

The Development of Malignant Tumors of the Face in Rats after Prolonged Treatment with Thiourea

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Earlier studies have shown that it is possible to induce a mitotic wave in the livers of rats by three consecutive intraperitoneal injections of large doses of thiourea (14). A subsequent study was undertaken to observe the effect on the liver of prolonged treatment with thiourea. No changes were found after such treatment. However, in five out of six rats which survived for more than 1 year, malignant tumors of the face were observed. The morphology and pathogenesis of these tumors are described below.

MATERIALS AND METHODS

Twelve albino rats of both sexes weighing from 78 to 126 gm. were used for the experiments. The animals were fed on Purina Chow Checkers (Ralston Purina Co., St. Louis). The rats received each week intraperitoneal injections of a 10 per cent aqueous solution of thiourea, in doses of 3.0, 4.0, 4.0 cc. on 3 consecutive days, for a period of 6 months. After this period thiourea was administered in drinking water in a concentration of 0.2 per cent. At certain intervals the animals were sacrificed. The organs were fixed in Carnoy's or Zenker's fluid and imbedded in paraffin. Sections 5 μ in thickness were stained with hematoxylin-eosin. Some sections were stained for connective tissue fibers according to Heidenhain's azan method or Laidlaw's silver stain.

RESULTS

Six out of twelve experimental animals died or were sacrificed 8 weeks to 8 months after the beginning of the experiment. In these animals no tumors were found.

Six animals survived for 1 year. Of these six rats five developed tumors. Two of these rats were males, one was a female, and for two the sex was not recorded. These rats were killed 1 year to a year and 4 months after the beginning of treatment. In all animals the tumors were localized on the face between the ear duct and the orbit. In one

rat the tumors were bilateral, in the others they were found on the right side of the face. In one rat the facial tumor was connected with a second larger tumor in the region of the larynx. The size of the facial tumors was small, approximately 1 cm. in diameter. In three animals the tumors were ulcerated. On dissection, four tumors were found to contain spaces filled with a whitish, foul smelling, granular material. One tumor was hard and compact.

On microscopic examination the three ulcerated tumors proved to be squamous-cell carcinomas. One tumor (rat 785) showed typical proliferation of squamous cells with cornified pearls in the center (Fig. 1). The cells were polymorphous, and numerous mitoses were seen. There was extensive invasion into the dermis. A number of large, cornified bands were seen between the cell strands.

Another tumor of this type (rat 818) contained broad cuffs of neoplastic cells, in the middle of which were found blood vessels or aggregates of connective tissue. Keratinized eosinophilic material occurred between the cellular strands. The cells and nuclei varied widely in size and shape. There were numerous mitoses, many of them atypical (Figs. 2 and 3).

The third tumor of this kind (rat 814) presented at some sites a solid growth of squamous epithelium, often in mitosis (Fig. 4). At other sites a tubular or pseudoglandular structure of neoplastic cells around a core of connective tissue was seen (Fig. 5).

Another tumor (rat 816) consisted of a mixed structure of squamous-cell carcinoma and sarcoma. In some places prickle cells were concentrically grouped around an eosinophilic homogeneous center. The cells were polymorphous, and mitotic figures were numerous (Fig. 6). These islets of squamous cells were surrounded by a tissue very rich in cells of varying size, shape, and nuclear configuration. There were gradations from small spindle cells to giant cells and from small nuclei to giant nuclei. Some cells had several nuclei. The

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cells were irregularly arranged in a pale, slightly eosinophilic matrix. Numerous small vessels were present, some with endothelial lining, some without endothelium, bordered by tumor cells. Mitoses were numerous (Fig. 7).

One tumor (rat 811) was a mixed-cell sarcoma of the same type as the sarcomatous part of the tumor previously described (Fig. 8). No metastases were found in other organs. The thyroid glands of two of the tumor-bearing animals were moderately hyperplastic. The thyroids of the other tumor-bearing animals were not examined. The thyroids of animals which died or were sacrificed after 2–11 months were also only moderately hyperplastic.

DISCUSSION

The appearance of malignant tumors of the face in five out of six rats surviving more than a year's treatment with thiourea, and the absence of spontaneous tumors of this kind in our rat colony,¹ suggest that these tumors were caused by the treatment with the antithyroid drug. The treatment in our experiments differed from the usually adopted continuous oral administration of small doses of thiourea in that intermittent large doses of thiourea were injected intraperitoneally during the first 6 months, followed by continuous administration of small doses of thiourea in drinking water. The observed tumors were situated between the ear duct and the orbit and were adherent to the outer wall of the ear duct. Three of them were squamous-cell carcinomas, one was a carcinosarcoma and one a sarcoma. Whether the squamous-cell carcinomas originated from the skin or from the sebaceous gland of the ear duct could not be ascertained, since the tumors were ulcerated and the tissue showed large necrotic areas.

Spontaneous tumors of this type classified as "face tumors near the orbit" were described by Dunning *et al.* (3). These authors stated that of 249 spontaneous tumors in rats of their large colony, 89 were skin tumors of the face near the orbit. Of these 89 malignant tumors, 57 were squamous-cell carcinomas, one contained squamous and glandular epithelium, 21 were sarcomas, and ten were mixed tumors made up of sarcoma and squamous-cell carcinoma.

The development of ear-duct tumors in the rat

¹ The rat colony of the Hebrew University produces approximately 15,000 albino rats per year. Of these about 200 are kept for more than 1 year. In these rats no tumors of the face were observed in the last 20 years. At present, 38 rats, 1 year old, which are under our personal supervision show no tumors of this kind. The rats are kept on a diet which consists of sprouted wheat, barley, bran, skim milk powder, and the seasonal vegetables. Sometimes cod liver oil is added. This diet contains 20 per cent protein.

after exposure to carcinogenic substances is not a rare event. Such tumors were observed after treatment with 2-amino- and 2-acetylaminofluorene (AAF) (18) and after treatment with 9,10-dimethyl-1,2-benzanthracene (5). As far as we know, no tumors of this kind have been previously observed after treatment with thiourea or similar antithyroid drugs. It is known that tumors of the thyroid, benign or malignant in character, may arise after prolonged oral treatment with thiourea, thiouracil or its derivatives (6, 7, 9, 11, 12).

Adenomas of the pituitary gland of mice were described by Moore, Brackney, and Bock (8) after treatment with propylthiouracil; and adenomas of the pituitary, one cancerous, as well as an adenoma of the adrenal cortex were seen by Seifter *et al.* (17) after administration of antithyroid compounds to rats. Besides these tumors which occurred in endocrine organs, tumors of the liver were observed. Fitzhugh and Nelson (4) reported the induction of hepatomas in rats by oral administration of thiourea for more than 2 years.

The face tumors, observed by us after thiourea treatment, comprise an addition to the list of tumors arising in organs which do not belong to the endocrine system.

According to Bielschowsky it is not thiourea which is responsible for the induction of the tumors of the thyroid, but the overproduction of TSH, brought about by the treatment with thiourea. This assumption is based on experiments in which it was shown that AAF alone induces no tumors in the thyroid. If, however, AAF acts on the residue of the thyroid after partial thyroidectomy, tumors appear (2). Partial thyroidectomy diminishes the production of thyroxin and thus enhances the production of TSH. The same effect is caused by thiourea administration.

Thus far it is difficult to define the role of thiourea in the induction of the described tumors. Thiourea may act indirectly by causing an overproduction of TSH. The hormonal imbalance would then be responsible for tumor production. A second possibility is that thiourea is a carcinogen as assumed by Fitzhugh and Nelson (4) with respect to its induction of hepatomas. A carcinogen may act along varied lines. It may diminish the growth-restraining forces of the environment, it may act as a mutagen, or may influence intracellular processes in a still unknown manner. We know that thiourea is a substance acting on the cell. It possesses the ability to induce mitoses; this effect was observed in the thyroid (10) and in the liver (14). Another effect of thiourea is the arresting of mitosis in the metaphase. The latter effect was seen in the liver (13), in the bone marrow (15),

and in cultures of fibroblasts treated with thiourea (16). It is possible that both factors, namely, the alteration of the environment as well as a direct effect on the cell, may be responsible for the appearance of tumors.

The fact that tumors of this kind arose in our random-bred rat strain and did not occur in rats treated with antithyroid drugs in other laboratories may be due to genetic differences or, perhaps in part, to the different method of treatment. We hope to be able to answer these questions from the results of further experiments.

SUMMARY

Malignant tumors of the face were observed in five out of six rats treated for more than a year with thiourea. The treatment consisted of intermittent intraperitoneal injections of large doses of thiourea for 6 months followed by continuous administration of 0.2 per cent thiourea in the drinking water. The pathogenesis of the tumors is discussed.

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ADDENDUM

After completing this work similar tumors appeared 1 year after the beginning of the experiment in two animals from a second experimental series of twelve male rats treated with thiourea as reported above. At the time of reading the proofs of this paper two further tumors appeared in this experimental series (14 and 15 months from the beginning of the experiment).

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

FIG. 1.—Rat 785. Squamous-cell carcinoma with cornified pearls. $\times 360$.

FIG. 2.—Rat 818. Proliferation of squamous cells forming

cuffs around blood vessels or connective tissue cores. $\times 95$.

FIG. 3.—High power view of tumor shown in Figure 2. $\times 385$.

FIG. 4.—Rat 814. Solid growth of squamous epithelium showing numerous mitotic figures. $\times 600$.

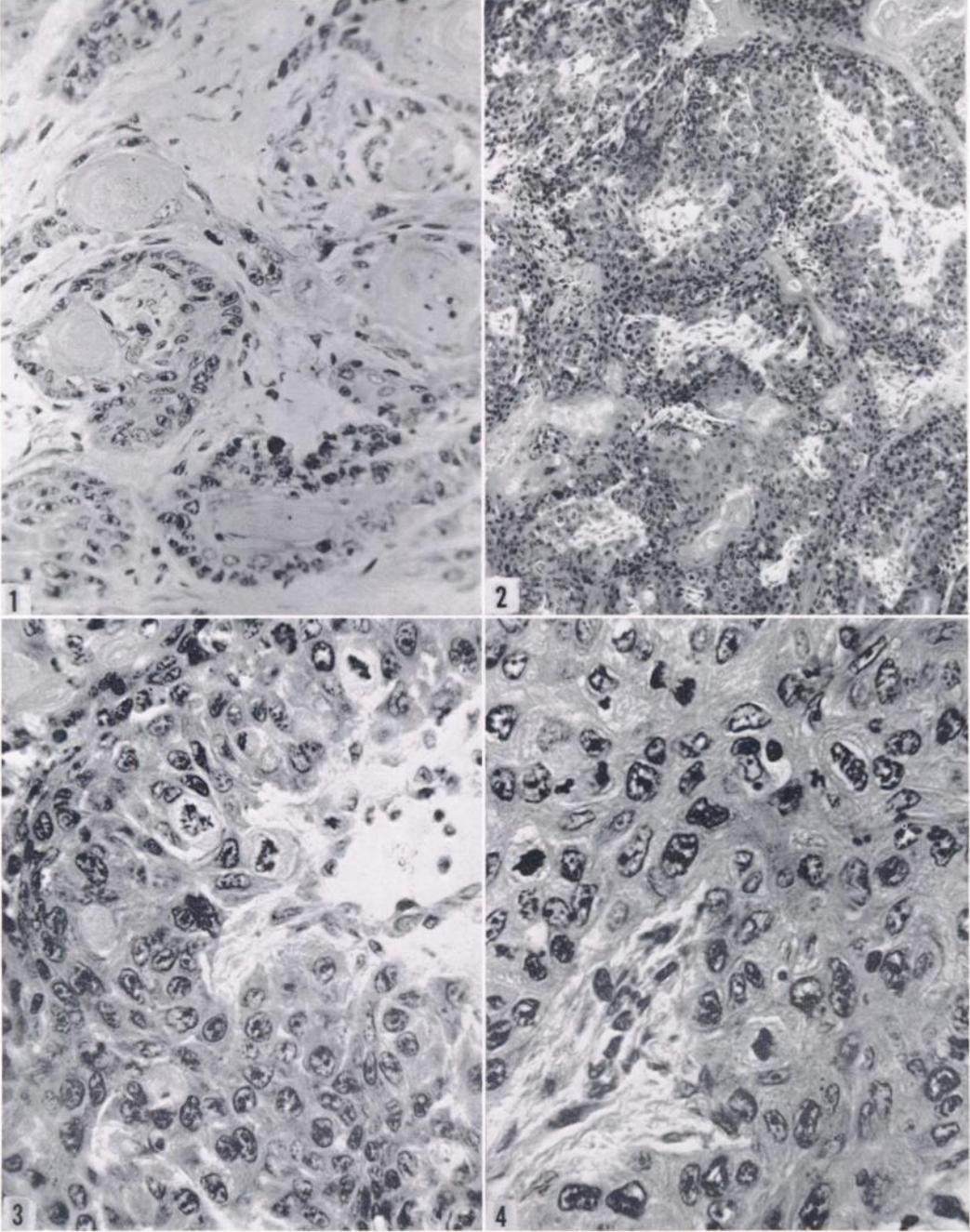


FIG. 5.—From the same tumor as Figure 4. Pseudoglandular formations. $\times 160$.

FIG. 6.—Rat 816. Prickle cells grouped around eosinophilic homogeneous center. $\times 560$.

FIG. 7.—From the same tumor as Figure 6. Sarcomatous tissue showing cells of various size and shape. $\times 560$.

FIG. 8.—Rat 811. Sarcoma showing great polymorphism of cells. $\times 560$.

