THE INTENSIFYING EFFECT OF GLUCOSE ON THE PROTECTIVE ACTION OF GLYCINE AGAINST THE HEAT-INACTIVATION OF COMPLEMENT

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The addition to serum of glycine to a final concentration of 7·5 %, and occasionally of 5 %, was shown by Gordon (1953) to prevent the normal inactivation of complement by heating at 55° C. for 30 min. Most of the complement is, however, destroyed by heating at 57° C. This paper reports experiments which show that this protective action of glycine is intensified in the presence of glucose and other sugars.

The concentration of glycine required to prevent heat inactivation of complement at 55° C. is reduced to 1 % when glucose to a final concentration of 10 % (0·55 M) is added, although glucose alone has no protective effect. When a large amount of glycine (10 %) is added with glucose to serum, complete protection is obtained at 57° C. At higher temperature these substances afford little protection and heating at 58° C destroys most of the complementary activity.

EXPERIMENTAL

Glycine was dissolved with gentle heating for a few seconds either in distilled water or in sugar solutions of appropriate strength and the pH adjusted to 7·5 with NaOH.

0·5 ml. of the solution under test was added to 0·5 ml. of fresh undiluted guinea-pig serum, heated for 30 min., and after cooling diluted 1 in 5 in saline. The mixture was then tested for complementary activity by the addition of 0·1, 0·2, 0·3, 0·4, 0·5, 0·6, 0·7, 0·8 and 0·9 ml. respectively to 0·2 ml. of sensitized sheep red blood corpuscles.

The effect of aqueous solutions of glycine was compared with that of glycine dissolved in 20 % glucose by adding either substance under test to fresh guinea-pig serum and heating the mixture at 55° C. for 30 min. Table 1 shows that in the presence of glucose a final concentration of 1 % glycine is sufficient to afford protection to complement, compared with about 5 % when glycine alone is used.

To discover how much glucose was required to increase the protective activity of glycine, 5 % glycine, not itself protective, was used in the presence of concentrations of 5, 10 and 20 % respectively of glucose, and as shown in Table 2, at least 5 % of the sugar is required.

Similar results to those with glucose were obtained with a number of carbohydrates (Table 3). On the whole, equimolar concentrations were about equally effective, although the pentose monosaccharides were a little less active than expected.

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The degree of heat resistance achieved using glycine-glucose mixtures was tested as shown in Tables 4–6; 20 % glycine alone confers only slight protection at 57° C., whereas with the addition of 10 % or 20 % glucose full protection is obtained. At 58° C., glycine alone gives no protection at all, whereas with 20 % glucose some complementary activity is preserved.

Table 1. *The action of glucose in reducing the concentration of glycine required to protect complement heated at* 55° C *for* 30 *min.*

Addition to 0·5 ml. of fresh guinea-pig serum	Haemolytic effect of the addition of 0·2 ml. of sensitized sheep R.B.C.'s to the following amounts of the mixture heated at 55° C. for 30 min. and then diluted 1 in 5								
	0·1	0·2	0·3	0·4	0·5	0·6	0·7	0·8	0·9
0·5 ml. of saline	0	0	0	0	0	0	0	0	0
0·5 ml. of 20 % glycine in aq. dest.	4	4	4	4	4	4	4	4	4
0·5 ml. of 15 % glycine in aq. dest.	4	4	4	4	4	4	4	4	4
0·5 ml. of 10 % glycine in aq. dest.	0	0	1	1	2	4	4	4	4
0·5 ml. of 5 % glycine in aq. dest.	0	0	0	0	0	0	0	0	0
0·5 ml. of 5 % glycine in 20 % glucose	4	4	4	4	4	4	4	4	4
0·5 ml. of 4 % glycine in 20 % glucose	4	4	4	4	4	4	4	4	4
0·5 ml. of 3 % glycine in 20 % glucose	4	4	4	4	4	4	4	4	4
0·5 ml. of 2 % glycine in 20 % glucose	0	0	1	1	3	3	3	4	4
0·5 ml. of 1 % glycine in 20 % glucose	0	0	0	0	0	0	0	0	0

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 concentration of glucose required to enable* 5 % *glycine to protect complement heated at* 55° C. *for* 30 *min.*

Addition to 0·5 ml. of fresh guinea-pig serum of	Haemolytic effect of the addition of 0·2 ml. of sensitized sheep R.B.C.'s to the following amounts of the mixture heated at 55° C. for 30 min. and then diluted 1 in 5								
	0·1	0·2	0·3	0·4	0·5	0·6	0·7	0·8	0·9
0·5 ml. of 5 % glycine in aq. dest.	0	0	0	0	0	0	0	0	0
0·5 ml. of 5 % glycine in 20 % glucose	4	4	4	4	4	4	4	4	4
0·5 ml. of 5 % glycine in 10 % glucose	0	1	2	3	4	4	4	4	4
0·5 ml. of 5 % glycine in 5 % glucose	0	0	0	0	1	1	2	2	3

SUMMARY

The addition of glucose considerably reduces the amount of glycine required to protect complement against heat-inactivation.

A similar effect has been demonstrated with other sugars.

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Table 3. *The action of some carbohydrates in reducing the concentration of glycine required to protect complement heated at* 55° C. *for* 30 *min.*

Addition to 0·5 ml. of fresh guinea-pig serum of	Haemolytic effect of the addition of 0·2 ml. of sensitized sheep R.B.C.'s to the following amounts of the mixture heated at 55° C. for 30 min. and then diluted 1 in 5								
	0·1	0·2	0·3	0·4	0·5	0·6	0·7	0·8	0·9
Saline, 0·5 ml.	0	0	0	0	0	0	0	0	0
5 % glycine in 0·5 ml. aq. dest.	0	0	0	0	0	0	0	0	0
5 % glycine in 0·5 ml. 12 % arabinose	0	1	2	3	4	4	4	4	4
5 % glycine in 0·5 ml. 8 % arabinose	0	0	0	1	1	2	3	3	3
5 % glycine in 0·5 ml. 4 % arabinose	0	0	0	0	0	0	0	0	0
5 % glycine in 0·5 ml. 12 % xylose	0	0	1	2	3	3	4	4	4
5 % glycine in 0·5 ml. 8 % xylose	0	0	0	1	1	2	2	3	3
5 % glycine in 0·5 ml. 4 % xylose	0	0	0	0	0	0	0	0	0
5 % glycine in 0·5 ml. 10 % glucose	0	1	2	3	4	4	4	4	4
5 % glycine in 0·5 ml. 5 % glucose	0	0	0	0	1	1	2	2	3
5 % glycine in 0·5 ml. 10 % galactose	0	1	2	2	4	4	4	4	4
5 % glycine in 0·5 ml. 5 % galactose	0	0	0	0	1	1	2	3	3
5 % glycine in 0·5 ml. 10 % mannose	0	0	1	2	3	4	4	4	4
5 % glycine in 0·5 ml. 5 % mannose	0	0	0	0	0	0	0	0	0
5 % glycine in 0·5 ml. 20 % sucrose	3	4	4	4	4	4	4	4	4
5 % glycine in 0·5 ml. 10 % sucrose	0	0	0	1	2	2	3	3	3
5 % glycine in 0·5 ml. 20 % lactose	4	4	4	4	4	4	4	4	4
5 % glycine in 0·5 ml. 10 % lactose	0	0	1	1	3	3	4	4	4
5 % glycine in 0·5 ml. 20 % maltose	2	4	4	4	4	4	4	4	4
5 % glycine in 0·5 ml. 10 % maltose	0	0	0	0	0	0	0	0	0
5 % glycine in 0·5 ml. 30 % raffinose	4	4	4	4	4	4	4	4	4
5 % glycine in 0·5 ml. 15 % raffinose	0	1	1	2	3	3	3	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.

Table 4. *The action of glucose in increasing the protective power of glycine on complement heated at* 56° C. *for* 30 *min.*

Addition to 0·5 ml. of fresh guinea-pig serum of	Haemolytic effect of the addition of 0·2 ml. of sensitized sheep R.B.C.'s to the following amounts of the mixture heated at 56° C. for 30 min. and then diluted 1 in 5								
	0·1	0·2	0·3	0·4	0·5	0·6	0·7	0·8	0·9
Saline, 0·5 ml.	0	0	0	0	0	0	0	0	0
5 % glycine in 0·5 ml. aq. dest.	0	0	0	0	0	0	0	0	0
5 % glycine in 0·5 ml. 10 % glucose	0	0	0	0	0	1	2	3	3
5 % glycine in 0·5 ml. 20 % glucose	4	4	4	4	4	4	4	4	4
10 % glycine in 0·5 ml. aq. dest.	0	0	0	1	3	4	4	4	4
10 % glycine in 0·5 ml. 10 % glucose	4	4	4	4	4	4	4	4	4
10 % glycine in 0·5 ml. 20 % glucose	4	4	4	4	4	4	4	4	4
20 % glycine in 0·5 ml. aq. dest.	4	4	4	4	4	4	4	4	4
20 % glycine in 0·5 ml. 10 % glucose	4	4	4	4	4	4	4	4	4
20 % glycine in 0·5 ml. 20 % glucose	4	4	4	4	4	4	4	4	4

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Table 5. *The action of glucose in increasing the protective power of glycine on complement heated at* 57° C. *for* 30 *min.*

Addition to 0·5 ml. of fresh guinea-pig serum of	Haemolytic effect of the addition of 0·2 ml. of sensitized sheep R.B.C.'s to the following amounts of the mixture heated at 57° C. for 30 min. and then diluted 1 in 5								
	0·1	0·2	0·3	0·4	0·5	0·6	0·7	0·8	0·9
5 % glycine in 0·5 ml. aq. dest.	0	0	0	0	0	0	0	0	0
5 % glycine in 0·5 ml. 10 % glucose	0	0	0	0	0	0	0	0	0
5 % glycine in 0·5 ml. 20 % glucose	0	0	0	1	2	2	3	3	3
10 % glycine in 0·5 ml. aq. dest.	0	0	0	0	0	0	0	0	0
10 % glycine in 0·5 ml. 10 % glucose	0	0	0	0	2	2	3	3	3
10 % glycine in 0·5 ml. 20 % glucose	3	4	4	4	4	4	4	4	4
20 % glycine in 0·5 ml. aq. dest.	0	0	0	0	0	0	1	2	2
20 % glycine in 0·5 ml. 10 % glucose	0	1	2	2	3	3	4	4	4
20 % glycine in 0·5 ml. 20 % glucose	4	4	4	4	4	4	4	4	4

Table 6. *The action of glucose in increasing the protective power of glycine on complement heated at* 58° C. *for* 30 *min.*

Addition to 0·5 ml. of fresh guinea-pig serum of	Haemolytic effect of the addition of 0·2 ml. of sensitized sheep R.B.C.'s to the following amounts of the mixture heated at 58° C. for 30 min. and then diluted 1 in 5								
	0·1	0·2	0·3	0·4	0·5	0·6	0·7	0·8	0·9
5 % glycine in 0·5 ml. aq. dest.	0	0	0	0	0	0	0	0	0
5 % glycine in 0·5 ml. 10 % glucose	0	0	0	0	0	0	0	0	0
5 % glycine in 0·5 ml. 20 % glucose	0	0	0	0	0	0	0	0	0
10 % glycine in 0·5 ml. aq. dest.	0	0	0	0	0	0	0	0	0
10 % glycine in 0·5 ml. 10 % glucose	0	0	0	0	0	0	0	0	0
10 % glycine in 0·5 ml. 20 % glucose	0	0	0	0	0	0	0	0	0
20 % glycine in 0·5 ml. aq. dest.	0	0	0	0	0	0	0	0	0
20 % glycine in 0·5 ml. 10 % glucose	0	0	0	0	0	0	0	0	0
20 % glycine in 0·5 ml. 20 % glucose	0	0	0	0	1	2	2	2	3

REFERENCE

GORDON, J. (1953). The protective action of some amino-acids against the effect of heat on complement. *J. Hyg., Camb.*, **51**, 140.

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