Effect of Prednisolone on the Randomization of Glucose Incorporated into Glycogen

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SUMMARY

Prednisolone administration stimulated glucose incorporation into glycogen with no change in the degree of randomization of carbon atoms 1 and 6 of the glucose residues incorporated. From this result it is concluded that the hormone produces an effect on the direct pathway from hexose to glycogen.

It was recently reported that there was a severalfold increase in the rate of incorporation of a number of labeled precursors into rat liver glycogen in the second to third hour after glucocorticoid administration (1). The magnitude of the effect was the same whether the precursor entered the glyconeogenic pathway at the pyruvate, fructose, or glucose level. Three separate reactions in the glyconeogenic pathway have been focused upon as likely sites of control, namely, the pyruvic carboxylase, the fructose diphosphatase, and the glycogen synthetase reactions (2). The conversion of pyruvate to glycogen requires all three, while that of fructose would appear to require only the last two[1] and of glucose only the third of these reactions. However, the earlier experiments (1) did not rule out the possibility that the hormone stimulation even in the case of glucose was at an early step in the reaction—for example, at the pyruvic carboxylase reaction—since most of the glucose carbon which ultimately appeared as glycogen might conceivably have been broken down to pyruvate first, rather than incorporated directly into glycogen. To test this possibility we have determined the degree of randomization of carbon atoms 1 and 6 of glucose molecules incorporated into glycogen and the hormonal effect thereon.

Procedure. ^{14}C-glucose labeled in carbon 1 (7.5×10^6 dpm) was injected either intraperitoneally or intravenously into 200–250 g male rats which had been fed ad libitum or starved overnight. An hour later a sample of liver was placed in hot 30% KOH and glycogen was isolated according to the procedure of Hassid and Abraham (4). Glucocorticoid-treated animals were injected with 2 mg of prednisolone acetate either intraperitoneally or intravenously 2 hours prior to the administration of labeled glucose.

After isolation of the glycogen, it was hydrolyzed in 0.66 N HCl. The solution was evaporated to dryness twice, and the glucose was taken up in a known volume of water. Carbon 6 and carbons 1 plus 6 of the glucose were isolated from separate

[1] The pathway of fructose conversion to glycogen is still uncertain and may involve, at least in part, a direct phosphorylation to fructose 6-phosphate (3). In this case the fructose diphosphatase reaction would be by-passed.

aliquots by the method of Bloom (5). After recrystallization of the dimedon derivatives, they were filtered on a Millipore filter, dried, and weighed. The filter was placed in a scintillation solution consisting of 15 ml of naphthalenedioxane (6) and 0.5 ml of water, which dissolved the precipitate. Another aliquot of the hydrolyzed glycogen sample was counted in the same solvent. Counting was carried out in a liquid scintillation counter equipped with an external standard.

The results are presented in Table 1.

TABLE 1

Effect of prednisolone on the randomization of glucose incorporated into glycogen

Data are given with standard errors. Numbers in parentheses are the number of animals in the group.

Sample	Dpm in glycogen per gram liver per 10^6 dpm injected[a]	% of counts in C-6[b]	% recovery in C-1 plus C-6[b,c]
Normal	363 ± 124 (9)	8.0 ± 1.7 (5)	110 ± 13 (5)
Hormone-treated	5330 ± 1750 (11)	7.9 ± 1.1 (7)	96 ± 4 (7)

[a] A quenching calibration curve was determined with ^{14}C-toluene as a standard and counts were corrected to dpm on this basis. Efficiency was about 70%.

[b] The radioactive glucose used in these experiments was carried through the degradation procedure to confirm the position of the ^{14}C. One per cent of the counts were found in C-6 and 99% and 95% were recovered in C-1 plus C-6 in two experiments. The percentage of counts in C-6 in the table have not been corrected for the 1% contamination in C-6 of the glucose injected.

[c] This value was calculated from the ratio of counts present in the dimedon derivative obtained from carbons 1 and 6 to that in an equivalent amount of undegraded glucose.

Since no obvious difference was apparent due to the route of injection or of an overnight starvation, all the experiments have been included together in the table.

The marked stimulation by prednisolone of glucose incorporation into glycogen is apparent from the table in confirmation of previous results (1). The greater scatter in these experiments may be attributed to the disparity of procedures which were employed. However, the isotope distribution data were very similar in all cases and showed clearly that there was very little randomization of the glucose carbons incorporated into glycogen in either the normal animals, as has previously been reported (7), or in the hormone-treated animals.[2] Thus it may be concluded that the bulk of the glucose converted to glycogen proceeded via a direct pathway, not involving prior breakdown to trioses, and that the hormonal effect was on this pathway.[3] Moriwaki and Landau have reached a similar conclusion on the basis of a different experimental approach (8). These results do not, of course, rule out an *additional* glucocorticoid effect elsewhere, for example at the pyruvic carboxylase reaction. In fact, a hormone effect *only* on the pathway leading directly from glucose to glycogen might be expected to produce a decrease in the degree of randomization, since a proportionally smaller amount of carbon atoms would come from the triose pool under these conditions. Such a decrease did not occur.

[2] On the basis that the appearance of 50% of the counts in C-6 would represent complete randomization, it can be concluded that about 15% of the glucose carbon *appearing in glycogen* had been previously converted to trioses via the Embden-Meyerhof pathway and resynthesized to hexoses. Another smaller fraction would presumably be formed from trioses arising via the pentose cycle. Since this fraction would be unlabeled, it would not affect the isotope distribution ratio.

[3] An effect on the interconversion of fructose 6-phosphate and fructose diphosphate might also account for the results.

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Further Studies on the Solubilities of Xenon and Cyclopropane in Blood and Protein Solutions

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SUMMARY

The solubility of xenon and krypton in whole blood and plasma has been measured chromatographically and shown to vary from species to species. Some physical-chemical parameters of another nonhydrogen bonding gas–protein system (cyclopropane–serum albumin) have been studied, and no changes in the degree of protein–gas interaction could be observed with changes in pH, temperature, or ionic strength of the solutions.

The interactions of certain nonhydrogen bonding gases with bovine proteins have been demonstrated by Featherstone *et al.* (1) and Muehlbaecher *et al.* (2) and discussed by them in a review published in 1963 (3). Calculations based on the data presented in these papers show that the molar ratio of gas to protein (Table 1) varied when aqueous buffered solutions of different proteins were equilibrated with several pure gases at 37° at atmospheric pressure. Approximately four molecules of cyclopropane associated with each molecule of hemoglobin and serum albumin. Under the same conditions, hemoglobin associated with only one molecule of xenon. These three systems were selected for further study.

Chromatographically pure xenon, krypton, and cyclopropane were used. The several times recrystallized bovine serum albumin is claimed by the manufacturer (Pentex Corporation, Kankakee, Illinois) to be electrophoretically homogeneous. Whole, heparinized blood and protein solutions were saturated at atmospheric pressure in a Van Slyke manometric apparatus, thermostated at either 37° or 25°; aliquots of the saturated solutions were removed anaerobically for analysis in a Beckman GC-2A gas chromatograph. The latter was fitted with a reaction chamber of a Fisher clinical gas partitioner, which permitted direct analysis of the aqueous samples. Analyses of xenon and krypton were carried out by using a 1-foot molecular sieve 13× (42/60 mesh) column, maintained at 220°. A 4-foot column of di-*n*-decyl phthalate (30% on C-22 Firebrick, 42/60 mesh) at 100° was used for cyclopropane measurements. Known volumes of pure gas were

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