THE PROTECTIVE EFFECT OF SOME CARBOHYDRATES AGAINST THE INACTIVATION OF COMPLEMENT BY HEAT, TOGETHER WITH A NOTE ON THE EFFECT OF GLYCOLAMIDE (AN ISOMER OF GLYCINE) ON HEAT-INACTIVATION

By the late J. GORDON and G. C. TURNER

Department of Bacteriology, School of Medicine, Leeds

Gordon & Turner (1955) reported that glucose and other sugars intensify the protective action of glycine against the heat-inactivation of complement, so that in their presence the concentration of glycine required to protect complement against inactivation at 55° C. is greatly reduced. Moreover, when high concentrations of glycine (e.g. 10 %) were used, higher temperatures were required to inactivate complement if the glycine was dissolved in a high concentration of a sugar. They also showed that although this intensification is a property of a wide range of sugars, it is not shown by the corresponding alcohols, although inositol is exceptional in causing marked intensification. No explanation was offered for these findings and further experiments have been undertaken to throw light on them.

Gordon & Turner (1956) stated that none of the carbohydrates they tested had any protective activity alone at 55° C., and indeed, so far as is known, only amino-acids have this property. There remains, however, the possibility that some degree of protection by carbohydrates might be demonstrable at temperatures below 55° C. We have found that as tested in our experiments complement is completely inactivated when heated for 30 min. at 53° C. (but not at 52° C.). The experiments reported here show that the carbohydrates previously found to intensify the protective action of glycine do themselves possess to a varying degree protective activity at 53 and 54° C.

EXPERIMENTAL

Gordon & Turner found that all the sugars they tried, whether monosaccharides or disaccharides, reducing or non-reducing substances, were intensifying agents, but no alcohols other than inositol had this action. A few of these substances, glucose, sucrose, dulcitol and inositol, were tested for *protective*, as opposed to intensifying, activity at 53° C.; the experimental details were as previously described (Gordon & Turner, 1955). As Table 1 shows, glucose, sucrose and inositol are protective, with high concentrations (a final concentration of 10 % in each case), but dulcitol is not. This corresponds to the action of the first three in intensifying the protective activity of glycine and of dulcitol in failing to do so.

More detailed experiments were carried out to determine what minimum concentration of each of the three protective substances was required to preserve demonstrable complement after heating at 53 and 54° C. respectively. Tables 2

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and 3 show that at 53° C. 15 % glucose, 20 % sucrose and 7 % inositol were required, but at 54° C., although 17 % inositol was effective, no protection was obtained with 20 % glucose and 20 % sucrose.

Table 1. *The effect of glucose, sucrose, dulcitol and inositol on complement heated at* 53° C. *for* 30 *min.*

Addition to 0·5 ml. of fresh guinea-pig serum of 0·5 ml. of:	Haemolytic effect of the addition of 0·3 ml. of sensitized R.B.C.'s to the following amounts of the mixture heated at 53° C. for 30 min. and then diluted 1 in 5 in saline								
	0·1	0·2	0·3	0·4	0·5	0·6	0·7	0·8	0·9
saline	0	0	0	0	0	0	0	0	0
20 % glucose	0	0	0	0	1	2	2	3	3
20 % sucrose	0	0	0	0	0	0	1	1	2
20 % dulcitol	0	0	0	0	0	0	0	0	0
20 % inositol	4	4	4	4	4	4	4	4	4

Control unheated mixtures of sensitized red cells and fresh guinea-pig serum diluted 1 in 10 with saline showed complete haemolysis at all concentrations of complement used.

In this and succeeding tables, the figures represent: 4, complete haemolysis; 3, almost complete haemolysis; 2, partial haemolysis; 1, trace of haemolysis; 0, no haemolysis. The tests were incubated at 37° C. and read after 2 hr.

Table 2. *The effect of various concentrations of glucose, sucrose or inositol on complement heated at* 53° C. *for* 30 *min.*

Addition to 0·5 ml. of fresh guinea-pig serum of 0·5 ml. of:	Haemolytic effect of the addition of 0·3 ml. of sensitized R.B.C.'s to the following amounts of the mixture heated at 53° C. for 30 min. and then diluted to 1 in 5 in saline								
	0·1	0·2	0·3	0·4	0·5	0·6	0·7	0·8	0·9
saline	0	0	0	0	0	0	0	0	0
20 % glucose	0	0	1	1	2	3	3	4	4
18 % glucose	0	0	0	0	0	1	2	3	4
15 % glucose	0	0	0	0	0	0	1	2	2
14 % glucose	0	0	0	0	0	0	0	0	0
20 % sucrose	0	0	0	0	0	0	1	1	2
19 % sucrose	0	0	0	0	0	0	0	0	0
20 % inositol	4	4	4	4	4	4	4	4	4
15 % inositol	4	4	4	4	4	4	4	4	4
10 % inositol	0	1	3	4	4	4	4	4	4
8 % inositol	0	0	0	1	2	2	3	4	4
7 % inositol	0	0	0	0	0	0	1	2	2
6 % inositol	0	0	0	0	0	0	0	0	1
5 % inositol	0	0	0	0	0	0	0	0	0

DISCUSSION

The fact that these carbohydrates are themselves slightly protective should be taken into account in assessing the significance of the intensification by sugars and by inositol of the protective effect of amino-acids against the heat-inactivation of complement. The protection is slight and is not demonstrable at 55° C., but is

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evident at 53° C. in the case of glucose, sucrose and inositol, and 54° C. in the case of inositol only. It occurs in the case of the monosaccharide glucose which is a reducing sugar and of the disaccharide sucrose which is not a reducing sugar. Inositol, the alcohol with a ring structure, is protective, whereas the straight-chain alcohol dulcitol is not. These findings are in parallel with those previously reported (Gordon & Turner, 1955, 1956) for intensification. Quantitative comparison of the two sugars in protection tests shows that 20 % glucose is more active than 20 % sucrose, a state of affairs similar to that observed in intensification tests, in which equimolar concentrations of these sugars are about equally effective, and in consequence twice the concentration of sucrose is necessary to produce the same effect as glucose. Inositol is much more active than glucose (the molecular weights being the same), and this again corresponds to the strong intensifying effect previously obtained with this alcohol.

Table 3. *The effect of various concentrations of glucose, sucrose or inositol on complement heated at* 54° C. *for* 30 *min.*

Addition to 0·5 ml. of fresh guinea-pig serum of 0·5 ml. of:	Haemolytic effect of the addition of 0·3 ml. of sensitized R.B.C.'s to the following amounts of the mixture heated at 54° C. for 30 min. and then diluted 1 in 5 in saline								
	0·1	0·2	0·3	0·4	0·5	0·6	0·7	0·8	0·9
saline	0	0	0	0	0	0	0	0	0
20 % glucose	0	0	0	0	0	0	0	0	0
20 % sucrose	0	0	0	0	0	0	0	0	0
20 % inositol	0	0	2	4	4	4	4	4	4
18 % inositol	0	0	0	0	1	1	2	3	3
17 % inositol	0	0	0	0	0	0	1	1	2
16 % inositol	0	0	0	0	0	0	0	0	0

SUMMARY

Glucose, sucrose and inositol fail to protect complement against heat inactivation at 55° C.

Complement is inactivated by heating at 53 and 54° C. Glucose and sucrose afford protection against heat-inactivation at 53° C. but not at 54° C. Inositol exerts a greater degree of protection and is effective at 54° C.

These findings should be taken into account in considering the significance of the fact that a wide range of sugars and also inositol but not other alcohols intensify the protective activity of amino-acids at 55° C. and higher temperatures.

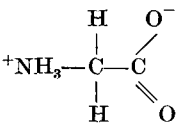
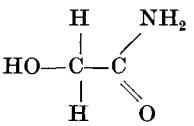
A NOTE ON THE EFFECT OF GLYCOLAMIDE ON THE HEAT-INACTIVATION OF COMPLEMENT

It is not known why, as Gordon (1953) reported, complement is not inactivated when heated at 55° C. in the presence of high concentrations of glycine and other amino-acids. The effect is intensified in the presence of some carbohydrates (Gordon & Turner, 1955), probably as a result of reinforcement from the slight

protective activity of these substances not demonstrable at 55° C., but, as we have shown above, observed at slightly lower temperatures. Apart from that, protective activity has only been demonstrated in the case of amino acids and we have attempted to discover the significance of this.

Gordon (1953) showed that certain derivatives of glycine had no protective action; in this paper we report similar negative results with glycolamide. This substance has the same empirical formula as glycine but a different molecular structure with a much lower melting-point and it does not ionize in solution. The differences between the two solutions are summarized in Table 4.

Table 4. *A summary of the properties of glycine and glycolamide*

	Glycine	Glycolamide
Molecular weight	75·07	75·07
Melting-point	230° C.	119° C.
Molecular structure		
	Zwitterion formation in solution	No ionization in solution

Glycolamide was prepared by the method suggested by Gucker, Ford & Moser (1939) and our sample had a melting-point of 117° C. A 20 % solution in distilled water was adjusted with NaOH to pH 7·5 and compared with a similar concentration of glycine tested by the method described by Gordon (1953).

Table 5. *The effect of* 20 % *glycolamide and* 20 % *glycine on the heat-inactivation of complement at* 55° C.

Addition to 0·5 ml. of fresh guinea-pig serum of 0·5 ml. of:	Haemolytic effect of the addition of 0·3 ml. of sensitized sheep R.B.C.'s to the following amounts of the mixture heated at 53° C. for 30 min.								
	0·1	0·2	0·3	0·4	0·5	0·6	0·7	0·8	0·9
saline	0	0	0	0	0	0	0	0	0
20 % glycine	4	4	4	4	4	4	4	4	4
20 % glycolamide	0	0	0	0	0	0	0	0	0
20 % glycolamide in 20 % glucose	0	0	0	0	0	0	0	0	0
20 % glycolamide in 20 % inositol	0	0	0	0	0	0	0	0	0

No protective activity was demonstrable with glycolamide at 55° C. even when dissolved in glucose or inositol (Table 5). In view of the possibility that it might possess a slight degree of protective power demonstrable only at a temperature lower than 55° C. the experiment was repeated at 53° C. but again with completely negative results.

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