EXPERIMENTAL HEPATOPATHIES AND MAMMARY GLAND CARCINOMA IN RATS

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Carbon tetrachloride was administered to female noninbred albino rats in a dose of 0.1 ml/100 g body weight twice a week for 2 years. The glucuronic acid concentrations in the blood and urine and biological activity of estrogens in the urine were determined. The excretion of glucuronic acid with the urine was constantly reduced in the experimental rats, whereas the biological activity of estrogens was increased as the experiment progressed. Against the background of these changes 75% of the experimental animals developed tumors of the mammary glands, some of them malignant.

After experimental injury to the liver by CCl_4 rats develop benign tumors of the fibroadenoma type [3, 6], while CC57 mice develop mammary gland carcinoma [2]. The appearance of these tumors is attributed to a disturbance of the inactivation of steroid hormones in the liver.

Inactivation of hormones, especially estrogens, takes place by their conjugation with glucuronic acid (GA) in the liver.

The object of the present investigation was to study the relationship between GA and estrogens and the possible effect of a disturbance of this relationship on the development of mammary gland tumors in rats.

EXPERIMENTAL METHOD

The experiments were carried out on 30 noninbred female albino rats aged 5-6 months and weighing 150-220 g. Twice a week the animals received a subcutaneous injection of CCl_4 in a sublethal dose (0.1 ml/100 g body weight). Fifteen female rats of the same age and weight acted as the control.

The GA concentration in the 24-h specimen of urine was determined for each rat separately every month [1]. At the 8th, 11th, and 15th months of the experiment the GA concentration in the blood and in the protein-free filtrate of the serum (fraction 1), the protein-bound GA (fraction 2), and the total GA also were investigated. The blood GA level of all the experimental animals was determined in the same test [9].

The biological activity of estrogens isolated from the urine of the rats [7] was investigated at the same times by means of the Allen–Doisy test. The biological activity of the estrogens was expressed as the ratio between the number

TABLE 1. GA Concentration in the Blood (in mg%), Its Excretion With the Urine (in mg/day), and Biological Activity of Estrogens (in percent) In Healthy Rats and in Rats Poisoned With CCl_4

Time of investigation from beginning of experiment	GA excretion with urine	GA concentration in blood			Biological activity of estrogens
		fraction 1	fraction 2	total GA	
8th month					
Control	2,5	1,1	2,1	3,2	30,9
Experiment	1,8	0,3	0,5	0,8	36,2
11th month					
Control	2,9	1,4	3,4	4,8	35
Experiment	1,6	0,6	0,3	0,9	61,6
15th month					
Control	1,8	0,6	1,4	2,0	30
Experiment	1,1	0,7	1,2	1,9	62,5

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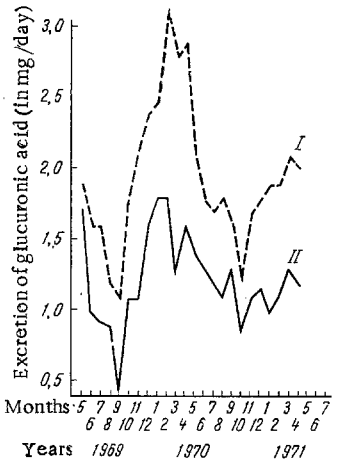

Fig. 1. Excretion of glucuronic acid with the 24-h urine of healthy rats (I) and rats poisoned with CCl_4 (II).

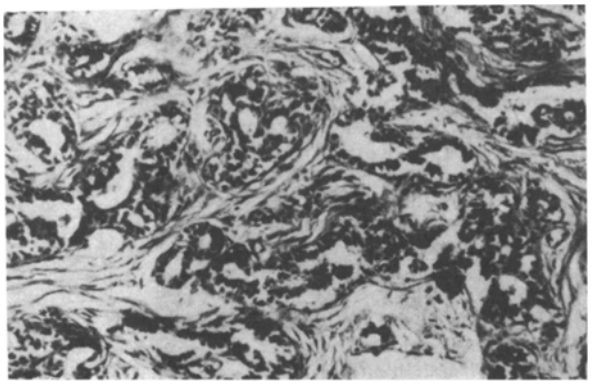

Fig. 2. Adenocarcinoma of the mammary gland. Hematoxylin-eosin, 90×.

of castrated mice in which injection of rat estrogens induced estrus and the total number of mice tested, and was expressed as a percentage [4].

The rats in both groups died naturally. The organs of 28 experimental and 12 control animals were fixed in 10% neutral formalin. Paraffin sections of the mammary glands, uterus, liver, kidneys, spleen, and lungs were stained with hematoxylin-eosin.

EXPERIMENTAL RESULTS

The initial 24-hourly excretion of GA was virtually identical in the experimental and control groups ($P > 0.1$). The GA excretion in the urine was reduced in the experimental group 1 month after the beginning of the experiment. This decrease remained constant throughout the experiment and, with rare exceptions (3rd and 16th months) it was statistically significant (from $P < 0.05$ to $P < 0.001$). The excretion of GA varied with the time of year: it was higher in the winter months than in the summer. The same fluctuations in the excretion of GA were observed in the experimental animals as in the control, but the range of these fluctations was smaller and the mean daily GA excretion remained constantly lower in the experimental rats than in the control (Fig. 1).

The GA excretion in the urine correlated with its blood level (Table 1). In the control group the amount of GA excreted with the urine depended on its blood level ($r = 0.97$), whereas in the experimental group the GA excretion by the kidneys was reduced despite a relative increase in the total GA in the blood ($r = -0.98$). A compensatory mechanism aimed at restricting the excretion of GA because of its reduced synthesis in the liver, was evidently brought into play in this case.

The disturbance of GA excretion was combined with changes in the estrogenic activity of the urine. In the control group, for instance, the biological activity of the estrogens was about the same at all times of the experiment. In the experimental group, however, it increased with the duration of the experiment (Table 1). The difference between the estrogenic activities in the control and experimental animals became significant in the 11th month of the experiment ($d = 4.4$) and remained so until its end. Consequently, in the experimental rats an increase in activity of the estrogens was combined with a decrease in the GA excretion in the urine.

Against this background the animals developed morphological changes in the mammary glands. Macroscopically mammary gland tumors were found in six experimental animals. The first tumor appeared in the 13th month of the experiment, grew for the next 6 months, and was shown histologically to be an adenocarcinoma (Fig. 2). In two other mammary glands the same rat developed an intraduct papillary adenocarcinoma and hyperplasia with atypia of the epithelium. The tumors were investigated after death of the animals approximately 7-8 months after the appearance of the tumor nodule. Five of the six tumors demonstrable visually were adenocarcinomas and scirrhus or solid carcinomas, while one was a fibroadenoma. Besides these five tumors, microscopic examination of the mammary glands revealed carcinoma in another three rats. Consequently, more than one-quarter of the animals developed malignant tumors of the mammary glands.

Benign tumors of the fibroadenoma and adenoma type, and fibrocystic disease of the mammary gland were found in five, one and two rats respectively. Hyperplasia of the epithelium with features of atypical growth were found in the mammary glands of five animals. In two rats hyperplasia was unaccompanied by atypia. No changes were found in the mammary glands of only five animals.

Fibrocystic disease of the mammary glands was found in one rat of the control group. Spontaneous precancerous states of the mammary glands in rats of this type have also been described by other workers [5].

Signs of endometriosis were observed in the uterus approximately equally often in the two groups.

Granular and vacuolar degeneration were observed in the liver of the experimental animals, and carcinoma of the intrahepatic bile duct was found in one rat. Granular degeneration was slight in the liver of the control rats.

No significant changes were found in the kidneys and spleen of either group, but a bronchogenic carcinoma was found in the lungs of one experimental rat, with metastases in the lymph glands.

Against the background of the reduced excretion of GA and increased activity of estrogens, 75% of the experimental rats thus developed changes in the mammary glands, in some cases with the appearance of malignant tumors. These changes probably took place because in rats poisoned with CCl_4 the synthesis of GA in the hepatocytes [8, 10] and its conjugation with various substances [11], including with estrogens, are disturbed with the result that an excess of unbound physiologically active estrogens arises. Consequently, the endogenous hyperestrogenemia which is regarded as playing the leading role in the development of mammary gland tumors in animals and man may be due, besides to other causes, to a disturbance of the glucuronic acid forming function of the liver.

LITERATURE CITED

1. I. A. Aksamitnaya and V. V. Tatarskii, Lab. Delo, No. 6, 21 (1961).
2. V. M. Berggol'ts and V. I. Gel'shtein, in: Problems in Oncology [in Russian], No. 7, Moscow (1954), p. 35.
3. A. N. Gorevaya, Proceedings of the Third Congress of Oncologists of the Ukrainian SSR [in Russian], Kiev (1967), p. 115.
4. Ya. M. Kabak, Textbook of Practical Endocrinology [in Russian], Moscow (1968).
5. E. L. Prigozhina, Arkh. Pat., No. 1, 109 (1956).
6. N. M. Turkevich, A. N. Gorevaya, L. K. Kunitsa, et al., Abstracts of Proceedings of the Eighth International Cancer Congress [in Russian], Moscow (1962), p. 179.
7. E. Furlong, B. Krichesky, and S. J. Glass, Endocrinology, 45, 1 (1949).
8. H. Kröner and W. Staib, Z. Klin. Chem., 6, 318 (1968).
9. J. Marrog, Ärz. Forsch., 12, 987 (1959).
10. D. Rubinstein, Am. J. Physiol., 203, 1033 (1962).
11. M. Yukutake, Okajama Igakkai Zasshi, 80, 969 (1968).