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Deleterious Effects of Pancreas in the Hyperthyroid Rat.*

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In preliminary experiments on the effects of feeding various tissues on symptoms of thyrotoxicosis in the rat, it was observed that immature rats fed diets containing both desiccated thyroid and pancreas lived but a fraction of the time of animals fed similar diets with either thyroid or pancreas omitted. The present experiment was undertaken to obtain further data on this interrelationship.

Procedure and Results. Four basal rations were employed in the present experiment: diets A, B, C, and D. Diet A was a synthetic ration. Diets B, C, and D were similar in composition but contained 10% pancreas, activated pancreas† or papain, added in place of an equal amount of sucrose. All 4 rations were supplemented with 0.0 and 5.0 g of U.S.P. desiccated thyroid per kg of diet. Sixty-four female rats of the Long-Evans strain were selected at 21 to 23 days of age and an average weight of 45.4 g for the present experiment. Animals were kept in metal cages with raised screen bottoms to prevent access to feces, and were fed *ad lib.* the diets listed in Table I. Feeding was continued for 28 days or until death, whichever occurred sooner.

Results are summarized in Table II. Findings indicate that immature female rats fed diets containing both pancreas and desiccated thyroid lived but a fraction of the time of animals fed similar diets with either pancreas or thyroid omitted. On diet A_1 100% of the rats survived the experimental period of 28 days. On diets B_1 and C_1 (containing pancreas and activated pancreas respectively) the mortality rate was 100% with an average survival time of 15.5 and 7.4 days respectively. Findings with papain (diet D_1) were substantially the same as those with pancreas preparations. A marked retardation in growth was observed in animals fed diets B_1, C_1 and D_1. In the activated pancreas series (diet C_1) 9 of the 10 rats in this group lost weight during the first week of feeding. On diets B_1 and D_1 the average gain in weight during the first week of the experiment was less than 10 g in contrast to an average gain of 24.7 g on diet A_1 and approximately 20 g for rats fed control rations (diet A_2, B_2, C_2 and D_2). No significant difference in growth was observed on the 4 diets employed in the absence of dietary thyroid, although gain in body weight was somewhat greater on the synthetic ration (diet A_2) than on diets containing pancreas or papain. All rats fed thyroid-free rations survived the experimental period of 28 days.

No data are available concerning the cause of death in animals fed diets containing desiccated thyroid and either pancreas or papain. Since both of the latter substances contain proteolytic enzymes and since activated pancreas (in which the trypsinogen is converted into trypsin) was more active than the ordinary desiccated material, it was felt that the deleterious effects of pancreas or papain in the thyroid-fed rat may have been due to

* This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, and has been assigned number 216 in the series of papers approved for publication. The views or conclusions contained in this report are those of the author. They are not to be construed as necessarily reflecting the views or indorsement of the Department of the Army. Contribution No. 193 from the Department of Biochemistry and Nutrition, University of Southern California.

† Activated pancreas is desiccated pancreas whose trypsinogen has been converted into trypsin. We are indebted to Mr. Ezra Levin of the VioBin Corporation, Monticello, Ill., for these preparations.

1 Sure, B., *J. Nutrition*, 1941, **22**, 499.

TABLE I.
Composition of Experimental Diets.

Dietary component	Diet A_1 and A_2	Diet B_1 and B_2	Diet C_1 and C_2	Diet D_1 and D_2
Pancreas*	0	10	0	0
Activated pancreas†	0	0	10	0
Papain‡	0	0	0	10
Casein§	22	22	22	22
Salt mixture‖	4.5	4.5	4.5	4.5
Sucrose	73.5	63.5	63.5	63.5

To each kg of the above diets were added the following synthetic vitamins: thiamine hydrochloride 72 mg, riboflavin 9 mg, pyridoxine hydrochloride 15 mg, calcium pantothenate 67.2 mg, nicotinic acid 60 mg, 2-methyl-naphthoquinone 10 mg, and choline chloride 1.2 g. Each rat also received 3 times weekly the following supplement: cottonseed oil (Wesson) 500 mg, alpha-tocopherol acetate 1.5 mg, and a vitamin A-D concentrate¶ containing 50 U.S.P. units of vitamin A and 5 U.S.P. units of vitamin D.

U.S.P. desiccated thyroid (Armour) was incorporated in diets A_1, B_1, C_1, and D_1 at a level of 5.0 g per kg of diet, replacing an equal amount of sucrose.

* Desiccated Pancreas, VioBin Corporation, Monticello, Ill.

† Activated Pancreas, VioBin Corporation, Monticello, Ill.

‡ Frenco's Papain Absolute—Powder, Frenco Laboratories, Nogales, Ariz.

§ Vitamin Test Casein, General Biochemicals, Inc., Chagrin Falls, Ohio.

‖ Sure's Salt Mixture No. 1.[1]

¶ Nopco Fish Oil Concentrate assaying 800,000 U.S.P. units of vit. A and 80,000 U.S.P. units of vit. D per g.

TABLE II.
Effects of Pancreas Feeding on the Survival Time of Hyperthyroid Rats.

Dietary group	No. of animals	Initial wt (g)	Gain in body wt 28-day period* (g)	% surviving†	Avg survival time of decedents* (days)
			Desiccated thyroid series.		
A_1	10	45.1	65.9 ± 4.6 (10)	100	—
B_1	10	45.2	— —	0	15.5 ± 1.7
C_1	10	45.4	— —	0	7.4 ± 0.6
D_1	10	45.2	25.0 ± 4.3 (2)	20	18.6 ± 1.2
			Control series.		
A_2	6	45.5	91.8 ± 4.5 (6)	100	—
B_2	6	45.2	77.1 ± 3.1 (6)	100	—
C_2	6	45.0	76.3 ± 2.8 (6)	100	—
D_2	6	45.5	73.7 ± 4.7 (6)	100	—
			Thyroxin series.		
A_2‡	6	45.5	82.8 ± 1.9 (6)	100	—
C_2‡	10	45.7	— —	0	11.6 ± 0.6

The values in parentheses indicate the number of animals which survived and on which averages are based.

* Including standard error of the mean calculated as follows: $\sqrt{\frac{\Sigma d^2}{n}} / \sqrt{n}$ where "d" is the deviation from the mean and "n" is the number of observations.

† Experimental period, 28 days.

‡ Each rat in this series received intraperitoneal injections of 200 γ thyroxin 3 times weekly during the course of the experiment.

greater digestion and absorption of the thyroid material. Such, however, does not appear to be the case for subsequent findings indicate the deleterious effects of pancreas feeding may be demonstrated following parenteral administration of thyroxin. The latter experiment was conducted with 16 female rats of the Long-Evans strain similar in age and weight to those employed in the earlier studies. Six of these rats were fed the synthetic ration (died A_2); the remaining 10 were fed a diet containing the activated pancreas preparation (diet C_2). Three times weekly each rat received intraperitoneal in-

jections of 200 γ thyroxin.‡ Injections were continued for 28 days or until death, whichever occurred sooner. All animals fed the synthetic ration (diet A_2) and receiving the thyroxin injections survived the experimental period of 28 days. All animals fed activated pancreas (diet C_2) and administered thyroxin died (average survival time 11.6 days). These findings indicate that the deleterious effects of pancreas feeding in the thyroid-fed rat were not due to increased digestion or absorption of thyroactive material.

‡ Thyroxin (Synthetic Cryst.), Roche-Organon, Inc., Nutley, N. J. The material was dissolved in .1 N NaOH, adjusted to a pH of 8.0 and diluted to a volume containing 200 γ thyroxin per cc.

Summary. Immature female rats failed to survive when fed purified rations containing both pancreas and desiccated thyroid. Length of survival was significantly prolonged if either pancreas or thyroid were eliminated from the experimental ration. The deleterious effects of pancreas in the thyroid-fed rat were not due to increased digestion or absorption of thyroactive material.

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Production of Hemagglutinin by Mumps and Influenza A Viruses in Suspended Cell Tissue Cultures.*

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The property of agglutinating red blood cells characteristic of certain viruses such as mumps and influenza has provided a simple method of estimating their concentration in infected materials. The procedure however, insofar as we are aware, has not been applied to viruses propagated in tissue cultures. Accordingly, experiments were undertaken with the purpose of ascertaining (a) whether the mumps virus would proliferate in suspended cell tissue cultures of the Maitland type and (b) whether this virus and influenza A virus would in such media produce detectable levels of hemagglutinin.

Methods. Preparation and maintenance of cultures. Cultures were prepared in a manner similar to that described by the Maitlands.[1] The nutrient medium consisted routinely of 3 parts of a balanced salt solution made up according to the formula of Hanks† and 1 part of Simm's ox-blood serum ultrafiltrate.‡ This mixture will hereafter be referred to as "HS." In the experiments with mumps virus, the tissue employed consisted of amniotic membrane from 8- or 9-day-old chick embryos. The membranes were suspended in a small amount of "HS", minced with scissors to obtain fragments approximately 1 mm in diameter, and centrifuged for 2 minutes at 800 r.p.m. in a graduated centrifuge tube. The fragments were then resuspended in a total volume of "HS" six times that of the sedimented tissue. Using a large bore Pasteur pipette, 3 drops of this

* Aided in part by a grant from the National Foundation for Infantile Paralysis, Inc.

1 Maitland, H. B., and Maitland, M. C., *Lancet*, 1928, **2**, 596.

† The balanced salt solution as recommended by Dr. J. H. Hanks, contained the following ingredients expressed as g/l: NaCl, 8.0; KCl, 0.4; $MgSO_4 \cdot 7\ H_2O$, 0.1; $MgCl_2 \cdot 6\ H_2O$, 0.1; $CaCl_2$, 0.14; Na_2HPO_4, 0.06; KH_2PO_4, 0.06; glucose, 1.0. To each liter, 5 cc 0.4% phenol red was added. After autoclaving at 10 lb. for 10 minutes, 0.5 cc 1.4% isotonic $NaHCO_3$ was added per 20 cc of the above.

‡ Purchased from Microbiological Associates Laboratories, Flemington, N. J.