Production of Acute Pancreatitis with Ethionine and its Prevention by Methionine.*† (18062)

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It has been repeatedly shown(1-4) that a low protein high fat diet produces, in experimental animals, morphological changes in the pancreas, in addition to fatty infiltration of the liver. In one study, methionine reversed these changes(4). In addition, malnutrition in young children in tropical zones results in a fatty liver and fibrotic changes in the pancreas, as observed in South Africa(5), Central Africa (Kwasiorkor)(6,7), and the Dutch West Indies(8). Recently, it was reported from Hungary(4) that infants on deficient diets revealed a decreased pancreatic secretion, relieved by the administration of milk. The relationship of a deficiency of animal protein to those syndromes in children has been discussed(9).

In previous studies(10,11), it was observed that ethionine, (α-amino-γ-ethylthiolbutyric acid), a presumed antagonist of methionine, inhibits the incorporation of radioactive methionine and glycine into the proteins of some tissues *in vivo* (protein synthesis) and induces a rapid fatty infiltration of the liver, without concomitant necrosis. The fatty liver occurs in adult female but not in adult male rats(12). A similar sex difference was also found in the inhibition of protein synthesis in the liver(13). All these effects of ethionine were prevented by the administration of methionine. During the course of these studies, pancreatic lesions were observed.

Experiments and results. Rats, previously fasted for a period of 12 to 16 hours, were injected intraperitoneally with ethionine, (1 mg per g body weight) in 3 divided doses. Within 24 hours the pancreas became enlarged, prominent and yellowish white. Histologically, the basal and perinuclear basophilia had almost completely disappeared; the eosinophilic cytoplasm was swollen and crowded with fine zymogen granules. The normally seen pyroninophilic material of the acinar cells was also strikingly diminished. At the base of a few cells, vacuoles were seen which failed to stain with fat stains (Fig. 1B). This lesion was entirely prevented by the simultaneous administration of methionine, but not by glucose (which also inhibits the fatty liver) nor by cysteine. It appears in equal severity in males and females, despite the absence of the fatty liver in the former (Table I). Forty-eight hours after the administration of ethionine, the acinar structure was obscured by spotty coagulation necrosis of the cytoplasm of some of the acinar cells and marked vacuolization of others. Only a few of the vacuoles gave a

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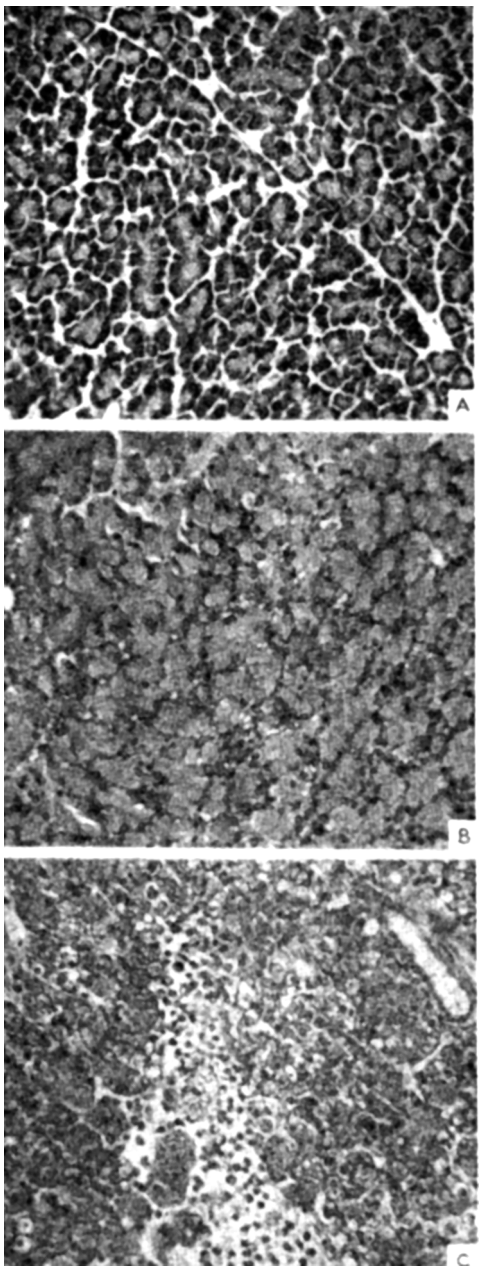

FIG. 1.

Photomicrographs of rat pancreas (×200).

A. Control (after 24 hours fasting). B. 24 hours after ethionine administration. Loss of basal basophilia of acinar cells, which are swollen and contain many zymogen granules. C. 48 hours after ethionine administration. Necrosis of acinar cells, loss of acinar structure and interstitial inflammatory infiltration.

staining reaction for fat. The severe cytoplasmic alterations were not accompanied by conspicuous nuclear changes except for occasional chromatolysis. They were associated with focal infiltration of the edematous interstitial tissue with histiocytic cells intermixed with a few neutrophilic segmented leucocytes (Fig. 1C). These lesions were more marked and frequently associated with fat necrosis in rats sacrificed after longer intervals. The islets and ductal cells revealed no characteristic alterations.

Discussion. It is safe to assume that the acute pancreatitis produced by ethionine is due to some interference with the normal utilization of methionine. Analogy with the other organs(10), invites the suggestion that inhibition of protein synthesis (due to methionine deficiency) causes the lesion in an organ which is known to be very active in protein synthesis. The observed loss of basophilia of the acinar cells, early in the process, is of interest, in view of the recently emphasized relation between cytoplasmatic basophilia (associated with ribonucleic acids) and protein metabolism(14). This aspect deserves further investigation. The rapidly developing pancreatitis, as a result of interference with methionine (and possibly protein) metabolism tends to confirm the opinion that the pancreatic lesion due to malnutrition in the tropics is caused by protein deficiency. The presented findings may also suggest that episodes of disturbed protein metabolism cause the initial stage of the commonly encountered chronic relapsing and of the acute pancreatitis in the temperate zone. It should be stressed that the pancreatic lesion, though often occurring simultaneously with the fatty liver, can develop independently of it and need not respond to the same treatment as the liver does.

Summary. The administration of ethionine produces in rats loss of cytoplasmic basophilia of the pancreatic acinar cells, followed by diffuse pancreatitis. The lesion is prevented by methionine but not by cysteine or glucose which latter prevents fatty liver following

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TABLE I.
Effect of Various Compounds Upon Pancreatic and Hepatic Changes Produced by Ethionine.

No. animals	Sex	Duration of exp., hr	Administered		Pancreas		Total liver lipids* % (wet wt) ± Standard dev.
			Ethionine*	Additional	Loss of basophilia	Necrosis	
3	f	24	—	—	0	0	5.1 ± 0.5
3	m	24	—	—	0	0	5.2 ± 0.7
3	f	24	+	—	+	0	14.6 ± 0.1
3	m	24	+	—	+	0	5.0 ± 1.2
3	f	24	+	Methionine‡	0	0	4.9 ± 0.1
3	f	24	+	Cysteine‡	+	0	14.4 ± 2.3
6	f	24	+	Glucose§	+	0	5.3 ± 0.3
3	f	24	+	Choline chloride‖	+	0	13.5 ± 2.5
6	f & m	48	—	—	0	0	5.2 ± 0.2
6	f	48	+	—	+	+	18.2 ± 5.8
3	f	48	+	Methionine‡	0	0	4.8 ± 0.3

* 1 mg per g body weight intraperitoneally.
† Determined as described previously (11).
‡ Administered intraperitoneally in amounts equimolar to and simultaneously with ethionine. In addition, one-half molar quantities were administered every 12 hr until time of sacrifice.
§ 2.5 g by stomach tube, in 3 divided doses, simultaneously with ethionine and 1.5 g 12 hr later.
‖ 42 mg intraperitoneally in 3 divided doses, simultaneously with ethionine and 10.8 mg 12 hr later.

ethionine administration. The lesion is interpreted as a result of interference with methionine (and possibly protein) metabolism.

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Plasminogen Purification by Acid Extraction.* (18063)

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Several methods have been reported for the partial purification of the active form of serum protease (see Tagnon *et al.*(1) and Rocha e Silva and Rimington(2) for references). Relatively few attempts have been made to isolate the naturally-occurring inactive form (plasminogen, profibrinolysin, serum tryptogen, etc.) in a state of high purity. The preparations commonly used have consisted essentially of the euglobulin fraction of serum or plasma, obtained by salting out, dialysis, or dilution and acidification (Milstone(3), Christensen(4,5), Christensen and MacLeod(6), Kaplan(7), Holmberg(8), MacFarlane and Pilling(9), Ratnoff (10).) In these preparations the enzyme

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