Oncopathological Research Institute, Budapest (Director, Prof. B. KELLNER)

SIMULTANEOUS HISTOLOGICAL, AUTORADIOGRAPHIC AND BIOCHEMICAL EXAMINATION OF EXPERIMENTALLY INDUCED THYROID TUMOUR

K. LAPIS and L. VEKERDI

(Received May 13, 1960)

Since the first application of effective goitrogens, numerous authors have been engaged in the experimental induction of thyroid tumours. BIELSCHOWSKY [4], later HALL [13] and PASCHKIS et al. [21] administered in addition to the goitrogens acetylaminofluorene for that purpose and succeeded in accelerating thyroid tumour development in rats. According to GORBMAN [11], no such effect of acetylaminofluorene can be seen in mice.

The view has been generally accepted that a low iodine diet and the administration of goitrogens cause tumour formation by reducing the level of thyroid hormones in the blood and thereby increasing the thyrotrophic hormone production of the anterior pituitary [5, 24, 15, 19].

The action mechanism of the carcinogen used simultaneously with the goitrogens has not been satisfactorily explained (NAPALKOV, 20). ESMARCH [7] induced thyroid tumour by injecting methylcholanthrene directly into the thyroid. 9-10 dimethyl 1-2 benzanthracene used in combination with goitrogens also produced thyroid tumours (GNATISHAK, 10).

In the present studies the various phases of thyroid tumour development have been examined by morphological and biochemical methods. A low iodine diet, a goitrogenic substance and 2-acetylaminofluorene (2-AAF) were applied simultaneously to induce cancer, and the individual effects of the above-mentioned factors have been examined separately.

Methods

In the course of the experiments 185 inbred albino rats of the Debrecen and CB strains, of both sexes, each weighing 130 g on the average, were used. Their age ranged between 2½ and 4 months. They were divided in 5 groups. Group 1 served as a control. Group 2 was maintained on a low iodine diet as described by HALL [13]. Group 3, in addition to the diet, received drinking water containing 0.01 per cent 4-methyl-2-thiouracil (MT) and food made up with such water throughout the experimental period. Group 4 received, in addition to the low iodine diet, 2.5 mg 2-AAF suspended in water through a stomach tube three times weekly during the first 6 weeks of the experimental period. After the initial 6 weeks only the diet was maintained. Group 5, the largest of the lot, was maintained on a low iodine diet following the initial 6-week 2-AAF treatment and was in addition treated on the same lines as Group 3 (see Table 1)

Starting three months after beginning the experiments, rats belonging to the various groups were killed at intervals of 4 to 6 weeks. Their thyroid glands were weighed and worked up partly for biochemical, partly for histological examination. One hour before killing the

Table I

Group	Type of Treatment	No. of Animals
1	Controls	20
2	low iodine diet	10
3	low iodine diet + MT	25
4	low iodine diet + 2-AAF (for 6 weeks)	30
5	2-AAF (for 6 weeks) + low iodine diet + MT	100

animals, 50 μc ^{131}I was injected into the tail vein. In the course of the histological preparation the thyroid glands were fixed for 48 hours in 4 per cent neutral formalin or ice-cold acetone and subsequently embedded in paraffin. Sections 7—8 μ thick were made and autoradiographic pictures taken of the unstained specimens. Subsequently the specimens were stained with haematoxylin-eosin. Simultaneously the periodic acid — Schiff (PAS) reaction and in some cases

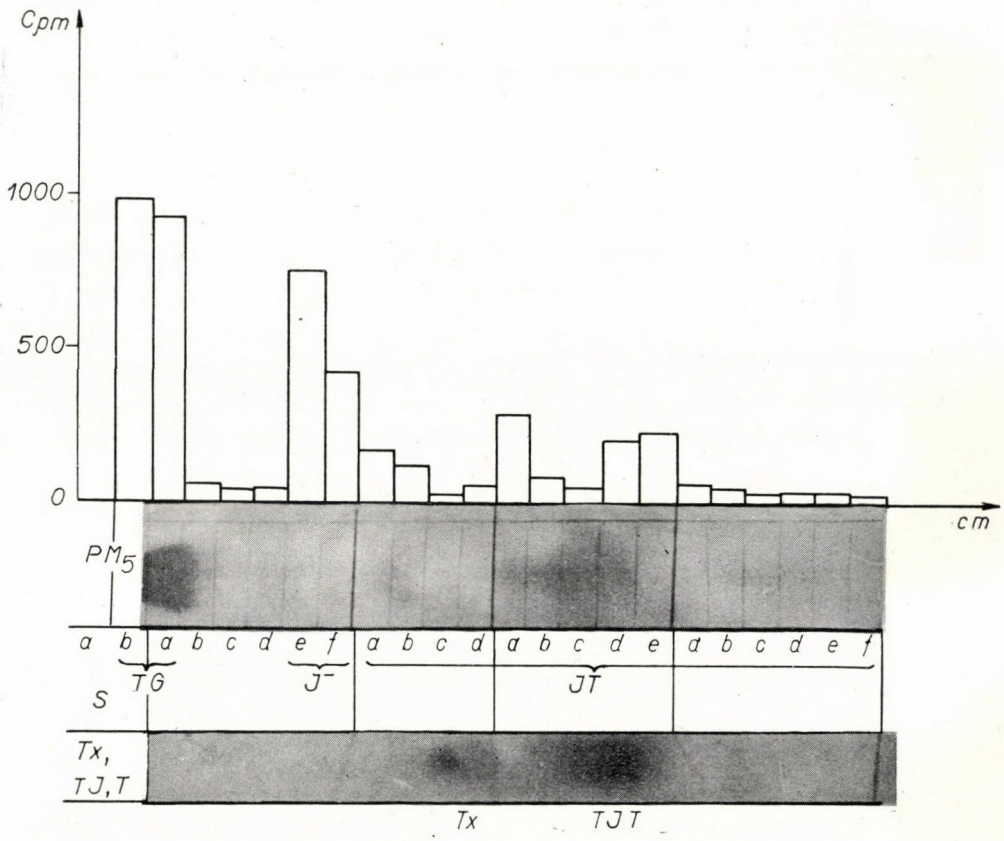


Fig. 1. Radiochromatogram of butanol extract of rat thyroid. Colums represent the radioactivity of different parts of the chromatogram (cutting 1 cm. in width). TG — thyreoglobulin, I^- — inorganic iodine and iodide, JT — iodthyronines

Gomori's alkaline phosphatase reaction, Kossa's calcium reaction and Gomori's silver impregnation were applied.

Histological work usually lasted a week. It was found that the correct exposure time for autoradiographs of the section was 24 hours. It was noted during the autoradiographic process that in the course of histological preparation (alcoholic-aqueous treatment) all the active materials contained in the thyroid sections were dissolved with the exception of those bound to protein. The radioactivity traces shown in the autoradiographs mark the ^{131}I built into the thyroglobulin.

For biochemical work a minimum of 30—40 mg of thyroid tissue is required. Such a quantity was not usually available from a single rat, so the thyroid glands of 2—3 animals belonging to the same group were worked up simultaneously. The thyroid glands were homogenized in 0.5 ml of distilled water to which was added one drop of saturated $Na_2S_2O_3$. The pH of this mixture was set to 3 by adding HCl. Subsequently the mixture was extracted three times each with 5 ml of butanol. The combined extracts were evaporated, paying attention all the time to prevent the temperature rising over 40°C. The residue was taken up in 0.2 ml of ethanol.

The material obtained was subjected to chromatography according to GROSS and PITT-RIVERS [12], TAUROG, CHAIKOFF and TONG [27] and TONG, TAUROG and CHAIKOFF [28].

0.1 ml of the residue dissolved in ethanol was chromatographed on Schleicher and Schüll paper No. 2043/b in n butanol—dioxan —2n ammonium hydroxide, 4 : 1 : 1, at 19°—20° C. The chromatograms were stained with ninhydrin and cut up into rectangles measuring 1 cm by 4 cm. The radioactivity of each of these rectangles was determined by an end-window GM tube with the positions kept constant throughout. The number of transmutations per rectangle per minute (Cpm) was expressed in the percentage of the Cpm for the whole chromatogram.

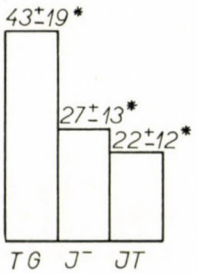
The chromatogram can be divided into three characteristic areas from the point of view of radioactivity. These are:

(1) At the spot where the material had been applied, the portion between Rf —0.05 and +0.05 corresponds to the thyroid protein remaining at the start line (TG).

(2) The activity at Rf 0.4 constitutes the area of the inorganic iodide and iodine.

(3) The activity at the area between Rf 0.45 and 1 contains the iodine compounds with thyronine structure and various other non-determined iodine compounds. As characteristic of thyroid function has been considered the ratio of the activities of areas No 1 and No 2 [18] (Figs. 1 and 2). Fig. 2 indicates that in spite of the wide scattering, in most control animals the amount of inorganic ^{131}I did not significantly exceed that of ^{131}I built into TG. A functional alteration was inferred to in case of a significant change in the relation TG/I.

Percentual distribution of I^{131} activity in buthanolic exract of untreated rat thyroids



*SD= Standard Deviation

	Percentual distribution of I^{131} activity						
N°	TG	J^-	JT	N°	TG	J^-	JT
1	26	47	15	11	39	33	17
2	78	7	7	12	22	45	25
3	49	25	12	13	52	25	21
4	29	36	19	14	54	18	22
5	38	30	22	15	38	10	43
6	48	25	12	16	35	14	41
7	40	26	20	17	20	33	39
8	86	3	2	18	21	36	45
9	62	26	12	19	15	37	33
10	34	51	18	20	54	8	24

Fig. 2. Characterisation of thyroid function by the ratio of activities of the three areas (TG, J^-, JT)

Results

The thyroid glands of the control rats maintained on a normal diet weighed 10 to 18 mg, and were composed of wide follicles filled with homogeneous eosinophilic colloid and lined with low cuboidal epithelium. The autoradiograph showed intensive focal ^{131}I uptake corresponding to the follicular areas (Figs. 3*a*, *b*). The ratio between the respective activities of thyroglobulin, iodide, and iodine compounds with thyronine structure is shown in Figs. 1 and 2. Specially characteristic of thyroid function is the ratio between the iodide built into TG and the unbound inorganic iodide. Under normal conditions the radioactivity of unbound ^{131}I does not exceed the radioactivity of the ^{131}I built into the thyroid proteins.

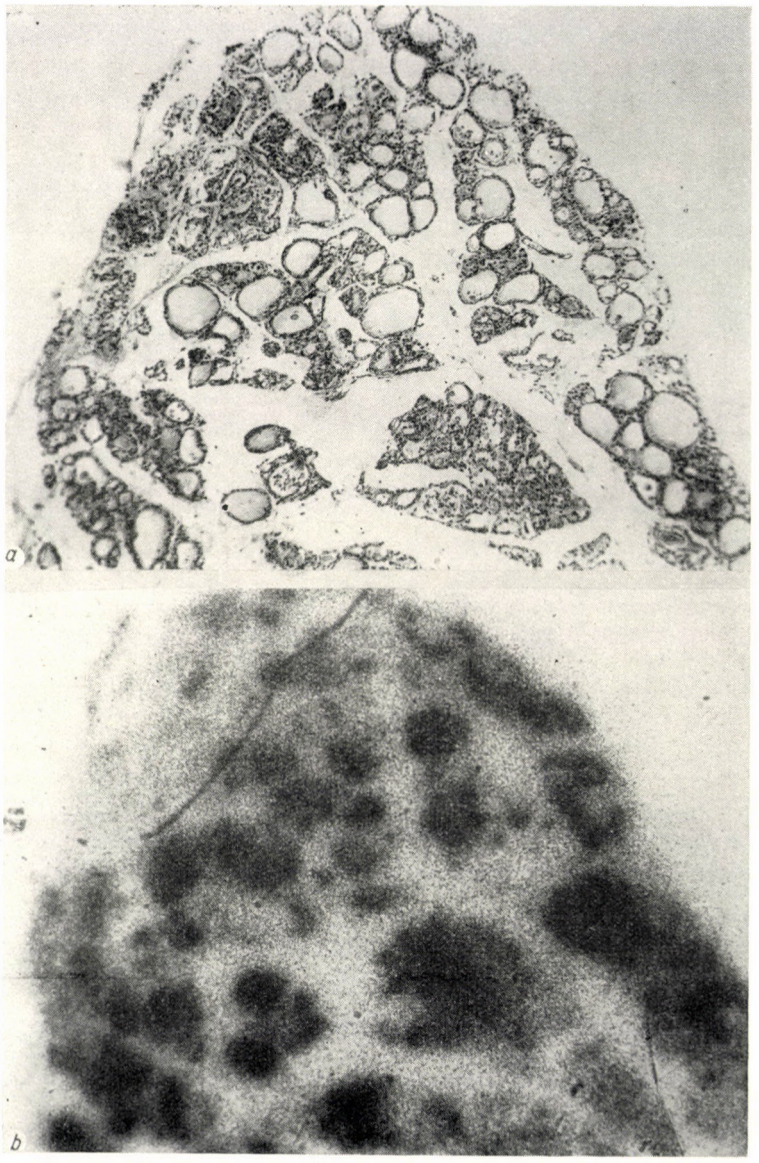
As a result of the low iodine diet the weight of the thyroid gland increased moderately, to 12—56 mg, showed diffuse hyperplasia, with acini lined with high cuboidal epithelium and containing little or no colloid. According to the autoradiograph, ^{131}I uptake slightly decreased but corresponding to the retained follicular structure the distribution of radioactivity was of focal character (Figs. 3*c*, *d*). The paper chromatogram indicated a slight decrease in the amount of ^{131}I built into thyroglobulin.

Under the effect of MT the weight of the thyroid gradually increased (70—400 mg) and pronounced diffuse hyperplasia occurred. The follicular epithelium became high cuboidal and then vacuolated cylindrical. The follicles were constricted and practically free of colloid. In the enlarged glands extensive vascularisation occurred. During the observation period of more than 400 days a circumscribed microadenoma was detected in one rat of the 22 belonging to this group.

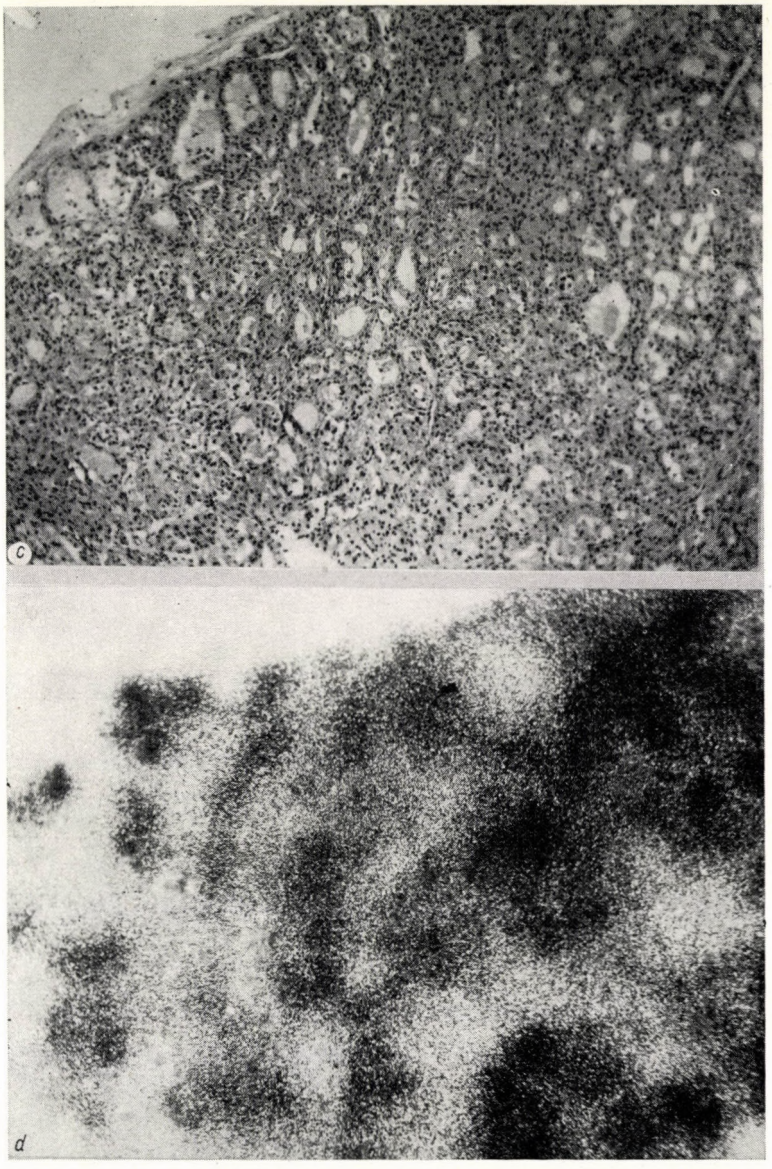
The autoradiographs showed decreased and rather diffuse radioactivity which had lost its follicular character (Figs. 4*a*, *b*). According to the chromatogram, no ^{131}I was built into TG (Fig. 5*a*), the quantity of inorganic iodine was several times that of ^{131}I built into TG.

On treatment by 2-AAF in combination with a low iodine diet the weight of the thyroid moderately increased (16—80 mg; mean, 48 mg). The follicular structure disintegrated in some areas, the epithelium of the persisting follicles was high and cuboidal, as in Group 3, but contained some colloid. ^{131}I incorporation was intense. Later on the epithelium underwent desquamation in many places and a number of acini became poor in colloid. In other places, basophilic concrements of concentric structure appeared in the dilated follicles; these concrements gave a positive Kossa's reaction and a negative one after decalcination. The rate of ^{131}I incorporation hardly decreased in spite of the morphological changes (Figs. 4*c*, *d*).

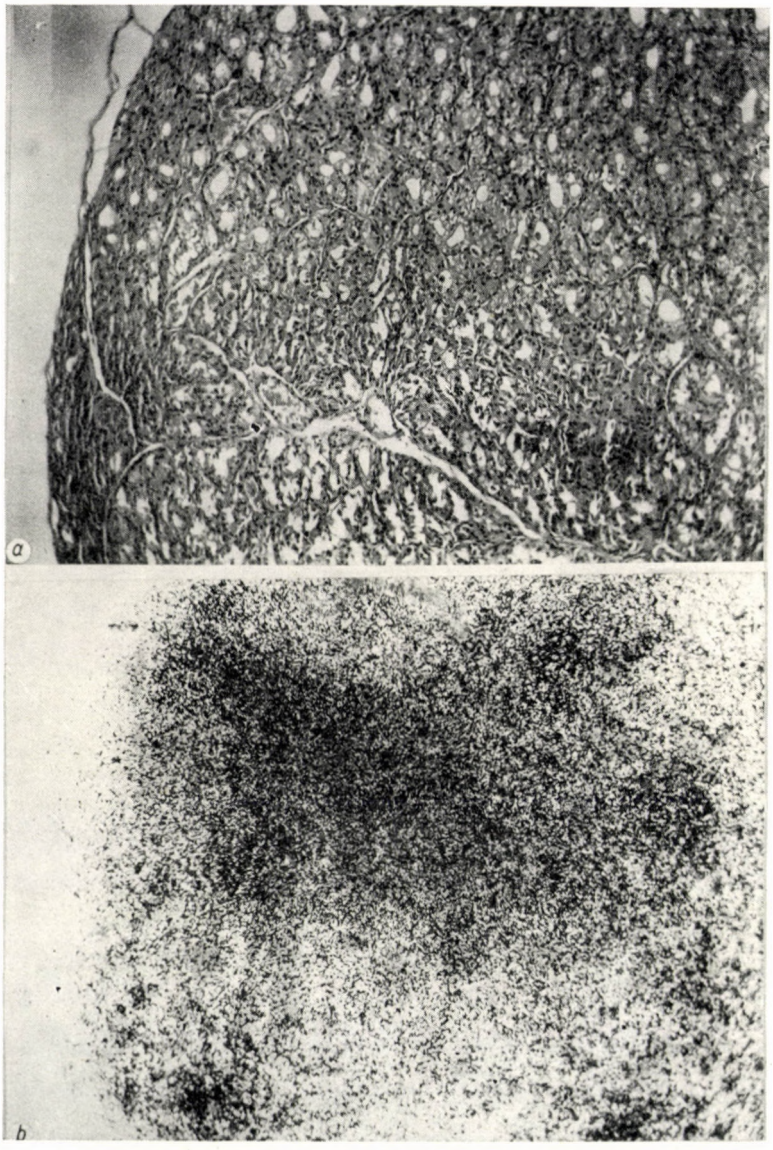
Paper chromatography showed a slight decrease in ^{131}I incorporation, and a slight increase in inorganic ^{131}I uptake (Fig. 5*h*).



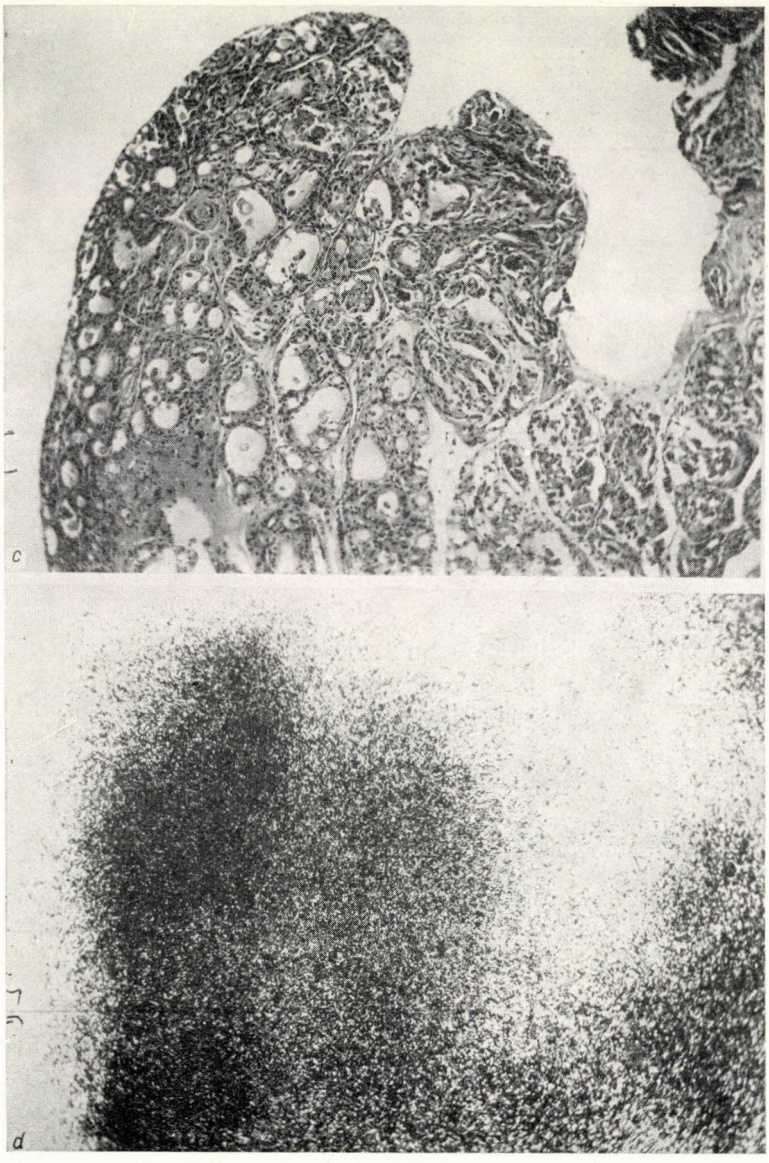
Figs. 3a and *b*. Thyroid of control rat: *a* — histological picture: wide follicles filled with homogeneous eosinophilic colloid and lined with low cuboidal epithelium. H. E. 85 × *b* — autoradiograph: intensive focal ^{131}I uptake corresponding to the focal areas



Figs. 3c and *d.* Thyroid of rat kept on low iodine diet (No. 9, duration of experiment: 118 days). *c* — histological picture: diffuse hyperplasia, with acini lined with high cuboidal epithelium containing little or no colloid. H. E. 85 × *d* — autoradiograph: slightly decreased ^{131}I uptake showing normal follicular distribution



Figs. 4a and *b*. Thyroid of rat treated with MT (No. 28, duration of experiment: 258 days) *a* — Histological picture: pronounced diffuse hyperplasia. Constricted follicles lined with high cuboidal epithelium and free of colloid. H. E. 85 × *b* — autoradiograph: decreased diffuse ^{131}I uptake



Figs. 4c and *d*. Thyroid of rat treated with 2-AAF. (No. 16, duration of experiment: 258 days). *a* — Histological picture: disintegrated follicular structure. The epithelium of persisting follicles is high cuboidal. In some areas the epithelium is desquamated or shows a fibrous swelling. In other places concrements of concentric structure containing calcium are seen in colloid-free dilated acini. H. E. 85 × *d* — autoradiogram: hardly decreased ^{131}I uptake showing some loss of follicular character

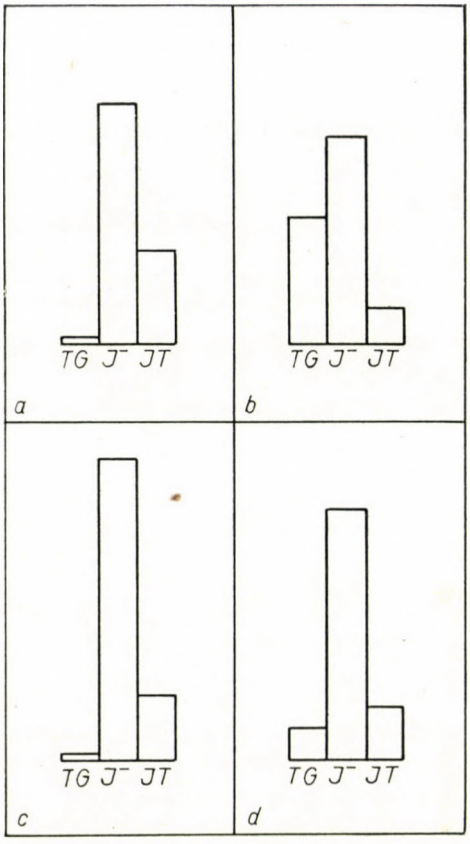
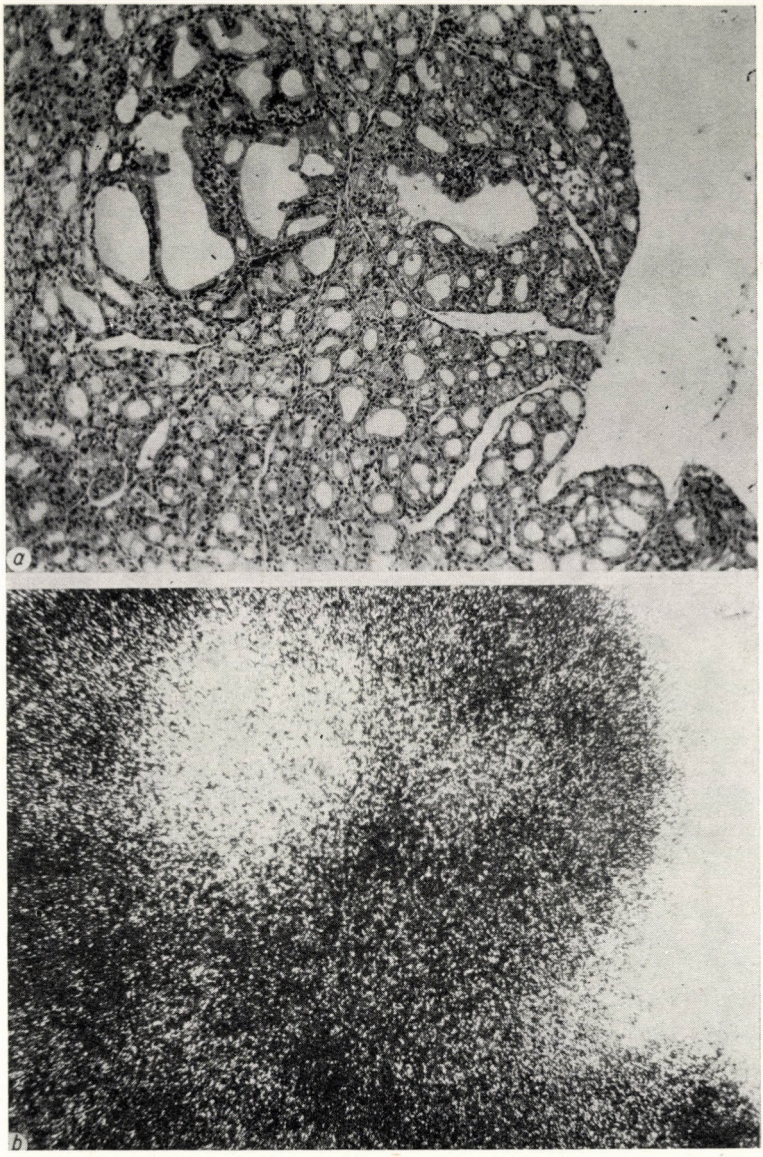


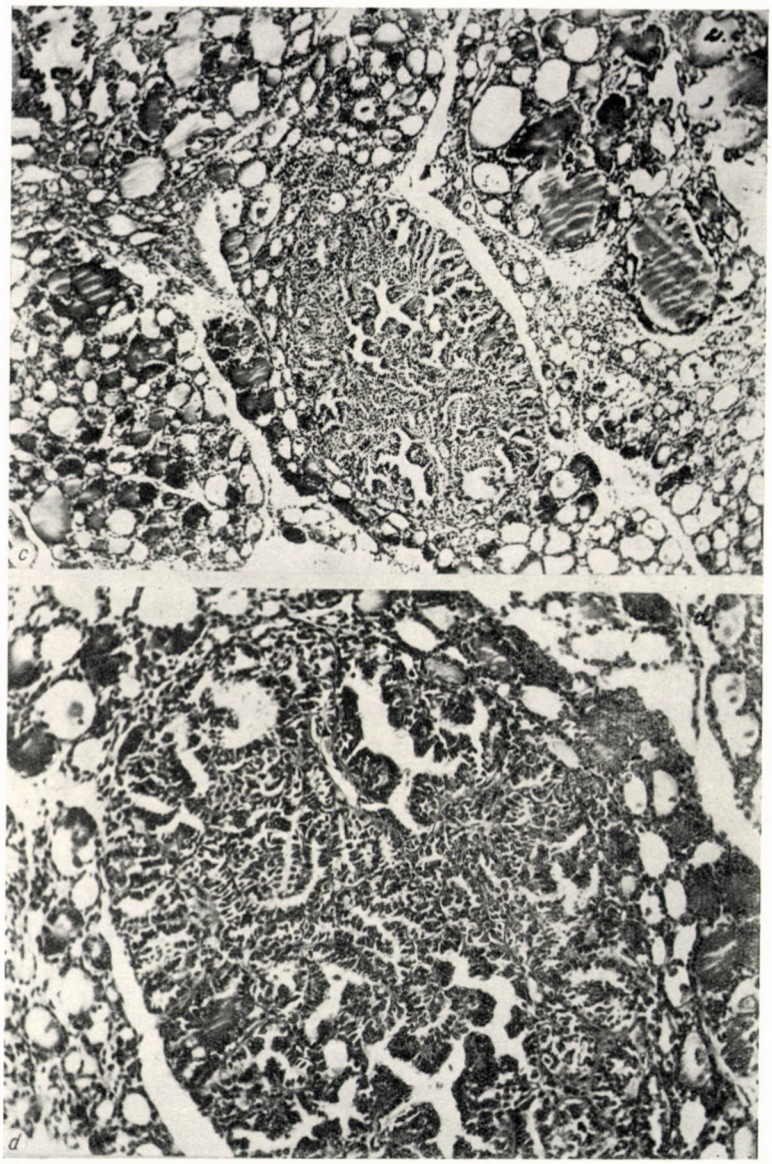
Fig. 5. Percentual distribution of total activity in areas TG, J^- and JT of radiochromatograms, after treatment with *a* — MT, *b* — 2-AAF, *c* — MT and 2-AAF, *d* — MT, 2-AAF and thyroxin

Under the combined effect of 2-AAF, MT, and a low iodine diet the weight of the thyroid increased with the duration of treatment (44 to 720 mg; mean 232 mg). As early as 200 days after the beginning of treatment, microadenomata, often a number of them, appeared in the hyperplasic environment. The environment often showed extensive round-cell infiltration, indicating previous tissue damage. The microadenomata were composed of follicles of varying size, lined by high cuboidal or cylindrical epithelium with nuclear hyperchromasia and a homogeneous eosinophil cytoplasm, and some of them contained a faintly eosinophilic PAS-positive colloid and desquamated epithelial cells, while some others were empty (Fig. 6*a*).

Later on some follicles of the adenomata became cystically dilated and the lining showed papillary proliferation. Such follicles were generally empty though some contained pale colloid.



Figs. 6a and *b*. Thyroid of rat treated with 2-AAF and MT (No. 61, duration of experiment: 258 days). *a* — histological picture: diffuse hyperplastic environment with adenomatous node composed of intensely stained wide follicles with high cuboidal or cylindric epithelium. Some follicles contain faintly stained eosinophilic colloid. H. E. 85 × *b* — autoradiograph: intense diffuse ^{131}I uptake in the hyperplastic thyroid tissue due to the unusual long exposure. No uptake in the adenomatous area



Figs. 6c and *d*. Thyroid of rat treated with 2-AAF, MT, and with thyroxin — 4 (10 μg s. c. daily for 35 days and 100 μg s. c. daily for 10 days before killing). (No. 76, duration of experiment: 301 days). *c* — papillary cystadenoma with hyperchromic high cuboidal epithelium. The surrounding thyroid tissue is composed of follicles with low cuboidal epithelium and PAS-positive colloid. T-4 treatment induced the formation of multilayered epithelium and hyperchromatic staining of nuclei in the adenoma. PAS-stain, *d* 85 × — *c* 190 ×

Radioautography showed practically no indication of ^{131}I uptake in the adenomata, cysts, or cystadenomata, even if the exposure was significantly prolonged, in order to intensify the contrast between the adenomata and their environment (Fig. 6*b*).

After the lapse of 400—500 days the adenomata were greatly enlarged and in some places coalescing. They were composed of irregularly shaped, distorted glandular ducts of varying width, lined by single or multiple-layered cylindrical epithelium with nuclear hyperchromasia. Some were empty while some contained colloid. In numerous follicles the epithelial lining formed ramifying papillas proliferating into the glandular ducts. In some places, however, the epithelium proliferated in the form of solid bundles. Neither atypia, nor polymorphism, vascular invasion or invasion through the capsule of the thyroid were observed. The area of adenomatous foci was intensively alkaline phosphatase positive, in contrast to the surrounding hyperplasic tissue and to the intact thyroid of the control subjects.

Autoradiography did not show ^{131}I accumulation in this phase.

Paper chromatography indicated a considerable decrease in all phases of ^{131}I incorporation, with a simultaneous increase in inorganic ^{131}I activity (Fig. 5*c*). In the cases where the adenoma occupied the major part of the gland, the ^{131}I content was considerably reduced.

In the course of papillary adenoma formation we examined the effects of suspending MT treatment and thyroxin administration. When MT had been discontinued for a protracted period, the weight of the thyroid decreased considerably (30—60 mg) and in the areas unaffected by adenomata the hyperplasia ceased, the follicles were again lined by single-layered cuboidal epithelium and contained approximately normal amounts of colloid. The adenomata themselves were reduced in size but retained their characteristic structure.

On the prolonged administration of thyroxin in doses of from 10 mg to 100 mg daily for 160 days, the follicles in the adenoma-free areas dilated, their epithelium was low cuboidal, and the lumina contained strongly PAS-positive colloid. The adenomata became reduced in size. Following massive doses of thyroxin, the papillary cylindrical epithelial lining of the adenomata became multiple-layered, with an increase of nuclear hyperchromasia. There was marked alkaline phosphatase positivity in the adenomatous areas. Out of seven cases in none was a regression or disappearance of the adenomata noted following the prolonged administration of thyroxin (Fig. 6*c*, *d*).

The administration of thyroxin greatly reduced the ^{131}I uptake. In accordance with the well-known effect of thyroxin, paper chromatography showed a great decrease of ^{131}I incorporation. The inorganic ^{131}I uptake was slight so that the gland as a whole became poor in iodine (Fig. 5*d*).

Discussion

The formation of experimentally induced thyroid tumours is generally attributed to the prolonged TSH overproduction by the anterior pituitary, irrespective of the mode of induction [13, 24, 15, 8]. The TSH overproduction is brought about by the reduction of the serum thyroxin level in all the carcinogenic methods used. On similar lines, regression of thyroid tumours was achieved by the administration of thyroid hormones. Further, PURVES et al. [22, 23] described the involution of a pulmonary metastasis of thyroid tumour following the administration of desiccated thyroid gland. BIELSCHOWSKY and GRIESBACH [6] administered 5 μg of d,l-thyroxin per 100 g of body weight during the last three weeks of the experimental period and observed a complete regression of the MT-induced thyroid hyperplasia and the proliferation of basophilic cells in the pituitary. The response was different to 2½ μg of d,l-thyroxin daily, which amount constitutes the normal daily requirement of healthy animals. This caused a regression of the thyroid changes while if 2-AAF was administered in addition, such regression did not occur. It therefore seems that 2-AAF causes a kind of change in the thyroid, which cannot be reversed by the low thyroxin dose corresponding to the normal daily requirement. The nature of this change has not been clarified.

Some authors hold the contrasting view that the administration of thyroxin or thyroid tissue is not only inadequate for causing the regression of existing adenomata, but in some cases can even lead to their malignant transformation [16, 10].

In the course of our own experiments the prolonged administration of thyroxin causing the inhibition of excessive TSH secretion failed to bring about the regression of the adenomata and, in fact, led to their increased proliferation. As a result of this treatment the hyperchromasia of the cells increased, the epithelial lining became multiple layered and entered papillary proliferation and the alkaline phosphatase positivity increased, in the adenomata.

At the same time the effect of thyroxin was clearly observable in the areas unaffected by adenomata. In these areas the epithelial lining assumed a cuboidal form and the dilated follicles became filled with PAS-positive colloid.

Most authors observed a high rate of adenoma formation in the animals treated with thiouracil derivatives [22, 23, 20, 17]. For instance, MONEY et al. [16] found 100 per cent adenomata after 500 days of thiouracil treatment.

We, on the other hand, found in the 500-day observation period a single case of microadenoma formation among the surviving 25 animals maintained on a low iodine diet containing MT.

For increasing the carcinogenic potency of MT BIELSCHOWSKY [4] applied 2-AAF. The result was a frequent and early adenoma formation. His

results have been confirmed by numerous authors [13, 21, 26, 1, 20]. According to HALL [13], under the effect of 2-AAF latent neoplastic cells are formed in the thyroid and it is from these cells that tumour formation later starts under TSH stimulation evoked by goitrogens [13, 14]. BERENBLUM [3, 6] regards these results as yet another proof of his two-phase theory of carcinogenesis. NAPALKOV contests this view [20], on the basis that such a correlation cannot possibly exist between the two drugs because either act as carcinogenic agent in the context of the thyroid.

In the course of the present experiments the administration of 2-AAF in itself has been found not to impair thyroid function considerably although it caused extensive morphological changes. This finding corresponds to BIELSCHOWSKY's observation according to which following 2-AAF treatment considerably higher thyroxin doses are required for the inhibition of TSH secretion. This would mean that 2-AAF treatment does not diminish thyroid function and, in fact, would in some cases increase ^{131}I incorporation.

In the present material exclusive 2-AAF treatment did not produce tumour formation in any one of 30 animals, on combined treatment (2-AAF MT), however, adenoma formation occurred in all animals surviving for 5 months. This means that under the effect of this combined treatment adenomata had developed at an earlier date, in greater numbers and in all the treated animals.

There is a fundamental difference between the action mechanisms of the two agents. 2-AAF, although causing considerable tissue damage, does not significantly influence thyroid function. MT, on the other hand, deeply influences thyroid function without causing serious anatomical changes. The intensification and acceleration of tumour formation is the sum of the two effects.

The administration of 2-AAF in small doses and for short periods is only followed by tumour formation if it is accompanied by some other chemical or hormonal carcinogenic effect over a prolonged period. This is the explanation which can be offered concerning the tumour-like structures in the ovaries of some animals which had received combined treatment. It is well known that MT does not only cause TSH overproduction but FSH overproduction, too, on a smaller scale. This associated FSH overproduction in combination with the effect of 2-AAF provides the explanation for the focal proliferations and tumour-like structures in the ovaries.

Similarly can be interpreted the observation that if the administration of 2-AAF is followed by paradimethylaminoazobenzene treatment, liver tumours occur, while if the administration of 2-AAF is followed by croton oil painting of the skin, the development of skin tumours and the acceleration of the development of such tumours, respectively, ensues as a result.

Summary

Morphological, histochemical, autoradiographic and biochemical examinations have been carried out in various stages of experimental thyroid cancer induced in rats kept on a low iodine diet by the administration of 2-acetylaminofluorene and 4-methyl-2-thiouracil.

The low iodine diet alone caused moderate hyperplasia. Low iodine diet and methyl thiouracil resulted in extensive diffuse hyperplasia. Thyroid activity was greatly reduced in both these groups and more diffuse than focal in character.

Acetylaminofluorene produced tissue damage but hardly influenced thyroid function.

None of the factors by itself induced tumour during the 400 days of observation. Combined treatment (low iodine diet + acetylaminofluorene + methylthiouracil), brought about adenomata in all animals in about 250 days. According to the autoradiographs there was practically no ^{131}I uptake in the area of the adenomata.

The following morphological stages could be distinguished in the induced tumours (i) Aspecific tissue damage (due to acetylaminofluorene). (ii) Diffuse hyparplasia (due to prolonged TSH overproduction caused be methylthiouracil). (iii) Multiple microadenomata. (iv) Papillary adenomata and cystadenomata.

Following the intravenous injection of 50 μc of ^{131}I, the amounts built into the thyroid proteins and inorganic iodine were separated by paper chromatography. The ratio of the two fractions seemed to be characteristic of thyroid function. Acetylaminofluorene brought about marked morphological changes but did not considerably influence the ratio of the two ^{131}I fractions. Methylthiouracil diminished the incorporation of ^{131}I with the parallel increase of the inorganic fraction. Little, if any, ^{131}I was incorporated by the adenoma developing as a result of the combined treatment.

None of the animals receiving combined treatment developed a malignant tumour within 500 days. On the other hand, the adenomata lost their hormone dependence as seen from the fact that regression could be achieved neither by discontinuing methylthiouracil treatment nor by the administration of high thyroxin doses. No ^{131}I was incorporated by the adenomata although they contained colloid. This colloid was obviously different from that present in normal thyroids.

REFERENCES

1. Axelrad, A. A., Leblond, C. P.: (1953) Thyroid Tumor Inducation in Rats by a Low Iodine Diet with and without 2-acetylaminofluorene. Proc. Amer. Ass. Cancer Res. **1,** 2. — 2. Berenblum, I.: (1954) In Advances in Cancer Research. **2,** 129. Academic Press New York. — 3. Berenblum, I.: (1957) Ann. Roy. Coll. Surg. Eng. **21,** 339. Cit. Napalkov, 20. — 4. Bielschowsky, F.: (1944) Tumours of Thyroid Produced by 2-acetyl-aminofluorene and Allyl-thiourea. Brit. J. exper. J. Path. **25,** 90. — 5. Bielschowsky, F., (1955) Neoplasia and Internal Environment. Brit. J. Cancer. **9,** 80—116. — 6. Bielschowsky, F., Griesbach, W. E.: (1950) Effect of Acetaminofluorene on Thyroids of Rats Treated with Methylthiouracil and Thyroxine. Brit. J. Cancer **4,** 135—138. — 7. Esmarch, O.: (1942) Deposition of Methylcholanthrene in some Organ of the Rats. Acta path. microbiol. scand. **19,** 79—99. — 8. Forgács, P., Vekerdi, L., Reviczky, A., Feuer, Gy., Szántó L.: (1959) A hypophysis pajzsmirigyműködést szabályozó hatásának vizsgálata ^{131}I inkorporációval. Kísérl. Orvostud. Budapest, **11,** 586—590. — 9. Furth, J.: (1953) Conditioned and Autonomous Neoplasms: Review Cancer. Res. **13,** 477—492. — 10. *Гнатышак А. И.:* (1957) Опухоли щитовидной железы в эксперименте и сопутствующие им изменения в некоторых органах внутренней секреции. Вопр. Онкологии **6,** 659—666. — 11. Gorbman, A.: (1946) Thyroid Changes Induced by Prolonged Feeding of Thiourea. Proc. Amer. Ass. Cancer Res. **6,** 492. — 12. Gross, J., Pitt-Rivers, R.: (1951) Unidentified Iodine Compounds in Human Plasma in Addition to Thyroxine and Iodine. Lancet **2,** 766—767. — 13. Hall, W. H.: (1948) The Role of Initiating and Promoting Factors in the Pathogenesis of Tumours of the Thyroid. Brit. J. Cancer **2,** 273. — 14. Hall, W. H., Bielschowsky, F.: (1949) The Development of Malignancy in Experimentally Induced Adenomata of the Thyroid. Brit. J. Cancer, **3,** 534—541. — 15. Leathen, J. H.: (1958) Goitrogen-induced Thyroid Tumours. Ciba Fundation. Colloquia on Endocrinology **12,** 50. — 16. Money, W. L., Fitzgerald, P. J., Godwin, J, T., Rawson, R. W.: (1953) Effect of Thiouracil on Collection of Radioactive Iodine in Experimentally Induced Thyroid Tumours. Cancer, **6,** 111—120. — 17. Money, W. L., Rawson, R. W.; (1947) Cit. 16. — 18. Morris, H. P.: (1955) The Experimental Development and Metabolism of Thyroid Gland Tumours. Advances in Cancer Research III. Academic Press,

New York. — 19. MÜHLBOCK, O.: (1960) Der Einfluß von Hormonen auf den Tumor. Berliner Symposion über Fragen der Carcinogenese. Akademie Verlag, Berlin. — 20. *Напалков, Н. П.:* (1949) Экспериментальные опухоли щитовидной железы, Вызванные комбинированным Воздействием 6-Метилтиоурацила и 2-Ацетиламинофлуорена. Вопр. Онкологии **5**, 25—33. — 21. PASCHKIS, K. E., CANTAROW, A., STASNEY, J.: (1948) Influence of Thiouracil on Carcinoma Induced by 2-Acetaminofluorene. Cancer. Res. **8**, 257. — 22. PURVES, H. D., GRIESBACH, W. E.: (1946) Studies on Experimental Goitre; Thyroid Carcinomata in Rats Treated with Thiourea. Brit. J. exp. Path. **27**, 294—297. — 23. PURVES, H. D., GRIESBACH, W. E.: (1947) Studies on Experimental Goitre; Thyroid Tumours in Rats Treated with Thiourea. Brit. J. exp. Path. **28**, 46—53. — 24 PURVES, H. D., GRIESBACH, W. E., KENNEDY, T. H.: (1951) Studies in Experimental Goitre; Malignant Change in Transplantable Rat Thyroid Tumour. Brit. J. Cancer. **5**, 301—310. — 25. SAFFIOTTI, U.: (1960) Kombinierte Effekte von verschiedenen und entfernt wirkenden Carcinogenen. Berliner Symposion über Fragen der Carcinogenese. Akademie Verlag, Berlin. — 26. *Шабад, Л. М.:* (1954) Некоторые экспериментальные данные к этиологии и патогенезу опухолей. Вестник Хир. **74**, 3, 6—17. — 27. TAUROG, A., CHAIKOFF, I. L., TONG, W.: (1950) Nature of Plasma Iodine as Revealed by Filter Paper Partition Chromatography. J. biol. Chem. **184**, 99—104. — 28. TONG, W., TAUROG, A., CHAIKOFF, I. L.: (1951) Nonthyroglobulin Iodine of Thyroid Gland; free Thyroxine and Diiodothyrosine. J. biol. Chem. **191**, 665—675.

ПАРАЛЛЕЛЬНЫЕ ГИСТОЛОГИЧЕСКИЕ, АВТОРАДИОГРАФИЧЕСКИЕ И БИОХИМИЧЕСКИЕ ИССЛЕДОВАНИЯ КАРЦИНОГЕНЕЗА ЩИТОВИДНОЙ ЖЕЛЕЗЫ

К. ЛАПИШ и Л. ВЕКЕРДИ

1. Проводились сравнительные гистологические, авторадиографические и биохимические исследования на крысах в различных стадиях карциогенеза щитовидной железы.

Опухоли вызывались у крыс при режиме питания с недостатком иода и одновременным введением 2-ацетил-аминофлюорена (2—ААФ) и 4-метил-2-тиоурацила (**МТ**). Эффект отдельных воздействий исследовался также обособлено.

2. Введение 2—ААФ само по себе ни в одном случае не вызывало образования опухоли. Среди животных, содержанных при режиме питания с недостатком иода и получивших **МТ** в течение 500 дневного периода, всего лишь в одном случае наблюдалось образование микроаденомы.

В случае комбинированного воздействия уже начиная с 5 месяца у всех животных образовались аденомы.

3. 2—ААФ, несмотря на поражение тканей, не оказал существенного влияния на функцию щитовидной железы. **МТ**, в значительной мере изменяет функцию щитовидной железы без тяжелых морфологических изменений. Образование опухоли является результатом совместного эффекта двух воздействий различного типа.

4. Продолжительное введение больших доз тироксина не вызывало обратного развития аденом.

PARALLELE HISTOLOGISCHE AUTORADIOGRAPHISCHE UND BIOCHEMISCHE UNTERSUCHUNG DER SCHILDDRÜSENKARZINOGENESE

K. LAPIS und L. VEKERDI

1. Vergleichende histologische, autoradiographische und biochemische Untersuchungen wurden an Ratten in verschiedenen Stadien der Karzinogenese der Schilddrüse vorgenommen.

Die Geschwülste wurden bei an jodarmer Diät gehaltenen Ratten mittels Verabreichung von 2-Acetylaminofluor (2-AAF) und 4-Methyl-2-thiouracil (MT) hervorgerufen.

2. Die Behandlung mit 2-AAF allein führte in keinem einzigen Fall zu Geschwulstbildung. Unter den an jodarmer Diät gehaltenen und mit MT allein behandelten Tieren würde

während der 500tägigen Beobachtungszeit nur in einem einzigen Fall die Bildung von Mikroadenomen festgestellt.

Bei kombinierter Behandlung hingegen traten Adenome bereits nach 5 Monaten bei sämtlichen Versuchstieren auf.

3. Die Behandlung mit 2-AAF hat trotz der Gewebsschädigung die Schilddrüsenfunktion nicht wesentlich beeinträchtigt. Die Behandlung mit MT rief jedoch eine tiefgehende Veränderung der Schilddrüsenfunktion hervor, ohne schwere morphologische Schädigungen zu verursachen. Die Geschwulstbildung war das Ergebnis der Summation der zwei verschiedenen Wirkungsarten.

4. Chronische Verabreichung hoher Thyroxindosen bewirkte keine Rückbildung der entstandenen Adenome.

Dr. Károly LAPIS
Dr. László VEKERDY
Budapest XII. Ráth Gy. u. 5., Hungary