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REACTIVITY OF THE LIVER TO GLUCOCORTICOIDS DURING CHEMICAL HEPATOCARCINOGENESIS

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Liver cells during chemical carcinogenesis (3'-methyldimethylaminoazobenzene) and cells of primary hepatomas retain the property of reacting to partial hepatectomy by increased incorporation of ^{3}H-thymidine into DNA, just as under normal conditions this process is inhibited by dexamethasone. The inducibility of tyrosine aminotransferase (EC 2.6.1.5) likewise remained unchanged, whereas induction of tryptophan pyrrolase (EC 1.13.11.11) in primary hepatomas was abolished. The adequacy of a model of chemical carcinogenesis of an organ if the heterogeneity of its cell populations is disregarded is discussed.

KEY WORDS: dexamethasone; hepatocarcinogenesis; enzymes; DNA.

It is now widely recognized that among the properties of neoplasms as a whole primary and secondary properties must be distinguished [2]. The primary properties, possessed by both benign and malignant tumors, include uncontrolled cell proliferation, whereas the secondary properties include systemic action of the tumor on the host arising in the course of its progression and characterizing only tissue which has undergone malignant change, ability to produce metastases, chromosomal anomalies, and absence of control over specific functions belonging to the homologous tissue. It is evident that during the investigation of uncontrolled cell proliferation characteristic of a neoplasm attention must be directed to concrete control mechanisms. The object of this investigation was to study the action of glucocorticoids, which inhibit DNA synthesis and cell division both in the liver, in which they induce the synthesis of various enzymes, notably tyrosine aminotransferase (TAT) and tryptophan pyrrolase (TP), and in the tissues in which they give a catabolic effect (lymphocytes, fibroblasts, etc.) [2, 11, 13]. It was hoped to elucidate changes in the regulation of DNA synthesis and induction of enzyme synthesis by glucocorticoids during chemical carcinogenesis.

EXPERIMENTAL METHOD

Male Wistar rats weighing 150-250 g, kept on an ordinary diet or on a special diet including 3'-methyldimethylaminoazobenzene (3'-MDAB) (diet No. 3 according to [9]), were used. Animals with Zajdela ascites

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TABLE 1. Dependence of Inhibition of Incorporation of ^{3}H-thymidine into DNA of Liver Cells on Time of Administration of Dexamethasone after Partial Hepatectomy

Time of administration of dexamethasone after operation, h	Radioactivity of DNA, cpm/μg
1	40±13*
10	60±20
20	100±47

*Deviation of mean values corresponds to 0.95 level of probability in Student's test.

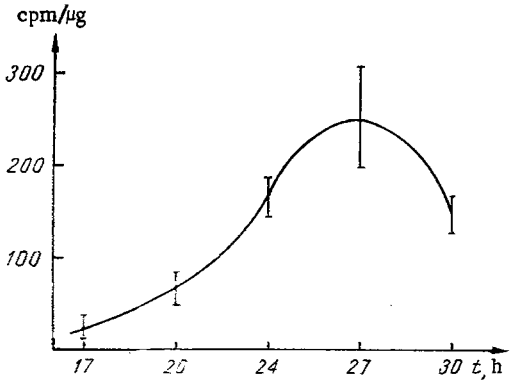

Fig. 1. Kinetics of incorporation of ^{3}H-thymidine into DNA of rat liver cells after partial hepatectomy. (Deviation of mean values here and in Figs. 2 and 3 corresponds to 0.95 level of probability in Student's test). Abscissa, time (in h); ordinate, cpm/μg.

hepatoma (ZAH) were used on the 4th day after transplantation of the tumor. Hepatectomy was performed by Higgins' method [7] between 8 and 10 a.m. in order to reduce to a minimum diurnal fluctuations in mitotic activity. Dexamethasone (the proprietary product Dexasone) was injected intraperitoneally in a dose of 1 mg/100 g body weight 1 h after the operation. ^{3}H-thymidine (12.8 Ci/mmole) was injected 1 h before sacrifice of the rats, 26 h after hepatectomy. Each animal received 100 μCi. Nucleic acids were precipitated from a 25% (by volume) liver homogenate by the addition of $HClO_4$ at 0°C to a final concentration of 0.2 M. The residue was washed three times with 0.2 M $HClO_4$ until the radioactivity disappeared from the washings. The residue was dissolved in 0.5 M $HClO_4$ and hydrolyzed for 15 min at 70°C. DNA in the supernatant was determined by Burton's method. Radioactivity was measured in "Ria-Sol" liquid scintillator on a Mark II counter (Nuclear Chicago).

TAT and TP are liver enzymes with a high turnover rate and are widely used as convenient models with which to study the molecular and physiological mechanisms of enzyme induction [10, 13]. During investigation of the various factors influencing TAT and TP activity the animals were decapitated and the liver quickly removed, minced, and homogenized in a glass homogenizer in 3 volumes of 0.1 M sodium-phosphate buffer, pH 7.6, containing 0.14 M KCl. The homogenate was centrifuged for 15 min at 15,000g. The supernatant was used to determine enzyme activity quickly after it had been obtained. In the case of TAT the effect of the glucocorticoids was assessed not only from changes in activity of the enzyme but also from changes in its absolute quantity, determined by the immunoprecipitation in agar method with specific antibodies [1]. TAT activity was determined by a modified Briggs' method [10]. The reaction mixture contained 10 mM α-ketoglutarate, 4 mM L-tyrosine, 40 μM pyridoxal phosphate, 50-200 μl of the test supernatant, and 0.3 M sodium-phosphate buffer, pH 7.6. The final volume of the mixture was 3 ml. Incubation was carried out for 1 h at 37°C. The reaction

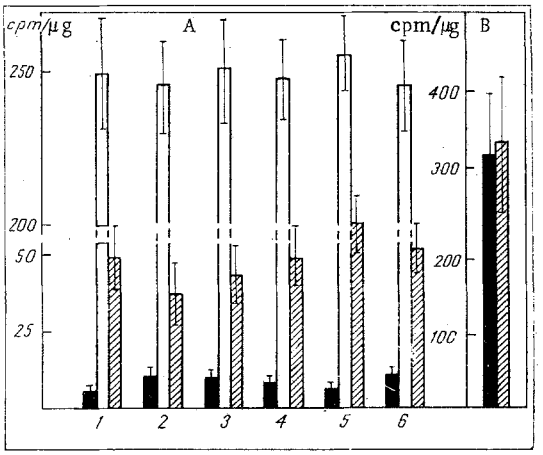

Fig. 2

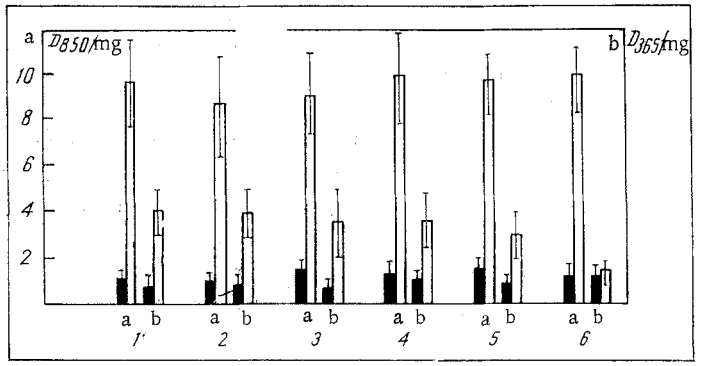

Fig. 3

Fig. 2. Effect of dexamethasone on incorporation of ^{3}H-thymidine into DNA. A) After partial hepatectomy: 1) rats received ordinary diet, 2-6) rats received diet with 3'-MDAB: 2) for 14 days, 3) 30 days, 4) 60 days, 5) 90 days, 6) primary hepatomas (120 days). Black columns – control; unshaded column – hepatectomy; obliquely shaded column – hepatectomy + dexamethasone. B) in ZAH cells. Black column – control, obliquely shaded column – dexamethasone. Ordinate, cpm/μg.

Fig. 3. Effect of dexamethasone on induction of TAT and TP during chemical carcinogenesis; a) TAT level; black columns – control, unshaded column – 4 h after administration of dexamethasone; b) TP level. 1) Rats received ordinary diet; 2-6) rats received diet with 3'-MDAB: 2) for 14 days, 3) 30 days, 4) 60 days, 5) 90 days, 6) primary hepatomas (120 days). Ordinate: a) D_{850}/mg, b) D_{365}/mg.

was stopped by the addition of 0.2 ml 50% TCA. The residue was removed by centrifugation and 2 ml of the supernatant was treated with 1 ml 1% KH_2PO_4 and 1 ml 3% ammonium molybdate in 5 M HCl. The optical density of this solution was measured 1 h later at 850 nm on the SF-16 spectrophotometer.

TP activity was determined by a modified Abdulla's method [3]. To 3 ml of the test supernatant 1 ml of 0.03 M L-tryptophan, 3 ml of 0.3 M sodium-phosphate buffer, pH 7.6, 5 ml H_2O and methemoglobin in a final concentration of $3 \cdot 10^{-6}$ M were added at 0°C. The reaction mixture (3 ml) was incubated for 75 min at 37°C. The reaction was stopped by the addition of 0.2 ml 50% TCA. To 2 ml supernatant 0.3 ml of 1 M NaOH was added. The optical density was measured 30 min later at 365 nm on the SF-16 instrument.

EXPERIMENTAL RESULTS AND DISCUSSION

The time corresponding to maximal incorporation of ^{3}H-thymidine into DNA and the conditions under which the action of glucocorticoids on this process was studied differed in investigations by different workers [4, 5, 12]; accordingly the optimal condition for detection of each of these effects were first determined (Fig. 1; Table 1). In accordance with the results of these preliminary experiments, in the main experiments both control rats and animals receiving 3'-MDAB with their diet were killed 27 h after the operation and dexamethasone was injected 1 h after partial hepatectomy. As Fig. 2 shows, liver cells during chemical carcinogenesis, and even cells of primary hepatomas separated from the remaining liver tissue, still preserved their ability to react to partial hepatectomy by an increase in the incorporation of ^{3}H-thymidine into their DNA; just as normally, this process also was inhibited by dexamethasone. The results now obtained do not agree with Fodge's hypothesis [6]; this worker, having shown that glucocorticoids inhibit DNA synthesis in cultures of normal fibroblasts but do not affect it in cells transformed by virus, concluded that this disturbance is specific for neoplasms. According to data in the literature, during hepatocarcinogenesis liver cells lose their ability to respond to partial hepatectomy by a burst of mitoses [4, 5, 12]. Since stimulation of DNA synthesis after partial hepatectomy was preserved both in the liver throughout the period of carcinogenesis and in primary hepatomas, it is natural to suggest that the block preventing these cells from starting mitosis retains them in the G_2-phase.

The inducibility of TAT was unchanged during hepatocarcinogenesis, whereas ability to respond to dexamethasone by the induction of TP was lost in the primary hepatomas (Fig. 3). These observations agree with the view that the disturbance of differentiation is secondary, i.e., is unnecessary for neoplasms. The absence of changes in TAT induction is in good agreement with the observations of Laishes [8], who, using a different

carcinogen – 2-acetylaminofluorene – likewise found no changes in the inducibility of TAT by dexamethasone. It is interesting to note that ZAH cells, cells of a transplantable and highly malignant hepatoma, completely lost their ability to respond to dexamethasone, for neither TAT nor TP could be induced in them and, as is clear from Fig. 2, dexamethasone did not affect the incorporation of ^{3}H-thymidine into the DNA of this tumor. Dmitrieva et al. [14], in the writers' laboratory, showed previously that disappearance of inducibility of TAT in ZAH by the hormone is due to disturbance of translocation of the hormone–receptor complex from cytoplasm into nucleus of the hepatoma cells. The absence of induction of TP in primary hepatomas while the response to TAT was preserved can hardly be explained by a disturbance of this translocation. A more likely explanation is that specific acceptor zones in chromatin responsible for activation of the gene coding TP lose their ability to react with the hormone–receptor complex. The possibility likewise cannot be ruled out that different receptors are necessary for different enzymes to be activated by the same hormone.

In conclusion the inadequacy of the data so far as they related to the understanding of the mechanisms of neoplastic transformation must be emphasized, for both the normal liver and the liver during chemical carcinogenesis are highly heterogeneous cell populations. Nevertheless, this model can be used for another purpose, namely to study the causes of conversion of hormone-dependent tumors into hormone-independent ones.

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