to allow for normal hematopoietic regeneration. Since lead protection of either the spleen or the appendix of the rabbit during irradiation allows a more rapid regeneration of the irradiated hematopoietic tissue than in rabbits without such protection, it is obvious that neither is it the spleen specifically nor the ability of the lead protected tissue to produce ectopic blood formation that accounts for the observed phenomenon. These findings strongly suggest that lymphatic tissue exerts a humoral control over hematopoiesis. It might be postulated that the bone marrow which is not normally lymphopoietic, and the thymus would produce a comparable effect were it possible to test this postulate by exclusive protection of these tissues during irradiation. Such experiments are probably necessary before one can assume that the entire hematopoietic system functions as a unit in the maintenance of a steady state. Colonization or seeding from the lead protected lymphatic tissue may actually provide cells from which the irradiated marrow and lymphatic tissue become repopulated. If this latter suggestion were true, then it would follow that the lymphocytes coming from a protected lymphatic organ such as the appendix and going to the bone marrow were capable of giving rise to all types of hematopoietic cells.

If the irradiated lymphatic tissue is acting to temporarily suppress hematopoietic regeneration and recovery under the conditions of these experiments, then the process may be related to the "indirect" therapeutic effect observed in myelogenous leukemia when the spleen alone is irradiated.

It should perhaps be pointed out that lead protection of lymphatic tissue of irradiated animals may possibly reduce "radiation toxicity" in a non-specific manner and that a more rapid regeneration of tissue in the body other than hematopoietic tissue such as skin, gonads, and intestinal mucosa will be apparent on examination.

*Summary and conclusions.* Young adult female mice were exposed to 1025 r whole-body X radiation. The spleens of these mice were mobilized surgically through an abdominal incision and lead protected during irradiation in one group and mobilized but not lead protected during irradiation in another group.

The bone marrow and lymphatic tissues were destroyed and no regeneration was as yet apparent on the tenth day in the animals irradiated without lead protection of the spleen. In the animals with lead protection of the spleen during irradiation, these tissues were never depleted of free hematopoietic cells and the bone marrow and the lymph nodes were normal in cellularity by the eighth day after irradiation.

Several possible interpretations of the role of the spleen under the conditions of these experiments are discussed.

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## Effects of Vitamin $B_{12}$ and Liver Residue on Growth of Hyperthyroid Male Rats.* (17712)

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Available data indicate that requirements for a number of nutrients are markedly increased in the hyperthyroid animal(1). This is particularly true for some of the B vitamins. An increased requirement for thiamine(2,3), pyridoxine(4), pantothenic acid

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(4), folic acid (5) and, more recently, vitamin $B_{12}$(6-8) has been demonstrated following the administration of large doses of thyroactive substances. In addition to the above, requirements are increased for at least one additional factor as well. This substance which has been termed the "antithyrotoxic factor of liver" is present in considerable concentration in the water-insoluble fraction of liver (liver residue) and is apparently distinct from any of the known nutrients(9-11). When immature female rats were fed purified rations containing casein as the dietary protein, a marked retardation in growth and inhibition of ovarian development was observed following the administration of massive doses of desiccated thyroid. These effects were completely counteracted by the administration of liver residue but not by supplements of any of the known nutrients including vitamin $B_{12}$ (9,11,12). Betheil and Lardy, however, found that male rats on a similar ration supplemented with thyroid gained significantly more weight following vitamin $B_{12}$ administration than they did if vitamin $B_{12}$ were omitted(8). Since differences in sex may have been responsible at least in part for the diverse growth response, the present experiment was undertaken to determine the comparative effects of vitamin $B_{12}$ and liver residue on the growth and gonadal weight of male rats fed massive doses of thyroid in conjunction with a purified casein-containing ration.

*Procedure and Results.* The basal ration employed in the present experiment consisted of sucrose, 73.0%; casein,† 22.0%; salt mixture,‡ 4.5%; and U.S.P. desiccated thyroid,§ 0.5%. To each kg of the above 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, 5 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.¶ In addition to the basal ration the following diets were also employed, consisting of basal ration plus each of the following supplements: (1) 30 $\mu$ of vitamin $B_{12}$ per kg of diet, and (2) 10% liver residue powder,** added in place of an equal amount of sucrose. A control ration was also employed consisting of the basal ration with thyroid omitted and replaced by an equal amount of sucrose. Forty male rats of the Long-Evans strain were selected at 22 to 25 days of age and an average weight of 50.6 g for the present experiment. Animals were kept in metal cages with raised screen bottoms to prevent access to feces and were fed the above diets *ad lib* (10 rats per group). Feeding was continued for 28 days. Results are summarized in Table I.

Findings are in agreement with those previously reported for the female rat(11,12). Massive doses of thyroid retarded the gain in

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

‡ Sure's Salt Mixture No. 13.

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§ Thyroid Powder, U.S.P., Armour and Co., Chicago, Ill.

‖ In view of the increased requirements for thiamine, pyridoxine, and pantothenic acid in the hyperthyroid rat(4), the B vitamins in the present experiment were administered in excessive amounts in order to assure an adequacy of these factors in the diet.

¶ 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.

** Extracted Liver Residue, Wilson Laboratories, Chicago, Ill. This liver fraction consists of the coagulated, water-insoluble material remaining after the removal of the extractable water-soluble substances.

TABLE I.
Effects of Vitamin $B_{12}$ and Liver Residue on the Body and Gonadal Weight of Immature Male Rats fed Massive Doses of Desiccated Thyroid, 10 Rats in Each Experiment.

| Supplements fed with basal ration | % thyroid | Initial body wt, g | Avg gain in body wt after 28 days feeding, g* | Avg wt of testicles, mg* |
|---|---|---|---|---|
| None | .5 | 52.3 | 62.1 ± 5.4 (9) | 1.22 ± .12 (9) |
| Vit. $B_{12}$ | .5 | 50.6 | 67.6 ± 3.1 (9) | 1.46 ± .10 (9) |
| Liver residue | .5 | 50.1 | 133.9 ± 4.3 (10) | 2.19 ± .09 (10) |
| None | .0 | 49.5 | 116.3 ± 6.1 (10) | 2.07 ± .07 (10) |

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 $\sqrt{\frac{\overline{\epsilon d^2}}{n}} / \sqrt{n}$ where "d" is the deviation from the mean and "n" is the number of observations.

body and gonadal weight of immature male rats. These effects were completly counteracted by the administration of liver residue at a level of 10% of the diet. Crystalline vitamin $B_{12}$ at a level of 30 $\mu$ per kg of diet was without significant effect. It is apparent that the growth retardation and inhibition of testicular development of male rats fed massive doses of thyroid was due, under conditions of the present experiment, to a deficiency of some factor other than vitamin $B_{12}$. As in the case of female rats, water-insoluble liver residue was a potent source of the missing factor.

In view of the increased nutritional requirements of the hyperthyroid animal, it is recognized that unless precautions are taken to the contrary a multiple dietary deficiency may readily develop as a result of thyroid feeding. Under such circumstances growth may be stimulated by the administration of any one of the missing nutrients, but gain in body weight will not reach normal levels until all the essential nutrients are present in adequate amounts. Betheil and Lardy(8) found that vitamin $B_{12}$ partially counteracted the growth retardation of immature male rats fed massive doses of thyroid in a ration similar to that employed in the present experiment. Gain in body weight was significantly less, however, than that occurring in animals fed a similar diet supplemented with 10% whole liver. It would appear, therefore, that Betheil and Lardy were dealing with a multiple nutritional deficiency. Vitamin $B_{12}$ was one of the deficient factors, but optimum growth was not obtained unless animals were supplied with at least one additional nutrient, present in whole liver powder and presumably identical with the "anti-thyrotoxic factor of liver". Since significant amounts of vitamin $B_{12}$ can be stored in the tissues of the young rat during the period of pregnancy and lactation(14), the occurrence of a vitamin $B_{12}$ deficiency and its extent following thyroid feeding is dependent in considerable degree on the vitamin $B_{12}$ content of the pretest dietary regime. It would appear that differences in the pretest dietary regime or differences in strain requirements for vitamin $B_{12}$ were responsible, at least in part, for the diverse results obtained by Betheil and Lardy from those reported above.

*Summary.* Immature male rats were fed massive doses of thyroid in conjunction with a purified ration containing casein as the dietary protein. A marked retardation occurred both in body and gonadal weight. These effects were completly counteracted by the administration of liver residue. Crystalline vitamin $B_{12}$, however, was without significant effect.

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