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Diet and Pancreas

SAMIR S.A. RASHWAN, Ph.D;
EDWARD I.A. SAAD, Ph.D.; HODA A. HASSAN, Ph.D.
and SHERIEF A. GALAL, Ph.D.

*The Departments of Histology and Anatomy, Faculty of Medicine,
Cairo University and the Department of Growth and
Nutrition Requirements, Nutrition Institute.*

Abstract

The effect of low protein diet on pancreatic function was investigated in two experimental groups of weanling male Sprague-Dawley rats, thirty each. They were followed for three months and were fed ad libitum diets containing 20% and 50% casein for the control and low-protein groups respectively. Ten rats from each group were sacrificed every month and pancreatic weights were measured. Histological and histochemical changes together with enzymatic pattern of pancreases were investigated. Low-protein fed group showed a reduction in absolute pancreatic weights all over the periods of the experiment. Acini of the pancreas of this group showed a diminution in size, loss of their tight packing inside lobules as well as poor staining of the nuclei and cytoplasm of acinar cells. Both acinar and islet cells of the same group showed a decrease in total protein, RNA contents and alkaline phosphatase activity, while acid phosphatase activity increased. Also degranulation of islet cells cytoplasm was detected. These changes pointed to hypofunction of exocrine and endocrine portions of pancreas in malnourished rats.

Introduction

NUTRITION is important for human being in improving and maintaining good health and as well as in improving poor health. Nutrition plays a vital role in the function of endocrine glands and most mammalian tissues and organs are dependent for their normal development, growth and function on endocrine glands [1,2]. Among the hormones which have attracted much attention in nutritional studies are thyroid hormones, adrenal steroids, anterior pituitary polypeptides and the secretion of the endocrine portion of pancreas [3].

Unbalanced diet may affect both exocrine and endocrine functions of the pancreas [4].

Eisentein et al. [5] has emphasized the importance of nutrient intake in secretion and release of several hormones. Malnutrition may alter tissue enzyme systems both quantitatively and qualitatively. Leatham [6] and Waterlow [7] reported that alteration and depletion of enzyme systems may be the cause of endocrine dysfunction and failure.

Although protein-energy malnutrition

had been investigated extensively in both biochemical and morphological fields, little has been made of the histochemical studies of these deficiency conditions, although they are valuable in localization of these metabolic disorders.

The present study was undertaken to elucidate the structural as well as the enzymatic variations of the pancreas in low protein fed rats.

Material and Methods

Three experimental periods were conducted on 60 young male growing albino rats of Sprague-Dawley strain with initial body weight (55-gms). The composition of the diet [8,9,10] is presented in Table I.

The rats received food and water ad libitum for one, two and three months. The rats were kept individually under standardized conditions. At the end of every period the animals in each group were fasted for 10 hours prior to killing. They were sacrificed by cervical decapitation to avoid the harmful effect of chloroform on the enzymatic activity of the pancreas in the histochemical studies. The pancreases were removed by dissection, dried with filter paper and weighed using the torsion balance. The obtained pancreases were freshly cut by cryostat and impregnated in paraffin in the usual manner after fixation in 85% alcohol at 4°C for 24 hours in the refrigerator or in chilled acetone for the same period. Seven microns thickness serial paraffin sections were obtained and 15 μ cryostat sections were performed.

The following techniques were used:

- 1- Hematoxylin and Eosin to detect histological changes.
- 2- Mallory's azan for islet cell differentiation [11].

- 3- Modified Gomori's technique for alkaline phosphomonoesterases using different substrates [12].
- 4- Modified Gomori's technique for acid phosphomonoesterases using different substrates [12].
- 5- PAS reaction for neutral mucopolysaccharides [13].
- 6- Brachet reaction for RNA and DNA contents [14].
- 7- Bromophenol blue reaction for the total protein content [15].

Results

I- Pancreas weight:

Table (2) shows the mean absolute weights of pancreases at the end of the different experimental periods. The mean absolute pancreatic weight of low protein fed group decreased significantly by 45%, 50% and 52% of the corresponding weights of the control group after the first, second and third months of feeding respectively.

II-Histological and histochemical changes:

Different histological and histochemical patterns were observed in the pancreases of rats fed low protein diet as compared to the control (Fig. 1). A common feature was detected in the structure of pancreases of animals fed low protein diet, it was the widening of interlobular septa with increase in fibrous tissue and the congestion of the interlobular blood vessels. Also, a diminution in the acinar sizes with loss of their normal tight packing inside the lobules were noticed (Fig. 2). The nuclei of acinar cells were poorly stained and their cytoplasm were homogeneously stained both in the basal and supranuclear regions of the cells and appeared faint red by Hematoxylin and Eosin (Fig. 3) and pinkish by Mallory azan (Fig. 4).

On the other hand, the islet cells (alpha and beta) lost their characteristic normal staining by Mallory azan, so the identification of the cell type was difficult (Fig. 4).

Mild alkaline phosphatase activity was observed in the nuclei of acinar and islet cells, while faint reaction was observed in their cytoplasm (Fig. 5). On the contrary, acid phosphatase activity is increased in both acinar and islet cells of these animals (Fig. 6).

Total protein and RNA content is decreased in acinar cells as well as in islet cells over the periods of the experiment (Fig. 7 and 8). Glycogen content of pancreatic acini is decreased (Fig. 9).

Table (1): Composition of Control and Low Protein Diet.

Ingredients	Control* (20%)	Low protein (5%)
Salt mixture**	4	4
Vitamin mixture***	1	1
Cotton seed oil	10	10
Casein	20	5
Corn starch	60	75
Cellulose	5	5

* Control diet according to Campbell [8].

** Salt mixture according to Hegsted et al. [9].

*** Vitamin mixture according to Bunce and Bloomer [10].

- 5 ml of 20% choline chloride/kgm of diet was added to the mixture including 16 drops of cod liver oil providing approximately 2000 I.V. vitamin A and 500 I.U. vitamin D₂/100 gms diet. Vitamin (E) was supplied by cotton seed oil.

Table (2): The Mean Absolute Weights of Pancreases (mgms) at the End of the Different Experimental Periods.

Duration	Pancreatic weight (mgm)	
	Control	Low protein
1 month	7.75±0.8	4.25±0.2*
2 months	8.9±1.6	4.45±1.2*
3 months	9.0±1.6	4.32±0.7*

Results are expressed in the term of Mean ±S.E.M.

* Highly significant ($p < 0.01$).

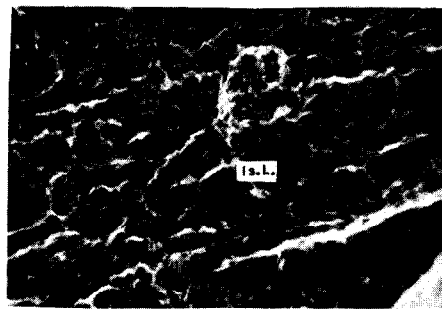


Fig. (1): Pancreas of a rat fed control diet for two months (Hx. & E. x 500).



Fig. (2): Pancreas of a rat fed low protein diet for one month showing widely separated pancreatic lobules and loss of normal tight packing (Hx. & E. x 300).

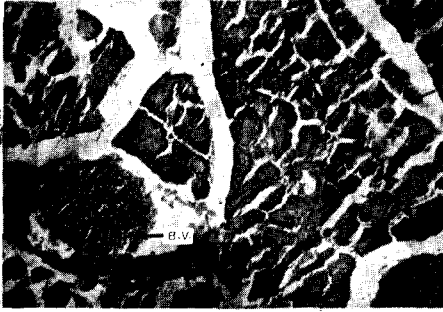


Fig. (3): Pancreas of a rat fed low protein diet for three months showing faintly stained pancreatic acinar cells (Hx. & E. x 500)

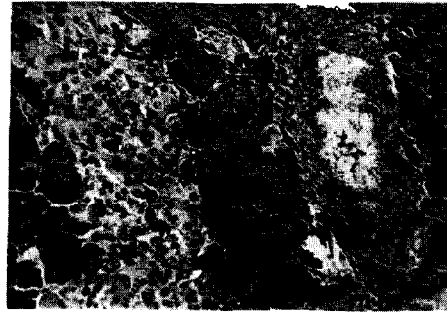


Fig. (4): Pancreas of rat fed low protein diet for three months. The normal characteristic staining of the islet cells is not evident (Mallory azan x 750).

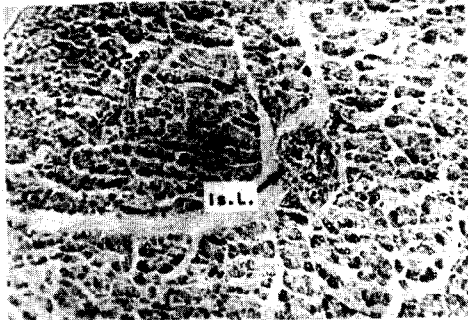


Fig. (5): Pancreas of rat fed low protein diet for three months showing mild alkaline phosphatase activity (Gomori reaction x 300).



Fig. (6): Intense acid phosphatase activity in the pancreas of rat fed a low protein diet for three months (Gomori reaction x 300).

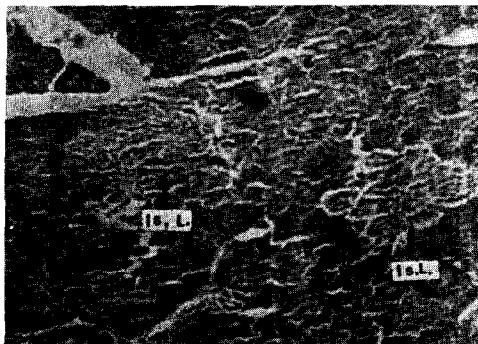


Fig. (7): Decreased total protein content of pancreas of rat fed a low protein diet for three months (Bromophenol blue x 500).

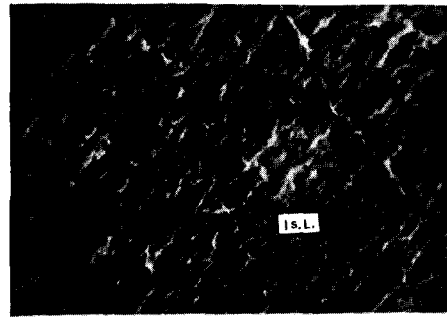


Fig. (8): Pancreas of a rat fed low protein diet for three months showing RNA content (Brachet reaction X 500).



Fig. (9): Pancreas of a rat fed low protein diet for three months showing faint PAS reaction in pancreatic acini. (PAS x 500).

Discussion

Pancreas has an important role in the digestion where the major portion of the ingested food is digested by enzymes secreted by exocrine pancreas. Also, it has a major role in the maintenance of the plasma glucose level by glucagon and insulin. On the other hand, diet affects pancreatic functions and this is evident from the results obtained in this study. After feeding low protein diet to rats, a reduction in pancreatic weights was observed in this work. It is confirmed by Gallagher [16] in rats, Platt and Stewart [17] in pigs and Deo et al. [18] in monkeys. This reduction in pancreatic weight may be due to atrophic changes observed in this study and is manifested in the form of fibrosis and diminution of acinar size. Similarly, fibrosis had been reported in human pancreas during malnutrition [19,20]. Vegheleyi et al. [21] attributed this fibrosis to chronic inflammatory process as a result of decreased globulins in the sera of rats. This extensive fibrosis may give rise to what had been called malnutrition related-independent diabetes [22]. This is very important, in view of the high prevalence of diabetes in some tropical countries.

The decrease in alkaline phosphatase activity of both acinar and islet cells detected in the study is attributed to improper synthesis of the enzymes due to lack of essential protein proenzymes [23], while the increase in acid phosphatase is attributed to the degenerative changes occurred in acinar cells and formation of autophagosomes [24].

Stewart and Heard [25] and Volk and Lazarus [26] attributed the degranulation of beta cells to the lack of essential amino acids for insulin synthesis. Cook and Hutt [27] and Milner [28] found impairment of glucose tolerance in malnourished children. Also Kabadi et al. [29] attributed alpha cells degranulation to the absence of the main stimulant for glucagon secretion (high protein in diet).

These results clarified the role of low protein diet in causing hypofunction of exocrine and endocrine portions of pancreas. So, diet must be adequate in quality and quantity to minimize the risk of diseases related to nutrition and to maintain a good state of health.

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