

to influence the development of disintegrative changes.

Discussion. The data presented are of interest from the point of view of preservation of monkey testicular tissue in the refrigerator for prolonged periods of time (5 to 8 weeks). This fact should prove helpful in those laboratories where such tissues are used for the isolation and identification of poliomyelitis viruses. The mechanism of action of albumin as a protector of fibroblasts has not been investigated. An analogous situation is the stimulating effects of either whole serum or the albumin fraction on growing tubercle bacilli as originally observed by Youmans and confirmed by others(3-6). In this instance the albumin was thought to neutralize toxic fatty acids and other substances(3,4). To the authors' knowledge human albumin has not been previously used as a constituent of media for the cultivation of cells. Sanford, *et al.*, (7) have recently reported an unsuccessful attempt to fortify a medium containing chick-embryo extract and horse serum ultrafiltrate with 6% crystalline bovine albumin. This mixture was not better than whole horse serum for the quantitative cultivation of the L strain

of mouse cells. We have utilized mixtures of bovine albumin and horse serum for growing human fibroblasts. Proliferation was equal to that seen with human albumin but degenerative changes appeared earlier.

Summary. Minced immature rhesus testicular tissue remained viable for a period of 8 weeks when refrigerated at 5°C in Hanks' basic salt solution containing 2.5% salt-poor human albumin. Some observations on the use of human albumin as a constituent of tissue culture media have been recorded.

1. Frisch, A. W., *PROC. SOC. EXP. BIOL. AND MED.*, 1952, v81, 545.
2. Youngner, J. S., Ward, E. N., and Salk, J. E., *Am. J. Hyg.*, 1952, v55, 291, 301.
3. Youmans, G. P., *PROC. SOC. EXP. BIOL. AND MED.*, 1944, v57, 122.
4. Dubos, R. J., and Davis, B. D., *J. Exp. Med.*, 1946, v83, 409.
5. Middlebrook, G., *Am. Rev. Tuberc.*, 1947, v56, 334.
6. Frisch, A. W., *PROC. SOC. EXP. BIOL. AND MED.*, 1952, v79, 281.
7. Sanford, K. K., Waltz, H. K., Shannon, J. E., Jr., Earle, W. R., and Evans, V. J., *J. Nat. Canc. Inst.*, 1952, v13, 121.

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Thyroid and Vitamin B₁₂ Interactions in the Mouse.* (20198)

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Low doses of thyroid-active substances have been shown to significantly increase the rate of growth in young mice for limited periods of time(1-3). Daily injections of .01-.03 mg of crystalline thyroxine sodium or feeding .04-.32% of iodinated casein in the ration for

a 5-week period increased body weight gains by about 28% over untreated control mice(2). This was accompanied by an increase in tissue protein and water, a decrease in fat and greater body length. Excessive doses of thyroid-active materials however, inhibit body growth in young mice, an effect which can largely be counteracted by supplementing the diet with "animal protein factor" or vit. B₁₂ (4,5). It was of interest therefore, to determine in mice the interactions between a dose of a thyroid-active substance which would increase growth rate and vit. B₁₂.

Methods. A total of 36 immature, male Rockland mice and 45 immature, male Car-

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worth mice were used in 2 separate experiments. The animals were divided into uniform groups by weight and were fed the following vit. B₁₂-deficient ration: yellow corn meal, 35%; ground wheat, 25%; linseed oil meal, 10%; soybean oil meal,§ 20%; alfalfa leaf meal, 6%; brewers' yeast, 3%; and table salt, 1%. In the first experiment, 4 groups of Rockland mice were treated as follows: controls, untreated; 100 µg vit. B₁₂|| per kilo of ration; .025% Protomone;¶ 100 µg vit. B₁₂ and .025% Protomone. In the second experiment, 5 groups of Carworth mice were similarly treated except that two levels of vit. B₁₂ were fed together with the Protomone. Body weights were recorded every 2 days in all the mice and food consumption was measured only in the mice of the first experiment. All mice were housed in metal cages with raised screen bottoms in an air conditioned room maintained at 75 ± 2° F.

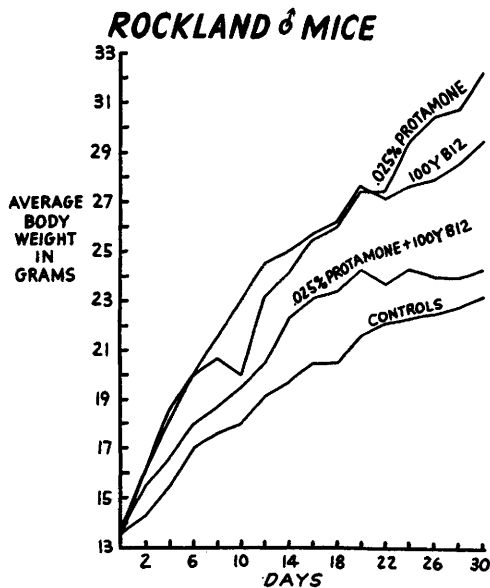


FIG. 1. Growth curves of Rockland mice receiving vit. B₁₂ and/or Protamone.

§ Low fiber, solvent extracted, containing 50% protein. Manufactured by Archer-Daniels-Midland Co., Minneapolis, Minn.

|| Vit. B₁₂ was made available through the courtesy of Dr. L. Michaud of Merck and Co., Rahway, N. J.

¶ Iodinated casein product containing thyroxine. Kindly furnished by Cerophyl Labs., Kansas City, Mo.

CARWORTH ♂ MICE

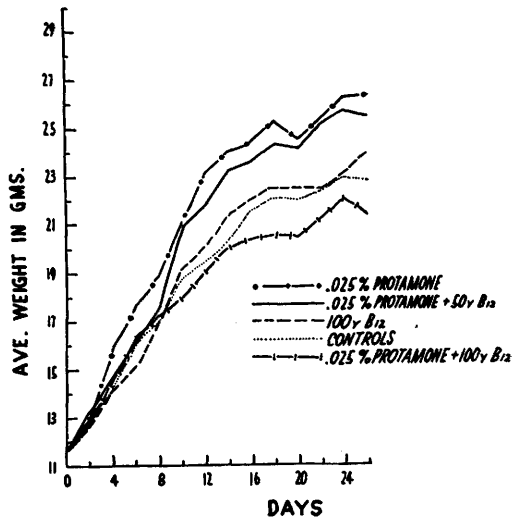


FIG. 2. Growth curves of Carworth mice receiving vit. B₁₂ and/or Protamone.

Results. The growth curves of the mice in the first experiment are shown in Fig. 1. The average body weight per mouse at the beginning of the experiment was 13.6 ± 0.5 g. The average body weight and total food consumption per mouse at the end of 30 days were as follows for each group: controls, 23.2 ± 0.8 g and 96.0 g; .025% Protamone, 32.3 ± 1.4 g and 161.7 g; 100 µg vit. B₁₂, 29.5 ± 1.5 g and 120.3 g; .025% Protamone and 100 µg vit. B₁₂, 24.3 ± 1.8 g and 121.2 g. The average food intake in grams per gram of body weight gain for each of the 4 groups was as follows: 10.0, 8.7, 7.5 and 11.5. Thus the efficiency of converting food into body weight gains was greatest for the groups receiving either Protamone or vit. B₁₂ and least for the group receiving both substances.

The growth curves of the mice in the second experiment are shown in Fig. 2. These mice weighed 11.7 ± 0.4 g each at the beginning of the experiment. The average final body weight per mouse for each group was as follows: controls, 22.6 ± 0.6 g; 100 µg vit. B₁₂, 24.0 ± 0.8 g; .025% Protamone, 26.3 ± 0.8 g; .025% Protamone and 50 µg vit. B₁₂, 25.7 ± 0.6 g; .025% Protamone and 100 µg vit. B₁₂, 21.6 ± 1.0 g. It can be seen that even though 100 µg of vit. B₁₂ did not signi-

ificantly increase the growth rate of these mice, this amount of vit. B₁₂ fed together with Protamone completely eliminated the favorable action of the latter on growth.

Discussion. These data are in agreement with previous reports that a mild degree of hyperthyroidism increases the growth rate of young mice(1-3). A maximum difference in average body weight of about 2.5 g between thyroid-treated and untreated mice was noted in these earlier studies, whereas differences of 8.9 g and 3.8 g were seen in the present 2 experiments. An analysis of the rations used by the previous investigators showed that they contained considerable quantities of "animal protein" and presumably of vit. B₁₂, whereas the basal ration used in the present study was deficient in vit. B₁₂. It seems probable therefore, that the differences in the growth response of these mice to thyroid-active substances were influenced by the levels of vit. B₁₂ in the diets. Swine may represent another species in which a growth response to a mild degree of hyperthyroidism is enhanced if the ration is deficient in vit. B₁₂. Beeson(6) reported that a small dose of Protamone was very effective in stimulating increased growth in pigs when the ration was deficient in vit. B₁₂, but that the same dose of Protamone was completely ineffective in stimulating growth in the presence of adequate vit. B₁₂.

No satisfactory explanation can be given at present for the apparent antagonism observed between vit. B₁₂ and Protamone in these mice. It has been suggested that a limiting factor or factors in the diet, *i.e.* methionine, may ac-

count for this phenomenon (Dr. A. White, personal conversation), but further research will be necessary to resolve this question.

Summary. 1. The effects of a mild degree of induced-hyperthyroidism or vit. B₁₂ or both on growth rate were determined in 81 immature male mice fed a vit. B₁₂-deficient ration, in two experimental trials of 26 or 30 days duration. 2. Feeding .025% Protamone (iodinated casein) or 100 µg of vit. B₁₂/kilo of ration each increased the growth rate of the mice, and Protamone was more effective than vit. B₁₂ in this respect. Each substance increased food intake and enhanced the efficiency of converting food into body weight gains. When Protamone and vit. B₁₂ were fed together however, the favorable effects of either on growth were largely eliminated and efficiency of food utilization was reduced. No satisfactory explanation can be offered for this phenomenon at this time.

1. Robertson, T. B., *Austral. J. Exp. Biol. and Med.*, 1928, v5, 69.

2. Koger, M., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bull.*, 1943, 377.

3. Miller, J. K., Master's Thesis, Michigan State College, 1948.

4. Bosshardt, D. K., Paul, W. J., and Barnes, R. H., *J. Nutrition*, 1950, v40, 395.

5. Bosshardt, D. K., Paul, W. J., O'Docherty, K., Huff, J. W., and Barnes, R. H., *J. Nutrition*, 1949, v37, 21.

6. Beeson, W. M., Cited in Annotated Bibliography of the Biological Literature on Iodinated Protein and Thyroxine, Cerophyl Labs., Inc., Kansas City, Mo., 1951.

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