Summary. Seven rabbits immunized with acetone dried *Salmonella typhosa* produced both 19S and 7S agglutinins for the typhoid somatic antigens although the 19S antibodies predominated in most serum samples even after repeated injections of vaccine. The results suggest that the route of injection and amount of antigen injected may influence the relative amounts of 19S and 7S antibodies appearing in the serum.

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Hypoglycemia Following Endotoxin Administration in Animals with Liver Damage. (29051)

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Previous studies in this laboratory(1,2) have demonstrated that guinea pigs pretreated with a sublethal dose of carbon tetrachloride (CCl_4) are remarkably susceptible to the lethal action of bacterial endotoxin. Endotoxin susceptibility is greatest when hepatic necrosis is maximal (about 48 hours after CCl_4 administration). It was also observed that CCl_4-treated animals became prostrate and died sooner than normal animals given an endotoxin dose of comparable lethality. These findings suggested that a different type of response to endotoxin might occur in these animals with severe liver damage. This study documents the occurrence of progressive and profound hypoglycemia in CCl_4-treated guinea pigs given a lethal dose of endotoxin, a phenomenon which was not seen in normal animals.

Materials and methods. Female Hartley strain of guinea pigs weighing between 300 and 400 g were used. Endotoxin of *E. coli* 026-B6 (Boivin type) was obtained from Difco Laboratories. The intravenous route was used for all endotoxin injections. CCl_4 treatment consisted of the subcutaneous injection of 0.15 ml 48 hours before endotoxin challenge. This dosage caused severe centrilobular hepatic necrosis but was not lethal(2). The animals were either allowed Purina rabbit

chow and lettuce *ad lib* until 12 hours prior to endotoxin injection or were fasted for 48 hours before endotoxin injection as described in the experiments. Blood samples for glucose determination were obtained either by cardiac puncture or by bleeding from the orbital plexus using capillary tubes calibrated to contain 0.05 ml (Kensington Scientific Corp., Berkeley, California). Glucose was determined by the Nelson modification of Somogyi's method(3). Liver glycogen was measured by the method of Good, Kramer and Somogyi(4).

Results. The LD_{90-100} of this endotoxin was 15 mg/kg for normal animals and 0.063 mg/kg for animals pretreated with CCl_4. This marked (235-fold) increase in susceptibility of CCl_4-treated animals is consistent with the findings of previous studies with endotoxins of *S. flexneri* and *S. marcescens*(1,2). Following intravenous injection of one LD_{90-100}, the median survival time was 12 hours for normal animals and 7 hours for CCl_4-treated animals (Fig. 1). Most of the CCl_4-treated animals appeared prostrate within 1-2 hours after challenge, whereas normal animals did not appear to be in shock until 6-8 hours after endotoxin injection. These findings confirm the previous clinical impression of early deterioration and death in CCl_4-treated animals as compared with normal animals given an endotoxin dose of comparable lethality.

A striking difference in the blood sugar response of normal and CCl_4-treated animals was observed following injection of endotoxin (Fig. 2). In this experiment both groups were fasted for 12 hours and received 15 mg/kg. Normal animals showed an initial hyperglycemia, presumably due to epinephrine release (5), followed by a gradual decline to normal levels and below. By 8 hours the mean blood glucose level was 50 mg/100 ml, and only one animal was severely hypoglycemic. In contrast, an immediate and progressive fall in blood glucose was observed in CCl_4-treated animals. At 4 hours the mean blood glucose level was 4 mg/100 ml, and the highest value observed was 14 mg/100 ml. All animals in this group died before 8-hour blood samples could be taken. CCl_4-treated animals which received no endotoxin showed no fall in blood sugar during the period of the experiment.

An identical hypoglycemic response was seen in CCl_4-treated animals when the endotoxin dosage was reduced to 0.063 mg/kg, an amount just sufficient to kill 90-95% of the animals (Fig. 3). In this and in subsequent experiments all animals were fasted during the 48-hour period just prior to endotoxin injection, to eliminate differences in dietary intake between normal animals and CCl_4-treated animals, which were usually quite anorectic. A fast of this duration did not in itself significantly alter susceptibility to endotoxin. The more prolonged hyperglycemia in the normal animals in this experiment as compared with the previous one may be due to the diminished carbohydrate tolerance associated with starvation. By 12 hours the mean blood glucose in this group was 41 mg/100 ml, and the lowest value was 30 mg/100 ml. Since 60% of the animals were dead by this time, it is unlikely that hypoglycemia was a significant factor in the death of these normal animals.

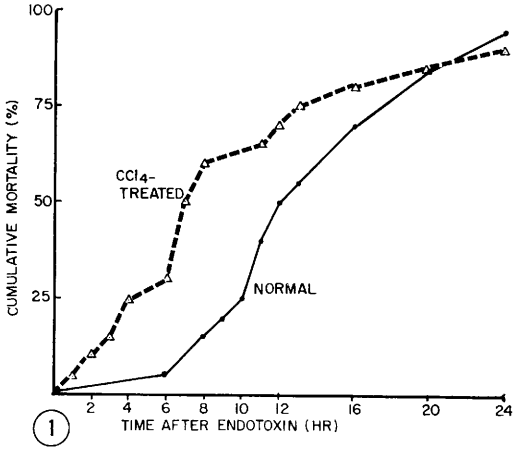
Experiments were next performed to determine if the hypoglycemia seen in CCl_4-treated animals given endotoxin was due to an increase in rate of removal of glucose from the blood by peripheral tissues, or to a decrease in input of glucose into the blood from the liver and/or gut. The rate of disappearance of glucose from the blood following an intravenous glucose load (50 mg/100 g) in CCl_4-treated animals was no different in those receiving endotoxin than in those not receiving endotoxin (Fig. 4); thus an increase in rate of peripheral removal of glucose following endotoxin could not be demonstrated.

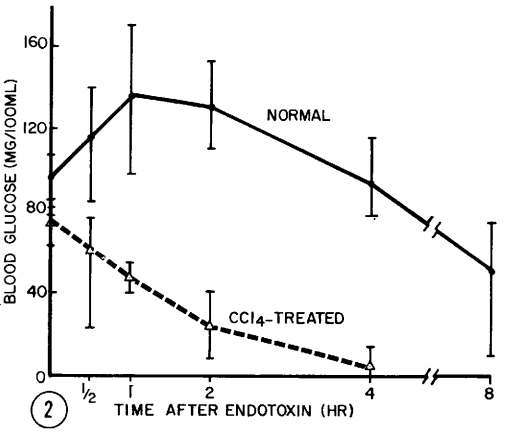
Of the 2 major sources of blood glucose,

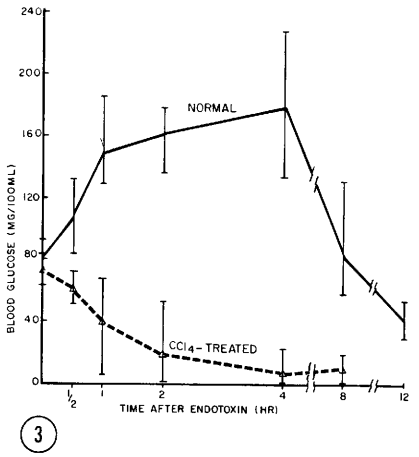
TABLE I. Effect of Glucose Infusion on Survival Time of CCl_4-treated Guinea Pigs Given Endotoxin ($3LD_{90-100}$).

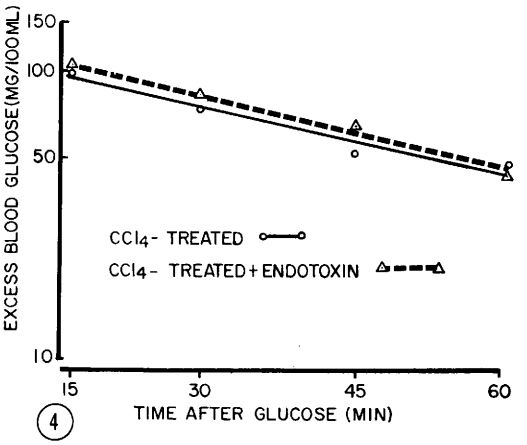
Infusion solution	No. animals	Mean survival time (hr) ± S.E.
Glucose*	6	10.3 (± 1.5)
Saline	6	7.1 (± 1.2)

* 50 mg/100 g/hr, as 20% aqueous solution (approx. 1.2 ml/hr). Animals fasted for 48 hr prior to endotoxin injection.









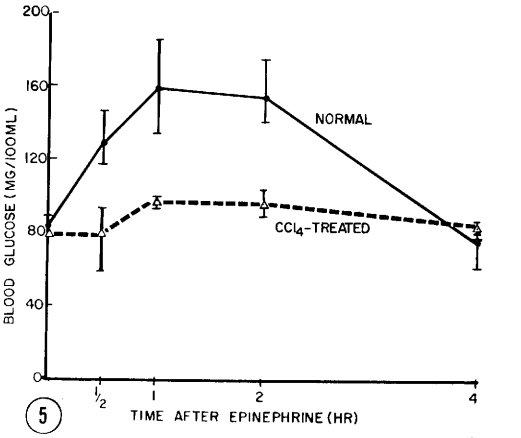


FIG. 1. Cumulative mortality of guinea pigs following intravenous administration of one LD_{90-100} of *E. coli* endotoxin. Normal animals received 15 mg/kg; CCl_4-treated animals received 0.063 mg/kg. Twenty animals per group.

FIG. 2. Effect of endotoxin (15 mg/kg) on blood glucose of normal and CCl_4-treated guinea pigs. Each point represents mean of at least 4 samples, with range in brackets. Animals allowed food *ad lib* until 12 hr before endotoxin injection.

FIG. 3. Effect of one LD_{90-100} endotoxin on blood glucose levels of normal and CCl_4-treated guinea pigs. Normals received 15 mg/kg; CCl_4-treated received 0.063 mg/kg. Each point represents mean of at least 4 samples, with range in brackets. Animals fasted for 48 hr prior to endotoxin injection.

FIG. 4. Blood glucose levels of CCl_4-treated guinea pigs following intravenous glucose loading (50 mg/100 g). Endotoxin group received 0.063 mg/kg *E. coli* endotoxin 1 hr before glucose loading. Each point represents mean of 5 samples. "Excess blood glucose" is difference between value obtained and fasting blood glucose of that animal. Animals fasted for 48 hr prior to glucose loading.

FIG. 5. Effect of intravenous epinephrine (10 μg) on blood glucose of normal and CCl_4-treated guinea pigs. Each point represents mean of at least 4 samples, with range in brackets. Animals fasted for 48 hr prior to epinephrine injection.

the gut was eliminated as a significant factor by fasting the animals for 48 hours before endotoxin injection. Therefore, a breakdown in hepatic glucose production following endotoxin injection in these animals with preexisting liver damage appears to be the most likely explanation for the hypoglycemia. That CCl_4-treatment does impair glucose production is shown by Fig. 5, in which the response of normal and CCl_4-treated animals to epinephrine is shown. An intravenous injection of epinephrine (10 μg) sufficient to double the blood sugar level of fasted normal animals resulted in a barely significant rise in the blood sugar of fasted CCl_4-treated animals. This difference is not due simply to differences in liver glycogen content. The mean liver glycogen concentration of fasted CCl_4-treated animals (4.6 mg/g) was actually somewhat higher than that of fasted normal animals (2.8 mg/g). These low glycogen values indicate that normoglycemia is maintained in these fasted animals not by glycogenolysis, but by glucose production from protein and fat (gluconeogenesis), and it is this function which is disrupted by the combined effects of CCl_4 and superimposed endotoxin shock.

Since hypoglycemia was implicated as an immediate cause of death following endotoxin administration in these animals with liver damage, the effect of maintaining the blood glucose by intravenous infusion was studied. CCl_4-treated animals were given $3LD_{90\text{-}100}$ of endotoxin, and either 20% glucose (50 mg/100 g/hr) or physiological saline was continuously infused *via* a polyethylene catheter inserted in a foreleg vein, using a dual infusion pump (Harvard Apparatus Co., Inc., Dover, Mass.). The animals receiving glucose showed consistent glycosuria, so it may be assumed that the infusion maintained the blood glucose at hyperglycemic levels. The mean survival time was 10.3 $\pm$ 1.5 hours in the glucose-treated group and 7.1 $\pm$ 1.2 hours in the saline controls. This difference of 3.2 hours may well be biologically important but was not statistically significant with the small number of animals used ($p \approx 0.10$ when tested by means of t test). Thus glucose appeared to prolong survival but did not prevent death following a dose of endotoxin which was only 1/75 of an $LD_{90\text{-}100}$ for normal animals.

Discussion. The finding that CCl_4-treated guinea pigs are highly susceptible to the lethal action of bacterial endotoxin, and that they deteriorate and die sooner than normal animals given an endotoxin dosage of comparable lethality, suggested that a different mechanism of death might be involved in these animals with severe liver damage. In this study animals with liver damage due to CCl_4 were found to develop severe and progressive hypoglycemia following relatively small doses of endotoxin. The hypoglycemia was evidently due to a decrease in the rate of hepatic glucose production. It seems likely that when the circulatory abnormalities of endotoxin shock are superimposed upon the damage caused by CCl_4, hepatic glucose production falls below the critical level required to maintain normoglycemia. Maintenance of the blood sugar level by intravenous infusion of glucose appeared to increase survival time but did not prevent the ultimate death of the animals from quite small doses of endotoxin. Thus, while hypoglycemia appears to be responsible for the early death of CCl_4-treated animals following endotoxin injection, it is not the factor underlying the extreme susceptibility of these animals to the lethal effects of small doses of endotoxin.

Normal animals given a lethal dose of endotoxin showed an initial and fairly prolonged hyperglycemic response, followed by a fall in the blood glucose to below normal levels. The severe hypoglycemia seen in CCl_4-treated animals was not observed, even at 12 hours, when 60% of the normals had died. Thus hypoglycemia does not appear to be a significant factor in the death of normal animals. It might be postulated that maintenance of the blood sugar level of CCl_4-treated animals by glucose infusion simply allows them to live long enough to die of endotoxin shock, as the normal animals do.

It has been stated that patients with advanced liver disease who develop bacteremia due to gram-negative organisms have an unusually poor prognosis(6). Hypoglycemia

should be looked for in such patients, since, if present, its correction might prolong life so that measures directed toward the underlying process of endotoxin shock could be effective.

Summary. An immediate and progressive fall in blood glucose level occurs in CCl_4-treated guinea pigs given a lethal dose of bacterial endotoxin. The hypoglycemia is evidently due to a decrease in rate of hepatic glucose production. Maintenance of the blood sugar level appears to prolong survival but does not alter the extreme susceptibility of these animals to endotoxin. Hypoglycemia should be looked for in patients with severe liver disease who develop gram-negative bacteremia, since if uncorrected it could render all other therapeutic measures ineffective.

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Relationship of Plasma Aldadiene Levels and Antimineralocorticoid Effects of Spironolactone in the Laboratory. (29052)

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Oral administration of spironolactone,* a steroidal spirolactone(1) and an antagonist of the renal electrolyte effects of mineralocorticoids(2), produces measurable plasma concentrations of a fluorogenic, Δ^6-metabolite (3), 3-(3-oxo-17β-hydroxy-4,6-androstadien-17α-yl)propanoic acid lactone(1) or aldadiene. Such data on plasma aldadiene have been applied in study of gastrointestinal absorption with various pharmaceutical preparations of spironolactone(3-7). In both experimental animals and man, data have suggested a correlation of aldadiene titers with renal electrolyte manifestations after spironolactone administration(4,7). However, as aldadiene obtained synthetically also possess antimineralocorticoid properties(1,8), an important question relates to relative influence of the Δ^6-metabolite on renal properties of spironolactone. As one approach, therefore, spironolactone and aldadiene were examined comparatively in rats and dogs for plasma aldadiene levels and urinary electrolyte effects; some data believed to be pertinent to the question are summarized below.

Material and methods. In rats (175-225 g), spironolactone or aldadiene was orally administered concurrently with 12 μg of deoxycorticosterone acetate (DCA) subcutaneously, as solutions of corn oil. Four-hour samples of urine were collected in metabolism cages (4 rats/cage) in one series of animals for Na and K determination. Antimineralocorticoid activity of the spirolactones was assessed by reversal of the urinary Na/K response to DCA according to previously described procedures(2). DCA alone induces Na retention and K loss, to produce thereby a reduction in the ratio of urinary Na/K. Other rats treated identically were sacrificed under ether at 1, 2, 3 and 4 hours after treatment, and blood specimens from the abdominal aorta collected in heparinized tubes using a plastic cannula. Plasma was separated by centrifugation for aldadiene analysis.

Trained female mongrel dogs (14-19 kg)

* 3-(3-Oxo-7α-acetylthio-17β-hydroxy-4-androsten-17α-yl)propanoic acid lactone; Aldactone® is the trademark of G. D. Searle and Co. for brand of spironolactone.