THE RELATION BETWEEN INVASION OF THE DIGESTIVE TRACT BY PARATYPHOID BACILLI AND DISEASE.

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(Received for publication, October 1, 1926.)

The large group comprising the colon, paratyphoid, and typhoid bacilli have certain characters in common which have led most students of this group to assume a close genetic relationship. The production of disease by members of the colon group resides in a capacity to multiply in the lower small intestines with coincident production of a toxin or toxins. These are absorbed and produce certain effects leading to extreme congestion of the capillary system and hemorrhage especially marked in the kidneys. The capacity to produce disease among the highly parasitic types of the paratyphoid group, such as the hog cholera bacillus, consists in the power to penetrate the mucosa, lodge and multiply in certain viscera, chiefly spleen, lymphoid tissue of the intestines, and liver, where a toxin similar to that produced by *B. coli* comes into intimate contact with certain vulnerable cells. Between these extremes we may assume that there exist many varieties either approaching one or the other of these types or so balanced that multiplication in the intestinal tube and the invasion of the tissues and multiplication therein both contribute to the disease.

The extensive literature dealing with epidemics of food poisoning and with the clinical paratyphoid disease in man serves to trace the different types of disease. Some are evidently due chiefly to gastrointestinal intoxication; others to invasion of the body, in which respect they approximate the behavior of the typhoid bacillus. If the above grading of the colon-typhoid group is true, it follows that the invasion of the digestive tract in itself may or may not be followed by clinical manifestations. In the colon bacilli it would be necessary that they multiply abundantly in the tube itself in order to produce

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enough toxin; for the other extreme, that they penetrate the mucosa and find no resistance to temporary multiplication in the blood or lymph channels or even within certain cells. The immediate bearing of the disinfecting action of the stomach and its relation to penetration of bacteria through the mucosa of mouth and throat instead of the intestinal wall need not enter into the problem, nor the obvious fact that different host species will react differently when invaded by the same type of bacteria. Moreover, certain abnormal states of the digestive tract may modify bacterial behavior.

The general question here raised has an important bearing upon an understanding of vaccinations against this group of bacteria. In the case of human typhoid the subcutaneous injection of bacilli killed by heat has the support of statistics as to its efficiency. The injection of dead bacteria tends to protect the body whose digestive tract is invaded by typhoid bacilli. Does the typhoid bacillus multiply in the lower small intestine after introduction or only later in the disease after it has been discharged through the bile and from local ulcers into a tube whose protective mechanism has been disorganized by the disease process? Are there races of typhoid, some capable of multiplying in the intestines at the start, others promptly invasive? The vaccine probably has no effect on bacilli in the tube but only after they have penetrated the mucosa. Disease might conceivably be produced by races simulating the colon type even after vaccinal protection.

In approaching questions of this kind experimentally, the nearer our experiment to natural conditions, as far as known, the nearer our results approach the solution of the natural or practical problem. At the same time any other combination of experimental procedures may assist in analyzing the problem and thus provide material for a future synthesis approaching practical conditions.

The experiments to be reported were begun in 1921 before the extensive researches on mouse typhoid by Flexner and Amoss, Topley, and Webster[1] had appeared, and were closed in August, 1923. They are incomplete but they furnish some data of value in the final

[1] For a good bibliography of this work up to 1926 see Lockhart, L. P., *J. Hyg.*, 1926, xxv, 50.

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formulation of factors governing the rise and fall of mortality in this group of diseases.

Methods.

To bring the problem within the field of experiment, the hog cholera bacillus and the white mouse were chosen. The susceptibility of gray mice to this bacillus had been studied by one of us in 1885. At this time the breeding of white mice for experimental purposes had scarcely begun. The white and the gray mouse have about the same degree of susceptibility towards the hog cholera bacillus. The gray mouse, however, as usually obtained in traps is a more variable subject often showing lesions of the kidneys and other organs due to various parasites and possibly poisons. The strain of the hog cholera bacillus used in the following experiments had been under cultivation for about 6 months when the experiments were begun.[2]

It is obvious that this relationship of hog cholera to white mice is artificial and that an analysis of it can cover only a narrow portion of the entire field. The minimum fatal dose, given subcutaneously, varied considerably from case to case. An 18 to 24 hour bouillon culture in which the final clouding is fairly constant was used in all cases. In 1921 the surely fatal dose was about 0.05 cc. Nearly 50 per cent succumbed when 0.02 cc. was injected. A small number died from doses down to 0.002 cc. Death resulting from a minimum dose usually occurred on the 7th to 9th day, rarely later. Doses representing multiples of the minimum dose killed within 4 days. In later experiments (1922–23) the minimum fatal dose was evidently somewhat larger. In an animal as small as the mouse the skill with which the infecting dose is deposited in the subcutis in each mouse, not too deep into the muscular tissue or even the peritoneal cavity nor into the skin itself, probably determines in part the death rate. No attempt was made to increase by passages the virulence of the strain used. Each experiment was started with the stock culture grown on sloped agar and kept at about 38–40°F. in the refrigerator after each monthly transfer had been incubated 1 or 2 days.

EXPERIMENTAL.

From the 50 or more mice used in preliminary trials which received subcutaneous doses of hog cholera bacilli and survived, the injected bacilli were demonstrated in the spleens in all cases up to the 75th day, the longest period tested. The spleens, usually larger than in the normal condition, were torn into three or four pieces which were placed in tubes of sloped agar. There was evident a gradual diminution in the number of colonies as the period between inoculation and

[2] This strain was kindly sent by Dr. Dimock of the University of Kentucky.

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killing widened. In mice killed when sick in the early days up to the 10th the colonies were numerous. In those active and apparently well when chloroformed they were scarce even in bits of spleen as large as split peas.

After considerable experimentation with one lot of white mice, a

TABLE I.

Mouse No.	Subcutaneous dose	Died (d.) or killed (k.)	Cultures (spleen)	Remarks
	cc.			
1	0.05	d. 7 days	Many colonies	
2	0.05	d. 9 "	" "	
3	0.01	k. 25 "	Few "	Some pneumonic foci
4	0.01	k. 25 "	" "	" " "
5	0.01	d. 9 "	Many "	
6	0.01	d. 10 "	" "	

TABLE II.

Mouse No.	Dose fed	Died (d.) or killed (k.)	Spleen cultures
	cc.		
1	6	d. 11 days	Many colonies
2	6	k. 25 "	Few "
3	6	d. 13 "	100 "
4	6	k. 15 "	Growth only in condensation water
5	6	d. 7 "	Many colonies
6	6	k. 26 "	1 colony
7	6	d. 11 "	Many colonies
8	6	k. 15 "	" "

pneumonia appeared which entered as a disturbing factor as Table I indicates.

It will be noted that of the four mice receiving 0.01 cc. subcutaneously the two affected with pneumonia survived. Another lot of mice was raised from a small group obtained from an isolated colony and this remained free from the pneumonia.

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Inasmuch as the problem before us concerned the relation of the digestive tract to infection, feeding of cultures was resorted to.

An 18 to 24 hour bouillon culture was used in a definite amount to moisten a small piece of dog biscuit which was placed in a solid watch-glass in the container, a battery jar with wire mesh cover. The mice were of the nearly uniform age of 2 months when used. They were all of the new lot which was free from mouse typhoid and pneumonia. The food consisted of corn, oats, and dog biscuit, with a constant supply of water. Before infecting the mice they were fasted overnight.

TABLE III.

New Stock of Mice.

Mouse No.	Bouillon culture fed	Died (d.) or killed (k.)	Spleen cultures	Remarks
	cc.			
47	0.2	d. 7 days	Many colonies	
48	0.5	d. 7½ "	" "	Cysticerci in liver*
49	0.5	d. 6 "	" "	" " "
50	1.0	d. 7 "	" "	
51	2.0	k. 9 "	" "	" " "
52	3.0	d. 7 "	" "	Very sick when killed
		Old Stock.		
59	0.2	k. 28 days	+−−**	
60	0.5	d. 10 "	Many colonies	
61	0.5	k. 28 "	+++	
62	1.0	d. 9 "	Many colonies	
63	2.0	k. 34 "	+++	
64	3.0	k. 34 "	++−	

* Only the original stock of new mice were infested. The offspring were free.
** 3 pieces of spleen in 3 tubes of agar. 2 remained sterile.

As a rule the food placed before them was entirely consumed within 18 to 24 hours. In the earlier experiments two mice were fed in a jar and kept together. The irregular results pointed to the possibility that one of the two mice had eaten the bulk of the infected food. This surmise is borne out by the experiment shown in Table II.

It will be seen from the table that only one of every pair died. The other must have obtained some of the food, for spleen cultures were positive 15 to 26 days after the feeding when the surviving, still

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active mouse of every pair was chloroformed. Thereafter only one mouse was fed in a jar.

Before giving up the old stock of mice a test was made of their resistance to the hog cholera bacillus as compared with that of the new stock. Six mice each were fed as described and each kept by itself in a jar.

It will be noted that five out of six of the new stock died. The sixth was very sick when chloroformed and would have probably died. The tapeworm infection of the original mice of the new stock, as observed in other mice of this lot, did not influence the outcome. Of the old stock only two out of six died. The remaining four were killed after 28 to 34 days. It will also be observed that the result did not go parallel with the dose fed.

TABLE IV.

Mouse No.	Dose fed	Died (d.) or killed (k.)	Spleen cultures
	cc.		
17	7	d. 6 days	Many colonies
18	Control	k. 25 "	Sterile
19	7	k. 19 "	A few colonies
20	Control	k. 25 "	Sterile
21	7	d. 7 "	20 colonies
22	Control	k. 25 "	Sterile
23	7	d. 6 "	Many colonies
24	Control	k. 31 "	Sterile

As a preliminary test the degree of infectivity of mice fed with hog cholera bacilli to other mice associated with them was tried in the following way.

Mice were distributed one in a jar and, after withholding food, fed with dog bread moistened with a fresh bouillon culture. A very large dose was given the mice purposely to bring about as heavy a discharge of bacilli as possible. On the following day the fed mice were removed to clean jars and a fresh mouse added to each jar. All but one of the fed mice died, with the usual postmortem appearances and positive spleen cultures. The one survivor was killed on the 19th day. The spleen culture was positive. The exposed mice remained active and were killed 25 to 31 days after the beginning of the exposure. The spleen cultures made with large bits of tissue remained sterile.

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In a second test, after feeding, the fed and the control mice were placed together in a wire cage. They were killed in pairs after 4, 5, 6, and 8 days respectively. In none of the four exposed mice were hog cholera bacilli detected, in the spleen, abdominal cavity, duodenum, ileum, and feces. Thus far the experiments indicated in all cases an invasion of the body after feeding cultures as shown by the presence of hog cholera bacilli in the spleen in all survivors for an indefinite period up to $2\frac{1}{2}$ months. Contacts with mice fed large doses did not carry bacilli in their spleens or digestive tracts.

It was next decided to analyze in some detail the condition of the digestive tract following feeding.

To ensure uniformity the mice used were from the new stock bred originally from only a small number of individuals. They were all about 2 months old and weighed from 17 to 23 gm. The diet and method of feeding remained the same. The culture was fed on a small piece of dog biscuit.

Cultures were made from certain regions of the alimentary tract and in some experiments the material was weighed after suspension in a definite amount of bouillon and a calibrated loop of this rubbed over agar plates in order that some rough conception of the numbers of hog cholera bacilli present might be gained. Cultures from the intestinal contents required careful manipulation since hog cholera bacilli were present in the abdominal cavity of most, if not all, infected mice. The portion of intestine desired was cut out and removed to a sterile petri dish. With sterile instruments the tube was cut longitudinally. In most cases the walls curled outward, exposing the contents, so that they could be removed with forceps without difficulty. The contents were suspended in bouillon. Feces were removed by holding the living mouse as if for injection and gently rubbing the abdomen with the finger. When feces were ejected, they were taken with sterile forceps and put into bouillon and shaken. From these shaken suspensions of intestinal contents and feces, plates were made directly with one loopful for each plate. The suspension was then kept at room temperature overnight. If the hog cholera organism did not appear on the first set of plates, the next day duplicate ones with malachite green were made from the incubated material. Two agar plates were made from each specimen, one contained 0.3 cc. of a 1 per cent aqueous solution of malachite green per 10 cc. of agar. The plain agar plate made possible a general survey of the intestinal flora of each mouse, and the malachite green agar inhibited growth of nearly all but the hog cholera bacillus. Occasionally there was found on the plates an organism which grew like the hog cholera bacillus. A large bubble of gas in lactose broth and negative agglutination tests with a specific rabbit serum sufficed to distinguish this organism from the hog cholera bacillus.

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The first series of tests to be described determined roughly the number of organisms found in the stomach after the beginning of feeding and the survival time. The 18 to 24 hour bouillon culture

TABLE V.

Bacteria in Stomach of Fed Mice.

Mouse No.	Killed after feeding	Bacteria in 0.15 gm. stomach contents	Calculated total in stomach contents	Stomach contents actually plated
		First Test		
	hrs.	*millions*	*millions*	*gm.*
113	2	65	261	0.00005
114	3	195	716	0.00005
115	4	15	37	0.00005
116	5	68	157	0.00005
117	26	0	0	0.00005
118	29	0	0	0.00005
119	48	0	0	0.0005
		Second Test		
120	2	233	570	
121	3	74	294	
122	5	83	168	
123	6	77	116	
124	8	231	231	
125	18	16	28	
126	24	0	0	Incubated contents also negative
127	51	0	0	
		Third Test		
		thousands	*thousands*	
168	18	24	151	0.0009
169	20	91	427	0.0009
170	22	<1	<1	0.0009
171	24	0	0	Incubated contents also negative

was added in amounts of 0.5 cc. to a gm. of dog biscuit and each mouse fed separately in a mouse jar. Three separate tests were made. In the two first the mice were left in the jars in which they

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had been fed. In the third they were removed to clean jars and fresh food given after 17 hours.

The results agree in demonstrating the presence of large numbers of hog cholera bacilli during the early hours after feeding. A decline occurs about the 18th hour and in 24 hours the fed bacilli are no longer detected in the contents.

Hydrogen ion determinations of stomach contents, according to Brown's drop method,[3] were made on three mice killed $1\frac{1}{4}$ hours after a meal of corn and oats. The pH readings were 5.4, 5.6, and 5.8.

In a second series of experiments comprising three lots of mice, 10, 10, and 12 in number, respectively, an attempt was made to follow the fed bacteria beyond the stomach into the lower digestive tract, the peritoneal cavity, and the spleen.

In the first series the mice were fed each 0.5 cc. of bouillon added to 3 gm. of dog biscuit. The 1st mouse was killed 24 hours after the beginning of the meal, and one on each succeeding day, except the 5th day. The 10th mouse was therefore killed on the 11th day after feeding. Spleen cultures were positive in all but the 1st mouse. Cultures from the peritoneal cavity were positive in all but the 2nd and 4th mouse. The fed bacilli were obtained on plain and malachite green agar plates from contents of the ileum in all cases; in the 4th and 7th mouse, however, only after incubating the contents in bouillon overnight. On the plain agar plates the fed bacilli were overgrown by colon bacilli in the 3rd, 6th, and 9th mouse.

In the second and the third series the examination was extended to duodenal contents and feces and to the 21st day after feeding.

The results agree with those of the first series. Two mice died in the third series after 9 and 11 days. One mouse in the second series was negative throughout. In addition to these series, two mice were fed and kept 28 days. The spleen cultures were positive. Plates from duodenum, cecum, and feces contained colonies of hog cholera bacilli in both mice. A few bacilli were found in contents of ileum of one only.

The prompt invasion of the peritoneal cavity and spleen by way of the digestive tract suggested a final experiment in which the reverse

[3] Brown, J. H., *J. Lab. and Clin. Med.*, 1923–24, ix, 239.

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route was to be traced. Three small groups of mice received subcutaneously 0.02 cc. of a 24 hour bouillon culture in 0.1 cc. fluid. They were chloroformed up to 41 days after injection and the contents of ileum, cecum, colon, and discharged feces spread on plain and malachite green agar plates.

TABLE VI, *a*.

Killed, days after feeding	Cultures			No. of organisms per 0.016 gm. of ileum contents
	Spleen	Duodenum	Feces	
1	−	−	+	11,600
2	+	−	−	2,227
3	+	+	+	6,032
4	+	−	+	39,771
6	−	−	−	0
7	+	+	+	18,560
8	+	+	+	185,600
9	+	+	+	659,569
10	+	−	+	2,735
11	+	−	−	0***

TABLE VI, *b*.

11	+	+	+	5,290
12	+	+	+	3,648
13	+	−	+	214
14	+	−	+	4,690
15	+	+	+	21,458
16	+	−	−	0
18	+	−	+	0***
19	+	−	+	426
20	+	−	+	95
21	+	+	+	0***

*** Recovered on plates after incubating material in bouillon overnight.

Among the first group of eight, one died within 24 hours of some undetermined cause. Invasion of the intestinal tract was evident after 3, 7, and 15 days. Mice killed after 2, 5, and 21 days respectively were negative in this series. In the second group of eleven mice, five died. These mice were smaller than those used hitherto. All

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but one of the six survivors were sick when killed. In all the intestinal tract contained hog cholera bacilli. The third group of

TABLE VII.

Killed, days after injection	Cultures				Condition of mouse when killed
	Ileum	Cecum	Large intestine	Feces	
First Group					
2	−	−	−	−	Well
3	+	−	−	−	"
5	−	−	−	−	Sick
7	+	+	+	−	"
8	−	−	−	+	Well
15	+	+	+	+	"
21	−	−	−	−	"
Second Group					
7	+	+	+	+	Sick
8	+	+	+	+	Very sick
9	+	+	+	−	" "
10	+	+	+	+	" "
13	+	+	+	+	" "
16	+	+	+	+	Well
Third Group					
12	−	−	−	−	Well
13	+	−	−	−	Sick
14	+	+	−	−	Slightly ill
16	+	+	+	+	Well
17	+	−	+	+	"
19	−	−	+	−	?
21	+	+	+	+	Well
37	−	−	+	−	"
39	−	−	−	−	"
41	+	−	+	+	"

twelve mice was more resistant. Only two died in 4 and 9 days, respectively. In two mice killed after 12 and 39 days respectively injected bacteria were not recovered.

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A control experiment was made to eliminate the chance that the injected culture dilution might ooze out locally, be lapped by the mouse, and introduced into the digestive tract directly. During the injection the needle was inserted under the skin about 1 cm. The skin was subsequently washed with 95 per cent alcohol, dried, collodion applied to the punctured area and allowed to dry before the mouse was caged. Six mice received 0.02 cc. culture fluid in 0.1 cc. bouillon dilution. Two died after 6 and 7 days, respectively. The rest were killed after 7, 8, 9, and 12 days. All had the injected bacteria in contents of cecum, colon, and in the feces. In all but one (9th day) bacteria were found also in the ileum.

DISCUSSION.

Before we attempt an interpretation of the experimental work, a brief statement of the lesions following the feeding or injection of hog cholera bacilli in mice may be helpful. Following a minimum fatal dose injected subcutaneously mice die in 7 to 9 days, rarely later. If the mouse is chloroformed when manifestly sick, the mucosa of the intestinal tract will be found with nearly intact epithelium. The gross lesions observed involve liver and spleen. Examination of fixed and hardened tissue show involvement of lymph nodes, mesenteries, and peritoneum generally and to a certain degree of the mucosa of the large and more rarely of the small intestine.

The lesions of the liver are the most constant and characteristic. Hog cholera bacilli just isolated from swine produce sharply defined necroses, 1 to 2 mm. in diameter.[4] These rarely appear following inoculation with strains under prolonged cultivation. The lesions always encountered comprise the presence of cells of endothelial type in the sinusoids. The entire liver is involved in this condition. The sinusoids may contain a few cells not encroaching on the liver tissue, or many closely packed, tubercle-like, and causing disappearance of surrounding liver cells. The spleen is always large, dark red, firm. The tissue changes differ according to the severity of the induced disease. In the fatal cases, there is much hemorrhage or at least marked distention of the pulp vessels and the focal accumulation of endothelial cells. In recovering cases the lymph follicles are large. The mesenteries are broadened by cell accumulations of endothelial and lymphoid type. The lymph nodes are enlarged and the changes like those going on in the spleen.

[4] Boxmeyer, C. H., *J. Med. Research*, 1903, ix, 146.

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The intestines interest us more particularly in view of the special problem of this communication. In the presumably fatal cases there may be found here and there in the sections of the large intestine accumulations of endothelial cells in the mucosa, forcing the crypts apart or else causing them to disappear. The infiltrated zones are as a rule still covered with epithelium. Actual ulcers were very rare in our cases. The large intestine is the dominant seat of such infiltrations, although they may also be found in the small intestine. There is no evidence that Peyer's patches or lymphoid follicles are involved to any degree. They are not the necessary loci for the changes. The feeding of large doses does not lead to diffuse injury to the surface epithelium or the free portions of the villi such as might be looked for when a toxin is freely discharged from multiplying bacteria and such as we note when poisons have been ingested. Nor do we observe the severe congestions often leading to hemorrhages which are associated with unrestricted multiplication of *B. coli* in the small intestine of calves.

The hog cholera organism is evidently of a more highly adapted, parasitic type than the cultures used by Webster and Topley. The disease follows subcutaneous inoculation and intraperitoneal injections are unnecessary. Deaths earlier than 4 days are rare. They tend to occur between the 7th and 9th day more or less independently of the dose within limits. These facts indicate a greater penetration and resistance to destruction and a more stabilized march of the disease process. Acute intoxication and early death is produced only by many multiples of the surely fatal dose.

The main outcome of these studies is the demonstration of the readiness with which hog cholera bacilli pass into the blood and lymph circulation after ingestion in food and the uniformity with which they make their appearance in the intestinal tract after subcutaneous injection in surviving mice. The second point to be noted is the persistence of the fed or injected bacilli in the spleen, even though in very small numbers. The prompt dissemination may be ascribed in part at least to the small size of the animal, the delicate tissues, and the short distances to be travelled. On the other hand, the universal invasion without marked illness or death suggests that, to compensate for this early entrance, the mouse is endowed with a high degree of

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fundamental resistance, or natural immunity, which represses multiplication. This is evident when the mouse is compared with the rabbit which succumbs to the minimum lethal dose of the mouse.

Clinical disease manifested in the mouse by crouching on top of the bedding with staring coat and somnolent behavior is not followed by recovery. If the mouse is killed at this time, many bacteria are found in the spleen, and the obvious inference is that disease is associated with multiplication of the bacilli in the viscera and when this has reached a certain momentum reversal of the process and recovery are no longer possible. The relation of feeding to clinical disease and death is not cleared up by the experiments reported. As we trace the fed bacteria down, we note during the first hours large numbers in the stomach contents which completely disappear within 24 hours. Some have probably passed into the duodenum. The numbers found in the ileum may represent those passed into the small intestine from the stomach and they may be the residuum of a process of destruction and multiplication going on side by side. 2 weeks after feeding there are no bacilli found or else only a few in this section of the small intestine. Their discharge in the formed feces goes on parallel with their presence in the digestive tract and spleen. On the whole the assumption is warranted that clinical disease depends on the numbers that penetrate into the body proper and multiply there. The conditions favoring penetration are unknown. The passage of bacilli outward may be due in part to the causes operating in favoring penetration inward, but in view of the uniform and diffuse involvement of the liver it is more probable that they are discharged in the bile. Lesions of the intestinal tract characterized by ulceration of the mucosa are very rare and they may play a very subordinate part in the outward discharge.

If multiplication in and consequent intoxication from the alimentary tract were factors when large doses are fed, we should expect the appearance of symptoms within 2 days, allowing for mass destruction in the stomach during the 1st day. This does not occur. The evidence points to penetration and subsequent multiplication reaching a climax within a week. The marked irregularity in the effects of small and large doses of hog cholera bacilli fed indicates that the presence of such bacilli in the intestinal tract of mice does not imply a

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corresponding absorption into the body proper, although such absorption takes place since bacilli are always detected in the spleen later on. A large dose fed may evidently be followed by slight absorption and if we assume that it is the numbers absorbed that determine the clinical manifestations, the marked absence of agreement between the doses fed and active disease is satisfactorily explained. The concept of absorption includes both penetration into the body and capacity to multiply. Here the individual immunity of the host must come into play. In these studies no attention has been paid to the possible relation of the minute nematodes of the cecum to the appearance of disease after feeding, nor were the intestinal contents examined for the overmultiplication of flagellates, occasionally observed in mice.

The passage of bacteria into the system from the digestive tract may stand in some causal relation to motility. To the writers' knowledge attention has not been called to this possible relationship. This function is remarkably persistent in the paratyphoid group under cultivation and is possessed by all pathogenic members of the group. In fact its persistence in bouillon cultures over 24 hours old, in the sense that it is shared by most organisms in the culture, is presumptive evidence that the organism belongs to the above group rather than to *B. coli*. Its persistence in a pathogenic group strongly argues for its usefulness and in the penetration of the normal mucosa it may explain the invasiveness of the hog cholera bacillus as compared with *B. coli*. After its entry into the system in small numbers by this method even in the presence of large numbers in the alimentary tract, a slight increase in resistance may suppress multiplication, whereas the introduction parenterally even of small doses may soon exhaust the normal, antibacterial forces. Experiments to test the significance of motility are under way.

CONCLUSIONS.

Hog cholera bacilli fed to mice disappear from the stomach within 24 hours, but remain and perhaps multiply in the ileum for at least several weeks. They promptly penetrate the mucosa and may be found in the spleen. Bacilli introduced subcutaneously quickly pass

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into the intestinal tract where they may be found for some weeks. Infected mice may harbor bacilli in the spleen for several months.

Mice possess a relatively high degree of natural resistance towards hog cholera bacilli which gives way to large doses. Disease is probably the result of the invasion of the viscera from the digestive tract following feeding, but the relation between the dose fed and the numbers penetrating the mucosa is a variable one and the conditions favoring such invasion not determined. Contact with mice discharging bacilli failed to cause recognizable invasion of the digestive tract or the viscera.

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