Anti-Tumor Activity of Glucagon

IRVING S. JOHNSON AND HOWARD F. WRIGHT

(Lilly Research Laboratories, Indianapolis, Ind.)

The report of Salter, Meyer, and Best (2) that glucagon alone affected growth of the Walker carcinosarcoma and that combinations of insulin and glucagon were even more effective in inhibiting this tumor in the rat has led us to evaluate the activity of these hormones against a limited spectrum of transplantable murine neoplasms.

MATERIALS AND METHODS

Ascites tumors were implanted intraperitoneally (I.P.) with 0.1 ml. of a 1:5 dilution of packed cells except for the L1210 leukemia, for which 0.5 ml. of a $10^{-4}$ dilution of ascitic fluid was used. The P1534 leukemia was implanted with 0.5 ml. of a $10^{-4}$ spleen suspension. Implants of all other tumors, approximately 1 cu. mm., were implanted subcutaneously by trocar. Insulin, glucagon in corn oil, and in one instance adrenalin were administered subcutaneously. Treatment was generally for 10 days, with insulin administered once a day and glucagon 3 times. The controls in all cases received equivolumes of 1 per cent horse serum in 0.85 per cent sodium chloride solution. This dilute protein solution was used, since protein could have been supplied in the insulin and glucagon preparations. Life span was an end-point in the leukemias, and mean tumor diameter was used for the solid tumors.

RESULTS

An initial experiment was employed with the use of Sarcoma 180, P1534 leukemia, and Adenocarcinomas 755 and E 0771. The levels of hormones were in the range, per kg/day, of that reported by Salter et al. in the treatment of rats. Treatment started 34 hours after implantation, with insulin being given first, in the combination treatment, followed by two treatments with glucagon, after which the sequence was repeated. Glucagon alone was given twice a day and insulin once. The levels were 0.75 mg/kg/dose for glucagon and 1.2 u/kg/dose for insulin. The average daily dose was 0.975 mg/kg and 0.84 u/kg for glucagon and insulin, respectively. Controls were treated once a day with the dilute serum. On this regimen there was good weight gain of all animals and no indication of anti-tumor effect.

A second experiment with Sarcoma 180, Adenocarcinoma 755, Mecca lymphosarcoma, and L1210 leukemia was run at the levels at which the hormones were administered, being considerably higher per kg than in the rat as suggested by Salter. In general, glucagon alone appeared to be more favorable in its anti-tumor effect than a combination of insulin and glucagon (Table 1). This result was partially due to the higher mortality rate of animals on the combination therapy, with a resultant lack of tumor measurements.

A third experiment was performed with a larger spectrum of mouse tumors and including the Walker carcinosarcoma 256 (Table 2). The over-all results appeared to be essentially the same as those observed by Salter et al., except that, in all cases in which tumors were implanted I.P. in a cell suspension, no effect was seen. This is in direct contrast to results obtained by Goranson on a single ascites tumor, a lymphoma of DBA/2Ha mice.

The high weight loss and anti-tumor effect of glucagon might be attributed to a decrease in food intake. In Salter's experiments, however, food intake of control animals was restricted to that of the glucagon-treated animals, with no diminution of anti-tumor effect. This does not rule out a possible difficulty in utilization of food by animals treated with glucagon.

Histologically, the only normal tissue which appeared to be affected in glucagon- or insulin-glucagon-treated animals was the thymus. This organ was much smaller than in insulin-treated or control animals; this might correlate with a general "stress" phenomenon. There was no sign of hemorrhagic streaks in the stomach of glucagon or of insulin-glucagon animals, however, although these are frequently seen in stressed rats. The tumor tissue itself in glucagon-treated animals showed no histological differences which could not be attributed to decreased rate of growth, i.e., less necrosis, etc. The rate of tumor growth of the Walker 256 increased markedly after glucagon treatment was terminated (Chart 1); in this regard glucagon behaved like any other carcinostatic agent.

1 Iletin, Insulin, Lilly.

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On the basis that stress alone could play a part one experiment was performed in which adrenalin was used to replace glucagon in the treatment regimen (Table 3). Although mortality was similar to the results with insulin and glucagon, no significant anti-tumor effect was seen; this would indicate that the mechanisms of the two types of stress are quite different as to effect on tumors or tumor-bearing hosts.

Goranson reported no effect with glucagon on the growth of Earle's strain L cells in culture. Similar tissue culture studies were made in which the effect of insulin and glucagon at 10 μg., 5 μg., 1 μg., and 0.1 μg./ml of tissue culture fluid were compared separately and in combinations on four human cell strains of both normal and malignant origin. The malignant cells were HeLa (epidermoid carcinoma of cervix) and J96 (peripheral blood of monocyteic leukemia). The normal cells were Earle's NCTC 2414 (squamous epithelial cell from skin) and LLCHI (embryonic connective tissue). Growth of replicate cultures was measured by the hematocrit method during 1 week of incubation in Eagle's medium containing 10 per cent horse serum. No significant effect either of inhibition or stimulation was seen.

**DISCUSSION**

While a hypothesis according to which the hormones are regulating growth of the tumors by affecting or controlling availability of glucose to the metabolic machinery of the malignant cell is attractive, there is as yet no direct evidence that this is the mechanism by which growth is being affected. Goranson was also unable to find any effect of glucagon on oxygen or glucose con-

<table>
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<th>Tumor</th>
<th>Host</th>
<th>Treatment†</th>
<th>Wt. Change (g.)</th>
<th>Mean Tumor Diam. (mm.)</th>
<th>Mortality</th>
<th>Per Cent Inhibition</th>
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* Control (C) animals received equivolume injections of 1 per cent horse serum in physiological saline. Treatment was initiated 3 days after implantation. Insulin (I) at 60 μg/kg was given once a day at 9 A.M.; glucagon (G) at a level of 2.25 mg/kg/dose was given 3 times a day at 9 A.M., 3 P.M., and 9 P.M.; and treatment lasted for 10 days. Animals were kept for an additional 4 days and then discarded. Mean tumor diameters were derived from a 2-dimensional caliper measurement. Weight changes were based upon differences between weights on the 1st day of treatment and 6 days later.

† I = insulin; G = glucagon; C = control.
TABLE 2
THE EFFECT OF DAILY ADMINISTRATION OF INSULIN AND GLUCAGON UPON THE GROWTH OF A SPECTRUM OF MURINE NEOPLASMS*

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* Treatment levels and schedules identical with those in Table 1, except that the rats received 11.2 u/kg of insulin and 2.1 mg/kg/dose of glucagon.
† Ascites production positive.
Cancer Research Vol. 19, June, 1959

Announcement of slices of rat diaphragm or Novikoff hepatoma in routine Warburg experiments, although this tumor was inhibited by glucagon in vivo. Tissue culture experiments along with animal experiments would suggest that the effect is on the host or mediated through the host rather than directly upon the malignant cell per se, a conclusion also reached by Goranson. 8

It should be emphasized that the amounts of glucagon used in these animal experiments are in the order of 150 times the per kilogram dose used to elicit a hyperglycemic response in man and more than 300 times that which will elicit such a response in rats.

The high mortality and weight loss would make glucagon used in these animal experiments appear of only academic interest at present. The possible importance, however, of a reasonably broad spectrum of anti-tumor activity by two naturally occurring hormones with somewhat opposite metabolic activities prompted this report. The effects of combinations of glucagon with other hormones and with known anti-tumor drugs are under study.

SUMMARY

1. Glucagon alone and in combination with insulin markedly inhibited a spectrum of transplantable murine neoplasms implanted subcutaneously. Tissue cultures and murine tumors implanted intraperitoneally by cell suspension were unaffected.

2. Although there was indication that a generalized stress phenomenon was involved, adrenalin could not be substituted for glucagon.

3. These data suggest that the anti-tumor response was due to an effect on the host or was at least host-mediated, rather than being the result of a direct effect upon the malignant cell.

4. The anti-tumor effect was one primarily of carcinostasis rather than tumor eradication.

ACKNOWLEDGMENTS

The authors are indebted to Misses Barbara Mattas, Janet Vlantis, and various members of the cancer screening group for technical assistance. We also wish to thank C. G. Culbertson, M.D., for the examinations of histological material and W. W. Bromer, Ph.D., for the supply of glucagon.

TABLE 8

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<th>Tumors</th>
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<th>Treatment</th>
<th>W., change (gm.)</th>
<th>Mean tumor diam. (mm.)</th>
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* Insulin and glucagon levels and injection schedule identical to those of Tables 1 and 2. Adrenalin (A) was substituted for glucagon at a level of 0.2 mg/kg/dose.

† Not considered significant.

REFERENCES


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