industry, 12th and 13th Ann. Rep., 1895–96*, 1897, 161.