THE PROTECTIVE ACTION OF SOME AMINO-ACIDS AGAINST THE EFFECT OF HEAT ON COMPLEMENT

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That complement is labile at 55° C. is well known. Any alteration of this lability should be of interest. Preliminary observations showed that if glycine was added in high concentrations to guinea-pig serum and the mixture was heated at 55° C. for 30 min. it still possessed complement activity. This finding was extended by experiments with other amino-acids, derivatives of glycine, some 'protective colloids' and a few other substances.

EXPERIMENTAL

Action of amino-acids

Aqueous solutions of glycine, adjusted to pH 7·5 with dilute NaOH, were added in 1 ml. amounts to each of a series of tubes, each of which contained 1 ml. of guinea-pig serum. The glycine concentrations used were 20, 15, 10 and 5 %. The mixtures were allowed to stand on the bench for half an hour, heated at 55° C. for half an hour, cooled and then diluted 1:5 with normal saline. These solutions were then added in volumes of 0·1, 0·2, 0·3, 0·4 and 0·5 ml. to 0·3 ml. of 4 % sensitized ox red blood cells and incubated at 37° C. for 2 hr. The results of such an experiment are given in Table 1. With concentrations of 20 and 15 % glycine, the complement was not destroyed by heating at 55° C. for half an hour. Glycine always had this protective effect at 20 and 15 %, sometimes it protected at 10 %. In the experiments recorded in this paper 1 ml. of the test substance at a stated concentration was always added to 1 ml. of guinea-pig serum so that the resulting mixture was always half the original concentration. In a parallel series of experiments in which the same concentrations of glycine were added to the serum, no complement activity survived heating to 60° C. for half an hour. Control tubes showed that glycine in these concentrations was not in itself haemolytic. The minimum haemolytic dose of complement was not increased if 1 ml. of a 20 % glycine solution was added to 1 ml. serum, the unheated mixture being then diluted 1:10 with normal saline.

The temperature at which the complement was destroyed in a serum-20 % glycine mixture was next determined. Sensitized red cells were haemolysed when heated at 55° C. for half an hour, at 56° C. the reaction was much slower, at 57° C. most of the complement activity was destroyed, and at 58° C. it was completely destroyed.

Similar effects to glycine were obtained, with β-alanine (see Table 2), L-alanine and DL-alanine.

Other amino-acids were tested at concentrations roughly corresponding mole for mole to 20 % glycine, e.g. enough DL-glutamic acid to make 10 ml. of a 40 %

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solution was neutralized with NaOH and made up to volume; this gave a 46 % solution of sodium glutamate. Using such a solution of the amino-acid at high concentrations of 40 and 30 % the complement was not destroyed when heated at 55° C. The results are given in Table 3. A similar effect was obtained using similar concentrations of DL-aspartic acid (see Table 4).

Table 1. *The effect of glycine on the heat-lability of complement*

	Volumes of solutions used				
	0·1 ml.	0·2 ml.	0·3 ml.	0·4 ml.	0·5 ml.
1·0 ml. fresh guinea-pig serum + 1·0 ml. 20 % solution of glycine in distilled water, allowed to stand on bench for ½ hr., heated 55° C. for ½ hr. and then diluted 1:5 with normal saline	4	4	4	4	4
1·0 ml. fresh guinea-pig serum + 1·0 ml. 15 % solution of glycine in distilled water, allowed to stand on bench for ½ hr., heated 55° C. for ½ hr. and then diluted 1:5 with normal saline	4	4	4	4	4
1·0 ml. fresh guinea-pig serum + 1·0 ml. 10 % solution of glycine in distilled water, allowed to stand on bench for ½ hr., heated 55° C. for ½ hr. and then diluted 1:5 with normal saline	0	0	0	0	0
1·0 ml. fresh guinea-pig serum + 1·0 ml. 5 % solution of glycine in distilled water, allowed to stand on bench for ½ hr., heated 55° C. for ½ hr. and then diluted 1:5 with normal saline	0	0	0	0	0
1·0 ml. fresh guinea-pig serum + 1·0 ml. 20 % solution of glycine in distilled water, allowed to stand on bench for ½ hr., heated at 60° C. for ½ hr., then diluted 1:5 with normal saline	0	0	0	0	0
1·0 ml. fresh guinea-pig serum + 1·0 ml. 20 % solution of glycine in distilled water, stood for 30 min., diluted 1:5 with normal saline	4	4	4	4	4
1·0 ml. normal saline + 1·0 ml. 20 % solution of glycine in distilled water, stood for 30 min., diluted 1:5 with normal saline	0	0	0	0	0

The figures in Tables 1–4 represent: 4 = complete haemolysis; 3 = almost complete haemolysis; 2 = partial haemolysis; 1 = trace of haemolysis; 0 = no haemolysis.
0·3 ml. of sensitized ox cells added to each tube, incubated at 37° C. and readings taken after 2 hr.

No protective effects were, however, obtained using 20 % solutions in distilled water of DL-α-amino-n-butyric acid and DL-α-amino-iso-butyric acid. These substances at this concentration did not interfere with the action of complement, nor did they have any haemolytic action by themselves on sensitized red blood cells.

Derivatives of glycine

Derivatives of glycine were next tested. Glycine ethyl ester hydrochloride when used in the same concentrations as glycine gave no protection against the destruction of complement by heat. Acetyl-glycine neutralized with caustic soda was used in concentrations of 30, 23 and 15 % (the 30 % being roughly equimolar with the 20 % glycine) at these concentrations complement activity was not interfered with but no protection against heat-inactivation resulted.

Benzoyl-glycine neutralized in a similar way and used in concentrations of 47,

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30 and 20% (the 47% being roughly equimolar with the 20% glycine) interfered with the action of complement, and it was only at concentrations of 10 and 5% that complement was not interfered with, but at these concentrations benzoyl-glycine gave no protection against the destruction of complement by heat. Glycyl-glycine neutralized to pH 7·5 was used in concentrations of 30, 23 and 15%; at these concentrations it had very little effect on complement but it did not protect complement against heat inactivation.

Table 2. *The effect of β-alanine on the heat-lability of complement*

	Volumes of solutions used				
	0·1 ml.	0·2 ml.	0·3 ml.	0·4 ml.	0·5 ml.
1·0 ml. fresh guinea-pig serum + 1·0 ml. 20% solution of β-alanine in distilled water, allowed to stand on bench for ½ hr., heated 55° C. for ½ hr. and then diluted 1:5 with normal saline	4	4	4	4	4
1·0 ml. fresh guinea-pig serum + 1·0 ml. 15% solution of β-alanine in distilled water, allowed to stand on bench for ½ hr., heated 55° C. for ½ hr. and then diluted 1:5 with normal saline	2	3	4	4	4
1·0 ml. fresh guinea-pig serum + 1·0 ml. 10% solution of β-alanine in distilled water, allowed to stand on bench for ½ hr., heated 55° C. for ½ hr. and then diluted 1:5 with normal saline	0	0	1	2	3
1·0 ml. fresh guinea-pig serum + 1·0 ml. 5% solution of β-alanine in distilled water, allowed to stand on bench for ½ hr., heated 55° C. for ½ hr. and then diluted 1:5 with normal saline	0	0	0	0	0
1·0 ml. fresh guinea-pig serum + 1·0 ml. 20% solution of β-alanine in distilled water, allowed to stand on bench for ½ hr., heated at 60° C. for ½ hr. and then diluted 1:5 with normal saline	0	0	0	0	0
1·0 ml. fresh guinea-pig serum + 1·0 ml. 20% solution of β-alanine in distilled water, allowed to stand on bench for 30 min. and then diluted 1:5 with normal saline	4	4	4	4	4
1·0 ml. normal saline + 1·0 ml. 20% solution of β-alanine in distilled water, allowed to stand on bench for 30 min. and then diluted 1:5 with normal saline	0	0	0	0	0

Some substances that might be regarded as 'protective colloids'

Gum acacia. To 1 ml. quantities of guinea-pig serum were added 1 ml. amounts of either 20, 15, 10 or 5% solution of pulverized gum acacia. At none of these concentrations did gum acacia give any protection against the usual heat-lability of complement nor did it in itself inactivate complement.

Dextran (Bengers), and also a fraction of dextran, the latter approximately of a molecular weight of 200,000 (kindly provided by Dr T. D. Day), were used in the same concentrations as the previous substance. A concentration of 20% appeared to be about its saturation point. On heating these substances with serum at 55° C., the complement was destroyed, no protection being observed, these substances in themselves having no action on unheated complement. Similar results were obtained with *gelatin*, *dextrin* and *glycerol* in the same concentrations, these

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substances themselves having no anticomplementary or haemolytic effect in the concentrations used.

Starch was used at a concentration of 5 %. No protection was obtained.

Effect of other substances on the heat-lability of complement

Egg albumin, whether from a dried preparation or as fresh egg-white, showed no protective action when used at a concentration of 20 % (dried) or as 50 % egg white. A sample of bovine serum albumin (9 %) also gave a negative result.

Table 3. *The effect of* DL-*glutamic acid on the heat-lability of complement*

	Volumes of solutions used				
	0·1 ml.	0·2 ml.	0·3 ml.	0·4 ml.	0·5 ml.
1·0 ml. fresh guinea-pig serum + 1·0 ml. 40 % solution of DL-glutamic acid in distilled water, allowed to stand on bench for ½ hr., heated 55° C. for ½ hr. and then diluted 1:5 with normal saline	4	4	4	4	4
1·0 ml. fresh guinea-pig serum + 1·0 ml. 30 % solution of DL-glutamic acid in distilled water, allowed to stand on bench for ½ hr., heated 55° C. for ½ hr. and then diluted 1:5 with normal saline	4	4	4	4	4
1·0 ml. fresh guinea-pig serum + 1·0 ml. 20 % solution of DL-glutamic acid in distilled water, allowed to stant on bench for ½ hr., heated 55° C. for ½ hr. and then diluted 1:5 with normal saline	0	0	0	0	0
1·0 ml. fresh guinea-pig serum + 1·0 ml. 10 % solution of DL-glutamic acid in distilled water, allowed to stand on bench for ½ hr., heated 55° C. for ½ hr. and then diluted 1:5 with normal saline	0	0	0	0	0
1·0 ml. fresh guinea-pig serum + 1·0 ml. normal saline, allowed to stand on bench for ½ hr., heated 55° C. for ½ hr. and then diluted 1:5 with normal saline	0	0	0	0	0
1·0 ml. fresh guinea-pig serum + 1·0 ml. 20 % solution of glycine in distilled water, allowed to stand on bench for ½ hr., heated 55° C. for ½ hr. and then diluted 1:5 with normal saline	4	4	4	4	4
1·0 ml. fresh guinea-pig serum + 1·0 ml. 40 % solution of DL-glutamic acid in distilled water, allowed to stand on bench for ½ hr., and then diluted 1:5 with normal saline	4	4	4	4	4

Urea and glucose. Concentrations of 10, 20 and 30 % added to serum gave no protection against heating at 55° C., these substances at these concentrations did not interfere with complement.

Para-amino salicylic acid (neutralized to pH 7·5). Owing to its marked anti-complementary effect in very high concentrations, only 6, 10 and 15 % were used and at these concentrations complement was not interfered with, and on mixing these concentrations of the substance with serum and heating at 55° C. the complement was destroyed.

Para-amino-benzoic acid (converted to the sodium salt at pH 7·5). Owing to its anticomplementary power above 10 %, concentrations of 10 and 5 % were added to serum and heated at 55° C. The complement was completely destroyed and no protective effect was observed.

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Acetamide. Using concentrations from 40 % down to 5 % no anticomplementary effect nor any protective effect was observed.

Table 4. *The effect of* DL-*aspartic acid on the heat-lability of complement*

	Volumes of solutions used				
	0·1 ml.	0·2 ml.	0·3 ml.	0·4 ml.	0·5 ml.
1·0 ml. fresh guinea-pig serum + 1·0 ml. 40 % solution of DL-aspartic acid in distilled water, allowed to stand on bench for ½ hr., heated 55° C. for ½ hr. and then diluted 1:5 with normal saline	4	4	4	4	4
1·0 ml. fresh guinea-pig serum + 1·0 ml. 30 % solution of DL-aspartic acid in distilled water, allowed to stand on bench for ½ hr., heated 55° C. for ½ hr. and then diluted 1:5 with normal saline	4	4	4	4	4
1·0 ml. fresh guinea-pig serum + 1·0 ml. 20 % solution of DL-aspartic acid in distilled water, allowed to stand on bench for ½ hr., heated 55° C. for ½ hr. and then diluted 1:5 with normal saline	0	0	0	0	0
1·0 ml. fresh guinea-pig serum + 1·0 ml. 10 % solution of DL-aspartic acid in distilled water, allowed to stand on bench for ½ hr., heated 55° C. for ½ hr. and then diluted 1:5 with normal saline	0	0	0	0	0
1·0 ml. fresh guinea-pig serum + 1·0 ml. 20 % solution of glycine in distilled water, allowed to stand on bench for ½ hr., heated 55° C. for ½ hr. and then diluted 1:5 with normal saline	4	4	4	4	4
1·0 ml. fresh guinea-pig serum + 1·0 ml. normal saline, allowed to stand on bench for ½ hr., heated 55° C. for ½ hr. and then diluted 1:5 with normal saline	0	0	0	0	0
1·0 ml. fresh guinea-pig serum + 1·0 ml. 40 % solution of DL-aspartic acid in distilled water, allowed to stand on bench for ½ hr. and then diluted 1:5 with normal saline	4	4	4	4	4

40 % aspartic acid as Na salt (5 g. DL-aspartic acid neutralized to pH 7·5 and made up to 12·5 ml.).

SUMMARY

1. Glycine, alanine, and several isomers of alanine, DL-glutamic acid and DL-aspartic acid, when added to fresh guinea-pig serum and allowed to stand on the bench for half an hour, will protect the complement of this serum from destruction by heating at 55 and 56° C. for half an hour, but most of the complement activity is destroyed by heating at 57° C. and it is completely destroyed at 58° C. after half an hour.

2. Derivatives of glycine do not have any protective effect.

3. Various substances of high molecular weight, that might be described as 'protective colloids' do not have any protective effect.

4. How these amino-acids when added to serum alter the heat-lability of the complement is not understood.

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