EFFECT OF ADMINISTRATION OF HEPATOCARCINOGENS AND THE HERBICIDE MONURON ON INTENSITY OF GLYCOLYSIS AND ACTIVITY OF ITS REGULATING ENZYMES IN RAT LIVER HYALOPLASM

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After administration of p-dimethylaminoazobenzene and thioacetamide, and of the herbicide monuron to rats with the food, a definite parallel is observed between changes in the intensity of glycolysis and in the activity of phosphofructokinase in the liver hyaloplasm. No such relationship is found for hexokinase. Carcinogens of different structures and monuron produced similar changes in the intensity of glycolysis and activity of the glycolytic enzymes. In the degree of severity of the changes produced, monuron more closely resembled thioacetamide.

Comparison of biochemical changes in the liver produced by hepatocarcinogens of different chemical structure can reveal the general features in the character of their action and provide an important approach to the study of carcinogenesis.

The object of the present investigation was to study the dynamics of changes in glycolysis and in the activity of enzymes regulating its velocity, namely phosphofructokinase (2.7.1.11) and hexokinase (2.7.1.1) in the liver hyaloplasm of rats during carcinogenesis induced by p-dimethylaminoazobenzene (DAB) or thioacetamide (TA). In addition, other rats received with their food another urea derivative, the herbicide monuron [N-(4-chlorophenyl)-N',N'-dimethylurea], for which a carcinogenic action has been suggested.

This investigation was a continuation of previous work along similar lines in the writer's laboratory [4, 5].

EXPERIMENTAL METHOD

Experiments were carried out on sexually mature male albino rats kept on a special diet [1]. The rats of groups 1 and 2 received the carcinogens DAB or TA [5] with their diet, the rats of groups 3 received monuron (450 mg/kg), and those of group 4 were controls. The animals were sacrificed in batches of 8-10 from each group at a time 2, 10, 18, and 24 weeks later for biochemical and histological tests. At the last of these times, tumors were found in the liver of all rats receiving the carcinogens.

Biochemical tests on the liver of rats receiving monuron continued for 18 weeks. No tumors were found in the liver. However, bearing in mind the possibility that this compound may have a weaker carcinogenic action, observations continued on the rats until the end of their lives.

Liver homogenates were prepared in a medium consisting of 0.05 M tris-buffer and 0.25 M mannitol solution (pH 7.4) in the cold, and by differential centrifugation [6] the nuclei and mitochondria were successively isolated, thus yielding the hyaloplasm. Glycolytic activity in the hyaloplasm was estimated from

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TABLE 1. Activity of Glycolysis and Enzymes Regulating Its Velocity in Liver Hyaloplasm of Rats in Control Series and during Administration of Carcinogen and Monuron (M±m)

Index	Group of rats	Duration of experiment (in weeks)			
		2	10	18	24
Activity of glycolysis (in mg lactic acid/g protein/30 min at 37°)					
Deficiency of coenzymes	Control	12.1±1.6	13.0±2.3	13.0±2.3	10.7±1.8
	1	7.9±1.5	10.2±2.4	44.5±5.7	17.6±2.6
	2	7.9±1.9	7.0±1.5	32.4±5.0	18.8±2.1
	3	9.2±1.6	7.22±1.9	20.8±1.9	
Excess of coenzymes	Control	21.4±2.7	22.9±0.9	22.9±0.9	35.2±3.0
	1	18.5±3.9	17.3±2.7	68.9±10.0	41.3±5.6
	2	12.7±0.5	20.9±1.8	67.7±10.1	50.9±6.7
	3	18.2±3.9	23.9±3.6	41.7±6.2	
Phosphofructokinase activity (in μmoles NAD · H_2/g protein/min at 25°)	Control	7.3±0.9	12.5±2.5	12.5±2.5	10.0±1.6
	1	8.7±0.8	7.5±1.0	19.8±2.7	12.6±1.0
	2	6.4±0.5	4.8±0.8		14.8±2.8
	3	4.8±0.6	4.3±0.8	20.9±3.3	
Hexokinase activity (in μmoles NADP/mg protein/30 min at 37°)	Control	0.096±0.01	0.164±0.03	0.164±0.03	0.101±0.02
	1	0.117±0.03	0.150±0.01	0.112±0.03	0.230±0.02
	2	0.133±0.02	0.191±0.03	0.163±0.03	0.211±0.01
	3	0.137±0.01	0.192±0.03	0.118±0.03	

the increase in content of lactic acid in the presence of glucose during 30 min at 37°, under the conditions of either an excess or a deficiency of glycolytic coenzymes [2]. Lactic acid was determined by the color reaction with p-hydroxydiphenyl. Activity of the enzymes was determined spectrophotometrically: phosphofructokinase (PFK) by the method of Underwood and Newsholme [8], and hexokinase (HK) from the rate of reduction of NADP in the presence of glucose, ATP, and an excess of glucose-6-phosphate dehydrogenase [7]. All calculations were carried out relative to protein, the content of which was determined by the biuret reaction [3]. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The results given in Table 1 show that in the presence of a deficiency of coenzymes of glycolysis, the intensity of this process in the liver hyaloplasm of rats in the first stages of carcinogenesis and during administration of monuron showed a tendency to diminish by comparison with the control. After 18 weeks, glycolytic activity showed a sharp increase during administration of both carcinogens ($P < 0.01$) and, to a lesser degree, of monuron ($P < 0.05$), so that it exceeded the initial level in the liver of rats with tumors (24 weeks). Almost identical changes were observed in the presence of an excess of glycolytic coenzymes (ATP, NAD).

The PFK activity in the liver hyaloplasm of rats receiving carcinogens for 2 weeks was essentially indistinguishable from the control, but it was reduced during administration of monuron ($P < 0.05$). After 10 weeks the decrease in activity of the enzyme also became marked during carcinogenesis, especially during administration of TA ($P < 0.02$).

Meanwhile a decrease in the intensity of glycolysis was observed in the presence of a deficiency of its coenzymes. At the time of maximal increase in glycolysis (after 18 weeks), PFK activity during

administration of DAB and monuron rose sharply. In the last stage of carcinogenesis, both PFK and glycolytic activity were slightly higher than initially. Determination of HK activity showed significant differences between the control and experimental series only at the last stage of carcinogenesis: in the liver hyaloplasm of rats with tumors. Under these circumstances the actvity of this enzyme rose sharply during adminstration of both carcinogens ($P < 0,02$). At the preceding stages of the investigation, neither the carcinogens nor monuron produced significant changes in hexokinase activity.

These investigations thus indicate a definite parallel between changes in the activity of glycolysis and of PFK, the enzyme regulating its velocity, in the liver hyaloplasm of rats during carcinogenesis and administration of monuron. No such relationship could be established for HK, which can also limit glycolysis if glucose is used as the substrate [9, 10]. Carcinogens of different structure, and also monuron, produced similar changes in the activity of glycolysis and glycolytic enzymes in the liver hyaloplasm of rats. In the degree of severity of the biochemical changes produced, monuron more closely resembled TA than DAB.

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