dent upon this particular ovarian hormone. Furthermore, the fact that lesioned animals whose vaginae opened after considerable delay exhibited a constant anestrous state, suggests that the hypophysis was incapable of elaborating or secreting those hormones responsible for initiation and maintenance of sexual cycling.

On the other hand, rats with anterior hypothalamic lesions have exhibited precocious puberty(1,2) and persistent estrus(5,6,7). Correlation of all observations suggests the possible existence of a dual "gonado-hypophytropin" controlling system: 1) an inhibitory mechanism located in the anterior hypothalamus, and 2) a stimulatory one located in the posterior hypothalamus. Activity of these 2 hypothetical "sex centers" should determine net gonadotropic activity of the hypophysis, and influence time of puberal initiation. The effects of circulating gonadotropic and ovarian hormones upon hypothalamic function must also be considered, although further discussion of such feedback mechanisms is beyond the scope of this study. In view of previous evidence, the hypophysis is, theoretically prepared to produce its gonadotropins at a prepuberal age. The reason for puberty not normally occurring earlier may be due to a differential immaturity of the hypothalamus itself.

Physiological evidence for ventromedial nuclei destruction was indicated by the obese patterns in these animals, confirming reports of Brobeck(8). It is interesting to note the similarity between delayed puberty, sexual infantilism and obesity resulting from posterior hypothalamic destruction and the characteristic signs of Frohlich's syndrome. In effect, Frohlich's syndrome has been experimentally produced in these animals.

Summary. Eleven 21-day old female albino rats (Holtzman) were subjected to bilateral lesions of the area from the mammillary body to the ventromedial nucleus. These animals showed definite signs of delayed puberty, reproductive immaturity, viciousness and imminent obesity. Five had vaginae that never opened. It appears evident that in the prepuberal rat, factors (neurosecretory substances), which prod the pituitary into a functional secretory state, and which permit the mammal to attain reproducive maturity, are not present, or, are in a state of preparation. The results indicate the importance of hypothalamic function to puberal onset, and to the structural and physiological success of the reproductive-sex complex.

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Effect of Methionine upon Ethionine Intoxication of the Rat.* (25462)

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Ethionine is an analogue and metabolic antagonist to methionine(1,2,3), and ample evidence has been presented that the features of ethionine intoxication including cancer are prevented by administration of methionine(4). Nevertheless, the possibility of a toxic effect

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of ethionine is not excluded. To supplement available information, the effects of various ratios of ethionine and methionine in the diet upon the subacute hepatic changes in the rat liver were studied. Particularly the effects of such ratios upon liver cell injury and ductular cell reaction were investigated, as they are the 2 most distinctive features of sub-acute ethionine intoxication(5).

Methods. Female Sprague-Dawley rats, weighing approximately 175 g, were placed in individual cages and kept on synthetic diet containing vitamin free casein, 160 g; corn oil, 50 g; sucrose, 750 g; salt mixture U.S.P., for depletion diet, 40 g/kg; and adequate vitamin supplements, except for riboflavin, 4 mg; and choline hydrochloride, 30 mg. The rats were divided into 16 groups each containing 10 animals. Five of these groups received each supplements of 0.5%, 0.75%, and 1% ethionine, respectively, whereas the last group served as overall control. In each of the 3 experimental groups, one subgroup received no supplement of methionine, whereas the other 4 in each group received supplements varying from 0.4 to 1.4% methionine (Table I). After 35 days all rats were sacrificed, and liver tissue was fixed in buffered 10% formalin and some in Carnoy's fixative. Paraffin sections were stained with hematoxylin-eosin, Gomori's silver impregnation for reticulum and subjected to Schiff's periodic acid reaction after removal of glycogen by diastase. Degrees of hepatic cell degeneration, of ductular cell proliferation, and reticulum increase were arbitrarily graded by 2 independent observers from 0 to 4 plus, without knowledge of the experimental history according to criteria previously used(6).

Results. Rats on ethionine alone exhibited severe damage to parenchymal cells of the liver associated with necrosis and regeneration of single cells (Fig. 1). Liver cells were often arranged in small groups as a result of formation of several cells thick plates. Between liver cell plates ductular cells accumulated frequenty without apparent arrangement into tubules. They were surrounded by mononuclear cells and segmented leucocytes, the combined lesion of ductular cells and inflammatory cells being designated interstitial cell reaction. The argentophil reticulum fibers were conspicuously increased, many of them arranged around ductules (Fig. 1). The described changes were increasingly severe as concentration of ethionine in the diet rose. In rats receiving methionine supplements slightly below ethionine levels, the hepatocellular changes were decreased in intensity. This became more pronounced as methionine supplements were raised to one-and-a-half or twice those of ethionine without, however, being completely normal. In contrast, ductular cell proliferation and the less regular accumulations of inflammatory cells were abolished by the smallest amount of methionine used. This also held true for the increase in reticulum fibers.

TABLE I. Arbitrary Grades (Average of 10 Rats per Group) of Histologic Features in Liver of Rats on Synthetic Diet for 35 Days with Varying Supplements of Ethionine and Methionine.

g/100 g of synthetic diet				
Ethionine	Methionine	Parenchymal degeneration	Interstitial cell reaction	Reticulum
0	0	±	0	0
.5	0	3-4+	2+	2+
"	.4	3+	0	0
"	.6	2+	0	0
"	.8	1+	0	0
"	1.0	"	0	0
.75	0	4+	3+	3+
"	.6	2+	0	0
"	.8	1-2+	0	0
"	1.0	"	0	0
"	1.2	±	0	0
1.0	0	4+	4+	4+
"	.8	1-2+	0	0
"	1.0	"	0	0
"	1.2	1+	0	0
"	1.4	±	0	0

Discussion. Intensity of histologic features of the sub-acute ethionine intoxication depends upon amount given and similarly, in confirmation of multiple observations, methionine suppresses the effects of intoxication almost completely. Thus, even high levels of ethionine failed to produce conspicuous changes if covered by increasing amount of methionine. The basal synthetic diet contains approximately 0.4 g methionine/100 g which has to be taken into consideration in evalu-

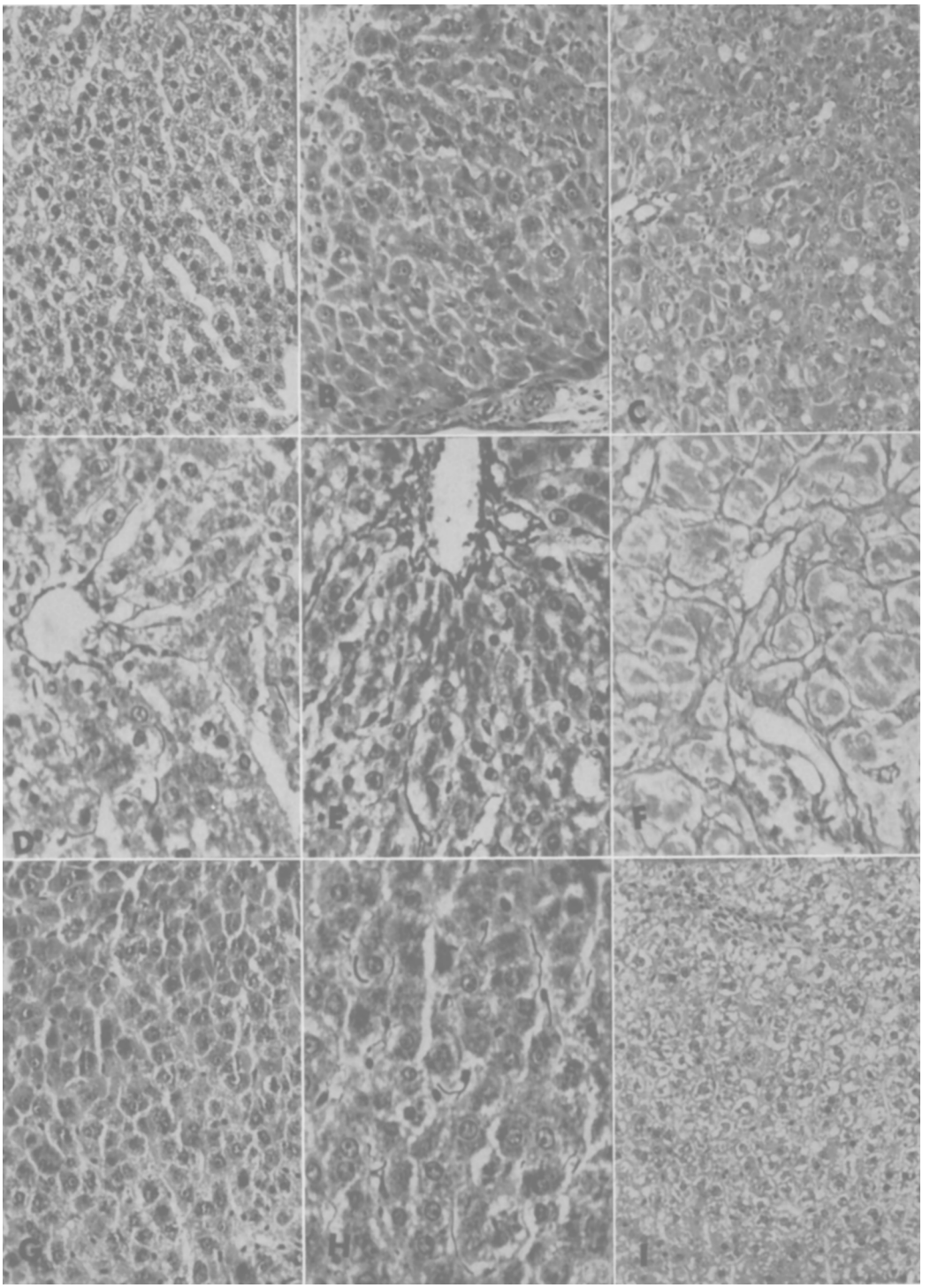

FIG. 1. Photomicrographs of livers of rats 35 days on various synthetic diets. A. Basal diet. Almost normal appearance of liver cells. HE 120×. B. 0.5% ethionine. Damage of liver cells and moderate interstitial reaction. HE 120×. C. 1% ethionine. Severe damage of liver cells and extensive interstitial reaction, composed of ductular and mesenchymal cells. HE 120×.

D. Basal diet. Small amount of reticulum. Gomori's silver impregnation 240×. E. 0.5% ethionine. Moderate increase of reticulum. Gomori's silver impregnation 240×. F. 1% ethionine. Conspicuous increase of reticulum, mainly arranged around ductules. Gomori's silver impregnation 240×. G. 0.5% ethionine and 0.4% methionine. Damage of liver cells without interstitial reaction. Compare with B. HE 120×. H. 0.5% ethionine and 0.4% methionine. Almost normal amount of reticulum. Compare with E. Gomori's silver impregnation 240×. I. 1% ethionine and 1.4% methionine. Slight irregularity of cytoplasmic staining of liver cells. No interstitial cell reaction. HE 120×.

ating methionine supplements. However, in view of the established differences in metabolic utilization between ethionine and methionine, it is difficult to calculate the effective ratio in the body. With this reservation it appears that higher absolute values of methionine are more effective in protection, in spite of the presence of larger quantities of ethionine. The results support the assumption of metabolic competition rather than toxicity for the ethionine effect.

Whereas on the same level of ethionine, the degree of liver cell damage reflects the amount of methionine supplemented, the interstitial cell reaction is prevented by all levels of methionine administered. Thus, in subacute ethionine intoxication the ductular cell proliferation and liver cell damage are dissociated in animals receiving methionine, similar to what may be observed when cortisone is administered to ethionine intoxicated rats(7). The discrepancy is particularly apparent in animals receiving relatively small methionine supplements. In animals receiving large supplements, routine histologic examination fails to show significant differences from control animals.

The parallelism in degree of interstitial cell reaction or ductular cell proliferation and increase of reticulum fibers is a further strong support for the concept that ductular cell proliferation is the most important stimulus for formation of fibers in ethionine intoxication in the rat(7,8).

Summary. Liver cell injury of subacute ethionine intoxication of the rat is accentuated by rising ethionine levels in the diet. Methionine supplements have an increasingly inhibitory effect depending on concentration. That effects of even high levels of ethionine may be almost completely eliminated by high methionine levels speaks for the role of ethionine as a metabolic competitor of methionine rather than as a primary toxic agent. Methionine inhibits ductular cell proliferation much more effectively than the injury to hepatic cells, thus dissociating both lesions. Ductular cell proliferation and fiber formation run parallel.

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