Gluconeogenesis in the Kidney and Liver Slices of a Lizard, *Uromastix hardwickii*

By M. ZAIN-UL-ABEDIN* AND B. KATORSKI*

Department of Biochemistry, University of Karachi, Karachi, West Pakistan

(Received 5 May 1966)

1. The liver and kidney of the lizard *Uromastix hardwickii* have much higher contents of carbohydrate than have been reported for the corresponding rat tissues. Most of this carbohydrate still remains in the tissue even after a long preincubation. 2. Kidney slices of this lizard release both glucose and other carbohydrates into the medium. Hence glucose release alone, as demonstrated for rats, cannot be used as a good criterion of gluconeogenesis in this lizard. Moreover, the results obtained by glucose release did not agree with those in which the total carbohydrate was estimated in the slice and medium. 3. L-Glutamate, L-aspartate, DL-valine, L-proline, L-cysteine, L-lactate and succinate stimulated gluconeogenesis in the kidney slices, whereas citrate, L-alanine, L-serine, glycine, L-arginine and L-leucine did not. In liver slices only L-glutamate increased gluconeogenesis. 4. New carbohydrate formation in the kidney and liver slices after incubation with various substrates indicated that gluconeogenesis as well as the amino acid metabolism in this animal may be somewhat different from that of mammals.

Gluconeogenesis has been extensively studied in mammalian tissues. The liver (Renold, Teng, Nesbitt & Hastings, 1953; Renold, Hastings, Nesbitt & Ashmore, 1955) and the kidney (Krebs, 1964) are the tissues where this process goes on to a considerable extent and are thus good systems for the study of gluconeogenesis. Krebs, Bennett, de Gasquet, Gascoyne & Yoshida (1963) indicated that, for short incubation periods, kidney slices of the rat are a better system for the study of this phenomenon because there is less preformed carbohydrate in this tissue than in the liver and hence small increments can be detected easily. Since glucose is the end product of gluconeogenesis in the rat kidney and most of this glucose is released into the medium, Krebs *et al.* (1963) successfully demonstrated that increased glucose release into the medium is a good criterion of gluconeogenesis when kidney slices are incubated with different substrates.

The present investigation on gluconeogenesis was carried out on a local desert lizard that hibernates during the colder months of the year (November to April). The capacity of liver slices to synthesize carbohydrate from various substrates was determined. Similarly the gluconeogenic capacity of kidney slices was also analysed both by measuring

the glucose release into the incubation medium as well as by measuring the total carbohydrate synthesis. The validity of these two methods for determining gluconeogenesis is discussed with respect to this system.

MATERIALS AND METHODS

Experimental animals. Local desert lizards, *Uromastix hardwickii*, were collected from the field without regard to sex, size and weight. The weights ranged between 100 and 450 g. They were kept in wooden boxes for several days before the experiment. During this period they were not given any food, since most of these studies were done during their hibernation period, when they do not consume any food.

Determination of glucose. A portion of the sample was deproteinized with equal amounts of 2% (w/v) ZnSO₄ and 0.1N-NaOH, and glucose estimated by the Glucostat method (Worthington Biochemical Corp., Freehold, N.J., U.S.A.).

Determination of total carbohydrate. Tissues were first digested with 30% (w/v) KOH. Samples were diluted and portions analysed with anthrone (Fales, 1951).

Determination of tissue carbohydrate. Animals were killed by cervical dislocation. The liver and kidneys were removed immediately, rinsed in 0.59% NaCl (Maher, 1964) and transferred to preweighed beakers containing 30% KOH. The beakers were reweighed and placed on a boiling water bath for 30 min. to digest the tissue. The digest was diluted and total carbohydrate estimated as described above.

To estimate the free glucose, glycogen and total carbohydrate of the kidney, one of the kidneys was transferred

*Present address: Depart of Biological Chemistry, University of Utah, Salt Lake City, Utah, U.S.A.

immediately after removal from the animal to 30% KOH and the other was placed in 0.59% NaCl kept chilled in an ice bath. The kidney in KOH was digested and diluted to 4 ml. and then divided into two equal portions. Glycogen was isolated from one portion by ethanol precipitation and then dissolved and estimated by the anthrone method. The other portion was diluted and a fraction taken directly for total carbohydrate determination. The other kidney was homogenized in 0.59% NaCl, deproteinized and analysed for glucose.

Incubation procedure. The liver and kidneys were removed and placed in cold 0.59% NaCl. These tissues were sliced free-hand between two frosted slides by the method of Deutsch (1936), rinsed in reptilian Ringer phosphate buffer, pH 7.4 (Maher, 1964), blotted on filter paper and weighed on a torsion balance. The slices were placed in flasks containing 2.5 ml. of the above buffer and incubated in a water bath at 40° at a shaking rate of 90 oscillations/min. with air as the gas phase.

Time-course of carbohydrate release. Kidney slices weighing 100–200 mg. were incubated as described above for 30, 60, 120 and 240 min. At the end of the incubation, the tissue slices were analysed for total carbohydrate and the medium was analysed for glucose and carbohydrate. Some slices were also analysed directly without incubation for the initial carbohydrate content of the tissue.

Glucose release as affected by substrate. Slices from the kidneys of each animal were divided so that half were incubated in buffer and the rest in buffer containing substrate at a concentration of 0.01 M. Lactate, succinate, citrate, L-glutamate and L-aspartate were added in these experiments.

Carbohydrate synthesis in liver and kidney. Liver slices (200–500 mg.) and kidney slices (100–300 mg.) from each animal were preincubated separately for 30 min. in 5 ml. of buffer at 40°. At the end of this time the slices were removed, blotted, divided in half and weighed. Half were placed in a flask containing 2.5 ml. of buffer alone and the other half in a flask containing 2.5 ml. of buffer containing substrate at a concentration of 0.01 M, except for DL-amino acids where the concentration was 0.02 M. All flasks were incubated for a further 2 hr. The contents of each flask were then digested with 1 ml. of 80% (w/v) KOH, the digest was diluted and a fraction was analysed for carbohydrate.

Statistical analysis. Significance of the difference between control and experimental groups was determined by paired comparison (Hill, 1961).

RESULTS

Tissue carbohydrate. The total carbohydrate content of the liver was found to be 6.52 ± 1.48 g./100 g. wet wt., whereas that of the kidney was

0.504 ± 0.019 g./100 g. wet wt. (Table 1). The kidney carbohydrate content was about the same in all the samples analysed, whereas that of the liver varied greatly. Free glucose and glycogen together account for the total carbohydrate of the tissue.

Glucose and total carbohydrate release from kidney slices. A large amount of glucose was released in the first 30 min. of incubation and thereafter up to 4 hr. the release was very slow (Table 2). Similarly the loss of total carbohydrate from the slices was greatest in the first 30 min. and less in the subsequent periods.

Release of glucose from kidney slices in the presence of substrate. The time-course of glucose release from the kidney slices incubated in buffer with or without 0.01 M-lactate was followed. After 1 hr. the slices incubated with lactate released $45.7 \mu\text{g.}/100 \text{ mg.}$ wet wt. above the value obtained with slices incubated in buffer alone. At 2 hr. this value increased to $62.2 \mu\text{g.}/100 \text{ mg.}$ wet wt. and at 3 hr. to $67.1 \mu\text{g.}/100 \text{ mg.}$ wet wt. Since there was little difference between the release at 2 hr. and 3 hr., in the studies with various substrates a 2 hr. incubation period was selected.

Lactate and glutamate when added at a concentration of 0.01 M increased the glucose release by 35% and 109% respectively above the control level, whereas citrate, succinate and aspartate did

Table 2. *Time-course of glucose and total carbohydrate release from Uromastix-kidney slices*

The slices were incubated in reptilian Ringer phosphate buffer, pH 7.4, at 40°. The medium was analysed for glucose and total carbohydrate at the end of the incubation period. The values represent the means of values obtained in three experiments. Initial values for glucose and total carbohydrate in the medium were zero.

Time of incubation (min.)	Glucose ($\mu\text{g.}/100 \text{ mg.}$ wet wt.)	Total carbohydrate ($\mu\text{g.}/100 \text{ mg.}$ wet wt.)
30	57	277
60	68	290
120	67	320
240	69	354

Table 1. *Carbohydrate content of the liver and kidney of Uromastix*

Each result is expressed as the mean $\pm$ s.e.m. of the number of determinations in parentheses.

Tissue	Wet wt. (g.)	Glucose (g./100 g. wet wt.)	Glycogen (g./100 g. wet wt.)	Total carbohydrate (g./100 g. wet wt.)
Liver	9.02 ± 1.8 (10)	—	—	6.52 ± 1.48 (10)
Kidney	0.701 ± 0.120 (4)	0.196 ± 0.019 (4)	0.318 ± 0.023 (4)	0.504 ± 0.019 (4)

not stimulate glucose release (Table 3). As shown in Table 3 there is a wide range of values among the controls. These animals varied not only in age and sex but also in their physiological states. In some of these experiments the animals used were freshly collected and in others they were kept for some time in the laboratory. Other than this we do not know of any reason for this variation.

In the initial studies on gluconeogenesis the glucose released from the tissues into the incubation medium was determined by the Glucostat method, the reaction being allowed to proceed until completion. In some experiments we observed that the

colour intensity in the samples did not develop until the end of the normal reaction period. According to the manufacturer of the Glucostat reagent, the enzyme preparation contains slight traces of other enzymes that would hydrolyse polysaccharides when the reaction is carried out for a long time. So it is very likely that some of the glycogen or its degradation products released into the medium was hydrolysed by these enzymes, thus giving false values for free glucose released. The release studies with kidney indicated that the total carbohydrate released into the incubation medium was about five times the free glucose released. The results obtained by measuring glucose release into the medium did not agree with those in which the total carbohydrate content of the slices and the medium was determined. Hence in subsequent studies we measured total carbohydrate of the tissue and medium at the end of the incubation as an indication of gluconeogenesis in the presence and absence of substrate, since glucose release into the medium was not a valid criterion of gluconeogenesis.

Carbohydrate synthesis in liver and kidney slices.

In these experiments a 30 min. preincubation period was selected, since after 30 min. there is very little increase in the amount of carbohydrate released from kidney slices (Table 2). Thus this time was adequate to deplete the kidney of most of its carbohydrate stores that are readily mobilized. For convenience the same preincubation time was also used for the liver slices.

Carbohydrate formation in the presence of substrate was noticeably more in kidney slices than in

Table 3. *Glucose release from Uromastix-kidney slices in the presence of various substrates*

The kidney slices from each animal were incubated in reptilian Ringer phosphate buffer, pH 7.4, at 40° for 2 hr. with or without substrate (0.01M). At the end of the incubation the medium was analysed for glucose. Each result represents the mean $\pm$ S.E.M. of the number of observations in parentheses.

Substrate	Glucose released (μ g./100 mg. wet wt.)	
	Buffer only	Buffer with substrate
L-Glutamate	35 $\pm$ 6 (2)	75 $\pm$ 6 (4)
L-Lactate	145 $\pm$ 41 (2)	221 $\pm$ 30 (4)
L-Aspartate	84 $\pm$ 15 (10)	63 $\pm$ 4 (16)
Citrate	42 $\pm$ 7 (3)	39 $\pm$ 4 (6)
Succinate	87 $\pm$ 17 (4)	66 $\pm$ 9 (4)

Table 4. *Total carbohydrate formation in Uromastix-kidney slices*

The slices were incubated in reptilian Ringer phosphate buffer, pH 7.4, at 40° for 2 hr. with or without 0.01 M-substrate (0.02 M for DL-amino acids). At the end of the incubation the total contents of the flask were analysed for carbohydrate. Each result is the mean $\pm$ S.E.M. of the number of determinations in parentheses. Positive and negative values for the percentage change represent the increase and decrease respectively from the control value. N.S., Not significant.

Substrate	Total carbohydrate (μ g./100 mg. wet wt.)		Change (%)	Value of P for paired comparison
	Control	With substrate		
L-Lactate	405 $\pm$ 26 (12)	450 $\pm$ 32 (11)	+ 11.1	< 0.05
Succinate	457 $\pm$ 30 (6)	574 $\pm$ 33 (6)	+ 25.6	< 0.05
Citrate	482 $\pm$ 34 (6)	441 $\pm$ 32 (6)	- 8.5	N.S.
L-Aspartate	429 $\pm$ 16 (6)	492 $\pm$ 16 (6)	+ 14.7	< 0.05
L-Glutamate	430 $\pm$ 12 (6)	509 $\pm$ 23 (6)	+ 18.4	< 0.05
L-Alanine	404 $\pm$ 13 (6)	394 $\pm$ 27 (6)	- 2.4	N.S.
L-Serine	447 $\pm$ 20 (9)	472 $\pm$ 12 (9)	+ 5.6	N.S.
Glycine	400 $\pm$ 14 (6)	421 $\pm$ 21 (6)	+ 5.3	N.S.
L-Proline	408 $\pm$ 8 (9)	449 $\pm$ 9 (9)	+ 10.0	< 0.05
L-Cysteine	437 $\pm$ 34 (6)	490 $\pm$ 39 (6)	+ 12.1	< 0.01
DL-Histidine	377 $\pm$ 13 (6)	394 $\pm$ 16 (6)	+ 4.5	N.S.
L-Arginine	517 $\pm$ 98 (6)	496 $\pm$ 73 (6)	- 4.0	N.S.
L-Leucine	369 $\pm$ 17 (8)	398 $\pm$ 27 (8)	+ 7.8	N.S.
DL-Valine	394 $\pm$ 57 (5)	451 $\pm$ 45 (5)	+ 14.5	< 0.01

liver slices. In the kidney glutamate, aspartate, cysteine, valine, proline, succinate and lactate increased the carbohydrate formation significantly when the results were statistically analysed by paired comparison, whereas histidine, serine, glycine, alanine, leucine, arginine and citrate did not have any significant effect (Table 4).

When liver slices were incubated under similar conditions with these substrates an increase in carbohydrate formation was observed only with glutamate. The value increased from $6334 \pm 835 \mu\text{g./100mg. wet wt.}$ to $6876 \pm 939 \mu\text{g./100mg. wet wt.}$ with a P value of 0.02 for paired comparison.

DISCUSSION

The carbohydrate content of the kidney of *Uromastix* is about ten times that of the normal rat (Krebs *et al.* 1963; Marsh & Miller, 1953) and the glycogen content is greater than that reported for severely diabetic rat kidney (Teng, 1959). The distribution of glucose and glycogen is also different. Krebs *et al.* (1963) reported values of 0.01% (wt./wet wt.) of glycogen and 0.05% (wt./wet wt.) of free glucose in the rat kidney, but we found 0.3% (wt./wet wt.) of glycogen and 0.2% (wt./wet wt.) of glucose in this lizard. This accounts for all of the total carbohydrate of the kidney, which is 0.5% (wt./wet wt.). Glucose and glycogen are thus the two major carbohydrates in this tissue.

The average liver carbohydrate of *Uromastix* was found to be about 6.5% (wt./wet wt.). These studies were conducted primarily during the hibernation period, when the animal does not ingest food. When these animals come out of hibernation the content was found to be 2.85% (wt./wet wt.). Maintenance of liver glycogen with a fall during arousal from hibernation has been reported in hamsters (Lyman & Chatfield, 1955). Normal rat liver contains about 4% of glycogen (Weber & Cantero, 1954; Long, 1961), which can rise to 10% after glucose infusion (Froesch, Ashmore & Renold, 1958) or after feeding with certain diets (Tepperman & Tepperman, 1958). In liver slices of this lizard none of the substrates except glutamate stimulated gluconeogenesis in detectable amounts. Even after preincubation of the tissue for 30 min. to remove mobilizable carbohydrate, a considerable amount of carbohydrate still remains in the tissue. This makes it difficult to measure a slight change; in this respect liver tissue is not a suitable system to study gluconeogenesis (see also Krebs, 1964).

In kidney slices succinate and lactate increased gluconeogenesis as expected, whereas citrate surprisingly did not. It is possible that the citrate added to the incubation medium instead of entering the tricarboxylic acid cycle was metabolized by an

alternative path. It has recently been demonstrated that citrate can be incorporated into fatty acids (Kornacker & Lowenstein, 1963; Spencer & Lowenstein, 1962). Moreover, citrate also stimulates fatty acid synthesis by activating acetyl-CoA carboxylase (Martin & Vagelos, 1962). The syntheses of fat and carbohydrate are generally regarded as alternative metabolic pathways and usually do not occur concurrently. Therefore if fatty acid synthesis were stimulated gluconeogenesis might be suppressed. This could explain the result observed with citrate in these experiments.

Although all the amino acids tested in this system except leucine are classified as gluconeogenic (Fruton & Simmonds, 1958), only valine, cysteine, proline, aspartate and glutamate were effective in kidney slices. It is surprising that alanine, serine and glycine did not stimulate gluconeogenesis. Apparently these amino acids are not entering into the pathway followed by lactate through pyruvate to glucose. Similarly glutamate and proline, which generally proceed through α -oxoglutarate, increased carbohydrate formation, whereas histidine and arginine, which are also known to go through the same pathway, did not have any effect. Krebs & Yoshida (1963) studied gluconeogenesis in kidney slices of a number of mammalian and two avian systems after the addition of various substrates. Although they reported considerable differences in the rates of gluconeogenesis in different species there were no qualitative differences. The observed qualitative differences in this lizard in comparison with mammalian and avian systems suggest that the gluconeogenic amino acids as well as the metabolic pathways of individual amino acids may be different in this lizard, a poikilotherm, from those of the homeothermic mammalian and avian systems.

REFERENCES

- Deutsch, W. (1936). *J. Physiol.* **87**, 57P.
- Fales, F. W. (1951). *J. biol. Chem.* **193**, 113.
- Froesch, E. R., Ashmore, J. & Renold, A. E. (1958). *Endocrinology*, **62**, 614.
- Fruton, J. S. & Simmonds, S. (1958). *General Biochemistry*, 2nd ed., p. 764. New York: John Wiley and Sons Inc.
- Hill, A. B. (1961). *Principles of Medical Statistics*, 7th ed., p. 150. New York: Oxford University Press.
- Kornacker, J. & Lowenstein, J. M. (1963). *Biochem. J.* **89**, 278.
- Krebs, H. A. (1964). *Proc. Roy. Soc. B*, **159**, 545.
- Krebs, H. A., Bennett, D. A. H., de Gasquet, P., Gascoyne, T. & Yoshida, T. (1963). *Biochem. J.* **86**, 22.
- Krebs, H. A. & Yoshida, T. (1963). *Biochem. J.* **89**, 398.
- Long, C. (1961). *Biochemists' Handbook*, p. 677. Princeton, N.J.: D. Van Nostrand Co. Inc.

- Lyman, C. P. & Chatfield, P. O. (1955). *Physiol. Rev.* **35**, 403.
- Maher, M. J. (1964). *Endocrinology*, **74**, 994.
- Marsh, J. B. & Miller, K. L. (1953). *Science*, **118**, 416.
- Martin, D. B. & Vagelos, P. R. (1962). *J. biol. Chem.* **237**, 1787.
- Renold, A. E., Hastings, A. B., Nesbitt, F. B. & Ashmore, J. (1955). *J. biol. Chem.* **213**, 135.
- Renold, A. E., Teng, C. T., Nesbitt, F. B. & Hastings, A. B. (1953). *J. biol. Chem.* **204**, 553.
- Spencer, A. F. & Lowenstein, J. M. (1962). *J. biol. Chem.* **237**, 3640.
- Teng, C. T. (1959). *Arch. Biochem. Biophys.* **48**, 415.
- Tepperman, H. M. & Tepperman, J. (1958). *Diabetes*, **7**, 478.
- Weber, G. & Cantero, A. (1954). *Science*, **120**, 851.