protein *in vitro*(12): tetraiodothyroacetic acid > L-thyroxine > 3,5,3′-triiodothyropropionic acid > 3,5,3′-L-triiodothyronine > DL-thyronine, with D-thyroxine being equally as effective as L-thyroxine. It is of interest to note that this order of effectiveness is the same as the order of relative binding affinities exhibited by these analogs for albumin† (4). It may be that thyroxine must first be bound at a specific site in a similar way to its binding to albumin in order for the hormone to exert its stimulatory action in the amino acid-incorporating system from liver (12). This suggests the possibility that, in addition to bilirubin toxicity itself(13,14), high intracellular concentrations of bilirubin may interfere with an action of thyroxine on protein biosynthesis.

Summary. The effect of bilirubin on the binding of thyroxine by human serum albumin has been studied by means of equilibrium dialysis. The results show that bilirubin can displace thyroxine from binding sites on albumin.

† Tetraiodothyroacetic acid exhibits the same relative binding affinity as tetraiodothyropropionic acid (4) for thyroxine-binding sites on albumin.

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Hematocrit Changes in Endotoxin Shock.* (30075)

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It is well established that endotoxin shock in dogs is accompanied by a marked rise of the hematocrit of venous or arterial blood(1, 2). The purpose of the present experiments was to study the mechanisms underlying such an increase of blood cell percentage (H) caused by endotoxin. Thus the role of splenic contraction was investigated by comparing the arterial cell percentage (H_a) with the portal venous cell percentage (H_p) in dogs with intact spleen and by comparing the H_a of such dogs with the H_a of splenectomized dogs. The influence of transcapillary fluid exchange in various regions was evaluated by comparing the cell percentage of various venous blood samples with H_a and by following the plasma protein concentrations. The effect of acidosis on erythrocyte size and hence the H of various blood samples was studied by measuring the changes in blood pCO_2 and pH and by determining the mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC).

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Methods. Forty-five healthy mongrel dogs, including 33 with intact spleens and 12

chronically splenectomized (at least one month before endotoxin experiment), were used in these experiments. The dogs were anesthetized with intravenous injection of sodium pentobarbital (33 mg/kg). A femoral artery was cannulated for recording of arterial pressure and for sampling of arterial blood. Femoral venous blood samples were taken through a polyethylene tubing inserted into a femoral vein without obstructing the blood flow in the vein (6 dogs with spleen and 2 splenectomized). Innominate venous blood was obtained from a catheter placed near its junction with the left external jugular vein just below the entrance of the thoracic duct (2 dogs with spleen and 2 splenectomized). In 10 of the animals with intact spleens and 4 of the splenectomized dogs, the abdomen was opened with a midline incision and a polyethylene tubing was inserted into the portal vein either *via* a branch of the splenic vein or directly, through a needle puncture. Renal venous samples were obtained in 5 laparotomized animals (including 2 splenectomized) from a catheter placed in the right renal vein *via* a femoral vein.

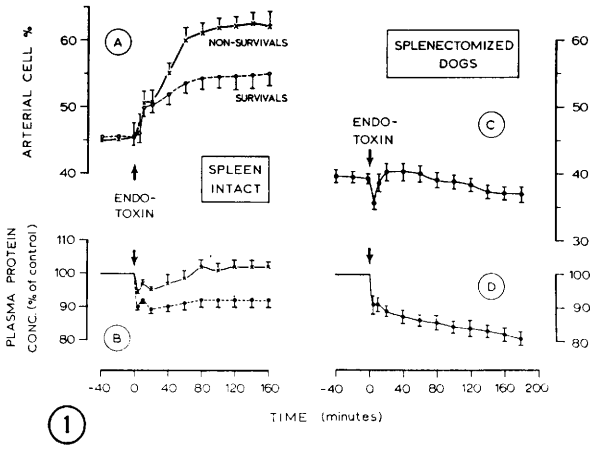
Hematocrits of the heparinized blood samples were determined either in Wintrobe hematocrit tubes centrifuged at 1,500 $\times$ *g* for 30 minutes or in microcentrifuge tubes spun at 15,000 $\times$ *g* for 5 minutes. The hematocrit values were corrected for plasma trapping(3) to yield the cell percentage (H) of the blood. The concentration of plasma proteins, (P.P.), was determined with a refractometer(4). In some experiments hemoglobin concentration(5), red blood cell counts (Coulter electronic counter, Coulter Electronics, Hialeah, Florida) and pCO_2 and pH (meters manufactured by Instrumentation Laboratories, Boston, Mass.) of the blood samples were determined.

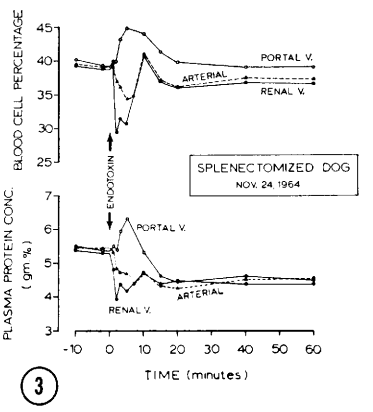
After control blood samples had been taken, *Escherichia coli* endotoxin (3 mg/kg, obtained from Difco Laboratories, Detroit, Mich.) was injected intravenously. Thereafter blood samples were drawn at frequent intervals for approximately 3 hours.

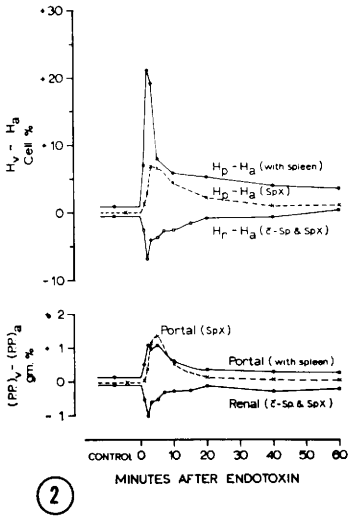
Results. In the dogs with spleen intact, the arterial cell percentage (H_a) averaged 45.5% in the control period and increased sharply after endotoxin (Fig. 1A). Twelve of the 33 dogs with spleen died within 200 minutes after endotoxin and 21 were still surviving at the end of this period. In the dogs that died during the first 200 minutes after endotoxin, the initial rise of H_a was followed by a secondary increase (20 to 60 minutes after endotoxin), which was much less pronounced in the surviving dogs (Fig. 1A). Thus the non-survivals eventually had higher H_a values, although the control H_a was almost identical in the 2 groups. The arterial plasma protein concentration, $(P.P.)_a$, of all dogs decreased immediately after endotoxin. In the survivals, $(P.P.)_a$ remained low throughout the period of observation, whereas in the non-survivals $(P.P.)_a$ increased progressively to the control level (Fig. 1B).

All 12 splenectomized dogs survived at 200 minutes after endotoxin. The control H_a averaged 39.5% and was significantly lower than that of the dogs with spleen (Fig. 1, A and C). H_a decreased significantly ($P<0.01$) 5 minutes after endotoxin injection, but it returned to the control level at 10 minutes (Fig. 1C). Throughout the 3 hours of observation, H_a never rose significantly above the control values, but rather showed a steady trend of decline after the first hour. Following endotoxin injection, $(P.P.)_a$ of the splenectomized dogs decreased and remained low (Fig. ID).

For all the dogs studied, the cell percentage of the portal venous blood (H_p) was not significantly higher than H_a during the control period. In the dogs with spleen, H_p increased markedly after endotoxin (Fig. 2). The degree and the speed of the increase in H_p varied in individual dogs. In some dogs, H_p reached values higher than 80% cells within 2 or 3 minutes and the difference between H_p and H_a exceeded 30 hematocrit %. When H_p increased so promptly after endotoxin, the initial increase of H_a also occurred early. Conversely, when the increase in H_p was delayed to 3 minutes or later after endotoxin, H_a declined initially as in the splenectomized dogs, but then rose later after H_p had increased. There was no significant difference between the (H_p-H_a) of the non-survivals and that of the survivals, and all the results obtained on dogs with spleen are







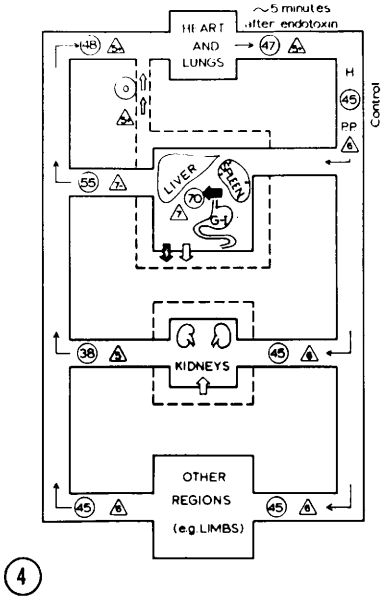


FIG. 1. Effects of *E. coli* endotoxin on arterial cell percentage and plasma protein concentration in dogs with spleen intact (A and B) and in splenectomized dogs (C and D). Vertical bars represent standard errors of mean. In the dogs with spleen (A and B), the results on survivals (circles) and non-survivals (crosses) are separately presented. Whenever P value for the difference in the 2 means is less than 0.05, closed circles are used for survivals.

FIG. 2. Effects of *E. coli* endotoxin on portal and renal venous blood. Cell percentage (upper panel) and plasma protein concentration (lower panel) of these samples are compared with those of the simultaneous arterial sample and differences are plotted. Note marked rise in portal venous cell percentage over arterial cell percentage in dogs with spleen. Changes in renal venous values were not different whether the spleen was present or not, hence data obtained on all dogs are grouped together as one curve.

FIG. 3. Effects of *E. coli* endotoxin on arterial, portal venous and renal venous blood in a splenectomized dog. Note similarity in initial changes of cell percentage and plasma protein concentration for each type of blood. The decrease of renal values was earlier in time and greater in extent than the lowering of arterial values.

FIG. 4. A diagrammatic summary of fluid and cell shifts occurring in a dog with spleen during the first few minutes after injection of *E. coli* endotoxin. Solid arrow indicates release of cells by spleen. Stippled arrow represents movement of proteins whereas blank arrow depicts that of protein-free fluid. Values in circles represent cell percentage and those in triangles depict plasma protein concentration (g %). Fluid and cell shifts in various regions account for the increase of arterial cell percentage (from 45 to 47%) and decrease of plasma protein concentration (from 6 g % to slightly over 5 g %) during these few minutes. See text for detailed explanation.

grouped together. In the splenectomized dogs, H_p rose to a much lesser extent, and therefore the increase in $(H_p\text{-}H_a)$ was smaller in magnitude than that found in the dogs with spleen (Fig. 2). The plasma protein concentration of the portal venous samples, $(P.P.)_p$, increased following endotoxin (Fig. 2), and the difference between $(P.P.)_p$ and $(P.P.)_a$ was indistinguishable between the splenectomized dogs and the group with spleen.

The cell percentage of the innominate venous blood (H_i) obtained below the entrance of the thoracic duct decreased after endotoxin. This decrease was found in both the splenectomized dogs and the dogs with spleen, and it was most pronounced when the left external jugular vein had been ligated. Although H_i became much lower than the simultaneous H_a, the plasma protein concentration of the arterial and the innominate venous samples remained almost equal.

In both the splenectomized dogs (Fig. 3) and the dogs with intact spleen (Fig. 2), the renal venous cell percentage (H_r) and plasma protein concentration, $(P.P.)_r$, decreased within 2 or 3 minutes following endotoxin injection, and these measurements became significantly lower than the simultaneous arterial values. Within 10 minutes after endotoxin, however, H_r and $(P.P.)_r$ rose again and approached the corresponding arterial values.

After endotoxin, the femoral venous cell percentage (H_f) and plasma protein concentration followed the corresponding arterial values. During the first 15 minutes, when H_a changed rather abruptly, H_f was parallel to H_a but the H_f curve showed a delay in time by 1-4 minutes, which probably reflected the slow circulation time through the limb under these circumstances. H_f determined later than 20 minutes after endotoxin showed no consistent difference from H_a. In 3 of the 8 dogs studied, H_f was found to be substantially higher than H_a. In these experiments, the femoral venous samples also had lowered pH, raised pCO_2, decreased MCHC (Table I) and increased MCV. Such changes were either slight or absent in the arterial samples.

Discussion. Since the initial phase of the H_a increase was observed in all the dogs with spleen regardless of their fate, but not in the splenectomized dogs, it can be concluded that the initial rise was due to the contraction of the spleen. This contention is strengthened by the finding that in dogs with spleen, the initial H_a rise was always preceded by a marked increase in H_p which was far more pronounced than that seen in the splenectomized dogs (Fig. 2 and 3). Furthermore, there is evidence that endotoxin causes the stimulation of the sympathetico-adrenal system(6,7,8) which would result in contraction of the spleen.

TABLE I. Effects of Endotoxin on Femoral Venous Blood.

	pH	pCO_2 (mm Hg)	H (%)	Hb (g %)	MCHC (g %)	P.P. (g %)
			Control			
Arterial	7.34	32	39.0	14.4	36.9	6.30
Femoral venous	7.31	43	38.5	14.0	36.4	6.35
			40 minutes after endotoxin			
Arterial	7.33	31	42.2	15.1	35.8	5.35
Femoral venous	7.24	49	47.2	16.2	34.3	6.02
			160 minutes after endotoxin			
Arterial	7.33	23	43.0	15.6	36.3	5.37
Femoral venous	7.18	47	47.0	16.0	34.0	6.07

Changes in blood cell percentage could result not only from alterations in the volume and distribution of the cells, but also of the plasma. In earlier experiments(2), it has been demonstrated that endotoxin causes a marked increase in the flow of protein-rich lymph from the thoracic duct, indicating an extravasation of plasma fluid. The loss of plasma fluid from the mesenteric circulation was probably the reason why $(H_p\text{-}H_a)$ increased in the splenectomized dogs. The increase in $[(P.P.)_p\text{-}(P.P.)_a]$ would indicate that the fluid lost did not contain as much protein as the plasma. A portion of the extravasated fluid was drained back into the blood circulation *via* the thoracic duct, as indicated by the decrease in the cell percentage of the innominate blood. Nevertheless, a considerable amount of the extravasated fluid must have been accumulated in the extravascular space of the splanchnic region and this *per se* would tend

to increase H_a. However, an analysis of the post-endotoxin changes in the concentration of several macromolecules in the plasma and in the thoracic duct lymph(2) indicates that the outward movement of protein-rich fluid from the blood capillaries is accompanied by a simultaneous influx of protein-poor fluid from the extravascular space. In the present experiments during the first few minutes after endotoxin, renal venous cell percentage and plasma protein concentration became considerably lower than the corresponding arterial values, indicating an influx of extravascular protein-poor fluid into the renal circulation. In this connection, it is interesting to note that the kidney weight decreases after endotoxin(9). The influx of protein-poor fluid may originate in part from the renal interstitial space but probably also was the result of back filtration of urine owing to a reversal of the pressure gradient between the renal capillaries and the tubular lumen. If endotoxin causes a release of antidiuretic hormone, the increase in free water absorption in the distal nephron would also aid in the dilution of renal venous blood. There may also be other sources of fluid influx than the kidney. The influx of protein-poor fluid would dilute the circulating plasma protein concentration and counteract the rise in H_a caused by the loss of plasma fluid from the splanchnic circulation. Since in the splenectomized dogs H_a fell rather than rose, it seems that the influx of protein-poor fluid was sufficient to compensate for the volume of protein-rich fluid that escaped from the splanchnic circulation. The replacement of protein-rich fluid by protein-poor fluid, however, accounts for the decrease in arterial plasma protein concentration. From the above discussion, the initial rise in H_a seen in dogs with spleen can be attributed entirely to the contraction of the spleen. At this time, the total fluid fluxes across capillary walls were such that they would tend to minimize, rather than to exaggerate, this initial rise of H_a.

The second phase of H_a rise seen primarily in the dogs which died within 200 minutes after endotoxin must be explained by mechanisms other than the contraction of the spleen. The value (H_p—H_a) at this time became smaller (Fig. 2) and can be accounted for largely by the escape of plasma from the splanchnic circulation and possibly also by the swelling of erythrocytes passing through this region. Since the second phase of H_a increase seen in the non-survivals was accompanied by a higher $(P.P.)_a$, it was probably the result of the extrasplanchnic extravasation of protein-poor fluid in addition to the splanchnic loss of protein-rich fluid. It is pertinent to note that plasma volume has been shown to decrease in later stages of endotoxin shock(6,10, unpublished observations by authors). The site of this extrasplanchnic extravasation has not been demonstrated in the present experiments.

The MCV and MCHC of the arterial blood did not change significantly after endotoxin. Therefore the changes in H_a were not due to a swelling of the erythrocytes. On the other hand, the femoral venous blood in some experiments showed a rise in MCV and a decrease in MCHC after endotoxin, indicating that the cells emerging from the venous side of the circulation had swollen. The swelling of the cells in the femoral venous blood can be explained by the chloride shift resulting from the rises of pCO_2 and acidity, which were higher in the venous than in the simultaneous arterial samples (Table I).

The changes in blood cell percentage during the first few minutes after endotoxin are schematically summarized in Fig. 4.

Of the 33 dogs with spleen, 12 died within 200 minutes. On the other hand, all of the splenectomized dogs survived at the end of this period. It is possible that the higher cell percentage resulting from splenic contraction is deleterious. In this connection, it is interesting to note that splenectomized dogs have been found to survive hemorrhagic shock better than dogs with spleen intact (11). Furthermore, the present experiments show that among the dogs with spleen, the arterial cell percentage was significantly higher in the non-survivals. It is possible that the high viscosity resulting from a higher cell percentage could lead to a vicious cycle involving the impairment of blood flow through the microcirculation and the loss of plasma fluid into the extravascular space.

Summary. Intravenous injection of *Esche-*

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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