

Effects of Cortisol on the Metabolism of Glucose by Lymphoid Tissue*

MELVIN BLECHER

From the Department of Biochemistry, Schools of Medicine and Dentistry, Georgetown University, Washington 7, D. C.

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Jedeikin and White (1) reported that the over-all oxidation of uniformly labeled glucose- ^{14}C to carbon dioxide by suspensions of rat thymic lymphocytes was inhibited by 31% by cortisol (10^{-4} M); similar depressions of glucose oxidation were observed in suspensions of rat lymphosarcoma cells. In addition, injection of cortisol into normal or adrenalectomized rats led to a decreased oxidation of glucose by thymocytes studied *in vitro* thereafter (1). Extension of these studies by Blecher and White (2) with malignant lymphoid tissue led to the suggestion that loci of action of adrenal corticoids in inhibiting the over-all oxidation of glucose resided in intermediate steps of glycolysis.

Bartlett, Morita, and Munck (3) reported that the injection of cortisol into adrenalectomized rats inhibited by about 30% the incorporation of injected glucose into thymus tissue fractions as determined shortly ($1\frac{1}{2}$ hours) after substrate administration. In addition, Morita and Munck (4) reported that, 2 hours after administration *in vivo* of cortisol to fasted, adrenalectomized rats, the ability of thymocytes *in vitro* to take up glucose from the incubation medium was depressed by 40%. Furthermore, the presence *in vitro* of cortisol (10^{-5} M) was reported (4)¹ to inhibit the uptake of glucose by rat thymocytes by about 28%; the inhibitory effects of lower concentrations of cortisol (10^{-6} to 10^{-7} M) were less significant (22 and 7%, respectively).

Since the loci of action of adrenal glucocorticoids in depressing glucose utilization by rat thymocytes remained in doubt (inhibition of uptake or of further metabolism), the present studies were undertaken to examine the effects of these steroids on the uptake, oxidation to carbon dioxide, and conversion to lipids and cell protein of glucose-6- ^{14}C by suspensions of thymic lymphocytes obtained from normal and adrenalectomized rats. It was observed that all parameters of glucose metabolism, except that of uptake of glucose by the cells, were inhibited by cortisol *in vitro*, and increased by prior adrenalectomy of donor animals; the uptake of glucose was unaffected by cortisol *in vitro* over a wide range of concentrations, or by prior adrenalectomy of donor animals.

EXPERIMENTAL PROCEDURE

From 300 to 600 $\times 10^6$ rat thymic lymphocytes, prepared as described previously (5) from 16-hour fasted normal or adrenalectomized (used 7 to 8 days postoperatively) male rats (Holtzman strain), were incubated in 2.0 ml (final volume) of a modified Krebs-Ringer-phosphate buffer (pH 7.4) containing 5.7 μmoles

of glucose-6- ^{14}C (375,000 c.p.m.) and cortisol (Chemed, Inc.; added in 50 μl of propylene glycol solution) in 25-ml flasks fitted with a serum cap-hanging glass center well assembly.

After 2 hours of incubation with shaking at 37° in an aerobic atmosphere, 0.25 ml of 1 M Hyamine hydroxide in methanol was injected through the serum cap into the center well, and 0.1 ml of 2 N H_2SO_4 injected into the incubation medium. After an additional 30-minute period of shaking to ensure quantitative absorption of radioactive carbon dioxide by the Hyamine, the contents of the center well were transferred to vials containing 10 ml of 0.4% 2,5-diphenyloxazole-0.05% 1-4-bis-2'-(5'-phenyloxazolyl)benzene in toluene, and radioactivity was assayed in a Nuclear-Chicago model 725 liquid scintillation counter; quench corrections were made by the channel ratio method.

Incubation mixtures, following chilling at ice bath temperatures, were centrifuged to reisolate and wash thymocytes free of materials contained in the incubation medium. The combined supernatant fluids were assayed for glucose concentration by a specific glucose oxidase procedure developed in this laboratory which is sensitive down to about 2 μmoles per ml of glucose (6).

After suspension of reisolated cells in 20 volumes (usually 5 ml) of chloroform-methanol (2:1) for 16 to 24 hours at 57° in screw-cap (Teflon-lined) centrifuge tubes with periodic agitation by a Vortex mixer, the residue was isolated by centrifugation ($1000 \times g$ for 15 minutes) then re-extracted with the same volume of solvent mixture. The pooled organic phases were washed with 0.2 volume of 0.73% NaCl solution, then twice with 2-ml portions of a chloroform-methanol-0.73% NaCl mixture (3:48:47) according to Folch, Lees, and Sloane Stanley (7). The washed organic phase, containing the total lipids, was taken to dryness under nitrogen, and the residue extracted with 5.0 ml of chloroform-methanol (2:1). Following removal of insoluble material by centrifugation, 4.0-ml aliquots were evaporated to dryness in vials, and the residue was dissolved in 10 ml of the scintillator solution noted above for liquid scintillation counting; no quenching was found with this procedure.

Tissue, remaining after extraction of lipids, was extracted, in succession, twice with 2 ml of cold 7% trichloroacetic acid, once with 5 ml of 95% ethanol, and finally, twice with 5 ml of absolute diethyl ether; washings were discarded. The protein residue was dissolved in 0.5 ml of 1 M Hyamine hydroxide by brief warming at 57°, and the yellow solutions of Hyamine proteinate were added to 10 ml of the scintillator solution described above for assay of radioactivity by liquid scintillation counting; quench corrections were made by the channel ratio method.

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¹ A. Munck, personal communication.

RESULTS AND DISCUSSION

Lymphocytes, isolated from thymuses of normal or adrenalectomized male rats, were incubated with glucose-6-¹⁴C in the presence of varying concentrations of cortisol. The amount of glucose utilized was about 25% of that initially present. As indicated by the representative data of Table I, uptake of glucose by cells was not significantly affected either by prior adrenalectomy of donor rats or by the presence of 1.5×10^{-4} to 1.5×10^{-6} M cortisol in the incubation medium. In contrast, prior adrenalectomy increased, in control incubation mixtures, the oxidation of glucose-6-¹⁴C to radioactive carbon dioxide and the conversion of glucose carbon to tissue lipids and protein. In all cases, the presence of cortisol in incubation mixtures decreased the utilization of glucose carbon for oxidation to carbon dioxide and conversion to lipid and protein; the cortisol effects increased with increasing concentrations of the steroid.

The present results, in contrast to those reported by Morita and Munck (4), indicate that cortisol is without influence upon the uptake *in vitro* of glucose by rat thymic lymphocytes obtained from either normal or adrenalectomized animals, but that cortisol inhibits the further metabolism of glucose by these cells to carbon dioxide, lipids, and proteins. This interpretation is supported by observations made with thymocytes obtained from adrenalectomized, fasted animals. In such cells, the prior absence of circulating adrenal steroids increased, when compared to cells from normal rats, the base-line values for conversion of glucose carbon to carbon dioxide, lipids, and proteins (Table I); the presence *in vitro* of cortisol lowered the values for these parameters of glucose metabolism to values approaching those observed with thymocytes from normal animals. Prior adrenalectomy led to no significant change from normal in the rate or extent of uptake of glucose by thymocytes. No explanation is as yet known for the contrast between the present results and those reported by Morita and Munck (4), although it is known¹ that considerable differences exist between the two laboratories in methods of preparation of thymic cell suspensions and addition of steroids to incubation vessels, as well as in the means used to measure glucose uptake; these differences are being investigated. In addition, it can be calculated from the data of Morita and Munck (4) that the amount of glucose taken up in the absence of steroid was about 5% of the initial substrate concentration. Since this is only about 0.2 of the control glucose uptake in the present studies, it is likely that differences exist in the metabolic activity of thymocytes as prepared in the two laboratories.

Experiments with another type of lymphocyte, *i.e.* isolated from rat blood, appeared to indicate an inhibition *in vitro* by

cortisol of glucose uptake, but no effect on the formation of lactate from glucose (8). However, from the data reported (8), it can be calculated that in control vessels only about 0.7% (standard error of the mean of $\pm 14\%$) of substrate glucose was utilized (in contrast to 25% in the present studies), and that the amount of glucose taken up in the presence of cortisol apparently fell to 0.3% (standard error of the mean of $\pm 50\%$). In view of the extremely limited utilization of glucose, the small difference in utilization of glucose in the presence of cortisol, and the relative insensitivity of the method employed by these workers for the assay of glucose, the conclusion that cortisol inhibited glucose uptake by blood lymphocytes may have been unwarranted.

The conclusions drawn from the present study are supported by previous reports of inhibition of glycolysis in intact rat lymphocytes by adrenal steroids (9) and the depression of protein synthesis in intact rat thymocytes by adrenal steroids (10). Since the observations made in the present study could also be explained by cortisol inhibition of the further metabolism of pyruvate derived from glucose, it is of interest that Jedeikin and White (11) observed the inhibition *in vitro*, by adrenal steroids, of the oxidation of pyruvate, as well as that of glucose, by cell-free preparations derived from rat thymus. Experiments *in vivo* by Glenn *et al.* (12) with the rat support a view that at least one locus of action of cortisol involves decreased utilization of pyruvate derived from glucose, and that cortisol does not inhibit glucose oxidation by prevention of the entry of glucose into cells.

Studies are in progress to determine loci of action of adrenal glucocorticoids in inhibiting the metabolism of glucose in thymic lymphocytes obtained from normal rats and in those with endocrinopathies.

SUMMARY

The effects *in vitro* of cortisol on glucose uptake, oxidation of glucose to carbon dioxide, and conversion of glucose carbon to lipids and protein were examined in thymic lymphocytes obtained from normal and adrenalectomized rats. All parameters of glucose metabolism measured, except that of glucose uptake by thymocytes, were inhibited by cortisol *in vitro*, and increased by prior adrenalectomy of donor rats; the uptake of glucose was unaffected by cortisol *in vitro* (10^{-4} to 10^{-6} M), or by prior adrenalectomy of donor animals.

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TABLE I

Effect of cortisol on metabolism of glucose-6-¹⁴C by thymocytes

Procedural details are described in "Experimental Procedure." The final concentrations of cortisol are given in the table. All values are normalized to a standard 10^9 cells.

Cortisol	Glucose uptake		CO ₂		Total lipids		Protein	
	Normal	Adrenalectomized	Normal	Adrenalectomized	Normal	Adrenalectomized	Normal	Adrenalectomized
μM	$\Delta\mu\text{moles}$		<i>c.p.m.</i>		<i>c.p.m.</i>		<i>c.p.m.</i>	
Control	1.28	1.35	6,550	12,000	632	968	6,360	11,900
1.5	1.21	1.25	5,640	9,100	465	844	5,340	9,540
15.0	1.25	1.49	5,410	8,330	453	814	5,030	9,120
150.0	1.29	1.49	4,530	5,950	430	752	4,400	8,100