tivity. Failure of FAC to increase the viral susceptibility of murine embryonic and macrophage cells in culture also points to a humoral factor in this phenomenon.

The ability of EDTA, a chelating agent, to protect newborn mice against MHV was of considerable interest. Although we have assumed that this was due to a reduction of serum iron levels and essentially the converse of the effect of parenteral FAC, no biochemical studies were performed and one cannot rule out a possible decrease in ionized calcium or other ions in extracellular fluids as a factor in its prophylactic action.

Infectious hepatitis in man is distinguished by profound derangements of iron metabolism. To quote Peterson (10), "Acute hepatitis is the only disease in human beings consistently associated with a marked hypersideremia. There is no satisfactory evidence to explain this." Further, there is a significant decrease in cytochrome oxidase in acute viral hepatitis (11). Whether a reduction of serum iron by the administration of a chelating agent will alter susceptibility or the course of IH in man merits investigation.

Summary. The administration of ferric ammonium citrate to newborn mice enhanced their susceptibility to MHV. Intraperitoneal inoculation of a chelating agent, EDTA, protected a significant proportion of newborn animals against MHV but only when administered shortly after birth.

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Endotoxin-Induced Inhibition of Renal Function in the Mouse (33387)

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The literature dealing with the effects of endotoxin on kidney function is surprisingly limited. In a number of recent reviews on the action of endotoxin on the host, the involvement of the kidney in endotoxemia has not been taken into consideration (1–3). Renal dysfunctions, however, have been reported to occur in dogs and monkeys following an injection of endotoxin (4, 5), and in humans after the administration of blood contaminated with gram-negative bacilli (6). In mice, Berry and Smythe (7) found that endotoxin inhibits the ACTH-induced increase in urinary nitrogen excretion, and, in a later study (8), they showed that these findings were related to impaired renal function.

The present study not only confirms and extends the findings of Berry and Smythe that renal function is depressed in mice treated with appropriate doses of endotoxin, but also demonstrates that mice can be rendered tolerant to the renal inhibitory effects of endotoxin. In addition, experiments are described which show that the renal effects of endotoxin can be quantitatively determined by measuring the blood, brain, and liver concentrations of urea nitrogen, the quantity of urea nitrogen excreted, and the renal clear-

ance of inulin and of para-aminohippurate (PAH).

Methods. Carworth Farm male albino mice (18–21 g) were used in this study. All injections of endotoxin (lipopolysaccharide B from *E. coli,* Difco, 026:B6, lot nos. 115458 and 500158) were by the intraperitoneal route, the material being injected in a final volume of 0.2 ml of pyrogen-free saline; control mice received 0.2 ml of saline. Blood samples were withdrawn from the ophthalmic venous plexus with Dispo micropipettes (Scientific Products) according to the method of Riley (9).

During urine collection periods, the mice were deprived of food but were allowed drinking water *ad libitum.* The water bottles were arranged on the metabolic cages so that no water entered the urine. In addition, a fine-meshed screen at the bottom of the cages and a wad of glass wool inserted into the stem of the collection funnel were used to obtain urine free of fecal materials.

Urea nitrogen was determined by the method of Coulombe and Favreau (10), using 0.5-ml aliquots of protein-free solutions obtained from blood, urine, brain, and liver. The supernatant solutions from blood and urine were prepared by pipetting 0.05 ml of blood or 0.02 ml of urine into 1 ml of water, and the proteins were precipitated from the hemolyzed blood or the diluted urine by the addition of 0.2 ml of 0.66 *N* sulfuric acid and 0.2 ml of 10% sodium tungstate. Each brain and liver was homogenized in 2.5 and 8 ml, respectively, of a 1:1 mixture of 0.66 *N* sulfuric acid and 10% sodium tungstate by the use of a tight-fitting, all-glass homogenizer. All of the preparations were clarified by centrifugation at 1500*g* for 15 min.

Inulin and PAH clearances were determined by measuring the quantity of these substances in the blood 15 min after an intravenous injection of inulin (50 mg) or sodium para-aminohippurate (7.2 mg) dissolved in 0.5 ml of saline. The inulin and PAH determinations were performed on 0.05 and 0.02 ml of blood, respectively. Each blood sample was pipetted into 1 ml of water, and the proteins were precipitated from the hemolyzed blood by the addition of 0.2 ml of 10% zinc sulfate and 0.2 ml of 0.5 *N* sodium hydroxide. After centrifugation, 0.75 ml of supernatant fluid was analyzed for inulin (11) or PAH (12). The results were expressed as percentage inhibition of inulin or PAH clearance, and were calculated according to the equation

$$\frac{(E/C - 1)}{(N/C - 1)} \times 100 = \% \text{ inhibition of inulin or PAH clearance,}$$

where E, N, and C equal the blood concentration of inulin or PAH in endotoxin-treated mice, bilaterally nephrectomized mice, and control (saline-treated) mice, respectively. The above equation is based on the assumption that 100% inhibition of inulin or PAH clearance occurs in bilaterally nephrectomized mice, and no inhibition (0.0%) in controls.

Mice were nephrectomized under ether anesthesia, and were used for clearance studies about 1 hr after their righting reflexes were restored. At the conclusion of each experiment the abdominal cavity was examined for blood or fluid accumulation to rule out inulin or PAH loss through this route. Statistical calculations (t test) were performed according to Snedecor (13).

Results. Urea nitrogen levels in blood, brain, and liver were significantly increased ($p<0.001$) 18 hr after the administration of endotoxin to mice in the dose range of 5–20 mg/kg; lower doses produced no significant changes (Fig. 1). The urea nitrogen content of the liver was increased to a greater extent than that of the blood, which, in turn, was increased to a greater extent than that of the brain.

The renal clearances of inulin and PAH, which were determined 18 hr after mice were injected with endotoxin, were both significantly inhibited ($p<0.001$) to approximately the same extent at endotoxin doses between 6 and 24 mg/kg, lower doses producing no significant inhibition (Fig. 2).

The urinary excretion of urea nitrogen and the urine volume were both significantly inhibited ($p<0.001$) during an 18-hr period after mice were injected with endotoxin in the dosage range of 1.25–24 mg/kg (Figs. 1 and

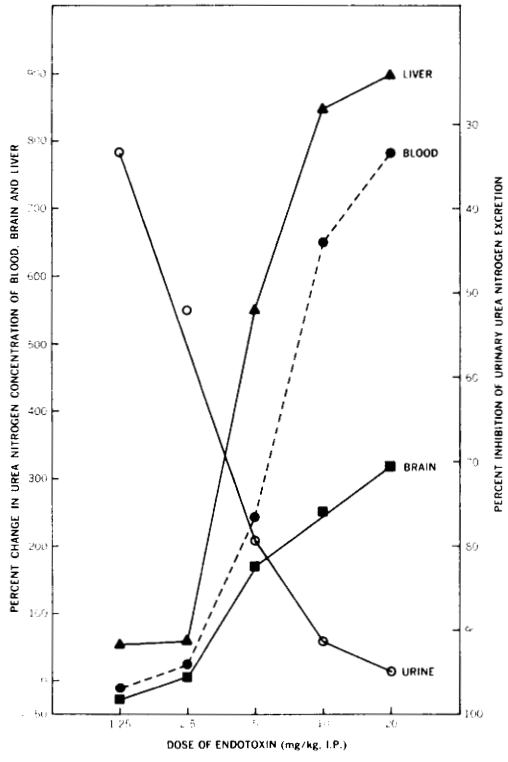

FIG. 1. Blood, brain, and liver concentrations of urea nitrogen, and the urinary excretion of urea nitrogen, in mice treated with graded doses of endotoxin. The control values (mean ± SE) for urea nitrogen were 7.70 ± 0.12 mg/100 ml of blood, 1.05 ± 0.07 mg/brain, and 1.32 ± 0.05 mg/liver; controls excreted 22.0 ± 0.8 mg/mouse/18 hr. Between 11 and 23 mice were used for each point when the blood, brain, and liver levels of urea nitrogen were determined; 3–6 groups of mice (8–10/group) were used for each point on the urinary urea nitrogen curve.

2). It is noteworthy that endotoxin doses lower than 5–6 mg/kg, which were ineffective in elevating the blood, brain, and liver levels of urea nitrogen and in inhibiting the renal clearances of PAH and inulin, were remarkably effective in decreasing the output of urine and urinary urea nitrogen.

Mice were rendered tolerant to the renal inhibitory effects, as well as to the lethal effects, of endotoxin by injecting them for 8 successive days with endotoxin at a daily dose which neither impaired renal function nor produced death. The results are shown in Table I. In mice pretreated with saline for 8 days and challenged on the ninth day with endotoxin (15 mg/kg), the blood urea nitrogen concentrations were markedly elevated, and the renal inulin clearances were substantially inhibited. In addition, an 85% mortality (72 hr) was observed in these mice. These changes, however, were not demonstrated in mice pretreated with endotoxin (1 mg/kg).

Discussion. The present data indicate that renal function is impaired in endotoxin-treated mice. This conclusion is based on the following changes noted after treatment with relatively large doses of endotoxin: (i) Elevation of the blood, liver, and brain levels of urea nitrogen; (ii) decreased excretion of urinary urea nitrogen; (iii) diminished output of urine; and (iv) inhibition of the renal clearances of inulin and PAH. It is noteworthy that endotoxin, at the highest dose tested, does not completely inhibit renal function since the kidney clearances of inulin and PAH were never inhibited by more than 70%.

It is important to note that the renal inhibitory effects of endotoxin were observed at

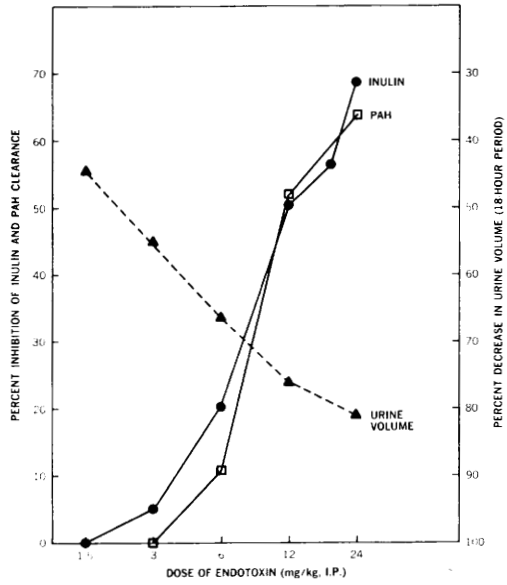

FIG. 2. Effect of endotoxin on the renal clearances of inulin and PAH, and on the urine volume excreted. The values for each point were obtained from 6 to 11 mice for the inulin and PAH clearances, and from 4 groups of mice (8–10/group) for the urine volumes.

TABLE I. Development of Tolerance to the Renal Inhibitory and Lethal Effects of Endotoxin.[a]

Group no.	Pretreatment 8 days	Treatment on Day 9	Renal inulin clearance (% inhibition)	Blood urea nitrogen (mg/100 ml)	Mortality (72-hr) (no. dead /no. used)
1	Saline (0.2 ml/mouse)	Saline (0.2 ml/mouse)	0	10.3 ± 0.5	0/38
2	Saline (0.2 ml/mouse)	Endotoxin (15 mg/kg)	67.8 ± 5.1	61.8 ± 3.3	34/40
3	Endotoxin (1 mg/kg)	Endotoxin (15 mg/kg)	5.3 ± 2.2	11.6 ± 0.6	1/41

[a] Values are means ± SE. Each value for inulin clearance and blood urea nitrogen was obtained from 12 mice (2 expts). Statistical significance: $p < 0.001$ when group 2 was compared with group 1, and group 3 compared with group 2.

dosages that are far greater than those required to increase nonspecific resistance to infections in mice (14). For example, Berger and Fukui (15) have reported that 0.25–1.0 mg/kg, i.p., of endotoxin (*E. coli,* Difco, 026:B6) significantly protected mice from death induced by experimental microbial infections. In our experiments, these dosages of endotoxin did not interfere with normal renal function.

The increased concentrations of urea nitrogen that were found in the blood and tissues of endotoxin-treated mice may be attributed not only to a decreased excretion of urea nitrogen resulting from impaired renal function, but also to an increased catabolism of liver proteins. This explanation seems plausible since the urea nitrogen concentration of the liver was increased to a greater extent than that of the blood or brain, and since hepatic lesions have been observed in mice following a single injection of endotoxin (16, 17).

Decreased output of urinary urea nitrogen and decreased excretion of urine were observed in mice treated with endotoxin doses (less than 5 mg/kg) that were ineffective in elevating the blood and tissue levels of urea nitrogen and in inhibiting the renal clearances of inulin and PAH. These effects may be ascribed to a diminished fluid intake, since mice dosed with endotoxin (1.5 mg/kg) excreted the same amount of urine and urea nitrogen as saline-injected mice when both groups were deprived of drinking water. In contrast, when drinking water was constantly available, mice treated with endotoxin (1.5 mg/kg) excreted significantly less urine and urea nitrogen than those treated with saline. It is of interest that decreased fluid intake has been found in mice treated with relatively low doses (approximately 1/1000 of the LD_{50} dose) of endotoxin (18, 19).

Renal clearances of inulin and PAH were inhibited to the same extent by the same dosages of endotoxin. Since inulin clearance is a measure of glomerular filtration, and PAH clearance a measure of tubular secretion and glomerular filtration, it may be concluded that both tubular secretion and glomerular filtration are inhibited to the same extent by endotoxin. These findings suggest that endotoxin inhibits both of these renal processes by a common mechanism, e.g., a strong and prolonged constriction of the renal vasculature, which may be brought about by the enhanced synthesis and/or the release of a substance with vasoconstrictive properties. This substance does not appear to be endotoxin per se, since it took 3–4 hr following a single intravenous injection of endotoxin (25 mg/kg) to inhibit the renal clearances of inulin and PAH. Although the nature of the substance(s) responsible for inhibiting kidney function remains to be determined, norepinephrine, epinephrine, serotonin, and histamine may not play an important part since the administration of alpha- and beta-adrenergic blocking agents (phentolamine, dibenamine, propranolol, and 3,4-dichloroisoproterenol), serotonin antagonists (cyproheptadine, methysergide), and antihistaminic drugs (phenindamine, chlorpheniramine) to endotoxin-treated mice did not reverse the inhibition of PAH and inulin clearances.

Summary. The effect of endotoxin on

mouse renal function was studied. The following dose-related changes were noted 18 hr after the intraperitoneal injection of endotoxin to mice: (i) Elevation of the blood, brain, and liver levels of urea nitrogen; (ii) decreased excretion of urinary urea nitrogen; (iii) diminished output of urine; and (iv) inhibition of the renal clearances of inulin and PAH. These findings clearly indicate that renal function is impaired in mice treated with relatively large doses of endotoxin. It is noteworthy that these doses exceed those usually required to demonstrate nonspecific resistance to experimental microbial infections in mice. Mice were rendered tolerant to the renal inhibitory effects and to the lethal effects of endotoxin by injecting them with a relatively low dose of endotoxin for 8 consecutive days prior to the administration of a high dose of endotoxin, which would ordinarily reduce renal function and produce 85% mortality in 72 hr.

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Effects of High Altitude on Lipid Components of Human Serum (33388)

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A significant decrease in body weight has been repeatedly observed in man during acute high-altitude exposure (1–4), and available evidence seems to indicate this decrease results primarily from a loss of body fat. Thus, Hannon *et al.* (5), on the basis of skinfold and body-weight measurements found an appreciable loss of subcutaneous fat in women exposed to high altitude for a period of 2.5 months. In subsequent studies, Hannon and Chinn (6) employing body-density measurements observed a similar fat loss in men exposed to 14,000 feet for 15 days. Further data on the fat reduction at high altitude are found in the report of Surks *et al.* (7) who estimated body fat content by measurements of body density, creatine excretion, and total body K^{40}. Long-term res-