The Effect of Glucagon on Tumor Growth

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Salter, De Meyer, and Best (6) recently reported that giving glucagon to Wistar rats bearing the Walker 256 adenocarcinoma significantly retarded growth of the tumor. It was therefore thought to be of interest to see whether this effect of glucagon was exerted on another kind of rat tumor and on an isologous tumor in a pure strain of mice and, if so, to determine whether the effect observed is a direct one on tumor cells or an indirect one upon the host bearing the tumor. Four experiments were designed to provide information on these points.

PROCEDURE AND RESULTS

In Experiment 1, eighteen female Wistar rats with initial body weights of approximately 150 gm. were used. They were fed ad libitum on powdered Purina chow containing 5 per cent corn oil, and daily checks were made of the food consumed. The animals were given inoculations intraperitoneally, with the use of a 20-gauge needle, of 0.5 ml. of a 20 per cent suspension of minced freshly obtained Novikoff tumor tissue in sterile isotonic saline. Treatment with glucagon[1] was initiated 3 days later, each animal receiving 150 μg/day in a 0.1 per cent suspension in 0.9 per cent saline in three equal doses at 6-hour intervals. Injections were given for 4 days, and the experiment was terminated on the 5th day because at this time two untreated tumor-bearing control animals died. The results, summarized in Table 1, indicate that glucagon had a significant effect in suppressing the growth of the Novikoff tumor.

In Experiment 2 (Table 2) the effect of glucagon was tested on the growth of the DBA/2 lymphoma ascites tumor which is strain-specific for DBA mice (2); this was done to eliminate the possibility of glucagon's modifying the reaction of the host to a homologous tumor and also because the use of this tumor permits a better quantitative estimation of tumor growth. The animals for the most part were 6-month-old female DBA/2Ha mice, averaging 21 gm., although in one experiment 30-day-old mice averaging approximately 10 gm. were used. All were inoculated intraperitoneally with ascites fluid from donor animals bearing 7-day lymphomas, the ascites fluid being diluted tenfold with sterile phosphate saline buffer at pH 7.2. In a preliminary experiment the growth of the lymphoma was found to be linear after an initial lag period of 24 hours following the injection of 5×10^7 cells, and this was approximately the inoculum dose used. The method of Klein (3) was used in determining the total tumor cell population. Treatment with glucagon was begun 48 hours after implantation; a total of 150 μg/day was administered subcutaneously in two equal doses, 9 hours apart, to adult mice, and 100 μg/day to the immature animals. The number of lymphoma cells was determined at the start of the glucagon injections and after 3, 4, and 5 days' treatment with this agent when the groups were autopsied. However, one group of mice treated for 4 days was kept for 4 days after the glucagon injections were terminated to ascertain whether normal tumor growth would be resumed. As shown in Table 2, growth of the lymphomas in the groups receiving glucagon for the 3- and 5-day periods was 21.9 and 15.3 per cent, respectively, of that in the controls. Tumor cell growth in the third group of animals, which received glucagon at a lower dose level for a 4-day period, averaged 36.4 per cent of the controls. Four days after the cessation of glucagon injections, the number of tumor cells amounted to 137.8 per cent of the number in the control animals, 4 days earlier.

Experiments 3 and 4 consisted of *in vitro* tests to examine the possibility of a direct action of glucagon on tumor growth and metabolism. Dr. L. Siminovitch kindly tested the effect of glucagon in tissue culture, in concentrations of 7 μg and 10 μg/ml, on the growth of Earle's strain L cells grown in CMRL medium supplemented with 20 per cent horse serum. No change in the growth rate of those cells that were cultured in the presence of glucagon was seen during a 48-hour observation period.

In a number of experiments the effect of gluca-

[1] The glucagon used in these experiments was a crystalline beef preparation from the Connaught Laboratories, University of Toronto, and supplied to us through the courtesy of Dr. A. M. Fisher.

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TABLE 1

THE EFFECT OF GLUCAGON ON THE GROWTH OF THE NOVIKOFF HEPATOMA IN THE WISTAR RAT

Treatment	No. animals		Food consumption (gm/animal/day)		Body weight*		Tumor weight at 7 days (gm.±S.E.)	Inhibition of growth (per cent)
	Initial	Final	Initial	Final	Initial (gm.)	Final (gm.)		
Control	7	5	13.5	4	163	164	8.8± .7	
Glucagon†	11	11	10	2	157	134	5.3± .65	38

* Average body weights inclusive of tumors.

† Glucagon injected from the 3d to 6th day, inclusive, after implantation of tumors.

TABLE 2

THE EFFECT OF GLUCAGON ON THE GROWTH OF THE LYMPHOMA ASCITES TUMOR IN DBA MICE

Tumor inoculum dose ($\times 10^6$)	2-day tumor cell number* ($\times 10^6$)	Glucagon (μg/day)	Duration of glucagon injections (days)†	Final tumor cell number* ($\times 10^6$)	Growth as per cent of control‡
0.55	2.4 1.7	None		5.7 5.6	
		150	3	2.1 1.4 4.9	22
0.55	0.16 0.51	None		11.1 9.7	
		150	5	0.84 3.84 0.96	15
0.42	0.25 0.32	None		3.76 3.51	
		100	4	2.18 1.54 0.78	36
		100§	4	5.64 7.32 5.44	138

* Each value represents the average of replicate counts on a single animal.

† Glucagon injected subcutaneously in two equal doses, at 9-hour intervals, and totaling 150 μg or 100 μg/day as indicated. The injections commenced on the 2d day after the animals were given inoculations of tumor cells.

‡ Tumor cell counts were averaged, and tumor growth in the glucagon-treated animals was expressed as a per cent of nontreated controls after deduction of the 2-day cell count in each case.

§ Mice were given injections of glucagon for 4 days and sacrificed 4 days after cessation of treatment. All other animals were autopsied on the day following the last treatment.

gon on the respiration and glucose uptake of slices of the Novikoff hepatoma and of rat diaphragm muscle was investigated with the conventional Warburg technics. At levels of 2 μg and 4 μg of glucagon/ml in Krebs-Ringer phosphate containing 100 mg. per cent glucose, no change in oxygen or glucose consumption by these tissues was observed. This observation is in agreement with other evidence which indicates no clear-cut effect of glucagon on glucose utilization by tissues *in vitro* (1).

DISCUSSION

The absence of an effect of glucagon on either the growth of the L cells in tissue culture or the uptake of glucose by the tumor tissue *in vitro*, together with the rapid resumption of growth of the lymphoma *in vivo* following cessation of glucagon injections in the DBA mice, suggests that the effect of glucagon on tumor growth *in vivo* is due to some reversible systemic effect on the tumor-bearing host.

The hyperglycemia evoked in the intact animal in response to glucagon is attributed in part to liver glycogenolysis (4, 7) and in part to gluconeogenesis; the latter is reflected in a greatly increased nitrogen excretion (5). Attention is drawn to the fact that, while the reduction in food intake in the glucagon-treated animals did not differ materially from that of the control animals, the former showed a much greater weight loss. Such evidence as is available therefore suggests that the enhancement in protein catabolism and gluconeogenesis which occurs during the administration of glucagon may interfere with tumor growth by limiting the supply of nitrogenous materials required for this process.

SUMMARY

Glucagon given daily to Wistar rats bearing intraperitoneal transplants of the Novikoff hepatoma, and to DBA mice bearing lymphoma implants, significantly slowed tumor growth. Cessation of glucagon treatment resulted in the resumption of rapid tumor growth in DBA mice. Glucagon exerted no effect on the growth of Earle's strain L cells in tissue culture or on the glucose consumption of slices of the Novikoff tumor *in vitro*. The results suggest that glucagon inhibits tumor growth not by a direct action on the tumor but indirectly as a consequence of its effect on the metabolism of the host.

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