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REACTION TO METHYLTHIOURACIL OF THYROID AUTOIMPLANTS IN THE RAT

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Literature contains many reports to show that chronic treatment of mice and rats with methylthiouracil (MTU) induces the growth of adenomas and carcinomas in the thyroid gland through the mechanism, inhibited synthesis of thyroxin → increased synthesis of thyrotropin in the adenohypophysis → → thyroid hyperplasia [1, 2, 17, 19, 20, 21, 30, 31]. Increased synthesis of thyrotropin is known to manifest itself morphologically with an excessive proliferation of the "thyrotrophic" cells of the pituitary, and that adenomas consisting of characteristic "thyroidectomy"-cells may develop [2, 3, 9, 10, 11, 12, 13, 22, 24, 26, 27].

Both forms of tumour represent typical examples of hormonally induced neoplastic growth, the above-said hormonal, i.e. humoral factor presumably playing a decisive pathogenetic role.

We have found that autologous and — after adequate conditioning — even homologous transplants of rat thyroid are both morphologically and functionally nearly equivalent to the thyroid gland *in situ* [4, 5, 6].

The aim of the present investigations was to establish whether methylthiouracil treatment would produce the same indirect blastomatogenic effect on thyroid tissue *in situ* as on heterotopic, transplanted thyroid tissue. As a secondary aim, it was intended to study the development of hypophyseal adenomas as also the occurrence of tumours reported to arise from the Zymbal glands in the course of chronic methylthiouracil treatment [25, 29].

Material and method

Fifty-seven male albino rats (of a non-inbred Wistar strain) of 170 to 190 g body weight, raised on a mixed non-standard diet, were used in the experiments. After removing the right lobe of the thyroid gland under sterile conditions, as described earlier [4], we implanted it under the skin of the back during the same session. Administration of methylthiouracil (Basethyrin, Richter, Budapest) was started two weeks after the operation. Six times weekly, a daily dose of 0.01 g was mixed to a small amount of food, and the animals were not allowed to eat their full daily ration until having first consumed the mixture. The rats were kept in cages, two in each.

The animals were weighed every month. Rats losing weight and those found to be ill were exsanguinated in ether anaesthesia and dissected. The first animals were killed on the

155th day, and the last seven on the 678th day, irrespective of their condition. Forty-one animals received methylthiouracil for more than 400 days. Not only the transplants and the thyroid *in situ* were studied histologically, but — in the later course of the experiment — the pituitary, the salivary glands, Zymbal's gland, the kidney, lung and occasionally other tissues as well. The removed organs were fixed in a 10 per cent solution of neutral formalin, the pituitary glands in a mixture of formol and mercury bichloride. We made serial sections of both the transplanted and the remaining thyroid glands; the latter were worked up together with the larynx and the trachea.

Reslts

Both the autologous transplant and the remaining thyroid grew to a multiple of their original size. The measure of enlargement was usually proportional to the time of treatment, so that it was most pronounced in animals sacrificed on the 678th day (Fig. 1). The size of the transplant was 3 to 20 times that of the originally transferred fragment of thyroid tissue. Nodulation of the surface, an indication of neoplastic growth, was frequent in both the transplanted and the remaining glands.

Cachexia, making us to kill the animals, was mostly (in 34 cases) due to, usually bilateral, multilobar multiplex bronchiectasic abscess and consequential pneumonia. Autopsy failed to reveal the cause of cachexia in 10 animals, in 4 of which we found exceedingly large hypophyseal adenomas. Nine animals, showing no signs of cachexia, were sacrificed according to plan at the termination of the experiment. Cachexia was due to spontaneous malignant tumour in 4 animals; three were cases of mesenteric lymphosarcoma, the fourth a skin cancer in the scapular area with metastasis in the axillary lymph node. (All spontaneous malignancies observed in our rat colony were mesenteric lymphosarcomas.)

Histological analysis revealed tumours in 78.9 per cent of the thyroids *in situ*, and 43.8 per cent of the autologously transplanted lobes. The tumours belonged to various types; none of them showed pronounced histological or biological malignancy. Their number per animal varied from one to six. It was not merely in respect of the incidence of tumours that there was a difference between the transplanted and the remaining thyroid tissue; they were different also in respect of the time at which the tumours began to appear. They were observed as from the 400th day in the glands *in situ* (Table 1).

The quantitative and the chronological discrepancy are shown together in Table 2; these differences persisted throughout the experiments.

According to their histological structure, it was possible to divide the tumours into various types, although transitory forms, too, were observed.

There was no difference between the transplanted and the remaining thyroid tissue either in respect of the type of the tumour or in that of the type of hyperplasia encountered in the non-tumorous parenchyma.

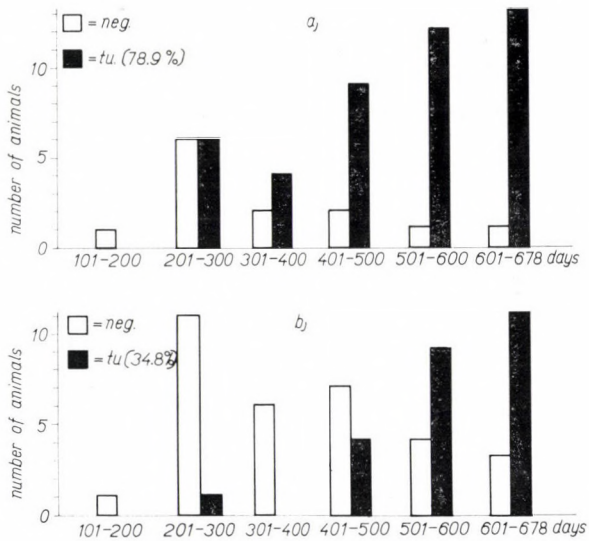
We distinguished the following types of tumour:

(1) The follicles composing the adenoma differed from those of the surrounding hyperplastic parenchyma only in size, while there was no difference as regards the nucleus and cytoplasm of the epithelial cells. The nucleus was clear, had a vesicular structure and contained a conspicuous nucleolus. The cylindrical eosinophilic cytoplasm was foamy, and many of the lumina con-

Fig. 1. Larynx and trachea of 5 animals killed on the 678th day, and those of a normal rat. Note considerably enlarged left thyroid lobe, in some cases with nodulous surface. Treated animals are devoid of right lobe. The hyperplastic left lobe extends from backward to the right side in animal No. 170, while the site of the right lobe is occupied by the displaced oesophagus in animal No. 191 V

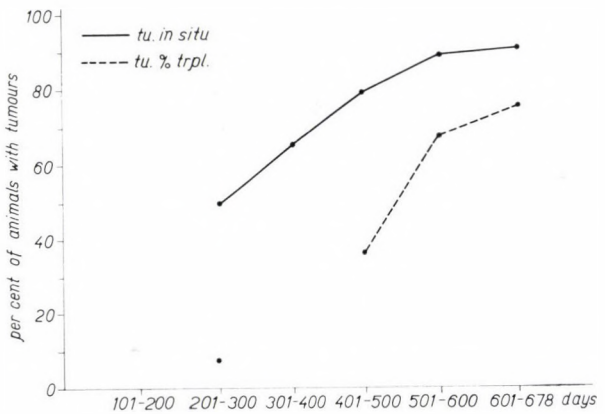
tained colloid. The adenoma was sharply circumscribed but without separate capsule. This type occurred rarely and showed no connection with the other types.

Table 1



Abscissa, days of treatment. Ordinate, number of animals. White columns tumorous animals. a = tumour in thyroid gland *in situ*. b = tumour in graft

Table 2



Abscissa, days of treatment. Ordinate, percentage of tumorous animals. Solid line shows percentage of tumours *in situ*, dotted line tumours in grafts

(2) The follicles composing the adenoma were, as a rule, larger than those in the non-tumorous tissue, sometimes cystic. Their lumen frequently contained colloid. The nucleus of the epithelial cells of the follicles was dark, circular or oval and pressed against the base; the cytoplasm was low, cylindri-

cal, eosinophilic and homogeneous. This type was the most common. It appeared in the form of small foci or as a large structure occupying nearly the entire lobe, — probably the result of a coalescence of several nodes.

(3) This type had developed from the preceding one. It revealed (a) multifocal haemorrhage and cicatrization; (b) distended veins with thickened walls, the latter consisting sometimes of nothing but follicles; (c) along with the development of scars, the follicles became diffuse and gave rise to large confluent cysts filled with colloid or desquamated, partly calcifying cells, the tumour was sometimes replaced by a huge cyst; (d) the proliferation of microfollicular, papillary, sometimes solid or trabecular epithelium was seen on the outer side of the cyst or growing towards the lumen. Cells in the solid parts were polymorphous, contained hyperchromatic nuclei, and divided but rarely. This type, too, was fairly common; it may be regarded as the regressive and progressive form of the preceding type.

(4) Small, solid tumours composed of polymorphous cells. We observed this type in 4 cases only.

(5) Tumours composed of cells containing empty vacuoles in their cytoplasm, with displaced nucleus, in solid or microfollicular form. We classified these structures under the head of adenomas in 2 cases only. They occurred otherwise in the form of small non-tumorous foci.

The ultimobranchial passages did not seem to be connected with the tumours. Most of the latter arose at the periphery of the lobes, so that a connection of the said kind was improbable also topographically.

A study of the hypophyseal changes was not a primary aim of our experiments, so that they were followed only in the 27 animals which were to be treated longer than 500 days. We found in 11 cases circular or oval, sometimes slightly lobate adenomas, between 3 and 7 mm in diameter. Invasive growth was not observed, the tumours impressed the base of the brain, and the sella appeared to have become broader and deeper. At cross examination there was a symmetrical enlargement of the pituitary in 16 cases. Histological examination revealed microadenomas in 9 of these glands, a diffuse proliferation of "thyroidectomy" cells (chiefly in the central portion of the anterior lobe) was observed in the other 7 cases. Thus methylthiouracil-treatment of more than 500 days induced adenoma in 20 out of 27 animals, i.e. in 74 per cent.

Some animals had hypophyseal tumour without simultaneous thyroid adenoma, so that no connection between the two seemed to exist.

The observed adenomas consisted of PAS-negative "thyroidectomy" cells with vacuolated cytoplasm; apart from signs of degeneration (necrosis, haemorrhage), marked polymorphism, giant cells and many mitotic forms were seen in large tumours.

Tissue from two hypophyseal adenomas, found on the 678th day, was homologously transplanted to white rats, part of which had previously been

thyroidectomized. The transplants took in all cases and were studied histologically after 21 and 42 days. Their size was not larger than that of the original grafts. We found groups of tumour cells surrounded by infiltrations; the cells were like the "thyroidectomy" cells of the original tumours.

Discussion

Chronic methylthiouracil-treatment has been found to induce adenomas more frequently and within a shorter time in the original than in the autologously transplanted thyroid tissue. This result was surprising, since what we expected was that tissues removed from their natural milieu would be governed by a changed mechanism which, in its turn, would result in an increased lability of cell-metabolism and render the transplanted tissues more susceptible to tumorous transformation in cases of excessive stimuli affecting their metabolism.

In connection with the thyroid, hormonal and neural influences are of prime importance. Of course, only changes in neural regulation have to be taken into account in cases of autologus transplantation, since the possibility of hormonal influence is undisturbed if the transplant has taken, as has been proved in earlier experiments [4, 5, 6].

In order to be able to explain why the remaining gland is more likely to develop tumours than the transplanted thyroid tissue, we have to examine the functional value of the latter and find out whether it is equivalent to the thyroid tissue *in situ*. As regards histological structure, both presented exactly the same picture during the entire experimental period. They were identical also in respect of methylthiouracil-induced hyperplasia as well as of the types to which the subsequent adenomas belonged. Transplanted and *in situ* tissues seemed to have the same functional value. This was in agreement with the results of earlier experiments [4] in which cortisone-conditioned thyroidectomized host animals with a homologous thyroid graft had shown normal development even after the lapse of 1 or 2 years. Examining the functional value of the homograft by means of radioiodine [5] as also on the basis of the response to endogenous TSH-stimulation [6], we found it approximately equivalent to that of the normal thyroid gland.

The diminished tendency of transplanted thyroid tissue to respond to blastomatogenic stimulation cannot, therefore, be due to reduced viability.

It was solely in innervation that we observed a difference between the two differently located tissues. Although the available literature does not contain data concerning a correlation between the innervation of thyroid tissue and its morphogenetic properties, the explanation of our results must nevertheless lie along this line. There are reports ot show that, by changing

the trophic conditions of tissues, it is possible to influence the growth of experimental tumours, and that by a transection of the sensory nerves it is possible to prevent the development of malignancy [14]. The reduced tendency of the grafts to neoplastic growth, as observed in our experiments, may have been due to their non-physiological trophic innervation.

Studying the genesis of tumours in autologous thyroid grafts, DONIACH and LOGOTHETOPOULOS [7] failed to find neoplastic growth after methylthiouracil treatment for 16 months.

Thyroid hyperplasia and adenoma, as induced by treatment with methylthiouracil, has formed the subject of many reports [2, 16, 17, 20, 28]. Since the growth of adenomas is not directly caused by methylthiouracil, other factors which promote TSH synthesis (e.g. iodine deficiency or radioiodine treatment) may also give rise to their development, especially in the thyroid gland of rodents.

Data regarding the frequency of malignant tumours are contradictory. FORTNER et al. [8] encountered cancers in 28 per cent of their material; ISRAEL and ELLIS [16] found one single case among a great number of animals. WILLIS [30] reported on an incidence of 15 to 26 per cent, while others [2, 17, 19, 31] did not see malignant growth in any of their cases. Most of the malignant tumours were forming metastases. We failed to observe malignant growth, although no experiment reported in the literature lasted longer than ours. We have mentioned in connection with the different types of tumours that there were four among our cases in which the adenoma (situated in the original gland in 3 cases, in the transplant in one case) had a solid structure and was composed of polymorphous cells. In the absence of other signs of malignancy we regarded them as benign tumours. That the incidence of malignant growths showed wide differences in the descriptions of the various authors may have been due to differences in strain. It is, for instance, well known that the frequency of thyroid carcinoma amounts to 30 to 35 per cent in rats of the Long-Evan strain [18].

The histological types of the adenomas, observed in our cases, were essentially the same as have been described by other authors [1, 17, 19, 20, 21, 30].

Development and morphology of hypophyseal adenomas have been extensively discussed in the literature [2, 3, 9, 10, 11, 12, 13, 20, 22, 24, 26, 27]. Our observations agree with those reported by other authors. Tissues transplanted from hypophyseal adenomas, examined 21 and 42 days later, were found to have taken without having grown. Transplanted tumours of this kind are known to have a long period (60 to 120 days) of latency [27]. These transplants showed the same picture whether or not they had been implanted to normal or thyroidectomized hosts; they may therefore, be regarded as hormonally independent tumours, a conclusion confirmed by the histology

revealing large-scale polymorphism and a great number of mitoses, i.e. symptoms of malignancy.

ROSIN and RACHMILEWITZ [25] observed the rise of malignant growths in rats after chronic methylthyouracil treatment, they did not exactly define the tissues from which the tumours had originated and wrote of facial tumours near the orbit. Histologically, they were cancers of the squamous epithelium. UNGAR and ROSIN [29] claimed that such tumours arose in the sebaceous glands (Zymbal's glands) of the external auditory meatus. We examined these glands in every case, but found no tumours in them. While their size was extremely variable, we failed to find either a tumour or the atypical hyperplasia described by other authors. Such differences in the observations are presumably due to differences in the strains.

Summary

Autologous grafting of a thyroid lobe into the skin of the back was performed in 57 male rats. The animals were then treated with methylthiouracil for periods up to 678 days. Adenoma developed in 78.9 per cent of the thyroid glands left *in situ*, and in 43.8 per cent of the grafted thyroid tissue. No cancers were observed. The difference in the susceptibility to tumorous transformation of the differently situated thyroid tissues may have been due to differences in their trophic innervation.

The incidence of hypophyseal adenoma was 74 per cent in animals that had been treated longer than 500 days.

No tumours were found in Zymbal's glands.

REFERENCES

1. AXELRAD, A. A., LEBLOND, C. P.: (1955) Induction of Thyroid Tumors in Rats by a Low Iodine Diet. Cancer **8,** 339. — 2. BIELSCHOWSKY, F.: (1955) Neoplasia and Internal Environment. Brit. J. Cancer **9,** 80. — 3. DENT, J. N., GADSDEN, E. L., FURTH, J.: (1955) On the Relation between Thyroid Depression and Pituitary Tumor Induction in Mice. Cancer Res. **15,** 70. — 4. DÉVÉNYI, I., CZENKÁR, B., ENDES, P.: (1957) Wirkung der Cortison-Adaptationsbehandlung auf das fetale und erwachsene Schilddrüsen- und Nebenschilddrüsen-Homoiotransplantat der Ratte. Frankfurt Z. Path. **68,** 418. — 5. DÉVÉNYI, I., KERTÉSZ, L., CZENKÁR, B.: (1958) I^{131}-uptake of Homotransplanted Rat Thyroids. Acta biol. Acad. Sci. hung. **8,** 326. — 6. DÉVÉNYI, I., MEGYERI, L.: (1959) Functional Reactivity of Homotransplanted Rat Thyroid. Acta morph. Acad. Sci. hung. **9,** 73. — 7. DONIACH, I., LOGOTHETOPOULOS, J. H.: (1956) The Long Term Survival and Reaction to Methylthiouracil of Thyroid Auto-implants in the Rat. Brit. J. exp. Path. **37,** 306. — 8. FORTNER, J. G., GEORGE, P. A., STERNBERG, S. S.: (1960) Induced and Spontaneous Thyroid Cancer in the Syrian (golden) Hamster. Endocrinology **66,** 364. — 9. FURTH, J.: (1954) Thyroid-pituitary Tumorigenesis. J. nat. Cancer Inst. **15,** 687. — 10. GORBMAN, A.: (1956) Pituitary Tumors in Rodents following Changes in Thyroid Function: A. Review. Cancer Res. **16,** 99. — 11. GRIESBACH, W. E., PURVES, H. D.: (1945) The Significance of the Basophil Changes in the Pituitary Accompanying Various Forms of Thyroxine Deficiency. Brit. J. exp. Path. **26,** 13. — 12. HALMI, M. S.: (1950) Two Types of Basophils in the Anterior Pituitary of the Rat and their Respective Cytophysiological Significance. Endocrinology **47,** 289. — 13. HALMI, M. S.: (1952) Two Types of Basophils in the Rat Pituitary: "Thyrotrophs" and "Gonadotrophs" vs. beta and delta Cells. Endocrinology **50,** 140. — 14. HARANGHY, L.: (1959) Általános kórbonctan. Medicina, Budapest. — 15. ISLER, H.: (1959) Effect of Iodine on Thyroid Tumors Induced in the Rat by a Low-iodine Diet. J. nat. Cancer Inst. **23,** 675. — 16. ISRAEL, M. S., ELLIS, I. R.: (1960) The Neoplastic Potentialities of Mouse Thyroid under Extreme Stimulation. Brit. J. Cancer **16,** 206. — 17. LAPIS, K., VEKERDI, L.: (1962) Simultaneous Histological, Autoradiographic and Biochemical Examination of Experimentally Induced Thyroid Tumour. Acta morph. Acad. Sci.

hung. **11,** 267. — 18. LINDSAY, S., POTTER, G. D., CHAIKOFF, I. L.: (1957) Thyroid Neoplasms in the Rat: A Comparison of Naturally Occurring and I^{131}-induced Tumors. Cancer Res. **17,** 183. — 19. MONEY, W. L., RAWSON, R. W.: (1950) The experimental Production of Thyroid Tumors in the Rat Exposed to Prolonged Treatment with Thiouracil. Cancer **3,** 321. — 20. MORRIS, H. P.: (1955) The Experimental Development and Metabolism of Thyroid Gland Tumors. Advances in Cancer Research. Vol. III. Academic Press, New York, P. 51. — 21. *Напалков, Н. П.:* (1959) Морфологические особенности экспериментальных опухолей щитовидной железы, вызванных у крыс 6-метилтуорацилом. Вопр. Онк. **11,** 578. — 22. PETREA, I.: (1961) Recherches sur la tumorigénese de certaines glandes endocrines (thyroide, hypophyse et surrénale). Neoplasma (Bratisl.) **8,** 145. — 23. POTTER, G. D., LINDSAY, S., CHAIKOFF, I. L.: (1960) Induction of Neoplasms in Rat Thyroid Glands by Low Doses of Radioiodine. Arch. Path. **69,** 257. — 24. PURVES, H. D., GRIESBACH, W. E.: (1951) The Site of Thyrotrophin and Gonadotrophin Production in the Rat Pituitary Studied by McManus—Hotchkiss Staining for Glycoprotein. Endocrinology **49,** 244.— 25. ROSIN, A., RACHMILEWITZ, M.: (1954) The Development of Malignant Tumors of the Face in Rats after Prolonged Treatment with Thiourea. Cancer Res. **14,** 494. — 26. RUSSFIELD, A. B.: (1957) The Endocrine System, in Analytical pathology. ed. Mellors, R. C. McGraw—Hill, New York. — 27. STEWART, H. L., SNELL, K. C., DUNHAM, L. J., SCHLYEN, S. M.: (1959) Transplantable and Transmissible Tumors of Animals. Atlas of Tumor Pathology. Section XII, Fasc. 40. Armed Forces Institute Pathology. Washington. — 28. SUGIYAMA, N.: (1960) Histological Studies of the Thyroid Gland of the Albino Rat with Methylthiouracil, with Special Reference to its Structure- and Growth-plan. Okajimas Folia anat. jap. **35,** 219. — 29. UNGAR, H., ROSIN, A.: (1960) The Histogenesis of Thiourea-induced Carcinoma of the Auditory Duct Sebaceous (Zymbal's) Glands in Rats. Arch. De Vecchi Anat. pat. **31,** 419. — 30. WILLIS, J.: (1961) The Induction of Malignant Neoplasms in the Thyroid Gland of the Rat. J. Path. Bact. **82,** 23. — 31. WOLLMAN, S. H.: (1961) Effects of Feeding Thiouracil on Thyroid Glands of Rats. J. nat. Cancer Inst. **26,** 473.

DIE REAKTION VON RATTENSCHILDDRÜSENAUTOTRANSPLANTATE AUF METHYLTHIOURACIL-BEHANDLUNG

I. DÉVÉNYI

Nach der Autotransplantation des einen Schilddrüsenlappens von 57 männlichen Ratten unter die Rückenhaut wurden die Tiere 678 Tage hindurch mit Methylthiouracil behandelt. In den verbliebenen Schilddrüsenlappen ließen sich bei 78,9% der Fälle, in den transplantierten Lappen hingegen nur in 43,8% der Fälle Adenome nachweisen. Es entstanden keine Karzinome. In der abweichenden Tumorbildung der in zwei verschiedenen Lokalisationen befindlichen Schilddrüsengeweben spielt vermutlich ihre unterschiedliche trophische Innervation eine Rolle.

Bei den länger als 500 Tage mit Methylthiouracil behandelten Tieren wurden in 74% der Fälle Hypophysenadenome gefunden.

In der Zymbal-Drüse wurden keine tumorösen Veränderungen beobachtet.

РЕАКЦИЯ АВТОТРАНСПЛАНТАТА ЩИТОВИДНОЙ ЖЕЛЕЗЫ КРЫСЫ НА ДАЧУ МЕТИЛТИОУРАЦИЛА

И. ДЕВЕНЬИ

Автор проводил у 57 крыс-самцов автотрансплантацию одной доли щитовидной железы под кожу спины и в течение 678 дней давал животным метилтиоурацил. В оставшихся in situ долях щитовидной железы в 78,9% случаев, а в автотрансплантированных долях только в 43,8% случаев наблюдались аденомы; карцином не развивалось.

Предполагают, что отклонение трофической иннервации обусловливает различия в образовании опухолей в тканях щитовидной железы, находящихся в двух различных локализациях.

У животных, получавших метилтиоурацил в течение свыше 500 дней, в 74% случаев обнаруживалась аденома гипофиза.

В железе Цимбала опухолевых изменений выявлено не было.

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