THE INFLUENCE OF THE CARCINOGEN ORTHOAMINOAZOTOLUENE ON THE REACTIVITY OF LIVER CELLS AFTER PARTIAL HEPATECTOMY

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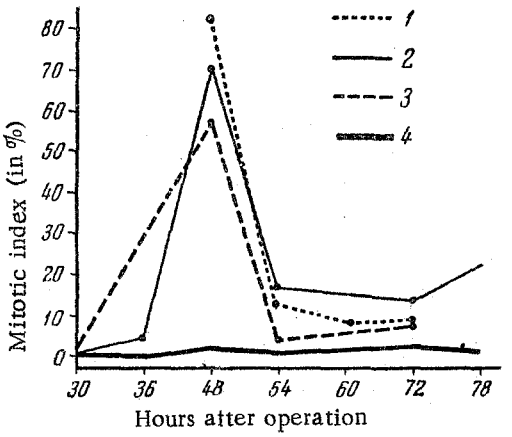
A study of the condition of reactivity of tissue exposed to the action of carcinogens is of great importance in revealing the fundamental mechanism involved.

One of the indices of the reactivity of hepatic cells is their ability to divide mitotically after hepatectomy.

It has been reported that after hepatectomy, regeneration of the liver proceeds differently according to whether the liver is normal, or whether the animal has been given hepatic carcinogens. However, the evidence is contradictory. Gershbein [3], and Hurowitz and his co-workers [5] observed that liver regeneration was suppressed by hepatic carcinogens, while Laird and Barton [6] found a stimulating effect. Laws [7] found that the type of regeneration depended upon the phase of carcinogenesis. Glinos and others [4] gave evidence that hepatectomy accelerated carcinogenesis if the operation was performed at the time when the tumor originated. Evidently, further work is required to determine the relationship of carcinogenesis to liver regeneration.



Change in the mitotic coefficient of liver cells after hepatectomy in control (healthy) mice and in mice treated with benzene, diethylaminoazobenzene, and orthoaminoazotoluene. 1) Control (untreated mice); 2) control (mice treated with benzene); 3) control (mice treated with diethylaminoazobenzene); 4) experimental group (mice treated with orthoaminoazotoluene).

We have therefore decided to study the response of mouse liver to hepatectomy carried out at various stages of a carcinogenesis induced by orthoaminoazotoluene (OAAT).

We here present the results of the response of liver of mice treated with minimal amounts of carcinogen, and hepatectomized shortly after treatment had been discontinued.

METHOD

The mice used were not pure bred. Every other day a 1% solution of OAAT in benzene was applied to the region between the scapulae. Control animals were divided into three groups. The first consisted of normal mice. The second was treated daily with pure benzene. The third (control) group were smeared daily with a 1% solution of diethylaminoazobenzene (a noncarcinogenic substance related to OAAT chemically).

On the fourth day after treatment, a partial hepatectomy was performed by the method of Higgins and Andersen, and the mice were killed, 3-6 at a time, 30, 36, 48, 54, 60, 72, and 78 hours after the operation. Fragments of liver obtained at operation, and when the animals were killed, were fixed in formalin or in Carnoy's fluid, and then embedded in paraffin. The preparations were stained in hematoxylin-eosin, and frozen sections were stained in Sudan III. As a rule the sections were cut 6 μ

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thick. From them counts were made of mitotically dividing cells. For the count we used a 90 × objective and a 7 × ocular, and a diaphragm area 7 × 7 mm. In each case, the total number of mitoses per 100 fields of view was counted. In the same preparation a count was made of the mean number of liver cells in one of every ten fields of view. We then calculated the mitotic index, representing the number of mitoses per 1,000 cells from the formula:

$$MK\frac{M_c \cdot 1000}{K_{cp} \cdot 100},$$

where MK = mitotic index; K_{cp} = mean number of cells per field of view; M_c = total number of mitoses per 100 fields of view.

The results obtained were treated statistically by the Fisher-Student method.

RESULTS

It can be seen from the figure that the mitotic activity of the cells of mice treated with OAAT is greatly reduced in comparison with those of the control mice, at all times after hepatectomy. In mice treated before operation with pure benzene or diethylaminoazobenzene, the mitotic activity of the liver cells attained its maximum value 48 hours after hepatectomy, as it did in the normal mice also, a result which agrees entirely with previous reports [1, 2].

Mitotic Coefficient (Mitoses per 1000 Cells) after Hepatectomy of Untreated Mice and Mice Treated with Benzene, Diethylaminoazobenzene, and Orthoaminoazotoluene

Time after operation (in hours)	Control		Experiment		Probability (P) of a significant difference between the means of the experimental and control groups (treated with benzene)
	Untreated mice	Mice treated with benzene	Treated with diethylaminoazobenzene	Treated with orthoaminoazotoluene	
30	—	0.15 ± 0.2	1.65 ± 1.36	0	
36	—	4.94 ± 3.6	—	0	
48	82.26 ± 17.68	69.27 ± 10.95	55.92 ± 10.28	2.61 ± 2.32	1.000
54	13.34 ± 5.3	17.71 ± 5.45	4.30 ± 3.49	1.74 ± 1.67	0.999
60	8.54 ± 6.09	—	—	—	
72	8.98 ± 3.8	14.73 ± 2.22	8.12 ± 2.25	3.78 ± 2.67	0.980
78	—	20.13 ± 2.96	—	2.16 ± 1.18	1.000

Statistical treatment of the results showed that the differences we found were statistically significant (see table).

A morphological study of the livers of experimental and control groups showed no significant differences between them.

Examination to determine the degree of fatty degeneration and infiltration showed that before the operation, in some cases, there was a focal formation of small fat droplets, which was equally well shown in both groups. After the operation, the degree of fatty degeneration, and particularly of infiltration was markedly enhanced. In reached a maximum 30 hours after hepatectomy, and then was gradually reduced. In this case the process proceeded at the same rate in both groups of mice.

Fatty infiltration was no hindrance to division. In cells dividing mitotically, we frequently observed a large amount of fat. In this connection, the results obtained by Sutherland [8] are interesting; they showed that in rats kept on a special diet which induced fatty degeneration of the liver, regeneration after hepatectomy was not impaired. Evidently, fatty infiltration does not impair the ability of liver cells to divide.

Our results also indicate that OAAT has a strong and evidently toxic influence on liver cells, with the result that they lose their ability to respond by mitotic division to such a strong stimulus as hepatectomy. We were not

able to demonstrate this action morphologically: before operation, after applying the smear of OAAT, the liver of the experimental mice could not be distinguished from that of the control animals.

Therefore from our experiments it follows that in the initial phase of the action of OAAT there is a marked suppression of the reactivity of liver cells, which shows up as an almost complete failure to divide mitotically in response to the stimulus of hepatectomy. Further work may be expected to throw light on the significance of this phenomenon and on its relationship to carcinogenesis.

SUMMARY

Mice (not pure bred) were painted three times with 1% O-aminoazotoluene in benzene. On the fourth day after the last painting, partial hepatectomy was performed. The mice were killed 30, 36, 48, 54, 60, 72, and 78 hours after the operation. O-aminoazotoluene inhibited the mitotic activity of liver cells in the hepatectomized mice, but no inhibition was observed in the control mice painted with benzene, or with the noncarcinogenic diethylaminoazobenzene.

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