THE EFFECT OF HEAT ON THE CLOTTING OF PLASMA BY STAPHYLOCOCCAL COAGULASE AND BY THROMBIN WITH PARTICULAR REFERENCE TO THE PROTECTIVE ACTION OF GLYCINE

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Gordon (1953) showed that the addition of glycine in certain concentrations to guinea-pig serum prevents the normal inactivation of complement by heating at 55° C. for 30 min. Even in the presence of glycine, however, a good deal of complementary activity is lost by heating at 57° C., and it is completely destroyed at 58° C. Duthie (1954), in a study of staphylococcal coagulase, noted that human or rabbit plasma heated at 56° C. for 4 min. was no longer clotted by the subsequent addition of 'free' staphylococcal coagulase or by thrombin. Experiments are reported in this paper which show that the heat lability of this latter phenomenon is also altered by glycine, the addition of which enables the plasma to withstand heating to 61° C.

EXPERIMENTAL

Plasma. Samples of plasma were collected by adding to 9 vol. of freshly drawn blood 1 vol. of a 1·34 % solution of sodium oxalate (Analar) and centrifuging to remove cellular elements.

Coagulase tests. 0·1 ml. of an overnight broth culture of the Oxford staphylococcus was added to 0·5 ml. of diluted plasma and the system incubated at 37° C. for 1 hr. The tubes were then inspected for the presence of a clot.

Thrombin. 0·1 ml. of a 1 % solution of dried thrombin, 5 units/mg. (Maw), was added to 0·5 ml. of diluted plasma.

RESULTS

The protective effect of glycine was demonstrated by heating two samples of diluted rabbit plasma, one in the presence of saline, the other in the presence of 20 % glycine. Coagulase tests on these samples and on similar but unheated mixtures were carried out as shown in Table 1.

Table 1. *The protective effect of* 20 % *glycine on the staphylococcal coagulase test using rabbit plasma*

Mixture	Effect of addition to mixture of 0·1 ml. of staphylococcal broth culture
0·5 ml. of 1/5 plasma + 0·5 ml. of saline	Clot
0·5 ml. of 1/5 plasma + 0·5 ml. of 20 % glycine	Clot
0·5 ml. of 1/5 plasma + 0·5 ml. of saline (heated together at 56° C. for 4 min.)	No clot
0·5 ml. of 1/5 plasma + 0·5 ml. of 20 % glycine (heated together at 56° C. for 4 min.)	Clot

To determine the concentration of glycine necessary to afford protection a series of tests was set up using concentrations between 1 and 20 %; and in addition to staphylococcal coagulase, thrombin was also used as a clotting agent. Smith & Hale (1944) suggested that free coagulase acted on prothrombin or some closely related substance, converting it into a product with a thrombin-like action on fibrinogen, so that it was considered to be of some interest to compare the results using the two clotting agents. The results are set out in Table 2.

Table 2. *Protective effect of various concentrations of glycine against the effect of heat on the coagulability of rabbit plasma by staphylococcal coagulase and by thrombin*

Addition to 0·5 ml. of 1/5 plasma of			Effect on mixture of addition of	
Saline (ml.)	Glycine (ml.)	Treatment of mixture	0·1 ml. overnight culture of Oxford staphylococcus	0·1 ml. of 1 % thrombin
0·5	—	Unheated	Clot	Clot
0·5	—	Heated at 56° C. for 4 min.	No clot	No clot
—	0·5, 20 %		Clot	Clot
—	0·5, 15 %		Clot	Clot
—	0·5, 10 %		Clot	Clot
—	0·5, 5 %		Clot	Clot
—	0·5, 4 %		Clot	Clot
—	0·5, 3 %		Clot	Clot
—	0·5, 2 %		No clot	No clot
—	0·5, 1 %		No clot	No clot

It was noticed that after a sample of plasma alone had been heated at 56° C. for 4 min., a marked floccular turbidity was present, which was also present in those heated plasma-glycine mixtures where the amount of glycine present was insufficient to protect the clotting power of the plasma from the action of heat. Heated plasma-glycine mixtures that were subsequently clotted by the addition of a suitable agent retained, despite the heat treatment, the uniform transparency characteristic of the unheated mixtures.

An attempt was next made to compare directly the protective action of glycine against the action of heat on clotting power with that reported by Gordon (1953) against the action of heat on complement. Rabbit plasma, however, is not a good source of complement, and guinea-pig plasma, which is more reliable from this point of view, was used in subsequent experiments. As Smith & Hale (1944) and Duthie (1954) have pointed out, however, guinea-pig plasma gives a poor or negative result with the staphylococcal coagulase test; thrombin was therefore used as the sole clotting agent.

As complement in serum is inactivated when heated in the presence of glycine at 58° C. for 30 min. (Gordon, 1953), experiments were undertaken to determine the temperature necessary to render plasma heated in the presence of glycine insensitive to thrombin, and also to ascertain to what temperature glycine pro-

tected complement in plasma heated for only 4 min. The results are set out in Tables 3 and 4.

Some of the complement titrations were repeated adding much larger volumes (up to 1 ml.) of the mixtures, heated to 59, 60 and 61° C., in order to discover whether traces of complement were present in those heated at these temperatures.

Table 3. *Protective effect of glycine against the effect of heat on the clotting power of guinea-pig plasma between* 56 *and* 62° *C.*

Addition to 0·5 ml. of 1/5 plasma of		Effect of addition to mixture of 0·1 ml. of 1 % thrombin after heating for 4 min. at			
Saline (ml.)	Glycine (ml.)	56° C.	60° C.	61° C.	62° C.
0·5	—	No clot	No clot	No clot	No clot
—	0·5, 20 %	Clot	Clot	Clot	No clot
—	0·5, 15 %	Clot	Clot	No clot	No clot
—	0·5, 10 %	Clot	No clot	No clot	No clot
—	0·5, 5 %	No clot	No clot	No clot	No clot

Table 4. *Protective effect of glycine against the effect of heat on the complementary activity of guinea-pig plasma between* 56 *and* 60° *C.*

Addition to 0·5 ml. of 1/5 plasma of		Haemolytic effect of the addition of 0·3 ml. of sensitized R.B.C.'s to 0·1, 0·2 and 0·3 ml. respectively of the mixture heated for 4 min. at													
Saline (ml.)	Glycine (ml.)	56° C.			57° C.			58° C.			59° C.			60° C.		
0·5	—	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
—	0·5, 20 %	4	4	4	4	4	4	4	4	4	0	1	2	0	0	0
—	0·5, 15 %	4	4	4	3	4	4	1	3	4	0	1	2	0	0	0
—	0·5, 10 %	4	4	4	0	3	3	0	2	2	0	0	1	0	0	0
—	0·5, 5 %	2	3	4	0	0	1	0	0	0	0	0	0	0	0	0

4 = complete haemolysis; 3 = almost complete haemolysis; 2 = partial haemolysis; 1 = trace of haemolysis; 0 = no haemolysis.

No complementary activity was however detectable in those mixtures heated at 60° C. The persistence of activity up to 59° C. fits in very well with the previously recorded end-point with 20 % glycine of 58° C., since in this case heating was for 4 min. only instead of half an hour.

In contrast with complementary activity, coagulability remained after heating in the presence of 20 % glycine at 61° C. Presumably the glycine exerts a protective effect on fibrinogen, which is otherwise rendered in some way insensitive to the action of thrombin when heated at this temperature.

SUMMARY

1. Plasma heated to 56° C. for 4 min. is not clotted by staphylococcal coagulase or thrombin, but when heated in the presence of glycine remains sensitive to the action of these clotting agents.

2. With glycine, plasma no longer clots after heating at 62° C.

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REFERENCES

DUTHIE, E. S. (1954). Evidence for two forms of staphylococcal coagulase. *J. gen. Microbiol.* **10**, 427.

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

SMITH, W. & HALE, J. H. (1944). The nature and mode of action of staphylococcus coagulase. *Brit. J. exp. Path.* **25**, 101.

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