

Chemical and Microscopic Nature of Pancreata from Chicks Fed Unheated Soybean Meal* (32991)

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The size of an organ may be related to its function and also may change in size in response to physiological mechanisms that regulate function and thereby control growth (5). The exocrine pancreas had the ability to generate functional units with the formation of new acini under appropriate physiological stimuli. It has been reported that hypertrophy in the pancreas occurred when unheated soybean meal was fed, but mitotic divisions were seldom noted (9). These results are in contrast to other evidence which favors hyperplastic changes in the pancreas of chicks fed the unheated soybean meal (11). Recently, working with rats, Konijin and Guggenheim (7) concluded that the enlargement was due to an increase in size of cells rather than the number of cells. The objectives of this investigation were to study the levels of DNA, RNA, and protein, and to study the microscopic nature of the pancreas to determine whether the enlargement of the pancreas was due to hypertrophy and/or hyperplasia when unheated soybean meal was fed to chickens.

Experimental. White Rock \times White Mountain chicks were fed the experimental diets containing either raw or autoclaved soybean meal (heated 30 min at 15 lb steam pressure) for 3 weeks. Composition of the basal diet is essentially the same as used by Saxena *et al.* (9). After 1, 2, and 3 weeks on the diets, chicks were slaughtered and pancreata were removed for measurements and analyses. Nucleic acids and proteins were determined on wet and dry basis on pancreata from these chicks. Nucleic acid determinations were made by the method described in Armstrong and Carr (2), using diphenylamine (National Aniline Division of Allied Chemical and Dye Corporation) for DNA,

and orcinol (5-methylresorcinol) \cdot H₂O C grade (Calbiochem) for RNA estimation. A Beckman DU spectrophotometer was used to measure the absorbancy. Nitrogen determinations were by the micro-Kjeldahl method. Dry extracts of the pancreata were prepared by homogenizing the pancreas in a 1:10 ratio in 10% trichloroacetic acid (TCA), centrifuging the homogenate at 3000g in a Sorvall RC-2 centrifuge for 10 min (4°C), washing the precipitate 3 times with a 3:1 ethanol to ethyl ether; the excess ether was removed by evaporation at room temperature. The extracts were dried under high vacuum overnight.

Excised pancreata for the wet weight determination were transferred in 0.9% NaCl. Moisture content was determined by desiccation in a force-draft oven at 104°C for a period of 24 hours. Pancreatic tissues were fixed in formol, cut at 10 μ and stained with eosin-hematoxylin for microscopic examination of the acinar cells and acinar size. The Cason stain was used for zymogen density studies (4).

Results and Discussion. The moisture content in pancreata from chicks fed the unheated or autoclaved soybean meal was not different. However, a slight decrease was observed with increasing age. The determination of nucleic acids was made both on wet and dried tissue to avoid the variance resulting from moisture content differences in pancreata from individual chicks (Table I).

At 1, 2, and 3 weeks of age, pancreata from the chicks were examined for DNA, RNA, and total nitrogen per unit weight on both a fresh and dried basis. Regardless of the type of diet, whether unheated or autoclaved, which had been fed, the amount of RNA, DNA, and total nitrogen were the same, thus the levels of these components does not appear to be affected by the type of diet consumed. The ratio of RNA/DNA did not

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TABLE I. Dry Weight Determinations of Nucleic Acids and Protein Levels in Pancreata of Chicks Fed Soybean Meal for 1, 2, and 3 Weeks.

Soybean meal	Age (weeks)	Moisture (%)	DNA ($\mu\text{g}/\text{mg}$)	RNA ($\mu\text{g}/\text{mg}$)	RNA/DNA	Protein ($\mu\text{g}/\text{mg}$)
Unheated	1	78.2 ^a	3.62 \pm 0.14 ^b	14.9 \pm 0.60	4.11 \pm 0.01	195 ^c
Autoclaved	1	77.8	3.58 \pm 0.70	14.1 \pm 1.20	3.93 \pm 0.40	186
Unheated	2	76.8	2.40 \pm 0.35	13.4 \pm 0.85	5.58 \pm 0.38	209
Autoclaved	2	77.2	2.50 \pm 0.30	12.7 \pm 0.95	5.08 \pm 0.23	195
Unheated	3	76.1	2.30 \pm 0.14	15.9 \pm 1.80	6.90 \pm 0.36	190
Autoclaved	3	76.8	2.25 \pm 0.21	16.6 \pm 2.60	7.30 \pm 0.40	181

^a Mean value of 15 pancreata.

^b Average deviation from the mean of 8-10 pancreata analyzed individually.

^c Mean value of 12 pancreata.

TABLE II. Wet Weight Determinations of Nucleic Acids and Protein Levels in Pancreata of Chicks Fed Soybean Meal for 1, 2, and 3 Weeks.

Soybean meal	Age (weeks)	DNA ($\mu\text{g}/\text{mg}$)	RNA ($\mu\text{g}/\text{mg}$)	RNA/DNA	Protein ($\mu\text{g}/\text{mg}$)
Unheated	1	15.4 \pm 1.9 ^a	57.6 \pm 6.5	3.74 \pm .04	881 ^b
Autoclaved	1	16.1 \pm 2.5	54.9 \pm 8.5	3.41 \pm .01	827
Unheated	2	13.8 \pm 0.7	54.5 \pm 4.8	3.94 \pm .15	879
Autoclaved	2	14.4 \pm 0.6	53.2 \pm 2.6	3.73 \pm .03	837
Unheated	3	11.4 \pm 1.4	48.3 \pm 2.8	4.20 \pm .28	882
Autoclaved	3	12.3 \pm 1.8	47.6 \pm 4.3	3.89 \pm .16	830

^a Average deviations from the mean from 8-10 pancreata analyzed individually.

^b Mean value of 12 pancreata.

vary significantly. However, there seems to be a small decrease in the DNA, RNA, RNA/DNA, and protein values as a function of age (Tables I, II). The data in Tables I and II show consistently that pancreata from chicks fed unheated soybean meal had slightly higher protein levels.

It has been assumed that the DNA level is constant per cell for normal cells in a given species and that the DNA levels increase or decrease as a reflection of the changes in cell number. The RNA metabolism of the pancreatic acinar cells is not responsive to diet (8) and there is no parallelism between protein synthesis and RNA synthesis in the pancreas (6). If the assumption regarding DNA holds true, then the enlargement caused by unheated soybean meal is a result of increase in the number of cells per se, rather than the size of the cell. These results support the observations reported earlier for the chick (11). In the case of the rat, data on histological studies of enlarged pancreata (3) indicate

that the hypertrophy was a result of enlargement of the basilar portion of the acinar cells. Furthermore, these workers reported that hyperplastic changes occurred in the pancreata of the rats because of increased mitotic division and decreased acinar tubule size. Recently, the DNA concentration in the pancreata of rats fed unheated soybean meal was shown to be lower than for similar pancreata taken from rats fed a commercially processed soybean meal, whereas the moisture, RNA, and total lipid levels were not different (7). It is worthwhile noting that the size of the pancreas is about two times as large for chicks fed unheated soybean meal compared with those fed autoclaved soybean meal. The enlargement observed in the rat was not this great.

Results on RNA, protein, and moisture content in the chick pancreas complement those of the rat, but the DNA concentration was not altered. This is indicative of a hyperplastic change. It is possible that the pancreas

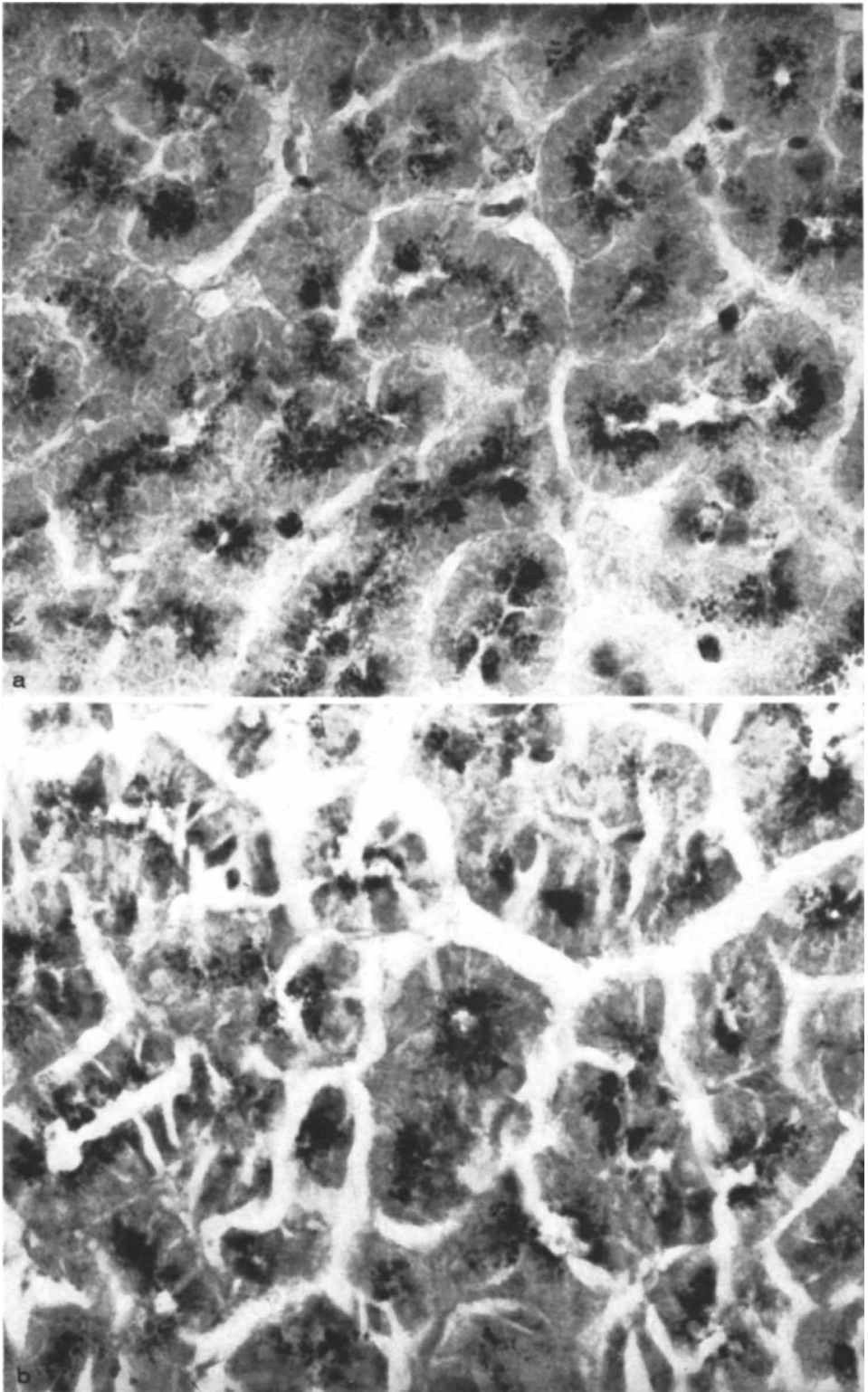


FIG. 1a and b. Photomicrographs of pancreata of 4-week-old chicks fed autoclaved and unheated soybean meal for 6 hours after 16-hours fasting period. 400 X.

hypertrophies as a result of hyperplasia at a lower level of organization, the cell, where proliferation occurs: the organ itself becomes enlarged by hypertrophy.

Histological studies of pancreata removed from chicks 6 hours postfeeding following a 16-hour fasting period were made. The diets fed to chicks during the previous 3 weeks and the 6 hours postfasting period were the same. In sections stained with eosin hematoxylin the relative size of the pancreatic acini was not different than that observed in pancreata from chicks fed either autoclaved or unheated soybean meal. Using the Cason stain, the observations revealed no differences in density of zymogen granules related to the feeding of unheated soybean or autoclaved soybean meal (Fig. 1a and b). This is in contrast with a previous observation (1) where a complete depletion of the pancreas from zymogen was reported as a result of feeding unheated soybean meal. In addition, chemical analysis of the intestinal contents from chicks fed unheated soybean meal indicated a lower level of protease activity than was found after feeding autoclaved soybean meal. Histochemical studies are of value in demonstrating the presence and site of the zymogen, but have the drawback of not giving quantitative information. From the study conducted on the zymogen granule density, it was evident that certain portions of the same section were almost completely depleted, compared with other areas that were moderately depleted. In almost all sections examined, the outer extremities of the exocrine pancreas showed areas that were totally devoid of the material which stained as zymogen. This observation is in support of Sjöstrand (19), who reported that the complete emptying of all secretory cells of the pancreas is extremely difficult to achieve, even by stimulation with both pancreozymin and secretin. He further stated that the pancreatic cells do not secrete in a synchronous way. After stimulation of the gland, some cells have emptied their zymogen granule content more or less completely, and some cells do not appear to have been affected by the stimulus at all, or have already finished a

secretory cycle. It is, therefore, impossible to conclude without further specific analysis whether there are any differences in the zymogen content of the pancreata from chicks fed unheated or heated soybean meal.

Summary. When chicks were fed diets containing 25% protein, those which had been fed unheated soybean meal developed pancreata which were much larger than pancreata from chicks which had been fed heated soybean meal. Histological examination did not indicate any variance between pancreata from either of the two treatments. The DNA, RNA, and protein levels, per unit weight, were not different whether unheated or autoclaved soybean meal was the source of protein in the diets. In addition, the moisture level in pancreata from both treatments was identical. These data indicate that the pancreas enlargement caused by feeding raw soybean meal is a result of hyperplastic rather than hypertrophic changes.

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