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Effect of Ethionine on Blood and Depot Lipids in Experimental Nephrotic Hyperlipemia.* (22378)

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The pathogenesis of nephrotic hyperlipemia is unclarified. An experimental approach to that problem has been facilitated since it was shown(1) that the renal disease induced in rats by the injection of anti-kidney sera obtained from rabbits simulates the nephrotic syndrome as observed in infants and children. Feinberg(2) and co-workers recently have shown that the oral administration of dl-ethionine markedly reduced the concentration of fatty acids, phospholipids and cholesterol in the blood of normal dogs. It thus seemed of interest to investigate the effect of this agent on the hyperlipemia that is regularly associated with the experimentally induced nephrotic syndrome in rats.

Procedures. Methods used to induce the nephrotic syndrome in rats have been described(1). All animals were kept in single cages, were fed Friskies and had unrestricted access to water. Determination of carcass and liver fat was carried out in rats that were fasted for 16-18 hours. They were exsanguinated from the heart under ether anesthesia. The liver fat concentration was determined by the method described by Payne (3) on approximately 1 g of liver tissue. The gastrointestinal tract was freed of its content by washing with saline solution. The entire carcass was then put through a meat grinder, dried at 100°C to constant weight (4-6 days) and ground to homogeneity with mortar and pestle. Three aliquot samples weighing approximately 1.5 g were placed in screw-cap bottles, covered with 50 cc petroleum ether and shaken intermittently for 48 hours. These were filtered under vacuum in fritted glass funnels lined with filter paper. The procedure was repeated until the filtrate was colorless. If not clear, the filtrate was centrifuged before evaporation and the fat residue was determined gravimetrically. 3.5 to 7.5 mg of dl-ethionine‡ per 100 g rat was given to 28 nephrotic rats by intraperitoneal injection twice daily for 5-21 days. A 2% saline solution of ethionine in 0.9% NaCl was used. Three additional rats were treated for 5 days with 13 mg ethionine and 12 mg methionine per 100 g rat per day. These agents were given in one syringe and their concentration was approximately equimolar.

Results. The effect of ethionine on blood lipids was studied in 12 nephrotic rats. A precipitous decline of the markedly increased total lipid and cholesterol values to normal or subnormal values was noted in all animals that received 11 mg or more of ethionine per 100 g rat per day (Fig. 1). When 7-10 mg was given, this effect was less regularly observed. The decrease occurred during the first 3-5 days of ethionine administration and subsided within a few days after its administration was discontinued. This effect was noted in male as well as in female rats. No other effect on the nephrotic renal disease was noted. Proteinuria, blood pressure and further course of the disease were not affected, and the microscopic appearance of the renal lesions was not different from that in

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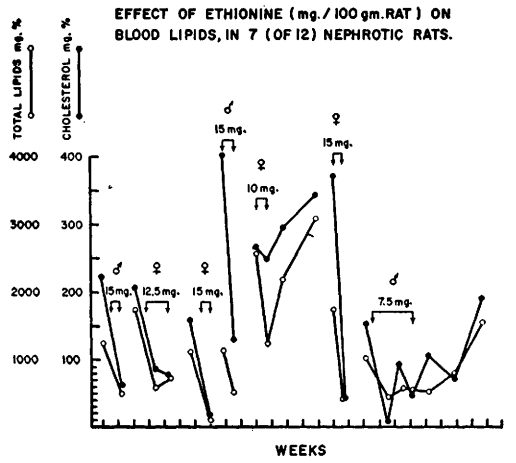

FIG. 1. Only the first 7 of 12 observations are reproduced. Connected arrows indicate duration of daily administration of ethionine.

nephrotic rats that were not given ethionine.

The amount of fat in the liver and the remaining carcass was determined in 28 untreated nephrotic rats, in 16 nephrotic rats that were treated with ethionine, and in 17 healthy controls of similar body weight.

The carcass fat values varied between 21 and 39.5 g per 100 g dry carcass. No significant difference between the 3 groups was noted.

Fig. 2 shows that when compared with values obtained in healthy controls, the liver fat is significantly decreased in nephrotic rats (4) ($p = <0.01$). In nephrotic animals treated with ethionine, this difference is no longer observed. The values for these rats are within the range obtained in control animals. In the nephrotic male rats that were treated with ethionine and dl-methionine,§ the total lipid and cholesterol concentration decreased in their plasma to subnormal values as rapidly as was observed when only ethionine was used. The liver fat values in these 3 rats were within normal range.

Discussion. It has been shown that dl-ethionine produces fatty livers in fasted female rats(5), while little or no change in the lipid content of the liver was noted in fasted male rats(6). It has also been reported that the simultaneous injection of methionine along with ethionine prevents the accumulation of lipids in the liver of the fasted female rat(6). The precipitous decline of hyperlipemic total lipid and cholesterol values in the plasma of nephrotic rats and the simultaneous increase of liver fat from subnormal to normal values in these animals, is neither sex-linked nor prevented by simultaneous injections of methionine. It thus seems probable that a different mechanism of ethionine action may be at play in the healthy, fasted female rat and in the nephrotic animal. The observation that ethionine decreases the concentration of plasma lipids in nephrotic animals to normal or even subnormal values with simultaneous and proportional increases of abnormally low liver fat values again suggests that the liver(7) plays an important role in the pathogenesis of the nephrotic hyperlipemia. This finding, and the observation

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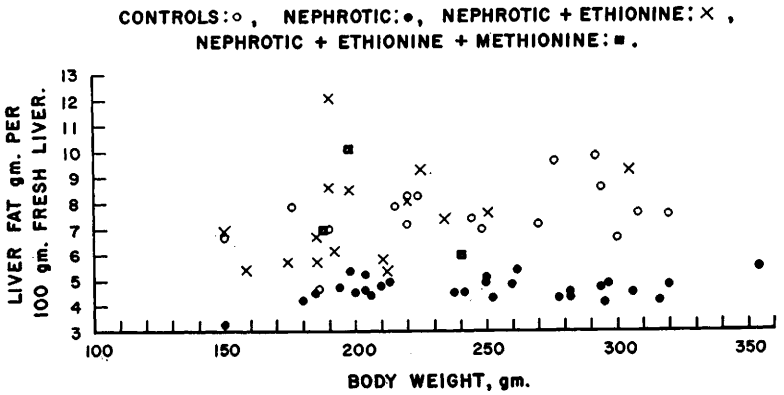

FIG. 2.

that the concentration of fats in the liver of untreated nephrotic animals is significantly diminished when plasma lipids are markedly increased, lends support to the hypothesis (8) that the nephrotic hyperlipemia may be due to an inability of the liver to take up plasma lipids. It has been proposed(9) that ethionine interferes with protein formation. Since practically all serum lipids are present as lipoproteins, it is conceivable that an effect of ethionine on lipoproteins in the liver explains both the increase of liver fat and concomitant decrease of plasma lipids in nephrotic rats.

It is of interest to note that the abolition of the nephrotic hyperlipemia remained without demonstrable effect on the course of the nephrotoxic renal disease in rats.

Summary. 1. Dl-ethionine reduces the markedly increased plasma lipid concentration of nephrotic rats to normal or subnormal values within 3-5 days. 2. The diminished liver fat values regularly noted in nephrotic rats increase to normal levels under ethionine administration while carcass fat values remain unchanged. 3. The effect of ethionine on hyperlipemia and liver fat of nephrotic rats was noted in female as well as male rats, and was not prevented by methionine. 4. These results lend support to the view that the nephrotic hyperlipemia may be due to an inability of the liver to take up plasma lipids normally.

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Effect of Pre-Treatment of $P^{32}O_4$ Solutions on Uptake and Release of P^{32} by Erythrocytes.* (22379)

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The presence of non-orthophosphate contaminants in $P^{32}O_4$ solutions has been recognized by several investigators. Causey and Harris(1), studying the uptake of $P^{32}O_4$ by skeletal muscle, attributed some of their erratic results to strongly adsorbable trace substances, and suggested that evaporation to dryness with acid reduced the interference by these compounds. Rouser and Neuman detected impurities in Oak Ridge $P^{32}O_4$ preparations, and recommended heating with nitric acid(2). Sato *et al.*(3) subsequently showed that these entities could be separated from orthophosphate by electrochromatography. In connection with studies on the release of phosphate from erythrocytes we were interested in learning whether we would have to use the rather tedious electrochromatographic separation to obtain a homogeneous preparation of orthoradiophosphate, or whether acid-evaporation would serve. A comparison of the two procedures in release studies gave results which prompted us to carry out further experiments on the uptake of phosphate by erythrocytes.

Methods. In the release studies, erythro-

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