the ketokinases from different sources differ so widely (liver <0.5 mM and rabbit muscle 0.2 M; Hers, 1955). The constant calculated for the locust-muscle enzyme, 5.5 mM, is of the same order as that found for hexokinase from yeast, 1.7 mM, by Slein *et al.* (1950).

We consider that locust muscle contains a single, non-specific hexokinase and that the enzyme is similar to that found in yeast and in brain in its general characteristics. In contrast to hexokinase from brain the locust enzyme is not sedimented by centrifuging at 18 000 *g* but, like the brain enzyme and in contrast to that from yeast, it is inhibited by the products of its action, G 6-P or F 6-P.

SUMMARY

1. Hexokinase activity has been demonstrated in extracts of thorax muscle and salivary gland of *Locusta migratoria*.

2. The characteristics of the enzyme are similar to those of the hexokinase present in brain extracts.

3. In the presence of adenosine triphosphate and Mg^{2+} ions, D-glucose, D-mannose, D-fructose and D-glucosamine are utilized.

4. Utilization of all substrates is inhibited by glucose 6-phosphate or fructose 6-phosphate, and that of fructose by glucose, mannose and N-acetylglucosamine.

5. An ester stable to acid hydrolysis, probably a mixture of glucose 6-phosphate and fructose 6-phosphate, has been identified as a product of phosphorylation of fructose.

6. Extracts of muscle and of salivary gland contain an amylase which hydrolyses glycogen present in extracts of fed insects.

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The Hexose Specificity of Hexokinase in Brain from Various Species

By MARGARET KERLY AND D. H. LEABACK*

Department of Biochemistry, University College London

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Geiger (1940) was the first to demonstrate hexokinase activity in extracts of rat brain, and later workers have shown the enzyme to be present in ox (Colowick, Cori & Slein, 1947), sheep (Slein, Cori & Cori, 1950) and mouse (Hesselbach & DuBuy, 1953)

* Present address: Institute of Orthopaedics, Stanmore, Middlesex.

brain. The studies of later workers [especially that of Sols & Crane (1954) on calf brain] indicate that the enzyme in mammalian brain resembles that from yeast in its ability to attack a number of sugars. The experiments described below show that an enzyme with a similar specificity is found in brain tissue of birds, fishes, amphibia and molluscs.

EXPERIMENTAL

Brain extracts were prepared by a method based on that of Weil-Malherbe & Bone (1951). Rats, pigeons, 4-day-old chickens and frogs were killed by decapitation or dislocation of the neck; the brain was removed immediately, placed in ice-cold 0.9% (w/v) NaCl and used at once for preparation of the extract. Brains were removed from marine animals at Plymouth and placed with ice in a vacuum flask for transit to London. Preliminary experiments with rat brain showed that this treatment caused no loss of activity. For preparation of the extracts one or more brains were removed from saline or ice, blotted and homogenized for 2 min. in 7.5 vol. of ice-cold water; the homogenate was centrifuged for 10 min. at 600 g at a temperature of 5°. The supernatant was poured off and the sediment suspended in a further 7.5 vol. of ice-cold water; it was centrifuged again and the supernatant added to that from the first centrifuging. Extracts prepared in this way were free from whole cells and large pieces of cell debris; the protein content was of the order of 6 mg./ml. Centrifuging of homogenates of pigeon brain at 18000 g for 30 min. caused sedimentation of about one-third of the enzyme. This is a smaller fraction than that reported by Crane & Sols (1953) for calf-brain and by Stern (1954) for rat-brain homogenates; possibly, as Stern has suggested, the amount of sedimentation depends on the technique used in preparation of the initial homogenate. In view of the results with pigeon brain, purification of homogenates from other species by high-speed centrifuging was not attempted. Hexokinase activity was assayed by estimation of the hexose utilized during incubation with adenosine triphosphate (ATP) and $MgCl_2$ as previously described (Kerly & Leaback, 1957).

RESULTS

Glucose and fructose were utilized at a high rate by extracts of all the brains examined (Table 1). There was no utilization without addition of ATP and it is concluded that the sugars were phosphorylated. The rate for glucose utilization was always greater than that for fructose but the ratio of glucose to fructose used varied from one homogenate to another. Phosphorylation of glucose was partially inhibited by the presence of an equimolecular concentration of fructose, but addition of glucose completely inhibited utilization of fructose. This suggested that the hexokinase present in the brains of these animals was, like that present in calf and rat brains, of the non-specific type which attacks both aldoses and ketoses. This view was supported by the results of further experiments in which the effect of alteration of substrate concentration on the utilization of fructose by pigeon- and rat-brain homogenate was compared (Table 2). The activity of the homogenates varied with the concentration of fructose, and from the data obtained the Michaelis constants for the enzymes from rat (1.7 mM) and pigeon (1.8 mM) brain were calculated by the method of Lineweaver & Burk (1934). The constants are similar to those found for the non-specific hexokinase in ox brain (1.6 mM, Sols & Crane, 1954) and in yeast (1.7 mM, Slein *et al.* 1950) and unlike those reported by Hers (1955) for the

Table 1. Utilization of glucose and fructose by brain homogenates from different animals

Homogenate (0.4 ml.) was incubated for 15 min. at 30° in a medium which contained veronal buffer (pH 7.9; 10 mM), $MgCl_2$ (5 mM), ATP (5 mM), NaF (50 mM) and hexose as shown. Total volume, 1 ml.

Substrate	Hexokinase activity		
	Glucose 2.77 mM (μ moles of glucose utilized)	Glucose 2.77 mM + Fructose 2.77 mM*	Fructose 2.77 mM (μ moles of fructose utilized)
Mollusca			
<i>Sepia officinalis</i> (squid)	1.14	1.09	0.57
Elasmobranch			
<i>Scyllium canicula</i>	1.06	1.02	0.48
<i>Raia brachyura</i>	0.83	0.67	0.69
Teleost			
<i>Scophthalmus maximus</i>	1.10	0.97	0.38
<i>Gadus merangulus</i>	0.94	0.84	0.78
Amphibia			
<i>Rana temporaria</i>	1.08	0.56	0.40
Avies			
<i>Columba</i> (Pigeon)	1.54	1.42	0.58
<i>Gallus</i> (Chicken)	1.48	1.15	0.72
Mammalia			
Rat	1.16	1.11	0.48

* When glucose and fructose were both present, no fructose was utilized by any of the homogenates.

ketokinase in liver (<0.5 mM) and in rabbit muscle (0.2 M).

Further evidence for the occurrence of a single enzyme is given by the effect of addition of mannose, *N*-acetyl-D-glucosamine and galactose on fructose utilization (Table 3); the two former, like glucose, caused inhibition whereas the last had no action. Phosphorylation of both glucose and fructose by homogenates of pigeon and chick brain was prevented by addition of glucose or fructose 6-phos-

phates, the reaction products of the non-specific hexokinase. This also supports the suggestion that only one enzyme is present. In this respect the hexokinase in these brains resembles that found in ox and rat brain and differs from that found in yeast, which is not inhibited by its reaction product. We conclude from these findings that the hexokinase present in pigeon brain and probably also that present in the brains of the other species examined resembles the enzyme found in mammalian brain in its specificity towards hexoses.

Table 2. *Effect on fructose utilization by brain homogenates of variation of concentration of substrate*

Homogenate (0.4 ml.) was incubated for 15 min. at 30° in a medium which contained veronal buffer (pH 7.9; 10 mM), MgCl₂ (5 mM), ATP (5 mM), NaF (50 mM) and fructose as shown. Total volume, 1 ml.

Fructose concn. (mM)	Hexokinase activity (μmoles of fructose utilized)	
	Rat brain	Pigeon brain
1.39	0.74	0.50
2.77	1.11	0.67
5.54	1.32	0.82
11.08	1.39	0.92

Table 3. *Effect on fructose utilization by brain homogenates of addition of glucose, mannose, galactose and N-acetyl-D-glucosamine*

Homogenate (0.4 ml.) was incubated for 15 min. at 30° in a medium which contained veronal buffer (pH 7.9; 10 mM), MgCl₂ (5 mM), ATP (5 mM), NaF (50 mM) and fructose (2.77 mM). Total volume, 1 ml.

Concn. of added hexose (mM)		Hexokinase activity (μmoles of fructose utilized)	
		Rat brain	Pigeon brain
None		1.10	0.95
Glucose	1.39	0.14	0.37
	2.77	0	0
Mannose	1.39	0.27	0.14
	2.77	0	0
Galactose	2.77	1.06	0.97
<i>N</i> -Acetyl-D-glucosamine	1.00	0.52	0.60
	2.00	0.31	0.36
	5.00	0.05	0

SUMMARY

1. Extracts of brains from pigeon, 4-day-old chick, two elasmobranch and two teleost fishes, frog and squid phosphorylate glucose and fructose.

2. In pigeon-brain extracts phosphorylation of fructose is inhibited by mannose and by *N*-acetyl-D-glucosamine; phosphorylation of both glucose and fructose is inhibited by glucose and fructose 6-phosphates.

3. The affinity for fructose of the enzyme present in rat and pigeon brain is similar to that of calf-brain hexokinase.

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