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Received May 31, 1961. P.S.E.B.M., 1961, v108.

Effects of Pretreatment with Antibiotics on Survival Following Mesenteric Arterial Occlusion in Rats. (26835)

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Recent studies have shown that changes in the intestinal tract are largely responsible for the irreversible course of the syndrome initiated by hemorrhagic, traumatic, and septic shock. Fine *et al.*(1,2) and Lillehei(3) have provided evidence indicating that the primary factor in this regard in the rabbit and dog is the systemic dissemination of bacterial products derived from the intestinal flora.

It appeared to us that the shock induced by temporary obstruction of the blood flow to the intestinal tract(3) would serve as an excellent test for this thesis. In particular, the reported ability of antibiotics(1,4) to afford protection by circumventing the usual intestinal bacteremia, required investigation in this type of shock.

Experiments in the rat reported herein do not support the concept of a systemic bacterial endotoxemia in bowel occlusion shock, but point rather to local factors within the intestine proper as the decisive elements in the fatal course of the syndrome.

Materials and methods. Two strains of rats, totaling 119 animals of both sexes, were used. These were German albino rats weighing 105 to 205 g and NAMRU stock rats weighing 110 to 300 g. In addition, 30 rats, which were excluded from the survival data, were used for observation of omental circulation, for measurements of mean arterial blood pressure, and for bacterial cultures. All rats were anesthetized with sodium pentobarbital (3 mg/100 g body weight, i.m.).

Shock was induced by temporary occlusion of the circulation to the intestinal tract. A midabdominal incision was made through the *linea alba* to minimize bleeding and the superior mesenteric artery was isolated. The artery was occluded with a wire serrafine clamp for 2 hours, and then released. The wound was closed and the rat returned to its cage. Aseptic precautions were taken throughout. Only those animals which survived the procedure are included in the data. Survival statistics are based on a 24-hour period of observation. Autopsies were performed routinely.

In representative members of each group of experimental animals, the blood was heparinized and mean arterial blood pressure was measured in the left carotid artery by a cannula leading to a conventional mercury manometer. Circulation of the omentum was also observed in these animals using Zweifach's visualization technic(5). Bacterial cultures (aerobic and anaerobic) were made on liver, spleen, intestine, and cardiac blood on selected animals (Table II).

Three series of experiments were conducted. In the first series, 6 groups of rats (77 animals) were given antibiotics. Five different antibiotics were employed, to pro-

TABLE I. Effect of Pretreatment with Antibiotics on Shock Following Occlusion of Superior Mesenteric Artery.

Agent	Amount*	Duration	Survival†		Strain
				(%)	
Controls—Water	.8 ml	6 days	4/18	22	German
"	.8 "	6 "	8/24	33	NAMRU
Terramycin	100 mg	6 "	8/11	73	German
"	100 "	Single dose	1/6	17	"
"	+ 2 " ‡	" "			
Gantrisin	.25 g	6 days	8/10	80	"
Aureomycin	100 mg	6 "	4/16	25	NAMRU
Neomycin	100 "	6 "	11/14	79	"
Chloromycetin	100 "	6 "	10/20	50	"

* Both water and antibiotics were given orally, in a volume of 0.8 ml.

† The significance of the survival data following antibiotic treatment was tested by the chi square method using Yates' correction factor. Only Terramycin, Gantrisin, and Neomycin values are statistically significant at 5% level ($\chi^2 > 3.841$).

‡ In 0.2 ml saline, inj. into portal vein following occlusion.

vide as wide an anti-bacterial spectrum as possible and to avoid the difficulty of possible pharmacological effects of particular substances over and above their antibiotic potentialities. Two of the materials (Aureomycin and Neomycin) have been previously found to protect dogs against a normally lethal episode of hemorrhagic shock(1). Terramycin and Gantrisin in general have a range of anti-bacterial effectiveness similar to the above 2 agents. Chloromycetin was included because it is particularly effective against gram-negative bacteria.

The animals were force fed by stomach tube 100 mg (in 0.8 ml water) of the following antibiotics for 6 days: Terramycin, Aureomycin, Neomycin, and Chloromycetin. Gantrisin (3,4-dimethyl-5-sulfamido-isoxazole) was given in 0.25 g doses. The animals were subjected to shock on the sixth day. Two control groups (42 animals) received only water. The 6-day period of treatment is similar to that employed by others(1,4) in the dog.

One group of animals received 100 mg Terramycin *per os* several hours before and 2.0 mg of antibiotic into the portal vein just after the superior mesenteric artery had been released (Table I).

The second and third series of experiments dealt with 2 forms of tolerance. In the second series, rats (20 animals) were made resistant to drum trauma by exposing them to progressively increasing numbers of rotations in the Noble-Collip drum(6). Resistance was induced by subjecting the rats on the first day to 100 rotations, on the third day to 200, on the fifth day to 400, on the seventh day to 500, and on the ninth day to 900 rotations. The rats were then subjected to bowel occlusion shock on the following day.

In the third series, 19 rats were made tolerant to endotoxin by serial intraperitoneal administration of *E. coli* endotoxin (Difco) beginning with 10 μg on day 1 and then at successive daily intrevals 20, 40, 80, 150, and 250 μg in 0.5 ml saline. The rats were subjected to the same occlusion shock 24 hours later. A group of 20 control rats received only saline (0.5 ml, i.p.) for a similar period.

Experimental findings. 1. *Survival.* The data (Table I) show that rats pretreated with Terramycin, Neomycin, or Gantrisin had the highest survival rates, 73%, 79%, and 80%, respectively, in contrast to the considerably lower survival rate of the controls (22% and 33%). It should be noted that Neomycin, one of the 3 effective antibiotics, is poorly absorbed, supporting the contention(1) that the protective effect of such pretreatment is due in the main to a local antibacterial action within the bowel proper. Unlike the reported efficacy of Aureomycin in hemorrhagic shock(1), no beneficial effect was observed against bowel occlusion shock. The Chloromycetin data were not consistent—in one run 3/10 survived, in another 7/10. The rats which received Terra-

TABLE II. Bacteriologic Findings.

Group	Rat No.		Liver	Spleen	Blood	Intestine
	43	No occlusion	—	—	—	E, Sa, Sta, Str, Sub, Y
	44	*Idem*	—	—	Sub	A, B, E, Sa, Sta
Control	40	Immed. after release	B, Sta, Sub, E	E, Sta	B, Sta	E, Sa, Sta, Pr
	41	*Idem*	B, Sta	—	Sta	B, E, Pr, Ps, Sta
	42	"	—	E	—	E, Pr, Ps, Str
	36	"	—	—	—	E, Pr, Ps, Str
Terramycin	37	"	Sta	—	B, Sta	E, Pr
	47	24 hr after	E, G, Sub	E	B, N, Sub	E, G, Pr, Ps, Sta
	38	Immed. after release	—	—	—	E, Sta
Gantrisin	39	*Idem*	—	—	—	B, E, Pr, Sta, Sub
	45	24 hr after	E, Ps, Sub	E, Pr, Sub	N, Pr, Sub	E, Pr, Ps, Sta
	46	*Idem*	E, Pr, Ps	B, E	B, E	B, E, Sta, Pr

Stool of #43: E, Pr, Sh, Sa.

Key: A = *Aerobacter aerogenes*, B = Bacteroides, E = *Escherichia coli*, G = *Gaffkya tetragena*, N = Neisseria, Pr = Proteus, Ps = *Pseudomonas aeruginosa*, Sa = Salmonella, Sh = *Shigella dysenteriae*, Sta = Staphylococcus, Str = *Streptococcus* sp., Sub = *Bacillus subtilis*, Y = Yeast.

mycin on the day of experiment survived less well than the controls (17%).

The 2 types of induced tolerance studied were found to have no significant protective action (40-42% survival). Here again, this finding is not in agreement with previous reports(1) on hemorrhagic and drum shock in rats where endotoxin and drum adaptation made the animals resistant to these forms of stress.

2. *Blood pressure.* The mean arterial blood pressure in fatal cases fell to shock levels (below 40 mm Hg) 2 hours after the clamp was released. The blood pressure rose slightly at time of occlusion to 150 mm Hg and remained at this level throughout the period of occlusion. Immediately upon releasing the clamp, the blood pressure dropped only slightly, about 10-25 mm Hg. In the antibiotic protected rats, the pressure rarely fell during the next 1-2 hours to below 100 mm Hg, whereas in the control group the pressure continued to decline during the initial 60-90 minutes and remained below 70 mm Hg. The rapid drop in pressure to 30 mm Hg occurred during the final 30 minutes of life.

3. *Bacteriology.* The results of bacterial cultures are summarized in Table II. Tissue and blood specimens from both control rats and those pretreated with antibiotics showed no growth (under aerobic and anaerobic incubation) when taken immediately after release of the clamp, except for samples from the intestines and stool. In contrast, all specimens taken at 24 hours after arterial occlusion were uniformly positive. Rats which survived were quite active and alert despite the positive bacteremia. There were no essential differences in the bacterial populations of unprotected controls which died and antibiotic protected rats which survived. Exact quantification was not attempted, since it was felt that by demonstrating the presence of gram-negative organisms, the point at issue was proved. It should be noted that bacterial species such as *Proteus, Bacteroides,* and *Pseudomonas* were uniformly present after shock, whereas they were either not encountered or present only in minor numbers in controls. The antibiotic regimen, although adequate for protection against lethal shock, did not significantly suppress the bacterial population in the intestinal lumen; nor was the incidence of bacteremia in blood, liver, and spleen after shock different in controls than in antibiotic treated rats.

4. *Pathological changes.* During the period of occlusion, the small intestines assumed a somewhat grayish or bluish appearance in all of the groups. Some of the antibiotic treated animals (especially those given Neomycin), showed a tendency toward intestinal edema. Upon release of the arterial

clamp, the intestine of the controls assumed a reddish, patchy appearance, whereas in the groups protected by antibiotics (Terramycin and Gantrisin), as well as after Aureomycin, the gut became hyperemic and showed no evidence of intraluminal hemorrhage.

In animals which succumbed, whether they had been treated or not, lungs were congested, adrenals enlarged, liver swollen and dark, and the spleen dark. Blood was usually found in the intestinal lumen. It was of interest that the endotoxin tolerant animals following bowel occlusion shock showed to a much lesser degree any of the above pathological changes. In particular, the intestinal tract had a more normal appearance. Drum tolerant rats showed an especially severe intestinal necrosis.

Upon sacrifice, animals which had survived, because of pretreatment with antibiotics, showed few or no abnormal findings, except for minor congestion of the lungs (probably the result of anesthesia).

5. *Omental observations.* Few changes either in distribution of blood, velocity of flow, or abnormal vasomotor behavior were seen during the period of arterial occlusion. Blood flow in the omentum was apparently maintained by the gastric branches of the celiac artery. After release of the arterial clamp, overall blood flow gradually diminished, the velocity of flow in the collecting venules being especially slowed within 45-60 minutes. Two hours after release of the clamp, there was no active circulation in most of the capillaries. The venules developed perivascular collars of hemorrhage and extensive stasis was present in the effluent capillaries.

In the Terramycin group, blood flow remained active throughout the 3-hour period of observation after clamp release. There was much less evidence of the viscous type of flow seen in fatal cases. Perivascular hemorrhage did not develop to any appreciable degree and capillary stasis was absent. Of the 4 animals observed in the antibiotic treated group, only one showed evidence of capillary stasis after 3 hours.

Discussion. There is good evidence that factors originating in the small intestines contribute to the circulatory derangement characteristic of various types of shock, including strangulation of the bowel(7-10). It has been suggested(11) that the bacterial flora in the intestines give rise to toxic products which, in the face of an impaired defense mechanism during protracted shock, are disseminated systemically and interfer with peripheral circulatory homeostasis. Apparently, in cases of intestinal strangulation, a lethal fluid is exuded(12) which is heavily infected with many of the bacterial flora present in the bowel.

The bacterial cultures in the present studies of bowel occlusion shock indicate that bacteremia *per se* may not be directly concerned with the lethality of the syndrome since even those rats which survived showed positive blood and tissue cultures 24 hours later.

Terramycin and Gantrisin (2 absorbable agents) and Neomycin (a non-absorbable agent) clearly showed a protective action. On the other hand, Aureomycin and Chloromycetin were not as effective. The basis for this distinction is not clear. Rats which had been pretreated with the various antibiotics uniformly showed less severe pathological changes in the intestines, despite the fact that 2 of the groups (Aureomycin and Chloromycetin) were not protected.

Although drum resistant rats have been found more tolerant of acute hemorrhagic and traumatic shock(13), they were not more resistant to bowel occlusion shock. Evidently, different mechanisms are involved in these several forms of shock. Rats made tolerant to endotoxin have been shown to withstand normally lethal episodes of hemorhagic and traumatic shock(14). They were not, however, able to tolerate the bowel ischemia following occlusion of the superior mesenteric artery. This circumstance by itself argues against endotoxemia as the major factor in the collapse of the circulation following bowel occlusion.

Passive transfer experiments(15) indicate that the blood of rabbits contains a lethal factor during shock after occlusion and release of the superior mesentric artery. However, as in the current study, this earlier report was unable to substantiate the presence

of endotoxic material in the blood stream during this type of shock.

Blood pressure masuremnts were not especially informative. Blood pressure dropped more abruptly in controls following removal of the arterial occlusion than it did in antibiotic protected animals. Apparently, the bowel was better able to withstand circulatory arrest when bacterial flora was suppressed by antibiotics. Subsequently, blood pressure fell progressively in those rats which ultimately died, but remained at near normal levels in survivors. These differences may be due to absence of swelling and plasma loss in the intestinal tract of antibiotic treated rats. Direct visualization of the omental circulation supports this possibility since the blood flow in protected animals did not show the hemoconcentration exhibited by the controls. Venular stagnation and precapillary hemorrhage, which were prominent features of the mesenteric circulation in fatal shock, did not develop to any significant extent in antibiotic protected animals.

In general, the protection afforded by orally administered antibiotics would appear to be a local circumstance which minimizes the pathological changes in the intestinal wall during shock and thereby avoids the attendant plasma loss and frank hemorrhage which are a prominent feature of bowel ischemia shock.

Summary and conclusions. Shock induced in rats by temporary occlusion of the superior mesenteric artery after pretreatment with various antibiotics and induction of resistance by drum training and by repeated administration of bacterial endotoxin was studied. Terramycin, Neomycin, or Gantrisin given orally for 6 days clearly protected rats, whereas those treated with Aureomycin or Chloromycetin were not protected. Adaptation to drum trauma did not circumvent the lethal effects of bowel occlusion. Tolerance to bacterial endotoxins was only moderately effective against occlusion shock.

It was concluded that the protective action afforded by certain antibiotics was probably duc to their local action in preventing pathological changes in the intestinal tract and not to prevention of a systemic bacteremia.

The author wishes to express his thanks to Dr. C. S. Yang and Mr. Y. C. Lin, Dept. of Bacteriology, Nat. Taiwan Univ. College of Med., for suggestions and assistance in preparing bacterial cultures; to Drs. J. T. Grayston and K. S. Pang, Dept. of Microbiology, NAMRU-2, Taipei, for supplying animals; and to Charles Pfizer & Co., Inc., New York, for Terramycin.

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Received May 31, 1961. P.S.E.B.M., 1961, v108.