HEPATIC GLUCONEOGENESIS OF THE RABBIT*

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SUMMARY

Rabbit hepatic mitochondria (m_w), incubated with pyruvate and HCO_3^- produce and liberate to the media sizeable amounts of malate, citrate, and aspartate (if given NH_4^+). M_w from fasted or diabetic rabbits produce and liberate larger amounts of malate. Even though rabbit liver m_w possess phosphoenolpyruvate carboxykinase(PEPCK), the amount of PEP formed and liberated is only a small fraction of the potential glucose carbon delivered to the medium as malate, citrate or aspartate. Demonstration of PEPCK in cytosol of fed rabbit liver plus the fact that fasting or alloxan diabetes both can cause a substantial increase in the total cytosolic activity of this enzyme without altering its total activity in m_w suggest that gluconeogenesis proceeds, in general, in a similar fashion in both rats and rabbits.

The process of gluconeogenesis and its regulation have recently been under intensive study although the majority of these studies have been pursued using the rat as the experimental animal. The laboratories of Lardy (1,2), Haynes (3) and others have produced a mass of evidence showing that oxalacetate formed in m_w by carboxylation of pyruvate (via pyruvate carboxylase) is transported in the form of malate, aspartate or citrate to the cytosol where these compounds are converted back to oxalacetate. The oxalacetate is then metabolized to PEP (via PEPCK) and ultimately to carbohydrate. The cytosolic conversion of malate or aspartate (via urea cycle enzymes and fumarase) back to oxalacetate can generate NADH which becomes available to satisfy the need for reducing equivalents for the reduction of phosphoglycerate to triose phosphate. Thus, in rat liver, the above-mentioned shuttle allows for the transfer of both carbon and reducing equivalents from their mitochondrial source of origin to the site

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of their utilization for gluconeogenesis in the cytosol. This concept of gluconeogenesis evolved after and most likely as an outgrowth of the observations that rat hepatic pyruvate carboxylase is located predominantly in m_w (4,5) while PEPCK is located mainly in the cytosol (6,7).

Data obtained with all other species examined thus far indicate that pyruvate carboxylase is always located predominantly in hepatic m_w (8). However, the cellular location of PEPCK has been reported to vary with species (6,8). For example, this enzyme is reported to be located only in m_w of rabbit liver, in both m_w and cytosol of guinea pig liver, and primarily in cytosol of rat liver. The reports of differences in the intra-cellular location of this key enzyme in various species have precluded the generalization that the above-mentioned shuttle system is operative in all its facets in all species.

Thus, our studies were designed to investigate the processes by which glucose is formed by an animal that, according to previous reports has a cellular distribution of PEPCK different from that of rats and that could, accordingly exhibit differences in its processes of carbohydrate formation. This report includes data related to metabolites formed in and liberated by isolated rabbit hepatic m_w given pyruvate and HCO_3^- as substrates. Data concerning the cellular location and activity of PEPCK in rabbit liver are also reported.

METHODS

Young, male rabbits of the New Zealand strain weighing approximately 2 kg were obtained from Gopher State Caviary, St. Paul, Minn. and were maintained on Purina Laboratory Chow and tap water *ad libitum*. Rabbits were made diabetic by a single injection of a solution of isotonic NaCl and alloxan monohydrate (dose 160 mg/kg) into the marginal vein of the ear (9). Diabetic rabbits were used approximately one week after receiving alloxan, provided that their blood sugar concentrations were at least 350 mg/100 ml. Blood glucose was estimated by the glucose oxidase method (Glucostat, Worthington Biochemical Corporation).

Rabbits were killed by a blow on the head and decapitation. Twice-washed hepatic m_w were prepared by the method of Schneider (10) and supernatant fraction was prepared by centrifugation at 105,000 X *g* for 60 min. The m_w were

resuspended in 0.25 M sucrose and used immediately in incubation experiments, or were frozen and thawed 4 times prior to being assayed for PEPCK activity.

PEPCK activity was assayed in the incubation medium described by Nordlie and Lardy (6) although the PEP formed was cleaved with mercuric ion (11) and the inorganic phosphate liberated was estimated by the method of Sumner (12). Protein was measured by the biuret method of Layne (13) as modified by Lee and Lardy (14).

For studies of metabolite production, m_w (0.5 ml, 5-7 mg N) were incubated for 10 min at 37° with 6.7 mM pyruvate as substrate in a medium containing 6.7 mM phosphate and 6.7 mM triethanolamine buffer (pH 7.4), 7.5 mM $MgSO_4$, 10 mM $KHCO_3$ and 0.25 M sucrose. The reactions were initiated by addition of m_w and were terminated by placing the flasks on ice. The m_w were separated from the media by centrifugation and the media plus the m_w washes were assayed for metabolites by standard, published methods (15).

Table I

PRODUCTION OF METABOLITES BY RABBIT HEPATIC MITOCHONDRIA

Mitochondrial Source	Pyruvate Used	Metabolites Formed		
		Malate	Citrate	PEP
		μ moles/gm liver/10 min		
Fed (9)[1]	6.28 ± 2.2[2]	1.06 ± 0.3	0.95 ± 0.35	0.19 ± 0.13
48 hr fasted(4)	7.03 ± 2.1	1.91 ± 0.6	1.12 ± 0.4	0.35 ± 0.10
	p>0.6[3]	p=0.05	p>0.5	p=0.1
fed diabetic(5)	8.82 ± 2.2	2.59 ± 0.9	1.16 ± 0.3	0.47 ± 0.17
	p<0.1	p<0.02	p=0.3	p<0.05

[1]Number of rabbits.

[2]Standard deviation.

[3]Significance of differences between sample means; data from fasted and diabetic rabbits tested against data from fed rabbits. p values of 0.05 or less are considered to be significant.

RESULTS

Data in Table I demonstrate the ability of m_w from fed rabbits to synthesize and liberate to the external media malate, citrate and PEP. Although not shown, the synthesis of these metabolites is dependent on the addition of HCO_3^- and is independent of ATP addition. The amounts of PEP found in the media are very small relative to the amounts of malate or citrate found.

Table II shows that the addition of NH_4^+ to the usual incubation mixture causes the appearance of aspartate in the media with a concomitant diminution in the production of malate and citrate. The addition of NH_4^+ also depresses substrate utilization although we can offer no explanation for this observation.

A comparison was made of the amounts of metabolites produced by m_w from fed, 48 hr fasted, and fed, diabetic rabbits and these data are also given in Table I. There is, generally speaking, an increased production of malate and PEP caused by fasting, and in fact the increased production of malate is statistically significant. There is an even greater increase in the production of malate and PEP if m_w from diabetic rabbits are used and in this case, the increased production of both metabolites is significant. However, just as with fed rabbits, the amount of PEP produced by m_w from either fasted or diabetic rabbits remains very small relative to the production of malate and citrate.

Table II

THE INFLUENCE OF NH_4^+ ON PRODUCTION OF METABOLITES BY RABBIT HEPATIC MITOCHONDRIA

	Pyruvate Used	Metabolites Formed			
		Malate	Citrate	PEP	Aspartate
		μ moles/gm liver/10 min			
Basic system	6.15(3)[1]	1.07	0.95	0.24	0
Basic system + NH_4^+	3.46(3)	0.29	0.45	0.25	0.83

[1]Number of experiments.

Table III

DISTRIBUTION AND ACTIVITY OF PEPCK IN LIVERS OF RABBITS

Condition	Liver Fraction			
	mitochondria		cytosol	
	Sp. act.[1]	Total act.[2]	Sp. act.	Total act.
Fed (9)[3]	41 ± 19[4]	90 ± 47	4.3 ± 3.5	30 ± 28
48 hr fasted(7)	97 ± 17 p<0.01[5]	83 ± 33 p>0.7	31 ± 9 p<0.01	122 ± 69 p<0.01
Fed diabetic(5)	78 ± 20 p<0.05	87 ± 37 p>0.9	37 ± 15 p<0.01	134 ± 60 p<0.02

[1] nmoles PEP formed/min/mg protein.
[2] μmoles PEP formed/min/total liver.
[3] Number of rabbits.
[4] Standard deviation.
[5] Significance of differences between sample means; data from fasted and diabetic rabbits tested against data from fed rabbits. p values of 0.05 or less are considered to be significant.

Due to this relative lack of mitochondrial formation and liberation of PEP, the location and activity of PEPCK in rabbit liver was reinvestigated and data obtained from fed, 48 hr fasted, and fed, diabetic rabbits are presented in Table III. Data are presented both in terms of specific activity and of total activity. It is apparent that there is a measurable amount of PEPCK in the cytosol of liver from fed rabbits. Even though the specific activity of the enzyme in cytosol is only about 10% of the activity in m_w, the total activity in the cytosol is about 30% of the total activity found in m_w.

It is also apparent that conditions conducive to gluconeogenesis, for example fasting or diabetes, are both sufficient to cause highly significant increases in the specific activities of PEPCK in both the m_w and the cytosol. However, these conditions cause a highly significant increase in the total activity of the enzyme only in cytosol and have no effect on the total activity of PEPCK in m_w.

DISCUSSION

It has been shown by various investigators (1,2,3) that rat liver m_w, given pyruvate and HCO_3^-, can synthesize and liberate to the media malate, citrate and aspartate (if a source of NH_4^+ is provided). It has recently been shown by Davis and Gibson (16,17) that rabbit liver m_w given pyruvate can also form malate, citrate and small amounts of PEP. The amount of PEP formed by their system is increased up to a point by the addition of uncouplers such as dinitrophenol or oleate. However, their procedures did not indicate which, if any of the metabolites formed, left the m_w, nor was the formation and liberation of aspartate suggested.

Data presented in this paper show that rabbit liver m_w, given pyruvate and HCO_3^-, can produce and liberate rather extensive amounts of malate and citrate and, under proper conditions, aspartate.

Although other investigators (17,18) have projected that PEP for gluconeogenesis (by rabbit liver) was formed in and liberated from m_w, we find that the amount of PEP formed in and liberated by m_w is only a small fraction of the total carbon leaving the m_w as PEP, malate and citrate. Even though our data indicate that m_w from fasted or diabetic rabbits can make and liberate larger amounts of malate and PEP, the amounts of PEP formed are still small relative to the amounts of other metabolites leaving the m_w.

In 1965, Nordlie and Lardy (6) published data indicating that PEPCK activity was non-demonstrable in cytosol of rabbit liver. Even though their data have generally been accepted, Ilyin, et al (19) published in 1966 data which indicated that there was nearly an equal distribution of PEPCK activity between the mitochondrial and cytosolic portions of rabbit liver. Their data also indicated that only the cytosolic PEPCK activity of rabbit liver could be extensively increased by fasting or by prolonged administration of hydrocortisone.

Some time prior to our knowledge of the data of Ilyin, et al (19), we initiated an investigation of the location and activity of PEPCK in rabbit liver. As indicated by data in this paper, we too, find demonstrable activity

of this enzyme in the cytosol of rabbit liver from fed rabbits although our data suggest that there is 3 times the amount of activity in the m_w as in the cytosol. We also find that fasting causes a substantial increase in the total activity of PEPCK only in the cytosolic portion of the liver. We have extended the studies of Ilyin, et al (19) to the diabetic rabbit and again find a substantial increase in the cytosolic PEPCK activity even though the total activity in m_w remains comparable to that of control rabbits.

Thus, the data presented here suggest that rabbit liver m_w given pyruvate and HCO_3^- form and liberate mainly malate, citrate and/or aspartate. A small amount of PEP is also produced and liberated. Although fasting or diabetes increases the mitochondrial production of malate and PEP, the amount of PEP produced still remains small relative to the amounts of other metabolites. We suggest, therefore, that the malate and/or aspartate can function as carriers of both potential glucose carbon and reducing equivalents to the cytosol where they are converted back to oxalacetate which can be, in turn, converted to PEP by the substantial amounts of PEPCK present in the cytosol under conditions conducive to gluconeogenesis.

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