Inhibition of Growth of Transplanted Hepatomas in Alloxanized Wistar Rats

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The high rate of glycolysis in cancerous tissues, first described by Cori in vivo (6) and by Warburg in vitro (22), suggests that a considerable part of the energy supply for the neoplastic process is derived from the “anaerobic” phase of carbohydrate catabolism. The low glycogen content characterizing a number of tumors (8—11, 15, 17) may also indicate that the substrate for this process is preferentially glucose. The accentuated rate of glycolysis has formed the basis of attempts, a number of them successful, to arrest or retard tumor growth in vivo by glycolytic blocking agents such as glyceraldehyde (14, 15, 18), fluoride, and iodoacetate (1, 2, 4, 21). A somewhat different approach to this problem has been the investigation of both the induction and growth of tumors in the alloxan-diabetic rat or mouse, the underlying rationale to this approach being that the high level of extracellular glucose, or the disturbance in carbohydrate and/or protein metabolism, may impose metabolic restrictions on neoplastic development (3, 5, 7, 12, 19).

In the present study on a rapid-growing transplantable hepatoma (Novikoff), evidence has been obtained of a marked inhibition of tumor development in the alloxanized rat. A significant reduction in hyperglycemia was observed in the alloxanized animals given injections of the tumor material. On the other hand, the administration of alloxan to rats bearing the hepatoma transplants was followed by regression or disappearance of the tumor.

EXPERIMENTAL

Wistar rats of both sexes, weighing between 160 and 180 gm., were used as the experimental animals. Throughout the experiments the animals were maintained on a standard laboratory diet and fed ad libitum. The alloxan was administered by intraperitoneal or subcutaneous injection, as indicated in Table 1, in a 5 per cent solution of alloxan monohydrate (Eastman) on the basis of 175 mg/kg body weight, without prior fasting of the animals.

The tumor used in these studies was a transplantable hepatoma (Novikoff). The tumor was removed, under aseptic conditions, from the donor animal 8—10 days after implantation, transferred to a sterile Potter-Elvehjem homogenizer tube, ground by hand, and diluted with 8 volumes of sterile 0.85 per cent saline. The coarse tumor homogenate was transferred to a sterile 10-ml syringe and injected into the test animals intraperitoneally in 0.5-ml amounts through an 18-gauge needle. The alloxanized and control rats were given injections alternately, to insure as uniform a concentration of the injected tumor material in the two groups as possible. The alloxanized animals were subdivided into four groups, one serving as a nontumor-transplanted control group. The three subgroups receiving the tumor implants were given injections of the hepatoma at intervals of 4 hours, 3 days, and 7 days, respectively, following the administration of the alloxan. The animals were placed under ether anesthesia 10 days after the tumor injections and following a 4-hour fast. Blood samples for sugar determinations were taken from the inferior vena cava, the tumors excised and weighed, and samples of the pancreas, adrenals, liver, and tumor removed for histological examination. Blood sugars were assayed by the method of Nelson (16).

The results of these experiments are presented in Table 1. Alloxanization of the rats prior to implantation of the tumor resulted in a marked reduction in the number of successful “takes” and in the rate of growth of the hepatoma transplants during the 10-day experimental period. The overall incidence of tumors in the alloxanized rats was 57.5 per cent of that in the nonalloxanized controls, the tumor weights in the former group averaging 38 per cent of those in the latter. The injection of tumor material into the alloxanized rats also resulted in blood sugar values significantly below those of the controls. This effect was
observed even in instances where the implanted tumor failed to develop.

The mortalities occurring in a series of ten alloxanized, eleven alloxanized tumor-injected, and eleven tumor control rats in an 11-day interval following tumor implantation are compared in Chart 1 (A, B, and C). In the alloxanized group the greatest number of mortalities occurred in the first 4 days after the alloxan. Mortalities in the alloxanized tumor-injected group, on the other hand, paralleled those in the tumor control group, and the nonsurvivors in these two groups possessed tumors of comparable size. At termination of the experiment 11 days after implanting the tumors, four of the alloxanized rats, four of the tumor controls, and six of the alloxanized tumor-injected rats were surviving. One tumor (6 gm.) was found in the six surviving alloxanized tumor-injected rats. All four tumor control animals possessed tumors, which weighed 4, 5, 13, and 16 gm., respectively.

Eight rats in the tumor control group, with large palpable tumors, were given intraperitoneal injections of alloxan on the 8th day after tumor implantation (Chart 1, D). The animals were sacrificed 6 days later following a 24-hour fast. One animal died on the 14th day after transfer of the tumor and was found to contain a highly necrotic tumor of 6 gm. No visible tumors could be found in the seven rats surviving on the 14th day. The fasting blood sugars in this group averaged 19 mg. per cent (S.D. ± 53).

### TABLE 1
TUMOR GROWTH AND BLOOD SUGAR LEVELS IN ALLOXANIZED WISTAR RATS
IMPLANTED WITH NOVIKOFF HEPATOMAS

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (gm.)</th>
<th>Blood sugar (mg. per cent)</th>
<th>Tumor weight (gm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alloxanized controls</td>
<td>220</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td>Alloxanized with tumor</td>
<td>200</td>
<td>75</td>
<td>15</td>
</tr>
<tr>
<td>Tumor controls</td>
<td>91</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alloxanized controls</td>
<td>220</td>
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</tr>
<tr>
<td>C</td>
<td></td>
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<td></td>
</tr>
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<td>15</td>
<td>1</td>
</tr>
</tbody>
</table>

Mean 165 ± 110
S.D. ± 64

Per cent successful transplants 46.4 per cent
Mean tumor weight (±S.D.) 5.5 ± 4.0

* Animals sacrificed on the 10th day after tumor implantation and following a 24-hr. fast.
† Body weights, inclusive of tumor.
‡ A: Tumor homogenates injected 7 days after alloxan (178 mg/kg body weight, intraperitoneally); B: tumor injected 3 days after alloxan (subcutaneously); C: tumor injected 6 hours after alloxan (intrapерitoneally).
DISCUSSION

Salzberg and Griffin (19) reported a reduced incidence of hepatomas induced by the administration of the carcinogenic dye 3'-methyl-4-dimethylaminoazobenzene in rats alloxanized 1 week prior to the administration of the dye in the diet. The azo dye-containing diet was maintained for a period exceeding 5 months. Carrie and Ham (5) likewise reported no reduction in the growth rate of Sarcoma 37 transplants in alloxan-diabetic Wistar rats and no change in the fasting blood sugar level of the animals with growing tumors.

In the work cited it may be pertinent to note that, where alloxanization of the animals was observed to inhibit tumor development or to reduce the mortality rate in tumor-transplanted animals, the tumors tested were carcinomas. In the studies in which the diabetic state was without significant effect on either tumor growth or survival of the cancerous animals, the tumors selected were sarcomas. These reported findings raise the interesting question whether the diabetic state induced by alloxan (or alloxanization of the animals per se) may differentially influence neoplastic development, depending on the type or site of tumor. In this connection it may be pointed out that Gottschalk and Grollman (11) reported inhibition in the growth of mammary carcinoma in cortisone-treated BALB/c mice, whereas cortisone was without inhibitory effect on the development of an ascites-producing sarcoma in these mice.

It is perhaps premature on the basis of the evidence at hand to speculate as to the nature of the inhibition of tumor growth in the alloxanized rats. It is not known whether the hepatoma cells implanted in the alloxanized animals failed to become established where no tumors were found, or whether the tumors regressed after once becoming established. It is not known whether the metabolic derangement in the tissues of the alloxan-diabetic animal involve the tumor tissue directly or whether limitation of tumor growth is due secondarily to the altered carbohydrate and protein metabolism in tissues other than the tumor in the host animal. The work of Sherman, Morton, and Mider (20) indicates that a growing tumor depletes tissues, other than liver and kidney, in a manner similar to starvation. The liver of the tumor-bearing animal undergoes an hypertrophy which, these authors suggest, might be an adjustment by the host to maintain the increased metabolism imposed by the tumor. Hence, an impairment in liver metabolism in the alloxanized animal may possibly restrict the supply of certain metabolites essential to the rapidly growing tumor.

With reference to the relatively low blood sugar values encountered in the alloxanized animals injected with the hepatomas, it has been noted (5) that human diabetics with certain tumors showed decreased glucosuria. Whether the lowered level of blood sugar represents some re-
mission in the diabetes produced by alloxan in these tumor-injected animals is as yet uncertain, although it is evident from Table 1 that the body weights of the alloxan-tumor group of rats were slightly higher terminally than those of the alloxanized group. It was observed that the polydypsia in the alloxan-tumor group was appreciably less than in the alloxanized controls, although still higher than normal. The histology of the pancreas, liver, adrenals, and tumor in these groups of animals will be discussed in a subsequent paper.

**SUMMARY**

A reduction in the incidence and size of tumors was observed in three groups of alloxanized Wistar rats transplanted with Novikoff hepatomas, intraperitoneally, at intervals of 4 hours, 3 days, and 7 days, respectively, after single diabetogenic injections of alloxan, and sacrificed 10 days after tumor implantation. The blood sugar values in the alloxanized tumor-injected rats were significantly lower than those in the alloxanized control animals. The injection of alloxan into eight rats with large, palpable hepatomas 8 days after implantation of the tumors was followed by complete regression of the tumors in seven animals.

**ACKNOWLEDGMENTS**

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**REFERENCES**

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