richia coli endotoxin (3 mg/kg) caused marked rises of arterial cell percentage in dogs with spleen but not in splenectomized dogs. The increase in arterial cell percentage can be divided into 2 phases. The initial phase (0 to 10 minutes after endotoxin) was seen in all dogs with spleen and is attributable to the contraction of the spleen, since the cell percentage in the portal venous blood was markedly elevated. In this initial phase, the extravasation of protein-rich fluid from the splanchnic circulation was accompanied by an influx of protein-poor fluid from the kidneys and possibly other areas, hence the circulating plasma protein concentration decreased. The second phase of increase of arterial cell percentage (20 to 60 minutes after endotoxin) was associated with a rise in arterial plasma protein concentration and is ascribed to an extravasation of protein-poor fluid. This second phase was more marked in dogs which died within 200 minutes after endotoxin. There were no significant alterations in the cell size of the arterial blood, whereas the cells in the venous blood swelled after endotoxin when pCO_2 and acidity increased.

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Effect of Endotoxin on the Transfer of Fibrinogen from Plasma to Lymph.* (30076)

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In previous experiments, the effects of endotoxin on capillary permeability to exogenous and endogenous macromolecules were studied by following the concentration of such substances in the plasma and in the thoracic duct lymph(1). These studies show that endotoxin increases the lymph-to-plasma ratio of many macromolecules, including the endogenous albumin and globulins. Since fibrinogen-fibrin conversion and intravascular coagulation have been found to play an important role in endotoxin shock(2,3,4), it was thought desirable to obtain information on capillary permeability to fibrinogen.

Methods. Ten healthy mongrel dogs, ranging in weight from 11 to 24 kg, were used. The operative procedures are similar to those described previously(1,5). Under sodium pentobarbital anesthesia (33 mg/kg, given intravenously), the thoracic duct was cannulated low in the neck with a polyethylene tubing. A femoral artery was cannulated. In 4 of the dogs the experiments were performed with abdomen intact. Lymph samples

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TABLE I. Control Measurements on Fibrinogen and Circulation.

	Intact abdomen (n = 4) Mean ± S.E.$_m$	With laparotomy (n = 6) Mean ± S.E.$_m$
Plasma fibrinogen concentration (mg %)	415 ± 61	355 ± 34
Lymph fibrinogen concentration (mg %)	136 ± 21	156 ± 17
L/P ratio for fibrinogen	.33 ± .02	.47 ± .06
Lymph flow rate (ml/min)	.22 ± .04	.36 ± .06
Systolic pressure (mm Hg)	172 ± 8	169 ± 8
Diastolic pressure (mm Hg)	125 ± 6	123 ± 4

were collected continuously. Five 20-minute lymph samples were taken before endotoxin, and 9 additional samples were taken beginning at 0, 5, 10, 20, 40, 60, 90, 120, and 160 minutes after endotoxin. The last sample was terminated at 200 minutes after endotoxin. Blood samples were drawn from the femoral artery at the mid-point of each lymph sampling period. Fibrinogen determination was carried out on 3 of the 5 pairs of control samples and all of the post-endotoxin samples. In the remaining 6 dogs, the experiment was performed with the abdomen opened and the portal vein catheterized *via* a branch of the splenic vein. In this group with laparotomy, one pair of control lymph and arterial blood samples (10 minutes prior to endotoxin) and 3 pairs of post-endotoxin (6, 30, and 95 minutes) samples were analyzed for fibrinogen.

The blood and lymph samples were collected with either heparin or potassium oxalate as an anticoagulant. In a given experiment, only one type of anticoagulant was used. The fibrinogen concentration in lymph and plasma samples was determined by the method of Ratnoff and Menzie(6). No anticoagulant was given to the animal, nor were the lymph samples returned. The *Escherichia coli* endotoxin used was obtained from Difco Laboratories (Detroit, Mich.) and a dose of 3 mg/kg was injected intravenously.

Results. The mean values and their standard errors for the control measurements are shown in Table I. The effects of endotoxin on these measurements in the 4 dogs with intact abdomen are depicted in Fig. 1. In the control period, fibrinogen concentrations in arterial plasma and thoracic duct lymph were rather constant, and the mean lymph-to-plasma (L/P) ratio was 0.33 (individual range 0.29 to 0.35). Almost immediately after administration of endotoxin, the fibrinogen concentration in the lymph and hence the L/P ratio rose significantly. Plasma fibrinogen concentration was unchanged at first, but began to decrease after approximately 30 minutes. Since the decrease in plasma fibrinogen concentration was accompanied by a parallel decline in lymph concentration, the L/P ratio remained high (approximately 0.8) throughout the experiment. The increases in lymph concentration and L/P ratio for fibrinogen were associated with an increase in lymph flow rate.

Control values for the 6 dogs with laparotomy are given in Table I. Although the mean fibrinogen L/P ratio and the mean lymph flow rate appeared to be higher than the corresponding values obtained in the dogs with intact abdomen, the difference is not statistically significant (Table I, P values for Student t test greater than 0.05 for all measurements). The effects of endotoxin on these 2 groups of dogs were similar (Fig. 2). Thus, lymph fibrinogen concentration, L/P ratio for fibrinogen and lymph flow rate all increased after endotoxin. In the dogs with laparotomy, the decrease in plasma fibrinogen concentration occurred earlier and was more marked than in the dogs with intact abdomen. Again, after the initial rise, lymph fibrinogen concentration tended to be parallel to plasma concentration, and the post-endotoxin L/P ratio for fibrinogen was thus maintained at approximately 0.7 (Fig. 2).

Discussion. In the control period, the mean L/P ratio for fibrinogen was 0.33 for the dogs with abdomen intact, 0.47 for those with abdomen open, and 0.41 (S.E.$_m$ 0.04) for all 10 dogs. These values are significantly lower than those for the other plasma proteins. Thus the mean L/P ratio for all the serum proteins combined (not including fibrinogen) averages 0.70 to 0.80 for dogs

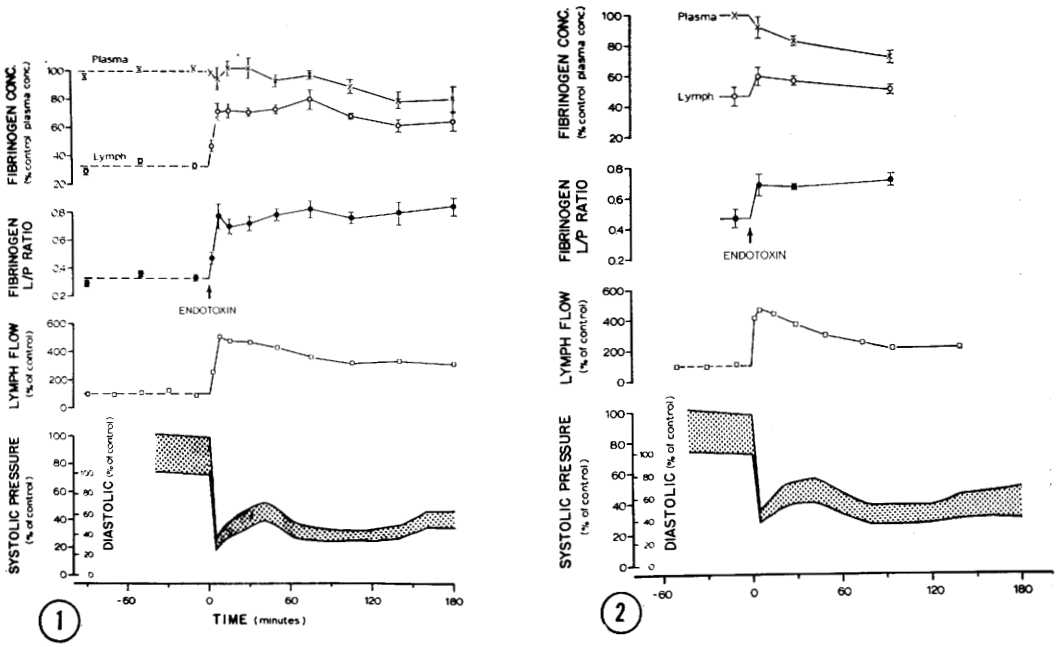

FIG. 1. Effects of *E. coli* endotoxin on fibrinogen concentrations in arterial plasma and thoracic duct lymph, fibrinogen L/P ratio, flow rate of thoracic duct lymph, and arterial pressure in 4 dogs with intact abdomen. Plotted are the mean values, with vertical bars representing standard errors. Lymph values are plotted at the mid-point of each sampling period. Plasma and lymph fibrinogen concentrations are calculated as % of control plasma values.

FIG. 2. Effects of *E. coli* endotoxin on 6 dogs with laparotomy. See Fig. 1 for detailed explanations.

with (unpublished observations) or without laparotomy(1,5). The low L/P ratio for fibrinogen is attributable to the relatively large molecular weight (approximately 340,000) and the elongated configuration of this protein.

The finding that the L/P ratio for fibrinogen increases markedly after endotoxin is similar to that found for the other plasma proteins(1). Since the increase in L/P ratio is accompanied by a significant rise in lymph flow, splanchnic capillary permeability to fibrinogen is markedly increased by endotoxin. For unknown reasons, the increase in lymph flow rate in the present experiments is more marked and prolonged than that reported earlier(1).

The post-endotoxin increase in splanchnic capillary permeability to fibrinogen is interesting from several aspects. Firstly, it demonstrates that the increase in capillary permeability is such that molecules as large as fibrinogen can move across capillary walls in significant quantities. Secondly, the extravasation of a large amount of fibrinogen, if accompanied by prothrombin and if encountering tissue thromboplastin, would make possible the conversion of fibrinogen into fibrin and the occurrence of coagulation in the extravascular space. Such extravascular coagulation, if immediately adjacent to the vascular wall could conceivably aid in occluding the vascular lumen due to either direct compression or vasospasm resulting from extravascular irritation, thus reducing blood flow through the region. Finally, the extravasation of fibrinogen-rich fluid from the splanchnic circulation in combination with the influx of protein-poor fluid from extrasplanchnic extravascular space(1,7) would lead to a decrease in plasma fibrinogen concentration. Therefore the post-endotoxin decrease in plasma fibrinogen concentration cannot be attributed exclusively to the usage of fibrinogen in intravascular coagulation. In our experiments, the fibrinogen concen-

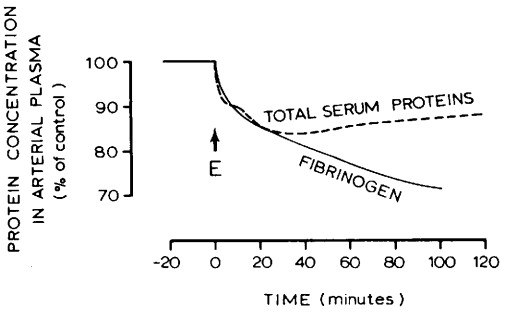

FIG. 3. Effects of *E. coli* endotoxin on concentrations of fibrinogen (as % of control, solid line) and total serum proteins (as % of control, broken line) in arterial plasma of 6 dogs with laparotomy. Note parallel decrease of the two concentrations during first 30 min. Thereafter the decrease in fibrinogen concentration exceeds that of the other proteins.

tration in arterial plasma decreases after endotoxin, although the change is not as prominent as that found by other workers (4,8). In the two groups of animals reported here, the post-endotoxin decrease in plasma fibrinogen concentration occurs earlier and is more prominent in the dogs with laparotomy (Fig. 2). However, determination of total serum protein concentration (micro-Kjeldahl method, 9) in these experiments shows that the serum proteins (not including fibrinogen) also decrease after endotoxin (Fig. 3). Fluid shifts alone would result in comparable changes in fibrinogen and the other proteins. Changes that are specific for fibrinogen, *e.g.*, due to its usage in intravascular coagulation, should result in a greater decrease in concentration of fibrinogen than that of the other proteins. As shown in Fig. 3, even for dogs with laparotomy, the specific decrease for plasma fibrinogen concentration commences only at approximately 30 minutes after endotoxin injection.

Summary. The fibrinogen concentration ratio between the thoracic duct lymph and the arterial plasma averages 0.41 in 10 dogs under pentobarbital anesthesia. Intravenous injection of endotoxin caused an increase of this L/P ratio to 0.7-0.8 which was sustained for at least 3 hours after endotoxin. The increase in the L/P ratio for fibrinogen is associated with a rise in lymph flow, indicating a marked increase in capillary permeability to this protein. The increase in L/P ratio after endotoxin is due primarily to an increase in lymph concentration, although there is a later decrease in plasma concentration. The decrease in plasma fibrinogen concentration is only partially due to fluid shifts and there is indication that specific decrease of fibrinogen concentration commences 30 minutes after endotoxin.

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