mechanism for this principle does not exist, with the factor being released into the circulation as rapidly as it is produced. The present results indicate that plasma may be the only site from which appreciable amounts of 'erythropoietin' are recovered. Experiments are in progress to determine whether the factor may actually be formed in plasma as the result of an enzymatic action upon substrates provided by the blood-forming organs.

Summary. Acidified boiled filtrates were prepared of plasma, liver, spleen, thymus, lung, brain, skeletal muscle, bone marrow and packed blood cells of rabbits made severely anemic by phenylhydrazine. When tested in normal rats, only the plasma extracts proved to be erythropoietic, as evidenced by significant elevations in peripheral red cell, hematocrit and reticulocyte values as well as by a marked increase in the percentages of marrow nucleated erythrocytes. The site of formation of circulating 'erythropoietin' remains unidentified.

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Canine Intestinal and Liver Weight Changes Induced by *E. coli* Endotoxin. (22557)

L. D. MACLEAN, M. H. WEIL, W. W. SPINK, AND M. B. VISSCHER.
Departments of Physiology, Surgery and Medicine, University of Minnesota Medical School, Minneapolis.

Shock has been observed in the human following transfusion of contaminated blood(1), the injection of purified products of bacteria (endotoxin) to induce hemorrhage in malignant tumors(2), and following the treatment of Gram-negative bacteremia with antibiotics. Irreversible hemorrhagic shock in the dog has also been related to the deleterious effects of bacterial products(3). Previous investigation(4) has shown that the intravenous injection of endotoxin prepared from *Escherichia coli* regularly results in a profound decline in systemic blood pressure with an accompanying elevation of portal vein pressure. This prompt hemodynamic reaction did not occur in the hepatectomized animal. Three possible explanations for this phenomenon are evident: 1) large quantities of blood are trapped in the liver, 2) the liver releases vasodepressor substances or 3) both 1 and 2 occur. Attempts

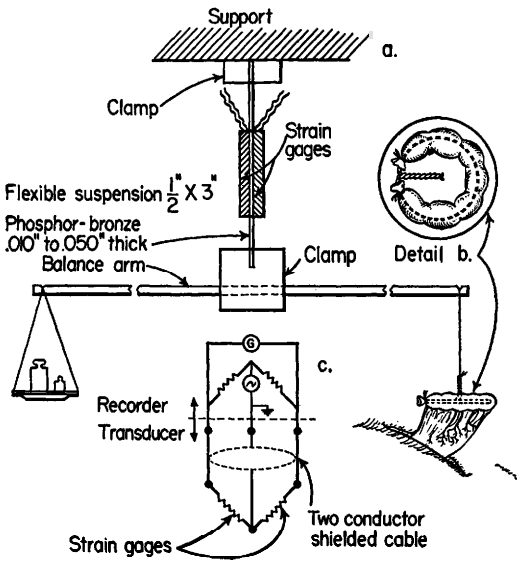

FIG. 1. Diagrammatic representation of weighing device including electrical circuit. See text.

to quantitate the volume changes of the liver following endotoxin administration by means of a plethysmograph were unsuccessful and led to the use of the method herein described. It has also been observed(4) that following the precipitous decline in blood pressure following intravenous endotoxin administration in the dog, a partial recovery occurs, coincident with return of portal vein pressure to normal, followed subsequently by a second decline in blood pressure leading to death. An estimation of pooling of blood in viscera other than the liver to account for the secondary hypotension was considered necessary. The small intestine was chosen and these results are included with a comparison of weight changes in the liver.

Methods. 1) Device for Continuously Recording Changes in Weight of Suspended Organs *in vivo*. The instrument illustrated in Fig. 1 has proved practical in estimating weight changes in organs *in vivo*. The performance characteristics demonstrate stability, sensitivity, and linearity adequate for the present physiological study(5). The weighing device consists of a rigid support and clamp from which is suspended a phosphor-bronze strip ½" x 3" and .01" to .05" thick, which in turn supports a balance arm 29" in length, at its midposition. There is provision for suspension of an organ to be studied at one end of balance arm and counterpoise at the opposite end. Strain gauge elements are cemented to each side of the phosphor-bronze strip so that any deflection of the balance arm will result in an increase in resistance in one strain element and a decrease in resistance in the opposite strain element; hence, an alteration in potential as measured by galvanometer G illustrated in C, which also includes a diagram of the resistances to electrical flow incorporated in strain gauges and in recorder. Differences in liver weight of 5 g and differences in small intestinal loop weight of one gram could be accurately detected. (2) Suspension of the Liver. The liver was exposed through a midline abdominal and sternotomy incision and mobilized by dividing the right and left triangular ligaments of the liver. The diaphragm was divided from the midline anteriorly to the inferior vena cava immediately cephalad to the liver. The right, left and quadrate lobes of the liver were elevated and enveloped in a net, the mouth of which was closed about the liver tightly enough for support only. The portal vein pressure was measured from a tributary of the splenic vein. All experiments were performed with a suspension which did not elevate portal vein pressure. All animals had a normal blood pressure at the beginning of the experiment suggesting that inferior vena cava flow was not compromised. The temperature of the liver was never less than 35°C. (3) Suspension of Intestine. A segment of small intestine from 25 to 60 cm in length with intact blood and nerve supply was isolated from the remaining bowel. Covered wire 0.5 cm in diameter was passed through the lumen of the isolated segment of bowel and utilized for support without tension on the mesentery and avoiding contact with other organs, (Fig. 1B). The loop was moistened with physiological saline at 37°C. During control observation periods of over one hour, the intestinal loops decreased in weight at the rate of one to 2 g per 25 cm per hour. All blood pressure records were made using strain gauges with optimal damping, recorded simultaneously and continuously with liver or intestinal

TABLE I. Changes in Liver and Intestinal Weight in the Dog following Systemic Injection of *Escherichia coli* Endotoxin.

Dog wt, kg	Type and dose of endotoxin	Min. from inj. to max incr. in liver wt	Max incr. in liver wt, g	Min. from ins. to return to normal liver wt	Simultaneous decline in mean systemic blood pressure, mm/Hg	Simultaneous incr. in portal vein pressure, mm/Hg	Min. from inj. to max incr. in intestinal wt and B.P. at that time	Max incr. in wt of intestinal loop, g/cm	Estimated storage of fluid in entire S. intes. tine, ml	Estimated storage of fluid in entire S. intest. at height of portal pressure rise, ml*
12	"Crude," .3 ml/kg	3	80	5.6	40	9	60 B.P. 25/0	18	770	55
19	"	1.5	350	25	100	19	50 B.P. 60/40	.8	340	170
12	"	1.5	90	5	35	15	50 B.P. 75/50	.5	210	56
15	"	1	80	5	50	19	55 B.P. 115/60	.5	196	77
13	Purified, 5 mg/kg	3	100	7	50	12	75 B.P. 110/80	.2	70	46
10	"	—	—	—	—	—	55 B.P. 100/65	.3	98	28

* Due to back pressure from the liver occurring at time of max increase in liver wt.

weight on a Sanborn Polyviso recorder. All animals were adult mongrel dogs. Anesthesia used was sodium pentobarbital 30 mg/kg. The preparation of endotoxin has been previously described(4).

Results. 1. *Changes in liver weight of dog due to* E. coli *endotoxin.* The results are illustrated in Table I, and Fig. 2. An increase in liver weight occurs within 3 minutes of endotoxin injection which is accompanied by a rise in portal vein pressure, and fall in systemic arterial blood pressure. The liver returns to normal weight in from 5 to 25 minutes during which time there is a return of arterial blood pressure in the direction of normal in most animals. While the quantity of blood trapped in the liver is enough to account for the decline in blood pressure observed in most experiments the possibility exists that the liver is producing a vasodepressor substance which contributes to the initial hypotension.

2. *Changes in small intestinal weight of the dog due to* E. coli *endotoxin.* The results are illustrated in Table I and Fig. 2. The storage of fluid in the small intestine due to back pressure from liver which occurs during the initial shock phase is in most experiments minor. A later large gain in intestinal weight occurred regularly at a time when the liver weight and portal pressure had returned to normal. Those animals with the lowest blood pressure at the time of maximal intestinal weight demonstrated the greatest increase in intestinal weight. The liver weight at this time (50 to 75 minutes) following endotoxin injection was at or below the preinjection level.

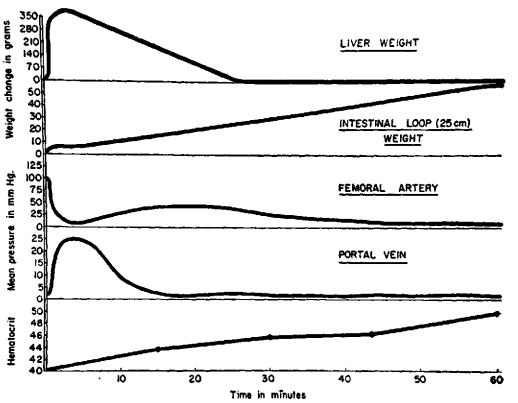

FIG. 2. Changes in liver wt, intestinal loop wt, femoral artery pressure, portal vein pressure and hematocrit for first hour following systemic *E. coli* endotoxin injection. Liver wt increased 350 g in a 19 kg animal within 1.5 min. A simultaneous increase in portal vein pressure and decline in femoral artery pressure occurred. A small elevation of intestinal loop (25 cm) weight occurred at this time, but a much larger rise in intestine weight occurred later, when liver weight had returned to normal. The latter rise is accompanied by a second decline in systemic blood pressure.

Discussion. There is some doubt that the component of the hypotension due to liver congestion is not species specific to the dog We have noted the same reaction as described for the dog in the rabbit and rat suggesting that this is not a species specific reaction. Since an elevation of portal vein pressure and hypotension can be produced with histamine, a bio-assay for this material was performed on the endotoxin utilized in this investigation. The "Crude" endotoxin contains between 0.5 and 1.5 μg of histamine per ml which is equivalent to a maximum of 10 μg in the largest dog studied and is not of significance in contributing to the marked weight gain observed in the liver or intestine. The purified endotoxin, Shear's endotoxin and crude endotoxin prepared from synthetic media do not contain histamine but give indistinguishable results.

A similar reaction to that described due to endotoxin (decline of systemic arterial pressure and elevation of portal vein pressure) has been noted following the systemic injection of certain peptone solutions of high protease content(6). While these reactions might be related, the shock, elevated portal vein pressure and increase in liver weight have been observed with the highly purified endotoxin of Shear and endotoxin grown on synthetic media both of which give a negative Biuret test for the peptide linkage(5). The site of obstruction leading to increase in liver weight is believed to be on the hepatic venous side of the liver sinusoids because microscopic examination of the liver reveals central vein congestion and vacuolization about the central vein. Additionally, when the isolated, weighed liver is perfused through the portal vein and hepatic artery using a constant flow pump, the addition of endotoxin results in increase in weight, an elevation of portal vein pressure, hepatic artery pressure and a decreased outflow during the period of weight increase. An increase in intestinal loop weight occurred at a time when the liver weight had returned to normal. The hematocrit invariably increases in these animals at this time, (Table I). The increase in small intestinal weight is believed to be due in considerable part to pooled or extravasated blood although fluid storage as edema is not excluded. The intestine is grossly hemorrhagic. This organ would appear to be the site of a vascular response induced by endotoxin possibly on a basis of previous sensitization.

Summary. 1. Systemic arterial blood pressure response of the dog to injection of *Escherichia coli* endotoxin is characterized by a sudden precipitous drop, a partial recovery and a subsequent slower decline over a period of hours. 2. Utilizing a device for quantitatively estimating changes in weight of organs *in vivo,* an increase in liver weight of from 80 to 350 g has been noted within one to 2 minutes following injection of *E. coli* endotoxin. 3. Simultaneous increase in weight of small intestine of lesser magnitude (28 to 170 g) was noted at the same time and is believed to be due to back pressure from the liver. Increase in liver and intestinal weight were believed to be quantitatively sufficient to account for the hypotension observed. 4. A difference in reaction of the liver and intestine was noted. Whereas the liver returns to normal weight within 5 to 25 minutes, the small intestine continues to increase in weight reaching a maximum of from 196 to 770 g approximately one hour following endotoxin injection. 5. The secondary hypotension due to endotoxin is believed to be at least partially the result of loss of blood in the small intestine due to vascular damage in this organ.

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