Histological Studies with Rats Fed Diets Containing Iodinated Casein and Different Levels of Vitamin B_{12}.* (20888)

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Earlier studies in our laboratories with rats have shown that the nucleic acid content and cell count per unit weight of liver were reduced, and that the liver weight to body weight ratio was increased in a vit. B_{12} deficiency(1). These studies have been extended to evaluation of the histological changes that occur in certain tissues and organs of the rat maintained on vit. B_{12}-deficient and supplemented diets. In the present work a corn-soybean oil meal basal diet plus 0.06% iodinated casein(2), used in the rat assay method for vit. B_{12}(3), was employed. Since iodinated casein was included in the diet, special attention was directed to studying histological changes in the thyroid gland.

Methods. Weanling albino rats of the Holtzman strain were fed the basal diet either with or without the addition of 50 μg of vit. B_{12} per kg of diet after a 2-week depletion period as described previously(2,3). After 4 weeks on experiment, animals from both groups and a control animal fed a stock diet† were sacrificed for histological study. Other animals were kept on experiment for 2 additional weeks to study the progression of changes noted. In addition, animals fed the basal diet for 4 weeks were fed the basal diet plus vit. B_{12} from the fourth to the sixth week in order to test the recovery of the animals deficient in vit. B_{12}. Seven animals had been fed the deficient diet, 5 the B_{12}-supplemented diet, and 2 animals the deficient diet for the first 4 weeks, and the supplemented diet from the fourth to the sixth week on experiment. The differences in weight attributed to dietary treatment (*i.e.*, an average weight of 182 g for the B_{12}-deficient group and 251 g for the B_{12}-supplemented group at 6 weeks on experiment) were slightly less than observed previously with this dietary regime(2,3). The following organs and tissues were taken for histological study: liver, thyroid, testis, bone marrow (femoral), and skeletal muscle (gastrocnemius). The tissues were fixed in freshly prepared Zenker-formol and processed by the standard celloidin technic. All organs were cut at 10 μ and stained with hematoxylin and eosin except muscle, which was stained with hematoxylin and Van Giesen's. Bone marrow tissues were examined under oil immersion for stages in red cell differentiation and other myeloid elements. The thyroid glands were sectioned in their entirety; and representative sections from the remaining 4 organs and tissues were prepared and studied.

Histological observations. No differences in the histological picture of the liver, muscle, and myeloid tissues were found between B_{12}-deficient and the control animals. Histological changes were present, however, in the thyroid and testis of the B_{12}-deficient rats. The following changes were observed in all animals studied in each group.

Two main changes were observed in the thyroid gland of the vit. B_{12}-deficient animals: 1) a reduction in the size of the secretory cells; and 2) accumulation of colloid in the follicles. Thus, the secretory cells were reduced to a low cuboid type in a gland taken from a rat fed the B_{12}-deficient diet for 4 weeks. The reduction was observed primarily in the cytoplasm of these cells. Practically all the follicles of the gland were filled with colloid, which took the hematoxylin stain uniformly and intensively. Vacuoles in these follicles were rather rare. These changes are indications of a reduced secretory activity of the gland.

Advanced degenerative signs of the gland were found in rats fed the B_{12}-deficient ration for 6 weeks. There was reduction in the size of the secretory cells over that observed at

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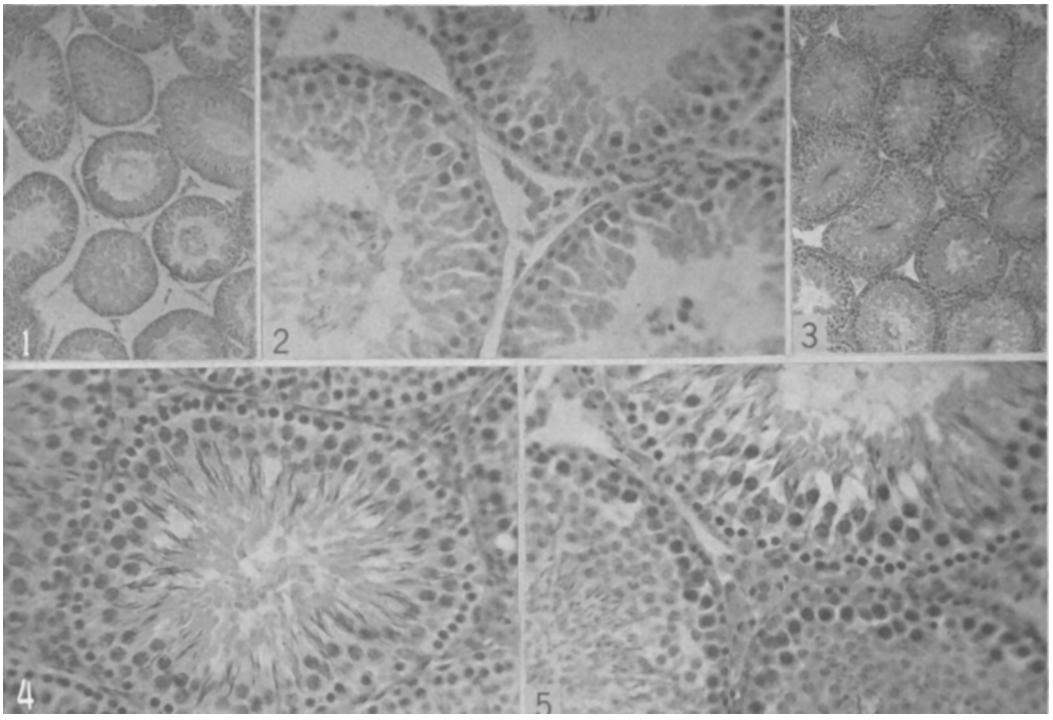
† Rockland rat pellets, complete.



Testis. FIG. 1—B_{12} deficient diet for 6 wk (× 50). FIG. 2—An enlarged portion of Fig. 1 (× 215). FIG. 3—B_{12} deficient diet for 4 wk then B_{12} supplemented ration from fourth to sixth wk (× 50). FIG. 4—An enlarged portion of Fig. 3 (× 215). FIG. 5—Stock diet (× 215). All Zenker-formol fixed and stained with hematoxylin and eosin.

4 weeks. These cells were quite flat and the nuclei were oriented parallel to the basement membrane of the follicle. The colloids persisted in these follicles and stained a dark blue with hematoxylin. These changes are highly indicative of a very low or non-existent secretory activity of the gland.‡ These results suggest that the gland of the B_{12}-deficient animal was not secreting any appreciable amount of thyroxine.

Some improvement in the thyroid gland occurred with vit. B_{12} supplementation from the fourth to sixth week on experiment as compared to results for animals fed the B_{12}-deficient diet for the entire 6 weeks, but the thyroid was not normal. In the group fed vit. B_{12} throughout the experiment, thyroid glands were examined after the animals were on experiment for 4 and also for 6 weeks. No marked differences were noted between animals on experiment for 4 and 6 weeks and, while the thyroids showed less abnormalities than those from the deficient animals of comparable age, they also were not normal. The histological pattern of the thyroids from animals fed the B_{12}-supplemented diet for the entire experiment was similar to that for animals fed the supplemented diet from the fourth to the sixth week of the experiment. It is possible that the level of vit. B_{12} fed (at least 3 times that required for optimum growth) may not have been sufficient to support normal thyroid activity or that supplementation with vit. B_{12} for a longer period would have resulted in complete recovery. It is more likely, however, that the proposed anti-thyrotoxic factor in liver (4,5) as well as vit. B_{12} is needed for complete recovery. In fact, measurement of histological changes in the thyroid may be a sensitive assay for the unknown factors in liver, particularly when low levels of thyroid-active materials are fed

‡ Probably such a gland would be smaller, although no attention was given to this at the time the animals were sacrificed.

and only small differences in growth rates with liver supplements are observed.

No difference was observed in the histology of the testes of the rats fed the basal ration plus 50 μg B_{12}/kg ration, as compared to the stock diet. The structure of both the seminiferous tubules and the cells of Leydig (interstitial tissue) in these testes indicated a perfectly functional state in spermatogenesis and male hormone production.

The testes of the B_{12}-deficient rats showed definitive signs of abnormality of a progressive nature. They consisted of: 1) shrinkage of the seminiferous tubules with the result of increased intertubular spaces; 2) decreased spermatogenetic activity; and 3) degeneration of the interstitial tissues. These histological changes were present in a mild degree in testes from animals fed the B_{12}-deficient diet for 4 weeks, but reached an advanced stage in animals fed the same diet for 2 weeks longer (Fig. 1 and 2). Spermatogenetic activity was greatly reduced after 6 weeks. In the 3 seminiferous tubules enlarged in Fig. 2, there were only a few spermatogonia left in the germinal epithelium. All other germinal cells higher in the process of differentiation had completely atrophied. The lumen of these tubules was filled with a mixed debris consisting of degenerate sperm cells and spermatids. The cells of Leydig were also highly pyknotic in appearance and scanty.

The testes of the animals fed a diet supplemented with vit. B_{12} from only the fourth to sixth week were normal (Fig. 3 and 4 as compared to 5). These results are in contrast to those observed for the thyroid.

Discussion. These results suggest that the thyroid gland and the testis were affected by the deficiency of vit. B_{12}. Atrophy of the thymus and changes in other organs observed when thyroid stimulants were fed(6,7) were corrected either by feeding vit. B_{12} or certain crude materials. In other studies(8) atrophy of the thymus gland induced by cortisone injections was largely counteracted by feeding vit. B_{12}. It is also quite possible that the observed disturbances of the thyroid gland and testis were reflections of a decreased output of certain of the anterior pituitary hormones which occurred in the vit. B_{12}-deficient animals. Experiments with the addition of liver preparations to the diet, and histological studies on the pituitary gland would be very valuable in evaluating these possibilities.

Summary. 1. A histological study was made of the thyroid, testis, liver, muscle, and bone marrow from rats fed diets containing iodinated casein and varying levels of vit. B_{12}. No marked changes were observed in the liver, muscle or bone marrow of the vit. B_{12}-deficient rats, while extensive changes were noted in the thyroid and testes. 2. The degenerative changes in the thyroid gland were more severe with increased time on experiment; however, these changes were only partially counteracted when vit. B_{12} was fed. 3. Decreased spermatogenetic activity, shrinkage of the seminiferous tubules, and degeneration of interstitial tissues of the testes observed in vit. B_{12}-deficient rats were completely counteracted by feeding vit. B_{12}. 4. The relationships of these observations to the anti-thyrotoxic factors from liver and pituitary function have been discussed.

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