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Effect of Azo-Dye Carcinogenesis on Hexosamine Synthesis in Rat Liver.* (25169)

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The enzyme which catalyzes formation of glucosamine from hexose-6-phosphate and glutamine was first observed in extracts of *Neurospora crassa*(1) and was subsequently demontrated in pig kidney extracts(2), rat liver extracts(3,4) and several tissues of the growing rabbit(5). Recently, hexosamine synthesis was demonstrated in a spectrum of transplantable tumors and it was observed that hexosamine synthesis by transplanted azo dye-induced hepatomas significantly exceeded the synthesis observed with normal liver(6). It was concluded from these observations that either carcinogenesis, subsequent transplantation or both, resulted in increased production of amino sugars by hepatomas when compared to the tissue of origin(6). The purpose of this investigation was to measure the extent of amino sugar synthesis in liver during azo-dye carcinogenesis to ascertain whether the increased amino sugar synthesis was associated with changes occurring during carcinogenesis or transplantation.

Materials and methods. Throughout an experimental period of ca. 150 days, female Holtzman rats were maintained on an *ad lib.* semi-synthetic, riboflavin-deficient diet described by Medes, *et al.*(7). The diet for half the animals contained 0.06% 3'-methyl-4-dimethylaminoazobenzene (3'-Me-DAB) during the first 90 days of the experiment.† Five animals from each group were sacrificed at 30-day intervals during the first 90 days. Then the dye was withdrawn from the diet and dye-fed animals were sacrificed individually when hepatomas were detected by palpation. Five control animals were sacrificed following last determination on dye-fed animals. Livers from both groups were perfused with cold isotonic saline, pooled, homogenized in 0.1 M phosphate buffer(9) pH 6.6, and diluted with buffer to give a tissue nitrogen content of 4 to 5 mg/ml in the reaction mixture. The reaction mixture contained 0.03 M glutamine, 0.02 M glucose-6-phosphate, phosphate buffer (pH 6.6) and homogenate. The conditions just described were optimum for hexosamine synthesis by liver homogenates(6). Aliquots were removed at 0 and 180 minutes and analyzed for total hexosamine content by the Blix(10) modification of the Elson-Morgan method(11). Tissue nitrogen content was determined by Kjeldahl procedure(12).

Results. Glucosamine synthesis appeared the same in livers of both groups throughout the 90-day azo-dye regimen, Table I. Pathological examinations‡ of the precancerous liv-

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† 3'-Me-DAB was synthesized by the method of Giese, *et al.*(8). The authors are grateful to Merle Maxwell for preparing 3'-Me-DAB.

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TABLE I. Hexosamine Synthesis by Rat Liver Homogenates during Azo-Dye Carcinogenesis.*

Days	Net hexosamine synthesis† (μg/mg tissue N) Control	Dye-fed
0	32.6	
30	32.6	22.5
60	44.7	21.4
90	28.0	31.5
140	35.3	
120–140 Adjacent liver‡		26.6 ± 3.0
120–140 Primary hepatoma‡		111.6 ±10.9

Reaction mixture: Glutamine 0.03 M, G-6-P 0.02 M, phosphate buffer 0.1 M, pH 6.6. Incubated 3 hr at 38°C.

* 3′-Methyl-4-dimethylaminoazobenzene, fed in a semi-synthetic diet.

† Final total hexosamine concentration minus initial concentration.

‡ Animals sacrificed after hepatomas were detectable by palpation. Values, avg of 3 rats. All other values from livers of 5 animals pooled.

ers indicated absence of neoplastic cells at 60 days on azo-dye and relatively small numbers of neoplastic cells at 90 days. From these data, it was apparent that 3′-Me-DAB exerted no pronounced change upon amino sugar synthesis in precancerous livers. When primary hepatomas and liver adjacent to primary hepatomas were tested for ability to form amino sugars, the results showed primary hepatomas to have activity exceeding either precancerous or control livers by a factor of 3- to 5-fold. The liver adjacent to primary hepatomas had essentially the same activity as control or precancerous livers. Therefore, 3′-Me-DAB carcinogenesis yielded a malignant tissue in which hexosamine formation was significantly elevated compared to its tissue of origin.

Since the magnitude of hexosamine synthesis in the primary hepatomas compared favorably with the synthesis previously observed with transplanted hepatomas(6), it appeared that increased amino sugar synthesis was attributable to biochemical changes induced by 3′-Me-DAB carcinogenesis rather than biochemical changes induced by subsequent transplantation of primary hepatomas. The present observation linking increased amino sugar synthesis with azo-dye carcinogenesis together with: (a) previous observations of mucopolysaccharide biosynthesis by neoplastic tissues(13,14) and (b) demonstration of hexosamine synthesis by transplanted tumors of both epithelial and connective tissue origin (6), supports an hypothesis that amino sugar metabolism constitutes a prominent biochemical feature in neoplastic tissues.

Summary. Rats were fed 3′-methyl-4-dimethylaminoazobenzene in a semi-synthetic diet to determine whether potential for hexosamine synthesis was altered in the precancerous liver and primary hepatoma. Throughout 90 days of dye feeding, no appreciable change was observed in livers of control or dye-fed animals. When primary hepatomas and liver adjacent to primary hepatomas were studied, amino sugar synthesis by primary hepatomas exceeded the synthesis of control or precancerous livers 3- to 5-fold. Activity in liver tissue adjacent to hepatomas was essentially the same as that observed in control or precancerous livers. It was concluded that increased amino sugar synthesis observed in hepatomas compared to liver was associated with biochemical changes occurring during carcinogenesis.

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