Summary. Intravenously administered bacterial endotoxin (LPS) is an effective emetic for cats. The site of emetic action in this animal was studied by determining the emetic responsiveness to LPS following different denervation procedures. Intrathoracic vagotomy and vagotomy + abdominal sympathectomy had very little influence on subsequent response to LPS. High LPS challenge doses elicited only prodromal signs of vomiting in cats subjected to spinal cord transection at T3-T4. Complete tolerance to emetic action of LPS resulted from abdominal deafferentation produced by vagotomy + spinal cord sectioning. The changes in sensitivity of the cat to the emetic action of intravenously administered staphylococcal enterotoxin were comparable to that for LPS for the different types of denervation.

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Glucose, Lactate and Potassium Metabolism in the Isolated Perfused Rat Liver.* (30759)

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Advances in the technique of liver perfusion have provided investigators with a new and fascinating tools. The perfused liver can metabolize proteins, fats, and carbohydrates anabolically or catabolically with such facility that one is sometimes tempted to think of the *in vitro* organ as being an *in vivo* system (1-5). However, one limitation is that the perfused liver forms glycogen at a slower rate than it does in the intact animal(6). The present investigation was designed to find out if glycogen formation might be limited by electrolyte deficiencies or by an inability to utilize substrates other than glucose.

Methods. The perfusion apparatus and dissection methods were similar to those used by Miller *et al*(7). Modifications permitted simultaneous sampling of both the affluent and effluent perfusate, adjustment of perfusion pressure, and measurement of flow rate. pH electrodes were placed in the bottom reservoir, making it possible to know the hydrogen ion concentration throughout the course of perfusion. All tubing was siliconized to help

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minimize hemolysis. The minimal circulating volume of the apparatus was 35 ml.

The average animal weight was 380 g (liver weight 11 g). Donors of blood and of livers were housed together and fed *ad libitum* until the time of the experiment except as indicated. Blood donors were lightly anesthetized with ether and bled from a neck incision. The heparinized blood was mixed in a 3:2 proportion with a solution containing NaCl 120, $NaHCO_3$ 21, KCl 4 meq/l. The final hematocrit ratio averaged .25 ($\pm$ S.D. $\pm$ .015), and did not change during perfusion. The starting volume averaged 79 ml. The perfusion medium was circulated in the apparatus and equilibrated with 95% O_2-5% CO_2 for approximately ½ hour before the liver was introduced into the circulation. The pH of the medium immediately before perfusion ranged between 7.45 and 7.52.

At the beginning of the dissection a tracheostomy was performed, and the rats breathed 100% O_2 which had been passed over ether. The use of 100% O_2 rather than room air decreased the initial losses of lactate from the perfused organ. The time for preparation of the liver from the beginning of anesthesia to the start of the perfusion averaged 21 minutes. During the last 4 minutes the liver was completely without circulation while the outflow catheter was put in place, the liver freed of its attachments, and the organ connected to the perfusion apparatus.

Methods of chemical analysis for glucose, potassium (K), and lactate have been described previously(8,9). Tissue water, sodium, and K concentrations were determined in samples of liver dried for 24 hours at 105°C and ashed at 500°C overnight, the ash being dissolved in 1 ml 1 N HCl. Tissue glycogen was determined as glucose from liver samples which had been digested with 30% KOH. Glycogen was precipitated overnight with 95% ethyl alcohol, washed twice with this reagent and redissolved in water.

The degree of hemolysis in each sample of perfusate was assessed by mixing a 1 ml aliquot of the perfusate supernatant with 4 ml of Drabkin's solution(10). The resulting color was read at 540 mμ in a Bausch and Lomb Spectronic 20 photometer and was compared to the color similarly produced from an appropriately diluted aliquot of hemolyzed whole perfusate. The results were expressed as per cent of hemolysis.

The K concentrations in the perfusate were corrected for the increase in K caused by hemolysis. In each experiment the K concentration in the red blood cells was calculated from determinations of K in whole perfusate, K in the supernatant, and the hematocrit ratio. From the per cent hemolysis the increment in K concentration which had been caused by the red blood cell destruction could be calculated and subtracted from the total supernatant K concentration.

The overall balances of glucose and lactate were calculated during each 20-minute interval as the product of changes in concentration of either substance and the circulating volume. That part of the overall balances which could be attributed to the red cells was subtracted and the remainder was considered to represent the movement of glucose and lactate in and out of the liver (Table II). These calculations were valid only when pH was constant, and the disappearance of glucose and production of lactate were linear functions.

Results. Circulating medium. In 12 experiments the perfusion medium was circulated without the liver. The observed K concentration increased 2.50 meq/l during the 3-hour period, but this was quantitatively accounted for by red cell hemolysis. The pH of the medium did not change. When the pH was between 7.40 to 7.45, glucose was utilized by the red cells at an average rate of .074 m moles/liter rbc/min. The red cells contributed significant amounts of lactate to the circulating medium, .17 m moles/liter rbc/min. Both glucose uptake and lactate production were linear functions during 3 hours. When the pH was increased (7.55) by adding $NaHCO_3$, glucose disappeared approximately twice as fast, and when decreased (7.20) by adding HCl it was utilized about one-half as rapidly as at the normal pH. Lactate production at the lower pH was also decreased.

Liver perfusions. Blood flow. As has been reported before, blood flow through the liver was slow during the first hour of perfusion

TABLE I. Volume 0.5 M $NaHCO_3$ (ml) Required to Maintain pH 7.40-7.44 in Circulating Medium.

Group	0-20 min.	20 min.-1 hr	1-3 hr	Total	N
I Fed rats	.7	.6	.4	1.7	6
IV Starved rats	0	1.1	.7	1.8	6

(11). However, it was noted that in the first 5 minutes of perfusion, blood flow was about 50% of the maximal rate, then decreased, attaining a minimum at about 15 minutes. Thereafter the rate increased exponentially becoming maximal at 70 minutes. The average maximal flow was 17.0 ml/min or 1.52 ml/min/g liver weight at perfusion pressures of 13-15 cm. At the end of 3 hours of perfusion the flow averaged 94% of maximal.

Hemolysis. By the time the liver was placed in the circulation about 1.1% of the red cells had been hemolyzed, and this increased to an average of 7.0% by the end of the perfusion. The liver did not increase the hemolysis, for this same degree of cell destruction was noted when the red cells were circulated alone.

Hydrogen ion concentration. In 3 perfusions during which no attempt was made to control the hydrogen ion concentration, the pH decreased from 7.40-7.45 to slightly less than 7.20 during the 3 hours. The largest decrease occurred during the first 20 minutes. In subsequent perfusions 0.5 M $NaHCO_3$ was added to the bottom reservoir in amounts necessary to maintain the pH between 7.40 and 7.44 (Table I). It was observed that the rate of base injection was greater during the first 20 minutes of perfusion than subsequently in perfusions of livers from fed rats. The initial change in pH was not noted during perfusion of livers from starved rats. The appearance of lactate in the medium did not completely account for the increase in concentration of hydrogen ions.

Glucose concentration. When no extra glucose was added to the perfusion medium (Group I), there was an initial increase in glucose concentration, but after 20 minutes there was a decrease (Fig. 1). Almost all of the decrease was accounted for by the utilization of glucose by the red cells (Table II). In Groups II and III 150 and 300 mg of glucose, respectively, were added to the circulating medium prior to introducing the liver. Added glucose did not prevent the initial glucose loss from the liver, but the subsequent rate of decrease in concentration after 20 minutes indicated a net uptake by the liver (Table II). There were no statistically significant differences in the final glucose concentrations between these three groups.

A possible interpretation of this observation might be that the liver was dead and was no longer able to accumulate glucose. That such was probably not the case is demonstrated by the following experiments, one of which is illustrated in Fig. 2. The liver

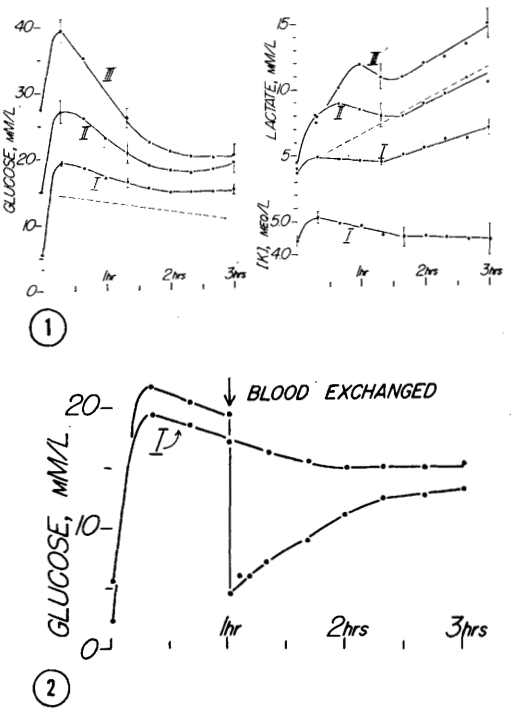

FIG. 1. Changes in glucose, lactate, and potassium concentrations in the circulating medium during 3 hr of perfusion after no extra glucose added (I), or 150 mg (II), and 300 mg (III) extra glucose added before beginning of perfusion. Dotted lines indicate changes in glucose and lactate concentrations when the medium alone was circulated. Vertical bars represent ± S.E. of mean concentrations.

FIG. 2. Mean changes in circulating glucose concentrations in livers from Group I compared to those from another type of experiment in which the medium was replaced with an equal quantity of fresh, unused perfusate. The exchange of blood at the time indicated by the arrow reduced glucose concentration, which subsequently increased as glucose was lost from the liver.

TABLE II. Balances of Glucose and Lactate Attributed to the Perfused Liver.

Gr*	n	Glucose m moles/kg 0-3 hr	Glucose m moles/kg 20 min-3 hr	Lactate m moles/kg 0-3 hr	Lactate m moles/kg 20 min-3 hr
I	6	− 97 ± 9†	+ 8 ± 5	+27 ± 16	+30 ± 15
II	4	− 63 ± 22	+30 ± 15	− 1 ± 12	+23 ± 8
III	4	+ 6 ± 22	+80 ± 18	−24 ± 12	− 4 ± 9
IV	6	+150 ± 11	+91 ± 14	+43 ± 9	+18 ± 8

* Gr I, II, III livers from fed rats: Gr IV livers from 24 hr starved rats: Gr I no extra glucose added: Gr II, III, IV, 150, 300, 500 mg glucose respectively added to medium before perfusion.

† ± S.E.

was perfused as usual for the first hour, at which time the circulating perfusate was replaced with a fresh aliquot of medium. The "exchange perfusion" drastically reduced the glucose concentration, and the liver responded by losing glucose in the medium. Thus the liver seemed to adjust the concentration of glucose in the medium, reducing concentrations greater than 15 mM and raising the concentration if it were less than this value.

Lactate concentration. There was a small initial loss of lactate from the liver during the first 20 minutes of perfusion as indicated by an increase in lactate concentration (Fig. 1). When no extra glucose was added to the medium (Group I), the lactate concentration remained less than that noted when the medium alone was circulated. The liver was able to utilize some of the lactate produced by the red cells. When 300 mg extra glucose was added (Group III), the increase in lactate concentration was greater than could be accounted for by red cell production. Some of the glucose taken up by the liver from these fed rats probably reappeared in the circulation as lactate.

Potassium concentration. The initial increase in the corrected K concentration noted after 20 minutes of perfusion was followed by a decrease. By the end of 3 hours the K concentration was not different from that at the beginning of the perfusion. Changes in K concentration were the same whether or not extra glucose was added.

Composition of the liver by direct analysis. The composition of the liver determined by direct analysis is presented in Table III. In the control experiments the liver was quickly removed from fed rats which were lightly anesthetized with ether. During perfusions a small lobe of the liver was removed within 4 minutes from the beginning of the circulation. The dissection caused some loss of glycogen and a small gain of tissue water. The gain in water accounted for the decrease in K concentration as calculated on a wet weight basis. After 3 hours of perfusion the water, K, and Na concentrations were well maintained at normal concentrations, and there was no significant further decrease in glycogen content.

Glycogen formation. The livers from rats

TABLE III. Composition of Liver by Direct Analysis.

	n	H_2O, ml/kg*	K meq/kg*	K meq/100 g* solids	Na meg/kg*	Na meq/100 g solids	Glycogen, %
Control (non perfused)	12	698 ± 2.0	90.6 ± .8	30.0 ± .3	27.9 ± .7	9.2 ± .2	3.65 ± .24
At beginning of perfusion	11	713 ± 2.7	84.3 ± 1.0	29.4 ± .5	31.9 ± .8	11.2 ± .4	3.04 ± .30
At end 3 hr of perfusion							
Group I†	6	701 ± 3.4	87.9 ± 1.2	29.4 ± .5	28.7 ± 1.0	9.6 ± .5	2.81 ± .36
" IV†	6	702 ± 2.0	97.3 ± 1.1	32.7 ± .6	33.8 ± 1.5	11.3 ± .7	1.18 ± .17

* Wet weight.

† Gr I livers from fed rats, no extra glucose added to medium. Gr IV livers from starved rats, 500 mg glucose added to medium ± S.E.

which had been starved for 24 hours were used for perfusions in Group IV. Five hundred mg of glucose was added to the medium at the beginning of the perfusion. The concentration of glucose decreased throughout the perfusion, but was greater than 25 mM at the end of 3 hours. There was a net uptake of lactate from the circulation. The glycogen content of the lobes removed at the beginning averaged .09% and at the end of perfusion 1.18%. The K concentration in the liver at the end of 3 hours was slightly greater than that in the liver tissue of Group I. The increase in liver glycogen indicated that about 35% of the glucose and lactate removed from the circulation was stored as glycogen.

One of the purposes of these experiments was to see if the rate of accumulation of stored carbohydrate, which is less in the perfused organ than in the intact animal, might be limited by the development of hypokalemia. Such was not the case. At the end of 1 hour of perfusion the corrected K concentration decreased to 3.4 meq/l, as compared to 4.6 meq/l during perfusion of livers from fed animals. In fact, the actual potassium available for uptake would also include that which had been released from red cells, so there was an excess of potassium at all times.

Discussion. The survival of the isolated perfused rat liver was confirmed by this series of experiments. During perfusion the water and electrolyte concentrations were well maintained and there was no swelling as noted by others(11). The perfused organ reduced the circulating glucose concentration when it was greater than about 15 mM and added glucose to the medium if the glucose concentration was less than this value. Others have indicated that the liver *in vivo* oscillates between glucose uptake and production depending upon the concentration of circulating glucose(12,13).

Whether glycogen formation in the perfused organ is the same as *in vivo* is open to question. The rate of formation is slower than *in vivo*(6), but this may be because certain hormones or even other organs are lacking in the perfusion system. Also the K content of the livers which formed glycogen was greater than observed in livers during glycogen deposition *in vivo*(14). Perhaps if the perfusions had been continued longer, the excess K would have been lost or water might have been gained to reduce the concentration.

Another observation which indicated that the conversion of glucose to glycogen might be limited in the perfused organ was the appearance of lactate in the circulation when added glucose was utilized by livers which contained moderate but not maximal amounts of stored carbohydrate (Group III). One would have expected that the extra glucose would have been converted to glycogen, for the latter had been depleted during the surgical preparation.

It was also interesting that uptake and loss of lactate by the liver seemed to vary with the concentration of liver glycogen. When extra glucose was added to the circulation during perfusion of livers from fed animals, there was a loss of lactate, but under similar conditions using livers from starved animals the organ gained lactate. These observations are similar to those reported for the intact animal(14).

Summary. 1. During the anesthesia and dissection preparatory to perfusion of the rat liver, there were small losses of tissue glycogen and a slight increase in water content. During the 3-hour perfusion, the water content returned to normal and the Na and K concentrations of the liver were maintained. 2. The perfused liver removed glucose from the circulation if the concentration were greater than 15 mM, and added glucose if it were less than 15 mM. The liver was able to utilize lactate produced from the circulating red cells. 3. During glycogen formation in livers from starved rats, both glucose and lactate were utilized. The rate of glycogen formation was not limited by a lack of K.

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Effect of Antigen Dose on Lymphatic Tissue Germinal Center Changes.* (30760)

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Earliest histologic changes observed during the immune response involve lymphatic tissue germinal centers, and various investigators have suggested that these centers are an integral part of the immune system(1-4). Three consistent histologic findings during the early period of spleen lymphatic tissue reaction to an antigenic stimulation of 1 ml of 10% sheep erythrocyte suspension have been reported(1,2). The first change is germinal center loss or dissociation; the second is the occurrence of large pyronin-staining cells throughout the lymphatic nodule and splenic red pulp. The presence and general significance of large pyronin-staining cells for reaction to antigenic material have been recognized for some time, and these cells are thought by many investigators to be the source of plasma cells and other immunologic phenomena(2,5,6). The third change is germinal center recovery, with hyperplasia beginning 2 days after antigen injection.

Autoradiographic and histologic studies have suggested that germinal center cells of spleen white pulp can be stimulated either directly or indirectly by antigen and that these stimulated cells emigrate from the centers to other areas of the spleen(7). This response ultimately results in what has been termed the dissociative growth of the center cells. These studies have also suggested that on a dynamic and morphologic basis, a correlation exists between germinal center cells and large pyronin-staining cells characteristically seen in lymphatic tissue during early intervals of the immune reaction.

Germinal center recovery with hyperplasia has been the most frequently described histologic alteration in lymphatic tissue during the immune response. The role of hyperplastic germinal centers, however, has not been resolved.

The purpose of this study was to investigate the effect of antigen dose on spleen germinal center changes and to correlate these changes with production of specific serum antibody during the first 30 days after primary antigenic stimulation. The results indicate a dose-response relationship between antigen dose and germinal center changes and a correlation between germinal center changes and specific serum antibody production.

Materials and methods. a) *Animals.* Male, $BC3F_1$ |(C3H/AN ♀ × C_{57} Bl ♂) F_1 Cum.| mice 13-16 weeks of age were used. They were kept 10 to a cage and given food and water *ad libitum.*

b) *Antigen.* Sheep blood was obtained fresh in modified Alsever's solution(8) and

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