Effect of Raw Soybean Flour on the Composition of Rat Pancreas. (32368)

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Raw soybean flour (sbf) contains heat-labile substances which cause an enlargement of the pancreas of chicken(1-5) and rats (6-8). Histological examinations(6,9) show pancreatic hypertrophy to be associated with an accumulation of zymogen in the acinar cells. The pancreatic enlargement is accompanied by an increased secretion of pancreatic enzymes(4,8,10,11). The present study is concerned with the chemical composition of rat pancreases enlarged by feeding raw sbf.

Methods. Young male rats of a local strain derived from Wistar rats weighing 30-40 g were used throughout. The basal protein-free diet consisted of cornstarch 91, vegetable oil 5 and salt mixture (U.S.P. XIV) 4. An appropriate amount of cornstarch was replaced by sbf to give 18% dietary protein. Two kinds of sbf were used: (a) Unheated flour obtained from hexane-extracted unprocessed flour with only enough heat to remove excess solvent; (b) Commercially processed (toasted) low-fat flour. Both flours were obtained from a local manufacturer. All diets were supplemented with the following vitamins (mg/kg): Thiamine 2.0, riboflavin 3.0, pyridoxine 1.6, calcium pantothenate 12.0, nicotinic acid 30.0, and choline chloride 1000. Each rat received 100 I.U. vitamin A and 4 I.U. vitamin D twice weekly in an oily solution.

After 3 weeks on diet the animals were killed by exsanguination without having previously been fasted. The pancreases were rapidly removed, freed from extraneous tissue, weighed and examined for moisture, total lipids, protein and nucleic acids. Moisture was determined by drying to constant weight in a vacuum oven, total lipids by extraction with alcohol-ether (3:1, v/v), protein according to Lowry, Rosebrough, Farr and Randall (12) following extraction of nucleic acids as described by Schneider(13). Bovine albumin (Fraction V, obtained from Nutritional Biochemicals Corp.) served as standard. RNA was examined by the method of Drury(14), ribose (Nutritional Biochemicals Corp.) being used as standard; and DNA, according to Burton(15). Highly polymerized DNA-sodium salt from calf thymus (Type I, Sigma Chemical Co.) was used as standard. Amino acids were examined in pooled samples each consisting of 12 pancreases. The samples were digested for 24 hours in 6 N HCl and examined in a Beckman-Spinco amino acid analyzer (Model 120). Nitrogen was determined in the hydrolysates with the micro-Kjeldahl method.

Results. Figures presented in Table I refer to rats maintained for 3 weeks on experimental diets. They indicate that the animals fed raw sbf gained significantly less weight ($P<0.001$) than those fed heated sbf. Weight of pancreases per 100 g body weight was significantly higher in rats fed raw sbf ($P<0.001$). Concentration of water, total lipids and protein were similar in pancreases of both groups. DNA concentration was less in "raw" pancreases ($P<0.001$). Therefore, more protein was found per mg DNA ($P<0.001$). Pancreases of rats which had subsisted on raw sbf contained, per 100 g body weight, more RNA ($P<0.001$) than those of rats fed heated sbf but similar amounts of DNA. Therefore, their RNA/DNA ratio was higher ($P<0.001$). Although figures of Table I were obtained after a 3-week observation period, differences in the composition of the pancreases could be discerned as early as 1-2 weeks.

It appears, therefore, that the enlargement of the pancreas in rats fed raw sbf is mainly due to an increase in cell size rather than to an increase in number of cells.

Figures on amino acid composition of pancreases are presented in Table II. They are expressed as μmoles/mg nitrogen.

There were some differences in the amino acid pattern of the two samples. Hypertrophied pancreases contained only 19% as much methionine and 76% as much lysine but 128% as much threonine and 123%

TABLE I. Effect of Feeding Soybean Flour on Weight Gain and Composition of Pancreas of Young Rats.*

	Raw soybean flour	Heated soybean flour
Wt gain in 3 weeks, g	44 ± 4 (18)	96 ± 4† (18)
Wt of pancreas, mg/100 g body wt	621 ± 20 (18)	462 ± 13† (18)
Moisture, mg/100 mg pancreas	76.2‡ (9)	80.7‡ (9)
Total lipids, mg/100 mg pancreas	3.0‡ (9)	2.9‡ (9)
Protein, mg/100 mg pancreas	12.3 ± 1.7 (11)	12.5 ± 2.1 (10)
mg/mg DNA	37.0 ± 1.1 (11)	29.5 ± 1.9† (10)
DNA, mg/100 g pancreas	326 ± 11 (12)	423 ± 5 † (11)
mg/100 g body wt	2.15 ± .09 (12)	1.96 ± .07 (10)
RNA, mg/100 g pancreas	1457 ± 27 (11)	1368 ± 21 (10)
mg/100 g body wt	9.50 ± .39 (11)	6.38 ± .28† (10)
RNA/DNA	4.42 ± .15 (11)	3.24 ± .19† (10)

* Means ± S.E.M. Figures in parentheses indicate number of animals.
† $P < .001$.
‡ Mean of 3 pooled samples each consisting of 3 pancreases.

as much valine as those of rats fed heated sbf.

Discussion. The results reported above indicate that the pancreatic enlargement following prolonged feeding raw sbf is due to a hypertrophy of the gland rather than to hyperplasia. It appears, therefore, that protein synthesis has increased in such pancreatic cells. These findings are in conformity with the minor histological changes reported by previous investigators(6,9).

Although it is difficult to explain the changes occurring in the pancreas following feeding raw sbf, it is tempting to correlate the decrease of concentration of methionine and the increase of threonine and valine to findings of other authors. Feeding unheated sbf to rats was found to increase the amount of expired $C^{14}O_2$ from radioactive methionine (16). Furthermore, the pancreas is a very active organ in the biosynthesis of cystine from methionine and its incorporation into enzyme protein. This conversion was observed to be increased by administration of soybean trypsin inhibitor(17). Both these responses to soybean trypsin inhibitor would contribute to the decrease of methionine in the pancreas.

TABLE II. Amino Acid Composition of Pancreas.*

	Pancreas of rats fed	
	Raw sbf	Heated sbf
Lysine	2.82	3.71
Histidine	.86	.69
Arginine	2.22	1.92
Aspartic acid	3.33	2.74
Threonine	1.20	.94
Serine	.98	.86
Glutamic acid	4.53	4.37
Proline	1.71	1.76
Glycine	4.32	4.24
Alanine	3.50	3.76
Half cystine	Traces	Traces
Valine	3.08	2.33
Methionine	.03	.16
Isoleucine	1.82	1.43
Leucine	3.32	2.82
Tyrosine	.87	.69
Phenylalanine	1.35	1.14
Ammonia	7.99	8.78

* μmoles/mg nitrogen.

Borchers(18) reported that rats fed raw sbf show an increased requirement for threonine and valine. Subsequent studies(19) demonstrated a block in the catabolism of these amino acids when raw sbf was fed. Although this effect dissipated a few hours after feeding, it is not impossible that it reflects an accumulation of these amino acids in the pancreatic proteins of rats fed raw sbf.

Summary. Young rats were fed for 3 weeks diets containing 18% protein derived from either raw or heated sbf. Rats maintained on the raw soybean diet gained less weight and had enlarged pancreases. The hypertrophied pancreases had similar concentrations of moisture, total lipids, protein and RNA as did the pancreases of rats fed the heated soybean diet but had a lower concentration of DNA. It is concluded that the enlargement results from an increase in cell size rather than from an increase in number of cells. Hypertrophied pancreases contained less methionine and lysine and more valine

and threonine than those of rats subsisting on heated sbf.

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Irreversibility of Major Chromosome Changes in a Mycoplasma-Modified Line of FL Human Amnion Cells.* (32369)

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Previously reported characteristic chromosome changes in cells of the FL human amnion line after mycoplasma (PPLO) infection (1) could be classified as 3 types: reduction in chromosome number; increase in chromosome aberrations; and the appearance of new chromosome varieties. The major changes developed very slowly. Thus chromosome numbers, predominantly between 70 and 76 for uninfected FL cells, continued to decrease in mycoplasma-infected cultures, and this was found to be true when counts were made even as late as the 8th and 10th months of cultivation (time of last report). Most chromosome aberrations in mycoplasma-infected FL cultures represented an accentuation of aberrations observed to a minor degree in uninfected FL cells. Three new chromosome varieties were seen: A very large teleocentric chromosome, which increased in frequency with time of culture and, after 10 months, was seen in 28.5% of metaphase plates in one of the mycoplasma-infected cell lines; in addition, a large metacentric chromosome and a large subteleocentric chromosome, both of which were seen with less frequency, although the latter in some preparations occurred in 20% of metaphase plates.

Studies have been continued to answer the two questions: 1) Would the mycoplasma-infected and -modified cells change further on continued cultivation with mycoplasma present? 2) What would be the effect on the modified chromosome picture if mycoplasma were eliminated from the culture? This report concerns these questions.

Materials and methods. Cell line F 138 has previously been described(1) as a line of FL human amnion cells exposed to myco-

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