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Gluconeogenesis from Lactate in the Developing Rat

STUDIES *IN VIVO*

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1. The specific radioactivity of plasma L-lactate and the incorporation of ^{14}C into plasma D-glucose, liver glycogen and skeletal-muscle glycogen were measured as a function of time after the intraperitoneal injection of L-[U-^{14}C]lactate into 2-, 10- and 30-day-old rats. 2. Between 15 and 60min after the injection of the L-[U-^{14}C]lactate, the specific radioactivity of plasma lactate decreased with a half-life of 20–33min in animals at all three ages. 3. At all times after injection examined, the specific radioactivity of plasma glucose of the 2- and 10-day-old rats was at least fourfold greater than that of the 30-day-old rats. 4. Although ^{14}C was incorporated into liver glycogen the amount incorporated was always less than 5% of that present in plasma glucose. 5. The results are discussed with reference to the factors that may influence the rate of incorporation of ^{14}C into plasma glucose, and it is concluded that the rate of gluconeogenesis in the 2- and 10-day-old suckling rat is at least twice that of the weaned 30-day-old animal.

Although numerous studies with tissue slices have indicated that the maximal rate of gluconeogenesis of the liver and kidney of the suckling rat measured *in vitro* is greater than that of the normal, fed adult (Ballard & Oliver, 1963; Yeung & Oliver, 1967; Vernon *et al.*, 1968; Zorzoli *et al.*, 1969; Vernon & Walker, 1970), the extent to which this capacity is realized *in vivo* is uncertain, as dietary amino acids are mainly used for growth in the developing rat (Hahn & Koldovský, 1966; Miller, 1970). The plasma concentrations of non-esterified fatty acids (Hahn *et al.*, 1963) and ketone bodies (Drahota *et al.*, 1966; Page *et al.*, 1971) are higher during the suckling period than in the normal adult and provide alternative sources of energy to glucose. There is also evidence that ketone bodies are metabolized more extensively *in vivo* in neonatal rats by tissues such as the brain, which utilizes glucose as its main source of energy in the adult (Hawkins *et al.*, 1971). In keeping with these observations, we have reported that the rate of glucose utilization *in vivo* of the suckling rat is only about half of that of the weaned rat (Vernon & Walker, 1972). It can be calculated, however, that even with this decreased rate of glucose utilization, the dietary carbohydrate supply of the suckling rat is insufficient to cover the glucose requirement (Vernon & Walker, 1972). In the present paper, therefore, we have investigated further the role of gluconeogenesis *in vivo* in the suckling rat by following the fate of isotopically labelled L-lactate administered by intraperitoneal injection.

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Materials and Methods

Animals

The rats were an albino Wistar strain; the normal dietary and weaning regimens were as described by Vernon & Walker (1968). After removal from the mother, 2-day-old and 10-day-old rats were kept in an incubator at 30°C.

Rats were injected intraperitoneally at approx. 0930h with 4μCi of L-[U-^{14}C]lactate (50Ci/mol) in a volume of 0.5ml of 0.9% NaCl/100g body wt. Animals were killed by decapitation at 15min intervals up to 60min after injection, and samples of blood, liver and skeletal muscle were removed in that order for analysis. Blood and tissue samples from four 2-day-old rats were pooled at each time, and from two animals for the 10-day-old rats. Thus each observation is a mean value for four animals for the 2-day-old rats and of two animals for the 10-day-old rats.

Materials

L-[U-^{14}C]Lactate was obtained from The Radiochemical Centre (Amersham, Bucks., U.K.). The sources of other materials are given in the preceding paper (Vernon & Walker, 1972).

Determination of the specific radioactivity of plasma lactate and glucose

Rat blood was collected in ice-cold heparinized tubes and immediately centrifuged at 1000rev./min for 4min at 4°C. Plasma was removed and stored at

−10°C until used (approx. 4h later). The plasma glucose and lactate concentrations and the radioactivity of the plasma glucose and lactate were measured as described in the preceding paper (Vernon & Walker, 1972).

Determination of liver and muscle glycogen specific radioactivity

Liver and muscle samples (approx. 1.0g) were digested with 2.0ml of 40% (w/v) KOH at 100°C. The glycogen concentration and radioactivity was measured as described in the preceding paper (Vernon & Walker, 1972).

Results

The three ages of rats used in this study were selected on the following basis. By 2 days after birth the immediate postnatal utilization of tissue storage materials and the concomitant changes in blood concentrations of metabolites will have occurred, and the rapid changes in the activities of key hepatic enzymes that follow birth will, in general, be complete. Ten days after birth represents a stage when considerable growth on a milk diet has occurred but well before weaning commences with its associated changes in diet and metabolic pattern. Thirty days represents an age when weaning is complete but before sexual differences in metabolic parameters begin to appear.

The body weights, liver weights and liver weight/body weight ratios of the rats at the ages studied are summarized in Table 1. At each age, the values of these parameters did not vary significantly between the groups of rats used at each time-interval after injection. Similarly, the plasma lactate and glucose concentrations, and the hepatic and skeletal-muscle glycogen contents of the rats at each age, did not change significantly during the experimental period; therefore only the mean values, obtained by pooling the values found at each time-interval, have been reported (Table 1).

Turnover of plasma lactate

There was no significant variation in the mean plasma L-lactate concentration with age (Table 1). At each age, the highest specific radioactivity of the plasma lactate during the time-period studied was found at 15min after the injection of labelled lactate (Fig. 1). Between 15 and 60min after the injection of L-[^{14}C]lactate, the specific radioactivity of the plasma lactate decreased with a half-life of 20min and 23min for the 2- and 30-day-old rats respectively. With the 10-day-old animals, a half-life could not be calculated because the regression of the log specific radioactivity with time was not linear at the $P=0.05$ level. The values for specific radioactivity of the plasma L-lactate in these 10-day-old animals at each time did not differ significantly, however, from those of the 30-day-old rats. On the other hand, the specific radioactivity of the plasma L-lactate in the 2-day-old animals was, at all times, approx. threefold greater than that in the 30-day-old rats; this difference was significant ($P<0.05$ or better).

At 15min after the administration of L-[^{14}C]lactate less than 15% of the ^{14}C injected remained in the lactate pool at any of the ages studied (Table 2). This statement assumes that the lactate space is equal to the total body water and that the concentration and specific radioactivity of the plasma lactate is as great

Table 1. *Body weights, liver weights, liver weight/body weight ratios, plasma glucose and lactate concentrations, and hepatic and skeletal-muscle glycogen concentrations of the rats at each age*

Results are expressed as means±S.E.M. Each individual result is the mean value of four pooled animals for the 2-day-old and of two pooled animals for the 10-day-old rats. Experimental details are given in the text.

	Age of rat		
	2 days	10 days	30 days
No. of observations	12	16	16
Body wt. (g)	7.8 ± 0.4	23.6 ± 0.6	88.4 ± 2.7
Liver wt. (g)	0.26± 0.01	0.70± 0.02	4.14± 0.14
100×liver wt./body wt.	3.38± 0.07	2.97± 0.03	4.68± 0.07
Plasma D-glucose (mM)	6.02± 0.23	7.78± 0.18	8.30± 0.16
Plasma L-lactate (mM)	1.73± 0.12	1.95± 0.18	1.37± 0.16
Hepatic glycogen (μmol of glucose/100g body wt.)	254.2 ±25.2	195.5 ±19.8	1010.6 ±43.7
Skeletal-muscle glycogen (μmol of glucose/g wet wt.)	14.9 ± 0.3	15.8 ± 0.7	12.6 ± 0.8

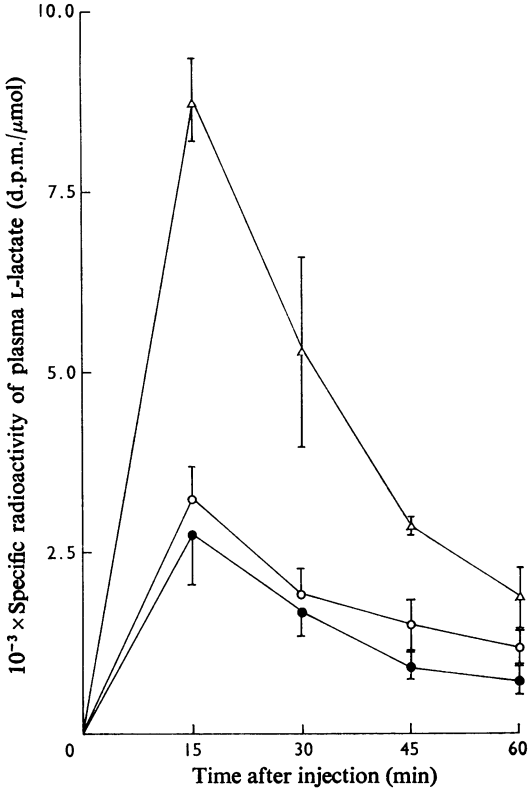

Fig. 1. *Time-course of specific radioactivity of plasma* L-*lactate*

L-[U-^{14}C]Lactate (4μCi/100g body wt.) was administered intraperitoneally at zero time to 2- (△), 10- (○) and 30- (●) day-old rats. The specific radioactivity is expressed as d.p.m./μmol of plasma L-lactate. Each point represents the mean of three or four determinations and the vertical bars show ±S.E.M. Further details are given in the text.

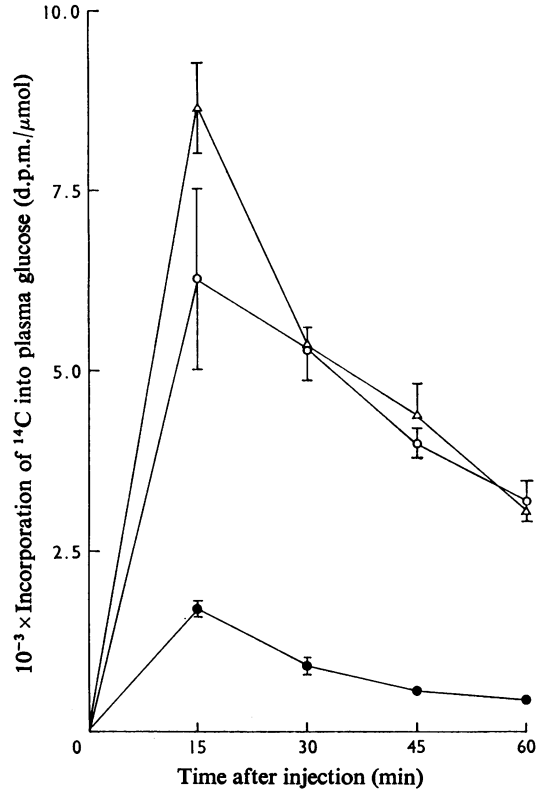

Fig. 2. *Time-course of incorporation of* ^{14}*C from* L-[*U*-^{14}*C*]*lactate into plasma* D-*glucose*

L-[U-^{14}C]Lactate (4μCi/100g body wt.) was administered by intraperitoneal injection at zero time to 2- (△), 10- (○) and 30- (●) day-old rats; further details are given in the text. The amount of ^{14}C incorporated is expressed as d.p.m./μmol of plasma D-glucose. Each point represents the mean of three or four determinations and the vertical bars show ±S.E.M. when large enough to record.

or greater than that of tissue lactate (Hohorst *et al.*, 1965). These results suggest that the half-life of the labelled lactate is much more rapid during this initial 15min period than during the subsequent 45min interval. Calculation of a lactate space from the decay curves of the specific radioactivity of the plasma L-[^{14}C]lactate by the method of Feller *et al.* (1950) gave spuriously high values, greater than 100ml/100g body wt.

Incorporation of ^{14}*C into plasma glucose*

The specific radioactivity of plasma glucose in animals at all three ages was found to be at a maximum 15min after the injection of L-[U-^{14}C]-lactate (Fig. 2). The specific radioactivity of plasma glucose at each time after injection in the 2- and 10-day-old rats was always significantly greater than that in the 30-day-old animals ($P<0.01$). At 15min after the injection of the labelled lactate, the specific radioactivity of the plasma glucose in the 2- and 10-day-old rats was 4- to 5-fold that in the 30-day-old animals; by 60min after injection of L-[^{14}C]lactate this difference had increased to 7-fold.

At all the times examined, the specific radioactivity per μmol of plasma glucose in the 2- and 30-day-old rats did not differ significantly from the specific

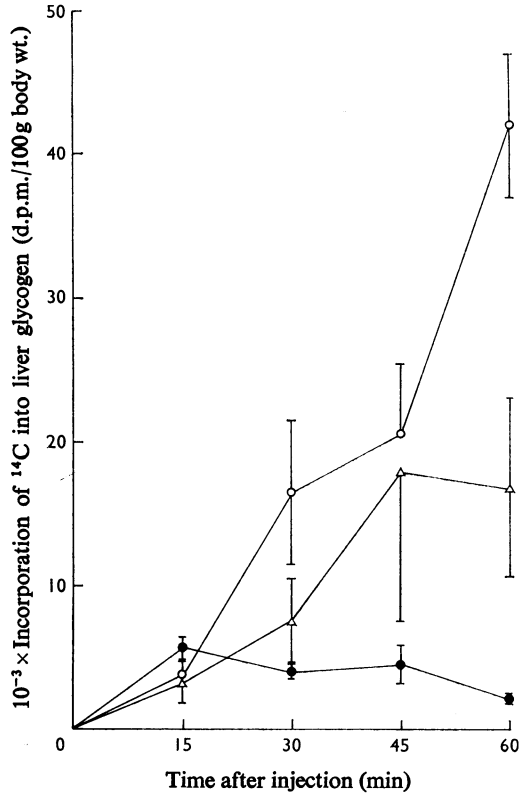

Fig. 3. *Time-course of incorporation of ^{14}C from L-[U-^{14}C]lactate into liver glycogen*

L-[U-^{14}C]Lactate (4μCi/100g body wt.) was administered by intraperitoneal injection at zero time to 2- (△), 10- (○) and 30- (●) day-old rats; further details are given in the text. The amount of ^{14}C incorporated is expressed as d.p.m. incorporated/100g body wt. Each point represents the mean of three or four determinations and the vertical bars show ± S.E.M. when large enough to record.

radioactivity per μmol of plasma lactate, but in the 10-day-old animals the specific radioactivity per μmol of plasma glucose was significantly greater ($P<0.05$) than that of the plasma lactate. However, the total amount of ^{14}C present in the glucose pool was in every case greater than that in the estimated lactate pool.

Incorporation of ^{14}C into glycogen

At 15min after the injection of the L-[^{14}C]lactate, the total radioactivity (d.p.m.) that had been incorporated into the liver glycogen, expressed per 100g body wt., was essentially the same in the animals at all ages (Fig. 3). This value decreased in the 30-day-old animals with time, but increased in the glycogen in the livers of 2- and 10-day-old rats. The highest value for the radioactivity present at any time in liver glycogen at any of the ages was less than 5% of that present in the glucose pool (Table 2).

At all three ages the specific radioactivity of the skeletal-muscle glycogen increased with time (Fig. 4). The rate of increase in its specific radioactivity was greater in the 2- and 10-day-old rats than in the 30-day-old animals. By using the value of 23–28g/100g body wt. for the weight of skeletal muscle of the developing rat (Miller, 1969) it can be calculated that the highest value for the amount of ^{14}C present in muscle glycogen at any time in animals at all three ages was less than 0.5% of the radioactivity administered.

Discussion

The results presented in Fig. 2 and Table 2 show that there is a highly significant increase in the amount of ^{14}C present in plasma glucose in the 2- and 10-day-old rats compared with that in the 30-day-old animals. The interpretation of these observations, however, is complicated by a number of factors. In particular, it is necessary to assess possible differences in the pool sizes and turnover rates of the precursor and product before the conclusion can be made that the results represent an increased flux of lactate carbon to glucose in the suckling rat (Friedmann *et al.*, 1967; Exton *et al.*, 1969).

We have therefore investigated both the pool size and the turnover rate of the precursor, lactate, to correct for possible differences with age. The specific radioactivity of the plasma lactate was found to be surprisingly low. This does not appear to be due to a slow rate of absorption of the administered lactate, since the specific radioactivity of both plasma lactate and glucose was greatest 15min after the injection of the labelled lactate. It has been shown that in dogs, into which [^{14}C]lactate was administered intravenously, the decay curve of plasma lactate could be divided into a fast and a slow component (Forbath *et al.*, 1967). The slow component was the dominant feature at 15–20min after tracer injection and corresponded to a half-life of 30min. A similar observation has been made in man (Kreisberg *et al.*, 1970). Both Forbath *et al.* (1967) and Kreisberg *et al.* (1970) found that values for the lactate space, calculated from the slow component of the decay curve, were greater than 100ml/100g body wt. It appears then that the rat resembles the dog and man in that a multicompartmental model must be used to describe the kinetics of L-lactate metabolism.

Table 2. *Distribution of* ^{14}C *at* 15 *and* 60 *min after the intraperitoneal injection of* L-[*U*-^{14}C]*lactate*

Results are expressed as a percentage of the total radioactivity administered and are means±S.E.M. of the number of observations in parentheses. The lactate space was assumed to equal the total body water and values of 80ml, 75ml and 70ml/100g body wt. (Spector, 1956) were used respectively for the 2-, 10- and 30-day-old rats. The glucose pool sizes, 357μmol, 292μmol and 268μmol/100g body wt. respectively for the 2-, 10- and 30-day-old rats, were taken from Vernon & Walker (1972).

	Time after the administration of L-[U-^{14}C]lactate					
	15min % of total radioactivity administered in:			60min % of total radioactivity administered in:		
Age of rats (days)	Lactate pool	Glucose pool	Liver glycogen	Lactate pool	Glucose pool	Liver glycogen
2	13.8±0.9 (3)	35.0±2.5 (3)	0.04±0.02 (3)	3.0±0.7 (3)	12.5±0.6 (3)	0.19±0.07 (3)
10	5.4±0.7 (4)	20.7±4.2 (4)	0.05±0.01 (4)	2.0±0.4 (4)	10.6±0.9 (4)	0.48±0.06 (4)
30	3.0±0.7 (4)	5.2±0.3 (4)	0.06±0.01 (4)	0.8±0.2 (4)	1.4±0.2 (4)	0.02±0.00 (4)

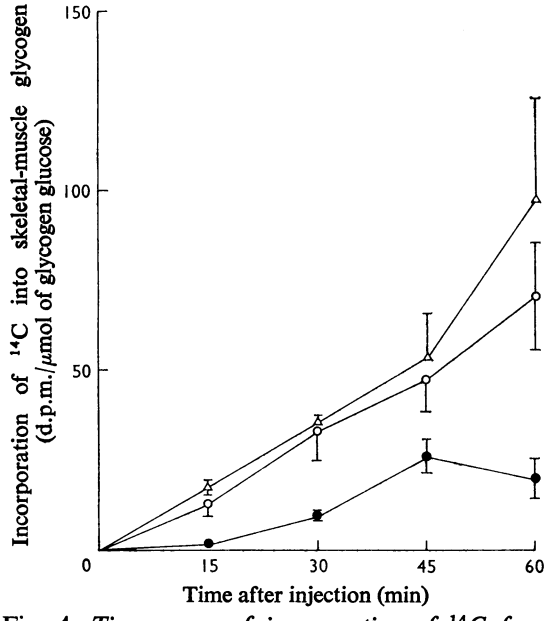

Fig. 4. *Time-course of incorporation of* ^{14}C *from* L-[*U*-^{14}C]*lactate into skeletal-muscle glycogen*

L-[U-^{14}C]Lactate (4μCi/100g body wt.) was administered by intraperitoneal injection at zero time to 2-(△), 10-(○) and 30-(●) day-old rats; further details are given in the text. The amount of ^{14}C incorporated is expressed as d.p.m./μmol of skeletal-muscle glycogen glucose. Each point represents the mean of three or four determinations and the vertical bars show ±S.E.M. when large enough to record.

Although the turnover rates and the sizes of the relevant lactate pools cannot be calculated for each age group, the results suggest that the kinetics of lactate metabolism are similar at all three ages studied. The only significant difference in the parameters measured was the specific radioactivity of the plasma lactate of the 2-day-old rat, which was significantly greater than that of the 10- and 30-day-old rats. The reason for this difference cannot be resolved from the available results and its effect on the rate of incorporation of ^{14}C into glucose is uncertain. It is pertinent to note, however, that although the specific radioactivity per μmol of plasma glucose and lactate of the 2-day-old and also the 30-day-old animals do not differ significantly, an equilibrium system is not indicated because the greater number of carbon atoms in the glucose molecule means that the specific radioactivity per carbon atom of plasma lactate will be approximately twice that of plasma glucose. In the case of the 10-day-old rat, the specific radioactivity per carbon atom of plasma glucose and lactate is similar, but again an equilibrium system cannot be postulated, because we have previously shown in analogous experiments with [6-^{14}C]glucose that the specific radioactivity of plasma glucose at all ages was greater than that of plasma lactate (Vernon & Walker, 1972).

The various assumptions that have to be made in calculating the percentage of ^{14}C remaining in the total body lactate pool render any conclusions at best approximate. The inherent complications that arise owing to the need to pool samples from several animals to obtain each value because of their small size make it difficult to obtain more precise information. The results suggest, however, that there is a greater loss of ^{14}C from the lactate pool during the 15min after injection in the 30-day-old compared with the younger rats. This could be due to either a faster lactate turnover rate during this period, or a more rapid rate of absorption of lactate from the peritoneal cavity in the 30-day-old as compared with the younger

animals. Both of these possibilities would favour a more rapid incorporation of ^{14}C into glucose in the 30-day-old animals, whereas the rate of incorporation of ^{14}C into glucose was greater in the 2- and 10-day-old rats.

We have reported (Vernon & Walker, 1972) that the glucose turnover rate, expressed as μmol of glucose/min per 100g body wt., of 2- and 10-day-old rats was approx. 50% of that of the 30-day-old animals. This factor, which accounts for differences in the glucose pool size and half-life, probably also accounts for half of the increased specific radioactivity of the plasma glucose of the suckling rats relative to the weaned animals and for the increase with time in the ratio of the specific radioactivity of the plasma glucose of the 2- and 10-day-old animals compared with that of the 30-day-old rats. When this factor has been taken into consideration, however, there is still a 2- to 3-fold increase in the rate of incorporation of ^{14}C into glucose in the suckling rats. The results strongly suggest, therefore, that there is an increased gluconeogenic flux in the suckling rat as compared with the 30-day-old rat.

The distribution of the ^{14}C incorporated from lactate between glucose and glycogen (Table 2) indicates that glucose is the major product of gluconeogenesis at all the ages studied. Similar results have been reported for adult rats under a variety of physiological and pathological conditions (Friedmann *et al.*, 1965, 1967). A comparison shows that changes in the radioactivity of liver glycogen after the administration of either [^{14}C]lactate (the present paper) or [6-^{14}C]glucose (Vernon & Walker, 1972) were similar, and are indicative of a net synthesis of hepatic glycogen in the 2- and 10-day-old rats, but a rapid labelling and turnover of a glycogen pool in the 30-day-old animals. The results also show that in the 2-day-old rat, lactate carbon is incorporated into liver glycogen twice as rapidly as glucose carbon. In the 10-day-old animals, lactate and glucose carbon are incorporated into glycogen at the same rate, whereas in the 30-day-old animals glucose carbon is incorporated into liver glycogen six times more rapidly than lactate carbon. These findings are in agreement with the conclusion that there is increased gluconeogenesis in the suckling rat, although the decreased rate of hepatic glucose phosphorylation in the 2- and 10-day-old rats (Ballard & Oliver, 1963), which can be ascribed to the absence of glucokinase (Walker & Holland, 1965), will probably contribute to the above differences in the rate of incorporation of lactate and glucose carbon into hepatic glycogen.

Because less than 10% of the glucose utilized in 2-, 10- and 30-day-old rats is recycled via the Cori cycle (Vernon & Walker, 1972), glucose carbon is probably completely oxidized to CO_2 by most tissues. We have now demonstrated that the flux of carbon atoms from lactate, and hence pyruvate, to glucose in the suckling rat is at least twice that of the weaned animal. The concentrations of lactate and pyruvate are considered to be at equilibrium in the liver and other tissues (Hohorst *et al.*, 1959; Krebs, 1969). The flow of ^{14}C from lactate to glucose will thus be equivalent to the flow of ^{14}C from pyruvate to glucose. Hence the increased gluconeogenic flux most probably reflects an increased flow of dietary carbon to glucose, pyruvate being an intermediate in the catabolism of several amino acids (Krebs, 1964). Although the suggestion that dietary amino acids may be a source of glucose in the suckling rat is questionable, it is pertinent to note that even when the nitrogen content of the diet is a limiting factor for growth, only 70–75% of the nitrogen is retained (Miller, 1970). The remainder of the dietary amino acids may thus be available for gluconeogenesis.

The results presented in this paper strongly support the conclusion drawn from studies with liver and kidney slices that there is an increased rate of gluconeogenesis in the suckling rat.

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