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Are Enteric Endotoxins Able to Escape From the Intestine? (38428)

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The problem at issue, never having been fully resolved, is whether enteric endotoxins are able to escape from the intestine and, if so, in what quantities. There is considerable controversy concerning this point because in the past there have been as many investigators who were unable to demonstrate its absorption from the normal intestine (1) as those who could (2).

Recently, a bioassay procedure of great sensitivity and reproducibility has become available for the detection of small amounts of endotoxin in the rat, an animal otherwise resistant to relatively large quantities of endotoxins. Selye *et al.* (3) first described the lead acetate-sensititized rat model; he and, subsequently, Filkens (4) demonstrated that it can detect the equivalent of 1 ng of a commercially available *E. coli* endotoxin preparation per 100 g rat body wt and they used this model for semiquantitative determinations. Its sharp end point is unique and, because of its noninvasive nature, the need for blood sampling is eliminated, thus preventing contamination inherent in most other techniques as a possible source of error. The procedure consists of sensitizing rats with an intravenously administered lead acetate solution, followed by intravenous injection of the material under investigation. The resultant animal mortality is related to the amount of endotoxin contained in the solution. Because of recently noted limitations of the Limulus lysate technique, we resorted to this model to determine whether endotoxin is or is not absorbed from the intestine (5).

Materials and Methods. In male Sprague–Dawley rats, weighing 250–275 g, a Thiry-Vella fistula (T-V-f), an isolated, bypassed, bile-free, ileal segment, was prepared. Elsewhere we have described the manner in which this is done (6).

One week after operation, after the animals had fully recovered as evidenced by return to normal food intake and weight gain, they were lightly anesthetized with ether, following which purse-string sutures were placed around both ileostomies. The distal ileostomy was closed off. Then, taking pains to avoid injury to the intestinal mucosa, a small soft rubber catheter was carefully threaded into the loop through the proximal ileostomy to introduce a mixture of 0.5 mg Cr^{51}-labeled and 4.5 mg unlabeled endotoxin to which methylene blue was added. Both agents were suspended in 3 ml of isotonic saline, distilled water, or hypertonic (50%) glucose in water. Then each animal was given 3.5 mg/100 g body wt of lead acetate intravenously, which, as was noted above, has been shown to sensitize the rat to endotoxin more than 10,000-fold by mechanisms that as yet have not been fully elucidated (7).

Endotoxin. Lipopolysaccharide B. *E. coli* preparations obtained from Difco Laboratories, Detroit, MI were used. Endotoxin was labeled with $Na_2Cr^{51}O_4$ as described by Braude *et al.* and modified by Chedid and co-workers (8).

Methylene blue. One-tenth milliliter of 0.25% solution was added to every 25 ml of endotoxin solution.

Lead acetate. Pb $(C_2H_3O_2)_2 \cdot 3H_2O$ (Matheson, Coleman and Bell, East Rutherford, NJ) was suspended in pyrogen-free water in a final concentration of 20 mg/ml. The material was drawn up in a new plastic, pyrogen-free tuberculin syringe and injected into the dorsal vein of the penis.

Autopsy. At time of death or sacrifice the animals were autopsied. Immediately after opening the abdomen, the peritoneal cavity was wiped clean with clean surgical sponges, before the bypassed intestinal segment was taken down. The sponges were placed in identical counting vials and counted in a well counter. The counts indicated represent the mean value (over background) ± 1 SD.

Results. In the first experiment the lethality of endotoxin alone, lead acetate alone, and their

combined effects were studied. Endotoxin (1 μg/100 g body wt) alone or lead acetate (3.5 mg/100 g body wt) alone are well tolerated. Injection of 1 ng of endotoxin/100 g body wt in lead sensitized rats resulted in death in two out of ten (20%); 10 ng in three out of ten (30%): 0.1 μg in six out of ten (60%); and 1 μg in eight out of ten rats (80%), results nearly identical to those previously reported by Selye *et al.* (3) and Filkins (4). Death is preceded by the characteristic symptoms of endotoxin shock as observed in this animal. In the next experiment 25-cm-long T-V-f's were prepared in two groups of rats, the first having undergone end-to-side portacaval shunt operations 1 week previously, allowing portal vein blood to bypass the liver which, as was shown previously, is able to clear and detoxify endotoxin from the portal vein blood (9). Animals of the second group had undergone only a sham operation consisting of cross clamping the portal vein and inferior vena cava for 30 min, the time required to complete a portacaval shunt in the rat.

In both groups of animals administration of lead acetate alone, 3.5 mg/100 g body wt, was well tolerated; none of the ten animals so treated showed any untoward effects. Also, if lead acetate was injected 4 hr after endotoxin was placed in the bypassed ileal segment, all ten sham-operated and ten portacaval shunt rats survived. However, if the lead acetate was injected at the *same* time the endotoxin was placed in the loop, one out of ten sham-operated rats and four out of ten portacaval shunt rats succumbed.

The finding that portacaval shunt rats tolerated lead acetate alone or lead acetate and endotoxin 4 hr apart would indicate that neither the portacaval shunt operation nor the lead acetate injection can be held responsible for the observed increase in mortality in these animals. Instead, it would suggest that portacaval shunt rats immediately sensitized with lead acetate are more susceptible to endotoxin than sham-operated animals. Because a portacaval shunt allows portal vein blood to bypass the liver, the increased mortality may be due to a diminished clearance by the liver of portal vein blood and the endotoxin it contains, suggesting that small amounts of endotoxin are indeed absorbed from a 25-cm ileal loop, and that at least part is transported via the portal vein. Certainly, the amount of endotoxin absorbed is minute; a 40% mortality in this assay system is consistent with absorption of approximately 10 ng of endotoxin per 100 g rat body wt, a rather insignificant amount, when compared to a lethal dose of endotoxin, which in the rat is in the range 2–4 mg/100 mg body wt (9).

The finding that none of the animals died if lead acetate was given 4 hr after the administration of endotoxin would indicate that at this time little or no further absorption of endotoxin takes place. This might suggest that, in the intestine, mechanisms operate that detoxify, alter, or otherwise retain the endotoxin within its lumen.

All subsequent experiments made use of T-V-f's in rats without portacaval shunts. In these animals the intestinal loop was made 40 cm long instead of 25 cm. As a result, the mortality rose from one rat in ten (10%), observed in animals with a 25-cm T-V-f, to five rats in 14 (36%), in those with a 40-cm T-V-f, indicating that, as the surface area increased, more endotoxin was absorbed.

Previous findings indicated that the capacity of the liver of the lead-sensitized rat to clear in one passage endotoxin from the portal vein blood and to detoxify it exceeds 1 μg endotoxin/hr/100 g body wt (9). The present findings suggest that, although the amount of endotoxin absorbed is small and considerably less than 1 μg/100 g body wt, not enough of it is cleared by the liver to save every animal. This would indicate that some of the endotoxin is able to bypass the liver to exert its systemic effects. The lymphatics are the only other available escape route. The question is which of the two routes is the more important one, particularly if intestinal permeability is increased?

Presently only a few circumstances have been shown to be associated with increased absorption of intestinal endotoxins, namely, total body irradiation (10), graft-vs-host reaction (11), and possibly treatment with chemotherapeutic agents. The question whether shock constitutes another example has never been unequivocally elucidated; hence there is presently as much evidence for (12) as against such a role (13). We introduced enhanced intestinal absorption by utilizing the effect of osmotic shock on the intestinal wall (14). This method was elected because of its ready accessibility, the ease with which it can be controlled, and the uniformity of the results provided.

The mortality rate of lead-sensitized rats was found to increase steeply when hypo- or

hyperosmolar solutions were used to resuspend the endotoxin prior to its introduction into a 40-cm T-V-f. Where five out of 14 rats (36%) died when endotoxin was resuspended in isotonic saline, 12 out of 19 (64%) succumbed when distilled water was used, and 13 out of 14 (93%) died within 5–6 hr if 50% dextrose constituted the suspension medium, suggesting that under those circumstances the amount of endotoxin that escapes from the T-V-f increased to more than 1 μg/100 g rat body wt. This rise in mortality was not related to the dehydrating effect of the hypertonic glucose solution because only one out of 20 nonsensitized animals died that received the same amount of endotoxin suspended in hypertonic glucose. At autopsy this animal showed a localized peritonitis, associated with the isolated, intestinal loop. Of ten lead acetate-sensitized rats that died after receiving endotoxin in hypertonic glucose and that were examined at the time of death, nine had significant amounts of radioactivity in the peritoneal cavity, suggesting that some of the labeled endotoxin had escaped from the intestinal lumen to the peritoneal cavity.

If no lead acetate was given, only five out of ten animals so examined were found to have radioactivity in the peritoneal cavity at the time of sacrifice, 6 hr after the start of the experiment. The relative amount of radioactivity noted in the group of animals that died as a result of endotoxemia, 2204 ± 803 cpm, was significantly larger than that found in the group of animals that was sacrificed, 191 ± 96 cpm, suggesting that either lead acetate itself or the condition that precedes death from endotoxemia, *e.g.*, shock, is associated with increased permeability of the gut wall. Presently, there is no agent that will sensitize rats to endotoxin to the same degree as lead acetate. Hence it has not been possible to examine whether lead acetate or shock could account for the observed differences.

Daniele *et al.* (15) previously demonstrated that, if *E. coli* endotoxin is placed in the peritoneal cavity, part of it remains while some escapes, predominantly via the thoracic ducts. This then would indicate that, if intestinal permeability increases, significant quantities of endotoxin are able to leave the intestine not by way of the portal vein but via the peritoneal cavity to enter the abdominal lymphatics and to be transported further via the thoracic ducts, suggesting that also under those conditions little endotoxin escapes directly into the portal vein circuit.

From these observations it would appear that a small amount of endotoxin is absorbed from a T-V-f loop. The mechanism whereby this occurs remains unknown.

Discussion. The data support the concept that the quantities of endotoxin absorbed from a normal isolated intestinal loop are limited indeed; the actual amount absorbed from a 40-cm T-V-f is in the range 1–10 ng. Of course absorption from longer segments is expected to be larger. Yet it is rarely expected to exceed the 100-ng level. It should not be construed that since it is absorbed from a T-V-f this amount is also absorbed from the intact intestine. The conditions under which absorption takes place from normal intact intestine are probably limited and remain to be defined. Were this also to occur in man, it would remain to be established whether the amount that could possibly be absorbed would be sufficient to inflict injury to organs in the manner previously postulated (16).

An abnormal intestine permits larger amounts of endotoxin to escape. The conditions associated with enhanced absorption and the possible consequences, however, also remain to be determined (17).

Summary. Endotoxin was introduced into a Thiry-Vella fistula (T-V-f) prepared in lead-sensitized rats. Of the 5 mg given to each rat an amount is absorbed into both the portal vein and intestinal lymphatics sufficient to kill a number of lead-sensitized rats. Most of the endotoxin escapes into the intestinal lymphatics, causing systemic manifestation and death because it is able to bypass the clearing and detoxifying functions of the liver.

The manner in which endotoxin is absorbed is presently not known. Presumably absorption occurs by passive diffusion, a process that is considerably enhanced as a result of the effects of osmotic shock. In fact, this condition causes additional transmural leakage of endotoxin, but even then the amount that escapes is so small that it is unlikely to be of any real significance.

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