FURTHER OBSERVATIONS ON THE PROTECTIVE ACTION OF GLYCINE AGAINST THE HEAT-INACTIVATION OF COMPLEMENT WITH PARTICULAR REFERENCE TO INTENSIFICATION BY SOME CARBOHYDRATES

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

Department of Bacteriology, School of Medicine, Leeds, 2

Gordon (1953) showed that guinea-pig serum to which glycine or certain other amino-acids had been added retained its complementary activity after heating at 55° C. for half an hour. Gordon & Turner (1955*a*) found that rabbit plasma heated at 55° C. for various times in the presence of glycine or other amino-acids was still clotted by staphylococcal coagulase or by thrombin, whereas plasma heated alone was no longer clotted by these agents. Later (1955*b*) these authors showed that the protective action of glycine against the heat-inactivation of complement was intensified in the presence of glucose and other sugars, although these substances are not themselves protective.

Experiments were devised to discover whether certain compounds derived from sugars, such as alcohols, acted in a similar way. Dulcitol, mannitol and sorbitol showed no intensifying action, unlike the sugars from which they are derived. Inositol, which is an alcohol structurally different from these, was however found to be at least as active as any of the sugars, and this will be discussed later in the paper.

EXPERIMENTAL

In the experiments reported here the method used for testing treated mixtures for complement activity was the same as that described by Gordon (1953).

0·5 ml. samples of serum were heated at 55° C. for 30 min. with 0·5 ml. of 5 % glycine, when inactivation took place, and with 0·5 ml. of 5 % glycine dissolved in 20 % glucose, which proved to be protective. A similar degree of protection was obtained with 20 % galactose, 20 % mannose and 20 % sorbose, but with the alcohols derived from these sugars by reduction, namely dulcitol, mannitol and sorbitol at the same concentration, only traces of complement activity were demonstrable after heating. (See Table 1.)

Another alcohol, inositol, structurally different from the foregoing, but readily available, was tested in the same way, and proved to have intensifying power similar to that of the sugars, and if anything, more powerful. Consequently, a further series of experiments was devised to compare its activity in this respect quantitatively with that of glucose.

Gordon & Turner (1955*b*) have reported that in the presence of 20 % glucose as little as 1 % glycine may be protective when added to serum. With 20 % inositol, 0·5 % glycine is sufficient to interfere with heat-inactivation (Table 2). Experiments were also undertaken to discover at what temperature complement was

inactivated in the presence of 20 % glycine in 20 % inositol. As Table 3 indicates, inositol reinforces the protective power of glycine to the extent of preserving complement activity after heating at 59° C. for 30 min. by comparison with an upper limit of 58° C. in the case of glycine and glucose and 57° C. with glycine alone.

Table 1. *A comparison of the action of various sugars and alcohols in intensifying the protective activity of glycine*

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 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	0	0	0	0	0	0	0	0
	aq. dest.	0	0	0	0	0	0	0	0	0
	20 % glucose	4	4	4	4	4	4	4	4	4
	20 % galactose	4	4	4	4	4	4	4	4	4
	20 % dulcitol	0	0	0	0	0	0	0	0	1
0·5 ml. of 5 % glycine in	20 % mannose	4	4	4	4	4	4	4	4	4
	20 % mannitol	0	0	0	0	0	0	0	0	1
	20 % sorbose	4	4	4	4	4	4	4	4	4
	20 % sorbitol	0	0	0	0	0	0	0	0	1
	20 % inositol	4	4	4	4	4	4	4	4	4

In this and subsequent 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. *A comparison of the actions of glucose and inositol in reducing the concentration of glycine required to protect complement from heat-inactivation*

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 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	0	0	0	0	0	0	0	0
2 % glycine in 20 % glucose	4	4	4	4	4	4	4	4	4
1 % glycine in 20 % glucose	0	0	0	1	2	3	3	4	4
0·5 % glycine in 20 % glucose	0	0	0	0	0	0	0	0	0
2 % glycine in 20 % inositol	4	4	4	4	4	4	4	4	4
1 % glycine in 20 % inositol	4	4	4	4	4	4	4	4	4
0·5 % glycine in 20 % inositol	0	0	1	1	3	3	4	4	4

The experiments on complement as a whole were also adapted to test whether similar phenomena were demonstrable in the case of the relatively heat-stable fourth component of complement described by Gordon, Whitehead & Wormall (1926). The fourth component was inactivated by adding 0·2 ml. of N-NH_4OH to 2 ml. of serum and incubating at 37° C. for 3 hr. No complement activity was demonstrable at this stage, but full activity was restored by the addition to 0·1 ml. of the ammonia-inactivated serum of 0·1 ml. of serum inactivated by heating at 55° C. for 30 min. This restoration did not occur if the serum was heated at 63° C.

for 30 min., i.e. at this temperature the fourth component is destroyed. As Table 4 shows, fourth component activity was preserved after heating at 63° C. for 30 min. in serum to which 20 % glycine was added, but inactivation occurred at 64° C. Glycine was more active in the presence of 20 % glucose, and even more so in the presence of 20 % inositol, when some degree of protection was conferred at 65° C.

Table 3. *A comparison of the actions of glucose and inositol in increasing the protective power of glycine on complement heated at* 58° *and* 59° *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 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
20 % glycine	0	0	0	0	0	0	0	0	0
20 % glycine in 20 % glucose	0	0	0	1	2	2	3	3	4
20 % glycine in 20 % inositol	4	4	4	4	4	4	4	4	4
As above, but heated at 59° C. for 30 min.									
20 % glycine	0	0	0	0	0	0	0	0	0
20 % glycine in 20 % glucose	0	0	0	0	0	0	0	0	0
20 % glycine in 20 % inositol	0	0	0	1	2	3	4	4	4

Table 4. *The effect of glycine-glucose and glycine-inositol mixtures in protecting the fourth component of complement from inactivation by heat*

Addition to 0·1 ml. of ammoniated serum of 0·2 ml. of serum heated with	Haemolytic effect of the addition of 0·3 ml. of sensitized R.B.C.'s to the mixture in the case of serum heated at					
	55° C.	62° C.	63° C.	64° C.	65° C.	66° C.
saline	4	4	0	0	0	0
5 % glycine	4	4	0	0	0	0
5 % glycine in 20 % glucose	4	4	0	0	0	0
5 % glycine in 20 % inositol	4	4	1	0	0	0
10 % glycine	4	4	0	0	0	0
10 % glycine in 20 % glucose	4	4	2	0	0	0
10 % glycine in 20 % inositol	4	4	4	0	0	0
20 % glycine	4	4	4	0	0	0
20 % glycine in 20 % glucose	4	4	4	4	0	0
20 % glycine in 20 % inositol	4	4	4	4	1	0

To attempt to discover whether any large complex was formed by the addition to serum of glycine and glucose and the subsequent application of heat, the effect of dialysis on the system was investigated. Dialysis of serum and consequent loss of salts causes precipitation of some protein, which is redissolved when enough sodium chloride is added to make the residue isotonic. This procedure does not impair the complementary activity of normal serum. Samples of serum with saline, with 20 % glycine or with 20 % glycine in 20 % glucose were dialysed through cellophane against distilled water for 24 hr. From the results set out in Table 5 it will be seen that although serum may be inactivated by heating at 55° C. for 15 min.,

and complementary activity not restored by dialysis, this activity is preserved when the serum is heated in this way with 20% glycine or with 20% glycine in 20% glucose and the system subsequently dialysed and 'reconstituted' with NaCl. If, however, heat is again applied at 55° C. for 15 min. after dialysis, complete inactivation occurs, although it does not do so if the system is not dialysed. In other words, the protective activity in systems made up of serum + glycine or serum + glycine in glucose is lost on dialysis.

Table 5. *The effect of dialysis on the protective action of* 20% *glycine and of* 20% *glycine in* 20% *glucose*

Mixture	Haemolytic effect of the addition of 0·3 ml. of sensitized sheep R.B.C.'s to the mixture: Heated at 55° C. for 15 min.	Heated at 55° C. for 15 min., dialysed and reconstituted with NaCl	Heated at 55° C. twice for 15 min.	Heated at 55° C., dialysed, reconstituted with NaCl and heated again
0·1 ml. of serum + saline (equal parts)	0	0	0	0
0·1 ml. of serum + 20% glycine (equal parts)	4	4	4	0
0·1 ml. of serum + 20% glycine in 20% glucose (equal parts)	4	4	4	0

DISCUSSION

The protective action of glycine and other amino-acids against the inactivation of complement by heat is intensified in the presence of sugars, but is not affected, or only very slightly, by the alcohols derived from certain of the sugars tested. Inositol, however, an alcohol with a cyclic structure, acts in this respect like the sugars by intensifying the action of glycine.

The mode of action of the amino-acids in affording protection to complement and of the sugars and inositol in intensifying the effect is a matter for conjecture. The fact that serum-glycine-glucose mixtures subjected to dialysis are inactivated at 55° C. as readily as untreated serum suggests that if a complex is formed between glycine, glucose and serum it is readily dissociable.

SUMMARY

1. The protection afforded by glycine and other amino-acids to guinea-pig complement against heat-inactivation is increased in the presence of sugars, although the latter are not themselves protective.

2. No significant increase in protective activity occurs in the presence of the alcohols derived from some of the sugars tested.

3. A marked exception amongst the alcohols is inositol, which reinforces the protective power of glycine at least as much as do the sugars.

4. The fourth component of complement is destroyed by heating at 63° C. for 30 min. It is, however, protected against heat-inactivation by glycine, and this protective effect is intensified in the presence of glucose and inositol.

5. The protection afforded to serum by the presence of glycine or glycine dissolved in glucose is removed when the system is dialysed, indicating that if a complex is formed it is readily dissociable.

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