Influence of Thiouracil on Carcinoma Induced by 2-Acetaminofluorene*

K. E. Paschkis, M.D., A. Cantarow, M.D., and J. Stasney, M.D.

(From the Jefferson Medical College, Philadelphia 7, Pa.)

(Received for publication February 18, 1948)

Bielschowsky (2) reported the occurrence of carcinoma in the rat's thyroid gland made hyperplastic by administration of allyl thiourea when the carcinogen, 2-acetaminofluorene (AAF) was administered simultaneously. Applying a similar principle, we investigated the effects of administration of this carcinogen to rats in which hyperplasia of target organs was produced by injection of male and female sex hormones. No evidence of malignancy was observed in these organs under these circumstances, but it was found that simultaneous administration of sex hormones and AAF enhanced the development of carcinoma of the liver (4). At the same time, experiments were conducted designed to repeat the work of Bielschowsky, with minor variations. His observation regarding the development of thyroid malignancy was confirmed. It was noted also that the liver of rats receiving thiouracil was protected against the carcinogenic effect of AAF.

The present communication reports (a) studies of the protective action of thiouracil against the hepatic lesions caused by AAF and (b) the effects on the thyroid gland of simultaneous administration of AAF and thiouracil. Inasmuch as thiouracil was found to protect the liver against carcinoma induced by AAF, and sex hormones enhanced the development of liver cancer, study of the protective action of thiouracil was extended to animals receiving AAF and sex hormones simultaneously. The effect of thiouracil on the development of liver cancer by p-dimethylaminoazobenzene was also investigated.

In an attempt to study the mechanism by which thiouracil exerts its protective action, the effect of methionine was investigated, inasmuch as it has been suggested that thiouracil protects against dietary cirrhosis by lowering the requirement for methionine (6). The possible role of hypothyroidism in this protective mechanism was studied in experiments on thyroidectomized rats and on rats receiving thiouracil and thyroxin simultaneously.

MATERIAL AND METHODS

The rats employed in this study were of the Sherman strain and Wistar descendants, weighing 75 to 100 gm. at the beginning of the experiment. They were given the carcinogenic diet and the various experimental supplements until death or sacrifice. AAF (0.03 per cent) was incorporated in a corn meal diet (16 per cent protein) reinforced by powdered brewers' yeast, as previously reported (4, 15). A motor-driven dough mixer was used to insure thorough mixture of the dietary ingredients. p-dimethylaminoazobenzene was given in a high-fat diet (20 per cent corn oil) described by Kline and his associates (7).

Thiouracil was administered in the drinking water in concentrations of 0.05 or 0.1 per cent.

Testosterone propionate was injected (in sesame oil) in the dosage of 0.5 mgm., three times weekly and estradiol dipropionate (in sesame oil), 10 gamma, three times weekly.

Methionine was dissolved in the drinking water. In order to insure complete intake, 40 mgm. were dissolved in 10 cc. of water and offered in the evening. During the following day, the empty drinking bottles were replaced by bottles containing tap water, which was, in the evening, again replaced by the methionine solution.

Crystalline 1-thyroxine was dissolved in slightly alkalized water and was injected subcutaneously daily in doses of 5 to 10 gamma.

The animals were killed by exsanguination under light ether anesthesia. Tissues were fixed in Bouin solution, imbedded in paraffin, sectioned, and stained with hematoxylin and eosin, and, in some instances, with Masson's trichrome stain. Organ weights were taken rapidly on a Roller-Smith torsion balance, on fresh tissues freed from fat and connective tissue.

RESULTS

The findings in the various experimental groups are presented in Tables I to IV.

Effect of thiouracil on hepatic carcinoma (Table I).—The histologic characteristics of the hepatic lesions produced by AAF have been described elsewhere (4, 15). The aggravating influence in this connection of testosterone is not readily apparent from the figures included in the table. Because of the high incidence of hepatic carcinoma in male rats receiving AAF alone for more than 200 days, this influence is exhibited chiefly in increased in-
cidence of cancer of the liver in males treated less than 200 days (58 per cent after AAF alone; 90 per cent in testosterone-treated) and in the greater average weight of the cancerous livers in the testosterone-treated animals (15). It is apparent that the protective influence of thiouracil against hepatic carcinoma induced by AAF is exerted also against the aggravating effect of testosterone. The incidence of carcinoma of the bladder, thyroid and ductus acusticus was not altered in males whereas that of mammary carcinoma in females was slightly but significantly decreased in the thiouracil-treated group.

Measurement of the food (and carcinogen) intake and increase in weight of the various experimental groups revealed no significant difference between the animals receiving AAF alone and those receiving also thiouacil.

Interesting differences were noted in the androgenic effects of testosterone in the rats receiving AAF with and without thiouracil. The AAF-treated group exhibited a relatively mild androgenic effect, as gauged by increase in prostatic weight (Table II) and inhibition of spermatogenesis. Mature sperms were present in the testes in 9 of 24 rats treated up to 254 days (Fig. 1). The androgenic response in the AAF-thiouracil group was essentially normal, the prostatic weight increasing considerably, as shown in Table II, and mature sperm appearing in the testes in only 2 of 40 animals (147 and 175 days respectively), the remainder exhibiting arrest of spermatogenesis (Fig. 2).

Cancer of the liver occurred in 15 of 17 (88 per cent) female Sherman rats receiving p-dimethylaminoazobenzene for 69 to 133 days and in 6 of 13 (46 per cent) receiving this carcinogen and thiouracil for 69 to 125 days. No difference was observed in males treated similarly.

Effect of methionine (Table III).—Administration of methionine had no influence upon the incidence nor the histologic characteristics of the hepatic neoplasms induced by AAF, with or without testosterone. However, the average weight of the cancerous livers in the methionine-treated group was lower than in those not receiving this supplement. One peculiar feature was a greater severity of cirrhotic changes in non-neoplastic portions of the liver in the methionine-treated animals as compared with those receiving only AAF.

Effect of thyroidectomy and of thyroxin (Table I).—None of the thyroidectomized rats survived for a long enough time to permit any conclusion regarding the influence of hypothyroidism per se upon the incidence of hepatic carcinoma due to AAF.

Attempts to prevent the development of hypothyroidism in rats receiving thiouracil by simultaneous administration of thyroxin were not entirely successful. Despite the fact that the dosage employed (5 to 10 gamma daily) is adequate to prevent hyperplasia (gross and microscopic) of the thyroid of normal rats receiving thiouracil, it did not do so in this series receiving also AAF. No liver cancer developed in the few females that survived sufficiently long to permit them to be included in these
statistics. In the males, the addition of thyroxine apparently overcame, to a considerable degree, the protection afforded by thiouracil against AAF-induced hepatic carcinoma.

**Thyroid lesions.**—In Table IV are presented data regarding the incidence of thyroid adenoma and carcinoma in rats receiving thiouracil alone (stock diet) and those receiving thiouracil and AAF (corn meal diet) with and without additional therapy. Inasmuch as no sex difference was observed, the data are not separated on this basis.

The thyroid was increased in size and weight in all animals receiving thiouracil; the degree of enlargement varied with the duration of treatment with this agent and was not significantly affected by simultaneous administration of AAF or sex hormones. The isthmus was usually involved in the enlargement. The glands were firm, sometimes lobulated and pale brown or gray in color.

Microscopic examination uniformly revealed the characteristic hyperplasia induced by thiouracil and similar goitrogenic drugs. Papillary proliferation appeared to be less marked after very long periods of therapy than in earlier stages, the process resembling the microfollicular rather than the macrofollicular and papillary hyperplasia of Graves’ disease (14). Some of the follicles, especially in the subcapsular region, contained pink-staining colloid. Occasional groups of acini were separated by connective tissue septa containing fibrocytes and lymphocytes.

Irregular nodular areas of two types were observed within the hyperplastic thyroid tissue. One consisted of islets or solid cords of cells. The other consisted of dilated acini, lined by low columnar or cuboidal cells and filled with red-staining colloid, with occasional finger-like projections into the lumen. These adenomatous lesions occurred more frequently and were more extensive in animals receiving AAF in addition to thiouracil than in those receiving the goitrogen alone, but were otherwise similar in appearance. (Figs. 3 and 4).

In no case was it felt possible to make a diagnosis of malignancy on the basis of purely morphological or cytological characteristics. Lesions were classified as malignant on the basis of infiltration between muscle fibers, invasion of blood vessels and metastasis to the lungs. (Figs. 5 and 6).

Administration of sex hormones had no appreciable influence upon the incidence of tumors of the thyroid. Estradiol was not well tolerated by rats receiving AAF and thiouracil, only 6 surviving 149 to 174 days and none surviving beyond this period. In the group of 47 rats receiving AAF and thiouracil, the incidence of adenoma was 93.6 per cent and of carcinoma 14.8 per cent as compared with 55 and 5 per cent, respectively, in the 20 animals receiving thiouracil alone. The one instance of thyroid malignancy in the latter group occurred after 884 days of treatment, whereas the 7 instances of malignancy in the AAF-treated group were observed after 232 to 312 days.

**DISCUSSION**

The data presented indicate (a) that thiouracil, administered in the drinking water, protects the liver of rats against the induction of cystic and neoplastic lesions by 2-acetaminofluorene and (b) that this protection extends also to the aggravating influence of testosterone upon these processes (4, 15). Similar, but less complete protection was observed against p-dimethylaminoazobenzene. Attempts to elucidate the mechanism of action of...
thiouracil in this connection were inconclusive. Three possibilities suggest themselves immediately:

1. Inasmuch as both the AAF and the thiouracil were ingested, the latter, perhaps as a reducing agent, might act upon the former in the gastrointestinal tract, converting it to an inactive compound.

2. The phenomenon may be dependent upon the state of hypothyroidism induced by thiouracil.

3. Thiouracil may act upon some enzyme mechanism in the liver cell in such a manner as to prevent the carcinogenic influence of AAF. The possibility of decreased intake of food and, therefore, of carcinogen, by animals receiving thiouracil was eliminated by measurement of food intake and weight gain, which did not differ significantly from the control (AAF) group.

The occurrence of tumors of the bladder, ductus acueticus, breast, uterus and thyroid in the absence of hepatic involvement in animals receiving thiouracil and also the restoration of carcinogenicity by injecting thyroxine, militate against the possibility of inactivation of the carcinogen in the bowel. This possibility is being explored further by administering AAF and thiouracil by different routes.

The role of hypothyroidism cannot be evaluated properly on the basis of the data obtained. Thyroidectomized rats did not tolerate the diet and carcinogen long enough to yield pertinent information. The fact that thyroxine, administered simultaneously, counteracted the protective influence of thiouracil is difficult to interpret. These agents exert no direct effect upon one another (1, 10). Although the quantity of thyroxine injected was adequate to have suppressed thyroid hyperplasia in rats receiving thiouracil (5, 11, 13), no such suppression was effected in the group receiving thiouracil and AAF. One cannot, therefore, attribute the restoration of hepatic carcinogenicity of AAF by thyroxine to prevention of thiouracil-induced hypothyroidism without additional information regarding the BMR in these animals. It is possible that the carcinogenic mechanism in the hepatic cell is responsive to quantities of thyroxine that are inadequate to maintain normal thyroid structure. On the other hand, it is also possible that thiouracil may exert a direct effect upon certain metabolic processes in the liver cell independently of its thyroid-depressing action (9). The failure of added methionine to combat the carcinogenic effect of AAF is of interest in this connection in view of the suggestion by György and Goldblatt (6) that the protective influence of thiouracil against dietary cirrhosis may be due to decreased requirement for (e.g., conservation of) methionine incident to the induced state of hypothyroidism.

The data presented indicate that, in rats receiving AAF, the hepatic "cocarcinogenic" effect of testosterone is accompanied by a decrease in its androgenicity, and that this phenomenon is reversed by simultaneous administration of thiouracil. This suggests that, in the presence of AAF, the metabolism of testosterone, perhaps in the liver, may be perverted so as to produce a metabolite that is cocarcinogenic and only slightly androgenic; when the liver is protected, by thiouracil, against the carcinogenic action of AAF, testosterone takes its normal metabolic pathway. The possibility was considered that the effect of thiouracil in increasing the androgenicity of testosterone in AAF-treated rats may be due to increased sensitivity of the target organs in the hypothyroid state, such as has been reported for estrogen (8). However, there is no evidence that this holds true for androgens, and, in unpublished experiments in our laboratories, administration of thiouracil to rats receiving testosterone (no AAF) had no perceptible effect on the androgenicity of the latter.

Our findings confirm the observation (2, 3, 12) that administration of goitrogenic drugs (allyl thiourea, thiouracil, etc.) leads to the formation of adenomas and, if continued for long periods, carcinoma of the thyroid in rats. Simultaneous ad-

**FIGURES 1 TO 6**

**Fig. 1.**—Testis of a rat which received AAF and testosterone propionate for 248 days, showing well preserved spermatogenesis. Hematoxylin and eosin stain. Mag. × 200.

**Fig. 2.**—Testis of a rat which received AAF, testosterone propionate and thiouracil for 164 days, showing degenerative changes in seminiferous tubules and absence of mature sperm. Hematoxylin and eosin stain. Mag. × 200.

**Fig. 3.**—Thyroid of a female rat treated with AAF and thiouracil for 312 days. Adenoma in the hyperplastic thyroid (microfollicular type). Hematoxylin and eosin stain. Mag. × 80.

**Fig. 4.**—Thyroid of a female rat treated with AAF, thiouracil and estradiol diprop. for 179 days, showing adenoma with hyperchromatic nuclei and irregular arrangement of acini. Hematoxylin and eosin stain. Mag. × 150.

**Fig. 5.**—Lung of female rat treated with AAF and thiouracil for 304 days, showing metastasis from thyroid cancer, resembling "benign metastasizing adenoma". Hematoxylin and eosin stain. Mag. × 150.

**Fig. 6.**—Thyroid cancer invading blood vessels and skeletal muscle fibers. Male rat treated with AAF and thiouracil for 293 days. Hematoxylin and eosin stain. Mag. × 115.
administration of AAF accelerates the development and increases the incidence of these lesions.

SUMMARY

1. Simultaneous administration of thiouracil protected the liver of rats against the carcinogenic action of 2-acetaminofluorene (AAF). This protection was exerted also against the hepatic cocarcinogenic action of testosterone. The incidence of liver carcinoma induced by $\beta$-dimethylaminoazobenzene was lowered in females by administration of thiouracil.

2. Possible mechanisms whereby this effect may be produced are discussed. The possibility that hypothyroidism plays an important role in this connection cannot be eliminated. However, a direct action of thiouracil upon liver cells, independent of the induced state of hypothyroidism, cannot be excluded from consideration.

3. Quantities of thyroxine adequate to prevent thyroid hyperplasia in rats receiving thiouracil alone did not inhibit hyperplasia in those receiving thiouracil and AAF.

4. Administration of methionine did not decrease the incidence of carcinoma of the liver induced by AAF.

5. Testosterone exerted only a mild androgenic effect (prostatic weight increase and inhibition of spermatogenesis) in rats receiving AAF. In those receiving also thiouracil, testosterone evoked the expected androgenic response. The hypothesis is suggested that, in the presence of AAF, the metabolism of testosterone is perverted so as to form a hepatic cocarcinogenic metabolite which is only weakly androgenic. When the action of AAF upon the liver is prevented by thiouracil, testosterone takes its normal metabolic pathway.

6. Thyroid adenomas and carcinoma developed in rats receiving thiouracil alone for protracted periods. Simultaneous administration of AAF hastened the development and increased the incidence of these lesions.

ACKNOWLEDGMENTS

We are indebted to Dr. E. Oppenheimer of the Ciba Pharmaceutical Products, Inc., and to Dr. E. Schwenk of the Schering Corp. for generous supplies of testosterone propionate and estradiol dipropionate. Thiouracil was supplied by the Lederle Corp., through the courtesy of Dr. St. Hardy, and 1-thyroxine by E. R. Squibb and Sons, through the courtesy of Dr. C. H. Mann.

We gratefully acknowledge the technical assistance of the Misses A. Masse, M. Del Bianco and E. Hay.

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Cancer Res 1948;8:257-263.

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