A reduction in the size and incidence of tumors has been shown to occur in alloxanized Wistar rats given injections intraperitoneally of homogenates of the transplantable Novikoff hepatoma (9, 10). Salzberg and Griffin (26) likewise demonstrated a reduction in the incidence of DAB-induced hepatomas in alloxanized rats, and Vangerov and McKee (80) have recently reported a diminution in the growth of Ehrlich ascites carcinoma cells in alloxan-diabetic C57/6BL mice.

A reciprocal effect of the Novikoff tumors in producing a significant lowering of the fasting blood sugar values in the alloxanized rats was also consistently found (9, 10).

Since Carrie and Ham, in an earlier report (8), observed no reduction in the fasting blood sugar levels in alloxanized Wistar rats given subcutaneous implants of Sarcoma 37 and no retardation in the growth of these transplanted tumors, in extending this work an investigation was made of the growth of both subcutaneous and intraperitoneal transplants of the Novikoff tumor in alloxanized rats. The influence of alloxan administered subsequent to as well as before implantation of the tumors was also tested, and a parallel study conducted with Walker 256 carcinoma.

MATERIALS AND METHODS

In the preliminary investigation (10), Wistar rats of both sexes were used as experimental animals, with standard laboratory rations fed ad libitum. Beach et al. (2) have reported that food consumption by the male alloxan-diabetic rat may be reduced on an unrestricted diet, in contrast to results with the diabetic female animal which exhibits an augmented food intake. To rule out the possibility that inhibition of tumor growth in the alloxanized rats fed ad libitum might be attributable to a decreased food intake, female Wistar rats averaging 150 gm. were utilized in the present study.

Serial transfers of the Novikoff hepatoma and of the Walker 256 carcinoma were carried out as previously described (10), with 6-day hepatoma and 12-day Walker tumor transplants from the donor animals; 0.5-ml. amounts of 50 per cent tumor homogenate (in sterile 0.85 per cent NaCl) were injected either intraperitoneally or subcutaneously (right subscapular region). Food and water consumptions were followed for 24-hour intervals. The daily water intake, expressed as a per cent of body weight, is recorded in Chart 1. The animals were sacrificed under ether anesthesia 10–15 days after implantation of the tumors and following a 24-hour fast. Blood samples for sugar determinations were withdrawn from the inferior vena cava, the tumors excised and weighed, and pancreatic tissue removed for histological examination with Bouin’s fluid used as fixative. Blood sugars were assayed by the method of Nelson (88), and differential staining of the pancreas was carried out by a modification of Gomori’s aldehyde fuchsin staining procedure (11, 12).

Chart 1 summarizes data on the growth of intraperitoneal and subcutaneous transplants of the Novikoff hepatoma in previously alloxanized rats. The alloxanized animals in this series received single intraperitoneal injections of 3.5 per cent alloxan monohydrate (Nutritional Biochemicals Corp.) in amounts of 175 mg/kg body weight, without prior fasting of the animals. The mean tumor weights (± S.D.), excluding “non-takes” and expressed as percentages of total body weights, are represented by the bars in the upper part of the chart, with the mean fasting blood sugar values (± S.D.) shown by the corresponding bars below.

Tumors implanted intraperitoneally 7 days (Group B) and 24 hours (Group C) after the administration of alloxan and removed 10 days later were significantly smaller than those in the non-alloxanized controls (Group A). The incidence of
tumors was 82, 73, and 56 per cent for Groups A, B, and C, respectively.

A significant reduction in the blood sugar values of fasted animals was also observed in the alloxanized rats receiving intraperitoneal transplants of the hepatoma. Associated with the decrease in blood sugars was a reduction in polydipsia, in polyphagia, and in weight loss.

Subcutaneous transplants of the hepatoma, implanted 24 hours after the injection of alloxan, also showed inhibition of growth in the 10-day period, the alloxanized group. The intraperitoneal Walker tumors also exerted an effect similar to that of the hepatoma in reducing the hyperglycemia.

One difference in the appearance of the subcutaneous tumors as compared with a number of the intraperitoneal tumors in the alloxanized animals was a greater vascularity, resembling more closely that of control tumors.

The converse of these experiments is presented in Charts 3 and 4, which illustrate the results when the alloxan was administered subsequent to im-

![Chart 1](chart1.png)

Chart 1—Growth of Novikoff hepatoma transplants in alloxanized Wistar rats. Mean tumor weights (±S.D.), in this and succeeding charts, are expressed as percentages of total body weights, and exclude animals in which the tumors failed to become established, with “tumors per cent” referring to incidence of tumors. Tumor size was measured 10 days after implantation. A and E refer to nonalloxanized control groups bearing intraperitoneal and subcutaneous transplants, respectively. B received tumor implants 7 days (intraperitoneally); C, 24 hours (intraperitoneally); and F, 24 hours subcutaneously, after intraperitoneal injection of alloxan (175 mg/kg). Number of animals per group is indicated above bars; 24-hour fasting blood sugar values (±S.D.) are represented by corresponding bars below tumor weights, with D and G indicating alloxanized control groups.

although the incidence of tumors was 100 per cent in each of the control and alloxanized groups. Some diminution in the fasting blood sugar values was also observed in the alloxanized rats bearing the subcutaneous transplants.

In similar experiments employing the Walker 256 carcinoma, comparable results were obtained (Chart 2). Tumors implanted intraperitoneally or subcutaneously 3 days after the administration of single diabetogenic injections of alloxan and removed 12 days later showed retarded growth. All animals receiving subcutaneous injections of the Walker tumor homogenate developed tumors. Rats implanted intraperitoneally with this tumor showed a 79 per cent tumor incidence in the control group and a 57 per cent tumor incidence in the alloxanized group. The effect of diabetogenic injections of alloxan, in graded amounts, on rats with 2-day intraperitoneal transplants of the Novikoff hepatoma is shown in Chart 3. No significant inhibition in tumor growth after 10 days was observed in animals receiving five successive subcutaneous injections of 100 mg of alloxan/kg/day, and there was also no appreciable elevation in the fasting blood sugar values (Group B). With a daily injection of 150 mg/kg of alloxan subcutaneously for 3 days, inhibition of tumor growth occurred with a marked increase in the average blood sugar values of fasting rats (Group C). Included in this chart is a group of animals with 7-day intraperitoneal tumors, in which a single intraperitoneal injection of alloxan (175
CHART 2.—Growth of Walker 256 carcinoma transplants in alloxanized Wistar rats. Intraperitoneal and subcutaneous tumors were transplanted 3 days after the administration of alloxan (175 mg/kg, intraperitoneally), and tumor weights were ascertained 12 days later. A and D, tumor control groups; B and E, alloxanized tumor-transplanted groups; and C, alloxanized control group (cf. Chart 1).

CHART 3.—The effect of alloxan on tumor growth in Wistar rats bearing Novikoff hepatoma transplants. Except for group D, the animals were sacrificed 12 days after tumor implantation. A and F, tumor control groups; B, C, and G, injected subcutaneously with alloxan 2 days after transplanting tumors; E and H, alloxan control groups. B received five subcutaneous injections of alloxan (100 mg/kg/day); C, three subcutaneous injections (150 mg/kg/day); G, a single injection (175 mg/kg). Animals in group D received one injection of alloxan (175 mg/kg, intraperitoneally) 7 days after inoculation with tumor homogenate, and were sacrificed 8 days later (cf. Chart 1).
mg/kg) resulted in a regression in tumor size after 8 days, with a pronounced hyperglycemia (Group D).

The administration of a single subcutaneous injection of alloxan (175 mg/kg) to rats with 2-day subcutaneous transplants of the hepatoma produced no significant retardation of tumor growth after 10 days, despite a marked elevation in the fasting sugar levels.

The inverse relationship between tumor size and degree of hyperglycemia indicated in these experiments and in the first series of experiments (Chart 1) was also strikingly apparent in tests with the Walker 256 carcinoma (Chart 4). A single injection of alloxan (175 mg/kg) administered intraperitoneally to rats with 4-day intraperitoneal transplants of the Walker tumor resulted in no diminution in tumor growth after 13 days, and no increase in the fasting blood sugar level (Group B). A significant inhibition of tumor growth in this same period and severe diabetes (Group F) were observed in animals with 4-day subcutaneous transplants of this tumor, similarly treated with alloxan. When the rats with 4-day intraperitoneal tumors were treated with larger amounts of alloxan, three successive intraperitoneal injections of 150 mg/kg/day, tumor inhibition and moderate diabetes occurred in the 13-day period.

In all instances in which alloxanized tumor-bearing animals showed fasting blood sugar levels within the normal range, islets with intact beta-cells were present in the pancreas, although in many of the islets a variable amount of peripheral damage was found.

**DISCUSSION**

The inhibition of growth of the transplantable tumors in the alloxan-diabetic rats may be due to (a) systemic changes with respect to carbohydrate and protein metabolism in the host animal which would indirectly restrict tumor development, (b) a direct involvement of the tumor cells in the metabolic abnormalities occurring in other tissue cells of the diabetic animal, or (c) a combination of these factors.

With regard to the first possibility, the work of Mider and his associates (18, 17) and of Babson and Winnick (1) indicates that a growing tumor acquires building materials, apart from dietary sources, by translocation from other body tissues, such as liver and muscle. In the diabetic organism this "tumor-host" relationship may be altered so as to restrict the supply of essential metabolites, nitrogenous materials in particular, and thus indirectly produce a limitation of tumor growth.

Concerning the second possibility, a biochemical characteristic of tumors, in general, is a high rate of glycolysis manifested under aerobic or anaerobic conditions. Associated with the high rate of glycolysis is a relative lag in the terminal oxidation of carbohydrate, suggesting that an important source of energy for tumor growth is in
the degradation of glucose to the 3-carbon stage. In tissues of the diabetic animal, on the other hand, experimental evidence supports the view that the biochemical lesion may involve the hexokinase system, directly or indirectly (28). An impairment in the entry of glucose into the metabolic cycle could conceivably restrict tumor growth by interfering with the supply of energy or of carbohydrate fragments for the neoplastic process. The accentuated glycolysis of tumor tissues has served as a basis for a number of attempts to arrest tumor metabolism or growth through the use of agents inhibitory to enzyme systems intermediate between glucose and lactic acid (3, 4, 6, 7, 10, 17, 24, 25, 29, 38).

Vangerov and McKee (30), measuring the oxidation of C14-labeled glucose, lactate, and alanine by Ehrlich ascites carcinoma cells from alloxan-diabetic mice reported that the only perceptible change was an increase in the oxidation of alanine. A comparative assay of glycolysis or of hexokinase activity was not performed. Burk and his associates (31) observed no enhancement in the oxygen consumption of S-91 mouse melanoma slices in the presence of insulin and added glucose, but did find an in vitro stimulation of both anaerobic and aerobic glycolysis by insulin in these slices.

The work of Young (39) and of Lukens and his colleagues (19) has demonstrated that a synergistic action of insulin is essential for the growth-promoting and protein-anabolic function of the anterior pituitary. These investigators have presented evidence indicating that, in the dog, overstimulation of the pancreatic islets by anterior pituitary extract or purified growth hormone leads to exhaustion of the beta-cells and consequent diabetes. Young observed that the growth response of dogs treated with anterior pituitary extract was inversely related to the hyperglycemia in these animals, a result which closely parallels the findings in the present study of an inverse relationship between tumor growth and severity of diabetes in the alloxanized tumor-bearing rats.

The fact that, experimentally, anterior pituitary growth hormone has been shown to be both tumorogenic (20-22) and diabetogenic has led Hertz (18) to suggest a positive correlation between diabetes and carcinogenesis. It should be pointed out, however, that growth hormone has not demonstrated diabetogenic action in the rat in which this principle has been shown to give rise to neoplasms. For the dog and cat, which are susceptible to the diabetogenic action of anterior pituitary extracts or purified growth hormone, evidence of the investigators cited above indicates that the growth-promoting function of these substances is abolished if diabetes develops. Moreover, growth hormone has not been shown to initiate the growth of neoplasms in these animals.

With reference to the statistical data of Jacobson (14), also suggesting a positive correlation between cancer and diabetes, Marble (15) has made the pertinent observation that the present-day therapy with insulin has served to reduce the difference between diabetic and nondiabetic groups. In this connection it might also be of interest to mention that in the decade preceding the use of insulin Braunstein (5) reported that diabetic patients with cancer often showed a remission of the diabetes, with symptoms reappearing following surgical removal of the tumors.

With regard to the relatively low blood sugar values encountered in the alloxanized rats bearing intraperitoneal tumors it would appear from histological examination that the presence of tumor tissue adjacent to, but not remote from, the pancreas may protect the beta-cells from injury by the alloxan or may exert a stimulatory effect on these cells. In many instances infiltration of the acinar tissue of the pancreas by tumor cells was observed. The possible role of sulfhydryl compounds in the tumor tissue in reversing the effect of alloxan on the pancreatic islets merits investigation.

Finally, it appears possible that a difference in the establishment of circulation may be involved in the higher incidence of subcutaneous compared to intraperitoneal tumors in nonalloxanized as well as alloxanized rats implanted with either the Novikoff hepatoma or the Walker 256 carcinoma.

SUMMARY

The administration of diabetogenic doses of alloxan prior to or after the intraperitoneal implantation of the Novikoff hepatoma or of the Walker 256 carcinoma in the female Wistar rat resulted in a reduction in the size and incidence of tumors, the rate of growth bearing an inverse relationship to the severity of the diabetes.

The effect of alloxan-diabetes on the growth of subcutaneous transplants of these tumors was, in general, less pronounced, with no effect on tumor incidence.

Conversely, tumors implanted intraperitoneally before or after the administration of alloxan exerted a reciprocal effect either in mitigating its diabetogenic action or in ameliorating the diabetes. Histological study suggested a protective or stimulatory effect of these intraperitoneal tumor transplants on the beta-cells of the pancreatic islets.

ACKNOWLEDGMENTS

We are indebted to the National Cancer Institute of Canada for a grant-in-aid in support of this work. Our thanks...
are due also to Dr. S. M. Friedman, Dr. P. Constantinides, Dr. I. Fiddes, and Dr. R. W. Radcliff in the Department of Anatomy, University of British Columbia, for supplying us with the stock Walker 256 carcinomas, for the use of histological and photomicrographic equipment and for the examination of slide material; to Dr. W. S. Hartroft, Department of Pathology, Washington University, St. Louis, for details of the staining procedure used in these studies, and to Mr. J. R. McBride and Mr. R. Gopaulsingh for technical assistance.

REFERENCES
12. Goranson and Tilsner—Alloxan-Diabetes and Tumor Growth
21. ———. Neoplasms in Rats Treated with Pituitary Growth Hormone. II. Adrenal Glands. Ibid., pp. 564—70.
22. ———. Neoplasms in Rats Treated with Pituitary Growth Hormone. III. Reproductive Organs. Ibid., pp. 548—56.
Studies on the Relationship of Alloxan-Diabetes and Tumor Growth

E. S. Goranson and G. J. Tilser