

Effect of Raw Soybeans on Levels of Protein and Nucleic Acids in the Rat Pancreas (34254)

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(Introduced by H. Busch)

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It has been known since 1948 that the feeding of diets, containing raw soybeans, to chicks results in pancreatic hypertrophy (1). The same effect has also been observed in rats (2, 3). The problem of whether this enlargement is due to an increase in the total number of cells or to an increase in the size of individual cells comprising the pancreas has recently been the subject of investigation. In an attempt to discover which of these two processes is operative in pancreatic hypertrophy, two groups of workers have performed essentially the same experiments based on the following reasoning. Assuming that the DNA content of pancreatic cells is the same, the ratios of the amounts of protein to DNA and of RNA to DNA in pancreases taken from animals fed heated and raw soybean diets ought to indicate whether the cells are on average of similar or dissimilar size. It was considered that closely agreeing ratios would show that enlargement had occurred by means of hyperplasia, whereas different ratios would probably reflect increases in cell size.

Konijn and Guggenheim (4), working with rats, obtained different ratios and concluded from these results that enlargement is due to an increase in cell size rather than to an increase in the number of cells. On the other hand, Salman *et al.* (5), working with chicks, obtained similar ratios and concluded that pancreas enlargement is a result of hyperplastic changes. In view of the contradictory nature of these published findings we have also investigated this problem and report below the results obtained.

Experimental Methods. Male weanling rats (30–40 g body wt) of the Wistar strain were used for all experiments. Three animals were

housed per wire-bottomed cage in a temperature-controlled room (22°) with free access to water and the diets. Two series of diets were prepared in both of which soybeans were the sole source of dietary protein. The first contained unextracted soybeans and the second hexane-extracted soybeans. Soybeans of the Geduld strain were ground to pass a 0.75-mm sieve and then solvent extraction was carried out where applicable. Heated soybeans were treated by autoclaving the ground beans at 15 psi pressure for 30 min. The unextracted soybean diets contained (g/100g): soybeans (heated or raw), 50.00; cornstarch, 38.41; salts (6), 5.00; vitamin mix (6), 0.25; cod liver oil, 1.00; choline chloride, 0.30; vitamin E, 0.04; and corn oil, 5.00. In the extracted soybean diets the amount of hexane-soluble material lost was made up by the addition of cornstarch. All diets had a protein content of 18% (N \times 6.25) as determined by the Kjeldahl procedure.

The rats were fed the diets for 21 days before they were killed, without prior starvation, by a blow on the head. The rat was placed in a weighed aluminum foil dish and the pancreas was carefully dissected out. The tissue was rinsed in ice-cold distilled water and mesentery, fat, lymph nodes, and the larger blood vessels were carefully and rapidly removed under a dissecting microscope (magnification \times 4.6). Two pancreases taken from rats fed the same diet were then pooled and homogenized at 0° in distilled water in a Douce all-glass homogenizer and made up to 25 ml. The DNA was determined on duplicate 5-ml samples of the homogenate according to the method of Burton (7) using highly polymerized calf thymus DNA (Nu-

TABLE I. Effect of Soybeans on the Dry Weights and Protein and Nucleic Acid Levels of Pancreases of Rats.

Soybeans in diet	Wt of pancreas (mg/100 g of dry body wt)	($\mu\text{g}/\text{mg}$) ^a			Protein/DNA	RNA-ribose /DNA
		Protein	DNA	RNA-ribose		
Heated, unextracted	356 \pm 16 ^b	492 \pm 7 ^b	22.2 \pm 0.4 ^b	24.5 \pm 0.4 ^b	22.2 \pm 0.5 ^c	1.11 \pm 0.03 ^c
Raw, unextracted	538 \pm 20 ^d	506 \pm 8	22.7 \pm 0.6	27.5 \pm 0.6 ^c	22.5 \pm 0.8	1.22 \pm 0.03 ^c
Heated, extracted	348 \pm 7	499 \pm 4	22.8 \pm 0.6	23.0 \pm 0.5	22.0 \pm 0.6	1.02 \pm 0.04
Raw, extracted	523 \pm 19 ^d	491 \pm 4	21.7 \pm 0.6	25.6 \pm 0.7 ^c	22.7 \pm 0.5	1.19 \pm 0.05 ^c

^a $\mu\text{g}/\text{mg}$ of dry pancreas.

^b Means of 9 pooled samples each consisting of 2 pancreases \pm SEM.

^c Means \pm SEM.

^d Significantly greater than corresponding heated soybean diet ($p < 0.001$); ^e ($p < 0.01$).

tritional Biochemicals Corp.) as standard. The DNA was determined essentially according to the method of Dische (8) on duplicate 0.3-ml samples, D-ribose (British Drug Houses Ltd.) being used as standard; and protein was determined according to the method of Lowry *et al.* (9) on duplicate 2-ml samples of a 50-fold dilution of the original homogenate. Five-times recrystallized ovalbumin (Koch-Light Laboratories) served as standard. Pancreatic dry weight determinations were achieved by drying to constant weight triplicate 3-ml samples of the homogenate in an oven at 90° for 72 hr. The rat carcass on the aluminum dish was dried under the same conditions. The dry weight of the pancreas was added to that of the rat to give the total dry body weight.

Results. The results in Table I show that the dry weight of pancreas per 100 g of dry body weight of rats fed the raw soybean diets was significantly greater ($p < 0.001$) than that of rats fed the corresponding heated soybean diets, irrespective of whether the soybeans had been extracted or not. The protein content of the pancreases taken from rats fed the different diets was essentially the same, as was the DNA concentration. The amount of RNA-ribose per unit weight of pancreas, however, was significantly greater ($p < 0.01$) in pancreases taken from rats fed the raw soybean diets than from rats fed the control heated soybean diets. Extraction of the soybeans with hexane did not alter the effect of the beans on the rats.

The ratios, protein to DNA and RNA-ribose to DNA, in pancreases taken from rats fed the different diets are also shown in Table I. No significant differences in the protein/DNA ratios were found. The RNA-ribose/DNA ratios for pancreases from rats fed raw soybeans, however, were significantly greater ($p < 0.01$) than those from the corresponding heated soybean-fed animals. This was due to the different RNA-ribose levels.

The results obtained, therefore, appear to be inconclusive. The amount of protein relative to that of DNA in the control and hypertrophied pancreases was almost the same, indicating that enlargement is probably due mainly to hyperplasia, but the RNA-ribose to DNA ratios were significantly different, thus favoring the conclusion that enlargement is due mainly to an increase in cell size.

Discussion. Although expressed differently, the measurements of the concentrations of protein and nucleic acids present in pancreases obtained by Konijn and Guggenheim (4), are of the same order as those obtained by the authors. The values reported by Salman *et al.* (5), using different assay procedures, are much lower. Konijn and Guggenheim found the level of DNA in the pancreases of rats fed raw soybean flour to be significantly lower than that in the pancreases from rats fed the control diet. This difference could not be detected in our laboratory, however, even though the experimental conditions employed were virtually identical to those used by Konijn and Guggenheim.

Salman *et al.* (5) similarly found no differences in the levels of DNA in the pancreases of chicks fed either heated or raw soybean meal for 1, 2, and 3 weeks.

Protein determinations carried out in the three different laboratories revealed that the feeding of different soybean diets to rats and chicks does not affect the total quantity of protein present per unit weight of pancreas. The result is perhaps surprising in view of the fact that raw soybean diets have been shown to cause a marked diminution in the amounts of digestive enzymes present in the pancreases of rats fed such diets for 3 weeks (10, 11).

Salman *et al.* (5) found that the levels of RNA in the pancreases of chicks fed heated or autoclaved soybean diets were essentially the same. The present study and that of Konijn and Guggenheim, however, has yielded results which show that in rats the feeding of raw soybean preparations results in a significant increase in the amount of RNA in the pancreas. A possible reason for this result follows from the observation (unpublished data) that the rate of protein synthesis in pancreatic tissue taken from rats fed a raw soybean diet for 3 weeks is greater than that of pancreatic tissue from rats fed the corresponding heated soybean diet. Hence the total amount of RNA present in the pancreatic cells of a rat fed raw soybeans is likely to be greater than that present in the pancreas of the heated soybean-fed rat, where the rate of protein synthesis is slower. While further study is needed to clarify the manner in which the pancreas of a rat fed raw soybeans hypertrophies, it is tentatively concluded that the evidence presented in this paper lends support to the view the enlargement is due mainly to hyperplasia.

Summary. The feeding of diets containing either unextracted or hexane-extracted soybeans to weanling rats for 21 days resulted in a significant increase in the weight of pancreas per 100 g of body weight when compared with rats fed the control diets contain-

ing soybean preparations previously autoclaved. All the diets contained soybeans as the sole source of dietary protein, fed at an 18% level. The protein and DNA contents per mg of dry weight of pancreases taken from rats fed the different diets were found to be the same, but the RNA-ribose content was significantly higher in those pancreases from rats fed the diets containing raw soybeans. For this reason the ratio of the amount of protein to that of DNA in pancreases from rats which had eaten the different diets was also the same. The RNA-ribose/DNA ratios, however, were significantly different and favored the raw soybean-fed rats. From these results and others it is tentatively concluded that pancreatic hypertrophy is most probably a consequence of an increase in the total number of cells rather than of an increase in the size of individual cells. Other workers have also investigated this problem and their results are discussed in relation to those reported here.

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