Thus all physiologic and metabolic studies in vivo and in vitro, in which crystalline reducing carbohydrates (possessing an aldehyde or ketone capable of forming anomeric hemiacetal), including isotopes, have been dissolved and utilized may results in variable findings depending on their determination, mutarotation and factors altering mutarotation such as temperature, pH, enzymes, and most important the selective nature of the reaction being studied for the α or β anomer of the carbohydrate.

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Hormon. Metab. Res. 7 (1975) 523-524

Lactate Dehydrogenase and Pyruvate Kinase Levels in the Liver of Fed and 24-Hour Fasted Suckling Rats

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In the suckling rats, the liver gluconeogenic capabilities develop in the first days after birth (*Yeung and Oliver* 1967, *Ballard* 1971) reaching a maximum in the preweaning period (*Vernon and Walker* 1972, *Aranda and Herrera* 1974) and stabilizing thereafter at adult levels. Lactate dehydrogenase (E.C. 1.1.1.27) has a double role as gluconeogenic and as glycolytic enzyme. Pyruvate kinase (E.C. 3.7.1.40) is an irreversible glycolytic enzyme the levels of which are related to the glycolytic capabilities of the liver. In the present study, we have determined the effect of fasting on the levels of these enzymes in the liver of suckling rats. We previously demonstrated that fasting produces an increase in the gluconeogenic activity of the adult but it is not altered in the suckling animals (*Aranda and Herrera* 1974). Newborn Wistar rats were maintained with their mother prior to 24-hour food deprivation. They were sacrificed by decapitation at 5, 10, 20 and 30 days of age. Livers were immediately removed, weighed and placed in chilled 0.25 M sucrose 0.1% Triton X-100. They were homogenized in glass homogenizers with teflon pestles in 9 volumes of sucrose-Triton medium. The homogenates were centrifuged in the cold for 10 minutes at 1500 x g. A 1:10 dilution of the supernatant in the same medium was used as the enzyme source.

Lactate dehydrogenase was assayed as described by *Bergmeyer and Bernt* (1974). Pyruvate kinase was determined according to *Llorente, Marco and Sols* (1970). All determinations were performed within 3 hours after the removal of the liver. A final volume of 0.7 ml in 50 mM imidazole 5 mM EDTA buffers was used in all determinations. Initial velocities were estimated and used to determine the specific activities. Since important changes in animal and liver size, cellularity and cellule size develope with the age of the rats (*Aranda, Blazquez and Herrera,* 1973), enzyme activities were expressed in nanokatals per gram of animal weight in order to permit comparisons between the different groups.

The results obtained with the liver extracts of suckling rats are shown in the Table. Pyruvate kinase levels were decreased

Table. Activities of lactate dehydrogenase and pyruvate kinase in the liver of fed and fasted suckling rats.

Age/days	Nutritional status	Pyruvate kinase nkat./gm of rat weight		Lactate dehydrogenase nkat./gm of rat weight	
5	fed	0.476 ± 0.106 ++	*	25.91 ± 1.03 +++	***
	24-h fasted	1.108 ± 0.181		46.98 ± 2.52	
10	fed	1.163 ± 0.153		45.07 ± 3.11 ++	
	24-h fasted	0.836 ± 0.050 ++		37.34 ± 1.56 +++	
20	fed	0.902 ± 0.022 ++		50.36 ± 2.95 +	
	24-h fasted	1.035 ± 0.061		42.25 ± 4.80 ++	
30	fed	0.984 ± 0.091		45.69 ± 3.46 ++	
	24-h fasted	0.980 ± 0.075		52.11 ± 3.92 +	
adults	fed	1.198 ± 0.065		63.73 ± 1.85	*
	24-h fasted	1.130 ± 0.051		88.59 ± 5.98 +	

All data are mean ± s.e.m. of 4–6 animals. Significance of fed vs. fasted: *$p < 0.05$;***$p < 0.001$ – Significance vs. adults: + $p < 0.05$; ++ $p < 0.01$; +++ $p < 0.001$.

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only in the 5 days old rats versus adults. In the 5 days old animals, the glycolytic system does not seem to be as competent in providing energy to the animal as the lypolytic one (*Schaub, Gutmann and Lippert* 1972). The adult levels of this enzyme are already attained in the 10 days old animals. Fasting produced significant increases only in the younger rats. It is interesting to note that fasting does not produce any change in gluconeogenesis at this age (*Aranda and Herrera* 1974). This is probably due to a concomitant increase in the glycolytic activity as these results seem to indicate. The activities of lactate dehydrogenase in liver show a pattern in close correspondance with previously published lactate liver levels in suckling rats (*Burch* 1965, *Aranda* 1973). There is a high rise in the 5 days old group of animals. The enzyme levels show a rather constant and slow increase with age and correspond with other comparable previously published data (*Stave* 1970, *Burch* et al. 1971). Fasting produces a significant increase in the lactate dehydrogenase levels both in 5 days old rats and adults. This probably occurs by enzyme induction, and corresponds with the concordant rise in lactate levels found both in blood and muscle (*Alemany*, unpublished) of the younger animals. Adult rats have a more massive lactate production in peripherical tissues when fasted. This corresponds with the increase shown here in the levels of lactate dehydrogenase with fasting since this enzyme must cope with the increased load of a higher lactate burden.

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Hepatic Metabolism of Glucose and Glycogen in Fed Rats

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Introduction

Experiments with the perfused liver of the rat have shown that in fasted animals, maximal rates of glycogen synthesis require the presence of glucogenic precursors as carbon source, and glucose (*Hems, Whitton and Taylor* 1972). The present experiments were designed to clarify the role and fate of glucose, and aspects of glycogen metabolism, in livers from fed rats.

Methods

Livers of rats (200 gm) were perfused (*Hems and Whitton* 1973), and glycogen and glucose analyzed (*Hems, Whitton and Taylor* 1972). Vasopressin and substrates were from Sigma Ltd.

Results and Discussion

In fed rats, hepatic glucose uptake was faster than in 48 hr-starved rats (Figure). The existence of net gluconeogenesis was demonstrated in livers from fed rats perfused with a mixture of glycerol and pyruvate, plus serine (see *Hems, Whitton and Taylor* 1972) or lactate (maintained by infusion: see *Hems and Whitton* 1973) which brought about net glucose output, in the absence of a decline in glycogen (Figure). In the presence of high glucose concentrations (30 mM), net glycogen synthesis is maximal (*Hems, Whitton and Taylor* 1972) and net breakdown minimal (*Glinsmann, Hern and Lynch* 1969). This condition might be expected to influence the action of hormones, such as vasopressin which has a potent glycogenolytic action at "autoregulatory" (9-12 mM) glucose concentrations (*Hems and Whitton* 1973). Indeed, at 30 mM glucose, glucose output due to the hormone was negligible (Figure), although net glucose uptake was halted. This effect presumably reflected the action of glucose in shifting the balance of the synthetase-phosphorylase system (*Hers* et al. 1970).

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