

Beneficial Effects of Fish Protein Concentrate on the Survival Time of Thyrotoxic Rats¹ (34209)

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Considerable data are available indicating that fish meal and fish protein concentrate (FPC) are sources of an unidentified nutritional factor or factors. One of the first studies indicating the presence of such a factor was that of Ritchey *et al.* (1). These workers selected day-old male chicks and fed them either a purified diet consisting of soy protein, glucose, salt mixture, corn oil, methionine, glycine, and the known vitamins or a similar diet supplemented with 10% defatted fish meal. In each of 3 separate experiments after 28 days of feeding the average weight increment of chicks fed the diet containing 10% fish meal was significantly greater than that of chicks fed the unsupplemented purified diet. In contrast to the above a supplement of 2% fish meal ash which corresponded to the ash present in the 10% fish meal supplement had little if any growth promoting effect. Additional data indicating the growth promoting effect in chicks of fish meal or FPC supplements when incorporated in purified diets have also been reported by Ritchey (2) and Rand *et al.* (3).

Available data indicate that immature rats fed a low fat purified diet containing casein as the source of dietary protein and sucrose as the dietary carbohydrate are extremely sensitive to thyroid feeding, with sensitivity manifesting itself in sudden death apparently due to cardiac failure (4). The length of survival of thyrotoxic rats fed the above diet could be significantly prolonged by a number of dietary supplements including yeast (4, 5), desiccated whole liver and water-insoluble liver residue (5), defatted liver residue (6), dried penicillin mycelia (7), aureomycin mash (7), whole egg (8), dried and defatted

pork (9), dried and defatted mutton (9), alfalfa meal (10) and others. Supplements of the known vitamins and minerals, cellulose and increased amounts of casein were without significant effect (5, 10). Length of survival could be significantly prolonged, however, by increasing the fat content of the diet in the form of cottonseed oil (11) or by supplements of fat-soluble substances such as cholesterol (12, 13) and liver fat (6). It is apparent that the survival time of thyrotoxic rats fed a low fat purified diet containing casein as the source of dietary protein and sucrose as the dietary carbohydrate can be prolonged by a number of substances unrelated chemically to one another. In the present communication data are presented indicating that substituting FPC for casein as the source of dietary protein in low fat purified diets containing sucrose as the source of dietary carbohydrate significantly prolonged the survival time of thyrotoxic rats.

Materials and Methods. Two basal rations were employed in the present experiment: Diet A which contained casein² and Diet B which contained FPC³ as the source of dietary protein. Each diet contained 5% salt mixture⁴, 2% cottonseed oil and the following vitamins per kg of ration: thiamine hydrochloride, 10 mg; riboflavin, 10 mg; pyridox-

² Vitamin-free test casein, General Biochemicals, Chagrin Falls, Ohio.

³ Fish protein concentrate prepared from whole fish by Alpine Marine Protein Industries, Inc., New Bedford, Massachusetts. This product was produced by azeotropic solvent extraction with ethylene dichloride as described by Levin and Finn (22). It had a protein content of 76.56% (12.25% N \times 6.25) and an ash content of 12.6%.

⁴ Wesson modification of Osborne-Mendel salt mixture, General Biochemicals, Chagrin Falls, Ohio.

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ine hydrochloride, 10 mg; calcium pantothenate, 60 mg; nicotinic acid, 100 mg; ascorbic acid, 200 mg; para-aminobenzoic acid, 200 mg; inositol, 400 mg; biotin, 1 mg; folic acid, 10 mg; vitamin B₁₂, 150 µg; 2-methyl-1,4-naphthoquinone, 5 mg; choline chloride, 2 g; vitamin A, 5000 USP units; vitamin D₂, 500 USP units; and alpha-tocopheryl acetate, 100 mg. Diet A contained 20% protein as casein; Diet B contained 20% protein as FPC. The balance of each diet consisted of sucrose. Tests were conducted with each of the basal rations fed alone (Diets A₁ and B₁) and supplemented with desiccated thyroid⁵ at levels of 0.25% (Diets A₂ and B₂) and 0.5% (Diets A₃ and B₃) of the ration. The desiccated thyroid was incorporated in the diets in place of an equal amount of sucrose.

One hundred and eight male rats of the Long-Evans strain averaging 52.3 g in body weight (range 44–60 g) and 108 female rats of the Long-Evans strain averaging 49.1 g in body weight (range 42–58 g) were employed in the present experiment. Animals were divided into 6 groups of comparable weight. Groups 1 and 4 which consisted of 12 male and 12 female rats each were fed Diets A₁ and B₁, respectively. Groups 2 and 5 which consisted of 18 male and 18 female rats each were fed Diets A₂ and B₂, respectively. Groups 3 and 6 which consisted of 24 male and 24 female rats each were fed Diets A₃ and B₃, respectively. Animals were kept in metal cages with raised screen bottoms (3 rats per cage) and were provided the various diets and water *ad libitum*. Animals were weighed weekly, and feeding was continued for 28 days or until death whichever occurred sooner. Surviving animals were killed after 28 days of feeding, and ventricular and adrenal weights were determined.

Results. Increment in body weight. In agreement with previous findings (4, 5, 11) desiccated thyroid when incorporated at levels of 0.25 and 0.5% in the low fat purified diet containing casein as the source of dietary protein and sucrose as the dietary carbohydrate resulted in a highly significant

⁵ Armour thyroid powder, USP, Reheis Chemical Co., Chicago, Illinois.

retardation in growth in both male and female immature rats. The retardation in growth was proportional to the level of thyroid fed and on a percentage basis was more marked in male than in female rats. Similar findings were observed on the FPC-containing diet. At each level of thyroid tested the increment in body weight of rats fed the FPC-containing diet did not differ significantly from that of rats fed the diet containing casein. Findings are summarized in Table I.

Ventricular and adrenal weights. Ventricular and adrenal weights particularly when expressed in term of mg per 100 g of body weight were significantly greater in both male and female rats fed the thyroid-containing diets than in those fed the unsupplemented basal rations. Similar findings were observed on both the casein and FPC-containing diets.⁶ The relative increase in adrenal weight of thyroid-fed rats over that of animals fed the unsupplemented basal rations was less for animals fed the FPC-containing diet than for those fed the diet containing casein. Findings are summarized in Table I.

Mortality. Data obtained in the present experiment confirm earlier findings that massive doses of thyroid result in a high mortality during the first 4 weeks of feeding in immature rats fed a low fat purified diet containing casein as the source of dietary protein and sucrose as the dietary carbohydrate (4, 5). In contrast to the above a highly significant reduction in mortality occurred in rats fed similar doses of thyroid in conjunction with the FPC-containing diet. At the 0.25% thyroid level the incidence of mortality which was 55.6 and 44.4% for male and female rats, respectively, after 28 days of feeding on the casein-containing diet was reduced to 0% on the FPC-containing diet. A significant reduction in mortality also occurred at the 0.5% thyroid level in rats of both sexes fed the FPC-containing diet. Findings are summarized in Table I.

Discussion. Available data indicate that re-

⁶ Additional findings indicate that the ovaries of thyroid-fed rats remained infantile in weight and microscope appearance on both the casein and FPC-containing diets.

TABLE I. Comparative Effects of Diets Containing Casein and FPC as Sources of Dietary Protein on the Weight Increment, Ventricular and Adrenal Weights and Percentage Survival of Rats Fed Toxic Doses of Desiccated Thyroid.*

Dietary group (Diet)	Thyroid (%) in diet	No. of rats	Initial body wt (g)	Av gain in body wt after 28 days of feeding (g)	Av ventricular wt (mg)	Ventricular wt (mg/100 g of body wt)	Av adrenal wt (mg)	Adrenal wt (mg/100 g of body wt)	Mortality over 28-day period (%)
Males									
A ₁	0.0	12	52.4	168.8	692	313	31.8	14.4	0.0
A ₂	0.25	18	52.3	105.6 (8)	925	586	47.0	29.8	55.6
A ₃	0.5	24	52.3	89.5 (6)	858	605	52.3	36.9	75.0
B ₁	0.0	12	52.4	166.4	658	301	31.2	14.3	0.0
B ₂	0.25	18	52.3	120.6	833	482	43.8	25.3	0.0
B ₃	0.5	24	52.3	102.7 (19)	892	575	49.5	31.9	20.8
Females									
A ₁	0.0	12	49.2	114.0	550	337	34.5	21.1	0.0
A ₂	0.25	18	49.2	93.2 (10)	867	609	43.3	30.4	44.4
A ₃	0.5	24	49.1	91.8 (9)	875	618	51.5	36.4	62.5
B ₁	0.0	12	49.2	113.6	492	302	35.7	21.9	0.0
B ₂	0.25	18	49.1	97.9	683	465	48.8	33.2	0.0
B ₃	0.5	24	49.1	83.1 (20)	760	575	46.8	35.4	16.7

* Values in parentheses indicate the number of animals which survived and on which averages are based when this number was less than the original number per group.

quirements for a number of nutrients are markedly increased in the hyperthyroid animal (14). This is particularly true for some of the B vitamins. An increased requirement for thiamine (15, 16), pyridoxine (17), pantothenic acid (17), folic acid (18) and vitamin B₁₂ (19, 20) has been demonstrated following the administration of large doses of thyroactive substances in the rat. In addition to the above, requirements are increased for at least one additional factor as well. Supplements of desiccated whole liver prolonged survival and counteracted the retardation in growth (5, 6) and inhibition of ovarian development (21) of immature rats fed massive doses of thyroid in conjunction with low fat purified diets containing casein as the source of dietary protein and sucrose as the dietary carbohydrate. Other supplements such as yeast (4, 5) and dried penicillin mycelia (7), however, were highly active in prolonging survival under conditions similar to the above but were without protective effect in counteracting the retardation in growth and inhibition of ovarian development. These findings suggest that the survival-prolonging factor may be distinct from that which promotes growth and ovarian development. Available data indicate that the protective factor or factors in the above supplements is distinct from any of the known nutrients (5, 10).

Present findings indicate that substituting FPC derived from whole fish for casein as the source of dietary protein in low fat purified diets containing sucrose as the dietary carbohydrate significantly prolonged the survival time of thyrotoxic rats. Since the retardation in growth and increment in ventricular weight of thyroid-fed rats on the FPC-containing diet were comparable to that of rats fed the diet containing casein, it would appear that the protective effect of FPC was not due to an interference with the absorption or utilization of the administered thyroid hormone. Further studies are indicated directed toward the concentration, isolation, and identification of the factor or factors in FPC responsible for its protective effect under conditions of the present experiment and its *modus operandi*.

Summary. A high incidence of mortality apparently due to cardiac failure occurred

within 28 days of feeding in immature rats fed massive doses of thyroid in conjunction with a low fat purified diet containing casein as the source of dietary protein and sucrose as the dietary carbohydrate. Substituting fish protein concentrate for casein in the above diet resulted in a highly significant reduction in mortality.

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