inhibition is, in fact, related to the diuresis. This places the locus of mercurial action on the proximal convoluted tubule—most certainly as regards its inhibition of succinic dehydrogenase activity and by implication also its diuretic action.

ADDENDUM: After manuscript was submitted a note appeared in *Science* describing a similar study by Wachstein and Meisel (*Science*, v119, 100). Their findings on localization of mercurial action are in essential agreement with those reported in the present paper.

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Relation of Dietary Protein Levels to Pancreatic Damage in the Rat. (20865)

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Extensive degenerative changes in the pancreas brought about by ethionine, a presumed methionine antagonist(1-3) have focused attention on the influence of dietary factors essential for the structural integrity of the pancreas. While the effects of obstruction of the pancreatic ducts as well as of other mechanical factors which are thought to be of importance in the etiology of human pancreatic necrosis have been extensively investigated(4), few workers have paid attention to the influence of nutrition on the pancreas. The feeding of diets low in protein and/or of poor quality and often high in fat was found to lead to pancreatic damage in the dog(5) and rat(6-9). In ducklings, a synthetic diet rich in sucrose often leads to pancreatic fibrosis(10).

It is the purpose of the present communication to describe briefly the changes that occur in the pancreas of the rat on a synthetic diet devoid of any protein as well as the influence of various protein levels and of methionine in such a synthetic diet on the microscopic structure of the pancreas.

Material and method. Young male rats of the Wistar strain weighing approximately 150 g were used. Sixty animals were given a basic protein free diet. Groups of 15 rats were fed the same diet supplemented with 2, 4, and 7.5% vitamin free test casein (Nutritional Biochemical Corp.) and 1% dl-methionine respectively. The basic diet contained 70% corn starch, 10% vegetable oil, 15% cellulose, 1% cod-liver oil and 4% salt mixture U.S.P. No. XIV. The following vitamin supplements were added per kg of food: Vitamin A concentrate 20,000 units, Viosterol 11,000 units, inositol 110 mg, alpha tocopherol 110 mg, choline chloride 1.65 g, Menadione 49 mg, para-amino benzoic acid 110 mg, niacin 100 mg, riboflavin 22 mg, pyridoxine 22 mg, thiamine 22 mg, calcium pantothenate 66 mg, biotin 0.44 mg, folic acid 2 mg, vit. B_{12} 0.03 mg. Animals on the basic protein free diet were allowed to live up to 75 days in order to study the development of the pancreatic lesions. Rats were sacrificed on the 5th, 7th,

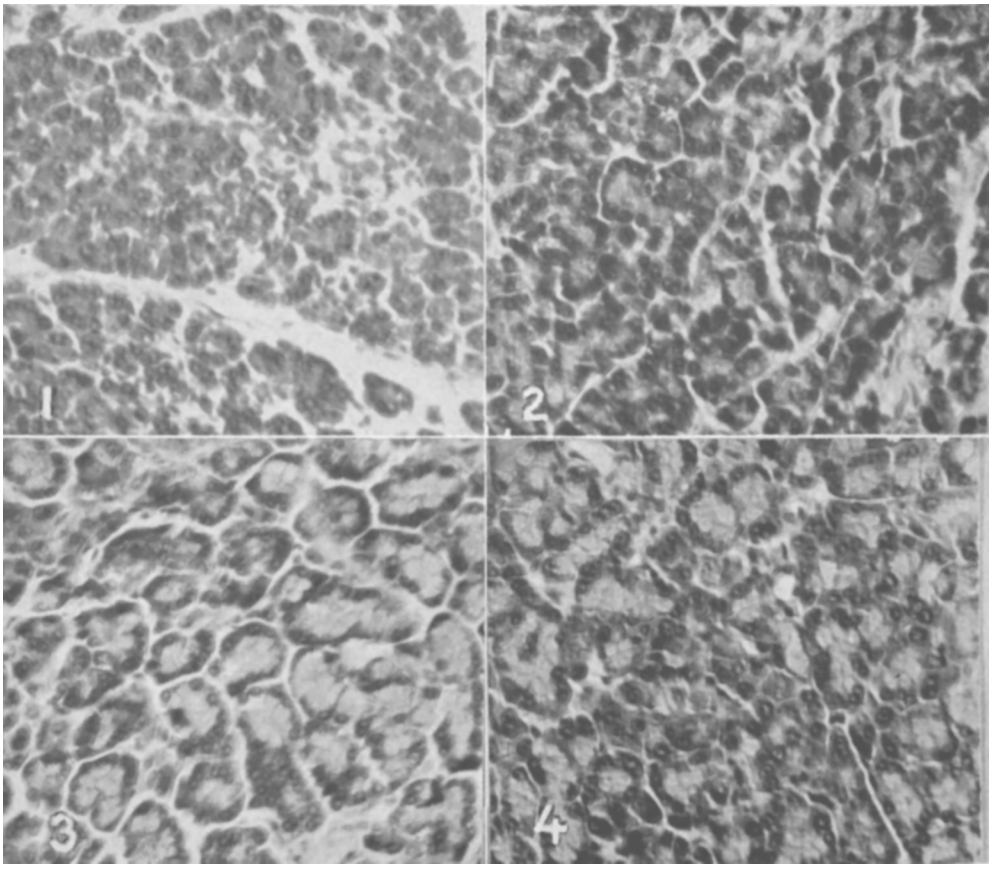

FIG. 1. Section of pancreas from a rat on the basic protein free diet for 45 days. Note marked atrophy of cells, disassociation of acini and focal areas of fibrosis. Hematoxylin-eosin × 300.

FIG. 2. Section of pancreas from a rat on the basic protein free diet supplemented with 4% casein, for 45 days. Atrophy of acinar cells and fibrosis is considerably less marked. Hematoxylin-eosin × 300.

FIG. 3. Section of pancreas from a rat on the basic protein free diet supplemented with 7.5% casein, for 45 days. The microscopic appearance of the acinar cells is almost normal. Hematoxylin-eosin × 300.

FIG. 4. Section of pancreas from a rat on the basic protein free diet supplemented with 1% dl-methionine, for 45 days. The microscopic appearance of the pancreas is similar to that depicted in Fig. 2. Hematoxylin-eosin × 300.

11th, 14th, 18th, 26th, 35th, 40th, 45th, and 75th day. Rats that died spontaneously in this or the other groups were not used. For the evaluation of the protein supplements the rats were kept for 45 days since within this time pancreatic damage in rats on the basic diet was quite marked. At the time of sacrifice pieces of liver, kidneys and pancreas were removed, fixed in Bouin's solution and stained with hematoxylin-eosin. In most cases sections were also stained with Gomori's chrome alum hematoxylin method. Other special stains were used when indicated.

Results. On the basic diet, changes developed after 10 to 12 days. There was seen gradual disappearance of zymogen granules and diminution in the size of acinar cells. Within the next weeks these changes became more pronounced. In rats sacrificed after 40 to 45 days the pancreas revealed marked atrophy of the acini (Fig. 1). Most of the zymogen granules were absent and many acinar cells consisted of nuclei with moderately prominent nucleoli which were surrounded by a markedly reduced amount of basophilic cytoplasm. Occasional cells appeared to consist of almost

naked nuclei. Due to the atrophy of the excretory cells the acini became dissociated from each other and were apparently separated by empty spaces. There was focal proliferation of fibroblastic cells which were surrounded by fine strands of connective tissue. There was only scanty infiltration by mononuclear cells. Some of the smaller ducts were dilated, but no cyst formation was noticed. In animals which were permitted to live for 75 days these changes became still more pronounced. By then almost every microscopic field showed evidence of marked atrophy of acinar cells with subsequent loss of the normal pancreatic architecture and focal connective tissue proliferation. There was, however, no change in the Langerhans islands and there was no degranulation of alpha or beta cells.

In the liver of animals which had been on the diet for 12 days the typical changes of protein depletion as described by Elman, Smith and Sachar(11) were seen. The liver cells appeared enlarged with prominent cell walls and rarified cytoplasm with only little stainable fat. In other livers, however, especially in rats which were on the diet for long periods of time, the liver cells showed considerable fatty infiltration. The average weight loss in 12 rats after 45 days was 67 g.

Basic diet plus 2% casein. The changes in the pancreas were as pronounced as on the completely protein free diet. Atrophy of single cells and dissociation of the acinar pattern was quite uniform. In many microscopic fields early fibrosis was noted. The liver cells were small and contained various amounts of stainable fat. The average weight loss of 7 rats in 45 days was 60 g.

Basic diet plus 4% casein. Pancreatic changes were considerably milder (Fig. 2). Many of the acinar cells contained zymogen granules and an almost normal amount of basophilic cytoplasm. Consequently the acinar pattern was considerably less disturbed than in the previously described two groups. Fibrosis was very scanty. The livers showed small cells with only traces of stainable fat. The average weight loss of 10 animals in 45 days was 42 g.

Basic diet plus 7.5% casein. The pancreas of these animals appeared almost normal (Fig. 3). The acini consisted of good sized cells with distinct perinuclear basophilia and well developed zymogen granules. Only occasional acini mostly in the vicinity of Langerhans islands showed a somewhat swollen cytoplasm with prominent zymogen granules and slightly decreased amounts of perinuclear basophilia. The liver cells appeared normal and contained only occasional traces of stainable fat. The average weight loss of 10 animals in 45 days was 3.5 g.

Basic diet plus 1% methionine. As in the group supplemented with 4% casein there was considerable protection of the pancreatic structure (Fig. 4). Most acinar cells showed perinuclear basophilia and a normal amount of zymogen granules. Atrophic cells and dissociated acini were only occasionally seen and there was no appreciable fibrosis. Liver cells were small without stainable fat. The average weight loss of 7 animals in 45 days was 56 g.

Comment. The basic changes seen in the pancreas on a completely protein deficient diet supplemented with the necessary vitamins consisted essentially of a slowly developing atrophy of acinar cells with loss of zymogen granules, with subsequent disassociation of acini and distortion of the normal architectural pattern. There was almost no inflammatory reaction but focal replacement fibrosis commenced after several weeks and became very marked within 75 days. Cystic degeneration as described by Kristal(8) and Véghelyi and coworkers(9) was not seen. It should, however, be noted that these investigators used diets containing various kinds of nutritionally inadequate proteins, often incomplete vitamin supplementation, as well as longer experimental periods(8).

Supplementation of the basic protein free diet with 2% casein was without any effect. Supplementation with 4% casein, however, was of considerable benefit. On a diet containing 7.5% casein the pancreas showed an almost normal microscopic pattern. It is interesting to note that at least 6% casein is necessary for the normal reproductive function of the rat. Pregnancies in rats given smaller amounts of casein terminated in reabsorption and abortion(12).

The considerable protective action of methionine described in this communication confirms and enlarges observations previously made by Véghelyi and his co-workers(9). The weight loss of animals on a methionine supplemented diet was of almost the same magnitude as that on the basic protein free diet with or without supplementation with 2% casein.

The changes on the basic protein free diet differ markedly from those induced by ethionine. The atrophic lesions induced by protein deficiency developed much slower. There is complete absence of any necrosis of acinar cells. Moreover the acinar cells do not show loss of perinuclear basophilia and de-differentiation which make them resemble fibroblasts (3). There is also a considerable difference in the effect on the liver since in protein deficiency necrosis of liver cells with subsequent diffuse fibrosis which follows the ingestion of methionine(13,14), is not observed. These considerable differences suggest that the effect of ethionine is probably not due to a conditioned methionine deficiency only, but is rather of a considerably more complex nature.

Summary. The development of typical atrophic lesions of the pancreas in the rat on a completely protein free diet is described. Supplementation of such a diet with 2% casein is without influence on the pancreas, while either 4% casein or 1% methionine have a marked beneficial effect. On a synthetic diet containing 7.5% casein the microscopic appearance of the pancreas is almost normal.

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Conversion of C^{14} Carotene to a Non-Saponifiable Substance or Substances in the Rat.* (20866)

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The conversion of carotene to vitamin A is a well established process; however, it remains to be demonstrated if this compound is a precursor for other body constituents. The object of this investigation was to determine if a part of the absorbed carotene was converted to some substance or substances in a carotene-free, non-saponifiable fraction in different tissues of the rat.

Method. A sample of uniformly labelled C^{14} carotene (90% beta)† having a specific activity of 0.4 microcurie per milligram, a total activity of 1.5 microcuries, and 4900 counts per minute was suspended in 3 ml of

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† The carotene was purchased from Nuclear Instrument and Chemical Corp., Chicago, Ill.