Renal Gluconeogenesis

THE EFFECT OF DIET ON THE GLUCONEOGENIC CAPACITY OF RAT-KIDNEY-CORTEX SLICES

By H. A. KREBS, D. A. H. BENNETT, P. DE GASQUET, T. GASCOYNE AND T. YOSHIDA
*Medical Research Council Unit for Research in Cell Metabolism, Department of Biochemistry,
 University of Oxford*

(Received 16 July 1962)

Gluconeogenesis is known to occur not only in the liver but also in kidney cortex. Renal gluconeogenesis from lactate, pyruvate, amino acids and intermediates of the tricarboxylic acid cycle was first observed in tissue slices by Benoy & Elliott (1937) and later confirmed by Weil-Malherbe (1938), Russell & Wilhelmi (1941), Barron, Lyman, Lipton & Goldinger (1941), Shipley (1944), and Teng (1954*a, b*). Evidence of gluconeogenesis in the kidney *in situ* was obtained on eviscerated animals by Bergman & Drury (1938), Reinecke (1943), Reinecke & Roberts (1944), Roberts & Samuels (1944), Drury, Wick & MacKay (1950) and McCann & Jude (1958).

As an experimental material for the study of gluconeogenesis, the kidney offers major advantages over the liver. The content of preformed carbohydrate is much lower in kidney than in the liver. Liver of well-fed rats may contain more than 10% (wt./wet wt.) of glycogen and more than 0.5% (wt./wet wt.) of glucose. The corresponding values for rat kidney are 0.01% of glycogen and 0.05% of glucose (Marsh & Miller, 1953; and our own measurements). Thus it is difficult to measure in the liver the net carbohydrate increases which occur after incubation for 1 or 2 hr. with precursors even when the preformed store of carbohydrate has been reduced by starvation. By contrast the increment of carbohydrate in kidney on incubation is relatively large.

The end product of gluconeogenesis in kidney is glucose rather than glycogen because the quantities of glycogen which can be stored in the kidney are very small, as shown by the observations of Froesch, Ashmore & Renold (1958). In kidney the glycogen content of normal rats rose from 0.48 mg./g. wet wt. to 1.0–1.5 mg./g. wet wt. after continued glucose infusion into the blood stream. Under the same conditions the liver glycogen value rose from 22 mg./g. wet wt. to 106–137 mg./g. Per unit of weight, liver thus stored about 100 times as much glycogen as kidney.

The present paper is concerned with measurements of the rates of gluconeogenesis from various precursors and the effects of the diet of rats and the

composition of the medium on the rates. Slices were used as the standard material because other tissue preparations failed to give high rates, for reasons which became obvious during the investigations.

EXPERIMENTAL

Treatment of tissue

Slices were cut free-hand by the method of Deutsch (1935). Usually a wet razor-blade was used and the slices were gently shaken for a few minutes in a saline medium (low in bicarbonate) at room temperature. This led to a loss of most of the preformed glucose. In some experiments where the initial glucose and glycogen contents were to be measured, slices were cut with a dry razor and the slices were weighed on a torsion balance. Incubation was at 40° in the saline medium of Krebs & Henseleit (1932) with O₂ + CO₂ (95:5) as the gas phase. Usually not more than 10 mg. of tissue (dry wt.) was suspended in 4 ml. of the medium and shaken for 1 hr. in a conical Warburg vessel. Modifications in the composition of the medium which were made to test the importance of individual ions are stated in the description of the experiments. At the end of the incubation the slices were removed and weighed after drying at 110°. The glucose in the solution was determined by the glucose-oxidase method. Preliminary experiments indicated that the amounts of glucose or glycogen in the tissue were usually negligible compared with the glucose content of the medium, and that glycogen was not discharged into the medium. Many tests were carried out in duplicate.

Analytical methods

Assay of glucose. Glucose was determined by the glucose-oxidase method (Keilin & Hartree, 1945, 1948) because of its high degree of specificity. The procedure adopted after many trials was based on those described by Huggett & Nixon (1957) and Jakobsen (1960). The methods of Miller & Van Slyke (1936) and Kingsley & Reinhold (1949) gave 20–50% higher values.

The reagents required were: 3N-HClO₄, prepared by diluting the commercial '60%' solution (which contains 923 g. of HClO₄/l.) with 2 vol. of water; 0.5M-sodium phosphate buffer, pH 7.0, prepared by mixing 4 parts of 0.5M-Na₂HPO₄ and 1 part of 0.5M-NaH₂PO₄; 1% (w/v) o-dianisidine in 95% (v/v) ethanol (which keeps for a few weeks in the dark); glucose-oxidase reagent prepared daily from 125 mg. of glucose oxidase (crude) (from Sigma Chemical Co.), 4 mg. of peroxidase (pure) (from C. F.

Boehringer und Soehne G.m.b.H.), 100 ml. of phosphate buffer and 0.5 ml. of *o*-dianisidine solution. Usually 1 ml. of solution, containing 0.1–0.8 μ mole of glucose, was mixed with 1 ml. of water and 0.2 ml. of 3N-HClO₄. When the glucose concentration of the unknown solution was very low, 2 ml. was used and water was omitted. After not less than 5 min. the mixture was centrifuged and 1 ml. of the supernatant was mixed in a 10 ml. stoppered tube with 2.5 ml. of the glucose-oxidase reagent, and incubated for 1 hr. at 37°. The extinction was then measured in glass cuvettes at 440 μ m. A standard curve was made for each set of determinations by replacing the unknown solution with 1, 1.5 and 2.0 ml. of a glucose stock solution containing 1 mg./ml. These stock solutions were diluted to 50 ml.; 2 ml. of the diluted solutions were mixed with 0.2 ml. of the 3N-HClO₄ solution and 1 ml. of this mixture was treated in the same way as 1 ml. of supernatant. Tests showed that the inorganic constituents of the saline medium did not affect the standard curve (see Salomon & Johnson, 1959).

A special feature of this procedure compared with others is the use of HClO₄ as a deproteinizing agent and the non-removal of the excess of the acid. Allowance is made in the buffer used in the enzyme reagent for the neutralization of the HClO₄.

Assay of glycogen. Glycogen was determined as glucose after enzymic hydrolysis by a commercial enzyme preparation (Diazyme, of Takamine Products, Miles Chemical Co., Clifton, N.J.) prepared from *Aspergillus* (see Pazur & Ando, 1959, 1960). The enzyme produces glucose from glycogen, starch, dextrins, maltose and intermediate oligosaccharides. It hydrolyses α -(1 $\rightarrow$ 6)-linkages as well as α -(1 $\rightarrow$ 4)-linkages but is stated to be low in transglucosidase activity. It contains traces of invertase. Glycogen was isolated by the method of Good, Kramer & Somogyi (1933) and dissolved in water. In the present work the required value was in many cases total carbohydrate rather than glycogen and in these cases glycogen was not isolated. According to the manufacturers, 1 unit of Diazyme forms from starch under standard conditions 1 g. of glucose/hr., and 1 g. of Diazyme is adjusted to contain 160 units. The enzyme can thus form 160 times its own weight of glucose/hr.

The pH of the sample to be analysed—either a solution or a homogenate or slices suspended in an aqueous medium—was roughly adjusted to 4.4 with N-HCl or N-NaOH, and for accurate adjustment sufficient M-acetate buffer, pH 4.4 (2 parts of acetic acid and 1 part sodium acetate), was added to bring the final buffer concentration to 0.05M. The amount of Diazyme used was about 0.2 mg./100 μ g. of glycogen; it was added in the form of an aq. 0.1% solution. An excess of Diazyme is undesirable because the enzyme preparation contains small quantities of apparent glucose or glucose precursors. The mixture was incubated for 90 min. at 50°. It was then cooled and protein was precipitated by the addition of 0.1 vol. of 3N-HClO₄. This mixture was further diluted with 0.27N-HClO₄ to obtain a solution containing between 10 and 40 μ g. of glucose/ml. Glucose was determined in 1 ml. of this material as described above. A control determined the blank glucose value.

The analysis of various samples of glycogen supplied by British Drug Houses Ltd., and of samples of *Ascaris* glycogen and of the liver of a case of 'glycogen storage disease', gave a yield of 90–95% which was not increased by repre-

cipitation of the glycogen with ethanol. Somewhat incomplete yields of the same order of the enzymic degradation of glycogen by enzyme mixtures have also been observed by Gunja, Manners & Khin Maung (1961).

Assay of L-lactic acid. L-Lactic acid was determined by an enzymic method based on that described by Lehmann (1938) and on information obtained from S. R. Elsdén (personal communication). The main modification is the replacement of ferricyanide as hydrogen acceptor by 2,6-dichlorophenol-indophenol, which increases the sensitivity about 10 times.

Lactate dehydrogenase was prepared from baker's yeast as described by Boeri, Cutolo, Luzzati & Tosi (1955). This gave an enzyme solution in neutral phosphate buffer containing EDTA (1 mM). Other reagents required are: 0.1M-sodium phosphate buffer, pH 7.4; 10 mM-HCN (65 mg. of KCN dissolved in 1 ml. of N-HCl and 10 ml. of 0.1M-sodium phosphate buffer, pH 7.4, and diluted with water to 100 ml.); 0.01% 2,6-dichlorophenol-indophenol, freshly diluted from a 0.1% stock solution.

Deproteinization was carried out as described for the glucose determination. In 1 cm. cuvettes were placed 1 ml. of deproteinized filtrate containing between 0.02 and 0.1 μ mole of lactic acid, 0.5 ml. of phosphate buffer, 0.4 ml. of HCN solution, 0.5 ml. of dichlorophenol-indophenol, and water to 2.8 ml. After these solutions had been mixed, 0.2 ml. of lactate dehydrogenase solution was added and readings were started. When the enzyme activity was low, up to 0.5 ml. was used, in which case the amount of water was decreased to keep the total volume to 3 ml. Readings were taken every 2 min. at 600 μ m against a blank in which the test solution was replaced by water until the rates in test and blank were the same. An extinction change (corrected for the blank) of 0.530 corresponds to 0.1 μ mole of lactate. The reaction was usually completed within 10–20 min., depending on the amount of lactate and the activity of the enzyme. The blank usually moved about 0.1 in 20 min.

Experimental animals

Wistar rats were used. Their standard food consisted of commercially available rat cubes [H. C. Styles (Bewdley) Ltd.] prepared according to the formula of Bruce & Parkes (1949). This diet contains approximately 16% of crude protein, 5% of crude fibre, 3% of fat, 2.5% of minerals and 1% of vitamin supplements. The remainder is mainly carbohydrate derived from wholemeal flour and ground oats. When it was noted during the investigation that the carbohydrate content of the diet affects the gluconeogenic capacity of the tissues, various low-carbohydrate diets were used for limited periods, usually not exceeding 5 days. They consisted of casein (3 parts) and margarine (1 part) plus vitamin and mineral supplements, or casein alone with vitamin and mineral supplements, or cooked sheep heart.

RESULTS

Time-course of carbohydrate synthesis. Rat-kidney-cortex slices, shaken in the presence of L-lactate, formed glucose steadily for about 2 hr. During the third hour the rate decreased (Table 1). The determination of glycogen in parallel experiments showed that the glycogen content of the

tissue fell slightly during the incubation but that this fall accounted for less than 5 % of the glucose formed.

Effect of the ionic composition of the medium on the formation of glucose from lactate. The highest rates of glucose synthesis were obtained when the medium of Krebs & Henseleit (1932) was used. Saline media which Buchanan, Hastings & Nesbett (1949*a, b*) and Hastings, Teng, Nesbett & Sinex (1952) found optimum for glycogen synthesis in liver proved inferior to the Krebs-Henseleit medium with kidney. The various media used by Teng (1954*a, b*) for kidney slices were also some-

what inferior. The following variations of the medium only slightly modified the rate of gluconeogenesis from lactate: omission of phosphate or addition of sodium phosphate buffer (0.1 M, pH 7.4) up to 8 mM final concentration; omission of Mg^{2+} ions; omission of bicarbonate from the medium and of carbon dioxide from the gas mixture and absorbing the respiratory carbon dioxide by 2N-sodium hydroxide in the centre well. On the other hand omission of Ca^{2+} ions reduced the rate of glucose formation from lactate (and fumarate) 60–70 % (Table 2); a Ca^{2+} ion concentration of 2 mM (the same as in blood serum) proved to be the optimum. Phosphate buffer at concentrations above 0.01 M was inhibitory, probably because of the precipitation of Ca^{2+} ions.

Effect of diet. Replacement of the standard diet which contains about 73 % of carbohydrate by a diet consisting of 3 parts of casein and 1 part of margarine, and containing only traces of carbohydrate, raised the rates of gluconeogenesis from various precursors substantially (Table 3). The increases were especially pronounced with L-lactate, pyruvate, α -oxoglutarate, L-glutamate, L-proline, fumarate and L-ornithine, and in the tissue not supplemented by a substrate. In these

Table 1. *Time-course of the formation of glucose from lactate in rat-kidney-cortex slices*

Experimental details are given in the text. About 10 mg. (dry wt.) was incubated with 0.01 M-L-lactate at 40°.

Time of incubation (min.)	Glucose found (μ moles/g. dry wt./hr.)
0	5.9
30	66
60	124
120	250
180	332

Table 2. *Effect of calcium ions on the rate of gluconeogenesis in rat-kidney-cortex slices*

Experimental details are given in the text. Slices were incubated for 60 min. in (a) Krebs-Henseleit saline or (b) the same medium without $CaCl_2$. The substrate concentration was 0.01 M. The results are given as means $\pm$ s.e.m. with the numbers of observations in parentheses.

Substrate	Glucose formed (μ moles/g. dry wt./hr.)		Inhibition due to absence of $CaCl_2$ (%)
	Standard saline	$CaCl_2$ -free saline	
L-Lactate	117 $\pm$ 8.4 (5)	38 $\pm$ 4.5 (5)	69
Fumarate	226 $\pm$ 33 (4)	58 $\pm$ 2.1 (4)	74

Table 3. *Effect of diet on the formation of glucose from various substrates in rat-kidney-cortex slices of young rats*

Experimental details are given in the text. Young rats, 3–4 months old and weighing 110–250 g., were used. The low-carbohydrate diet was given for 3–5 days. Slices were incubated for 1 hr. The substrate concentration was 0.01 M. The results are given as means $\pm$ s.e.m. with the numbers of observations in parentheses.

Substrate added	Standard mixed diet	Low-carbohydrate diet (casein-margarine)	Starvation for 48 hr.
None	12.6 $\pm$ 1.46 (14)	22.8 $\pm$ 1.0 (15)	25 $\pm$ 2 (6)
L-Lactate	110 $\pm$ 3.1 (24)	200 $\pm$ 4.8 (14)	200 $\pm$ 17 (6)
Pyruvate	225 $\pm$ 14.4 (13)	450 $\pm$ 23.4 (8)	342 $\pm$ 29 (6)
Fumarate	208 $\pm$ 16.6 (13)	361 $\pm$ 18.9 (11)	332 $\pm$ 20 (6)
Succinate	310 $\pm$ 18.2 (5)	433 $\pm$ 18.3 (5)	—
Glycerol	151 $\pm$ 9.7 (4)	186 $\pm$ 10.1 (5)	—
α -Glycerophosphate	113 $\pm$ 4.8 (4)	127 $\pm$ 3.2 (5)	—
Oxaloacetate	164 $\pm$ 14.8 (4)	248 $\pm$ 13.0 (6)	—
α -Oxoglutarate	220 $\pm$ 20.2 (7)	425 $\pm$ 17.2 (5)	—
L-Aspartate	90 $\pm$ 13.1 (4)	112 $\pm$ 11.2 (7)	—
L-Glutamate	157 $\pm$ 30.2 (6)	263 $\pm$ 12.7 (12)	202 $\pm$ 19 (6)
L-Proline	62 $\pm$ 2.1 (5)	146 $\pm$ 8.4 (5)	87 $\pm$ 11 (6)
L-Arginine	23 $\pm$ 1.8 (5)	37 $\pm$ 2.0 (5)	37 $\pm$ 2 (6)
L-Ornithine	36 $\pm$ 11.0 (4)	66 $\pm$ 4.0 (5)	46 $\pm$ 3 (6)

cases the increase was about 100 %. The effect of the diet was noticeable after 1 day and reached the maximum after 3 days. The results in Table 3 refer to young rats weighing 110–250 g. Older rats aged 12–15 months and weighing 400–500 g. gave similar values (Table 4).

Diets consisting of casein only or of cooked sheep heart caused the same effect as the casein-margarine mixture.

Starvation for 48 hr. also increased the rate of gluconeogenesis but substantially less so than the feeding of a casein-margarine diet, except when lactate or proline was the precursor (see Table 3).

Metabolism of glycogen in kidney cortex. As glycogen, unlike glucose, may be expected to remain within the slice, the measurement of glycogen metabolism requires an analysis of the slices. In order to determine both glycogen and glucose changes the slices, cut with a dry razor and weighed on a torsion balance, were analysed either directly or after incubation. In the latter case the slices were freed from the medium on filter paper before they

were placed in the potassium hydroxide solution as described by Good *et al.* (1933) for the glycogen determination, or into perchloric acid solution for the glucose determination. Results of an experiment in which lactate and fumarate served as substrates is shown in Table 5. Glycogen was formed in small quantities in the control to which no substrate was added as well as in the presence of lactate or fumarate. The absolute amounts of glycogen formed were almost independent of the substrate, so that glucose was virtually the only carbohydrate formed from the added substrate. The result is in agreement with previous findings (see Landau, 1960; Marsh & Miller, 1953).

DISCUSSION

General comment. The main object of the experiments reported in this paper was to establish kidney cortex as a favourable material for the study of gluconeogenesis *in vitro* and to find the experimental conditions under which the process is optimum. Some advantages of kidney have already been summarized in the introduction. Others are the stability of the tissue *in vitro* over several hours, the high rate of metabolic activity, and the greater permeability to the anions of the di- and tri-carboxylic acids.

The rates of glucose formation reported in this paper are considerably higher than those recorded by others (see Table 6). The values of Russell & Wilhelmi (1941) are the only ones which approach the present rates for rats fed on a standard diet (Tables 3 and 4). Those of Benoy & Elliott (1937) are about 50 % of the present values and those of Teng (1954*a, b*) and Landau (1960) only 15–20 %. The reason for the low values found by previous investigators is in all probability the inadequate oxygenation of the tissue. Teng used 400–700 mg. wet wt. in 3–4 ml. of medium and Landau 500–800 mg. in 8 ml. of medium. We used 30–50 mg. in 4 ml., after noting that the rates of gluconeogenesis fell when more tissue was used. In view of the high rate of respiration in the presence of gluco-

Table 4. *Effect of diet on the formation of glucose from various substrates in rat-kidney-cortex slices of older rats*

Substrate added	Standard mixed diet	Low-carbohydrate diet (casein-margarine)
None	11.2 ± 1.23 (6)	24.6 ± 4.2 (5)
L-Lactate	97 ± 10.1 (8)	181 ± 23.9 (5)
Pyruvate	250 ± 26.3 (4)	404 ± 50.4 (5)
Fumarate	273 ± 16.9 (6)	355 ± 21.5 (8)
Succinate	286 ± 18.6 (6)	293 ± 38.2 (5)
Glycerol	97 ± 9.8 (3)	140 ± 20.5 (3)
Oxaloacetate	155 ± 11.9 (3)	203 ± 17.3 (3)
α-Oxoglutarate	248 ± 20.4 (6)	340 ± 20.7 (5)
L-Aspartate	33 ± 4.3 (6)	73 ± 5.8 (5)
L-Glutamate	137 ± 10.0 (7)	269 ± 16.9 (8)

Table 5. *Formation of glucose and glycogen in rat-kidney-cortex slices*

Experimental details are given in the text. The rat was fed on the standard diet. The substrate concentration was 0.01M. Incubation was for 1 hr. Glycogen is expressed as glucose.

Substrate added	...	...	...	...	None	L-Lactate	Fumarate
Initial value (μmoles/g. dry wt./hr.)							
Glucose in slices					13.5	13.5	13.5
Glycogen in slices					1.2	1.2	1.2
Final value (μmoles/g. dry wt./hr.)							
Glucose in medium					33	104	151
Glycogen in slices					4.5	6.1	5.4
Change on incubation (μmoles/g. dry wt./hr.)							
Glucose					+19.5	+90.5	+137.5
Glycogen					+3.3	+4.9	+4.2

Table 6. *Rates of gluconeogenesis in rat-kidney-cortex slices observed by previous authors*

The values quoted are representative. Where the original papers mentioned wet weight only the values obtained were multiplied by 4.5. The results refer to normal rats fed on standard diets. The slices were suspended in a saline medium at body temperature.

Author	Substrate	Glucose formed (μ moles/g. dry wt./hr.)
Benoy & Elliott (1937)	DL-Lactate	50
	Pyruvate	160
	Succinate	70
Russell & Wilhelmi (1941)	Pyruvate	160
	L-Glutamate	220
	Succinate	160
Teng (1954 <i>a, b</i>)	Pyruvate	30
	Glycerol	33
	Pyruvate	35
Landau (1960)	Pyruvate	35

neogenic substrate (about 2000 μ moles/g. dry wt./hr.) this fall is not unexpected. The thinness of the slices is also of importance if the rate of oxygen supply is not to be a factor limiting the rate of gluconeogenesis.

The rates of glucose formation from lactate and pyruvate and some of the amino acids are comparable with those of glycolysis of many tissues. Brain, intestinal mucosa, testis, pancreas, salivary gland and diaphragm can glycolyse up to 100–500 μ moles of glucose/g. dry wt./hr. whereas kidney cortex can synthesize 450 μ moles of glucose from pyruvate and 200 μ moles from lactate in animals fed on a low-carbohydrate diet. It is noteworthy that the two tissues possessing the gluconeogenic enzyme systems—liver and kidney cortex (Benoy & Elliott, 1937)—have very low rates of glycolysis. In kidney cortex the rate of gluconeogenesis from lactate or glutamate can be 3–4 times greater than that of glycolysis.

Role of calcium ions. The fact that Ca^{2+} ions are necessary for maximal rates of gluconeogenesis may explain why attempts to obtain gluconeogenesis in tissue homogenates have been essentially unsuccessful. Such gluconeogenesis as has been observed in homogenates (Dierks, 1957) represents only a fraction of the original capacity of the tissue. Gluconeogenesis depends on a supply of ATP, and the generation of ATP in homogenates is inhibited by the concentration of Ca^{2+} ions needed for maximum gluconeogenesis. Thus 'compartmentation', keeping enzymes dependent on Ca^{2+} ions and those requiring them apart, must be essential. It remains to be investigated which stage of gluconeogenesis requires the presence of Ca^{2+} ions.

Effect of diet. The response of the kidney to a low-carbohydrate diet is an adaptive reaction, adjusting the metabolism to the needs of the

organism. That some enzymes involved in gluconeogenesis, e.g. glucose 6-phosphatase, increase their activity on a low-carbohydrate diet has been demonstrated by Freedland & Harper (1957, 1958) and by Mokrasch, Davidson & McGilvery (1956), who showed an increased activity of fructose 1,6-diphosphatase after giving a low-carbohydrate diet or treatment with cortisone (see also Landau, Hastings & Zottu, 1958). Long (1940) first showed that the gluconeogenic capacity of the body is under the control of the hormones of the adrenal cortex. Weber & Macdonald (1961) have demonstrated the role of the diet and hormones in the control of the level of glucose 6-phosphatase, phosphohexose-isomerase and phosphoglucomutase activities.

Since Knox (1951) first demonstrated the adaptive formation of liver enzymes in response to the feeding of tryptophan, many cases of adaptation and enzyme induction have become known in animal tissues (e.g. Ashmore, Hastings, Nesbitt & Renold, 1956; Schimke, 1962). Gluconeogenesis, involving at least 11 enzymes when lactate is the starting material and at least 17 when proline is the precursor, may prove a useful system for studying the mechanism of, and the role of hormones in, adaptation, the 'co-ordinate repression' of enzymes (Ames & Garry, 1959) and the identification of pacemaker steps (see also Monod & Jacob, 1961).

Gluconeogenesis from amino acids. In rat-kidney cortex L-glutamate, L-aspartate, L-proline, L-ornithine and L-arginine are the only L-amino acids that show rapid rates of gluconeogenesis (Tables 3 and 4; see also Russell & Wilhelmi, 1941; Hanson, Lindsay & Barker, 1961). Other amino acids known to be gluconeogenic in the whole organism yielded little or no glucose in rat-kidney cortex.

SUMMARY

1. The optimum conditions for the conversion of lactate, pyruvate, intermediates of the tricarboxylic acid cycle and amino acids into glucose in rat-kidney-cortex slices have been investigated. The balanced saline medium of Krebs & Henseleit (1932) was found to give maximal values. The rates of gluconeogenesis were much higher than those observed by previous investigators. Pyruvate yielded up to 450 μ moles of glucose/g. dry wt./hr.

2. Reduction of the concentrations of bicarbonate, carbon dioxide or Mg^{2+} ions, or increasing the concentration of phosphate to 8 mM, had no major effects on the rates of gluconeogenesis from lactate or fumarate, but omission of Ca^{2+} ions reduced the rate by about 70 %.

3. By keeping rats for 3–5 days on a diet low in carbohydrate the capacity of the tissue to syn-

thesize glucose from various precursors was almost doubled.

4. Starvation (for 48 hr.) also increased the gluconeogenic capacity, but not as much as the low-carbohydrate diet.

5. Of the gluconeogenic amino acids only glutamate, aspartate, proline, ornithine and arginine yielded glucose rapidly.

6. An enzymic method for the determination of glycogen is described.

7. Several advantages of kidney cortex over liver as a material for the study of gluconeogenesis are pointed out; a major one is the low content of pre-formed carbohydrate.

This work was aided by grants from the Rockefeller Foundation and from the National Institutes of Health, United States Public Health Service.

REFERENCES

- Ames, B. N. & Garry, B. (1959). *Proc. nat. Acad. Sci., Wash.*, **45**, 1453.
- Ashmore, J., Hastings, A. B., Nesbitt, F. B. & Renold, A. E. (1956). *J. biol. Chem.* **218**, 77.
- Barron, E. S. G., Lyman, C. M., Lipton, M. A. & Goldinger, J. M. (1941). *J. biol. Chem.* **141**, 957.
- Benoy, M. P. & Elliott, K. A. C. (1937). *Biochem. J.* **31**, 1268.
- Bergman, H. & Drury, D. R. (1938). *Amer. J. Physiol.* **124**, 279.
- Boeri, E., Cutolo, E., Luzzati, M. & Tosi, L. (1955). *Arch. Biochem. Biophys.* **56**, 487.
- Bruce, H. M. & Parkes, A. J. (1949). *J. Hyg., Camb.*, **47**, 202.
- Buchanan, J. M., Hastings, A. B. & Nesbitt, F. B. (1949a). *J. biol. Chem.* **180**, 435.
- Buchanan, J. M., Hastings, A. B. & Nesbitt, F. B. (1949b). *J. biol. Chem.* **180**, 447.
- Deutsch, W. (1935). *J. Physiol.* **87**, 56r.
- Dierks, C. (1957). D.Phil. Thesis: University of Oxford.
- Drury, D. R., Wick, A. N. & MacKay, E. M. (1950). *Amer. J. Physiol.* **163**, 655.
- Freedland, R. A. & Harper, A. E. (1957). *J. biol. Chem.* **228**, 743.
- Freedland, R. A. & Harper, A. E. (1958). *J. biol. Chem.* **230**, 833.
- Froesch, E. R., Ashmore, J. & Renold, A. E. (1958). *Endocrinology*, **62**, 614.
- Good, C. A., Kramer, H. & Somogyi, M. (1933). *J. biol. Chem.* **100**, 485.
- Gunja, Z. H., Mannars, D. H. & Khin Maung (1961). *Biochem. J.* **81**, 392.
- Hanson, R. W., Lindsay, R. H. & Barker, S. B. (1961). *Endocrinology*, **69**, 883.
- Hastings, A. B., Teng, C. T., Nesbitt, F. B. & Sinex, F. M. (1952). *J. biol. Chem.* **194**, 69.
- Huggett, A. St G. & Nixon, D. A. (1957). *Lancet*, ii, 368.
- Jakobsen, L. K. (1960). *Scand. J. clin. Lab. Invest.* **12**, 76.
- Keilin, D. & Hartree, E. F. (1945). *Biochem. J.* **39**, 293.
- Keilin, D. & Hartree, E. F. (1948). *Biochem. J.* **42**, 230.
- Kingsley, G. R. & Reinhold, J. G. (1949). *J. Lab. clin. Med.* **34**, 713.
- Knox, W. E. (1951). *Brit. J. exp. Path.* **32**, 462.
- Krebs, H. A. & Henseleit, K. (1932). *Hoppe-Seyl. Z.* **210**, 33.
- Landau, B. R. (1960). *Endocrinology*, **67**, 744.
- Landau, B. R., Hastings, A. B. & Zottu, S. (1958). *J. biol. Chem.* **233**, 1257.
- Lehmann, J. (1938). *Scand. Arch. Physiol.* **80**, 237.
- Long, C. N. H. (1940). *Endocrinology*, **26**, 309.
- McCann, W. P. & Jude, J. R. (1958). *Bull. Johns Hopk. Hosp.* **103**, 77.
- Marsh, J. B. & Miller, K. L. (1953). *Science*, **118**, 416.
- Miller, B. F. & Van Slyke, D. D. (1936). *J. biol. Chem.* **114**, 583.
- Mokrasch, L. C., Davidson, W. D. & McGilvery, R. W. (1956). *J. biol. Chem.* **222**, 179.
- Monod, J. & Jacob, F. (1961). *Cold Spr. Harb. Symp. quant. Biol.* **26**, 389.
- Pazur, J. H. & Ando, T. (1959). *J. biol. Chem.* **234**, 1966.
- Pazur, J. H. & Ando, T. (1960). *J. biol. Chem.* **235**, 297.
- Reinecke, R. M. (1943). *Amer. J. Physiol.* **140**, 276.
- Reinecke, R. M. & Roberts, S. (1944). *Amer. J. Physiol.* **141**, 476.
- Roberts, S. & Samuels, L. T. (1944). *Amer. J. Physiol.* **142**, 240.
- Russell, J. A. & Wilhelmi, A. E. (1941). *J. biol. Chem.* **140**, 747.
- Salomon, L. L. & Johnson, J. E. (1959). *Analyt. Chem.* **31**, 453.
- Schimke, R. T. (1962). *J. biol. Chem.* **237**, 459.
- Shiple, R. A. (1944). *Amer. J. Physiol.* **141**, 662.
- Teng, C. T. (1954a). *Arch. Biochem. Biophys.* **48**, 409.
- Teng, C. T. (1954b). *Arch. Biochem. Biophys.* **48**, 415.
- Weber, G. & Macdonald, H. (1961). *Exp. Cell Res.* **22**, 292.
- Weil-Malherbe, H. (1938). *Biochem. J.* **32**, 2276.