Hepatic Carcinogenesis in Thyroidectomized Rats: Apparent Blockade at the Stage of Initiation

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Summary

The protective effect of thyroidectomy against the induction of liver tumors by 2-acetylamino-fluorene in rats is confirmed. Evidence is presented indicating that the effect of thyroidectomy may be attributable to blockade of liver carcinogenesis at or before the stage of initiation rather than to retardation of tumor progression in such animals.

Introduction

The induction of liver tumors in rats fed 2-acetylamino-fluorene (AAF) was found by Paschkis et al. (17) to be partially inhibited by concurrent treatment with thiouracil. This result was confirmed by Leatham and Barksen (12) who also excluded, by pair feeding, the possibility that reduced food intake (25) in the goitrogen-treated animals might have accounted for the strikingly lower tumor incidences obtained. Paschkis et al. (18) later presented some evidence that the anticarcinogenic effect might be attributable to disordered uracil metabolism, but that it was in fact due to chronic hypothyroidism was shown by Bielschowsky and Hall (4, 5) who used surgically thyroidectomized rats. The inhibition of liver carcinogenesis by thyroidectomy in their experiments was apparently absolute: neither AAF nor 2-amino-fluorene (AF) treatments resulted in a single liver tumor in the completely thyroidectomized animals, whereas virtually 100% of their intact controls receiving the same total dose of carcinogen developed malignant hepatomas. The treatment produced extra-hepatic tumors (of Zymbal's gland, breast, lung, and other tissues) with approximately the same frequency in both the thyroidectomized and intact animals. With respect to the liver, the degree of thyroid deficiency was found to be crucial, since even a very small thyroid remnant permitted the development of small but significant numbers of liver tumors. These results have been recently confirmed (9, 10). An equally powerful and specific inhibition of hepatocarcinogenesis by azo dyes as well as by AF and AAF has been demonstrated in completely hypophysectomized rats (3, 11) and probably obtains also in adrenalectomized rats (19).

These experiments have raised several important problems which are still unresolved, such as the identity of the hormones involved as permissive agents in liver carcinogenesis, why the anticarcinogenic protective effect of the endocrine ablations is effective only in the liver, and at what stage of the carcinogenic process the inhibitory effect of the endocrine deficiencies is exerted. This paper is concerned with only the last of these questions. It has already been shown that, whereas thyroidectomy performed before administration of the carcinogen completely inhibited hepatocarcinogenesis, there was no definite effect of the operation if it was performed after an effective dose of AAF had already been given (5). On the other hand, liver tumors can be induced in thyroidectomized rats by AF if they are simultaneously treated with cortisone (7), iodide (10), or crude growth hormone preparation (2). The earlier phases of tumor development therefore seemed likely to be the stage affected and, in order to discriminate more clearly between the possibilities of failure of initiation, or, alternatively, an extreme retardation of subsequent progression of initiated liver cells in thyroidectomized rats, the following experiment was performed.

Materials and Methods

Male Wistar rats were surgically thyroparathyroidectomized at 4 weeks of age, and subsequently, with their intact controls, maintained on a special diet previously described (9, 10). Treatment with AF began at age 8 weeks in all groups of rats, except for untreated intact control animals; a total dose of approximately 270 mg of AF/animal was given by painting the shaved dorsal skin 3 times weekly for 30 weeks with a 4% solution of AF in acetone. Six thyroidectomized rats (Group 2), treated with AF, were left without further treatment to again confirm the inhibitory effect of thyroidectomy on liver carcinogenesis, and 6 intact control rats (Group 1) were similarly treated with AF to confirm the adequacy of the carcinogenic stimulus. No spontaneous hepatomas occurred in untreated control rats. The main group of 16 thyroidectomized rats (Groups 3a and 3b) were allowed to rest for 2 weeks after completion of a course of AF treatment, the same as for the 2 groups of controls, to permit excretion or metabolism of any residual carcinogen, and were then provided with new cages to insure complete removal from any traces of AF or its metabolites. From the 32nd week of the experiment onward, these animals were provided with thyroid digest in their drinking water continuously until death. The thyroid hormone preparation was made by the method of T. H. Kennedy (3). The amounts of the thyroid solution consumed were measured daily, and the amount of the digest taken provided the equivalent of approximately 1.25 µg of L-thyroxine/100-gm of rat/day, by calculation. The dosage administered was that previously found to permit optimal growth and weight gain in recently thyroidectomized rats (9). In addition, half of the thyroid-treated group (Group

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3b) was given daily doses of 0.5 mg of cortisone acetate p.o., as in an experiment described earlier (7). The experimental design is summarized in Chart 1. The intact rats were killed with carbon monoxide when liver tumors became palpable; the thyroidectomized rats, both the hormone-treated group and their controls, were kept until ill health or the size of extrahepatic tumors made it advisable to kill them, and the experiment was terminated 72 weeks after carcinogen treatment began. The histologic methods were those previously described (10).

Results

CONTROL GROUPS. The AF treatment regime induced multiple malignant hepatomas in all of the intact rats (Group 1), with a mean latent period of 46 weeks and the 1st tumor appearing by 29 weeks. The pathologic criteria of Stewart and Snell (23) were adopted, and the liver tumors conformed to their descriptions. Although the same amount of AF applied to thyroidectomized rats represented a dose rate more than twice as high per unit body weight, no neoplasms at all occurred in the livers of the control thyroidectomized animals (Group 2) up to the time of termination at 80 weeks of age. In addition, the livers of thyroidectomized animals failed to hypertrophy and showed virtually no sign of injury, having only a few very small solitary cysts lined by an extremely flattened epithelium. The complete suppression of hepatocarcinogenesis by thyroidectomy in spite of treatment with high doses of AF again confirms our previous results (4, 5, 7-9). Extrahepatic tumors still occurred, however (Table 1).

RATS WITH SECONDARY THYROID SUBSTITUTION. Since the additional administration of cortisone in Group 3b was found to have no significant effects on organ weights or morphology, the 2 groups of rats receiving thyroid hormone supplements (Groups 3a and 3b) are considered together. There was a latent period of about 2 weeks after addition of thyroid digest to the drinking fluid, and then somatic growth was resumed (Chart 2). The animals had almost doubled their weight and were still slowly growing when the rats were 80 weeks of age, at which time the experiment was terminated because of rapidly growing tumors of the external ear ducts (Zymbal's gland carcinomas) and deformation of the incisor teeth. Many of the Zymbal gland tumors had been present several weeks before thyroid substitution therapy began and, in several cases, there was a remarkable acceleration in the growth rate of these tumors after introduction of thyroid treatment.

The livers of the thyroid-treated animals of Group 3 were strikingly hypertrophied, but apart from a small number of tiny, solitary, atrophic biliary cysts similar to those seen in the thyroidectomized controls (Group 2), they showed no sign of AF action. Whereas body weight increased by more than 60% on the average during the period of thyroid treatment, absolute liver weights were nearly doubled. The amounts of fat and glycogen demonstrable histologically in the liver were normal, and the larger livers were considered to represent a true hypertrophy. Binucleate liver cells were unusually common, but no mitotic figures were seen. None of these livers contained tumors, nodules, or any other parenchymal lesion in histologic sections.

Treatment with thyroid digest also produced striking changes in the adenohypophysis, adrenals, testes, and seminal vesicles, all in the direction of normality. The pituitaries contained approximately 70% of the normal population of stainable acidophils, in contrast to the complete absence of stainable acidophils in the completely thyroidectomized rats of Group 2. The adrenals were pink-cream in color, and were considerably enlarged (Table 1). In the testes spermatogenesis was normal, and the interstitial cells were restored to normal proportions. That androgen secretion was restored was evident from the striking histologic repair of the seminal vesicles, which regained normal weight (Table 1) and contained abundant secretion. Bone marrow appeared normal, and the hematocrits returned to normal levels. General carcass fat and other tissue were present in normal proportions and no clinical evidence of thyrotoxicosis was present in life.
TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (intact + AF)*</th>
<th>Group 2 (Tx + AF)*</th>
<th>Group 3 (Tx + AF + Th)*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Liver</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gm</td>
<td>15.8</td>
<td>3.9</td>
<td>8.5</td>
</tr>
<tr>
<td>gm/100 gm body wt.</td>
<td>4.23 ± 0.12</td>
<td>3.36 ± 0.51</td>
<td>5.01 ± 0.73</td>
</tr>
<tr>
<td>Adrenal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mg</td>
<td>42.3 ± 0.83</td>
<td>23.5 ± 0.24</td>
<td>66.8 ± 3.41</td>
</tr>
<tr>
<td>mg/100 gm</td>
<td>12.60 ± 0.18</td>
<td>16.0 ± 0.17</td>
<td>19.7 ± 1.85</td>
</tr>
<tr>
<td>Seminal vesicle, mg/100 gm</td>
<td>422.3 ± 129.0</td>
<td>127.3 ± 22.1</td>
<td>431.0 ± 156.4</td>
</tr>
<tr>
<td><strong>Hematocrit, vol %</strong></td>
<td>45.8 ± 1.15</td>
<td>38.2 ± 0.98</td>
<td>42.5 ± 2.31</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Tumor incidences in rats with</strong></th>
<th>Group 1 (intact + AF)*</th>
<th>Group 2 (Tx + AF)*</th>
<th>Group 3 (Tx + AF + Th)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver carcinoma</td>
<td>6/6</td>
<td>0/6</td>
<td>0/16</td>
</tr>
<tr>
<td>Latency (wk): mean, range</td>
<td>46, 29-56</td>
<td>2/6</td>
<td>10/16</td>
</tr>
<tr>
<td>Zymbal's gland carcinoma</td>
<td>3/6</td>
<td>1/6</td>
<td>3/16</td>
</tr>
<tr>
<td>Lung adenoma</td>
<td>2/6</td>
<td>0/6</td>
<td>1/16</td>
</tr>
<tr>
<td>Breast carcinoma</td>
<td>1/6</td>
<td></td>
<td></td>
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</tbody>
</table>

* Tx, Thyroidectomized; AF, 2-aminofluorene; Th, thyroid hormone supplement.
+ Mean ± S.E.

Discussion

The point at which the inhibitory effect of thyroidectomy or hypophysectomy was exerted in the blocking of liver carcinogenesis in rats treated with aromatic amines or azo dyes was not established in previous work. Various authors have shown earlier that supplying increased amount of thyroid (13, 16, 20), pituitary (26), and adrenal cortical hormones (6, 8) to intact rats accelerated the appearance of chemically induced liver tumors, and there is converse evidence that relative hypothyroidism (1, 17, 22) tends to prolong the latent period for hepatomas. It was thus possible that previous results showing an anticanicogenic effect of thyroidectomy could be explained by a retardation in the rate of progression of any latent tumor foci initiated in the liver by treatment with AF. An alternative possibility was that treatment with AF was no longer sufficient to initiate the earliest neoplastic transformation of liver cells in the abnormal internal environment resulting from thyroidectomy. Since the argument turns upon a negative phenomenon proof can hardly be claimed, but it is considered that the present experimental results support the latter hypothesis indirectly, by strongly denying the alternative of retarded progression as an explanation for the effect of thyroidectomy. First, the complete absence of liver neoplasms in AF-treated thyroidectomized rats even when they were kept to age 80 weeks, compared with the hepatoma latencies in intact animals, indicated the slowing of progression, if that were the explanation, must have been extreme in degree. Such degree of retardation would seem irreconcilable with the previously reported minor effect of thyroidectomy when it was performed some time after an effective dose of carcinogen had been administered (5). Second, if initiation of neoplastic change had in fact occurred in the thyroidectomized rats of Group 3, then progression to form fully developed hepatomas could reasonably be expected during the 2nd phase of the experiment, since all of the conditions known to favor tumor growth (1, 9, 26) were evidently present during the period of almost 10 months of thyroid replacement therapy. The rats grew and were well nourished, their hematocrits returned to normal, and the endocrine organs secreted near normal amounts of pituitary, adrenal cortical, and gonadal hormones, as far as could be judged from their morphology and that of the target tissues. Indeed, the hepatomegaly (16) and adrenocorticalomegaly (15) in these rats may imply the presence of excess amounts of thyroid and adrenocortical hor-
mones, both of which have been shown to accelerate hepatoma induction in rats treated with liver carcinogens (8, 16), although Reuber (21) recently reported finding no enhancement of hepatocarcinogenesis by adrenocortical hormones in rats treated with N-2-diacylaminofluorene. The relative hepatomegaly, and acceleration of the growth of ear duct (Zymbal) tumors observed, also favor the view that conditions in the thyroid-treated rats of the present experiments were favorable for the progression of any latent foci of neoplastic cells that may have existed in the liver.

The fact that no tumors, nor any other parenchymal lesions, occurred in the livers of thyroidectomized rats treated 1st with AF and later with thyroid hormones, thus implies that the blockade of hepatocarcinogenesis due to thyroidectomy occurs at or before the stage of initiation. This view would be consistent with the previously quoted results of Bielschowsky and Hall (5), and also with recent results of Symeonidis (24) who found the inhibition of azo dye carcinogenesis by combined adrenalectomy and treatment with desoxycorticosterone acetate was only effective if the operations were done during the earlier weeks of carcinogen treatment. Lotlikar et al. (14) have shown that the metabolic conversion of AAF to the N-hydroxy metabolite, N-OH-AAF, is considerably less efficient in various endocrine-deficient states, which are also states in which inhibition of hepatocarcinogenesis has been found by others in long term experiments. Inefficient metabolism of AF towards the proximately carcinogenic molecular species therefore seems likely to provide at least a partial explanation for the protective effects of thyroidectomy and some other endocrine ablations. However the apparent specificity of the protection for the liver, while extrahepatic tumors still develop freely in thyroidectomized and hypophysectomized rats (3, 5, 9, 10) indicates that other mechanisms may also be involved in this anticarcinogenic phenomenon.

References
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