Pancreatic Fibrosis in Ducks on a Nutritional Basis.* (19557)

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Pathological changes occurring in the acinar tissue of the pancreas have been discussed recently by Wallace *et al.*(1), Baggenstoss (2), Davies(3), and Véghelyi *et al.*(4). In a series of 200 autopsies(1) on cases excluding diabetes mellitus, acute pancreatic necrosis and carcinoma of the pancreas, fibrosis of the pancreas was present in 42%, being more predominant in older age groups. Baggenstoss (2) reviewed 270 cases of uremia and frequently observed a "remarkable degree of dilation of the acini, flattening of the lining epithelial cells, and inspissation of secretion . . . neither age, sex nor degree or duration of azotemia appeared to play a significant role in the production of the lesion. In the control series of 200 cases in which uremia did not occur, the lesion was present in mild or moderate degree in 40 cases (20%)." In some cases (rarely) an entire acinus was destroyed and replaced by fibroblasts and reticulum fibers. Davies(3) reported the essential pathology of the nutritional disorder, Kwashiorkor, which is widespread in Africa. In the pancreas, "The cells undergo hyaline changes, the tubules are dilated; and a periacinar, intratubular, and perilobular fibrosis begins . . . The effect is that the acinar tissue is broken up by the fibrosis and lobular outlines are shown up by dense bands of fibrous tissue." Véghelyi(4) has described a progressive decrease and apparent cessation of the excretory function of the pancreas in infants nourished by a diet lacking in animal protein. Histologic observations on the pancreas of some of these infants revealed "that the acinar cells became small, atrophic and angular and separated from each other. A considerable part of the cytoplasm disappeared and only few, if any, secretory granules were visible. . . . The advanced stage of the pancreatic disorder observed in several infants who died after 8 to 12 weeks was distinct fibrosis around the lobules and invasion and intersection of the parenchyma by fine bands of connective tissue. In no instance were the islets seen to be affected." These authors(4) were able to produce similar pancreatic lesions in rats fed diets containing low quality protein. Other reports (5,6) describe pancreatic fibrosis in experimental animals similar to that observed in man.

In recent nutritional studies(7) fibrosis of the pancreas was observed in ducks fed a purified diet in which the corn starch was replaced by sucrose. It has been suggested that this lesion is the result of a nutritional deficiency(8).

Procedure and materials. White Pekin ducklings (2-3 days of age) were divided into groups of 5 having approximately the same average weight and placed in heated cages with free access to food and water. The purified diets are fully described elsewhere(8), but those to be considered here differ only in one respect, the nature of the carbohydrate. The control group received natural foods (Purina Duck Startena and Growena). Purified diet "A" contained corn starch (Argo), diet "B" contained sucrose, and diet "C" contained commercial dextrose as the only source of carbohydrate. No striking differences in food consumption and growth rate were observed among the groups. Birds fed either the control diet or diet "A" demonstrated markedly better feathering qualities. Usually the birds were sacrificed after 30-35 days on the experimental diets. However, in one experiment it was observed that the lesion described below appeared as early as the 12th to 17th day. Sections of the pancreas were removed from 74 ducks immediately following death by decapitation and put into either Bouin's solution or a 4% solution of formaldehyde. A section of the kidney was also taken for histological study from each of 35 ducks. Paraffin sections were prepared and stained routinely with

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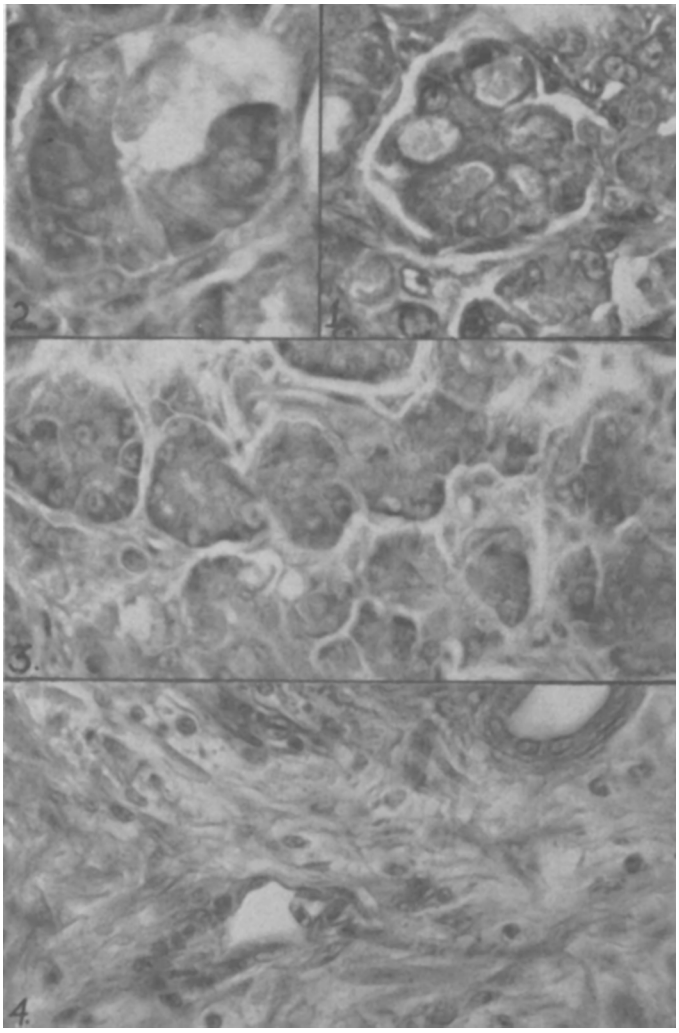

FIG. 1. Early degenerative changes in the cells of the acini, rated 1+. Hematoxylin and phloxine. × 855.
FIG. 2. More advanced changes, rated 2+. Hematoxylin and phloxine. × 855.
FIG. 3. Intralobular fibrosis, rated 3+. Hematoxylin and phloxine. × 559.
FIG. 4. Extensive pancreatic fibrosis, rated 4+. Hematoxylin and phloxine. × 559.

hematoxylin and phloxine.

Results. The pancreas from birds receiving the control diet or diet "A" appeared flesh-pink in color and was soft and pliable; it would lie over the finger like a wet string. The pancreas from birds receiving diet "B" appeared mottled-yellow to white in color; it felt hard to the touch and would hold its rigid form (as a lead pencil) when held vertically between the thumb and index finger.

The earliest microscopic changes observed in the pancreas were increased acidophilia and vacuoles within the cytoplasm of the acinar cells, pyknosis and karyorrhexis. Such lesions occurred within 12 days in ducks fed diet "B," Fig. 1. The zymogen granules coalesce to form acidophilic masses either within the degenerating cells or in the lumen of the acini, Fig. 2. These changes were usually diffuse, but occasionally focal. These degenerative changes have been rated as 1 and 2 plus in Table I.

Accompanying the degeneration of the acini there occurred a progressive increase in the

TABLE I. Relation of Diet to Incidence and Severity of Pancreatic Lesions.

Diet	Character of diet	No. of birds	Days on diet	Rating of pancreatic lesion (No. of birds)				
				Normal	1+	2+	3+	4+
Control	Natural foods	12	21	9	3			
A	Corn starch	29	30	19	8	2		
B	Sucrose	21	31	1	3	7	6	4
C	Dextrose	12	32	3	7	2		

fibrous stroma that normally separates the acini. This increase in stroma became conspicuous by the 17th day in the ducks fed diet "B." There was some variation in the quantity of stroma present in the pancreas of the different ducks, Fig. 3 and 4. The degree of fibrosis was rated 3 or 4 plus in Table I. The increased stroma apparently filled the spaces previously occupied by the acini. There was some variation in the quantity of fibrous tissue proliferation in different areas of the pancreas; a diffuse lesion was most commonly seen.

In ducks with extensive pancreatic fibrosis only a few acini could be found within the stroma. Fig. 4. The epithelial cells in these acini usually were smaller than the normal and their cytoplasm contained fewer, smaller zymogen granules. Sometimes the pancreatic capsule was markedly thickened by fibrous tissue when there was extensive fibrosis within the pancreas. It is possible that the cystic-like appearance in Fig. 4 was in reality a section through ductules or ducts. No pathologic changes were observed within the Islets of Langerhans of any duck fed the experimental rations. No significant lesions were present in the kidney of 35 ducks in which this organ was studied.

Table I is a summary of the pathologic findings in the pancreas as observed in 74 ducks. The pancreas was essentially normal in 12 ducks fed the Purina Startena and Growena control ration. Of the 29 ducks fed the corn starch diet (diet "A"), 19 had a normal pancreas; in 10 the pancreas showed early pathological changes. Of the 21 ducks fed the sucrose ration (diet "B") only one bird had a normal pancreas, while in 10 the pancreas showed extensive degeneration characterized by fibrosis. Of the 12 birds fed diet "C" 10 had essentially normal pancreases, while 2 showed a 2 plus lesion.

Discussion. The lesions in the pancreas are considered to be the result of undernutrition rather than that of any toxic effect associated with the assimilation of dietary sucrose. Some supporting evidence lies in the finding of essentially normal kidneys in all birds receiving a diet containing sucrose which demonstrates that unhydrolyzed sucrose is not entering the blood stream. This is in contrast to the observation that sucrose in the blood stream produces pathological changes in the kidney(9). Other data suggest that the severity of the pancreatic lesions was decreased when corn starch was added in increasing amounts to replace sucrose, or when certain fractions obtained during the commercial preparation of dextrose were added to diet "B." Lesions have been produced in the rat similar to those in the duck by 2 groups of workers using experimental diets(4,5) which were vastly different from each other but were similar in that the protein content of the diet was low and/or of poor quality.

It is interesting to compare pancreatic lesions observed in experimental animals to similar lesions observed in man, but one must do so with caution. It would be of considerable interest to study the nutritional history of the uremia cases reviewed by Baggenstoss (2), particularly in view of the observation that there is reasonably good correlation between vomiting and nausea, and the incidence of pancreatic degeneration which appears to accompany uremia. The pancreatic lesions described by Davies(3) and Véghelyi(4) are obviously the result of poor nutrition.

It is conceivable that the cases of pancreatic acinar tissue degeneration described by Wallace(1) and Baggenstoss(2), as well as those

described by Davies(3) and Véghelyi(4), all have a nutritional basis as a common denominator. Although it is not the purpose of this paper to discuss the similarities between this lesion and the ones found in celiac disease, fibrocystic disease of the pancreas, and sprue, it would seem worthwhile to consider a common etiology for all.

Summary. A degeneration of the acinar cells of the pancreas as the result of a nutritional deficiency has been described. The microscopic lesion was characterized by increased acidophilia and vacuoles within the cytoplasm of the acinar cells, pyknosis and karyorrhexis, which were followed by disintegration of the acinar structure and infiltration by fibroblasts. Finally, there was an increase in the perilobular and periacinar fibrous stroma and in some areas, large amounts of the parenchymal tissue were replaced by fibroblastic material. No changes were observed in the islet cells.

1. Wallace, S. A., and Ashworth, C. T., *Texas State Med. J.*, 1941-1942, v37, 584.
2. Baggenstoss, A. H., *Am. J. Path.*, 1948, v24, 1003.
3. Davies, J. N. P., *Lancet*, 1948, v1, 317.
4. Véghelyi, P. V., Kemêny, T. T., Pozsonyi, J., and Sós, J., *Am. J. Diseases Child.*, 1950, v79, 658.
5. Friedman, S. M., and Friedman, C. L., *Can. Med. Assoc. J.*, 1946, v55, 15.
6. Goldberg, R. C., and Chaikoff, I. L., *Arch. Path.*, 1951, v52, 230.
7. Miller, O. N., and Rigdon, R. H., *Fed. Proc.*, 1952, v11, 422.
8. Miller, O. N., in preparation.
9. Rigdon, R. H., and Cardwell, E. S., *Arch. Int. Med.*, 1942, v69, 670.

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Changes in Distribution of Potassium and Sodium in Rabbit Muscle Following Cold Injury.* (19558)

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Several observations may be found in the literature reporting alterations in the distribution of potassium and sodium in muscles of experimental animals following various forms of injury as prolonged ischemia, burns and trauma(1-4). Crismon and Fuhrman(5) observed, in the rabbit's muscle exposed to severe cold, a significant gain of sodium in the injured tissue ranging from 30 to 697% with an accompanying increase of extracellular water. In our experiments, the alterations in potassium and sodium distribution in relation to morphological changes in rabbit muscles subsequent to cold injury, were investigated.

Material and methods. Seven adult albino rabbits weighing 2350 to 2800 g were anesthetized with pentobarbital sodium. A standard injury was produced on one of the depilated hind legs by immersing for 4 minutes in a medium previously cooled by solid carbon dioxide to a temperature of —35°C to —37°C (6,7). The muscle biopsies for chemical analysis and cytological study were taken from anterior tibial muscle of both the exposed and unexposed leg under light ether anesthesia, using aseptic technic. The first group of animals (No. 15, 16, 17, 18) was sacrificed at 48 hours, the others (No. 1922, 2023, 2124) 24 hours after exposure to cold and portions of the same muscle were removed. The muscle tissue to be investigated was free from fat, tendons, larger blood vessels and nerves. Muscle specimens were dried to constant weight at a temperature of +105 to +110°C and digested in nitric acid. Sodium and potassium concentrations were determined by means of an internal standard flame photometer. The specimens for morphological study were preserved in Carnoy's fluid, embedded in paraffin and stained with hematoxylin and eosin and van Gieson stain.

Results in electrolyte alterations. The resulting changes are tabulated in Table I and

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