Some Properties of Rat-Liver Glucose–Adenosine Triphosphate Phosphotransferases

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In normal rat liver hexokinase (EC 2.7.1.1) activity usually accounts for not more than 30% of the total glucose–ATP phosphotransferase activity, the remainder being due to glucokinase (EC 2.7.1.2). In the present work it was found that in normal rat liver the relative activities of these two enzymes were occasionally very different from those usually found even though the total glucose–ATP phosphotransferase activity was within the normal range. In some cases almost the entire glucose–ATP phosphotransferase was accounted for by the low-K_m enzyme hexokinase. Some properties of this enzyme system are reported. It is suggested that this shift in favour of the low-K_m enzyme without change in the total glucose–ATP phosphotransferase activity may represent a regulatory mechanism.

It is now well established that rat liver contains two distinct enzymes catalysing the phosphorylation of glucose by ATP. The general term glucose–ATP phosphotransferase is used to describe the overall ability to catalyse this reaction and the name hexokinase is used for the enzyme with the high affinity for glucose ($K_m < 0{\cdot}1$ mM) (EC 2.7.1.1) and glucokinase (EC 2.7.1.2) for that with the low affinity for glucose (K_m 10 mM). These two enzymes can be distinguished by their many different properties. Hexokinase, in addition to having the high affinity for glucose, is markedly inhibited by glucose 6-phosphate and is precipitated by ammonium sulphate between 20 and 50% saturation. In contrast with this glucokinase is not so strongly inhibited by glucose 6-phosphate and is not precipitated until the ammonium sulphate saturation reaches 60–70%; these enzymes have also been separated on DEAE-cellulose columns and by starch-gel electrophoresis (Viñuela, Salas & Sols, 1963; Walker, 1963; Salas, Salas, Viñuela & Sols, 1965; Gonzáles, Ureta, Sanchez & Niemeyer, 1964; Katzen & Schimke, 1965).

In addition to these different physical properties these two enzymes show different physiological control. Although the activity of glucokinase undergoes very marked changes in different nutritional and hormonal conditions, hexokinase activity remains, in general, remarkably stable (Sharma, Manjeshwar & Weinhouse, 1964; Sols, Salas & Viñuela, 1964). For example, glucokinase is very low in alloxan-diabetic rats or starving animals (Salas, Viñuela & Sols, 1963; Sharma, Manjeshwar & Weinhouse, 1963; Blumenthal, Abraham & Chaikoff, 1964; Sols, Sillero & Salas, 1965). This enzyme is also low or absent in many hepatomas and in foetal liver (Shatton, Donnelly & Weinhouse, 1962; Sharma, Sharma, Donnelly, Morris & Weinhouse, 1965; Walker, 1963; Walker & Holland, 1965).

The total glucose–ATP phosphotransferase activity of rat liver in the presence of excess of substrate is approx. 2 μmoles of glucose 6-phosphate formed/g. of liver/min., and the relative contribution of hexokinase is normally in the region 10–20% of the total activity. These figures represent average values from those reported in the literature. In the present work it was observed that in normal rat liver the relative activities of these two enzymes were occasionally different from those usually found even though the total glucose–ATP phosphorylating activity was within the normal range. In the most extreme cases almost the entire glucose–ATP phosphotransferase activity was accounted for by the low-K_m enzyme. Some properties of this altered enzyme system have now been studied. On the basis of this work it is suggested that glucokinase may undergo some modification such that phosphorylation of glucose can occur at a much lower substrate concentration than is normally possible with this enzyme and that this might represent another type of mechanism for the control of glucose metabolism.

METHODS

Materials. Commercial reagents were used with the exception of 6-phosphogluconate dehydrogenase, which was a partially purified preparation from liver, prepared as

described by Glock & McLean (1953), and used in the assay of hexokinase and glucokinase. Glucose 6-phosphate dehydrogenase and NADP were obtained from C. F. Boehringer und Soehne G.m.b.H. (Mannheim, Germany). Mercaptoethanol was a product of L. Light and Co. Ltd. (Colnbrook, Bucks.); 3′,5′-(cyclic)-AMP was obtained from Sigma Chemical Co. (St Louis, Mo., U.S.A.).

Animals. Adult male albino rats of the Wistar strain were used. The rats were approx. 8 weeks old and weighed between 170 and 210 g. and were fed on the stock diet *ad lib.* They were killed by cervical dislocation.

Preparation of tissue extracts. Except where otherwise stated portions of liver were homogenized in an all-glass Potter homogenizer, in 3 vol. of ice-cold medium containing 150 mM-KCl, 5 mM-$MgCl_2$, 5 mM-EDTA and 10 mM-mercaptoethanol and adjusted to pH 7·4 with $KHCO_3$. This is similar to the extracting medium used by Sharma *et al.* (1963). This preparation was centrifuged at $100000 g_{av.}$ for 45 min. The supernatant fraction was dialysed against the same extracting medium for 2 hr. in the cold to decrease the blank value; this did not alter the glucose–ATP phosphotransferase activity.

Glucose–ATP phosphotransferase activity. The hexokinase and glucokinase activities of the dialysed high-speed supernatant fractions from rat liver and hepatoma were measured essentially according to the method of Sharma *et al.* (1963) with modifications as described by McLean & Brown (1966).

A unit of enzyme activity is defined as 1 μmole of glucose 6-phosphate formed/min. at 25°. The values of μmoles of NADPH produced were divided by 2 to convert them into μmoles of glucose 6-phosphate formed since the assay system contained excess of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase as previously described (McLean & Brown, 1966). Each assay consisted of three cells and the results have been calculated as described by Sharma *et al.* (1963) in the following way.

Cell *A*: high glucose concentration, 100 mM.
Cell *B*: low glucose concentration, 0·5 mM.
Cell *C*: *N*-acetylglucosamine blank.
Total glucose–ATP phosphotransferase = *A*–*C*.
Glucokinase = *A*–*B*.
Hexokinase = *B*–*C*.

Where the hexokinase activity is only 10–20% of the total glucose-phosphorylating activity the above method of calculation is not subject to great error. However, when the proportional contribution of hexokinase to the total activity is much greater than this, allowance must be made for the inhibition of hexokinase by high glucose concentrations when calculating the glucokinase activity and the total glucose–ATP phosphotransferase. The results in Table 4 show that under the conditions of the assay the inhibition of hexokinase in the presence of 100 mM-glucose was approx. 30% and thus at high glucose concentrations the hexokinase activity would not be fully manifest. Values for the total phosphorylating activity and for glucokinase may be readily corrected by recalculation of the hexokinase contribution in the presence of high glucose concentrations. This is only necessary where the hexokinase contribution is a large proportion of the whole and has been used in Table 5 and Fig. 2. Specific mention is made in the text where this method of correction has been used. Where other additions or alterations were made to the medium used for homogenizing the tissue or to the assay system these are given in the appropriate parts of the Results and Discussion section.

The rate of reduction of NADP was measured with a Unicam SP. 800 recording spectrophotometer with constant-temperature cell housing and scale expansion accessory.

RESULTS AND DISCUSSION

The occurrence of a glucose–ATP phosphotransferase enzyme with a low K_m which has a very high activity, accounting for 40% or more of the total glucose phosphorylation, has been observed repeatedly in the present experiments. In a series of 64 normal control rats, 12 fell within such a range, that is 20% of all values (see Table 1). The total phosphorylating activity in these groups was closely similar and it was only the percentage contribution of the low-K_m enzyme which distinguished them. In the largest group, comprising 23 rats, the total phosphorylating activity was

Table 1. *Relationship between the total glucose–ATP phosphotransferase activity, hexokinase and glucokinase in liver in a series of normal rats*

Values are given as μmoles of glucose 6-phosphate formed/min./g. of liver at 25°. The total number of rats used was 64. The results are given as means ± S.E.M.

% Contribution of hexokinase to total activity	Total glucose–ATP phosphotransferase activity	Hexokinase	Glucokinase	No. of animals
0–10	2·05 ± 0·17	0·17 ± 0·013	1·89 ± 0·15	11
11–20	2·11 ± 0·20	0·33 ± 0·025	1·79 ± 0·12	23
21–30	1·96 ± 0·16	0·50 ± 0·04	1·46 ± 0·13	13
31–40	2·18 ± 0·18	0·82 ± 0·08	1·36 ± 0·12	5
41–50	1·97 ± 0·30	0·89 ± 0·15	1·28 ± 0·15	5
51–60	2·04 ± 0·53	1·16 ± 0·16	0·89 ± 0·16	4
61–100	1·81 ± 0·07	1·46 ± 0·12	0·35 ± 0·18	3

2·11 ± 0·20 units/g. of liver, of which 10–20% of the total glucose 6-phosphate formation was catalysed by the low-K_m enzyme. At the opposite end of the scale, in another group of seven rats derived from the same population, the contribution of hexokinase was over 50% of the total glucose–ATP phosphotransferase activity. Although the total glucose–ATP phosphotransferase activity was clearly the same in each group there were highly significant differences in the hexokinase and glucokinase activities.

To eliminate the possibility that the large variation in hexokinase activity might be due to seasonal effects, 12 rats were taken from stock and the enzyme estimations were performed on the entire group on the same day (Table 2). This shows marked variation in the activity of hexokinase as a percentage of the total glucose–ATP phosphotransferase activity, which remains remarkably constant. The distribution of the normal hexokinase and the abnormally high hexokinase appears to be random within the group.

Examination of values quoted in the literature for hexokinase and glucokinase activity of liver reveals that the rate of glucose phosphorylation by this low-K_m enzyme is normally no greater than 0·6 μmole of glucose 6-phosphate/g. of liver/min. at 25°, being equal to a 30% contribution of hexokinase to the total rate of glucose phosphorylation (see Table 3). The values for hexokinase quoted by Sharma *et al.* (1963) are smaller by a factor of three than those of Viñuela *et al.* (1963), a point which the former authors investigated but for which they found no satisfactory explanation. These and other published values are shown in Table 3.

We have arbitrarily divided the present results into two groups, based on published figures and those collected from the group of normal rats reported here. (1) Liver supernatant preparations with hexokinase (low-K_m enzyme) activity less than 1 μmole of glucose 6-phosphate formed/g. of tissue/min. at 25°, i.e. contribution less than 40% of the total glucose–ATP phosphotransferase, were considered to be within the widest limits of the normal range and this is called henceforth hexokinase. (2) Preparations in which the low-K_m enzyme phosphorylated amounts of glucose greater than this were considered to be outside the normal range and to represent some activated form of glucose–ATP phosphotransferase activity. Since the total phosphorylating activity remained the same (see Tables 1 and 2) this suggested a conversion of glucokinase (the high-K_m enzyme) into one which would utilize glucose at a lower glucose concentration, typical of hexokinase, and this modified enzyme has been called hexokinase A. Some properties of normal hexokinase and hexokinase A are described below.

Effect of storage at 4°. The stability of hexokinase, hexokinase A and glucokinase was studied by measuring these activities in the liver supernatant fractions immediately after dialysis and after storage at 4° for periods up to 5 days. These results

Table 2. *Hexokinase and glucokinase activities of one group of normal rat livers*

Values are given as μmoles of glucose 6-phosphate formed/min./g. of liver at 25°. Rats were killed in the order given, livers were removed and homogenates prepared in the same medium (KCl–EDTA–mercaptoethanol–Mg^{2+}) for all 12 livers. The extracts were spun at 100000*g* for 45 min., dialysed for 2 hr. and estimated immediately for hexokinase and glucokinase activity also in the order shown. Samples 4, 6, 7, 9 and 10 were combined (normal hexokinase preparation) and samples 2, 3, 5 and 11 were combined (hexokinase A preparation). These were used for separation of hexokinase by $(NH_4)_2SO_4$ fractionation as shown in Table 4, and for the determination of effect of glucose concentration (Table 4), Michaelis constants for glucose and pH–activity curves (Fig. 1).

Rat no.	Total glucose–ATP phosphotransferase activity	Hexokinase	Glucokinase	% Contribution of hexokinase to total activity
1	2·54	0·64	1·90	25
2	2·60	1·46	1·14	56
3	2·36	0·94	1·42	40
4	2·22	0·42	1·80	19
5	2·44	1·30	1·14	53
6	2·68	0·50	2·18	19
7	2·18	0·50	1·68	23
8	2·30	0·84	1·46	37
9	2·20	0·46	1·74	21
10	2·38	0·36	2·02	15
11	2·44	1·10	1·34	45
12	2·42	0·78	1·64	32

Table 3. *Values for relative activities of hexokinase and glucokinase in normal rat liver*

Values are given as μmoles of glucose 6-phosphate formed/min./g. of liver at 20–25° and have been recalculated where necessary. Results of Sharma *et al.* (1963) have been divided by 1·7 (the factor given by these authors to convert the NADPH formed into glucose 6-phosphate). Values of Oliver & Cooke (1964) were converted from 37° to 25°, assuming Q_{10} approx. 2 for this enzyme. In most cases values for adult male rats fed on stock diet *ad lib.* have been used. The values of Salas *et al.* (1963) are for rats fed with glucose for 12hr. The present results are the means of the 64 normal rats, which are subdivided into smaller groups in the results reported in Table 1.

Hexokinase	Glucokinase	Total glucose–ATP phosphotransferase	% Contribution of hexokinase to total activity	Reference
0·31±0·13	1·06±0·25	1·37	23	Viñuela *et al.* (1963)
0·60±0·08	1·46±0·20	2·06	29	Salas *et al.* (1963)
0·09±0·006	0·72±0·07	0·81	11	Sharma *et al.* (1963)
0·45±0·10	1·28±0·44	1·73±0·47	26	Walker & Rao (1964), Walker & Holland (1965)
0·15±0·02	2·26±0·15	2·41	6	Oliver & Cooke (1964)
—	—	—	15	Ballard & Oliver (1964)
0·52	1·52	2·04	25	Present work

Table 4. *Recovery and properties of hexokinase and hexokinase A from rat liver with ammonium sulphate fractionation*

	Hexokinase	Hexokinase A
Average initial activity* (μmoles of glucose 6-phosphate/g. of liver/min.)	0·45	1·20
Recovery in $(NH_4)_2SO_4$ fractions (as % of initial activity)		
20–50% saturation	66	73
50–60% saturation	9	3
60–75% saturation	2	1
Total recovery	77	77
Activity of 20–50% $(NH_4)_2SO_4$ fraction (ΔE/1 min./0·1 ml. of enzyme)		
(*a*) Effect of glucose concentration		
0·5 mM-glucose	0·250	0·780
10 mM-glucose	0·190	0·490
Inhibition (%)	24	37
(*b*) Effect of 3′,5′-(cyclic)-AMP†		
0·2 mM	0·245	0·640
Inhibition (%)	2	18

* Hexokinase precipitated from pooled samples 4, 6, 7, 9 and 10 and hexokinase A precipitated from pooled samples 2, 3, 5 and 11 shown in Table 2.

† Enzyme preincubated with 3′,5′-(cyclic)-AMP at a final concentration 0·2 mM for 15 min. at 25° before assay of the enzyme activity. For method of assay and other details see the Methods section.

show that hexokinase and hexokinase A are stable for 5 days at 4°. This contrasts sharply with the glucokinase activity, which decreases approx. 25% after storage for 24hr. and has completely lost activity after 5 days. If these liver supernatant preparations are stored at −15°, both glucokinase and hexokinase activities appear to be retained almost unchanged for periods of several weeks.

Effect of high glucose concentration. In following the hexokinase and glucokinase activities of the stored liver extracts low and high substrate concentrations were used (0·5 mM- and 0·1 M-glucose respectively). When such measurements were made with extracts stored for 5 days at 4°, when only the low-K_m enzyme remained, it was found that high concentrations of glucose caused some inhibition of hexokinase. This inhibition was more clearly seen when hexokinase A was present since this had the higher activity. Similar results were obtained with hexokinase in the normal range of activity and with hexokinase or hexokinase A separated from glucokinase by fractionation with ammonium sulphate (20–50% saturation) (see Table 4). In a series of six experiments the average

inhibition of hexokinase by the high glucose concentration was 32%.

Effect of mixing hexokinase and hexokinase A. When liver supernatant preparations containing either hexokinase or hexokinase A are mixed the result is an exact summation of the two activities. Thus if an activator is responsible for the high activity of hexokinase A then this is not present in excess amounts such that the normal hexokinase can be further activated. Average values for two such experiments in terms of units of activity/g. of liver are: hexokinase, 0·18; hexokinase A, 1·41; mixed hexokinase + hexokinase A, 1·63. These two values, the mixed assay and the summated values, agree to within 3% of each other, indicating that no excess of activator is present.

Effect of metal ions. The effects of some metal ions on the activities of hexokinase and hexokinase A were tested to find if contamination of the liver supernatant fractions with trace metals during the preparation of the extracts could account for the high activity of hexokinase A. Chromium ammonium sulphate, final concentration 1 μM in the assay, had little or no effect; at ten times this concentration it caused a 20% inhibition of the activity of both hexokinase and hexokinase A. Aluminium ammonium sulphate (10 μM final concentration) caused less than 10% inhibition of hexokinase or hexokinase A.

Ammonium sulphate fractionation and some properties of hexokinase and hexokinase A. The recovery of hexokinase and hexokinase A from rat-liver supernatant preparations by fractionation with ammonium sulphate is shown in Table 4. It is clear that, irrespective of the initial activity of the enzyme or the proportion of the total phosphorylating activity that this represents, the major portion of the hexokinase A is precipitated at the same ammonium sulphate concentration as normal hexokinase, that is between 20 and 50% saturation (see Table 4).

The effect of glucose concentration on the activity of hexokinase in the 20–50% ammonium sulphate fraction was tested and it was found that inhibition of enzyme activity by high concentration of glucose (100mM) was obtained in both cases.

The K_m values for hexokinase and hexokinase A precipitated at 20–50% ammonium sulphate saturation were closely similar: hexokinase was $0{\cdot}8 \times 10^{-5}$M whereas hexokinase A was $1{\cdot}2 \times 10^{-5}$M. These correspond well with those already published for liver hexokinase (20–50% ammonium sulphate fraction) by Viñuela *et al.* (1963).

The pH–activity curves for glucokinase, hexokinase and hexokinase A, obtained by ammonium sulphate fractionation, are shown in Fig. 1. The optimum pH for all three enzyme preparations was at pH 8·5; there was little difference in activity between pH 7·5 and 9·0 but in each case the activity fell sharply below pH 7·5. The shapes of the curves for hexokinase and hexokinase A were similar. In general, values between pH 7·5 and pH 8·0 have been used in most studies on hexokinase and glucokinase, Sharma *et al.* (1963) and Walker & Holland (1965) both used glycylglycine, pH 7·5; Viñuela *et al.* (1963) used tris, pH 7·4, and Blumenthal *et al.* (1964) used tris with histidine at pH 8·0. Ballard & Oliver (1964), using tris buffer, have shown that the pH optimum for adult rat-liver glucokinase was 7·0 whereas at pH 9·5 only 20% of the maximum activity remained. The difference between their results and the present work may be due to the different buffer systems employed. The effect of 3′,5′-(cyclic)-AMP on the activity of hexokinase and hexokinase A was also tested by preincubating the enzyme with this nucleotide at a final concentration of 0·2mM for 15min. at 25° before assay of the enzyme activity. Some slight inhibition of hexokinase A occurred.

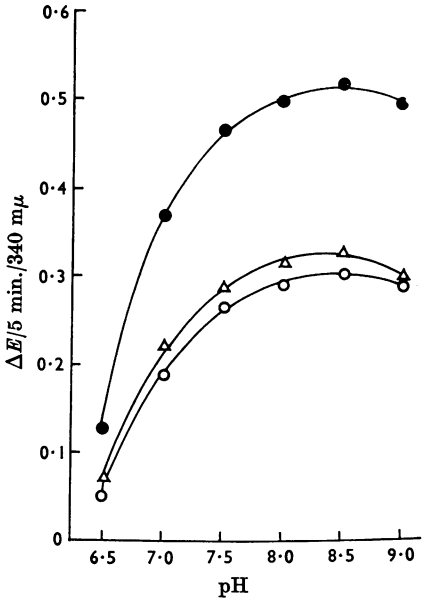

Fig. 1. pH–activity curves for hexokinase, hexokinase A and glucokinase of rat liver fractionated with $(NH_4)_2SO_4$. Hexokinase (○) was measured in the fraction precipitated with $(NH_4)_2SO_4$ (20–50% saturation) and glucokinase (△) in the fraction precipitated with $(NH_4)_2SO_4$ (60–75% saturation) from the pooled high-speed supernatant fractions from rats nos. 4, 6, 7, 9 and 10. Hexokinase A (●) was precipitated by 20–50% saturation with $(NH_4)_2SO_4$ from the pooled samples nos. 2, 3, 5 and 11 (see Tables 2 and 4). The buffer used was glycylglycine adjusted to the appropriate pH with KOH.

Attempts to produce liver extracts with a high proportion of hexokinase. The evidence from the

Table 5. *Recalculated values for the total glucose–ATP phosphotransferase activity of liver with correction for the inhibition of hexokinase by high glucose concentration*

Values are given as μmoles of glucose 6-phosphate formed/g. of liver/min. at 25°. Hexokinase activity is inhibited approx. 30% by high glucose concentration (100mM): see Table 4. Hexokinase activities measured at 0·5mM-glucose (column I) are recalculated to give the value which would be obtained at 100mM-glucose concentration (column II). The total rate of glucose–ATP phosphotransferase of rat-liver supernatant fraction measured with 100mM-glucose is given in column III. The corrected value for glucokinase is the difference between III and II. The total glucose–ATP phosphotransferase activity in column III is low because of the inhibition of hexokinase by 100mM-glucose; the last column shows the corrected value. The average value for the corrected total glucose–ATP phosphotransferase is 2·24μmoles of glucose 6-phosphate/g. of liver/min. The total number of animals was 64 and they were divided into groups as in Table 1. For details of calculations see the Methods section.

Group no.	No. of animals	Hexokinase 0·5mM-Glucose (I)	Hexokinase 100mM-Glucose (II)	Total glucose–ATP phospho-transferase 100mM-Glucose (III)	Corrected glucokinase 100mM-Glucose (III−II)	Corrected total glucose–ATP phospho-transferase 100mM-Glucose +0·5mM-glucose [(III–II)+I]
1	11	0·17	0·12	2·05	1·93	2·10
2	23	0·33	0·23	2·11	1·88	2·21
3	13	0·50	0·35	1·96	1·61	2·11
4	5	0·82	0·57	2·18	1·61	2·43
5	5	0·89	0·62	1·97	1·35	2·24
6	4	1·16	0·81	2·04	1·23	2·39
7	3	1·46	1·02	1·84	0·79	2·24

above studies suggested that hexokinase A might be formed from glucokinase. The enzyme activities of the groups given in Table 1 have been recalculated to allow for the inhibition of hexokinase by high glucose concentrations, the degree of inhibition being taken as 30% as shown in Table 4. The recalculated data are shown in Table 5 and when plotted as glucokinase activity versus hexokinase activity the inverse relationship between these enzymes is clearly seen (Fig. 2).

Attempts were made to produce conditions whereby normal rat-liver extracts could be converted into extracts with a high content of hexokinase. None of these methods would consistently produce extracts with activities of the hexokinase A level but they are listed below since they do at least eliminate some possible mechanisms for the present observations.

Normal rat liver was divided into two portions and one homogenized in the normal medium, 150mM-potassium chloride–5mM-EDTA–5mM-magnesium chloride – 10mM-mercaptoethanol, pH 7·4, with a loose-fitting all-glass homogenizer. The other part was homogenized in one of the following media: 150mM-potassium chloride–5mM-EDTA – 5mM-magnesium chloride – 10mM-mercaptoethanol, pH 5·0, 0·25M-sucrose or 0·25M-glycylglycine, pH 7·6, and the activities were determined after centrifugation at high speed and dialysis against the appropriate medium. No marked change in activity was observed. Neither the addition of charcoal (60mg. of Norit A/g. of liver) nor the addition of 20mM-sodium fluoride to the normal homogenizing medium altered the enzyme activity. The clearance between the

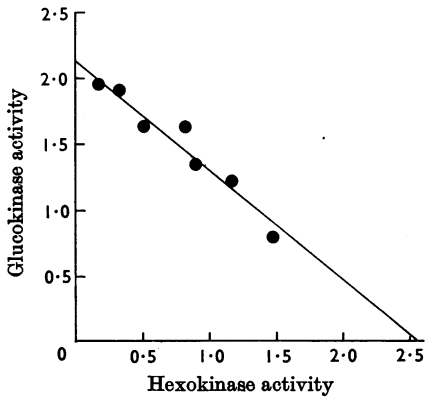

Fig. 2. Relationship between hexokinase and glucokinase activity in groups of normal rats with uniform glucose–ATP phosphotransferase activity. Results calculated in Table 5 are plotted to show the correlation between glucokinase and hexokinase activities. The position of the line was calculated by the method of least squares. For details see the Methods section. Points represent the mean activities of differing numbers of observations.

plunger and the barrel of the all-glass homogenizer did not appear to alter the proportion of hexokinase activity. It seemed possible that certain livers might contain variable concentrations of substrates such as glucose or citrate which might modify the hexokinase. Citrate has marked effects as an activator of acetyl-CoA carboxylase (Numa, Bortz & Lynen, 1965) and an inhibitor of phosphofructokinase (Passonneau & Lowry, 1964). Rat-liver homogenates prepared in the normal medium, in medium to which had been added sodium citrate (5mM final concentration) and in the medium containing glucose (0·025M final concentration) all showed the same hexokinase and glucokinase activity. In each case the substrates were present only during the period of homogenization and centrifugation and were removed by dialysis against the normal extracting medium before assay of the enzyme activities.

The possibility that the lipid content of different preparations might in part account for the results was tested by the addition of the lipid layer separated by high-speed centrifugation to the assay medium. This caused no change in hexokinase activity. Bovine serum albumin caused a small variable stimulation in hexokinase activity.

It seemed possible that a mechanism similar to that occurring in control of phosphorylase activity might be important in producing the abnormally high hexokinase activities observed in the present experiments. However, 3′,5′-(cyclic)-AMP and calcium used in concentrations known to activate phosphorylase (see Krebs *et al.* 1964) had little or no effect on the proportion of the hexokinase activity. Treatment of normal rats with 0·1mg. of adrenaline, by subcutaneous injection 20min. before killing the animals and preparing liver homogenates, also failed to raise the hexokinase activity to that classified here as hexokinase A.

In another series of unpublished experiments on the hormonal control of enzymes concerned with glucose metabolism, particular attention was paid to the occurrence of high hexokinase activity, that is where values exceeded 1·0μmole of glucose 6-phosphate formed/g. of liver/min. at 25°. From this work it may be tentatively stated that high hexokinase was not found in a series of 20 hypophysectomized rats but occasional high values were found in a series of starved and diabetic animals. However, a much larger series of animals would be needed before any correlation with hormonal status and appearance of high hexokinase activities could be established.

It appears that under certain circumstances rat-liver glucokinase can be altered in some way to give an enzyme catalysing the same reaction but with a much lower K_m for glucose. This transformation, which may be partial or almost complete, appears to occur immediately and is not readily reversed. It has not proved possible to induce this change by any of the methods noted above and, until this problem is solved, further study is greatly hampered since in our Laboratory only about 10% of the animals show this phenomenon spontaneously. The properties of the hexokinase A appear to be closely similar to hexokinase with respect to K_m, pH optimum and stability in the cold. The existence of isoenzymes of hexokinase in liver reported by Gonzáles *et al.* (1964) and by Katzen & Schimke (1965) are of particular interest in this context. The present results suggest a possible control mechanism for the phosphorylation of glucose by alterations in the relative proportions of hexokinase and glucokinase, the total glucose-phosphorylating activity remaining the same.

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