THE IMMUNITY PRODUCED BY THE GROWTH OF TETANUS BACILLI IN THE DIGESTIVE TRACT.

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In a previous paper (1) we pointed out that while tetanus spores can be found in the stools of about a third of the population in Peking, so far as we could determine tetanus as a disease is relatively rare. Upon examining the sera of carriers we found that an appreciable amount of antitoxin was present and our conclusion was that carriers must be relatively immune to tetanus. We felt, however, that the conclusion should be put to experimental test, and this and the following papers are the results of these tests. We early learned that in testing the immunity of carriers we had to consider the serological types of the bacillus as first described by Tulloch (2) and confirmed by one of the writers (3). Our results show that immunity is specific for the serological type of tetanus bacilli fed or injected and, much to our surprise, that the antitoxin content of the serum does not indicate the degree of resistance to infection.

As experimental animals we have used guinea pigs throughout our work for, as we have already reported (4), it is relatively easy to establish tetanus bacilli in their digestive tracts. When once established the sera of these carriers soon show agglutinins for the type fed while antitoxin appears more slowly. After some months however, this latter antibody can be found in relatively large amounts. Tulloch (2) has shown that the antitoxin produced by the injection of toxin formed by one type of bacilli will neutralize the toxins of all the other types. In other words antitoxin is not specific for type. Our findings agree with this work for, using the toxin formed by Type V bacilli, we find that the sera of animals fed the other types will have approximately the same neutralizing power as the sera from the animals fed Type V. If antitoxin were specific for type we would expect that the

sera of animals fed Type V would show a higher antitoxin content than those fed other types.

In contrast to much of the experimental work that has been done on tetanus we have not tested the immunity of our animals by the injection of toxin but have sought to produce an infection by the injection of spores free from this substance. As is well known spores alone will not produce an infection but some irritating substance must be introduced at the same time in order that they may germinate. Kitasato (5) used for this purpose a splinter of wood, Tulloch (2) saponin, and Bullock and Cramer (6) used $CaCl_2$. We tried all of these agents and were not satisfied with them. Infection and death appear so soon after the inoculation that the test of immunity is a very severe one. We wanted to produce a disease which would as nearly as possible resemble that in man in having an incubation period of several days and as long a duration as possible. After considerable search we selected the method given below. It is not ideal in that after it once appears the disease progresses rapidly but it is more satisfactory than any other we have tried.

Methods.

We have used the same methods in a number of studies and shall give them here in some detail.

Aleuronat is the irritating substance that we have used to enable the tetanus spore to germinate and grow. We make it up with starch as for the production of an exudate except that we use a 3 per cent instead of a 5 per cent suspension as the latter is too thick to be injected with ease. The factor that has probably helped us most in securing a long incubation period is that we have used a small number of spores (about 1000). We prepare our spore suspension as follows:

Bouillon cultures under vaseline of known types of tetanus bacilli that have been incubated 8 days and then placed in the refrigerator are the source of the spores. When wanted, about 10 cc. of culture is centrifugalized and the sediment is suspended in salt solution. This suspension is heated for 20 minutes at 70° and after cooling known dilutions are made in sterile salt solution. From the higher dilutions 1 cc. amounts are transferred from each to labeled sterile 1 inch test-tubes, duplicate tubes being made for each dilution. To each tube is now added 5 cc. of clear liver digest agar (7) that has been melted and cooled to 45°. The tubes are kept in water at 45° until the agar has been added to all, when they are taken out one at a time and Esmarch roll cultures made by rotating the tubes on a block of ice. The agar containing the spores is thus deposited as a thin film

on the sides of the tubes. Sterile melted agar at 45° is now carefully added to each of the tubes until it comes well above the level of the films, care being taken not to detach the latter from the sides of the tubes. After immersion in ice water until the agar has solidified the tubes are incubated. Usually in 24 hours but sometimes not until a day later, the spores that have been deposited on the sides of the tubes have developed and colonies large enough to count have been formed. Suitable tubes are counted and the number of spores per cc. of the original suspension is calculated. This method of determining the number of spores has proven quite accurate, it is easier than the direct microscopical count, and it has the additional advantage that it gives us the number of viable spores only. It seems to us that this might be a useful method of studying colony formation of anaerobes as the growth is just under the surface of the glass.

Experiments have shown that tetanus spores are not easily destroyed in salt solution as counts made after the suspensions have been stored 2 days in the refrigerator agree quite closely with those made on the day of the preparation of the suspension. Our practice therefore has been to place the suspensions in the refrigerator until counts have been made and the number of spores per cc. has been determined. The original suspension is then diluted until it theoretically contains 10,000 spores per cc. and from a 1:1000 dilution of this two anaerobic roll cultures as a check on the first count are made. To a flask containing 90 cc. of freshly sterilized and warm 3 per cent aleuronat plus 3 per cent starch in distilled water we add 10 cc. of the suspension supposed to contain 10,000 spores per cc. After mixing thoroughly, the flask containing the mixture is placed in a jar from which the air is then partially exhausted in order to remove the air bubbles from the aleuronat. This mixture which should contain, and as counts have shown does contain, approximately 1000 spores per cc. is then injected into the muscles of the hind legs of our animals, by means of a 1 cc. syringe and a No. 18 needle.

As a matter of fact it makes little difference whether 1000 or 10,000 spores are injected as shown in Table I. The results indicate that with aleuronat the injection of 1000 spores invariably produces tetanus while alone 50,000 spores failed to induce infection. In another experiment two guinea pigs that were each given 1 cc. of aleuronat suspension containing 160 Type V spores both developed tetanus on the 7th and 8th days respectively. The former died on the 12th day after the injection while the latter recovered. In all our experiments we keep animals that fail to develop tetanus under observation for 2 months before they are discarded. This is done because, as will be noted later, when we were using another method some of our animals developed tetanus a month after inoculation. With the method here described we have not had this difficulty.

An examination of the results given in this and the papers that are to follow will show that our control animals always developed tetanus and that symptoms usually appeared on the 5th though sometimes on the 4th day. In all these animals death followed on the 7th to the 10th

TABLE 1.

Test of Ability of 1 Cc. of 3 Per Cent Aleuronat to Produce Tetanus When Mixed with Different Types of Tetanus Spores.

Type of spores used.	Spores suspended in aleuronat.				Spores suspended in salt solution.			
	No. of spores injected.	Guinea pig No.	Weight.	Result.	No. of spores injected.	Guinea pig No.	Weight.	Result.
			gm.				*gm.*	
I	10,000	1	320	4th day—local tetanus. 7th " death.	50,000	5	300	No tetanus.
"	"	2	230	2nd " local tetanus. 6th " death.	"	6	330	" "
"	1,000	3	320	5th " local tetanus. 9th " death.	10,000	7	?	" " 2nd day—death; cause?
"	"	4	280	5th " local tetanus. 8th " death.	"	8	310	No tetanus.
II	10,000	9	310	5th " local tetanus. 7th " death.	50,000	13	290	" "
"	"	10	250	5th " local tetanus. 7th " death.	"	14	390	" "
"	1,000	11	220	5th " local tetanus. 7th " death.	10,000	15	420	" "
"	"	12	250	5th " local tetanus. 7th " death.	"	16	220	" "
III	10,000	17	280	4th " local tetanus. 7th " death.	50,000	21	280	" "
"	"	18	250	4th " local tetanus. 7th " death.	"	22	520	" "
"	1,000	19	410	5th " local tetanus. 8th " death.	10,000	23	390	" "

III	1,000	20	410	5th day—local tetanus. 7th " death.	10,000	24	290	No tetanus.
V	10,000	25	280	5th " local tetanus. 7th " death.	50,000	29	240	" "
"	"	26	330	5th " local tetanus. 7th " death.	"	30	380	" "
"	1,000	27	250	5th " local tetanus. 7th " death.	10,000	31	410	" "
"	"	28	290	5th " local tetanus. 7th " death.	"	32	290	" "

TABLE II.

Immunity of Carriers to Type III Spores.

Guinea pig.		Type of spores fed on Aug. 28, Sept. 3, Sept. 20, 1924.	Tests on serum.						Results of intramuscular injection of 1000 Type III spores suspended in 1 cc. of 3 per cent aleuronat emulsion. Feb. 20, 1925.
No.	Weight at time of inoculation.		Date of bleeding.	No. of M.L.D. of toxin neutralized by 0.25 cc. of serum.	Agglutination titre for type.				
					I	II	III	V	
	gm.								
33	920	III	Feb. 10	50	20—*	20—	160	20—	No tetanus during next 60 days.
34	610	"	" "	50	20—	20—	320	20—	Found dead on 10th day. No tetanus. Abscess at site of inoculation.
35	820	I	" "	50	160	20—	20—	20—	Generalized tetanus 6th day. Death 7th day.
36	910	"	" "	75	160	20—	20—	20—	" "
37	640	V	" "	50	20—	20—	20—	160	Local tetanus on 5th day. Death on 6th day.
38	600	"	" "	75	20—	20—	20—	160	" "
39	600	"	" "	50	20—	20—	20—	320	" "
40	480	Controls.	" 9	2—†	Not tested.				" "
41	480	"	" "	2—	"	"			" "
42	500	"	" "	2—	"	"			Local tetanus on 5th day Death on 7th day.
43	520	"	" "	2—	"	"			Local tetanus on 5th day. Death on 6th day.

* The figures are the highest dilution in which agglutination was observed. When followed by a minus no agglutination was present in this the lowest dilution used.

† 2 — means that 0.25 cc. serum failed to neutralize 2 M.L.D. of toxin.

TABLE III.

Immunity of Carriers to Type I Spores.

Guinea pig.		Type of spores fed on Aug. 28, Sept. 3, Sept. 20, 1924.	Tests on serum.						Results of intramuscular injection of 1000 Type I spores suspended in 1 cc. of 3 per cent aleuronat emulsion. Mar. 13, 1925.
No.	Weight at time of inoculation.		Date of bleeding.	No of M.L.D. of toxin neutralized by 0.25 cc. of serum.	Agglutination titre for type.				
					I	II	III	V	
	gm.								
44	680	I	Feb. 10	25	320	20—	20—	20—	No tetanus during next 60 days.
45	630	"	" "	10	160	20—	20—	20—	" " " " " "
46	1010	III	" "	50	20—	20—	160—	20—	Local tetanus 5th day. Death 7th day.
47	725	"	" "	25	20—	20—	160—	20—	" " " " " " "
48	800	V	" "	75	20—	20—	20—	320—	" " " " " " "
49	695	"	" "	75	20—	20—	20—	320—	" " " " " 6th "
50	390	Controls.	Mar. 2	2—	Not tested.				" " " " " " "
51	400	"	" "	2—	" "				" " " " " " "
52	435	"	" "	2—	" "				" " " " " 7th "
53	440	"	" "	2—	" "				" " " " " " "

TABLE IV.

Immunity of Carriers to Type V Spores.

Guinea pig.		Type of spores fed on Aug. 28, Sept. 3, Sept. 20, 1924.	Tests on serum.						Results of intramuscular injection of 1000 Type V spores suspended in 1 cc. of 3 per cent aleuronat emulsion. Mar. 20, 1925.
No.	Weight at time of inoculation.		Date of bleeding.	No. of M.L.D. of toxin neutralized by 0.25 cc. of serum.	Agglutination titre for type.				
					I	II	III	V	
	gm.								
54	610	I	Feb. 10	50	320	20—	20—	20—	4th day—local tetanus; 5th day—general tetanus; 6th day—death.
55	720	"	" "	50	160	20—	20—	20—	4th day—general tetanus; 5th day—death.
56	750	III	" "	75	20—	20—	160	20—	4th day—local tetanus; 6th day—general tetanus; 7th day—death.
57	760	"	" "	25	20—	20—	160	20—	" "
58	760	V	" "	50	20—	20—	20—	160	No tetanus during next 60 days.
59	720	"	" "	50	20—	20—	20—	160	" " " " " "
60	430	Controls.	Mar. 10	2—	Not tested.				4th day—general tetanus; 6th day—death.
61	430	"	" "	2—		" "			" " " " " " "
62	420	"	" "	2—		" "			4th day—local tetanus; 5th day general tetanus; 7th day—death.
63	440	"	" "	2—		" "			4th day—death.

day after inoculation. In order to secure such uniform results it is necessary to use for controls guinea pigs with stools that, on culture, show no tetanus-like bacilli and with sera free from antitoxin.

For the cultural test two or three fecal pellets from each animal are suspended in approximately 3 cc. of sterile salt solution and heated 20 minutes at 75°, after which the whole suspension is transferred to a deep tube of freshly heated cooked meat medium. If present, tetanus bacilli bearing the typical round terminal spores are found in the greatest numbers in this medium after 5 days incubation. Films are therefore made from the fecal cultures on the 4th and 5th days of incubation and if tetanus-like bacilli are found the guinea pig in question is discarded. About one-third of our guinea pigs carry tetanus bacilli in their digestive tracts and therefore are not satisfactory for this work. Those animals with negative feces for tetanus-like bacilli are bled from the heart and their sera tested as follows: 0.5 cc. of serum, 0.5 cc. of salt solution, and 1 cc. of a solution of carefully standardized dried toxin diluted so that it contains four minimal lethal doses for a hamster per cc. are mixed in a small test-tube and allowed to stand half an hour at room temperature. The hamster (*Cricetulus griseus*, M.-Eds.) has been used as a test animal in this work because it is the cheapest laboratory animal we have and it reacts quite uniformly to tetanus toxin. Each animal is injected subcutaneously with 1 cc. of the above mixture and is kept under observation for 4 days. If at the end of this time the hamster is alive, *i.e.* if 0.25 cc. of serum has neutralized 2 M.L.D. of toxin, the guinea pig that supplied this serum is not used for our work. Very rarely do we find antitoxin in the blood of a guinea pig which we have passed as having no tetanus-like bacilli in the stool. This test is therefore used as an additional precaution and a check on the cultural method.

Having developed methods for producing in the guinea pig, by the injection of spores, an infection that had an incubation period of approximately 4 days and that always ended fatally, we proceeded to test the immunity of animals that had been carrying known types of tetanus bacilli for a known period.

EXPERIMENTS.

Immunity Following the Feeding of One Type of Tetanus Spores.

In August, 1924, a number of guinea pigs were selected and, after it had been shown that their feces were negative for tetanus-like bacilli and their sera free from antitoxin and agglutinins for the various types of tetanus bacilli, they were divided into three equal lots. On 3 different days these animals were fed the heated washed sediment from old bouillon cultures of tetanus bacilli, one lot being given Type I, another Type III, and the third Type V. The cultures of Types I and III used for the feeding are Tulloch's key strains while the Type V

is a culture we had isolated. The feeding was done by withholding food from the animals for a day and then placing the spore material on their usual food. Some 6 months later, at dates which will be found in the tables, these animals were bled and their sera tested for antitoxin as well as for agglutinins for the various types of tetanus bacilli. The results of these tests which are given in Tables II to IV confirmed our previous report in that only agglutinins for the type bacillus fed were found in the sera and that antitoxin was present in all. When 0.25 cc. serum neutralizes 50 M.L.D. of our toxin, tests have shown that the serum contains 0.05 U. S. A. units of antitoxin per cc.

On the dates given in the tables the animals were injected with spores from known types of tetanus cultures, suspended in aleuronat prepared according to the method given above. The results are so clear-cut that little comment is necessary. When injected with a culture other than that fed the animals develop tetanus and die in the same time as the controls, in spite of the fact that their serum shows considerable antitoxin. When injected with the culture fed the animals fail to develop tetanus though in some cases their serum had a lower antitoxin content than did those fed other types. In other words, one culture of tetanus bacillus established in the digestive tract will produce in 6 months an immunity to this culture but not to cultures of other types and the immunity is not dependent upon the antitoxin content of the blood.

After our experiments were nearly completed we realized that we had made a mistake in using for inoculations the same cultures that were used for feeding. The only conclusion we could draw was that the animals were immune to the culture fed whereas we believe that they are immune to all organisms of the serological type present in the digestive tract. Unfortunately at this time we had only a few animals that had carried organisms for a sufficient length of time for immunity to develop but the results of the inoculation given in Table V show that the immunity is specific for the serological type. This point will be brought out more fully in papers which are to follow.

While we have only to a limited extent studied the development of immunity after feeding the results indicate that it comes on rather slowly. 4 months after feeding animals may or may not resist the culture used for the feeding though their serum will at this time show a considerable amount of antitoxin. After carrying one culture for from 6 to 8 months they are immune to this culture but not to cultures

of other types. Whether they would eventually develop an immunity to other types is a question we cannot positively answer though our results all indicate that immunity is strictly limited to the serological type. This too is a point that will be brought out in further papers.

That the immunity obtained by feeding is not absolute was shown in some experiments we made over a year ago. At that time we were producing tetanus by inserting under the skin glass capsules filled with a spore suspension, the capsule being crushed after insertion.

TABLE V.

Test of Immunity of Guinea Pig Carriers of Culture XIX Type V to Other Cultures of Same Type.

Each animal was injected with 1000 spores suspended in aleuronat. May 21, 1925.

Culture used.		Carrier guinea pigs.			Control guinea pigs.		
No.	Source.	No. of M.L.D. of toxin neutralized by 0.25 cc. serum.	Weight.	Result.	No. of M.L.D. of toxin neutralized by 0.25 cc. serum.	Weight.	Result.
			gm.			gm.	
190	Feces of soldier.	50	600	No tetanus.	2—	470	Local tetanus 5th day. Death 7th day.
5158	Wound, human tetanus.	50	790	" "	2—	350	" "
T V	Tulloch.	50	670	" "	2—	460	Local tetanus 5th day. Death 8th day.
146	Guinea pig feces.	25	770	" "	2—	480	" "

Our suspension was much more concentrated than the one we have been using in the recent experiments as each capsule contained over a hundred thousand spores. Tetanus developed in the controls and the animals died on the 7th or 8th day. The animals inoculated with types other than that fed also died with tetanus in the same time as the controls. The guinea pigs inoculated with the same culture which they had been fed showed no tetanus when the others were all dead, but from 12 to 25 days after the inoculation they, with two exceptions,

developed a fulminating type of infection and died usually on the day that symptoms were first noted. Our interpretation was that the constant irritation produced by the bits of glass in the subcutaneous tissues enabled the bacilli to grow and that gradually the immune substances were exhausted. Why the animals should show such a rapidly progressing disease we have not been able to explain. The

TABLE VI.

Immunity of Carriers of Several Types of Tetanus Bacilli.

Guinea pig.		Tests on serum.						Date of injection.	Type spores used.	Results of intramuscular injection of 1000 spores suspended in 1 cc. of 3 per cent aleuronat emulsion.
No.	Weight at time of inoculation.	Date of bleeding.	No. of M.L.D. of toxin neutralized by 0.25 cc. of serum.	Agglutination titre for type.						
				I	II	III	V			
	gm.									
64	720	Dec. 2	50	320	320	320	160	Feb. 20	III	Local tetanus; recovery. 5th day—local tetanus; 14th day—death.
65	880	" "	10	320	320	160	160	" "	"	
66	830	" "	50	640	1280	40	320	" "	"	No tetanus.
67	780	" "	50	320	640	80	320	" "	"	" "
68	970	" "	10	160	320	160	640	Mar. 13	I	" "
69	550	" "	25	160	640	160	640	" "	"	" "
70	875	" "	50	320	320	160	320	" "	"	" "
71	780	" "	50	320	160	320	320	" "	"	" "
72	960	Mar. 24	50	320	320	160	320	" 30	V	" "
73	980	" "	25	160	320	160	640	" "	"	" "
74	815	" "	50	320	320	160	160	" "	"	" "
75	930	" "	50	320	160	320	80	" "	"	Local tetanus, 5th day; death, 10th day.

For controls see Tables II to IV.

two guinea pigs that failed to develop the disease had by mistake been placed with a male and were in a late stage of pregnancy at the time they were injected. Living young were born a few days later and the mothers showed no evidence of infection though they were kept under observation for some months. Without further tests we should hesitate to say that pregnancy had raised the resistance to tetanus.

Immunity of Guinea Pigs Fed Several Types of Tetanus Spores.

In contrast to the above experiments in which it has been shown that feeding one type of spore protects against that type only are the results of the tests made on animals that were fed a mixture of types. It will be seen in Table VI that most of the animals were immune to all the types tested and that those that developed tetanus did so later than the controls, indicating that they had a partial immunity. The difference in weight between the animals under test and the controls cannot account for this difference for the results given in the previous tables show that weight plays little part in the resistance to infection.

These results are not strictly comparable to those obtained in the animals fed a single type, as these guinea pigs had been carrying tetanus bacilli in their digestive tracts for a much longer period. They were first fed a single type of spore in June, 1923, and in the spring of 1924 were fed a mixture of spores of all types. When finally inoculated on the dates given in the table care was taken to select for each experiment animals that received at the first feeding a type other than that used for the inoculation.

That more than one type had grown in the digestive tract is indicated by the results of the agglutination tests made with the sera of these animals. Whereas in the previous experiment animals fed a single type showed agglutinins for that type only, here all the types tested were agglutinated by the sera. Antitoxin was found in the sera in approximately the same amounts as in the animals fed a single type.

Resistance of Carrier Guinea Pigs to Tetanus Toxin.

Contrary to our expectation guinea pigs that carry tetanus bacilli in their digestive tracts show little, if any, resistance to tetanus toxin in spite of the fact that their sera contains antitoxin. Our study of this phase of the immunity was not made on animals that had been fed known types of spores but on guinea pigs from our stock whose feces showed tetanus-like bacilli in their stools and antitoxin in their blood.

The data pertaining to the animals as well as the effect of the injec-

tion of toxin are given in Table VII. The intravenous injections were made several days after the subcutaneous injections with the same toxin solution used for the first test. The solution had been kept in the refrigerator in the interval and it seems probable that some of the toxin had been destroyed for it will be noted that 0.00075 mg. injected subcutaneously kills while twice this amount injected intravenously produces no effect.

TABLE VII.

Test of Resistance of Carrier Guinea Pigs to Tetanus Toxin.

Guinea pig.	Result of examination of stool for tetanus-like bacilli.	Amount of toxin neutralized by 0.5 cc. serum.	Tetanus toxin injection.		
Weight.			Amount.	Route.	Result.
gm.		*mg.*	*mg.*		
440	Negative.	0.0006–	0.00075	Subcutaneous.	48 hrs. local tetanus; 6 days 5 hrs. death.
440	"	"	"	"	53 hrs. local tetanus; 5 days 4 hrs. death.
470	"	"	0.0015	"	46 hrs. local tetanus; 72 hrs. death.
440	"	"	"	"	46 hrs. local tetanus; 70 hrs. death.
490	Positive.	0.003	0.003	"	46 hrs. death.
380	"	0.0075	"	"	" " "
460	"	0.003	0.0075	"	33 hrs. general tetanus; 46 hrs. death.
350	"	0.0075	"	"	" "
310	Negative.	0.0006–	0.0015	Intravenous.	No tetanus.
300	Positive.	0.0075	"	"	" "
330	Negative.	0.0006–	0.003	"	40 hrs. death.
340	Positive.	0.0075	"	"	" " "

Our experiments do show that when 0.5 cc. of serum contains enough antitoxin to neutralize the amount of toxin injected, the animals are just as susceptible as those which have no antitoxin.

This phenomenon was first noted by Wladimiroff (8) and has been observed in laboratories where antitoxin is being prepared. We have no explanation other than that commonly given, that it takes some time for toxin and antitoxin to combine and that toxin has a greater affinity for nerve cells than for antitoxin.

The observation has a practical application for if guinea pigs that carry tetanus bacilli were resistant to toxin a considerable error would be introduced in the standardization of antitoxin. While our experiments are not extensive enough to rule out this factor absolutely they indicate that it is not of great importance.

DISCUSSION.

We have shown elsewhere (4) that soon after tetanus bacilli are fed to guinea pigs, agglutinins for the type fed appear in the blood, and that even after 6 months the agglutinins remain specific. Antitoxin appears more slowly but by the end of 6 months it amounts to about 0.05 U. S. A. units per cc. of serum. This is not a high content if one thinks of units but it will neutralize a considerable amount of toxin.

We assumed that this antitoxin would render the animals immune to tetanus bacilli as at the beginning of these experiments we regarded antitoxin as an adequate protecting antibody. The results given in this paper show this assumption not to be justified, for while the guinea pigs are shown to be immune to the type organisms fed they are just as susceptible to organisms of other types as are control animals. *We believe therefore that antitoxin plays little part in acquired immunity to tetanus but that there are other bodies, specific for type, which protect animals against infection.* We shall return to this point in a paper that is to follow on the protective action of sera of animals immunized by the injection of tetanus bacilli.

Guinea pigs can be made immune to more than one type of tetanus bacilli if these types are growing in the digestive tract. While we have never tried to determine how many types of these organisms could be isolated from the stool of a single human, examination of the sera of many individuals in Peking shows that most of them have agglutinins for several types of bacilli. This, together with the many chances for the introduction of tetanus spores into the digestive tract, makes us feel that man must often carry the common types and that he thereby acquires an immunity. Carrying the bacillus does not necessarily make man absolutely immune for we have seen two cases of infection in individuals from whose stools we obtained tetanus bacilli. In one of these cases the organism in the stool was of the

same serological type as that producing the infection. In the other case infection followed a rupture of the intestines and we were not able to recover the bacillus responsible for the tetanus. It is worthy of note that both cases recovered while of the four cases we have seen whose stools contained no tetanus bacilli, three died in spite of intensive treatment with antitoxin. While these findings seem to us suggestive we do not wish to draw conclusions from so few cases, and it will be some time before we can secure enough data to warrant any conclusions as to the protection afforded man by tetanus bacilli in the intestinal canal of man. Incidently it may be mentioned that we have been unable to detect any ill effects due to the presence of these organisms in the digestive tract. Although the tetanus bacillus is known to form an hemolysin, blood counts have failed to show an anemia in carrier animals, observations out of accord with the statement of Rabinowitsch (9) that marasmus and death resulted from feeding this organism.

SUMMARY AND CONCLUSIONS.

1. A method for the production of tetanus by the injection of a fixed number of spores is described together with the tests made in selecting animals for experimental work.

2. Guinea pigs fed a single serological type of tetanus bacilli will, after 6 months, show considerable amounts of antitoxin in their sera and will manifest immunity to the type fed. To other types they are just as susceptible as are controls.

3. Animals fed several types are immunized to each of these types. It is pointed out in the discussion that the digestive tract of man may carry several types and that he probably reacts in a manner resembling the guinea pig carriers.

4. Guinea pigs that carry tetanus bacilli and have antitoxin in their sera show little if any resistance to tetanus toxin.

5. As there is no relation between the amount of antitoxin in the blood and immunity to tetanus we believe that other bodies, specific for type, must occur and make for the immunity observed.

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