The Influence of Hyper- and Hypothyroidism on the Incidence of Lymphatic Leukemia of AKR Mice

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While much is known about the influence of the estrogens, androgens, and adrenocortical hormones on experimental leukemia, very little has been published on the effect of the thyroid gland on this disease. However, it has been assumed that the thyroid might play a role in leukemia, not much as lymphocytosis is frequently observed in hyperthyroid subjects; moreover, growth of lymphatic tissue occurs in thyroxine-treated animals, while thyroidectomy has the reverse effect (12, 13, 17). Several authors have also described elevated metabolic rates in leukemia (9, 11, 15, 21), some even claiming that the basal metabolic rate was a truer indicator of the severity of the process than the leukocyte count (9, 11, 15, 19). Furthermore, the blood iodine was described as being elevated in a considerable number of patients with lymphoid leukemia (21). Finally, thyroidectomy has been claimed to produce a striking remission in a case of chronic lymphatic leukemia (5).

While the reports thus far described have tended to suggest that the thyroid is hyperactive in lymphatic leukemia, the following findings contradict this: the serum cholesterol level of patients with lymphatic leukemia was normal on the average (21), as was also the thyroidal I\(^{131}\) uptake in fourteen of fifteen leukemic patients, ten of whom had increases in the basal metabolic rate (1). This suggests the nonparticipation of the thyroid gland in the elevation of the basal metabolic rate of leukemic patients, and some authors have suggested protein catabolism as the major factor in the increased gaseous metabolism (2). Furthermore, chronic thyroid administration did not significantly alter the incidence of radiation-induced tumors in C57BL mice (14), and thiouracil similarly failed to influence lymphoma growth in R3 mice (22) or in humans (3). Methylthiouracil was also ineffective in two cases of myelogenous leukemia (10). However, a preliminary experiment in our laboratory revealed that thyroxine decreased, while thiouracil increased the incidence of spontaneous leukemia of AKR mice (8). The experiments described in this report were designed to further elucidate the role of the thyroid gland in the leukemia of AKR mice, a strain in which a majority of mice develop lymphatic leukemia spontaneously.

MATERIALS AND METHODS

Because the level of thyroid function definitely influences the body weight and because of the evidence of a relationship between body weight and the incidence of leukemia in AKR mice (20), it was essential to determine whether any effects of the thyroid gland on the spontaneous leukemia of AKR mice were direct or mediated by its effect on body weight. Accordingly, three experiments were devised.

In Experiment 1, thyroxine, 5 \(\mu g\)/gm body weight, was administered to one group, causing body weight loss; 0.1 per cent thiouracil was administered in the drinking water and by injection to a second group in which it caused body weight gain. A control group received injections of the slightly alkaline medium in which thyroxine or thiouracil was administered to the other groups.

Experiments 2 and 3 were really two parts of the same experiment, in which an attempt was made to render hyperthyroid AKR mice heavier than hypothyroid mice. In Experiment 2, thiouracil was administered as in Experiment 1, but the animals in this group were fed Remington's iodine-deficient diet in place of Purina Fox Chow (18). Body growth was inhibited in this group. Another group received the same diet, but with 1-2 \(\mu g\) iodine daily/mouse in the place of thiouracil. A third group received only the Remington diet, while a fourth received Purina Fox Chow only. In Experiment 3, thyroxine was administered in the same dosage as in Experiment 1, and in addition large amounts of dietary supplements were given to prevent body weight loss (4, 6). A control group received the same diet, but with injections of the medium in which the

1The Purina Fox Chow was carefully mixed with food supplements in the following proportions: Purina Fox Chow, 990 gm.; extracted liver residue, 210 gm.; thiamine hydrochloride, 72 mg.; nicotinic acid, 60.0 mg.; 2-methyl-naphthoquinone, 5.0 mg.; choline chloride, 1.2 mg. In addition, each mouse received the following supplement in the food 3 times a week: cottonseed oil, 625 mg.; alpha-tocopherol acetate, 1.9 mg.; 83 units of vitamin A and 18 U.S.P. units of vitamin D\(_3\). Excessive amounts of these vitamins were administered to assure an adequacy of these factors in the diet of the hyperthyroid animal whose requirements were above normal (4, 6).

The liver residue was prepared by Wilson Laboratories, Chicago, Ill., and consisted of the coagulated water-insoluble material remaining after removal of the extractable water-soluble substances.
thyroxine was dissolved. The animals were allowed to live out their "natural" lives and at death were autopsied.

The influence of thyroxine on transplanted lymphatic leukemia in AKR mice was also investigated in two experiments. In the first one (Experiment 4), 0.2 cc. Locke's solution containing approximately 1 million cells from the thymus, lymph nodes, and spleen of an AKR mouse ill with lymphatic leukemia were injected intraperitoneally into each of 33 AKR mice. Thyroxine injections (1 µg/g body weight) were begun the day after transplantation in one group of seventeen mice, while the remainder received injections of the medium at the same time, and served as controls.

In Experiment 5, approximately 3½ million lymphatic leukemia cells in 0.1 cc. Krebs Ringer phosphate medium with added glucose and heparin were injected subcutaneously into the groin of each of 25 AKR mice. One hundred µg. of thyroxine was injected into thirteen of the mice twice weekly for 18 days before transplantation and once weekly thereafter; the medium was injected into the remaining twelve mice at the same time.

The administration of thyroxine and medium in Experiments 1, 2, and 4 was made subcutaneously once weekly until all the animals died. The DL-sodium salt of thyroxine1 was used in all experiments.

Female mice were used in Experiments 2, 3, and 5; both sexes were used in Experiments 1 and 4, in which the proportion of male to female mice of any one group was the same as that of any other group of the same experiment. Similarly, in all experiments, animals of any one group had litter-mates in the other groups of the same experiment and were all in the preleukemic stage—that is, younger than 5 months old when treatment was started.

Oxygen consumption determinations were made in all experiments by a method described previously (7). As expected, thyroxine increased and thiouracil decreased the oxygen consumption. In some experiments thyroid weights were also determined to assess whether thiouracil and the iodine-deficient diet were goitrogenic as expected.

Two types of statistical analysis were utilized: χ² tests in testing the significance of differences in incidence of spontaneous leukemia between different groups and t tests in testing the significance of differences in survival time.

1 Prepared by British Drug Houses.

RESULTS

Experiment 1.—Food consumption was least in thiouracil-treated mice and greatest in thyroxine-treated mice (Chart 1, P < 0.001).

The body weight data may be considered in two periods: one from the 2d to the 6th month of the experiment, the other part from the 6th month to the time when the determinations were stopped (Chart 1). In the first period, the control mice were the heaviest, and the thiouracil-treated mice the lightest, while the thyroxine-treated animals weighed only slightly more than the latter (P < 0.001). In the second period, the body weight of the thiouracil-treated mice increased until they became the heaviest group, while the thyroxine-treated mice were the lightest (P < 0.001). Most of the mice of this experiment died in this second period.

The loss of body weight which occurred in the untreated mice after about the 4th month of the experiment, that is, when they were around 8 months of age, was the usual loss that occurs in AKR mice during aging. On the other hand, the thyroxine-treated mice began to lose weight after the 2d month of the experiment, at which time they were 6 months old, and their body weight remained below that of the controls for the next 8 months. This was owing to the injection of large amounts of thyroxine.

In this experiment the incidence of spontaneous leukemia was highest in the thiouracil-treated mice and lowest in the thyroxine-treated animals, this difference being significant (Table 1, Experiment 1, 0.02 > P > 0.01). A treatment period of many months appeared to be necessary to reveal
differences in spontaneous leukemic incidence.

There was no significant difference in survival time between the mice of the various groups if all the mice in any one group were considered together, whatever the cause of death (Table 1, Experiment 1, $1.0 > P > 0.8$). In the control and thyroxine-treated groups, but not in the thiouracil-treated animals, mice that died from causes other than lymphatic leukemia lived longer (Table 1, $0.01 > P > 0.001$). Also, male mice in this experiment lived $386 \pm 14$ days as against $296 \pm 11$ for the females ($P < 0.001$).

Experiment 2.—The food consumption in gm/day/mouse measured for a 10-day period when the mice were about a year old was $3.5 \pm 0.6$, $3.2 \pm 0.5$, $3.4 \pm 0.4$, and $2.2 \pm 0.2$ for Groups I, II, III, and IV, respectively. There were no significant differences between the food intakes of Group I, II, and III ($0.9 > P > 0.6$). However, mice of Group IV (thiouracil-treated) ate significantly less than those of combined Groups I, II, and III ($0.01 > P > 0.001$) and had a consistently lower body weight (Chart 2).

There was no significant difference in the incidence of lymphatic leukemia between the control group and the groups which received the iodine-deficient diet alone or together with iodide in the drinking water (Table 1, $0.7 > P > 0.5$). On the other hand, the group which received both Remington's diet and thiouracil showed a significantly decreased incidence of lymphatic leukemia when compared with all the other groups in this experiment (Table 1, Experiment 2, $0.01 > P > 0.001$).

There was no significant difference in the survival time between the mice of the various groups if all the mice are considered together, whatever the cause of death (Table 1, Experiment 2, $P > 0.20$). Also, there was no significant difference in survival time between the mice dead from leukemia and those dead from other causes in the

<table>
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<th>EXPERIMENT</th>
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<tr>
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<td>Nonleukemic deaths</td>
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<td>1</td>
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<td>II. Thyroxine</td>
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<td>I. Control</td>
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<td>II. Iodine-deficient diet</td>
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<td>III. Iodine-deficient diet + iodine</td>
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<td>IV. Iodine-deficient diet + thiouracil</td>
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<td>I. Control</td>
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<td>II. Thyroxine</td>
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group receiving both thiouracil and an iodine-deficient diet (Table 1, Experiment 2, Group IV; 0.9 > P > 0.8). It was not possible to reliably assess the statistical significance of such differences in survival time in the other groups, inasmuch as in these too few mice died from causes other than leukemia.

The fact that body growth was permanently inhibited only when thiouracil and the iodine-deficient diet were given together and did not occur when fed separately suggests that hormonal synthesis in the thyroid gland was less efficiently inhibited by the iodine-deficient diet or by thiouracil fed separately. Support for this is provided by the following facts: (a) While Remington's iodine-deficient diet by itself significantly increased the thyroid weight of AKR mice from 3.4 ± 0.3 mg. to 10.5 ± 2.2 mg. (0.01 > P > 0.001), the additional administration of 0.1 per cent thiouracil in the drinking water caused a further and significant increase in thyroid size to 23.0 ± 1.8 mg. (P < 0.001). As expected, the addition of iodide to the iodine-deficient diet prevented the goitrogenic effect of the latter and yielded thyroids weighing 3.9 ± 0.4 mg., which is not significantly different from normal (0.4 > P > 0.8). (b) Thiouracil (0.1 per cent) administered by itself, as in Experiment 1, did not quite double the thyroid weight. This was considerably less than the seven-fold increase which occurred when 0.1 per cent thiouracil and Remington's iodine-deficient diet were fed simultaneously in Experiment 2. (c) The pituitary weights per 100 gm. body weight of the AKR mice receiving the iodine-deficient diet alone (7.6 ± 1.4 mg.) were not significantly different from those of the controls (7.9 ± 1.1 mg.) or from those that received iodide in addition to Remington's diet (8.1 ± 1.9 mg.) (0.9 > P > 0.8). On the other hand, the pituitaries of the group that received both Remington's diet and thiouracil had significantly larger pituitaries (19.8 ± 1.2 mg.) than did any of the other groups (P < 0.001).

Experiment 3.—The thyroxine-treated mice had a significantly higher body weight and a significantly higher food intake than the controls (Chart 3; P < 0.01). There was no significant difference either in the incidence of lymphatic leukemia between the two groups (Table 1, Experiment 3, P > 0.20) or in the mean survival time (Table 1, Experiment 3, 0.90 > P > 0.10).

While the addition of vitamins to the diet protected the AKR mice against the toxic effects of large doses of thyroxine as seen by the prevention of body weight loss, it not only failed to prolong life but actually shortened it. This was apparent when the survival time of the mice of Experiment 1 was compared with that of the animals in Experiment 3. The thyroxine-treated mice of Experiment 3 lived a significantly shorter time than did those of Experiment 1 (Table 1; P < 0.001), there being no significant difference in the survival times of the control mice of Experiments 1 and 3 (Table 1; 0.2 > P > 0.1). Presumably because the body weight of the vitamin-fed AKR mice was not decreased by the thyroxine treatment, they tended to develop spontaneous leukemia sooner and hence die sooner. This is of interest inasmuch as such dietary supplements not only restored growth to normal, but also prolonged the survival time of immature thyroid-fed rats (4, 6). However, these rats did not have the tendency to develop leukemia spontaneously.

Experiment 4.—There was no significant difference in survival time between the control and thyroxine-treated groups (Table 2).

Experiment 5.—At the time of implantation of leukemic cells, the thyroxine-treated mice in this experiment were hyperthyroid, as revealed by a significantly higher food and water intake and basal metabolic rate than were observed in the controls (P < 0.001). However, there was no significant difference in body weight between the two groups (P > 0.20).

After the implantation with cells, there was a significant decrease in food and water intake of the control mice, especially in the last 12 days of the experiment (P < 0.05). However, the body weight
did not decrease significantly in the same period. Similar comparisons of the food and water intake could not be made in the thyroxine-treated group, because, at the time when the leukemic cells were injected, the thyroxine injections were reduced from twice to once weekly. Thus, any decrease in food and water intake occurring thereafter could have been due either to the diminished amount of thyroxine administered or to the injection of leukemic cells.

There was no significant difference in survival time between the control and thyroxine-treated groups (Table 2).

**DISCUSSION**

When the thiouracil-treated mice weighed more than the thyroxine-treated mice (Chart 1), the to influence per se the incidence of spontaneous lymphatic leukemia of AKR mice. Rather, whatever effect these states appear to have on the incidence of this disease appears to depend on the effect these states have on the body weight, a decrease in body weight resulting in a decreased incidence.

In experiments on the influence of underfeeding on the inhibition of tumor formation, the question arises whether it is the underfeeding per se that has this effect or whether the consequent inhibition of body growth is of greater importance. This question could not be answered in experiments described by others where the B.M.R. was not altered, inasmuch as in such cases underfeeding leads inevitably to underweight. However, the results of Experiment 1 show that inhibition of body

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<td><strong>The Effect of Thyroxine on the Survival Time of AKR Mice Injected with Leukemic Lymphocytes</strong></td>
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<td><strong>Survival after transplantation</strong></td>
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Mean ± standard error (days) 17.5 ± 0.8 16.0 ± 0.4
Significance of difference (P) 0.2 > P > 0.1

Mean ± standard error (days) 34.7 ± 1.9 36.5 ± 2.0
Significance of difference (P) 0.6 > P > 0.5

The incidence of leukemia was higher in the former group (Table 1, Experiment 1, 0.01 > P > 0.001). On the other hand, when the thiouracil-treated mice weighed less than the thyroxine-treated animals (compare the body weight of the thiouracil-treated mice of Experiment 2 with that of thyroxine-treated mice of Experiment 3), then the incidence of leukemia was significantly lower in the thiouracil-treated animals (Table 1, Experiments 2 and 3, 0.05 > P > 0.02). Therefore, hypo- and hyperthyroidism of long duration do not appear to influence per se the incidence of spontaneous lymphatic leukemia of AKR mice. Rather, whatever effect these states appear to have on the incidence of this disease appears to depend on the effect these states have on the body weight, a decrease in body weight resulting in a decreased incidence.

In experiments on the influence of underfeeding on the inhibition of tumor formation, the question arises whether it is the underfeeding per se that has this effect or whether the consequent inhibition of body growth is of greater importance. This question could not be answered in experiments described by others where the B.M.R. was not altered, inasmuch as in such cases underfeeding leads inevitably to underweight. However, the results of Experiment 1 show that inhibition of body growth is more important for the inhibition of the lymphoid tumor formation than is underfeeding, inasmuch as the thyroxine-treated mice of that experiment weighed less and had a lower incidence of spontaneous lymphatic leukemia than did the thiouracil-treated mice, despite the fact that the former group of mice consistently ate more (Chart 1). However, the inhibition of body growth obtained in this experiment did not prolong the survival time of the thyroxine-treated animals over that of the controls.

Thyroxine treatment, whether started several weeks before or the day after the implantation of leukemic cells, failed to influence the survival time of the AKR mice. While no experiments were conducted in our laboratory on the effect of thyroid deficiency on the survival time of AKR mice with...
a transplanted leukemia, one report from another laboratory describes a significant prolongation of life in such mice when rendered hypothyroid by iodine-131. Apparently, this effect was not owing to the fact that the hypothyroid mice were eating less (16).

SUMMARY

AKR mice rendered hyperthyroid by thyroxine had a significantly lower incidence of spontaneous lymphatic leukemia than did AKR mice rendered hypothyroid by thiouracil. In this experiment, the hyperthyroid mice weighed less than the hypothyroid animals. However, when AKR mice were treated with thyroxine, but in addition were fed supplements of vitamins and liver to protect them against the toxic effects of the hormone, they had a higher incidence of spontaneous lymphatic leukemia than did AKR mice rendered hypothyroid by thiouracil and an iodine-deficient diet. In these experiments the hyperthyroid mice weighed more than the hypothyroid animals. Hence, the influence of hyper- or hypothyroidism on the incidence of spontaneous lymphatic leukemia of AKR mice depends on what effect these states have on the body weight.

Hyper- and hypothyroidism did not influence the survival time of AKR mice that were allowed to die "naturally," nor did hyperthyroidism significantly alter the length of survival of AKR mice with a transplanted lymphatic leukemia.

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The Influence of Hyper- and Hypothyroidism on the Incidence of Lymphatic Leukemia of AKR Mice

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