



TRACING 2.

Effect on blood pressure of intravenous injection of peritoneal fluid obtained from a dog dying of intestinal strangulation with a ruptured loop.

aerobic and aerobic organisms. For details of experiments see the table and tracings.

Conclusions. The peritoneal fluid obtained from dogs dying of strangulation obstruction is non-toxic, when injected intravenously into normal dogs providing the strangulated loop is not ruptured or about to rupture and the fluid does not give off a foul odor. We do not deny that the peritoneal fluid may contain toxic products, but they are not demonstrable, at least on injection into other dogs until rupture of the loop.

5976

Can Immunity to Tetanus be Produced by the Oral Route?

MELVILLE H. MANSON. (Introduced by Owen H. Wangensteen.)

From the Department of Surgery, University of Minnesota.

The problem of producing an acquired, systemic immunity to diseases of bacterial origin by the oral administration of organisms or their growth products has been studied sporadically since 1880. The majority of investigations have been undertaken in diseases that have either the portal or entry or their pathology in the gastrointestinal tract, as in typhoid fever, dysentery, and intestinal anthrax. This study was undertaken because of the undisputed opinion based on experimental evidence in the literature that a systemic immunity to tetanus could be produced by the oral administration of tetanus bacilli.

Ten Broeck and Bauer¹ observed that tetanus was less frequent among the Chinese people than those of the United States, reported the finding of tetanus bacilli in 34.7% of the stools of 78 Chinese. They later reported the examination of the blood sera of 26 individuals known to have tetanus bacilli in their digestive tracts, all of which had "appreciable amounts of antitoxin".² The blood sera of 30 persons in whom no tetanus bacilli were found in the stools contained no antitoxin, with 2 exceptions. In 40 specimens of serum from persons not examined for bacilli, they report the finding of antitoxin in 37.5%, which corresponded quite well to the previously reported 34.7% positive stools. In 1923, Ten Broeck and Bauer³ reported that they were able to produce an active immunity in guinea pigs by the oral administration of tetanus bacilli. As a preliminary procedure in their animal experiments they cultured the stools of a number of their stock guinea pigs and found that 27% had tetanus spores or bacilli in the intestinal tract and also antitoxin in the blood serum. They report the successful production of antitoxin in the blood to such an extent that 0.5 cc. protected against 10, 25, and 75 minimum lethal doses of toxin, appearing in approximately one month after the establishment of carriers.

Experiments. Two series of guinea pigs were used in the first set of experiments. Series I, 8 animals were fed tetanus bacilli each day. Stool cultures were made at intervals, the filtrate of which produced tetanus when injected into white mice, thus establishing that the animals had the organisms in their digestive tracts. Series II, 8 animals, were fed toxin each day. by sucking the fluid from a small pipette. Ten mg. of toxin was mixed with 10 cc. of saline, each animal receiving 1 cc. each morning. Before commencing the feedings the stools of all the animals were cultured in glucose brain broth. If, on microscopic examination, there appeared any organisms which were morphologically similar to the tetanus bacillus, the culture was filtered through a Berkefeld filter and the filtrate injected into a white mouse. None of the filtrates produced any symptoms of tetanus. Tests for immunity in the form of antitoxin in the blood serum were made either by directly injecting one m.l.d. of toxin (1/50 mg.) or more intramuscularly or by withdrawing heart's blood, pipetting off the serum and mixing this with tetanus toxin. The serum and toxin was allowed to stand at room temperature for one hour before injection. Fresh solutions were made each

¹ Ten Broeck, C., and Bauer, J., *J. Exp. Med.*, 1922, **36**, 261.

² Ten Broeck, C., and Bauer, J., *J. Exp. Med.*, 1923, **37**, 479.

³ Ten Broeck, C., and Bauer, J., *PROC. SOC. EXP. BIOL. AND MED.*, 1923, **21**, 267.

day from dried toxin, both for the daily feedings and for injection purposes.

PROTOCOL SUMMARY SERIES I.
Fed tetanus bacilli. Feedings started on February 14, 1931.

Pig	Wgt. gm.	Injected with	Time days	Dead in days	Control Dead in days
1	400	1 m.l.d.	37	3	6
2	300	1 m.l.d.	37	6	8
3	330	1 m.l.d.	40	4	9
4	370	Animal died 21st day after feedings were started. No tetanus.			
5	440	1 m.l.d.	40	6	8
6	300	1 cc. serum plus 1 m.l.d. into control pig		40	6
7	350	Animal died 18th day. No tetanus. No injection.			
8	380	1 cc. serum plus 1 m.l.d. into control pig		96	8

Pigs Nos. 6 and 8 from whom serum was taken were also injected with 1 m.l.d. of toxin. Died in 5 and 7 days respectively. Controls died in 4 and 10 days.

In Series I there were 6 intramuscular injections of 1 m.l.d. of tetanus toxin given to animals which had been fed tetanus bacilli from 37 to 96 days. None of the animals showed any evidence of resistance to the disease. Peculiarly, the majority died at an earlier date than the control animals. In 2 instances serum from the animal which had received bacilli by mouth when mixed with 1 m.l.d. of toxin failed to protect another animal.

SERIES II.
Fed tetanus toxin. Feedings started on January 23, 1931.

Pig	Injected with	Time days	Result	Control
1	1 m.l.d.	22	Dead 13 days	Dead 8 days
2	1 m.l.d.	35	Developed tetanus Alive 62 days	
3	1 m.l.d.	35	Developed tetanus Recovered	
			On 108th day, 1 cc. serum plus 1 m.l.d. toxin into control resulted in death 8th day	
4	1 m.l.d.	59	Dead 9 days	Dead 6 days
5	1 m.l.d.	59	Dead 6 days	Dead 7 days
6	Died 35th day. No tetanus.		No injection.	
7	2 m.l.d.	65	Dead 3 days	No control injected
8	Died 22nd day. No tetanus. No injection.			

In Series II the results of 6 intramuscular injections of 1 m.l.d. of toxin given to animals who had received 1 mg. of toxin by mouth for periods from 22 to 65 days were lethal to each of the pigs within a time comparable to the control animals except for Nos. 2 and 3. Pig No. 2 was unfortunately used for another experi-

ment and the opportunity to test his serum for the presence of anti-toxin was lost. The blood serum of No. 3 failed to neutralize 1 m.l.d. of toxin after 108 days of oral administration of toxin.

Because the experiments were so few in number and because the results were at such variance with those previously reported by others, the third series of experiments was made.

SERIES III.

Fed 1 mg. or more of toxin each day. Feeding started on Sept. 14, 1931.

Fig	Injected with	Time days	Result	Control
1	1 m.l.d.	55	Dead 13 days	
2	1 m.l.d.	55	Developed tetanus. Lived	
	2 m.l.d.	89	Dead 6 days	
3	1 m.l.d.	55	Developed tetanus. Lived	
	2 m.l.d.	89	Dead 6 days	
4	1 m.l.d.	90	Dead 5 days	2 control ani-
5	1 m.l.d.	90	Dead 7 days	mals injected
6	2 m.l.d.	90	Dead 4 days	1 m.l.d. toxin.
7	2 m.l.d.	90	Dead 6 days	Died 6 days.
8	2 m.l.d.	90	Dead 5 days	2 control ani-
9	2 m.l.d.	90	Dead 3 days	mals injected
				1 m.l.d. toxin.
				Died 6 days.
10	Pig developed an ulceration on side of face; a week after this was noticed the animal developed tetanus (October 16—33 days of toxin), from which it died in 9 days.			
11	1 cc. serum from No. 5 plus 1 m.l.d. toxin	55	Dead 9 days	
12	1 cc. serum from No. 7 plus 1 m.l.d. toxin	55	Developed tetanus. Lived.	

Pig No. 10 completed an experiment without having any injection of toxin. It was thought that the portal of entry was through the ulcer, contact being obtained from spillage at the time of its daily feedings. With the exception of one animal, 1 or 2 m.l.d.'s of toxin was lethal in every instance.

Comment. Eighteen intramuscular injections of tetanus toxin were made into guinea pigs which had received the same toxin orally for periods of from 22 to 108 days. There was no evidence that they had established any immunity to the disease. Six animals which had been fed tetanus bacilli for 96 days failed to show any immunity to tetanus either when injected with tetanus toxin or when their blood serum was mixed with toxin and injected into a normal guinea pig.

Conclusions. 1. Cultures of 16 stools from stock guinea pigs showed no tetanus bacilli. 2. Guinea pigs fed with tetanus bacilli do not develop an active immunity to tetanus. 3. Guinea pigs fed with tetanus toxin are as susceptible to tetanus as normal animals.