The structural difference between the two classes of hydroxynaphthoquinones discussed above is interesting with regard to various proposed mechanisms, of ATP synthesis[7,8] which involve the formation of rings by condensation of the side chain of a 3-alkyl quinone with O-4. A 2',3' double bond is required for this cyclization. The 2-hydroxyl group in the naphthoquinones used in this work, by contributing an orthoquinoid character, should affect the stability of the ring. The above argument presents an approach to the investigation of the proposed involvement of chromanols or related compounds in mitochondrial oxidative phosphorylation.

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The effect of glucose analogues on the hepatic glucose-phosphorylating enzymes

Recent work on the nature of the enzymic system catalysing the phosphorylation of glucose by rat liver[1,2] resulted in the demonstration that two enzymes are involved[2,3]. These two enzymes occur in different ammonium sulphate fractions of rat-liver supernatant fractions[4] and have been designated[3,4] as glucokinase (having a low affinity for glucose, $K_{m,1}$, of type EC 2.7.1.2 but not necessarily specific for glucose only[4]) and hexokinase (having a high affinity for glucose, $K_{m,2}$, and probably of non-specific type EC 2.7.1.1). Detailed characterization of the two enzymes will require first their isolation and purification, perhaps following the lead mentioned[4], but considerable information can be obtained by kinetic analysis along the lines used previously[3]. The purpose of this communication is to show how such procedures can be used for a system consisting of two enzymes catalysing the same reaction simul-

taneously and to report the effects of 2-deoxy-D-glucose, D-glucosamine and *N*-acetyl-D-glucosamine upon the glucokinase and hexokinase of rat liver.

The hexokinase can be studied alone by using the foetal liver, which contains no glucokinase[2,3], as source of enzyme. The kinetics of the two-enzyme system in adult liver can be analysed because the two enzymes have such widely differing apparent K_m values[3], thus enabling the properties of the glucokinase to be assessed. In the most general case where both enzymes are competitively inhibited by the analogue, by substituting $K_m(1 + I/K_i)$ for K_m for each enzyme, the observed velocity v is given by

$$v = \frac{V_1}{1 + \frac{K_{m,1}}{S}\left(1 + \frac{I}{K_{i,1}}\right)} + \frac{V_2}{1 + \frac{K_{m,2}}{S}\left(1 + \frac{I}{K_{i,2}}\right)} \qquad (1)$$

(*cf.* Eqn. 1 of ref. 3). In the range of glucose concentrations where S is large ($1/S$ is low), *i.e.*, where the substrate concentration is saturating the hexokinase, the application of the same treatment as before[3] leads to the equation

$$\frac{1}{v} = \frac{1}{V_1 + V_2} + \frac{1}{S}\left(\frac{V_1 K_{m,1}\left(1 + \frac{I}{K_{i,1}}\right) + V_2 K_{m,2}\left(1 + \frac{I}{K_{i,2}}\right)}{(V_1 + V_2)^2}\right) \qquad (2)$$

which represents a straight line cutting the ordinate at

$$\frac{1}{V_1 + V_2}\left(= \frac{1}{V}, \text{ where } V = V_1 + V_2\right)$$

and the abscissa (where $1/v = 0$) when

$$S = -\frac{V_1 K_{m,1}\left(1 + \frac{I}{K_{i,1}}\right) + V_2 K_{m,2}\left(1 + \frac{I}{K_{i,2}}\right)}{V_1 + V_2}$$

In the special case (as here) where $K_{m,2} \ll K_{m,1}$, then $1/v = 0$ when

$$S = -\frac{V_1 K_{m,1}\left(1 + \frac{I}{K_{i,1}}\right)}{V_1 + V_2} \qquad (3)$$

The implications of this analysis for the present special case are that (a) if the $K_{m,2}$-enzyme (hexokinase) only is inhibited,

$$S = \frac{V_1 K_{m,1}}{V_1 + V_2}$$

when $1/v = 0$, *i.e.*, no effect of the inhibitor is seen at high S values, and (b) if the $K_{m,1}$-enzyme (glucokinase) only or both the enzymes are inhibited, Eqn. 3 applies.

The sources of materials, methods of preparation of the tissue extracts and of assay of glucose phosphorylation were as used previously (WALKER[3]). The glucose analogues 2-deoxy-D-glucose and *N*-acetyl-D-glucosamine (Mann Research Labs. Inc.) and D-glucosamine hydrochloride (British Drug Houses Ltd.) (recrystallized, and neutralised just prior to use) were added in various concentrations to reaction mixtures.

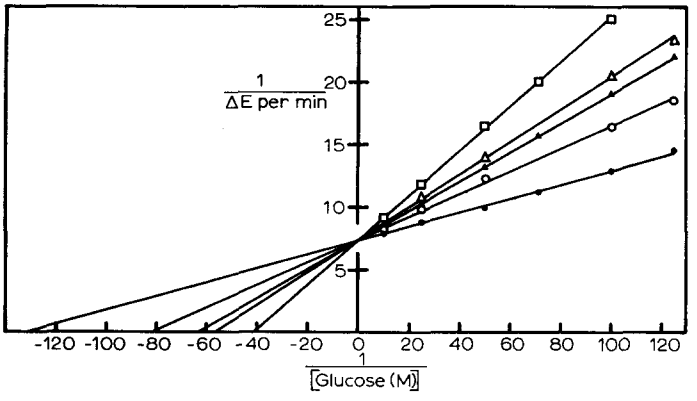

Fig. 1. Competitive inhibition of rat glucokinase by 2-deoxy-D-glucose and *N*-acetyl-D-glucosamine as demonstrated by LINEWEAVER–BURK plots. The reaction mixture (total vol 1.5 ml) contained (final concentrations): 50 mM sodium glycylglycine buffer (pH 7.5), 7.5 mM $MgCl_2$, 5 mM ATP, 0.5 mM $NADP^+$, 0.1 Kornberg unit glucose-6-phosphate dehydrogenase, 30 μl adult rat liver supernatant preparation dialysed for 20 h, concentrations of glucose ranging from 100 to 8 mM; together with: ●—●, no inhibitor; ▲—▲, 0.6 mM *N*-acetyl-D-glucosamine; ○—○, 10 mM 2-deoxy-D-glucose; △—△, 20 mM 2-deoxy-D-glucose; □—□, 30 mM 2-deoxy-D-glucose.

The three glucose analogues competitively inhibited both the hexokinase and glucokinase. Using foetal liver preparations (from both rats and guinea-pigs), typical reciprocal plots for competitive inhibition of the hexokinase were obtained and K_i values were assessed using three inhibitor concentrations by the methods of LINEWEAVER AND BURK[5] and DIXON[6]. Results with adult rat-liver preparations at glucose concentrations ranging from 100 to 8 mM showed that the three glucose analogues are also competitive inhibitors of glucokinase (Fig. 1). Although all three analogues are modified at C-2 compared to glucose, the K_i for 2-deoxy-D-glucose with the

TABLE I

COMPETITIVE INHIBITION OF THE HEPATIC GLUCOSE-PHOSPHORYLATING ENZYMES

Reaction mixtures were prepared as in Fig. 1. The results for the rat and guinea-pig hexokinases were obtained using foetal liver supernatant fractions dialysed as before[3] (50–100 μl per 1.5 ml incubation mixture) and those for rat glucokinase by similar preparations from fed adult male animals (20–40 μl per 1.5 ml incubation mixture). Substrate concentrations ranged from 100 to 8 mM glucose for glucokinase and 100–0.05 mM glucose for the hexokinases. Three inhibitor concentrations were used in each case and K_i values calculated as described in the text.

Compound	*K_i (mM)*		
	Rat glucokinase	*Rat hexokinase*	*Guinea-pig hexokinase*
D-Glucose*	10	0.04	0.03
D-Fructose*	—	2.4	1.7
2-Deoxy-D-glucose	14	0.3	0.6
D-Glucosamine	0.8	0.3	0.2
N-Acetyl-D-glucosamine	0.5	0.2	0.3

* K_m values (mM).

glucokinase is much higher than for the other two and is comparable in magnitude to the K_m for glucose (Table I) and mannose[4].

The present results thus extend the earlier observations and emphasize the need to use not only high glucose concentrations for the measurement of the maximum rate of processes depending upon the phosphorylation of glucose as the rate-determining step but also high concentrations of 2-deoxy-D-glucose if this analogue is to have a marked direct effect upon hepatic carbohydrate metabolism[7]. SPIRO[8] demonstrated that D-glucosamine and *N*-acetyl-D-glucosamine strongly inhibited the synthesis of glycogen in rat-liver slices in a competitive manner. In spite of the complexity of the system, it was possible to implicate competitive inhibition of a "non-specific hexokinase" by the glucose analogues as the operative mechanism[8]. KONO AND QUASTEL[9] studied the effects of the three analogues at concentrations up to 50 mM on "liver hexokinase" but the substrate concentration used was only 5 mM glucose. While their conclusion[9] that these analogues elevate hepatic phosphorylase activity thereby increasing glycogen breakdown remains valid, the present results show that the analogues can also have a marked effect upon glycogen synthesis by inhibiting the phosphorylation stage. These facts, together with the changes in the relative contributions of the two glucose-phosphorylating enzymes to the total activity which occur with changing glucose concentration[10], make the problem of assessing the effect of the inhibitors on complex systems such as found in tissue slices and *in vivo* extremely difficult.

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Separation of cellulases on Sephadex G-100

Cellulolytic culture filtrates from fungi and bacteria have been resolved into several cellulase components by ion-exchange chromatography and electrophoresis[1]. Both of these methods separate proteins mainly according to charge. The gel-filtration method newly adopted for the purification of cellulases from the basidiomycete *Polyporus*