Effect of Adrenocortical Adenocarcinoma on Glycogen Deposition In Rat Liver. (28019)

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The adrenocortical adenocarcinoma under study was one of 4 tumors observed in a group of female NIH-Osborne-Mendel rats fed a low-protein, low-riboflavin, semisynthetic diet containing 0.06% p-dimethylaminoazobenzene(1). Rats bearing this adenocarcinoma developed adrenocortical atrophy, sodium retention, increased potassium excretion, and polyuria(2). This tumor gave complete protection to adrenalectomized rats against a dose of potassium chloride lethal to comparable rats without tumor(3). It was of interest to know about other hormonal activities of this tumor. This paper reports the results of glycogen deposition in the livers of rats bearing this adenocarcinoma.

Materials and methods. The adenocarcinoma used in these studies was the 25th transplant generation of the original tumor (1). It showed no recognizable changes in its histological make-up from the 15th generation transplant used for K-tolerance studies(3) and it was just as effective in inducing adrenocortical atrophy in host rat as its 15th generation counterpart. The same adrenocortical tumor now (Sept. 1962) in its 45th generation still retains its histological make-up and its physiological effects on host(2).

Bilateral adrenalectomy was performed on male rats of NIH-Osborne-Mendel Strain (3). The rats were about a month old and weighed 150-175 g at time of operation. One month later tissue from the adrenocortical adenocarcinoma was transplanted to some of these rats and others served as controls. The adrenalectomized rats received 0.9% saline in place of drinking water. The saline was replaced with tap water, when transplants grew and were visible as subcutaneous nodules. This carcinoma was also transplanted into intact male rats about 2 months old.

All rats were housed in individual compartments in partitioned cages. Each animal had free access to diet of Purina chow pellets. Every rat in each group, except hepatoma-bearing rats, gained some weight during the experimental period. However, weight gains for tumor-bearing rats were only about ⅓ of those for their non-tumor counterparts.

Hepatoma originating in this laboratory in Osborne-Mendel rats and in its 25th generation was transplanted to some intact rats, and an osteogenic sarcoma in the 202nd generation acquired from Dr. H. L. Stewart, was transplanted in intact M-520 rats. These served as tumor-bearing controls.

The glycogen deposition tests were performed on all tumor-bearing rats when the transplants were about 4 cm or more in diameter. Comparable non-tumor rats were included in every test series. Rats were fasted for 18 hours before the test. Samples of blood for sugar determination were collected from fasting rats by cardiac puncture. Five percent glucose was administered intraperitoneally in 3 doses at hourly intervals, at the rate of 1 ml per dose per 100 g body weight. One hour after the last glucose dose rats were killed by cervical dislocation, blood drained out and weighed samples of liver dropped at once, in flasks with 30% KOH. Liver glycogen was precipitated and hydrolyzed by the method of Good *et al.*(4). All glucose determinations were made by the copper reduction method of Folin(5) and glycogen calculated as mg of glucose per g of liver. Some rats bearing adrenocortical tumor received intraperitoneal injections of 2 mg of cortisol acetate per 100 g of body weight in 3 divided doses with glucose injections.

As a rule, tumor-bearing rats and their control counterparts were littermates with starting weights within 30 g of each other. Each group contained rats from more than one litter, all born within a week of each other. Average starting weight of control and experimental rat was within 5 g of each other and no rat in a group weighed over 30 g more than another. All rats were weighed before

TABLE I. Liver Glycogen Deposition and Blood Sugar in Control and Tumor-Bearing Rats.

Treatment	No. of observations	Fasting blood sugar mg % ± S.D.	Liver glycogen mg/g ± S.D.	Liver glycogen % of control
Intact				
Control	15	134 ± 11	24.9 ± 3.8	100
Adrenal tumor	9	101 ± 9	9.0 ± 4.5	36.0
Hepatoma	6	108 ± 22	.6 ± .7	2.4
Osteosarcoma	6	99 ± 14	14.6 ± 7.6	59.5
Adrenal tumor + cortisol acetate	6	—	15.6 ± 3.8	62.5
Adrenalectomized				
No tumor	12	98 ± 13	10.3 ± 2.1	41.5
Adrenal tumor	15	74 ± 15	8.1 ± 2.5	32.6
" " + cortisol acetate	6	—	12.8 ± 2.3	51.5

the tests and tumor and liver weight noted and each animal examined for possible lesions. Fasting blood sugar(5) was determined on all the animals studied.

Results. The adrenocortical adenocarcinoma reached desirable size in 4 to 6 weeks in intact and 9 to 12 weeks in adrenalectomized rats. Tumor diameter and weight in intact rats varied from 3.5 to 5.0 cm and 35 to 50 g and in adrenalectomized rats from 3.5 to 4 cm and 25 to 35 g respectively. Osteogenic sarcoma reached right size in 4 to 6 weeks and its diameter and weight varied from 3.8 to 5.0 cm and 30 to 40 g. Hepatoma was ready in 3 to 4 weeks and varied from 4 to 5.5 cm in diameter and 35 to 55 g in weight.

The glycogen values as milligrams of glucose per gram of fresh liver and fasting blood sugar levels as mg % glucose for control and experimental rats are shown in Table I. The same relative values are obtained when the glycogen is calculated on unit body weight basis.

Fasting blood sugar in rats with transplants from any of the 3 tumors was lower than that in comparable animals without tumors. Blood-sugar values for rats with adrenocortical adenocarcinoma were not significantly different from those for animals with osteosarcoma or hepatoma. Blood sugar for adrenalectomized rats was in the same range as that for intact tumor-bearing rats. The growth of adrenal tumor in these rats reduced the blood sugar still lower.

Tumor transplants weighed from 30 to 50 g and averaged about 40 g. On gross examination osteosarcomas showed the least necrosis, adenocarcinoma had more necrosis and hepatoma had the most. The hepatoma was hemorrhagic and livers of the animals bearing this tumor were jaundiced and the rats anemic (corpuscular volume 10-15%).

Livers of rats bearing hepatoma transplants showed least glycogen deposition and these animals did not respond to cortisol administration, indicating that there were other things wrong with the glycogen deposition system of these rats besides lack of glucocorticoids. Taking glycogen deposition in the livers of intact control rats as 100, glycogen deposition was depressed by 40.5% in rats bearing osteosarcoma and by 64% in those bearing the adrenal tumor. Adrenalectomy depresses glycogen deposition by about 60% and the adrenocortical adenocarcinoma transplant does not increase glycogen deposition but seems to depress it.

Intact and adrenalectomized rats bearing this adrenal tumor respond to cortisol treatment by increase of 60-70% in glycogen deposition over the values for corresponding untreated rats.

Discussion. Britton and Silvette(6) showed that extracts of adrenal glands restored the blood sugar level and liver muscle glycogen in adrenalectomized rats and also influenced the carbohydrate metabolism in the normal intact animals. Reinecke and Kendall(7) developed a quantitative method for assay of adrenal hormones by determining glycogen deposition in livers of adrenalectomized rats.

Venning *et al.*(8) modified this method to detect and determine urinary excretion of small amounts of glucocorticoids. They used the adrenalectomized mouse as the test animal and administered the test material with 5% glucose solution in divided doses.

This principle was utilized to estimate glucocorticoid secretion by the adrenocortical adenocarcinoma under study.

It was observed that tumor growth suppresses glycosuria in diabetic rats(9,10). Low values of blood sugar in tumor bearing rats confirm these observations. Ashmore *et al.* (11) suggest that cortisol may act on circulating glucose to increase glycogen deposition in the liver. Thus tumor growth and glycogen deposition compete for the same substrate (blood glucose) for their respective activities. Deficiency of glucocorticoid induced by adrenalectomy lowered the blood sugar, (a finding reported before(9,12)) besides reducing the glycogen deposition. Transplantation of the adrenal tumor reduced both these values, further indicating that if there was any glucocorticoid secretion by this tumor, it was not enough to counterbalance the effects of tumor growth.

The adrenocortical adenocarcinoma under study induces significantly lower glycogen deposition in the liver of intact rats than that induced in comparable rats with osteosarcoma, a non-hormonal tumor. This again indicates lack of glucocorticoid secretion. Significantly lower glycogen deposition in the adrenal tumor bearing intact rat could be explained by atrophy of the cortical area of the adrenal glands induced by this tumor in the host rat(2). Capability of the adrenal adenocarcinoma bearing rat to respond to glucocorticoid is demonstrated by 60-70% increase in mean glycogen deposition in these rats after a single dose of 2 mg of cortisol per 100 g body weight. These differences in the mean values are statistically significant with a P value of less than 0.01. As the adrenal adenocarcinoma averaged about 40 g even 1% of this mass would give 400 mg of functioning tissue, about 8 times the weight of adrenal glands of a rat, and thus large enough to give detectable glycogen deposition even with a modest secretion of active hormone.

Summary. Liver glycogen was determined under standard conditions on intact and adrenalectomized rats with or without adrenocortical adenocarcinoma transplants. Some of these tumor-bearing rats were treated with cortisol acetate before glycogen determination. Liver glycogen was also determined on rats bearing an osteosarcoma or a hepatoma transplant. There was little glycogen deposition in the livers of the hepatoma-bearing rats. Livers of these animals were jaundiced and rats were anemic and did not respond to cortisol. The rats bearing adrenal adenocarcinoma had lower glycogen deposition than the rats bearing osteosarcoma, a non-hormone tumor. This indicates lack of glucocorticoid secretion by this tumor, detectable by liver glycogen deposition. In adrenalectomized rats, animals bearing adrenal adenocarcinoma has lower glycogen deposition than in those without tumors, again indicating lack of glucocorticoid secretion.

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