

TABLE II. Effect of Atropine on respiration supported by Alpha-glycerophosphate in Various Media.

Medium	Drug	% inhibition
I	10^{-3} M At*	41
II	10^{-2} M At	23
	10^{-2} M AA†	7
III	10^{-3} M At	15
	10^{-2} M At	41

Medium I. Ca-free Krebs-Ringer Phosphate with NaCl replaced by KCl; medium II. Krebs-Ringer Phosphate, Ca-free; medium III. Robinson's medium (see ref. 4) (similar to Krebs-Ringer but with somewhat higher buffer concentration). * Atropine sulfate; † Equimolar atropine sulfate and acetylcholine chloride together. Alpha-glycerophosphate was 0.01 M in all cases. All concentrations refer to final concentrations in the test system.

relate the spectroscopic changes to the ionization and corresponding respiration inhibition if such a relationship exists between all 3 factors. An obvious difficulty is the requisite demonstration of the manner in which ionization of the amine moiety affects the tropic acid group which is chiefly responsible for the absorption characteristics of the whole molecule(9). The importance of the ionic state

of inhibitors has been emphasized in studies on cyanide and azide(10,11,12) and on certain antiseptics(13).

Summary. (1) It was found that atropine in relatively high concentrations inhibited the respiration of rat brain homogenates by as much as 40% under optimal conditions. (2) The respiration inhibition was partially reversible by acetylcholine under certain conditions. (3) The inhibition was pH dependent, increasing with increasing pH. Corresponding changes in the atropine absorption spectrum with varying pH were observed.

9. Fischer, H., *Arch. exp. Path. Pharm.*, 1933, v170, 623.

10. Horecker, B. L. and Kornberg, A., *J. Biol. Chem.*, 1946, v165, 11.

11. Horecker, B. L. and Stannard, J. N., *J. Biol. Chem.*, 1948, v172, 589.

12. Stannard, J. N. and Horecker, B. L., *ibid.*, 599.

13. Simon, E. W. and Blackman, G. E., *Symp. Soc. Exp. Biol.*, 1949, v3, 253, Cambridge.

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The Growth-Promoting Action of Various Supplements in the Hypert thyroid Rat.* (18281)

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Studies on the deficiencies of rations composed principally of corn and soy-bean-oil meal for normal reproduction and lactation in rats and swine have been in progress at this station for several years. In the course of

these investigations, it was deemed desirable to undertake the development of an assay procedure that was less time-consuming and more sensitive than that involving reproduction. One of the procedures used by other workers has been assessment of the ability of a supplement to reverse the growth inhibition induced by feeding thyroid-active agents to the immature rat. In the presence of an ample supply of other nutrients, it has recently been shown by Emerson(1), by Betheil and Lardy(2),

* Published with the approval of the Director, Oklahoma Agricultural Experiment Station. Appreciation is expressed to Merck and Co., Rahway, N. J., for supplies of crystalline vitamins; to the Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y., for pteroylglutamic acid and APF concentrates; to Armour and Company, Chicago, Ill., for liver fractions; to U. S. Industrial Chemicals, New York, for DL-methionine, and to Cerophyl Laboratories, Kansas City, Mo., for iodinated casein.

1. Emerson, G. A., *PROC. SOC. EXP. BIOL. AND MED.*, 1949, v70, 392.

2. Betheil, J. J., and Lardy, H. A., *J. Nutrition*, 1949, v37, 495.

and by Frost *et al.*(3) that the administration of thyroid-active substances will induce a retardation in growth which is partially overcome by the supplementation of the ration with vit. B₁₂. Employing a somewhat different type of ration containing desiccated thyroid, however, Ershoff(4) was unable to demonstrate any positive effect of crystalline B₁₂ on growth but found that whole liver substance and various liver fractions were highly active. The basal ration employed in the studies reported here was designed to resemble as nearly as possible the corn-soybean meal ration used in previous reproduction studies (Watts *et al.*(5)) and yet be composed of materials of more uniform quality. The ration developed, therefore, used isolated soybean protein and corn starch to replace natural feedstuffs. In view of the recent suggestion by Lewis, *et al.*(6) that the variation in results obtained by different groups of workers may be due to the carbohydrate component of the ration, the results obtained with this ration, which differs in both source of protein and source of carbohydrate from that used by others, are of additional interest.

Experimental. Rats used in all these studies were from the local colony (Sprague-Dawley strain). Young were from females fed the following ration: ground yellow corn, 55.5%; soybean meal, 20; alfalfa leaf meal, 2; dried skim milk, 20; condensed fish solubles, 1; dried brewers yeast, 1; salt, 0.5; calcium carbonate, 0.5 and cod liver oil, 0.5. At weaning, the young were fed a basal ration of the following composition for a period of 10 days: soybean protein preparation (sodium proteinate, Archer-Daniels-Midland Company, Minneapolis, Minn.) to provide 20% protein; salts (Hegsted, *et al.*, *J. Biol. Chem.*, 1941,

v138, 460), 4.0; cottonseed oil, 5.0 and corn starch to make 100%. The following vitamins were added per kg of ration (excessive levels were maintained to provide for any increased need): thiamine, 8 mg; riboflavin, 12; pyridoxine, 6; calcium pantothenate, 40; nicotinic acid, 40; inositol, 40; p-amino benzoic acid, 40; pteroylglutamic acid, 2; choline chloride, 2 g. In addition, vitamins A and D and alpha-tocopherol were administered by dropper twice weekly. The thyroid-active material was omitted during this period, because, in our experience, more uniform response has been noted in rats weighing from 80-100 g than in smaller animals. For the ensuing 2 weeks the animals were allowed access to the above basal ration modified to contain 0.25% iodinated caesin (Protamone, Cerophyl Laboratories, Kansas City, Mo.). Supplements were added to this ration on the basis of nitrogen equivalence, replacing sodium proteinate.

The results of such supplementation are presented in Table I. These data represent the combined results of eleven separate trials with nine different supplements. Some supplements have been used repeatedly; others have been tested only once or twice. The growth of males and females during the trial period are presented separately, because we have frequently observed a sex difference in response to supplementation. It is recognized that interpretation of the results is rendered more difficult by presentation in such a condensed manner, but limitations of space will not permit tabulation of the results of each of the trials. These detailed results are summarized in the thesis of Bolene(7).

Before discussing some of the implications of these data, it seems well to point out that in our experience rats of the same strain obtained from another source (a commercial dealer) did not respond in the same manner. They were less sensitive to the growth-depressing effect of iodinated casein and did not respond to supplementation in like manner when the level of thyroxine-like materials was increased. It may be that the stock ration fed

3. Frost, D. V., Fricke, H. H., and Spruth, H. C., *PROC. SOC. EXP. BIOL. AND MED.*, 1949, v72, 102.

4. Ershoff, B. H., *PROC. SOC. EXP. BIOL. AND MED.*, 1949, v71, 209.

5. Watts, A. B., Swank, W., Ohman, R. J., Ross, O. B. and MacVicar, R. W., *J. Nutrition*, 1950, v41, 533.

6. Lewis, U. J., Tappan, D. V., Register, U. D. and Elvehjem, C. A., *PROC. SOC. EXP. BIOL. AND MED.*, 1950, v74, 568.

7. Bolene, Charles, *Studies on Antithyrototoxic Factors*, Thesis, Oklahoma A. and M. College, 1950.

TABLE I. Effect of Supplementation on Growth of Rats Receiving Toxic Levels of Iodinated Casein.

Supplement	Level in ration %	No. of animals	Avg gain per rat for 2 wk exp. period	Avg % change with respect to basal ¹
Males				
None	—	28	29.9	—
Fish solubles	1.5	6	44.8	77.7**
APF No. 3 (Merck)	0.5	10	48.8	50.1**
APF supplement (Lederle)	2	6	27.2	60.3 ²
1:20 liver powder (Wilson)	2	17	49.7	92.6**
Biopar A (Armour)	3	5	35.6	109.9 ²
Cryst. B-12	25 mcg/kg	21	45.0	50.7**
Liver residue	3	10	44.4	120.6**
Extracted liver residue	3	5	43.0	35.2 ²
Extract of liver residue	3	4	45.2	42.3 ²
Females				
None	—	32	30.8	—
Fish solubles	1.5	4	47.9	48.7 ²
APF No. 3 (Merck)	0.5	8	46.1	39.1*
APF supplement (Lederle)	2	12	32.8	28.3
1:20 liver powder (Wilson)	2	11	43.0	51.7**
Biopar A (Armour)	3	12	24.1	11.6
Cryst. B-12	25 mcg/kg	24	33.6	13.6
Liver residue	3	20	42.4	38.5**
Extracted liver residue	3	4	30.8	14.1 ²
Extract of liver residue	3	5	39.6	10.5 ²

¹ Comparisons made with respect to the basal ration in each trial in which supplement appeared, weighed for disproportionate numbers.

² Not subjected to statistical analysis due to small numbers.

* and ** indicate statistical significance by analysis of variance with respect to the basal ration at the 5 and 1% levels of probability.

the females producing the young in our colony provided a lesser amount of the factor antagonizing the growth-depressing effect than was provided by the commercial dealer's stock ration. Transfer of nutrients, (including vit. B₁₂) through the egg to the chick has been repeatedly shown to permit essentially normal growth on rations which are low in the particular factor unless the hens have been previously depleted. It seems likely that an analogous transfer may occur in the case of the factor(s) antagonistic to the growth-depressing effect of thyroid-active agents in the rat.

Examination of the effect of supplementation (Table I) shows that fish solubles, two commercial APF supplements (No. 3, Merck and Co. and Lederle), and 1:20 liver powder (Wilson), materials which have appreciable "animal protein factor" activity for the chick, are also effective in counteracting the growth-depressant effect of toxic levels of iodinated casein. Crystalline vit. B₁₂ gave a highly significant growth response in males, but was less

effective in females. Even in males, however, the growth response was distinctly less than that obtained with various preparations of liver and fish solubles. This suggests that the deficiency induced by hyperthyroidism is multiple in nature and that vit. B₁₂ is one of the components. These findings are essentially in accord with the observations of Bethell and Lardy(2) who used male rats of the same strain employed here. They are at variance with the findings of Ershoff(8) who was unable to demonstrate any "anti-thyrotoxic" effect of vit. B₁₂ in rats of the Long-Evans strain fed a basal ration of the sucrose and casein type. If it is assumed that thyroprotein induces a multiple deficiency, of which vit. B₁₂ is one component, then the divergence of results may be due either to the presence of some other factor in limited amounts in the rations employed by Bethell and Lardy(2) and by ourselves or to increased

8. Ershoff, B. H., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 459.

intestinal synthesis. We are presently unable to suggest any reason for the differences observed between males and females in their response to vit. B₁₂. It may be that there are sex differences in the requirement for some additional factor. This factor could be present in sufficient amounts to permit response on the part of males to vit. B₁₂ but so limit the growth of the females that no response to vit. B₁₂ supplementation was possible.

The effectiveness of hot-water insoluble liver residue in counteracting the growth depressing effects of thyroid toxicity has been previously observed by Ershoff (4,5). A preparation obtained as a by-product of the manufacture of liver extract (Armour and Company) was tested and found to give a positive response. This substance was more effective in males than in females, although the response with the latter was significant at the 5% level of probability. The hot-water soluble fraction of liver (Biopar A, Armour and

Company) was likewise more effective in males than in females but the number of animals was too small for reliability. Very preliminary efforts to fractionate the activity of the liver residue by exhaustive extraction with 70% ethanol yielded inconclusive results. Both the extract and extracted liver residue were moderately active for males and showed little activity in the case of the females. Again, however, the number of animals used has been very limited.

Summary. The growth depressant effect of toxic levels of iodinated casein in the ration of immature male and female rats was partially counteracted by the addition of fish solubles, liver extracts and a commercial APF supplement. Supplementation with crystalline vit. B₁₂ was more effective in males than in females, but maximum response was not obtained with this substance. The multiple nature of the "antithyrototoxic factor" is therefore suggested.

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Course of Experimental Tuberculosis in the Albino Rat as Influenced by Cortisone.* (18282)

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Previous studies in this laboratory have demonstrated certain quantitative differences in the cellular response of the rat and the rabbit to the intraperitoneal injection of mineral oil (1). It was shown that an oil-induced peritoneal exudate is strikingly different in the rabbit, naturally susceptible to tuberculosis, and in the rat, naturally resistant to the

disease. The rat responded to the intraperitoneal injection of mineral oil with an exudate richer in total cells, percentage of mononuclear cells, and in the absolute number of mononuclear cells than did the rabbit. The administration of cortisone depressed the total exudate in both species with the total and differential counts of the rat approaching those of the normal rabbit (2). These results suggested that tuberculosis in the naturally resistant rat might be altered by cortisone administration. The albino rat responds to infection with virulent human tubercle bacilli with a chronic, granulomatous, non-fatal pro-

* Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are the result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

1. Bloom, W. L., Cummings, M. M., and Michael, M., Jr., *Proc. Soc. Exp. Biol. and Med.*, 1950, v75, 171.

2. Cummings, M. M., Michael, M., Jr., Bloom, W. L., in press.