Intestinal Tissue Blood Flow in Shock Due to Endotoxin

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With the technical assistance of Louis M. Levy, B.S.

The small intestine has been implicated in irreversible shock due to endotoxin and hemorrhage by both clinical and experimental observations. Penner and Bernheim in 1939 described the pathology of the small intestine in shock that occurs clinically. They stated that there first appeared congestion, edema and hemorrhage in the submucosa with hemorrhagic necrosis of the mucosa as a later manifestation. Similar lesions have been produced experimentally by shock due to hemorrhage, various endotoxins and exotoxins, trauma from tumbling, and prolonged infusion of adrenalin. Lillehei showed that perfusion of the superior mesenteric artery was successful in preventing the usual irreversibility of hemorrhagic shock; however, perfusion of the liver via the hepatic artery or perfusion of the femoral artery with similar quantities of blood was not successful. Shock due to endotoxin has been demonstrated to be characterized by a marked increase in weight of the small intestine in 15 minutes to 4 hours after administration of the toxin. This "fluid loss" into the intestine was sufficient to account for the hypotension observed.

There is evidence that both severe hemorrhage and endotoxin produce these deleterious effects by inciting severe and lasting vasospasm in the small arteries and veins of the intestine. Drugs that prevent or counteract vasospasm have an ameliorating effect on the induced shock and on the characteristic pathological findings in the intestine.

The above findings indicate that the small intestine, and more particularly its blood supply, plays an important role in shock. For this reason, the present investigation was designed to determine the influence of shock due to endotoxin administration upon (1) the total blood flow to the small intestine and (2) the distribution of the blood flow to the component tissues (mucosa, submucosa, muscularis, mesentery) of the organ.

Methods

One of the simplest of the many methods available to measure the total blood flow through the intestine was used in this study: the collection of a timed sample of the total venous outflow in a graduated container.

For the measurement of the distribution of the total flow to the different tissues of the organ, no simple, direct method exists at present. The one used here is based upon the conservation of matter, commonly referred to as the Fick principle in blood flow studies. This is that the rate of uptake of a test substance is equal to the rate of inflow of that substance via the arteries minus the rate of outflow via the veins. The test substance used here was deuterium oxide.

\[
\frac{dN_t}{dt} = F_t C_A - F_v C_v
\]

where \(N\) = amount of D\(_2\)O, \(t\) = time in minutes, \(F\) = flow in ml. blood water/min., \(C\) = concentration of D\(_2\)O in water in mole per cent excess, and the subscripts, \(A\) and \(V\) refer to the tissue, arterial blood and venous blood, respectively. Assuming that the arterial inflow equals the venous outflow during an experiment, (1) becomes

\[
\frac{V_t dC_t}{dt} = F(C_A-C_V)
\]

where \(V\) = volume of water in the tissue in milliliters.

Although the experimental design may be so arranged as to keep \(C_A\) constant, both \(C_V\) and \(C_V\)
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will vary with time. To integrate equation (2) it is therefore necessary to know the relation between these 2 variables. For the case of any test substance whose rate of uptake by the tissue is limited by the blood flow through the tissue, this relation is simple: the concentration of the substance in its volume of distribution in the venous blood will at all times be equal to the concentration in its volume of distribution in the tissue. It is for this reason that D2O was used in the study described here. Johnson, Cavert and Lifson7 and Thompson, Cavert, Lifson and Evans8, 9 concluded that the distribution of D2O in dog heart and gastroenemius and in rat liver was largely flow limited. Weiner and Grin in this laboratory have found the same to be true to a reasonable approximation for the individual tissues of the intestine of normal dogs anesthetized with Nembutal in the first 1 to 2 minutes of D2O perfusion. On this basis, the assumption can be made that the concentration of D2O in the venous water is equal to its concentration in the water of the tissues. Equation (2) becomes

\[ \frac{F}{V_T} \int_0^t dt = \frac{dC_T}{C_T - C_T} \]

Integrating between \( t = 0 \), \( C_T = 0 \), and \( t = t \), \( C_T = C_T \), this becomes

\[ \frac{F}{V_T} = \frac{1}{t} \ln \left( \frac{C_T}{C_T - C_T} \right) \]

from which the tissue perfusion ratio, \( \frac{F}{V_T} \)

(the units of which are in ml. of blood water/min.-Gm. of tissue water) can be calculated, since all variables on the right side can be measured experimentally.

Since the water content of mucosa, submucosa, muscularis, and blood were all about 80 per cent (78 to 85 in this study) and since the specific gravity of blood was nearly one, the perfusion ratios for these tissues closely approximates the blood flow through the tissue in ml. of blood/min.-Gm. of tissue. In the case of the mesentery, this is not true, as the water content of this fatty tissue varied from 20 per cent to 60 per cent; the average blood flow/Gm. of tissue is therefore about 40 per cent of the perfusion ratio.

All experiments were performed in an air-conditioned laboratory on mongrel dogs of either sex using morphine analgesia (2 mg./Kg.). The femoral artery was cannulated for the purpose of monitoring the blood pressure and the femoral vein for administering endotoxin. After infiltration with procaine (1 per cent) the abdomen was entered and a loop of ileum exposed. The bowel was maintained at a temperature of 35 to 39 C. by means of a heating pad and kept moist with isotonic saline. The mesentery was sectioned on both sides of a major vascular arcade, and the artery and vein of the arcade cannulated with polyethylene catheters. The artery was perfused with heparinized blood from a reservoir in a constant temperature bath. In all instances, this blood was obtained from normal donor dogs immediately prior to experiment. The perfusion pressure was maintained constant in the reservoir by means of compressed air introduced into the reservoir through a tube having a side arm immersed in a vessel of mercury to an appropriate depth. The blood in the reservoir was stirred with a magnetic stirrer. Only after the perfusion had been established was the loop of intestine isolated from the rest of the bowel with intestinal clamps. In this way the loop was never without a blood supply. With the loop isolated, perfusion was rapidly changed, by means of a stopcock in the arterial cannula, to blood containing approximately 2 per cent D2O. This perfusion was contained for 30 to 60 seconds during which time the venous outflow was collected in a graduated tube. From this volume of venous blood and the weight of the loop, the total blood flow through the loop in ml./min.-Gm. was calculated. At the end of the perfusion period, and in rapid sequence, the loop was removed, drained of any intraluminal fluid, split along the mesenteric border, blotted with filter paper, weighed, and rapidly divided into mucosa, submucosa, muscularis, and mesentery. The mesentery was trimmed from the loop and the mucosa and muscularis were separated from the submucosa by scraping off the tissues with a scalpel. The individual tissues were weighed. The water content was determined for the individual tissues in many of the experiments by desiccation. Samples of tissue and perfusing blood containing approximately 0.2 ml. of water were prepared for D2O analysis by distilling in vacuo to dryness. The distillate was equilibrated with hydrogen gas at atmospheric pressure in the presence of a platinum oxide catalyst. The DH:HI ratio was determined with a mass spectrometer.

Three groups of experiments were performed. In the first, loops of intestine from normal dogs were perfused at 135 mm. Hg to provide controls. In the second group, loops from dogs which had been given a lethal dose (5 to 7.5 mg./Kg.) of endotoxin* 2 to 3 hours before experiment were used. These loops were also perfused at 135 mm. Hg. In the third group, loops from endotoxin shock dogs were perfused at the dog's blood pressure measured at the time of experiment. This pressure varied from 50 to 100 mm. Hg with a mean of 70 mm. Hg.

*Cendotoxin was kindly furnished by Dr. Wesley Spink of the Department of Medicine, University of Minnesota.
Table 1

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Mucosa</th>
<th>Submucosa</th>
<th>Muscularis</th>
<th>Mesentery</th>
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<tr>
<td>1</td>
<td>0.54</td>
<td>0.52</td>
<td>0.53</td>
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<tr>
<td>2</td>
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<td>4</td>
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<td>5</td>
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<td>10</td>
<td>0.24</td>
<td>0.44</td>
<td>0.77</td>
<td>0.44</td>
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<tr>
<td>Mean</td>
<td>0.38</td>
<td>0.50</td>
<td>0.66</td>
<td>0.58</td>
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</table>

Results and Discussion

In the control group, this mean total blood flow through the loops was 0.56 ml./min.-Gm. of tissue. This agrees with the usually accepted values for small intestinal blood flow. The perfusion ratios of the individual tissues of these control loops as calculated by means of equation (4) are shown in table 1. As stated earlier, the perfusion ratios of mucosa, submucosa and muscularis approximate the tissue blood flows in ml./min.-Gm. of tissue. Since the mean water content of mesentery was about 30 per cent, the blood flow to this tissue was only 40 per cent of the perfusion ratio, that is, about 0.23 ml./min.-Gm. In a representative gram of dog ileum, the fractional weights of mucosa, submucosa, muscularis and mesentery were 0.45, 0.15, 0.25 and 0.15, respectively. If the tissue blood flows are multiplied by these fractional weights, the volume of blood passing through each tissue in 1 Gm. of intestine per minute is obtained. They are 0.17, 0.08, 0.17 and 0.03 ml./min. respectively, with a total of 0.45, a value which is 20 per cent smaller than the observed total flow of 0.56 ml./min.

There are several possible explanations for this discrepancy. If the distribution of D₂O in the tissues was blood flow limited as assumed in the derivation of equation (4), then 20 per cent of the total blood flow bypassed capillaries and flowed through arteriovenous (A-V) anastomoses. In this case, the data correctly express the capillary blood flow rates in the individual tissues. On the other hand, it is possible that more than 80 per cent of the blood passed through capillaries, but that the distribution of the D₂O from the capillaries was not entirely flow limited in one or more of the tissues. There may have been barriers to the free diffusion of D₂O across the capillary wall or across nearby tissue cell walls. It is also possible that the anatomic distribution of capillaries in some of the tissues may have been such that some regions of the tissue were very poorly perfused or even not perfused at all. In either of these cases, the mean D₂O concentrations of the tissue water would have been less than the D₂O concentration of the venous blood water draining the capillaries of that tissue; that is, the assumption of flow limited distribution would be invalid, and the calculated perfusion ratios would be underestimates of the true capillary blood flows in the tissues so affected. There are some reasons for suspecting that there might be diffusion barriers or heterogeneity of capillary perfusion in 2 of the tissues. Gersh and Still have shown that the surface to volume ratio of capillaries and the density of the capillary bed are considerably smaller in fatty tissues like the mesentery than in fat-poor tissues; and, other studies in this laboratory have indicated that barriers to free diffusion of D₂O may exist in the mucosa. It is, therefore, possible that the capillary blood flow through these 2 tissues are somewhat higher than indicated by the data.

In the second group of dogs, those administered endotoxin and loops of which were perfused at 135 mm. Hg, the mean total blood flow was 0.52 ml./min.-Gm. The agreement of this value with that for the control loops shows that the total resistance to blood flow at normal perfusion pressure was unaltered by the endotoxin shock under the conditions of these experiments. It should not be inferred that the resistance would necessarily have been unchanged if the loops had had an intact innervation or if they had been studied imme-
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Table 2

<table>
<thead>
<tr>
<th>Tissue Perfusion Ratios in ml. Blood Water/Min.-ml. Tissue Water in Ileum of Dogs in Endotoxin Shock Perfused at 135 mm. Hg</th>
</tr>
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<tbody>
<tr>
<td>Expt.</td>
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<tr>
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</tr>
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<td>1</td>
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<td>10</td>
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<tr>
<td>11</td>
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<tr>
<td>12</td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>% of control</td>
</tr>
</tbody>
</table>

Immediately following the administration of the endotoxin.

The calculated perfusion ratios of the individual tissues in these loops are shown in table 2. In the last line of this table the mean tissue perfusion ratios are shown as percentages of the control values. Despite the fact that the total blood flow through the shock loops as directly measured was essentially the same as that through the control loops, the calculated perfusion ratios were decreased in all tissues except the mesentery. The reduction in perfusion ratio is most marked (nearly 50 per cent) in the mucosa. This may indicate a real decrease in tissue capillary blood flow; that is, a shift of blood flow from capillaries to A-V anastomoses. It may also have been the result of a decrease in capillary wall and/or tissue cell wall permeability or an increase in heterogeneity of capillary perfusion. Since it is usually thought that injury to tissues is accompanied by an increased not a decreased permeability, it seems more likely that the endotoxin produced a reduction in capillary flow either in parts of the tissues, leading to heterogeneous perfusion, or in all of the tissue, the capillary flow having been diverted to A-V anastomoses. Whatever the mechanism, the rate of appearance of D$_2$O, and by implication other water-soluble materials, was decreased in the mucosa, submucosa, and muscularis by the endotoxin. It might be said that the “effective” capillary flow was reduced to the extent indicated by the calculated perfusion ratios, even though the actual capillary flow may have fallen to a lesser extent.

In the third group of experiments, loops were obtained from dogs treated with endotoxin and perfused at the animal’s pressure as measured at the time of experiment. The mean perfusion pressure was 70 mm. Hg or slightly more than one-half of that used in the first and second groups. The total blood flow through these loops was reduced also by about one-half to 0.27 ml./min.-Gm., indicating that the resistance to flow was the same at both perfusion pressures. The calculated tissue perfusion ratios are shown in table 3.

The mean perfusion ratios expressed as percentages of the perfusion ratios found with shock loops perfused at 135 mm. Hg are shown in the next to last line of table 3. The reduction in perfusion ratios of the mucosa, submucosa, and muscularis agree within experimental error with the decrease in total blood flow. The simplest explanation of this observation is that the capillary blood flow through these 3 tissues was also reduced to one-half. This would certainly have been the

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case if D₂O distribution was flow limited in these tissues in the endotoxin shock loops whether perfused at 135 or 70 mm. Hg, for the calculated perfusion ratios would have equaled the real capillary flows in both instances. If D₂O distribution was not entirely flow limited, it is most likely that the reduction in real capillary flows were even greater than the fall in the calculated perfusion ratios. Thus, unless the perfusion at the lower pressures caused a marked increase in D₂O transport limitations or heterogeneity of perfusion, it can be concluded that the real capillary flows in the mucosa, submucosa and muscularis were reduced at least 50 per cent at the lower perfusion pressures.

The simplest explanation of the change in mesenteric perfusion ratio is that again the real capillary flow fell to one-half; but, because of considerable deviation from flow limited kinetics of D₂O distribution in this tissue, the calculated perfusion ratio was reduced to a lesser extent. It is, of course, possible that D₂O distribution was flow limited and that a decrease in capillary flow of only 25 per cent occurred.

In the last line of table 3, the mean tissue perfusion ratios of the endotoxin shock loops perfused at shock pressures are shown in relation to the same ratios for the control loops. These values demonstrate the profound effects of endotoxin shock on the perfusion ratios of the intestinal tissues, a reduction to one-third of normal in the mucosa and to less than one-half in the submucosa and muscularis. Although these reductions may have been due only in part to decreases in real capillary blood flow, some having been due to increases in D₂O transport limitations or perfusion heterogeneity, the fact remains that the rate of D₂O uptake by the tissues was reduced to the extent indicated by the calculated perfusion ratios. Thus, the “effective,” if not the real, capillary flow was greatly reduced. It seems reasonable to conclude that this may be the basis, at least in part, of the pathological changes observed, particularly in the mucosa and submucosa, in response to lethal doses of bacterial endotoxin.

**Summary**

An attempt has been made to measure the capillary blood flows through the individual tissues of the ileum of normal dogs and those which were in endotoxin shock. The technique used was based upon the assumption that the uptake of D₂O by the tissues was limited by the capillary flow. In loops from endotoxin shock dogs which were perfused at normal blood pressure (135 mm. Hg), the tissue perfusion ratios calculated on this assumption were reduced to about one-half of the control value in the mucosa and to about three-fourths in the submucosa and muscularis, despite the fact that total blood flow was unchanged. When the loops from shock dogs were perfused at the dog’s pressure (70 mm. Hg), there was a further decrease of about 50 per cent in both the total blood flow and the tissue perfusion ratios. It is concluded that this reduction in tissue capillary flow, especially in the mucosa, may be the basis of the pathological changes in the intestine in endotoxin shock.

**Summario in Interlingua**

Esseva interprendite le tentativa de mesurar lo fluxo de sanguine capillar per le tissus individual del ileum de canes normal e de canes in choc causate per endotoxiiia. Le technica usate se basava super lo these que le acceptation de D«O per le tissus es limitate per le fluxo capillar. In ansas ab canes in chock endotoxie, perfusionate con normal pressiones de sanguine (135 mm de Hg), le proportiones del perfusion tissular calculate super le base del mentionate these esseva redueite a circa un medietate in le caso del mueosa e a circa tres quartos in le caso del submucosa e del tunica muscular, in despecto del facto que lo fluxo de sanguine total non esseva alterate. Quando le perfusion del ansas ab canes in choc esseva effectuante sub lo pression existente in lo canes mesme (70 mm de Hg), il occurrera un declino addicional per circa 50 pro cento tanto in lo fluxo de sanguine total como etiam in le proportiones del perfusion tissular. Es consoludite que iste declino del fluxo in le capillares tissular, specialmente in le mueosa, es possibilemente le base del alterationes pathologic in le intestino in chock per endotoxina.

**References**

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