# CHANGES IN SUBSTRATE LEVELS IN LIVER DURING GLYCOGEN SYNTHESIS INDUCED BY LACTATE AND HYDROCORTISONE*

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The conversion of 3 and 4 carbon precursors to glycogen and glucose in liver and the influence of adrenal cortical hormones on such conversions have been extensively investigated (Cori, 1931; Buchanan and Hastings, 1946; Wood, 1946; Krebs, 1954; Ashmore et al., 1960; Long, 1960; Landau et al., 1963; Weber, 1963). However, information is limited as to concomitant changes in metabolic intermediates (Hohorst et al., 1962). This paper reports the changes in levels of metabolites of the Krebs cycle and glycolytic pathway after Na lactate and/or hydrocortisone administration. The results help to indicate the pathway and control points of glycogenesis.

## Methods

Male rats (180-200 g, Holtzman Strain) were anesthetized with Na phenobarbital, (150 mg per kg) and after experimental procedures, liver was frozen with Freon-12 at -150°, either in situ or immediately after excision. Samples were weighed at -20°, homogenized at -10° in 2 M $HClO_4$, diluted 3-fold with 5 mM aqueous EDTA, and centrifuged. The supernatant fluid was neutralized with $KHCO_3$.

Most of the assays were made by published fluorometric methods (Lowry et al., 1964). Malate (Fleming, Passonneau and Lowry, unpublish-

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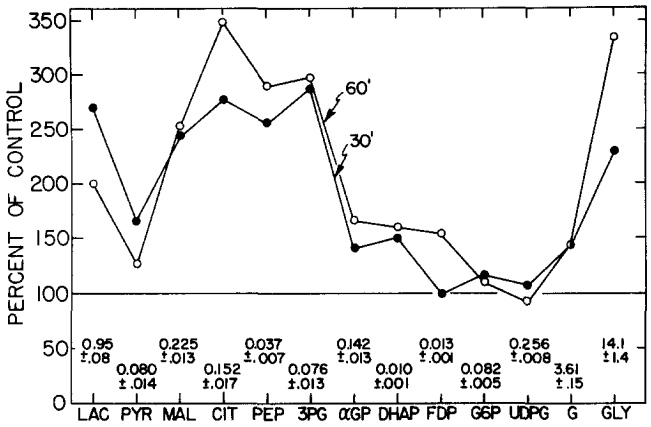


Figure 1. Changes in 13 constituents of rat liver following lactate administration. Rats were starved 24 hours, anesthetized with phenobarbital and 30 minutes later injected intraperitoneally with Na lactate (1.5 g per kg). Livers were excised 30 or 60 minutes later. Control levels, recorded as mmoles per kg, are the means ± standard errors for 9 rats, except that for αGP and UDPG the averages represent 4 rats. Control rats received intraperitoneal isotonic saline. Each curve represents the average for 4 to 6 animals except that on the 30 minute lactate curve the αGP point represents 2 rats and the UDPG point 1 rat. Plasma lactate values were 6.6 and 4.7 mmoles per liter at 30 and 60 minutes after lactate administration. Control levels averaged 3.2 mmoles per liter.

ed) and citrate (Goldberg, Passonneau and Lowry, unpublished) were also measured by enzymatic fluorometric methods. UDPG[1] was determined fluorometrically with UDPG dehydrogenase (Sigma Chem. Co.).

## Results

Rate of glycogen synthesis: Injection of 0.75 and 1.5 g of Na lactate per kg respectively produced glycogen synthesis in starved rats at rates of 26 and 35 mmoles $kg^{-1}$ $hr^{-1}$ (Fig. 1). Hydrocortisone increased glycogen levels 25 mmoles $kg^{-1}$ $hr^{-1}$ (Fig. 2) after a lag of 1 hour (not shown). Lactate plus hydrocortisone produced glycogen synthesis at a

[1] The following abbreviations are used in the figures or text: lactate, lac; pyruvate, pyr; malate, mal; citrate, cit; P-pyruvate, PEP; 3-P-glycerate, 3PG; α-glycero-P, αGP; dihydroxyacetone-P, DHAP; fructose-1,6-diphosphate, FDP; glucose-6-P, G6P; uridine diphosphoglucose, UDPG; glucose, G; glycogen, gly.

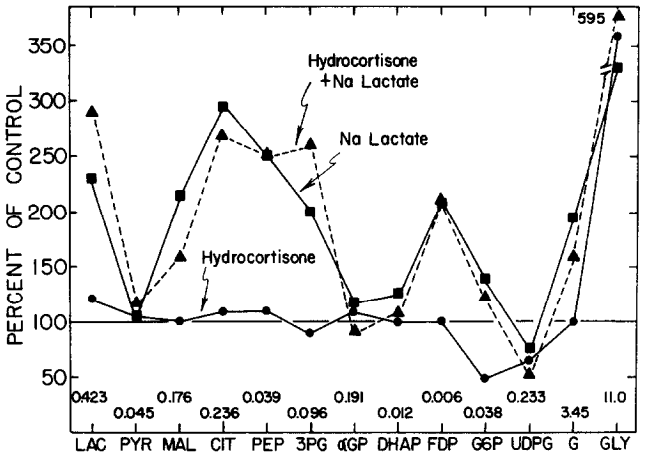


Figure 2. Changes in 13 constituents of rat liver following lactate and/or hydrocortisone administration. Rats were starved 24 hours and anesthetized with phenobarbital. Na lactate (0.75 g per kg) was given intraperitoneally 60 minutes before sampling. Hydrocortisone (50 mg per kg) was injected subcutaneously 2 or 3 hours prior to excision of the liver (0.5 or 1.5 hours before phenobarbital). Control rats received isotonic saline. Control levels, recorded as mmoles per kg, are the averages for 2 rats. Levels in lactate-treated rats are the averages for 2 rats; the hydrocortisone and hydrocortisone plus lactate levels are the averages for 4 rats.

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rate equal to the sum of the individual rates (Fig. 2).

Substrate levels after lactate administration: Within 30 minutes after lactate injection, 2- to 3-fold increases were observed in hepatic lactate, malate, citrate, PEP and 3PG (Fig. 1). Pyruvate, however, increased much less even though it must be presumed to be a precursor of the other 4 compounds. Fumarate, (not shown), increased in parallel with malate indicating equilibration between these two compounds. αGP, DHAP, and FDP (at 60 minutes only) increased 1.5 times over control levels (Fig. 1). Glucose-6-P and UDPG were both near control levels.

A lower dose of lactate induced similar changes (Fig. 2) but UDPG levels actually fell. Such a decrease after lactate administration has been confirmed by several subsequent experiments.

Substrate levels after hydrocortisone administration: Hydrocortisone administration resulted in a striking fall in hepatic G6P and UDPG concentrations (Fig. 2). No other intermediates changed significantly. Lactate plus hydrocortisone caused an even more pronounced decrease in the UDPG level. Changes in other substrates were similar to those with lactate alone. The unexpected effects of hydrocortisone on UDPG and G6P levels were studied as a function of time (Fig. 3). In starved animals, UDPG fell within an hour reaching a minimum 2 or 3 hours after hydrocortisone treatment. In contrast, UDPG in the livers of fed rats did not fall and was distinctly elevated at 6 hours. The changes in G6P were roughly parallel to those of UDPG.

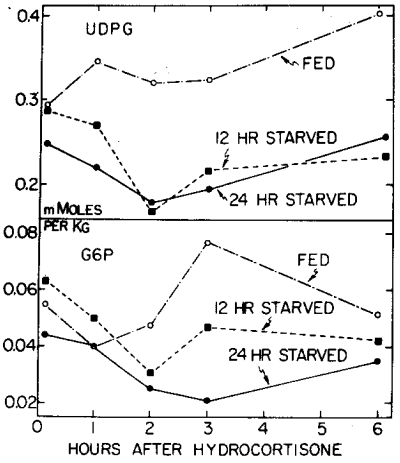


Figure 3. Effect of hydrocortisone on hepatic UDPG and G6P levels. Values are recorded as mmoles per kg. Rats were either allowed free access to food (fed) or starved 12 or 24 hours. Each point is the average for 3 rats.

## Discussion

A priori, an increase in concentration of some precursor might result in an elevated level of UDPG and hence push glycogen synthesis;

conversely facilitation of the final synthetase step would lower the level of UDPG and tend to pull precursor substrates from various pools. A pull mechanism of action for hydrocortisone is indicated by the consistent decrease in UDPG which it induces in starved animals. Since G6P levels also fall, the increased synthesis cannot be explained by G6P stimulation of glycogen synthetase as postulated by Steiner et al. (1961) and Hilz et al. (1963). These investigators observed significant increases in hepatic G6P content after hydrocortisone.[2]

Hydrocortisone, even when combined with lactate, did not alter the other intermediates, suggesting that its influence on possible intermediate steps (Lardy et al., 1964) may not be related to the stimulation of glycogen synthesis.

In the case of glycogen formation from lactate administration, although a push mechanism would have been anticipated, both a pull and push mechanism appear to be involved. UDPG levels either fell or failed to rise significantly during rapid glycogen synthesis. The other concomitant changes observed are more difficult to interpret. Lactate increased much more in proportion than did pyruvate. Since plasma levels were 1.5 or 2 times higher than liver concentrations (see legend to Fig. 1), it is probable that some of the excess lactate was extracellular. (In addition a decrease in the $DPN^+$ to DPNH ratio would not seem unlikely during rapid lactate assimilation.)

The observed decrease in the pyruvate to PEP ratio would seem to rule out a direct pyruvate kinase step on the lactate to glycogen pathway. Only an exceedingly low PEP to pyruvate ratio, combined with a very low ADP to ATP ratio would permit this reaction to procede towards PEP (Krimsky, 1959). The present data are compatible with the mechanism

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[2]The higher G6P levels reported by others may be explained by the fact that in fed animals G6P more than doubles after 1 minute of ischemia (unpublished data). Presumably this extra G6P is derived from glycogen. Since hydrocortisone increases glycogen content, more precursor becomes available to permit an artifactual increase in G6P if ischemia is not rigorously avoided.

proposed by Keech and Utter (1963) that pyruvate is converted to PEP via pyruvate carboxylase and P-pyruvate carboxykinase, but do not rule out a malic enzyme step. The fact that PEP increases more than pyruvate indicates facilitation at an intermediate step. Acceleration of pyruvate carboxylase by acetyl-CoA, also derived from pyruvate, would account for the rise in both PEP and citric acid cycle intermediates.

The failure of DHAP to rise in parallel with 3PG after lactate administration suggests that either an intermediate step is not fast enough to maintain equilibrium or that the equilibrium has been shifted by a change in concentration of a cofactor. The absence of change in the DHAP to 3PG ratio after hydrocortisone makes the second possibility seem the more likely.

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