Formation of Glucose from Hexoses, Pentoses, Polyols and Related Substances in Kidney Cortex

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1. Slices of rat kidney cortex, on incubation in a saline medium, formed D-glucose from the following substances: D-fructose, D-galactose, D-mannose, L-sorbose, L-arabinose, D-xylose, glycerol, *myo*-inositol, L-iditol, sorbitol, xylitol, ribitol, methylglyoxal, dihydroxyacetone, L-glyceraldehyde, D-glyceraldehyde, DL-glyceraldehyde, DL-glycerate. Values for the rates of glucose formation from these precursors are given. 2. No glucose was formed from L-rhamnose, D-arabitol, D-arabinose, D-ribose, L-fucose, D-lyxose, mannitol, dulcitol, D-glucuronate, propane-1,2-diol and propan-2-ol. 3. The pathways of glucose formation from the various precursors are discussed (Scheme 1). 4. L-Glyceraldehyde inhibited the formation of glucose from D-glyceraldehyde.

Measurements of the rate of formation of glucose from non-carbohydrate precursors by slices of kidney cortex have been reported previously (Krebs, Bennett, de Gasquet, Gascoyne & Yoshida, 1963; Krebs & Yoshida, 1963). The present paper reports that rat kidney cortex forms glucose also from several hexoses, pentoses, trioses, polyols and related substances. The rates of formation of glucose from these precursors in kidney cortex are of the same order of magnitude as those in liver, an organ that converts various carbohydrates into glucose (Cori & Shine, 1935, 1936; Cori, Ochoa, Slein & Cori, 1951; Mendeloff & Weichselbaum, 1953).

EXPERIMENTAL

The general procedure of measuring the formation of glucose in kidney-cortex slices was as described by Krebs *et al.* (1963). Male Wistar rats weighing 200–300 g. and fed on standard rat cubes were used. The washed slices were incubated for 1 hr. at 40°. The substrate concentration was 10 mM unless otherwise stated. D-Glucose was determined by the glucose-oxidase method, and all substrates were tested for contamination with glucose and whether they reacted with the glucose-oxidase reagents. The samples of mannose, xylose and galactose contained small amounts of glucose (1.5, 0.4 and 0.5% respectively) and corrections were made. Dihydroxyacetone gave a slight colour with the glucose-oxidase reagents for which an allowance was made in the calculation of the results.

RESULTS

Rate of formation of glucose from various precursors in rat kidney cortex. The rates of formation of glucose are shown in Table 1. Fructose formed

glucose more rapidly than any of the other substrates tested. Next in effectiveness and approximately equally active were D-glyceraldehyde and

Table 1. *Glucogenesis from carbohydrates and related substrates in rat kidney-cortex slices*

For experimental details see Krebs *et al.* (1963) and the text. About 10 mg. (dry wt.) of tissue was incubated in a Warburg vessel for 1 hr. at 40° in 4 ml. of the saline medium of Krebs & Henseleit (1932). The gas phase was O₂ + CO₂ (95:5). The substrate concentration was 10 mM. The results are given as means $\pm$ S.E.M. with the number of observations in parentheses.

Substrate added	Glucose formed (μ moles/g. dry wt./hr.)
None	15 $\pm$ 1.6 (8)
L-Lactate	103 $\pm$ 12.0 (5)
D-Fructose	423 $\pm$ 15.2 (4)
D-Galactose	56 $\pm$ 14.8 (4)
D-Mannose	50 $\pm$ 6.4 (5)
L-Sorbose	79 $\pm$ 7.6 (4)
L-Arabinose	24 $\pm$ 2.7 (4)
D-Xylose	40 $\pm$ 4.5 (4)
Glycerol	79 $\pm$ 6.6 (5)
<i>myo</i> -Inositol	41 $\pm$ 2.9 (4)
L-Iditol	25 $\pm$ 3.9 (3)
Sorbitol	80 $\pm$ 7.6 (4)
Xylitol	106 $\pm$ 6.8 (9)
Ribitol	36 $\pm$ 3.5 (4)
Methylglyoxal	33 $\pm$ 3.1 (4)
Dihydroxyacetone	268 $\pm$ 18.4 (4)
L-Glyceraldehyde	77 $\pm$ 7.3 (4)
DL-Glyceraldehyde	153 $\pm$ 5.6 (11)
D-Glyceraldehyde	322 $\pm$ 19.9 (4)
DL-Glycerate	72 $\pm$ 9.8 (4)

dihydroxyacetone. Less reactive, but still giving a relatively high rate, was xylitol, which formed glucose about as rapidly as did lactate. Sorbitol, D-galactose, D-mannose, L-sorbose and DL-glycerate gave lower rates of formation of glucose. Still lower were the rates with L-iditol, *myo*-inositol, L-arabinose, ribitol and methylglyoxal. No significant increases (not recorded in Table 1) were obtained with L-rhamnose, D-arabitol, D-arabinose, D-ribose, L-fucose, D-lyxose, mannitol, dulcitol, D-glucuronate, propane-1,2-diol and propan-2-ol.

Inhibition by L-glyceraldehyde. L-Glyceraldehyde reacted much more slowly than D-glyceraldehyde. As the initial stages of the pathways for the D- and L-isomers are different (see Scheme 1), the addition of the DL-compound might be expected to show an additive effect. In fact DL-glyceraldehyde (whether prepared by mixing the D- and L-isomers or used as a commercial preparation) gave lower rates than D-glyceraldehyde (Table 1). These findings indicate that L-glyceraldehyde inhibits the formation of glucose from D-glyceraldehyde. This is further illustrated for various conditions by the results given in Table 2. L-Glyceraldehyde (20mm) did not inhibit appreciably the formation of glucose from lactate and fumarate. Since L-glyceraldehyde does not affect the pathway from lactate to glucose, the site of its inhibitory effect might be the stage causing the phosphorylation of D-glycer-

aldehyde. It is also possible that L-glyceraldehyde is phosphorylated (Bublitz & Kennedy, 1954) and that the L-glyceraldehyde 3-phosphate, or free L-glyceraldehyde, condenses with dihydroxyacetone phosphate to form sorbose 1,6-diphosphate or sorbose 1-phosphate respectively, which may exert inhibitory effects (see Lardy, Wiebelhaus & Mann, 1950; Tung, Ling, Byrne & Lardy, 1954).

Sheep kidney cortex. The precursors that form glucose in rat kidney cortex do so also in sheep kidney cortex (Table 3). The rates are in most cases about the same as in rat kidney. Exceptions

Table 2. *Inhibition of gluconeogenesis by L-glyceraldehyde in rat kidney-cortex slices*

Experimental conditions were as described in Table 1. Means $\pm$ S.E.M. are given with the number of experiments in parentheses; otherwise individual values are given.

Substrate(s) added (final concn.)	Glucose formed (μ moles/g. dry wt./hr.)
D-Glyceraldehyde (10mm)	228 $\pm$ 18.8 (3)
L-Glyceraldehyde (10mm)	74 $\pm$ 9.7 (3)
D-Glyceraldehyde (10mm) + L-glyceraldehyde (10mm)	181 $\pm$ 24 (3)
D-Glyceraldehyde (10mm)	280 $\pm$ 12 (3)
L-Glyceraldehyde (20mm)	70 $\pm$ 33.9 (3)
D-Glyceraldehyde (10mm) + L-glyceraldehyde (20mm)	82 $\pm$ 34.3 (3)
D-Glyceraldehyde (10mm)	349
DL-Glyceraldehyde (20mm)	184
D-Glyceraldehyde (10mm) + DL-glyceraldehyde (20mm)	150
L-Lactate (10mm)	71
L-Glyceraldehyde (20mm)	96
L-Lactate (10mm) + L-glycer- aldehyde (20mm)	159
Fumarate (10mm)	127
L-Glyceraldehyde (20mm)	49
Fumarate (10mm) + L-glycer- aldehyde (20mm)	144

Table 3. *Formation of glucose from various precursors in sheep kidney cortex*

Conditions were as described in Table 1 for rat kidney-cortex slices. Freshly collected abattoir material was used. Values given are from single determinations.

Substrate added	Glucose formed (μ moles/g. dry wt./hr.)	
	Expt. 1	Expt. 2
None	28	23
D-Fructose	152	192
D-Galactose	85	86
D-Mannose	53	—
L-Sorbose	—	41
L-Arabinose	43	—
D-Xylose	41	—
Glycerol	70	—
<i>myo</i> -Inositol	—	47
Sorbitol	52	59
Xylitol	95	99
Dihydroxyacetone	167	149
D-Glyceraldehyde	—	100

Table 4. *Effect of substrate concentration on the rate of glucose formation from polyols in rat kidney-cortex slices*

Experimental conditions were as described in Table 1 except for the substrate concentration. Means $\pm$ S.E.M. are given with the numbers of experiments in parentheses; otherwise average duplicate determinations are given.

Substrate added (final concn.)	Glucose formed (μ moles/g. dry wt./hr.)
Sorbitol (5mm)	47 $\pm$ 4.6 (3)
Sorbitol (10mm)	78 $\pm$ 2.8 (4)
Sorbitol (20mm)	125 $\pm$ 12.1 (4)
Sorbitol (40mm)	134 $\pm$ 8.7 (3)
Xylitol (10mm)	120 $\pm$ 3.5 (3)
Xylitol (20mm)	160 $\pm$ 9.8 (3)
Xylitol (40mm)	169 $\pm$ 4.0 (3)
L-Iditol (10mm)	29
L-Iditol (20mm)	49
L-Iditol (40mm)	68

Scheme 1. Pathways of glucose formation from hexoses, pentoses, polyols and related substances. The intermediary stages leading from lactate, propionate, glucogenic amino acids and intermediates of the tricarboxylic acid cycle to glyceraldehyde phosphate are not shown (see Krebs, 1964). There are five steps leading from D-xylulose phosphate to fructose 6-phosphate and glyceraldehyde phosphate. They are catalysed by enzymes of the oxidative pentose phosphate cycle (xylulose phosphate epimerase, pentose phosphate isomerase, transketolase and transaldolase). The sum of these reactions is:

References to the reactions shown in the scheme are the following: Embden & Griesbach (1914), Blakley (1951), Hickman & Ashwell (1956), Touster, Reynolds & Hutcheson (1956), Hollmann & Touster (1957), McCormick & Touster (1961), Touster & Shaw (1962) and Exton & Edson (1964) on hexitols and pentitols; Cadenas & Sols (1960), Kattermann *et al.* (1961) and DiPietro (1964) on fructose; Hers (1962), Kattermann *et al.* (1961) and Landau & Merlevede (1963) on glycerol, trioses and glycerate; Anderson & Coots (1958), Burns *et al.* (1959) and Charalampous (1960) on *myo*-inositol; Kalekar (1958) and Abraham, Fitch & Chaikoff (1961) on mannose and galactose. Most of the evidence is derived from experiments on liver.

are fructose, dihydroxyacetone and D-glyceraldehyde, which react more rapidly in rat kidney.

Effect of substrate concentration. In the previous experiments where lactate, amino acids, glycerol and intermediary metabolites were precursors of glucose (Krebs *et al.* 1963) optimum rates were obtained with 10mM-substrate. The rate of formation of glucose from polyols, in contrast, increases markedly when their concentration rises above 10mM (Table 4). Since the K_m of polyol dehydrogenase for the substrate is about 1mM (Smith, 1962), the effect of the high substrate concentration cannot be accounted for by a low affinity of the enzyme for the substrate. A rate-limiting factor for the formation of glucose may be the entry of the substrate into the cells, or alternatively the effect of the substrate concentrations could be explained by the presence in the tissue of another polyol dehydrogenase with a higher K_m . A multiplicity of NAD-dependent polyol dehydrogenases has been suggested by observations of Hollmann & Touster (1957).

DISCUSSION

It is already well established (see Cori & Shine, 1935, 1936; Exton & Edson, 1964; Kattermann, Dold & Holzer, 1961) that various polyols (sorbitol, xylitol), hexoses (galactose, mannose, fructose), pentoses (xylose, ribose, xylulose), trioses (dihydroxyacetone, glyceraldehyde) and *myo*-inositol can form glucose in the mammalian body, and that liver contains the required enzymes. The pathways leading from the various precursors to glucose are also known and are summarized in Scheme 1. The experiments reported in this paper show that kidney cortex is a highly active site of the formation of glucose from all substrates that are glucogenic in liver. Several of the necessary enzymes have been shown previously in kidney cortex. These include polyol dehydrogenase (L-iditol-NAD oxidoreductase, EC 1.1.1.14) (Blakley, 1951), the enzymes for the conversion of inositol into fructose (Burns, Trousof, Evans, Papadopoulos & Agranoff, 1959) and some of the enzymes initiating the metabolism of C₃ precursors, namely glycerol kinase or L-trio-kinase (Kalekar, 1938; Wieland & Suyter, 1957) and D-glycerate kinase (Holzer & Holldorf, 1957). The rapid formation of glucose from xylitol indicates that the transaldolase, transketolase and xylulose-kinase reactions, which are intermediary stages in the formation of glucose from xylitol, can be relatively rapid in kidney cortex.

Polyols that are not attacked by polyol dehydrogenase in the liver (D-arabitol, mannitol, dulcitol; Blakley, 1951; McCorkindale & Edson, 1954) did not form glucose in kidney cortex. The failure of propane-1,2-diol to form glucose is relevant to the

hypothesis of a 'propanediol pathway' (Shull & Miller, 1960) of conversion of ketone bodies into carbohydrate.

The observed reaction rates are in general agreement with the pathways of Scheme 1 in that the postulated intermediates, whenever tested, react at least as rapidly as the starting materials. This applies to the pathways initiated by L-idoitol, sorbitol and xylitol.

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