Some experiments were carried out with muscle α-glucan phosphorylase. Either a purified preparation[8] or the above-mentioned glycerol extract (which also has phosphorylase activity) were used, and as shown in Fig. 1, the results were very similar to those obtained with UDPG–glycogen glucosyltransferase.

Several synthetic nucleoside diphosphate sugars were tested as donors. ADP-glucose was found to be 50% as effective as UDPG of the same molarity, while CDP-glucose, IDP-glucose, and ADP-maltose did not serve as substrates for the enzyme.

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The inhibition of haemolysis by phloridzin

Delayed haemolysis in hypotonic glucose solutions is inhibited by phloridzin[1]; since delayed haemolysis also occurs in hypotonic solutions of malonamide[2] the effect of phloridzin on this system was examined by methods already described[3,4].

Fig. 1 illustrates the action on malonamide-induced haemolysis of 0.1% (w/v) phloridzin, in which concentration the osmotic contribution of the inhibitor is approximately equivalent to 0.05 atm.; the graphs show that although the osmotic contribution of phloridzin is about one tenth that of glucose and glycine, the inhibition it produces is much greater. The effect of temperature on the inhibited process was also examined and the combined results are recorded in Table I.

Glucose stabilizes the water lattice through the promotion of extensive hydrogen bonding and because a system of this nature is very heat sensitive, glucose inhibition

has an increased energy of activation over the control; this is in agreement with the increased entropy of activation, which implies greater order in the initial state compared with the control. Glycine, on the other hand, disrupts the water lattice, so the effect of heat is less and the entropy of activation is less in the inhibited system. Both glycine and glucose penetrate the cell membrane, but whereas the former stabilizes the internal structure and promotes water retention, the latter disrupts the intracellular phase and promotes the loss of water; cell swelling is less and inhibition therefore greater with glycine.

From these considerations it appears that phloridzin is a structure-breaking solute since both the energy of activation and the entropy of activation are lower than those of the control. However, when the effect of the inhibitors on osmotic haemolysis is examined, a rather different picture emerges, as shown in Table II.

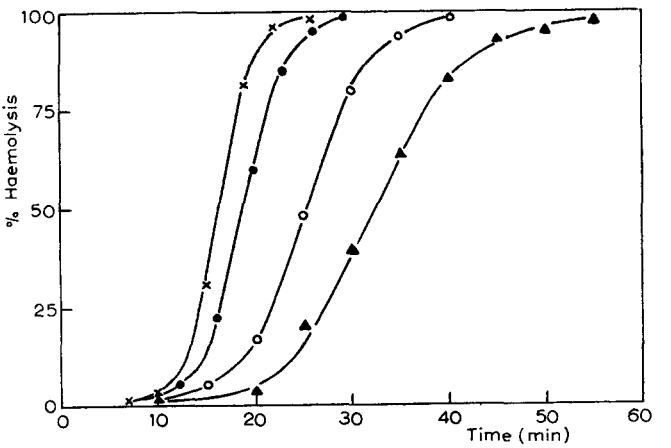

Fig. 1. The inhibition of malonamide-induced haemolysis. ×—×, control, 3.5 atm. malonamide; ●—●, with 0.5 atm. glucose; ○—○, with 0.5 atm. glycine; ▲—▲, with 0.05 atm. phloridzin.

TABLE I

THE INHIBITION OF MALONAMIDE-INDUCED HAEMOLYSIS

Inhibitor	*Inhibitor osmotic increment (atm.)*	*Degree of inhibition*	*Energy of activation (kcal)*	*Entropy of activation (cal/deg)*
None	0	0	10.9	63.6
Glucose	0.5	0.16	14.5	74.1
Glycine	0.5	0.57	9.5	51.0
Phloridzin	0.05	1.04	10.3	55.3

TABLE II

THE EFFECT OF THE INHIBITORS ON OSMOTIC HAEMOLYSIS

Inhibitor	*Inhibitor osmotic increment (atm.)*	*Degree of inhibition*	*Energy of activation (kcal)*	*Entropy of activation (cal/deg)*
None	0	0	5.1	35.5
Glucose	0.5	0.06	5.9	38.1
Glycine	0.5	0.18	4.0	31.1
Phloridzin	0.025	0.62	5.4	37.7
	0.05	1.41	6.2	39.7

While the order of effective inhibition remains the same, the effect of heat is greatest with phloridzin and the entropy of activation is highest in this system; this indicates that in osmotic haemolysis phloridzin behaves as a promotor of water structure and, allowing for the differences in concentration, the structure-promoting activity of this substance is very high indeed.

The results of the experiments are apparently contradictory, but it is possible to account for this in terms of structure interaction.

Accepting the evidence of the simpler experiments that phloridzin is a powerful promoter of water structure, and remembering that malonamide itself is a structure promoter, it seems likely that, in a system containing both solutes, the stabilizing forces will be in competition; since the action of phloridzin is much greater this predominates and malonamide is effectively a structure breaker. A structural equilibrium is therefore established in which the overall stability of the water lattice is slightly less than that which obtains in the presence of malonamide alone.

Competitive stabilization is not the only factor involved because, if it were, the degree of inhibition would be very much less, in keeping with the comparatively small change in the equilibrium water lattice.

Structure stabilization is essentially a long-range phenomenon, but lattice disruption tends to be a local effect, depending largely on the physical dimensions of the structure-breaking unit—including primary hydration. In the malonamide–phloridzin system, although the total number of intact hydrogen bonds is slightly less than it is in aqueous malonamide and the heat sensitivity is consequently less, it seems likely that these bonds will be preferentially orientated to occlude the disrupting units, for it is only in this way that overall lattice stability—which is still much greater than the stability of the pure water lattice—could be achieved. In this circumstance the principal constraint is exerted on the malonamide molecules and so these move more slowly into the cell; intracellular water retention is less, swelling is slower and haemolysis is slower also.

In these experiments therefore, through competitive stabilization, the equilibrium water lattice is little changed but the structure is so modified that a constraint is imposed on the movement of malonamide.

It may be that a similar mechanism is concerned in the action of phloridzin on the resorption of glucose by the kidney tubule, for even compared with glucose this substance is a much stronger promoter of structure in the water lattice.

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