Role of the spleen and portal hypertension in canine endotoxin shock

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Recent activity in the field of experimental shock has focused attention on intestinal factors in shock potentiation and protection.[4-6, 8] The presence or absence of intraluminal proteolytic enzymes and amino acids, and the metabolic state of the intestinal mucosa have become important factors in considering the mechanisms of shock progression in the dog. It is apparent, however, that the full significance of these biochemical events requires a more definite understanding of the behavior of the splanchnic circulation during the shock state.

Our initial goal in studying this problem was to define the dynamic relationship between hematocrit and pressure changes in the portal and systemic circulations following endotoxin administration. Changes in arterial and portal venous hematocrit and plasma protein concentration following large doses of endotoxin have been previously reported by Chien and associates.[7] However, in our experience, the use of large doses of endotoxin tends to be associated with more inconsistent hemodynamic effects which might mask some of the mechanisms involved. An LD_{50} dose of endotoxin was used in the hope of eliciting a wider spectrum of effects which would aid in a determination of the underlying mechanisms.

METHODS

Mongrel dogs of either sex weighing between 30 and 35 pounds were used. Sterile implantation of polyethylene portal venous catheters was performed 10 to 14 days prior to the initiation of the shock experiment. The catheters were inserted via the inferior pancreaticoduodenal vein, or the left gastric vein in dogs with spleen intact, and the splenic vein in splenectomized dogs. The veins were irrigated with heparinized saline solution daily. On the day of the shock experiment, the fasted animals were anesthetized by means of intravenous pentobarbital (28 mg. per kilogram of body weight), with endotracheal intubation used to maintain an adequate airway. Polyethylene catheters were inserted into the aorta and inferior vena cava via the femoral artery and vein. The catheters were used for obtaining arterial and portal venous blood samples, and for direct observation of blood pressure. Mean arterial pressure was monitored with a mercury manometer; portal and central venous pressures were measured with saline manometers. Hematocrits of the heparinized blood samples were determined in duplicate by means of the microhematocrit technique. The hematocrit values were not corrected for plasma trapping.

After obtaining control pressure recordings and blood samples a dose of a single lot of *Escherichia coli* endotoxin* (0.5 mg. per kilogram of body weight, previously established to be an LD_{50} in this laboratory) was injected intravenously in a solution of 30

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c.c. of isotonic saline. Arterial and portal venous blood samples were drawn at 1, 3, 5, 10, 15, 30, and 60 minutes after endotoxin administration. Blood pressures were monitored and recorded throughout the period of observation, which lasted from 1 to 2 hours following endotoxin injection, after which the animals were sacrificed and autopsied. Catheter position was noted at the time of postmortem examination.

The present report covers 3 experimental groups:

Group I. Splenectomy and portal vein catheterization performed 10 to 14 days prior to shock (7 dogs).

Group II. Spleen intact and portal vein catheterization performed 10 to 14 days prior to shock (6 dogs).

Group III. Acutely prepared end-to-side portacaval shunt and splenectomy performed with portal vein catheterization (4 dogs). Tributaries of the portal vein near the liver hilus that could not be included in the shunt were divided to insure complete diversion of splanchnic flow. The experiment was performed only if the portal occlusion time was less than 20 minutes, and the bowel regained normal color soon after release of the vascular clamps. The position of the portal venous catheter just beyond the junction of the splenic and portal veins, approximately 2 cm. proximal to the vascular anastomosis, was ascertained at postmortem examination.

RESULTS

Following the intravenous injection of 0.5 mg. per kilogram of body weight of endotoxin to the pentobarbitalized dog, portal venous pressure rapidly rose and became maximal within 1 to 3 minutes. There was a precipitous fall in mean arterial pressure, which reached a minimal level 1 to 4 minutes after portal pressure became maximal in splenectomized dogs. In dogs with spleen intact, the reaction was simultaneous with the maximum portal pressure rise, or 1 to 2 minutes later. There was a definite tendency for the minimum level of mean arterial pressure to be reached later in the splenectomized dog.

Maximum changes in portal venous hematocrit occurred from 3 to 5 minutes after endotoxin injection in both splenectomized and intact dogs. In both groups, these changes occurred 1 to 3 minutes after the maximum rise in portal vein pressure, and was concomitant with, or occurred 1 to 3 minutes after, the minimum level of mean arterial pressure had been reached. The relationship between arterial and portal venous pressure changes and portal venous hematocrit change is illustrated in Tables

Table I. *Maximum pressure and portal venous hematocrit change following administration of LD_{60} endotoxin to splenectomized dogs*

Dog no.	*Fall in mean arterial pressure (% control)*	*Portal vein hematocrit increase (%)*	*Maximum portal pressure (mm. saline)*	*Duration portal pressure > 220 mm. saline (min.)*
1216	67	21	370	9
1400	59	13	260	4
1107	73	30	280	3
722	41	28	315	4
1075	19	1.6	220	–
1183	3.6	0	100	–
1152	65	0	220	–

Table II. *Maximum pressure and portal venous hematocrit change following administration of LD_{60} endotoxin to dogs with spleen intact*

Dog no.	*Fall in mean arterial pressure (% control)*	*Portal vein hematocrit increase (%)*	*Maximum portal pressure (mm. saline)*	*Duration portal pressure > 220 mm. saline (min.)*
1080	60	90	230	2
1294	50	79	285	9
1117	52	35	350	7
856	19	15	260	1
771	18	21	260	1
1241	9.5	0	180	–

and II. Representative experiments are shown in Figs. 1 and 2.

In seven splenectomized dogs (Table I), there was a maximum fall in mean arterial pressure of from 3.6 to 73 percent. Significant changes in portal venous hematocrit occurred only if the maximum increase in portal pressure was greater than 220 mm. of saline. At maximum portal pressures of 260 to 370 mm. of saline, increases of 13 to 30 percent in portal venous hematocrit were observed (4 dogs). Portal pressure was sus-

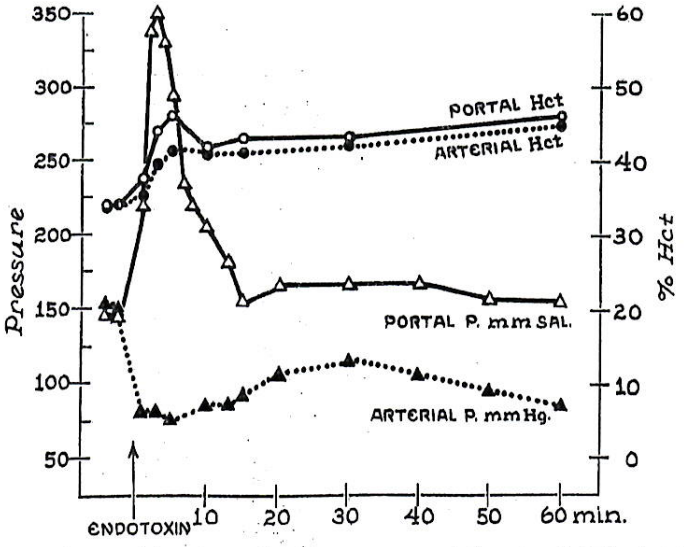

Fig. 1. Changes in arterial and portal vein pressures and hematocrit following *Escherichia coli* endotoxin in dog with spleen intact (No. 1117).

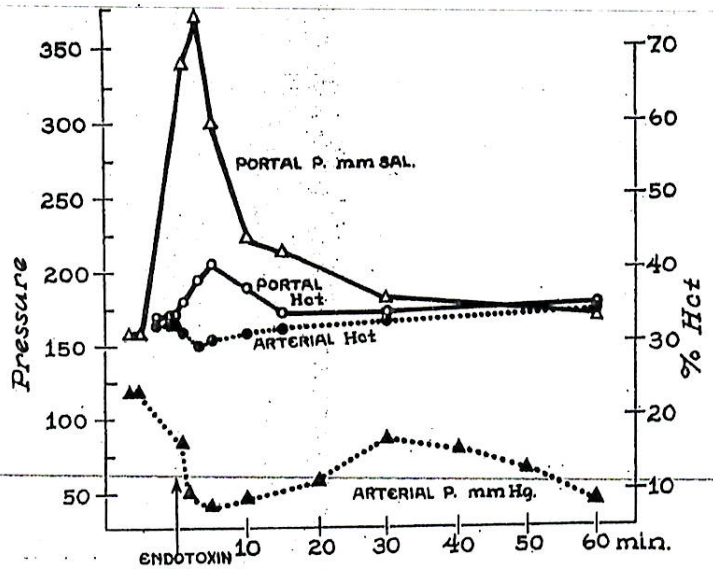

Fig. 2. Changes in arterial and portal vein pressures and hematocrit following *Escherichia coli* endotoxin in splenectomized dog (No. 1216).

tained above 220 mm. of saline for 3 to 9 minutes. The relationship between hematocrit change and the degree of arterial hypotension was not as consistent.

In three dogs with intact spleen (Table II), a maximum fall in mean arterial pressure of 50 percent or greater was associated with a 35 to 90 percent maximum rise in portal venous hematocrit. Portal pressures ranged from 230 to 350 mm. of saline. In 2 dogs with a maximum fall in arterial pressure of approximately 20 percent (Nos. 856 and 771) hematocrit changes of only 15 and 21 percent were observed. In these 2 animals, portal pressures were above 220 mm. saline for only 1 minute. In 1 animal (No. 1241) with very small pressure changes, no hematocrit change occurred.

Spearman rank order correlations were computed for portal vein hematocrit increase and maximum portal pressure in splenectomized dogs, and relating maximum fall in mean arterial pressure to portal vein hematocrit increase in dogs with intact spleen. The rho for the former relationship was 0.83, and for the latter, 0.89. Both coefficients are significant at less than the 5 percent level (one tailed test). Evidence is thus provided that a linear relationship exists between the pairs of variables under consideration.

After maximum levels of portal hematocrit had been reached, they fell, and then tended to equilibrate with the arterial hematocrit in both splenectomized and intact dogs. Therefore, significant differences existed between the portal and arterial hematocrits during the first 3 to 5 minutes after endotoxin injection.

The mean percentage change in arterial hematocrit for splenectomized and intact dogs is shown in Table III. Only time intervals where complete observations were available are shown. In splenectomized dogs, the arterial hematocrit decreased significantly 1 and 3 minutes after endotoxin injection ($p = <.001, <.05$), and never rose appreciably above control values during the first hour of observation. The arterial hematocrit of dogs with intact spleens increased sharply during the first 5 minutes after endotoxin administration, with a secondary rise at 60 minutes following injection.

An analysis of variance was performed on the percentage change scores for arterial hematocrit in the two groups of dogs. This demonstrated that the curves for changes in arterial hematocrit over a period of time were different in shape for splenectomized and intact dogs. The group by time interval interaction was significant at less than the 0.5 percent level. A t-test done on the mean percentage change in arterial hematocrit between splenectomized and intact dogs showed the differences to be of borderline significance at 3 minutes following endotoxin injection, but significant from the 5 minute point onward (Table III).

Because of the possibility that the acute rise in portal venous hematocrit in splenectomized dogs was related to portal hypertension, four dogs were prepared with end-to-side portacaval anastomoses, and splenectomized. There was no significant increase in the portal venous pressure after endotoxin injection in these animals. The portal pressure usually fell to well below control levels during the first 5 minutes after in-

Table III. *Mean percentage change in arterial hematocrit after administration of LD_{50} endotoxin*

	Minutes after endotoxin					
	1	*3*	*5*	*15*	*30*	*60*
Splenectomized	−3.04	−1.70	−0.591	1.14	−1.00	1.30
Intact	2.26	9.29	14.27	15.07	19.21	30.04
t	.88	1.25	2.46	2.30	3.35	4.75
p	NS*	NS*	<.05	<.05	<.01	<.001

*NS = Not significant.

tion, with a gradual decline thereafter. 3 dogs, the arterial pressure gradually ll throughout the period of observation, thout the precipitous early drop seen in gs without portacaval anastomoses. In e dog (No. 800), a rapid fall in arterial ssure preceded the fall in portal pres- re, both showing some recovery within 5 to 60 minutes, but remaining well below ntrol levels. There was no increase in the rtal vein hematocrit during the first 5 inutes after shock, and both the arterial nd portal hematocrit values tended to re- ain at or below control levels during the rst hour of observation. However, dog No. 00 began to show mild hemoconcentration both circulations 10 minutes after endo- xin.

DISCUSSION

The hemodynamic events of canine endo- xin shock are well known, and have been extensively investigated.[1, 17] The three stages of blood pressure alterations that have been described were observed to occur to variable degrees in all of our chronically prepared animals. By using a sublethal dose of endo- xin, we hoped to achieve a spectrum of responses which would better define the total hemodynamic reaction.

In both splenectomized and intact dogs, he rise in portal venous pressure, fall in arterial blood pressure, and increase in por- tal vein hematocrit occur sequentially, and all within 5 minutes following endotoxin in- jection (Figs. 1 and 2). The intact dog is easily distinguished from the splenectomized dog by the parallel rise in arterial hemato- crit which follows the increase in portal vein hematocrit, suggesting that an increase in circulating red blood cells has occurred (Figs. 1 and 2, Table III). In splenectomized dogs, the arterial hematocrit decreases 1 and 5 minutes after endotoxin, and never rises appreciably above control values.

The canine spleen is a contractile organ of variable volume which concentrates red blood cells. The red cell content of splenic blood is 1.7 times greater than that of venous blood.[2] With pentobarbital anesthe- sia, as used in typical shock experiments, the spleen becomes greatly dilated, and may contain from 18 to 29 percent of the dog's total red blood cells.[11, 22]

In order to test the possibility that acute splenic contraction occurs during the early phase of arterial hypotension as a result of adrenal humoral release, 5 dogs were acutely bilaterally adrenalectomized, and given an LD_{60} dose of endotoxin intravenously ½ to 1 hour later.[3] Mean arterial pressure fell to 55 to 75 percent of control, and portal venous pressure rose to 165 to 360 mm. saline soon after endotoxin administration. The acute rise in portal venous hematocrit noted in the dog with intact adrenals and spleen did not occur in 4 of the 5 animals. Instead, a progressive rise in portal venous hematocrit, followed by a progressive rise in the arterial hematocrit began 5 to 10 minutes after endotoxin administration, and persisted for the 1 hour period of observation. This suggested a slow and continuing release of the splenic reservoir instead of the acute release noted in the dog with intact adrenals.

With the administration of endotoxin, there is an increased release of epinephrine into the adrenal vein and, thus, the systemic circulation,[19, 21] which would result in contraction of the spleen. Nykiel found a significant reciprocal relation between the minute output of epinephrine from the innervated adrenal and the level of arterial blood pressure.[19] When endotoxin was given as a slow intravenous infusion, there was no rise in epinephrine output until a significant decrease in the arterial blood pressure occurred.[19]

These experiments strengthen the contention that the initial rise in portal venous and arterial hematocrits following endotoxin administration in the dog with intact spleen is due primarily to splenic contraction. The observation that the degree of hematocrit rise tends to follow the degree of arterial hypotension suggests that the stimulus to adrenal release of epinephrine in endotoxin shock is mediated via a baroreceptor mechanism.

In the splenectomized dog there is nothing to suggest that a significant increment in circulating red blood cells has occurred. The rise in portal vein hematocrit is associated with a significant fall in arterial hematocrit, and the portal hematocrit soon returns to control levels. Increases in portal vein hematocrit occurred only when the rise in portal pressure was greater than 220 mm. saline, and sustained above that level for at least 3 minutes. These data suggest that the increase in portal hematocrit in the splenectomized dog is a result of an acute fluid shift from the portal circulation. This concept is strengthened by previous studies demonstrating that, following injection of endotoxin, there is an increase in liver weight, a smaller increase in the weight of the small intestine,[18] increased hepatic and thoracic duct lymph flow,[1] and increased portal (but not arterial) plasma protein concentration,[7] all occurring during the time interval of the portal hematocrit changes observed in the present study. The abolition of both portal hypertension and acute changes in portal vein hematocrit by means of portacaval anastomosis in the splenectomized dog seems to establish this point definitively. Acute rises in portal vein hematocrit in the intact dog would, therefore, seem to result primarily from splenic contraction, and, to a lesser extent, from acute fluid shifts from the splanchnic circulation.

Of interest in this regard is the observation of Alican and Hardy,[1] that when the characteristic initial hemodynamic changes of endotoxin shock are abolished by portacaval shunting, there is an absence of the early hepatic component of increased lymph flow in the thoracic duct, and a tendency for the intestinal component to occur later than in dogs without shunts. Presumably, it would take longer to accumulate enough fluid in the small intestine to increase lymphatic flow when portacaval shunting abolishes portal hypertension and the acute shift of fluid out of the splanchnic circulation.

In order to determine a maximum fluid shift out of the prehepatic portal circulation, 2 additional dogs were acutely splenectomized, and the portal vein cross clamped above the highest tributary.[3] The portal venous hematocrit rose to as high as 80 percent after 10 minutes, concomitant with a fall in arterial hematocrit. Both returned to control levels within 5 minutes after release of portal occlusion. These experiments seem to reflect in an exaggerated form the pattern of hematocrit change observed in the splenectomized dog after endotoxin injection.

The fall in portal vein pressure in dogs with Eck fistula during the first 5 minutes after endotoxin injection may be a reflection of the profound fall in superior mesenteric artery flow. Using noncannulating electromagnetic flow meters, the flow in the superior mesenteric artery has been observed to fall to very low levels during the first few minutes after endotoxin injection.[16]

Of considerable interest is the sustained elevation of arterial hematocrit noted in the dog with intact spleen. Vessel size, pressure gradient, and the viscosity of blood determine flow in the circulatory system. Blood viscosity is known to fluctuate dramatically in response to changes in red cell concentration, plasma protein concentration (especially that of fibrinogen), velocity, and temperature. Since the increase in blood viscosity that occurs in experimental endotoxin shock[10] is associated with a depression in blood fibrinogen level,[9, 12, 13] the most important factor affecting the viscosity at any given rate of shear (velocity gradient) or temperature would be the hematocrit.

In the dog with intact spleen, significant and sustained changes in arterial hematocrit occur early due to release of the concentrated splenic reservoir. This may well have a deleterious effect on the animals' ability to survive the shock episode by increasing blood viscosity, and impairing blood flow through the microcirculation, thus favoring the development of tissue hypoxia and metabolic acidosis. This is especially important, since the effects of changes in hematocrit on blood viscosity are more pronounced at low shear rates,[10] and the relationship between hematocrit and viscosity changes from a

linear to an exponential one as the hematocrit exceeds 50 percent.[20] Hardaway has found increased survival of splenectomized dogs after lethal hemorrhagic shock.[14] A survival study utilizing an LD_{50} dose of endotoxin might demonstrate increased survival in splenectomized dogs versus those with intact spleens.

In this same regard, it may be possible to demonstrate an increased survival rate in chronically prepared Eck fistula dogs if an LD_{50} dose of endotoxin is used, rather than the larger doses that have been administered heretofore.[1] Vascular changes and the extravasation of fluid from the splanchnic circulation as a result of the acute rise in portal venous pressure may contribute to the metabolic depression of the intestinal mucosa, and its subsequent autodigestion.[5, 6, 8]

The spleen does not serve the same reservoir function in the human as it does in the dog. The possibility has been suggested that its role in the progression of canine shock is deleterious. We would therefore suggest that splenectomy should be included as part of any canine experimental shock model procedure.

SUMMARY

Changes in portal venous pressure and hematocrit compared with arterial blood have been determined in the dog following an LD_{50} dose of *Escherichia coli* endotoxin. Acute portal hemoconcentration was observed in the intact, and to a lesser extent, in the splenectomized animal. This phenomenon is apparently related to at least two distinct mechanisms. Contraction of the splenic red cell reservoir causes an abrupt outpouring of concentrated blood directly into the portal vein, and thus the systemic circulation. This response is graded, and presumably related to a baroreceptor stimulus to the adrenals following acute arterial hypotension. The second mechanism is related to the transudation of fluid from the portal circulation during acute portal hypertension in endotoxemia. The magnitude of this latter effect is dependent upon the degree and duration of portal pressure elevation. The contribution of the first mechanism (splenic contracture) is eliminated by splenectomy. Both mechanisms are eliminated by splenectomy and portasystemic shunting.

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