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Prolonged Pancreatic Hypertrophy and Reversibility in Rats Fed Raw Soybean Meal. (29453)

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It was reported previously that 3 adverse effects were observed when raw soybean meal was fed to rats for 38 days as the sole source of dietary protein(1). These effects, namely, decreased growth rate, reduced feed efficiency, and pancreatic hypertrophy, were eliminated when heated soybean meal ($\approx$ 14% protein) replaced the raw soybean meal in the diet. The earlier report also showed that supplementation of the raw soybean meal diet with valine, tyrosine, methionine and threonine corrected the poor growth and improved the feed efficiency, but did not prevent pancreatic hypertrophy.

The importance and significance of pancreatic stimulation in the raw soybean meal syndrome was confirmed recently by Muelenaere(2). His findings indicated that the difference between the nutritive value of raw and autoclaved soybeans was due to an extra demand for protein made on the animal in order to keep up with the faster epithelial cell regeneration and the sharply increased digestive enzyme secretions.

The present work was undertaken to investigate the reversibility of pancreatic hypertrophy, and to determine whether continuous pancreatic stimulation for approximately one-quarter of the rat's lifespan would result in malignancy of the pancreas.

Materials and methods. The basal diet fed to control rats had the following percentage composition: dextrose 50.8, cornstarch 20.0, crude casein 17.2 (N $\times$ 6.25 = 14% protein), salts U.S.P. XIV 4.0, complete commercial vitamin mix 2.0, soybean oil 4.0, and powdered cellulose 2.0. Composition of the hexane-extracted raw soybean meal on a moisture-free basis was nitrogen 7.93% (protein 49.6%) and oil <0.5%. In the diet fed to experimental rats for 38 and 192 days, the raw soybean meal was substituted in the basal diet at a level of 28.4%, to provide 14% protein at the expense of 17.2% crude casein, 9.2% dextrose and 2.0% cellulose.

Albino rats from our colony were divided into uniform groups of 5 per dietary treatment and caged on wood shavings with free access to food and water. Weekly records of body weights and food intake were kept. At

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TABLE I. Effect of Raw Defatted Soybean Meal on Body Weight, Feed Efficiency, and Pancreas Weights of Rats.

Diet No.	Dietary protein	Duration (days)	Age & sex	Mean body wt (g ± S.E.) Start	End	Feed efficiency (38 days)	Mean pancreas wt (g/100 g body wt ± S.E.)
1.	Crude casein	38	weanling ♀	33	129 ± 4	.31	.34 ± .04
2.	Raw meal	38	"	32	81 ± 3*	.18	.65 ± .03*
3.	Crude casein	192	"	33	210 ± 4	.30	.35 ± .04
4.	Raw meal	192	"	33	184 ± 5*	.16	.61 ± .02*
5.	Raw meal	192†	"	33	230 ± 4*‡	.22	.41 ± .05
6.	Crude casein	38	adult ♂	298	336 ±16	—	.25 ± .03
7.	Raw meal	38	"	297	297 ±11‡	—	.46 ± .04*
8.	Crude casein	192	"	298	375 ±11	—	.26 ± .04
9.	Raw meal	192	"	297	358 ±12	—	.44 ± .02*

* Significant difference from control value, P = <.01. † Switched to casein basal after 38 days.
‡ Two rats died in Group 5; one rat died in Group 7.

autopsy the pancreas of each rat was carefully excised, placed in 10% aqueous formaldehyde for 48 hours, then trimmed of extraneous fat, blotted, and weighed. Paraffin sections of each pancreas were stained with hematoxylin-eosin and examined for histopathological changes. The student "t" test was used for statistical treatment of the data.

Results and discussion. When defatted raw soybean meal was fed to weanling female rats for periods of 38 and 192 days (Table I), pancreatic hypertrophy, reduced feed efficiency, and decreased weight gains were observed as compared with control rats fed the casein diet. However, when rats fed the raw soybean meal diet for 38 days were switched to the basal casein diet for an additional 154 days, there was a marked decrease of pancreatic weights toward normal values.

Significant pancreatic enlargement also occurred in adult male rats fed raw soybean meal for either 38 or 192 days (Table I). The body weights of these rats were not greatly reduced.

Since histopathological examination of the pancreas in both young and adult rats fed raw soybean meal for 6 months revealed no abnormalities other than those previously reported(1), and since the pancreatic enlargement showed a marked degree of reversibility, it appears that the pancreatic stimulation produced by raw soybean meal is a nontoxic physiological response.

The actual mechanism of the hypertrophic action of raw soybean meal on the pancreas is unexplained, but it has been established that the site of action involves only the acinar epithelium, whereas the pancreatic islets are not affected(1). Raw soybean meal causes pancreatic hypertrophy in chicks(3), as well as in rats(1), and it is not unreasonable to assume that it might exert a similar action in man. The heat labile protein isolated from raw soybean meal and possessing trypsin-inhibitor activity, has now been directly implicated as the pancreas stimulating factor(2, 4). Since in this present report the effects of raw soybean meal have been shown to be both reversible and nontoxic after continuous ingestion, the possibility suggests itself that the isolated soybean trypsin-inhibitor might have therapeutic value for the treatment of pancreatic insufficiency in man resulting from cystic fibrosis.

Summary. When defatted raw soybean meal was fed to weanling female rats for 192 days, pancreatic hypertrophy, reduced feed efficiency, and decreased body weight gains were observed. Pancreas enlargement was also observed when adult male rats were fed the raw soybean meal containing diet for the same period. The pancreatic hypertrophy in rats ingesting raw soybean meal for 38 days was reversed when the rats were switched to the control diet for 154 days. Pancreatic tissues, which had been hypertrophied for more than 6 months, showed no histopathological damage.

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Maternal-Fetal Mortality in Mice with Isoantibodies to Paternal γ-Globulin Allotypes. (29454)

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A situation analogous to the Rh maternal-fetal incompatability in humans leading to erythroblastosis fetalis was developed experimentally in rabbits; this involved a maternal-fetal interaction in mothers immunized with paternal γ-globulin allotype(1). Maternal isoantibodies to the paternal γ-globulin allotype were shown to inhibit substantially the ability of the young heterozygote to develop the paternal allotype; at the same time a compensatory increase of the γ-globulin allotype controlled by the allelic gene also occurred (1). In addition, a first litter of 3 offspring from one of the mothers immunized with paternal allotype was stillborn, suggesting that fetal mortality also might be induced; subsequent litters from the same doe were viable and exhibited the "suppression-compensation" phenomenon described above(2). The high fetal mortality as well as other breeding problems in our rabbit colony precluded further evaluation of the question of fetal mortality.

The recent identification of the mouse γ-globulin allotypes and the knowledge of their genetic control(3-7) have made it possible to determine whether the "suppression-compensation" phenomenon first seen in rabbits could also be produced in mice. In addition, the low fetal mortality occurring in some strains of inbred mice favored the determination of whether isoantibodies to γ-globulin allotypes do in fact contribute to fetal and possibly maternal mortality in these strains.

Thus C57BL/6 mice with γ-globulin Asa2 were immunized with the allelic Asa1 γ-globulin of BALB/c mice to produce anti-Asa1 and conversely BALB/c mice were selected and immunized with γ-globulin of C57BL/6 mice to produce anti-Asa2 isoantibodies(6). Since γ-globulin is known to cross maternal-fetal barriers, the effect of anti-Asa2 antibody in immunized BALB/c mice, and of anti-Asa1 antibody in immunized C57BL/6 mice, on the developing heterozygous offspring which are genetically predestined to synthesize both the Asa1 and Asa2 γ-globulins could be studied.

This communication is concerned with the first phase of a more extensive investigation, and reports findings on the maternal and fetal effects of isoantibody in pregnant mice.

Methods. Four-week-old BALB/c female mice were divided into 3 groups (Ia, Ib and Ic). Group Ia was immunized with C57BL/6 γ-globulin (allotype Asa2); Group Ib was injected in the same manner with BALB/c γ-globulin (allotype Asa1); and Group Ic was not treated. At 14 weeks of age when the immunized mice (Group Ia) were producing precipitating antibody to C57BL/6 γ-globulin (allotype Asa2), all the mice in the 3 groups were mated with C57BL/6 males of the same age by placing 2 females and one male in the same cage. Thereafter, all mice were observed daily.

The mice in Group Ia were immunized by injections of washed agglutinates containing C57BL/6 γ-globulin prepared by mixing 0.1 ml C57BL/6 anti-*Proteus mirabilis* ascitic fluid with packed 10^9 *P. mirabilis* cells. The agglutinates were mixed with either complete or incomplete Freund's adjuvant or with physiologic saline and .05 ml of the mixture