Malignant Tumors in the Eyelids and the Auricular Region of Thiourea-treated Rats

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During the administration of thiourea to rats for periods of 1 year or longer, squamous-cell carcinomas and sarcomas developed in the region of the face (10). Further experiments on larger groups of animals have now been completed and confirm the first results. In addition to the tumors previously described (10), located between the external ear duct and the eye, a certain number were observed on the eyelids, some of them destroying the entire orbital content. The pathology of these tumors and certain phases in their development are described in the present paper.

MATERIALS AND METHODS

Thirty-five male albino rats of a local random-bred strain (Hebrew University strain), weighing approximately 100 gm., were divided into two groups:

1. Sixteen rats received, on 3 consecutive days of every week, intraperitoneal injections of a 10 per cent aqueous solution of thiourea (purchased from “British Drug Houses,” Ltd., London) in doses of 3.4 and 4 ml., respectively. These injections were continued for a period of 6 months; after that time, the animals were given a 0.2 per cent solution of thiourea instead of drinking water.

2. Nineteen rats received thiourea in 0.2 per cent solution in the drinking water for the entire experimental period.

The animals were caged in groups of three to four and kept in quarters receiving moderate indirect daylight and were never exposed to direct sunlight. They were maintained for the first 6-9 months on a diet of Purina Chow Checkers (Ralston Purina Co., St. Louis, Mo.), and after that time on a diet consisting of sprouted wheat, barley, skimmed milk powder, and seasonal vegetables to which, occasionally, cod liver oil was added. This diet contained 20 per cent protein. At the end of 12-15 months the animals weighed about 300-350 gm., which is the average weight of untreated rats of the same age, in our colony.

The experimental animals grew at a rate identical with that of the controls.

The animals died or were sacrificed at various intervals of time, as stated below. Tissues were fixed in Carnoy’s fluid and in Zenker’s acetic acid. Paraffin sections were stained with hematoxylin-eosin and in some instances by Laidlaw’s silver method, counterstained with van Gieson’s mixture.

Twelve control male rats of the same age as the experimental animals were maintained under identical conditions during the period of the experiment.

RESULTS

INCIDENCE AND DISTRIBUTION OF TUMORS

Combined intraperitoneal and oral administration of thiourea.—Four rats died without tumors during the first 2-11 months of the experiment. Ten of the remaining twelve animals surviving longer than 1 year developed tumors which appeared from 12 to 18 months from the beginning of treatment. Three of the tumor-bearing animals died after 13, 21, and 22 months, respectively. The others were sacrificed between 12 and 19 months, shortly after the tumors were detected grossly.

In six animals the tumors were located near the ear ducts, in one instance being bilateral. In three animals the tumors were located only in the orbital region, and one animal showed tumors, both on the eyelids and near the ear ducts.

Administration of thiourea in drinking water.—Eighteen out of nineteen rats showed tumors on gross examination. The earliest tumor appeared at 4½ months after the start of the experiment. Ten of the remaining twelve animals surviving longer than 1 year developed tumors which appeared from 12 to 18 months from the beginning of treatment. Three of the tumor-bearing animals died after 13, 21, and 22 months, respectively. The others were sacrificed between 12 and 19 months, shortly after the tumors were detected grossly.

In six animals the tumors were located near the ear ducts, in one instance being bilateral. In three animals the tumors were located on the eyelids or in the orbit, and one animal showed tumors, both on the eyelids and near the ear ducts.

Administration of thiourea in drinking water.—Eighteen out of nineteen rats showed tumors on gross examination. The earliest tumor appeared at 4½ months after the start of the experiment, the remainder at periods of 13-20 months. The first tumor was located on the nose and was histologically of a myxomatous type. Its morphology and behavior in transplantation experiments have been described elsewhere (9).

Of the remaining seventeen tumor-bearing rats, six had tumors only near the ears, six had tumors only in the orbital region, and five had tumors in
various shapes and sizes. Scattered giant cells with marked anaplasia. In some of these tumors remnants of sebaceous cells were noted (Fig. 1). However, in the case of the rat showing destruction of half the face and secondary growth in the lungs, the histologic character of the malignancy was difficult to determine. Wide areas of necrosis were bordered by tissue rich in cells of various shapes and sizes. Scattered giant cells which often showed abundant cytoplasm and one or several hyperchromatic nuclei were present (Fig. 2). In some areas, large cells were found lying in strands (Fig. 3), the intercellular spaces not being apparent, while in other areas the cells were in disorderly pattern. Mitoses were numerous. The cells were embedded in a slightly eosinophilic matrix. With silver impregnation, a fine network of fibrils was seen. In brief, some areas of this tumor looked like an anaplastic carcinoma, whereas others suggested a mixed-cell sarcoma.

In the orbital region the ulcerated tumors were epidermoid carcinomas, occasionally anaplastic and in one instance of basal-cell type. In the animals sacrificed before destruction of the lids had occurred, carcinomas were regularly seen to originate in the Meibomian glands. The malignant change showed here two modes of development which sometimes were simultaneously present in the same eye. One type was as follows: The excretory duct of the glands was occluded by keratin masses arising from epidermization of the duct epithelium, presenting occasionally several chalazion-like cysts (Fig. 4). Examination of the surrounding epithelium revealed in many places various stages of canceration, beginning with simple hyperplasia and scattered mitotic figures and developing to epidermoid carcinoma which invaded the lid in the direction of the conjunctiva (Figs. 4, 5). Where this occurred, keratin masses protruded into the conjunctival sac, causing keratitis and leading to perforation of the cornea.

The second type of canceration appeared to arise from the basal cells in the depths of the glands. In those instances the acini of the Meibomian glands presented a regular structure, except in the most distal portions where carcinoma was found infiltrating into the surrounding tissues (Figs. 6, 8). At the point where the carcinoma bordered the intact portion of the glands, there was loss of sebaceous cells which were replaced by hyperplastic basal cells. In other instances the tumor had invaded the adjacent acini and was found among the remnants of sebaceous cells (Fig. 7). In several eyes hyperplasia of basal cells was present simultaneously with cystic dilatation of the excretory duct, which then showed epidermization and keratinization.

Sebaceous glands of the lashes were never affected by the changes described above. Except where the carcinoma had invaded the orbit, no histological changes were observed in the lacrimal and Horner's glands. Invasion appeared mainly to follow the orbital muscles and later to extend into the tissues surrounding the optic nerve and the intraorbital glands.
DISCUSSION

In the experiments reported in this paper, malignant tumors developed after oral administration of thiourea in eighteen out of nineteen rats and after combined oral and intraperitoneal administration in ten out of twelve rats which survived at least 1 year after the start of treatment. The tumors were located either in the auricular region or on the eyelids. One tumor, a myxoma, was found on the bridge of the nose and was discussed elsewhere (9).

Spontaneous "face tumors near the orbit" have been observed in certain strains of rats by Dunning et al. (3). No spontaneous tumors of the eyelids in rats have been reported. In the random-bred strain of the Hebrew University no tumors of this type have been observed (see number quoted in [10]); moreover, twelve rats kept for 2 years under the same conditions as the experimental animals have remained free of tumors. The proportion of tumors in our experiments is obviously high enough to exclude spontaneous occurrences. Accordingly, the present results confirm our previous observations on the development of malignant tumors of the face in thiourea-treated rats (10).

Induced tumors in the auricular region have been reported to arise in rats after treatment with 2-amino- and 2-acetylaminofluorene (1, 2, 5, 7, 8, 11, 14–16), and with 9,10-dimethyl-1,2-benzanthracene (4). Spitz, Maguigan, and Dobriner (12) observed tumors of the external ear duct after administration of benzidine to rats. In a study of serial sections through the head, these authors traced the development of the tumors from a sebaceous gland in the ear duct, originally described by Zymbal (17). The very early changes caused in this gland by 2-acetylaminofluorene were described by Laws et al. (7). In our material the study of the origin and histogenesis of the tumors in this area was not possible, since all rats were examined at a stage when widespread necrosis and infiltrative growth had taken place.

Tumors of the orbital region were found to have developed from the Meibomian glands of the lids, other structures adjacent to the eyes being only secondarily affected. No tumors of this origin are known in animals. In man, tumors of sebaceous glands are uncommon (13). Among them, tumors of the Meibomian glands are rare, only about 100 cases being reported up to 1955. They are known for their rapid invasiveness (6).

In the present experiments 26 out of 28 observed tumors were classified as carcinomas in various degrees of differentiation. One tumor of the auricular regions showed in some areas features of a sarcoma, in others of an anaplastic carcinoma, and one tumor on the bridge of the nose was a myxoma. In the experiments reported earlier (10), three out of five tumors were squamous-cell carcinomas, one was a sarcoma, and one was composed of elements of both carcinoma and sarcoma. All experimentally induced tumors of the auricular region cited here were reported as carcinomas. However, Dunning et al. (3) mentioned among spontaneous tumors of this region squamous-cell carcinomas, sarcomas, and carcinomas.

The assumption that canceration of the Meibomian glands occurred as a result of continuous irritation by thiourea spilled from the drinking water does not appear probable. Indeed, no irritation of the eyelids was ever observed during the course of the experiments. Moreover, the beginning of the canceration in the deeper portions can hardly be by external irritation. The animals would normally close the lids against chemical irritation, and one would therefore expect the tumors to originate at the lid margins or in the Zeiss glands.

The most likely explanation for the strictly localized origin of the carcinoma evoked by thiourea appears to be the excretion of a carcinogen through the Meibomian and Zymbal's glands. In view of the intactness of the other sebaceous glands of the skin and in particular of those which are attached to the eye lashes, it appears likely that the Meibomian glands and possibly also the Zymbal glands of the ear duct are functionally different from other glands of identical histological structure.
Fig. 5.—Area from the carcinoma shown in the preceding figure, near the cyst wall. X130.

Fig. 6.—Section through anterior portion of eye together with the lids, from rat showing no macroscopic lesion. Carcinoma is seen arising in Meibomian glands of the upper and lower lids. X26.

Fig. 7.—Epidermoid carcinoma of eyelid infiltrating into normal portions of Meibomian gland (seen at bottom of the figure). X460.

Fig. 8.—Early carcinoma in fundus of Meibomian gland of lower lid shown in Figure 6. X460.

All photomicrographs were made from sections stained with hematoxylin-eosin.

The photomicrographs were prepared by Mrs. H. Weinman.
SUMMARY

The present experiments confirm our earlier report concerning the development of malignant tumors in a high proportion of rats which were treated with thiourea for periods of 1–2 years.

The tumors were epidermoid carcinomas originating in the region of the external auditory duct and in the Meibomian glands of the eyelids.

REFERENCES

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