Effect of Raw Soybean Meal and Amino Acids on Pancreatic Hypertrophy in Rats. (25950)

A. N. Booth, D. J. Robbins, Wm. E. Ribelin and F. DeEds
Western Regional Research Laboratory, Albany, Calif.*

A number of physiologically active constituents have been reported in raw soybean meal, including a pancreas stimulating factor (1), trypsin inhibitor(2), goitrogenic substance(3), saponins(4), estrogenic substances (5), hemagglutinin(6), and anticlotting factor(7). However, when we fed raw soybean meal to rats as the sole source of dietary protein (14%), the only adverse effects noted were (a) poor growth, (b) reduced food efficiency and (c) pancreatic hypertrophy. Borchers(8) reported that growth inhibition in rats due to raw soybean meal can be counteracted by addition of 4 specific amino acids to the diet. In the present study enlargement of the pancreas was observed when rats were fed amino acid-supplemented raw soybean meal diet.

Methods. Weanling male albino rats from our colony were separated into 4 uniform groups of 5 according to weight. Animals were housed in individual wire-bottom cages and fed diets *ad lib.* Group 1 received control (casein) diet which contained in per cent: cerelose 50.7, cornstarch 20, crude casein 17.3 (N x 6.25 = 14.0% protein), salts (USP XIV) 4, complete commercial vitamin mix 2, and powdered cellulose 2. Group 2 received above diet with 28.4% raw soybean meal (N x 6.25 = 14.0% protein) substituted for casein (17.3%), cerelose (9.1%), and powdered cellulose (2.0%). Group 3 received the raw soybean meal diet plus 4 amino acids (0.6% L-tyrosine, 0.6% DL-methionine, 0.6% DL-threonine, and 0.2% DL-valine) substituted for equal amount of cerelose. Group 4 received 28.4% heated soybean meal (autoclaved at atmos. pressure for 30 min). Weekly records of body weights and food intakes were maintained. After 36 days rats were sacrificed and pancreatic tissue including extraneous fat was carefully excised, preserved in 10% aqueous formaldehyde for 48 hours, then carefully trimmed, blotted and weighed. Food efficiency was calculated for each rat by dividing grams gain in body weight by grams of food consumed during the 36-day period. Since all groups received the same dietary level of protein (14%), it follows that food efficiency and protein efficiency of utilization are directly related.

Results. The data in Table I show that significant growth inhibition, increased pancreatic weight and reduced food efficiency resulted when raw soybean meal was substituted for casein. When 4 amino acids were fed with raw soybean meal (Group 3), growth and food efficiency were no longer significantly different from the control group. However, hypertrophy of the pancreas was not prevented by amino acid supplementation. No adverse effects were observed in Group 4 receiving heated soybean meal.

The following tissues were examined microscopically: heart, lungs, thyroid, testes, spleen, liver, pancreas, kidney, adrenals and intestine. All organs were normal except the pancreases of animals fed raw soybean meal with or without amino acids, (Fig. 1 and 2). Pancreatic acini had lost their regular circular outlines and were jumbled and without distinct lumens. The basilar portion of the hyperplastic acinar epithelium was intensely basophilic and increased at the expense of the zymogenic portion. Ribonuclease digestion studies indicated this basophilic cytoplasm to consist predominately of ribose nucleic acid. Mitoses were occasionally encountered. Acini still spherical enough to be measured were significantly smaller ($P = <0.01$) than normal controls, apparently due to squeezing by adjacent hyperplastic cells. Nucleoli were significantly larger ($P = <0.05$); nuclei appeared larger but were not statistically so. Pancreatic islets appeared unaffected.

* A laboratory of Western Utilization Research and Development Division, Agri. Research Service, U. S. Dept. of Agriculture.

TABLE I. Effects of Raw and Heated Soybean Meal and Amino Acids on Growth, Pancreas Weights and Food Efficiency of Rats.

Dietary protein	Final body wt, g*	Pancreas wt, g/100 g body wt*	Food efficiency, wt gain/g food*
(1) Casein	147.40 ± 3.88	.56 ± .04	.31 ± .01
(2) Raw soybean meal	89.00 ± 3.54†	.85 ± .08†	.19 ± .007†
(3) *Idem* + amino acids	142.40 ± 7.11	.93 ± .06†	.28 ± .01
(4) Heated soybean meal	148.40 ± 3.27	.50 ± .02	.29 ± .006

* ± S. E. † P = <0.01.

Discussion. The original observation of pancreatic hypertrophy in chicks fed raw soybean meal(1) is now extended to include the rat. The observation of Borchers(8) that growth inhibition may be reversed by addition of tyrosine, methionine, threonine and valine to a raw soybean meal diet is confirmed. Since added amino acids did not prevent pancreatic hypertrophy, poor growth is obviously not the cause of pancreas enlargement. According to Lyman and Lepkovsky(9) and Lyman(10), excessive amounts of pancreatic enzymes are emptied into the intestine and excreted in the feces when either raw soybean meal or soybean trypsin inhibitor concentrate is fed to rats. These workers concluded that growth depression may be exerted through a loss of essential amino acids from endogenous sources rather than through depression of normal intestinal proteolysis. Support for the concept of fecal endogenous amino acid loss, rather than incomplete intestinal proteolysis, is the finding that decreased growth rate and protein utilization resulted when soybean trypsin inhibitor concentrate was fed with hydrolyzed casein to mice(11). Other workers(12,13) have reported that when 2 dietary levels of raw soybean meal are compared for inhibition of chick growth, the high level gives better growth than the low level. If it is assumed that maximal pancreatic stimulation occurs at the low level, then it is not surprising that the high level of raw soybean meal would support better growth by increasing dietary supply of amino acids to overcome endogenous loss of amino acids *via* the feces. Sources and levels of dietary nitrogen used may be the key to some of the conflicting conclusions that have been reported regarding toxicity of soybean fractions.

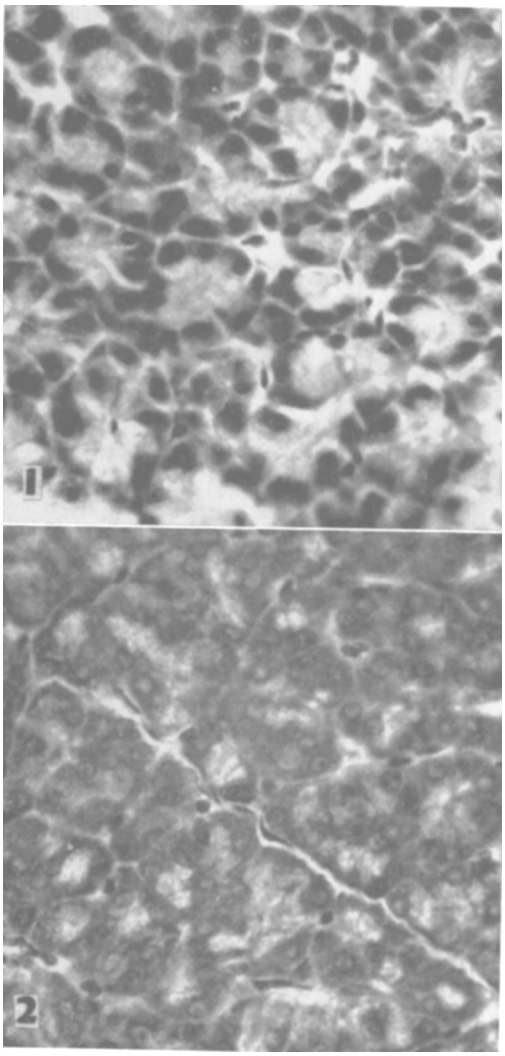

FIG. 1. Normal rat pancreas. Hematoxylin and eosin stain. ×540.

FIG. 2. Pancreas of rats fed 25% raw soybean meal for 22 days. Hematoxylin and eosin stain. ×540.

Summary. Pancreatic hypertrophy, poor growth and lowered food efficiency result from feeding raw soybean meal to rats as sole

source of dietary protein. Addition of 4 specific amino acids to raw soybean diet corrects poor growth and reduced food efficiency but does not prevent pancreatic hypertrophy. These results support the concept that decreased growth rate and protein efficiency caused by feeding raw soybean meal are due to direct stimulation of the pancreas resulting in excessive loss of critical amino acids contained in pancreatic enzymes excreted in feces.

1. Chernick, S. S., Lepkovsky, S., Chaikoff, I. L., *Am. J. Physiol.*, 1948, v155, 33.
2. Ham, W. E., Sandstedt, R. M., *J. Biol. Chem.*, 1944, v154, 505.
3. Sharpless, G. R., PROC. SOC. EXP. BIOL. AND MED., 1938, v38, 166.
4. Potter, G. C., Kummerow, F. A., *Sci.*, 1954, v120, 224.
5. Cheng, E., Story, C. D., Yoder, L., Hale, W. H., Burroughs, W., *ibid.*, 1953, v118, 164.
6. Liener, I. E., Pallansch, M. J., *J. Biol. Chem.*, 1952, v197, 29.
7. Balloun, S. L., Johnson, E. L., *Arch. Biochem. and Biophys.*, 1953, v42, 355.
8. Borchers, R., *Fed. Proc.*, 1959, v18, 517.
9. Lyman, R. L., Lepkovsky, S., *J. Nutr.*, 1957, v62, 269.
10. Lyman, R. L., *ibid.*, 1957, v62, 285.
11. Westfall, R. J., Bosshardt, D. K., Barnes, R. H., PROC. SOC. EXP. BIOL. AND MED., 1948, v68, 498.
12. Borchers, R., *J. Nutr.*, 1958, v66, 229.
13. Fisher, H., Johnson, D., Jr., *Arch. Biochem. and Biophys.*, 1958, v77, 124.

Received April 14, 1960. P.S.E.B.M., 1960, v104.

A Latex Agglutination Test for Anaerobic Diphtheroids. (25951)

ALFRED L. FLORMAN AND JEANNE L. SCOMA (Introduced by S. Stanley Schneierson)
Stanley Jay Lagin Pediatric Research Lab., North Shore Hospital, Manhasset, N. Y.

Serological observations with anaerobic diphtheroids have been limited because of the tendency of these organisms to grow in clumps and to agglutinate spontaneously. This communication is to report a latex (polystyrene) agglutination test for anaerobic diphtheroids which is reproducible, easy to read and not influenced by spontaneous bacterial agglutination.

Materials and methods. Organisms were characterized as anaerobic diphtheroids if they required an anaerobic environment for multiplication, had a pleomorphic rod-like or branching appearance, were Gram positive, produced catalase and did not ferment xylose, salicin or raffinose(1). All of our anaerobic diphtheroid strains were recovered and maintained in fluid thioglycollate medium. Most of the other bacteria mentioned in the text were obtained from the American Type Culture Collection and maintained in appropriate media. *The latex test* required an antigen-latex-buffer mixture which was prepared by mixing a suspension of washed bacteria (density to match McFarland #8 standard), 0.81 μ latex particles and glycine saline buffer (pH 8.2). (One part of the stock suspension of latex was diluted with 4 parts of distilled water to give the proper concentration of latex.) To prepare 5 ml of this mixture: 0.5 ml of bacterial suspension, 0.2 ml of diluted latex and 4.3 ml of buffer were mixed and allowed to stand at room temperature for at least 10 minutes. Sera which were to be tested were diluted 2-fold starting with a 1:10 dilution in glycine saline buffer to yield 0.5 ml in each tube. Clear test tubes measuring 10 x 75 mm were used. To each tube of diluted serum, 0.5 ml of the prepared antigen-latex-buffer mixture was added. Tubes were shaken, incubated for 1½ hours in 56°C water bath, centrifuged at 2300 rpm for 3 minutes and read. Appropriate nonspecific serum and antigen controls were included. Positive reactions were indicated by a clear supernatant, negative reactions by a turbid supernatant similar to that in the control tubes. *In bacterial agglutination test,* the same bacterial antigen was used