In vitro Metabolism of Specifically Labeled Glucose by Adipose Tissue of Mice Bearing Adrenocorticotropic Tumors.* (26353)

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(Introduced by G. W. Thorn)
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Adrenocorticotropic tumors, induced in mice surviving exposure to ionizing radiations from an atomic detonation, have been implanted in successive passages and are now autonomous in that they can be grown in non-adrenalectomized hosts(1). Normal mice grafted with such tumors are submitted to high levels of endogenous glucocorticoids and study of the metabolism of their isolated tissues might give valuable information on the mode of action of the natural adrenocortical secretion. Adipose tissue is a major site of hormonal regulation(2,3) and it seemed therefore worthwhile to compare the metabolism of this tissue in tumor-bearing mice and in untreated controls.

Material and methods. Male mice of the LAF_1 strain, fed Ralston-Purina laboratory chow *ad libitum,* had been injected 4 weeks previously in the thigh muscles with a saline suspension of tumor tissue. Terramycin HCl was added to their drinking water in order to control the infections which are frequently observed in mice with adrenocorticotropic tumors. The animals were approximately 14 weeks old at time of sacrifice. Untreated mice of the same strain and age served as controls. The epididymal fat pads were excised with minimal handling and the metabolic activity of one fat pad, incubated without insulin, was compared to the activity of its paired control, incubated in presence of insulin (0.1 U/ml of recrystallized insulin containing less than 0.1 μg glucagon per mg, obtained through the courtesy of Dr. W. R. Kirtley, Lilly Research Labs, Indianapolis, Ind.). Incubations were carried out at 37°C, in 3 ml of Krebs-bicarbonate buffer, for 3 hours. Glucose concentration was 20 mM/liter and glucose radioactivity was one microcurie per flask (chromatographically pure glucose-1-C^{14} or glucose-6-C^{14} obtained from Nuclear Chicago Corp.). The methods used in determining the amount of glucose carbon oxidized to CO_2 or incorporated into long chain fatty acids, alpha carbon of glyceride-glycerol, and glycogen, have been described (4,5). All values have been expressed in terms of micromoles of glucose carbon metabolized per mg of tissue nitrogen per 3 hours, as well as in terms of percentage of total amount of recovered glucose carbon.

Results. In the grafted mice, the adrenocorticotropic tumors measured approximately one cm in diameter. Adrenal weight was roughly double that of the controls. The effect on body weight was variable (Table I). However, mean weight of the 2 epididymal

TABLE I. Fatty Acid and Nitrogen Content of Epididymal Adipose Tissue from Mice with Adrenocorticotropic Tumors.

		Mean values for epididymal adipose tissue (both pads)				
		Weight		Fatty acids,	Nitrogen	
Type and No. of mice	Body wt range (g)	mg	% of body wt	% wet wt tissue	mg	% wet wt tissue
Controls (6)	21-31	340	1.2	78	.59	.17
Tumor-bearing (11)	20-35	530	2.0	79	.63	.12

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TABLE II. *In Vitro* Metabolism of Glucose-1-C^{14} and Glucose-6-C^{14} by Epididymal Adipose Tissue from Mice with and without Adrenocorticotropic Tumors. (Krebs bicarbonate buffer. Glucose concentration 20 mM. Insulin concentration, when present, 0.1 U/ml. Values expressed as micromoles (μM) of glucose carbon metabolized per mg of tissue nitrogen per 3 hr, or as percentage (%) of total recovery. Mean values ± S.E.M.)

Type and No. of mice	Insulin added *in vitro*	Carbon dioxide				Fatty acid				Glyceride-glycerol alpha carbons				Glycogen				Total			
		μM		%		μM		%		μM		%		μM		%		μM		%	
		C-1	C-6	C-1	C-6	C-1	C-6	C-1	C-6	C-1	C-6	C-1	C-6	C-1	C-6	C-1	C-6	C-1	C-6	C-1	C-6
Control (6)	—	1.53 ±.21	.58 ±.10	57	29	.46 ±.14	.70 ±.29	17	35	.67 ±.15	.66 ±.16	25	33	.03 ±.01	.05 ±.02	1	3	2.69	1.99	100	100
	+	9.90 ±2.31	.55 ±.05	66	5	3.57 ±.61	8.15 ±1.08	22	76	.92 ±.18	1.33 ±.23	6	12	.55 ±.09	.68 ±.18	5	6	15.1	10.7	100	100
Tumor-bearing (11)	—	.95 ±.14	.71 ±.09	58	44	.16 ±.05	.29 ±.10	10	18	.51 ±.14	.61 ±.15	31	38	.01 ±.01	.01 ±.01	1	1	1.63	1.62	100	100
	+	4.90 ±.89	1.49 ±.43	61	27	1.28 ±.26	1.73 ±.39	16	32	1.67 ±.33	2.16 ±.78	21	39	.17 ±.04	.15 ±.03	2	3	8.02	5.53	100	100

fat bodies was found to be somewhat greater in the tumor-bearing mice and represented 2.0% of total body weight, as compared with 1.2% in normal mice. Nitrogen content was lower in the epididymal adipose tissue of tumor-bearing animals, while fatty acid content was the same in both groups. The *in vitro* metabolism of carbon atom-1 and carbon atom-6 of glucose by isolated epididymal adipose tissue is shown in Table II. Total recovery of glucose carbon-1 and glucose carbon-6 was lower in the adipose tissue of tumor-bearing mice, being respectively 66% and 81% of that observed in control animals. Furthermore, the metabolism of these 2 glucose carbons was modified. Incorporation of glucose carbon-1 and glucose carbon-6 into glycogen was depressed, and their incorporation into fatty acids reduced to less than half. Recovery of both glucose carbons in the alpha carbon of glyceride-glycerol was normal. Oxidation of carbon-1 was depressed to 62% of the norm, while oxidation of carbon-6 was comparable to that observed with control animals. When these values are expressed on a percentage of recovery basis, the relative impairment in lipogenesis remains striking and a relatively high oxidation of glucose carbon-6 becomes apparent.

The effect of insulin added to the medium on metabolism of glucose was also different in the tissue from tumor-bearing and from control mice. In the adipose tissue of mice with adrenocorticotropic tumors, total recoveries of both glucose carbons were only 50% of the norm, and lipogenesis and glycogen synthesis were promoted to a lesser extent by the presence of insulin in the medium. Oxidation of glucose carbon-6, and glycerol synthesis from both carbons, however, were stimulated more markedly in the tissue from the tumor-bearing animals.

Discussion. The metabolic picture prevailing here is the result of a complex endocrine situation. In the tumor-bearing animals, adipose tissue is chronically submitted to high levels of endogenous ACTH, endogenous glucocorticoids (mostly corticosterone(6)), and perhaps also insulin, as suggested by marked hypertrophy of the Islets

of Langerhans(7). Total recovery of glucose carbon-1 and glucose carbon-6 metabolized by the adipose tissue of tumor-bearing animals was lower than normal, suggesting that overall glucose metabolism was less. Relatively greater suppression of oxidation of glucose carbon-1 than carbon-6, with relatively greater suppression of lipogenesis from glucose carbon-6, suggests relatively greater depression of the phosphogluconate-oxidative pathway in adipose tissue of tumor-bearing mice. On the other hand, normal capacity of this tissue for oxidation of glucose carbon-6 suggests relative integrity of the tricarboxylic acid cycle. The results showing an impaired lipogenesis are in line with those obtained in rats treated with cortisone. In these animals cortisone pretreatment has been shown to result in decreased lipogenesis in subsequently isolated adipose tissue (8,9) and also to depress the respiratory quotient of this tissue(10,11). A reduction in responsiveness of glycogenesis and lipogenesis to insulin was apparent when insulin was used in concentrations known to be maximally effective on mouse adipose tissue(5). The presence of altered insulin sensitivity is also suggested by the observation that, in spite of the indirect evidence of hyperinsulinism in the tumor-bearing mice(7), the metabolism of their isolated epididymal adipose tissue differed grossly from what one might expect as a result of chronic insulin overdosage.

It is known that adrenocorticotropic tumor-bearing mice contain 3 times as much extrahepatic fat as their controls, even when body weight is in the normal range(12). If the metabolic data obtained *in vitro* (Table II) may be considered as representative of the situation prevailing *in vivo,* it would seem that this increased accumulation of fat is not likely to be the result of accelerated lipogenesis. Other mechanisms must be involved. Accelerated hepatic lipogenesis with peripheral accumulation of the newly synthesized fat has been suggested by the experiments of Zomzely and Mayer(12). Delayed mobilization of fat from fat stores is also conceivable.

Summary. The *in vitro* metabolism of specifically labeled glucose by epididymal adipose tissue of mice bearing adrenocorticotropic tumors has been studied. Sluggish lipogenesis from glucose was found to be coupled with evidence suggesting decreased activity of the phosphogluconate oxidative pathway. Glyceride-glycerol synthesis was normal, and oxidation of glucose carbon in the tricarboxylic acid cycle appeared to proceed at a normal rate. Insulin added *in vitro* was less effective in promoting lipogenesis and glycogen synthesis than with tissue from control animals.

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