1. Dayton, S., *Circulation Research,* 1959, v7, 468.
2. Dayton, S., Dayton, J., Drimmer, F., Kendall, F. E., *Am. J. Physiol.,* 1960, v199, 71.
3. Schwenk, E., Werthessen, N. T., *Arch. Biochem. and Biophys.,* 1952, v40, 334.
4. Katz, L. N., Stamler, J., *Experimental Atherosclerosis,* 1953, Charles C. Thomas, Springfield, 127 ff.
5. Kritchevsky, D., *Cholesterol,* 1958, John Wiley & Sons, Philadelphia, 54 ff.

Received July 21, 1961. P.S.E.B.M., 1961, v108.

Hepatic Clearance of Endotoxin Absorbed from the Intestine.* (26909)

R. GREENE, T. WIZNITZER,† S. RUTENBURG, E. FRANK AND J. FINE
Yamins and Kirstein Laboratories for Surgical Research, Beth Israel Hospital, and Department of Surgery, Harvard Medical School, Boston, Mass.

Endotoxin absorbed from the gut has been demonstrated in the blood of animals in 3 types of traumatic shock(1). In the normal animal the endotoxin is extracted from the blood and destroyed almost immediately by the reticulo-endothelial system (R.E.S.)(2). But in the shocked animal the capacity of the system to extract or destroy endotoxin is severely impaired(2). Thus endotoxin becomes free to injure the circulation and to prevent recovery. The amount of endotoxin absorbed varies with the size of the pool of the gram-negative flora in the gut(3). Additional evidence to this effect is presented below.

Method. New Zealand rabbits (average wt 2 kg) with normal intestinal flora, as indicated in each instance by culture from rectal swabs, were anesthetized with nembutal intravenously, and the abdomen opened with aseptic precautions through a midline incision. The intestine was gently exteriorized, and 7-10 ml of blood withdrawn from the portal vein into heparinized tubes. Similarly, blood was withdrawn from the inferior vena cava. These bloods were cultured and were found to be uniformly free of bacteria. They were also tested for the presence of endotoxin by the method of Smith and Thomas(4). A positive test consisted of multiple hemorrhages and death of at least 4 of 6 ten-day-old chick embryos, to each of which 0.1 ml of the same sample of blood had been administered. Death of not more than 2 of 6 embryos was considered evidence of the absence of endotoxin. Because the cultures from rectal swabs do not always accurately reflect the pattern of the intraintestinal flora, cultures from the lumen of ileum and colon were obtained at the end of each experiments as a check upon the preoperative cultural data from rectal swabs.

Five groups of experiments were performed. In Group 1 the procedure as described above was carried out. In Group 2 the procedure was the same, except that 3 hours preoperatively 100 mg of an *E. coli* $0111B_4$ endotoxin (MLD/100 = 3-4 mg/kg) in 30 ml of normal saline was given by gavage. In Group 3 the procedure was the same as in Group 2, but in addition 2.75 ml/kg of Thorotrast was given intravenously when the endotoxin was fed. In Group 4 the procedure was the same as in Group 1, except that the rabbits used were coliform-free by the cultural data from preoperative rectal swabs. In Group 5 the procedure was the same as in Group 4, except that these rabbits were given neomycin (250 mg/kg) by gavage daily for 3 successive days, and again 3 hours preoperatively on the 4th day.

Results are listed in Table I. *Group 1.* The data of Group 1 indicate the presence of endotoxin in the blood of the portal vein in one-half, and in the blood of the systemic veins in one-sixth of rabbits with a normal intestinal flora. There was no instance in

* Aided by a grant from Nat. Inst. Health, Bethesda, Md., and by a contract with Office of the Surgeon General, U. S. Army.

† Nat. Inst. Health Research Fellow in Surgery, Instructor in Surgery, Hadassah Univ. Hospital and Hebrew Univ., Jerusalem, Israel.

TABLE I. Assay of Blood for Endotoxin by Chick Embryo Test.

Group	No.	Positive portal vein blood	Positive inf. vena cava blood
		%	
1. Normal coli-positive	30	50	17
2. Normal coli-positive + endotoxin by gavage	10	60	30
3. Normal coli-positive + Thorotrast	9	100	89
4. Coli-neg.*	10	40	0
5. Coli-neg. + preoperative neomycin	28	16	4

* Coli-negative signifies absence of coliform bacteria in cultures of preoperative rectal swabs. Cultures of the lumen of ileum and colon at time of laparotomy showed that of the 10 rabbits only 4 were, in fact, coli-negative. The number of coliforms in the other 6 was very small, however.

this or any other group in which the blood of a systemic vein was toxic when the blood of the portal vein was non-toxic.

Comment. These data are evidence of absorption of endotoxin from the intestine. They are consistent with previous data obtained by direct perfusion of the spleen with endotoxin, showing that an intact R.E.S. can extract some 80% of the endotoxin presented to it(2). This latter observation would allow for some toxin in the blood leaving the R.E.S. Hence the occasional presence of toxicity in systemic vein blood (17%) of normal rabbits is to be expected. Indeed the escape of endotoxin into the general circulation may account for occasional variations from the normal responses to states of stress in which endotoxin can become a critical determinant. *E. g.*, in any given series of animals exposed to severe hemorhagic shock, a few animals develop irreversibility to transfusion very much sooner than the majority. Contrariwise, some 10-15% do not develop irreversibility because of a better than normal detoxifying capacity of the R.E.S.(5). Animals with a low pool of endotoxin in the gut because of a coliform-free intestinal flora also do not develop irreversibility to transfusion(6). In them one would expect most of the bloods from the portal as well as those from the systemic vein to be non-toxic (See data of Group 4 below). But even when the flora is normal, absence of toxicity might be expected when the amount of endotoxin entering the portal vein at the time of sampling is less than can be detected by the chick embryo test. One can thus account for the fact that one half the samples in Group 1 were non-toxic.

Group 2: Comment. The data of Group 2 show a higher incidence of toxicity in the systemic blood than was found in Group 1, suggesting that an increase in size of the pool of endotoxin in the gut can increase the amount absorbed so as occasionally to overtax the liver's extracting capacity.

Group 3: Comment. The data of Group 3 demonstrate that Thorotrast eliminates the liver's capacity for extracting the endotoxin delivered to it from the intestine, confirming a previous observation by Cremer and Watson(7) and supporting some of our own later observations(6). This phenomenon doubtless accounts for the enormous increase in sensitivity to endotoxin shown by Thorotrast-pretreated animals when given endotoxin for eliciting the Shwartzman reaction. Such a spontaneously generated endotoxemia may explain the lethal property of Thorotrast when given alone in large doses(6).

Group 4: Comment. The data of this group require interpretation in the light of the cultural data obtained postmortem, showing that of the 10 rabbits considered coliform-free before operation, only 4 were coliform-free by direct culture of the ileum and colon. Of these 4, one yielded a positive portal vein blood. The remaining 6 showed some coliform bacteria in the ileum and colon, and of these 6, three yielded positive portal vein bloods. It is probable that the zero incidence of toxicity in the systemic vein blood of these 10 rabbits is due to the very much smaller pool of endotoxin in this group than in those with a normal coliform flora.

Group 5: Comment. The nearly total elimination of toxicity in portal as well as systemic blood as a result of giving neomycin to a group of coliform-free rabbits is strong support for the view that antibiotics given orally prevent endotoxemia, and therefore,

irreversibility to transfusion in severe and prolonged hemorrhagic shock as a result of sharply reducing the size of the intraintestinal pool of endotoxin(8). Thus it is clear that not only can the size of the intraintestinal pool be readily altered, but also that the amount of endotoxin which enters the circulation varies with the size of the pool.

Discussion. These observations supplement considerable published evidence that endotoxin is continuously absorbed from the intestine(9), and that a number of phenomena are influenced by this process in a hitherto unsuspected manner(6). Thus in 1957 we confirmed previous observations that mortality from a lethal dose of endotoxin is reduced by prior or simultaneous administration of an antibiotic. This was also the case when a non-absorbable antibiotic was given(10). From these data we concluded that an MLD/100 of endotoxin is not lethal *per se,* but by virtue of the participation of additional endotoxin absorbed from the gut following injection of the "lethal" dose. The endotoxin which is continuously absorbed from the gut may also account for the occurrence of the generalized Shwartzman reaction after a single dose of endotoxin in the pregnant animal (11,12), and for the sporadic occurrence of the local Shwartzman reaction in mice following a single dose of endotoxin(13). This is all the more likely in view of the observation that tetracycline given orally eliminates such responses until the intestinal flora acquire resistance to the antibiotic(14). The apparently wayward responses of rabbits, tested under comparable circumstances, to administration of 2 spaced doses of endotoxin for the purpose of inducing the generalized Shwartzman reaction may also find its explanation in variations in the size of the intraintestinal pool of endotoxin, and in the rate of its absorption into the circulation. That rabbits with a coliform-free intestinal flora subjected to hemorrhagic shock do not develop the endotoxemia observed in coliform-bearing rabbits(15), and the recent report of Schaedler and Dubos(16) that pathogen-free Swiss mice are more resistant to endotoxin than mice of the same breed bearing coliform bacteria, further support the hypothesis that the pattern of the intestinal flora, tolerance to endotoxin, and tolerance to traumatic shock are interrelated.

Summary. Data are presented showing that endotoxins absorbed from the intestine are present in the blood, and in much higher incidence in portal than in systemic vein blood. The incidence of endotoxemia in portal vein blood varies with the size of the intraintestinal pool of endotoxin. A reduced capacity of the R.E. system in the liver for extracting endotoxin allows endotoxin in the portal vein blood to traverse the liver and enter the general circulation.

1. Schweinburg, F. B., Fine, J., *J. Exp. Med.,* 1960, v112, 793.

2. Wiznitzer, T., Better, N., Rachlin, W., Atkin, N., Frank, E., Fine, J., *ibid.,* 1960, v112, 1157.

3. Wiznitzer, T., Schweinburg, F. B., Atkin, N., Fine, J., *ibid.,* 1960, v112, 1167.

4. Smith, T. R., Thomas, L., *ibid.,* 1956, v104, 217.

5. Smiddy, F. G., Segel, D., Fine, J., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 584.

6. Fine, J., Rutenburg, S., Schweinburg, F. B., *J. Exp. Med.,* 1959, v110, 547.

7. Cremer, N., Watson, D. W., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 510.

8. Jacob, S., Weizel, H., Gordon, E., Korman, H., Schweinburg, F. B., Frank, H., Fine, J., *Am. J. Physiol.,* 1954, v179, 523.

9. Ravin, H. A., Rowley, D., Jenkins, C., Fine, J., *J. Exp. Med.,* 1960, v112, 783.

10. Schweinburg, F. B., Davidoff, D., Koven, I. H., Fine, J., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 662.

11. Apitz, K., *J. Immunol.,* 1935, v29, 255.

12. McKay, D., Wong, T., Galten, M., *Fed. Proc.,* 1960, v19, 1.

13. Kelly, M. G., Smith, N. H., Woodlinsky, I., Rall, D. P., *J. Exp. Med.,* 1957, v105, 653.

14. Arndt, W. F., Schneider, H. A., *ibid.,* 1960, v112, 167.

15. Ravin, H., Schweinburg, F. B., Fine, J., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 426.

16. Schaedler, R. W., Dubos, R. J., *J. Exp. Med.,* 1961, v113, 559.

Received July 26, 1961. P.S.E.B.M., 1961, v108.