OX BILE SENSITIZATION IN MOUSE TYPHOID INFECTION.

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Earlier papers have emphasized the fact that in any series of laboratory mice a certain number is immune to mouse typhoid.[1] An explanation for this phenomenon was sought in an analysis of the biological environment at the normal portal of entry of the mouse typhoid bacilli, but it was found that the intestinal flora played no part in susceptibility to infection.[2] Attention was then directed to the intestinal wall itself in an effort to alter its permeability. Besredka, unable to infect mice with typhoid and paratyphoid bacilli, given by way of the mouth, found that sterile ox bile, administered 24 hours prior to the culture of organisms, rendered the animals susceptible to disease.[3] Acting on this information, we administered ox bile to mice before injecting them *per os* with a strain of mouse typhoid bacilli (*Bacillus pestis caviæ*) and endeavored to discover and analyze any differences in susceptibility between the animals so treated and the controls.

The following experiment was devised to ascertain the proper dose of ox bile, the time for its administration, and the optimum interval before injecting the mice *per os* with the living cultures.

Experiment 1.—42 mice, weighing from 16 to 18 gm. each, were treated as follows: 18 mice, called Series A, received *per os*, by stomach tube, 0.5 cc. of undiluted sterile ox bile; 18, called Series B, received 0.25 cc. of bile made up to 0.5 cc. volume in salt solution; 6 were used as controls. 9 of Series A and 9 of Series B received the bile on an empty stomach and were fed 6 hours later; the remaining 9 of Series A and Series B received the bile ½ hour after feeding.

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[1] Webster, L. T., *J. Exp. Med.*, 1922, xxxvi, 71.

[2] Webster, L. T., *J. Exp. Med.*, 1923, xxxvii, 21.

[3] Besredka, A., *Ann. Inst. Pasteur*, 1918, xxxii, 193; 1919, xxxiii, 557, 882.

TABLE I.

Protocols of Mice Used in Experiment 1.

Mouse No.	Bile dosage.	Time.	Condition of animal 24 hrs. after bile ingestion.	Time of inoculation.	Duration of life after inoculation.
	cc.				*days*
1	0.5	Full stomach.	Dead.	——	——
2	0.5	" "	"	——	——
3	0.5	" "	Severe diarrhea.	24 hrs. after bile treatment.	16
4	0.5	" "	" "	24 " " " "	Survived.*
5	0.5	" "	" "	48 " " " "	1
6	0.5	" "	" "	48 " " " "	15
7	0.5	" "	" "	48 " " " "	Survived.
8	0.5	" "	" "	Control.	5
9	0.5	" "	" "	"	5
10	0.5	Empty "	Dead.	——	——
11	0.5	" "	"	——	——
12	0.5	" "	"	——	——
13	0.5	" "	Severe diarrhea.	24 hrs. after bile treatment.	Survived.
14	0.5	" "	" "	24 " " " "	"
15	0.5	" "	" "	48 " " " "	6
16	0.5	" "	" "	48 " " " "	6
17	0.5	" "	" "	Control.	5
18	0.5	" "	" "	"	Survived.

19	0.25	Full stomach.	Slight diarrhea.	24 hrs. after bile treatment.	5
20	0.25	" "	" "	24 " " " "	6
21	0.25	" "	" "	24 " " " "	Survived.
22	0.25	" "	" "	48 " " " "	"
23	0.25	" "	" "	48 " " " "	"
24	0.25	" "	" "	48 " " " "	"
25	0.25	" "	" "	Control.	"
26	0.25	" "	" "	"	"
27	0.25	" "	" "	"	"
28	0.25	Empty "	" "	24 hrs. after bile treatment.	12
29	0.25	" "	" "	24 " " " "	Survived.
30	0.25	" "	" "	24 " " " "	"
31	0.25	" "	" "	48 " " " "	"
32	0.25	" "	" "	48 " " " "	"
33	0.25	" "	" "	48 " " " "	"
34	0.25	" "	" "	Control.	"
35	0.25	" "	" "	"	"
36	0.25	" "	" "	"	"
37	—	—	—	24 hr. series.	"
38	—	—	—	24 " "	"
39	—	—	—	24 " "	"
40	—	—	—	48 " "	10
41	—	—	—	48 " "	11
42	—	—	—	48 " "	19

* Survivors discarded after 30 days.

TABLE II.

Protocols of Mice Used in Experiment 2.

Mouse No.	Date.	Type of flora.	Stool culture.	Blood culture.	Agglutinins.	Condition of animal.	Mouse No.	Date.	Type of flora.	Stool culture.	Blood culture.	Agglutinins.	Condition of animal.
	1922							*1922*					
A-1	Mar. 27	A = C	0	0	0	Well.	A-6	Mar. 27	A = C	0	0	0	Well.
	" 28	Bile ingested.						" 28	Bile ingested.				
	" 29	Bacilli ingested.						" 29	Bacilli ingested.				
	" 30	C	?	0	0	Well.		" 30	C, few A	++	++	0	Well.
	Apr. 1	" few A	0	+	0	"		Apr. 1	——	——	+	0	Diarr.
	" 3	" = A	+	+	——	"		" 3	C	0	++	——	Sick.
	" 6	" few A	+	+++	——	Fair.		" 6	——	——	++	——	"
	" 8					D.*†		" 10					D.*†
A-2	Mar. 27	A = C	0	0	0	Well.	A-7	Mar. 27	A, few c	0	0	0	Well.
	" 28	Bile ingested.						" 28	Bile ingested.				
	" 29	Bacilli ingested.						" 29	Bacilli ingested.				
	" 30	C	0	+	0	Diarr.		" 30	C, few A	++	0	0	Well.
	Apr. 1	" few A	0	0	0	"		Apr. 1	" = A	+	0	0	"
	" 3	" " "	0	0	——	"		" 3	——	——	+	——	"
	" 6	" " "	+	+++	——	Fair.		" 6	——	——	+	——	Fair.
	" 10					D.*†		" 10					D.*†
A-3	Mar. 27	A few C	0	0	0	Well.	A-8	Mar. 27	A, few C	0	0	0	Well.
	" 28	Bile ingested.						" 28	Bile ingested.				
	" 29	Bacilli ingested.						" 29	Bacilli ingested.				
	" 30	C	0	0	0	Well.		" 30	C	0	0	0	Well.
	Apr. 1	" = A	0	0	0	"		Apr. 1	"	0	+	0	"
	" 3	" = "	0	0	——	"		" 3	A = C	0	++	——	Sick.
	" 6	" = "	0	0	——	"		" 5					D.*†

	Apr. 12	A, few C	0	0	0	Well.	A-9	Mar. 27	A, few C	0	0	0	Well.
	" 18	"	0	0	——	"		" 28	Bile ingested.				
	" 25	" few c	0	0	0	"		" 29	Control.				Sl. diarr.
	May 9	" " C and c	0	0	0	"		" 30	C	0	0	0	Well.
								Apr. 1	"	0	0	0	"
	" 29	A, few C and c	0	0	0	"		" 3	"	0	0	0	"
								" 6	C, few A	0	0	——	"
								" 12	" = A	0	0	0	"
A-4	Mar. 27	A, few c	0	0	0	"		" 18	A, few C	0	0	——	"
	" 28	Bile ingested.						" 25	" " "	0	0	0	"
	" 29	Bacilli ingested.						May 9	" " "	0	0	0	"
	" 30	C	+	0	0	Well.		" 29	" " "	0	0	0	"
	Apr. 1	A = C	0	0	0	"							
	" 3	" = "	0	0	——	"	A-10	Mar. 27	" " c	0	0	0	"
	" 6	" = "	0	+	——	"		" 28	Bile ingested.				
	" 12	" few C	0	0	0	"		" 29	Bacilli ingested.				
	" 18	C, " A	0	0	——	"		" 30	C	0	+	0	Well.
	" 25	" " "	0	0	0	"		Apr. 1	"	0	0	0	"
	May 4					D.*†		" 3	"	0	+	——	"
								" 6	A, few c	0	++	——	Fair.
A-5	Mar. 27	A = C	0	0	0	Well.		" 9					D.*†
	" 28	Bile ingested.											
	" 29	Bacilli ingested.					A-11	Mar. 27	A, few C and c	0	0	0	Well.
	" 30	C	++	++	0	Well.							
	Apr. 1	"	+	+	0	Sick.		" 28	Bile ingested.				
	" 2					D.†		" 29	Bacilli ingested.				

C indicates colon colonies; c, coccus colonies; A, *acidophilus* colonies; ——, test not performed; 0, test negative; +, 1 to 10 colonies; ++, 10 to 50 colonies; +++ more than 50 colonies. D. indicates dead; Diarr., diarrhea; Sl. diarr., slight diarrhea.

* Autopsy lesions typical of mouse typhoid.

† Heart's blood culture positive.

TABLE II—*Continued.*

Mouse No.	Date.	Type of flora.	Stool culture.	Blood culture.	Agglutinins.	Condition of animal.
	1922					
A-11	Mar. 30	C, few A	+	0	0	Well.
	Apr. 1	A	0	0	0	"
	" 3	C = A	0	0	——	"
	" 6	A, few c	0	0	——	"
	" 12	" " "	0	0	0	"
	" 18	"	0	0	——	"
	" 25	"	0	0	0	"
	May 9	" few c	0	0	0	"
	" 15					D.*†
A-12	Mar. 27	A = C	0	0	0	Well.
	" 28	Bile ingested.				
	" 29	Control.				Sl. diarr.
	" 30	C = A	0	0	0	Well.
	Apr. 1	" = "	0	0	0	"
	" 3	A, few C	0	0	——	"
	" 6	" = c Few C	0	0	——	"
	" 12	A = c Few C	0	0	0	"
	" 18	A = c Few C	0	0	——	"
	" 25	A = c Few C	0	0	0	"
	May 9	A = c Few C	0	0	0	"
	" 29	A = c Few C	0	0	0	"
B-4	Mar. 27	A, few C	0	0	0	Well.
	" 29	Bacilli ingested.				
	" 30	C, few A	0	0	0	Well.
	Apr. 1	A, " C	0	0	0	"
	" 3	" " "	0	0	——	"
	" 6	——	——	+	——	"
	" 12	C, few A	0	0	0	Fair.
	" 18	" " "	0	0	——	"
	" 25	——	——	+	0	Sick.
	May 9	——	——	++	0	"
	" 15					D.*†
B-5	Mar. 27	A = c	0	0	0	Well.
	" 29	Bacilli ingested.				
	" 30	A, few C and c	++	0	0	Well.
	Apr. 1	A = c	0	0	0	"
	" 3	" few C	+	++	——	"
	" 6					D.*†
B-6	Mar. 27	c	0	0	0	Well.
	" 29	Bacilli ingested.				
	" 30	A = c	0	0	0	Well.
	Apr. 1	" = "	0	0	0	"
	" 3	"	0	0	——	"
	" 6	" few C	0	+	——	"
	" 12	" = C	+	++	0	Sick.
	" 13					D.*†

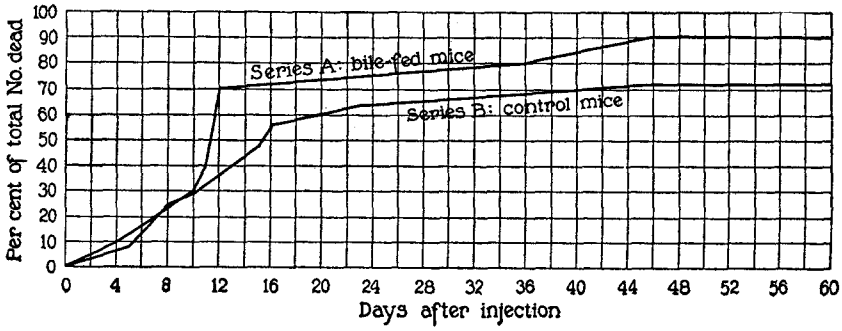
B-1	Mar. 27	A = C = c	0	0	0	Well.
	" 29	Bacilli ingested.				
	" 30	A = C	++	0	0	Well.
	Apr. 1	" = c	0	0	0	"
	" 3	" = C	0	0	—	"
	" 6	" few C	+	0	—	"
	" 12	—	—	+	0	Fair.
	" 14					D.*†
B-2	Mar. 27	A, few C	0	0	0	Well.
	" 29	Bacilli ingested.				
	" 30	A = C	0	0	0	Well.
	Apr. 1	—	—	0	0	"
	" 3	—	—	0	—	"
	" 6	C	0	+	—	"
	" 12	"	+	+++	0	Sick.
	" 13					D.*†
B-3	Mar. 27	A, few C	0	0	0	Well.
	" 29	Bacilli ingested.				
	" 30	A, few C	0	0	0	Well.
	Apr. 1	"	0	0	0	"
	" 3	"	0	0	—	"
	" 6	C, few A	0	0	—	"
	" 12	" " "	0	0	0	"
	" 18	A, " C	0	0	—	"
	" 25	" " "	0	0	0	"
	May 9	" " "	+	0	0	"
	" 29	" " "	0	0	0	"
B-7	Mar. 27	A = C	0	0	0	Well.
	" 29	Bacilli ingested.				
	" 30	A, few C	0	0	0	Well.
	Apr. 1	" " c	0	++	0	"
	" 3	" " C	+	+++	—	Sick.
	" 6					D.*†
B-8	Mar. 27	A = c	0	0	0	Well.
	" 29	Bacilli ingested.				
	" 30	A, few c	+	0	0	Well.
	Apr. 1	"	0	0	0	"
	" 3	"	0	0	0	"
	" 6	" few c	0	0	—	"
	" 12	" = C	0	++	0	"
	" 18	" = "	+	++	—	Fair.
	" 21					D.*†
B-9	Mar. 27	A = C	0	0	0	Well.
	" 29	Bacilli ingested.				
	" 30	A, few c	++	0	0	Well.
	Apr. 1	" " C	0	+	0	"
	" 3	" " "	0	++	—	"
	" 6					D.*†
B-10	Mar. 27	A, few C	0	0	0	Well.
	" 29	Bacilli ingested.				
	" 30	A	0	0	—	Well.
	Apr. 1	—	—	+	0	"
	" 3	—	—	+	—	Sick.
	" 6	—	—	+++	—	"
	" 8					D.†

TABLE II—*Concluded.*

Mouse No.	Date.	Type of flora.	Stool culture.	Blood culture.	Agglutinins.	Condition of animal.	Mouse No.	Date.	Type of flora.	Stool culture.	Blood culture.	Agglutinins.	Condition of animal.
	1922							*1922*					
B-11	Mar. 27	C	0	0	0	Well.	B-12	Mar. 27	C	0	0	0	Well.
	" 29	Bacilli ingested.						" 29	Bacilli ingested.				
	" 30	A = C	0	0	——	Well.		" 30	A = C	+	0	0	Well.
	Apr. 1	" = "	0	0	——	"		Apr. 1	"	0	0	——	"
	" 3	"	+	0	——	"		" 3	"	0	0	——	"
	" 6	" few C	0	0	——	"		" 6	" = C	0	0	——	"
	" 12	" " "	0	0	0	"		" 12	" = "	0	0	0	"
	" 18	C	+	0	——	"		" 18	C	0	0	——	"
	" 25	A, few C	0	0	0	"		" 25	A, few C	0	0	0	"
	May 9	C	0	0	0	"		May 9	C	0	0	——	"
	" 29	" few A	0	0	0	"		" 29	" = A	0	0	0	"

24 hours later, 2 mice from Series A, which had ingested the bile on a full stomach, were dead; 3 mice from Series A, which had received the bile on an empty stomach, had succumbed; the survivors of Series A were extremely sick with signs of severe diarrhea. From Series B none had succumbed but all showed evidence of slight diarrhea.

At this time, 2 mice of Series A, which received the bile on a full stomach, were inoculated *per os*, by stomach tube, with a 1:200 dilution of an 18 hour broth culture, taken from the stock agar slant; 3 were injected the next day, 48 hours after the ox bile treatment; 2 were used as controls. 2 mice of Series A, which had received the bile on an empty stomach, were given a similar dose of bacteria, 2 were injected the next day, and 2 were used as controls. In the same manner, 6 mice of Series B were injected 24 hours and 6, 48 hours after the bile treatment, while 6 were saved for controls. The original controls were similarly injected. Table I shows the division of mice and subsequent series of events.



TEXT-FIG. 1. Experiment 2. Comparison of the mortality curves of the mice of Series A and B.

From this preliminary experiment it was concluded that 0.25 cc. of bile per mouse was a maximum dose, that the bile might be administered on a full or empty stomach, and that the mouse might be injected 24 or 48 hours after the bile treatment, without grossly altering the course of disease.

Experiment 2.—24 mice, averaging 18 to 20 gm., were placed in separate jars and stool cultures were taken. The next day, 12 mice (Series A) received *per os* 0.25 cc. of sterile ox bile, made up to a volume of 0.5 cc. 24 hours later, 10 of these mice and the 12 remaining controls (Series B) received *per os* a 1:200 dilution of an 18 hour broth culture of *B. pestis caviæ* taken from the stock tube. 2 mice from Series A were saved as controls. The condition of the animals was noted. Stool cultures and blood cultures were taken and the blood was examined for agglutinins at short intervals. The technique for stool culturing has been de-

scribed.[2] Blood cultures were made by clipping the tail and expressing 3 drops to a 0.5 per cent dextrose agar plate. The technique used in agglutination tests has been described.[4] Table II shows the protocols for each mouse and Text-fig. 1 shows the mortality curves of Series A and B.

This experiment shows that ox bile, administered in doses large enough to cause diarrhea, increases the susceptibility of mice to mouse typhoid infection by slightly raising the death rate and shortening somewhat the duration of life of the susceptible animals.

DISCUSSION.

The above observations are of value in interpreting the recent work of Besredka on intestinal immunity to experimental typhoid, paratyphoid, and dysentery infection. He found that ox bile, given *per os* to laboratory animals, not only rendered them susceptible to enteric infection by this route but increased the efficacy of gastrointestinal immunization against a subsequent injection of the homologous strain *per os* or intravenously. We found that ox bile increased the susceptibility of mice to mouse typhoid infection only when given in maximum doses. It then seemed to raise the death rate and shorten the duration of life of the susceptible animals.

According to Besredka, bile injures the intestinal epithelium, allowing the pathogenic bacilli to gain easy access to the blood stream. And judging from the evident discomfort and diarrhea following bile ingestion in our animals, it seems quite probable that his interpretation is correct. But while host susceptibility to typhoid infection may be altered by injury to the intestinal epithelium, experiments so far have failed to justify his claim that all immunity to typhoid, paratyphoid, and dysentery infection, natural or acquired, is localized in the intestinal tract. On the contrary, in individual susceptibility[5] and in acquired immunity to mouse typhoid infection,[1] the intestinal wall is but one factor in a general mechanism.

CONCLUSION.

Sterile ox bile, administered *per os* in maximum doses to laboratory mice, tends to increase their susceptibility to mouse typhoid infection.

[4] Amoss, H. L., *J. Exp. Med.*, 1922, xxxvi, 45.

[5] Webster, L. T., *J. Exp. Med.*, 1923, xxxvii (in press).