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Sex Difference in Induction of Periportal Fatty Liver by Methionine Deficiency in the Rat.*† (24022)

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Ethionine, the ethyl analogue of methionine, was previously reported to induce periportal fatty liver within 10 to 12 hours after intraperitoneal administration to female but not male rats(1,2,3). Administration of testosterone propionate or other androgens to susceptible animals protected against this effect of ethionine(2,4). Simultaneous methionine administration, but not other amino acids, also protected the animals(1,5,6). In contrast, choline showed no protective effect (1,6). These results suggested that ethionine may be producing a fatty liver by interfering with some metabolic role of methionine. This would imply that methionine plays another role in prevention of some fatty livers, in addition to its ability to contribute methyl groups for synthesis of choline. The protective role of methionine would appear to be linked in some as yet unexplained manner with the action of androgens. However, before this suggestion could be seriously considered, it was important to observe whether animals given methionine devoid diets would develop fatty liver with lobular pattern and sex difference similar to that found with ethionine.

Induction of fatty liver by feeding rats diets devoid in methionine and adequate in choline has been observed(7) but lobular distribution of excess lipid and presence or absence of sex difference have not been reported heretofore. In the present study, liver lipid content and histologic distribution of excess lipid was determined in male and female rats, force-fed methionine devoid and complete diets. To determine specificity of changes observed with methionine deficiency, similar animals were force-fed a diet devoid in another essential amino acid, threonine. This amino acid was selected because diets devoid in threonine induced periportal fatty liver in immature rats (8).

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TABLE I. Liver Weight and Lipid in Male and Female Rats Force-fed Methionine Devoid or Complete Diets.

Diet	Sex	No. of rats	Liver wet wt, g/100 g body wt	Liver lipid g/100 g liver	Liver lipid mg/liver/100 g body wt
Complete	♂	10	4.28 .17*	6.6 .6*	275 28*
Methionine devoid	♂	9	4.23 .15†	7.1 .5†	292 14†
Complete	♀	13	4.21 .14	6.9 .7	289 26
Methionine devoid	♀	12	4.76 .20§	11.3 .9‡	587 98‡

* Mean ± stand. error of mean. † $P > .05$ (not significant). ‡ $P < .01$ (highly significant). § P between .05 and .01 (probably significant).

Materials and methods. White rats of both sexes of Wistar (Carworth Farms) and Sprague-Dawley strains, weighing 130 to 240 g, were maintained on Purina Laboratory Chow until beginning of experiments. The animals were placed in individual cages in air conditioned room and after overnight fast, were force-fed twice daily at 8:30 a.m. and 4:30 p.m. The method of Shay and Gruenstein(9) for tube feeding was used. The basal diet was similar to that of Forbes and Vaughn(7). It was composed of: essential amino acids 9.23%, non-essential amino acids 9.69%, salt mixture 4%, vitamin sucrose mixture 5%, corn oil 5%, cod liver oil 1.5%, and sucrose 65.58%. The essential amino acids consisted of: L-lysine HCl 1.24%, L-arginine HCl 0.75%, DL-tryptophan 0.20%, DL-phenylalanine 0.90%, DL-leucine 1.60%, DL-isoleucine 1.0%, DL-valine 1.4%, L-histidine HCl 0.54%, DL-methionine 0.67% and DL-threonine 1%. The non-essential amino acids consisted of: L-glutamic acid 2.0%, DL-serine 0.50%, glycine 0.7%, DL-tyrosine 2.8%, L-cystine 0.2%, L-proline 0.9%, L-asparagine monohydrate 1.39%, and DL-alanine 1.2%. The vitamin mixture contributed the following amounts in mg/100 g of diet: Thiamine HCl 0.25, riboflavin 0.5, pyridoxine HCl 0.25, calcium pantothenate 2, nicotinamide 1, choline chloride 100, biotin 0.01, folic acid 0.1, inositol 10, 2-methyl, 1, 4-naphthoquinone 0.1 and Vit. B_{12} 0.01. The mineral mixture was that of Hegsted *et al.*(10). The devoid diets contained added sucrose in place of the missing amino acid, methionine or threonine. The diet mix was blended with water so that 1 ml of diet mixture contained 1 g of diet. One group of animals was tube fed the complete ration while other groups were given the devoid diets. In some experiments, rats received from ½ to 2 days of force-feeding of complete diet before beginning experimental diet while in others, the experimental diet was fed after initial overnight fast. No differences were observed between the 2 groups. Each rat received a total daily intake of from 0.7 to 0.9 g of diet/10 g of initial body weight. Animals were allowed free access to water. In the methionine devoid experiments, rats were sacrificed at 3, 4, or 6 days after beginning the experiment. Since the results in each group were the same at different time intervals, the data were combined. In experiments with threonine devoid diet, the rats were killed after being on diets for 3 days. Animals were anesthetized with ether and sacrificed by exsanguination 18 hours after last feeding. For total liver lipid analysis, aliquots of liver were dried by grinding with anhydrous sodium sulfate and extracted with chloroform for 24 hours. The residue was extracted with petroleum ether and lipid was determined gravimetrically after evaporation of solvent. Pieces of liver were fixed in Bouin's solution and in 10% formalin. Paraffin sections were stained with hematoxylin-eosin and frozen sections with Sudan IV.

Results. Force-feeding of methionine devoid diet to adult rats rapidly induced fatty liver in females but not in males (Table I). In females on methionine devoid diet, livers showed average of 13% increase in total liver weight, a 64% increase in % lipid and 103% increase in total lipid/100 g body weight. These differences are statistically significant at high level of probability. In contrast, in the male, livers showed 1% decrease in liver weight, only 8% increase in % lipid and 6% increase in total lipid. These changes are not

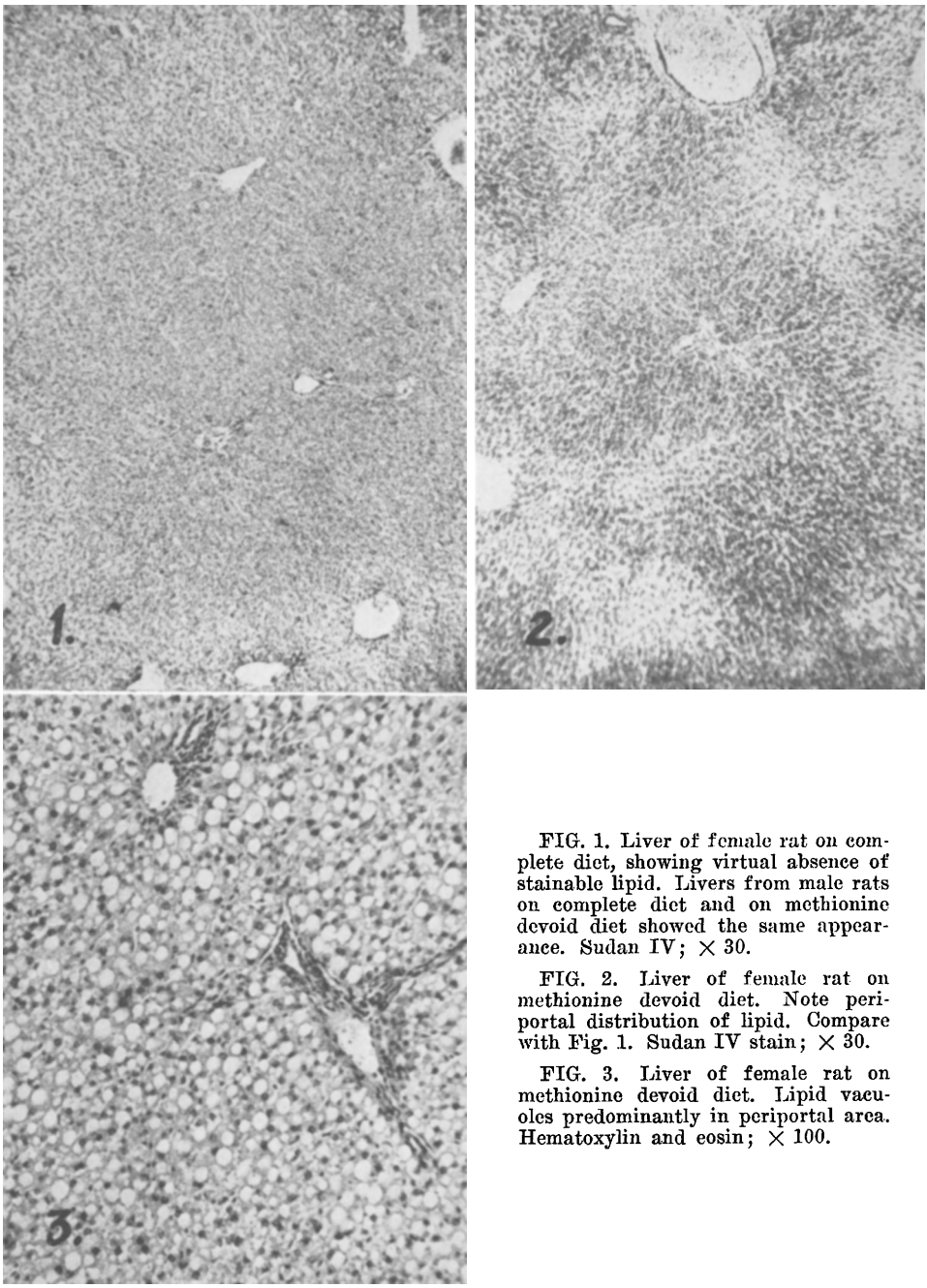

FIG. 1. Liver of female rat on complete diet, showing virtual absence of stainable lipid. Livers from male rats on complete diet and on methionine devoid diet showed the same appearance. Sudan IV; × 30.

FIG. 2. Liver of female rat on methionine devoid diet. Note periportal distribution of lipid. Compare with Fig. 1. Sudan IV stain; × 30.

FIG. 3. Liver of female rat on methionine devoid diet. Lipid vacuoles predominantly in periportal area. Hematoxylin and eosin; × 100.

significant. The average daily changes in body weight during experiments were as follows: Male controls, +3.1 g : male methionine devoid, −0.7 g : female controls, +1.7 g : and female methionine devoid, +0.7 g. These changes in body weight were small in comparison to total weights of animals and therefore do not influence to any significant degree the liver lipid values when expressed on the basis of liver weight/100 g body weight.

The sex difference found by chemical determination of liver lipid was confirmed by study of histologic sections (Fig. 1 to 3). When

TABLE II. Liver Weight and Lipid in Adult Rats Force-fed Threonine Devoid or Complete Diets for 3 Days.

Diet	Sex	Liver wet wt, g/100 g body wt			Liver lipid, mg/100 g body wt		
Control	♀	(4)*	4.03	.28†	(3)*	248	39†
Threonine devoid	♀	(3)	4.69	.18‡	(2)	440	21§
Control	♂	(6)	3.77	.13	(6)	256	13
Threonine devoid	♂	(6)	5.44	.38‖	(6)	413	25‖

* No. of animals in parentheses.
† Mean ± stand. error of mean.
‡ $P > .05$ (not significant).
§ P between .05 and .01 (probably significant).
‖ $P < .01$ (highly significant).

liver lipid was increased, its distribution was consistently periportal.

In contrast to results with methionine devoid diet, the livers of both males and females fed threonine devoid diet showed increase in liver lipid (Table II). Although the rise in liver lipid was statistically significant in both sexes, the increase in the male was significant at a higher level of probability.

Discussion. Our results clearly show that female rats, but not males, develop fatty liver when force-fed choline containing diets devoid of methionine. Excess lipid is periportal in distribution, in contrast to centro-lobular localization of fat in animals fed choline deficient low-methionine diets(11). Animals force-fed threonine devoid diets also develop a periportal fatty liver but without a sex difference.

Using a few adult rats, Spector and Adamstone(12) reported no sex difference in periportal fatty liver induced by force-feeding tryptophan deficient diets. Using force-fed tryptophan, isoleucine or phenylalanine devoid diets, Samuels and coworkers(13,14) also reported induction of periportal fatty liver in young adult male rats. Thus, although periportal fat deposits have been produced by a variety of single amino acid deficiencies, a sex difference has not been reported to occur with any amino acid except methionine.

That both methionine devoid diets and ethionine administration produce fatty livers in female but not in male rats lends further support to the thesis that ethionine acts by producing a functional methionine deficiency. Since fatty livers induced by ethionine or by methionine devoid diets are periportal rather than centro-lobular and are not prevented by choline, methionine must have some additional role in prevention of some forms of fatty liver, aside from its ability to contribute methyl groups for synthesis of choline. Since it has been fairly well established that the sex difference in production of fatty liver by ethionine is due, at least in part, to the presence of androgens in the male(2,4), additional metabolic property of methionine in fatty liver appears to be related in some as yet unknown manner with the action of androgens. Further support for this relationship is found in the common "protein sparing" action of both methionine(15) and androgens(16) and the sex difference, probably also androgen mediated, in the inhibition by ethionine of protein synthesis in the liver *in vivo* and *in vitro* (17, and unpublished). The observed correlation between inhibition of hepatic protein synthesis and subsequent appearance of fatty liver in rats given ethionine(17) suggests that the effects of methionine and androgens in preventing ethionine-induced fatty liver may be concerned with some common or interrelated effect of each on protein metabolism.

Summary. 1. Force-feeding of purified choline-containing diets devoid of methionine for 3 to 6 days induces fatty liver in adult female rats, but not in adult male rats. Excess lipid accumulates in the periportal region of liver lobule. 2. Force-feeding of threonine-devoid diet for 3 days to adult rats also induces periportal fatty liver but without a sex difference. 3. Occurrence of the same sex difference and the same lobular distribution of fat in the liver by force-feeding methionine devoid diets and by injection of ethionine lends further support to the thesis that ethionine exerts some of its effects through interfering with metabolic role of methionine. 4. Since choline does not protect liver against the effects of methionine deficiency, it appears that methionine must play some role in regulation of lipid content of liver aside from its contribution of methyl groups for synthe-

sis of choline. It is suggested that this role of methionine is related in some as yet unknown manner with the action of androgens.

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Cholinesterase Activity and Potassium Permeability in Human Erythrocytes. (24023)

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Since Dale's(1) suggestion that an enzyme was present in blood which brought about destruction of acetyl choline, and subsequent work which led to clarification of the nature of this enzyme as a specific acetyl cholinesterase(2-5), its exact role in metabolism of the erythrocyte has remained obscure. Lindvig, Greig, and Peterson(6) found that acetyl choline decreased the net exchange of sodium and potassium of human erythrocytes suspended in bicarbonate buffer, and also delayed hemolysis which was apparently due to increased intracellular cation and water. These processes were reversed by physostigmine. Production by normal erythrocytes of a substrate for cholinesterase, demonstrated by Holland and Greig(7), could necessitate a means for its removal upon completion of its function. Thiele and Pennell(8) confirmed the production of cholinesterase substrate in erythrocytes, which, at –1.5 - 0°C, greatly exceeded its hydrolysis. They postulated a cholinesterase-substrate-dextrose relationship important in synthetic and hydrolytic functions, and suggested also a contractile function of acetyl choline, alternately contracting and relaxing the fibrous structural proteins of the cell as a means of regulating membrane permeability. Taylor, Weller and Hastings(9), using radioactive potassium and calculating its concentration as the resultant of 2 exchange rates, showed a loss of the ion from human red cells when exposed to cholinesterase inhibitors in concentrations approximately 100 times that necessary to inactivate completely the enzyme. The relationship between enzyme inhibition and potassium loss was not readily apparent. Goodman, Marrone and Squire(10) found that the *in vivo* potassium gradient was unrelated to erythrocyte cholinesterase activity.

The following report describes our attempts to demonstrate altered permeability to potassium in human erythrocytes in the presence of acetyl choline, and cholinesterase inhibitors diisopropylfluorophosphate and physostigmine

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