

Mucolytic Enzyme Systems. V. Anaphylactic Effects on Hyaluronidase Inhibitor in Serum of Normal and Herpetic Rabbits.*

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In an earlier study,¹ it was demonstrated that poliomyelitis infections provoked a significant elevation in the concentration of hyaluronidase inhibitor in blood serum. Good and Campbell² showed that anaphylactic shock can precipitate an active encephalitis in rabbits in which *Herpes simplex* virus infection is latent after recovery from an attack of the disease. Accordingly, an investigation was undertaken to determine (1) the effect of *Herpes* infection on the level of the hyaluronidase inhibitor in the blood serum of rabbits,

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¹ Glick, D., and Gollan, F., *J. Inf. Dis.*, 1948, **83**, 200.

² Good, R. A., and Campbell, B., *Proc. Soc. Exp. Biol. and Med.*, 1948, **68**, 82.

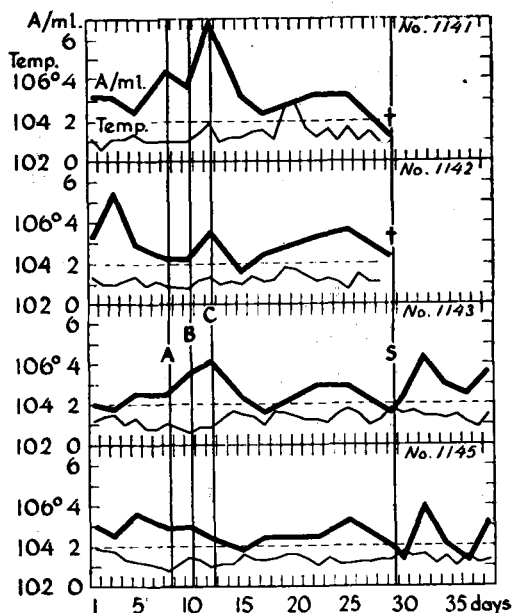


FIG. 1.

Effect of sensitization to egg white, and shock, on the level of hyaluronidase inhibitor in rabbit serum. A, B, C represent the time of sensitizing injections and S the time of the shock injection.

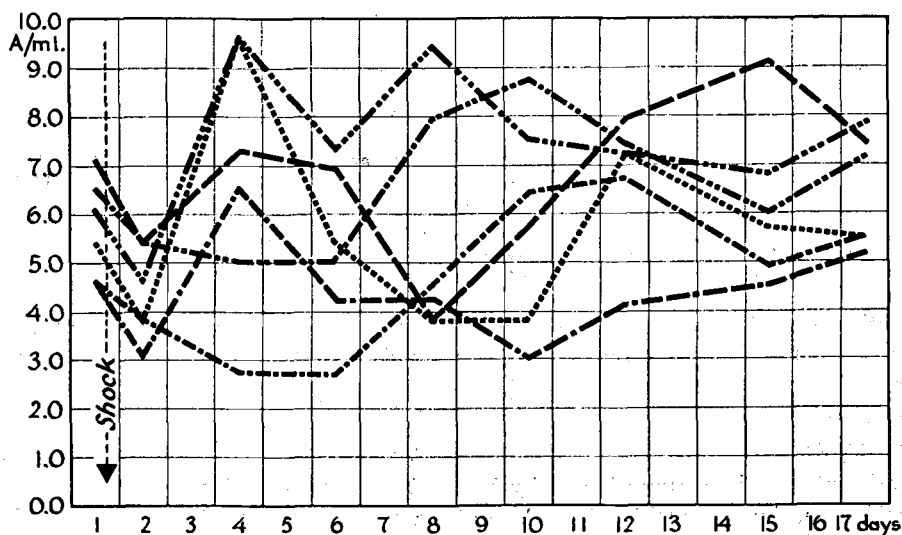


FIG. 2.

Effect of anaphylactic shock on the level of hyaluronidase inhibitor in rabbit serum.

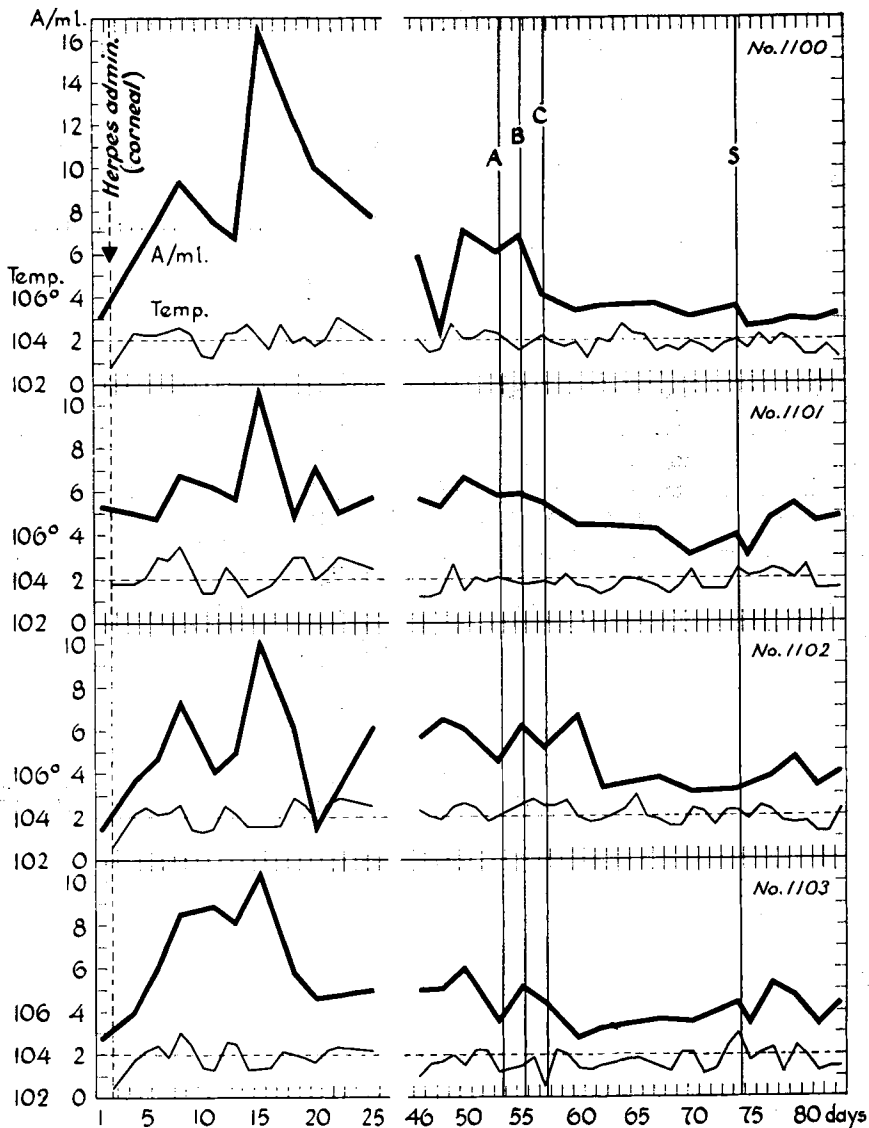


FIG. 3.

Effect of herpetic infection via the cornea, and subsequent sensitization to egg white and shock on the level of hyaluronidase inhibitor in rabbit serum. A, B, C represent the time of sensitizing injections and S the time of the shock injection.

(2) whether a precipitation of active encephalitis by anaphylaxis will be accompanied by a change in the level of the inhibitor, and (3) if an alteration in the latter will result from anaphylaxis *per se*.

Experimental. The preparation of hyaluronic acid from human umbilical cords and hyaluronidase from bull testes, and the viscosimetric measurement of hyaluronidase in-

hibition by serum were carried out as previously described.¹ The term employed to express the inhibiting property of the serum is $\frac{R - R_0}{R_0}$, designated as (A), where (R_0) represents the time in seconds for the viscosity of the reaction mixture to fall to one-half its initial value, and (R) is the corresponding term for the fall in viscosity in the presence of serum.

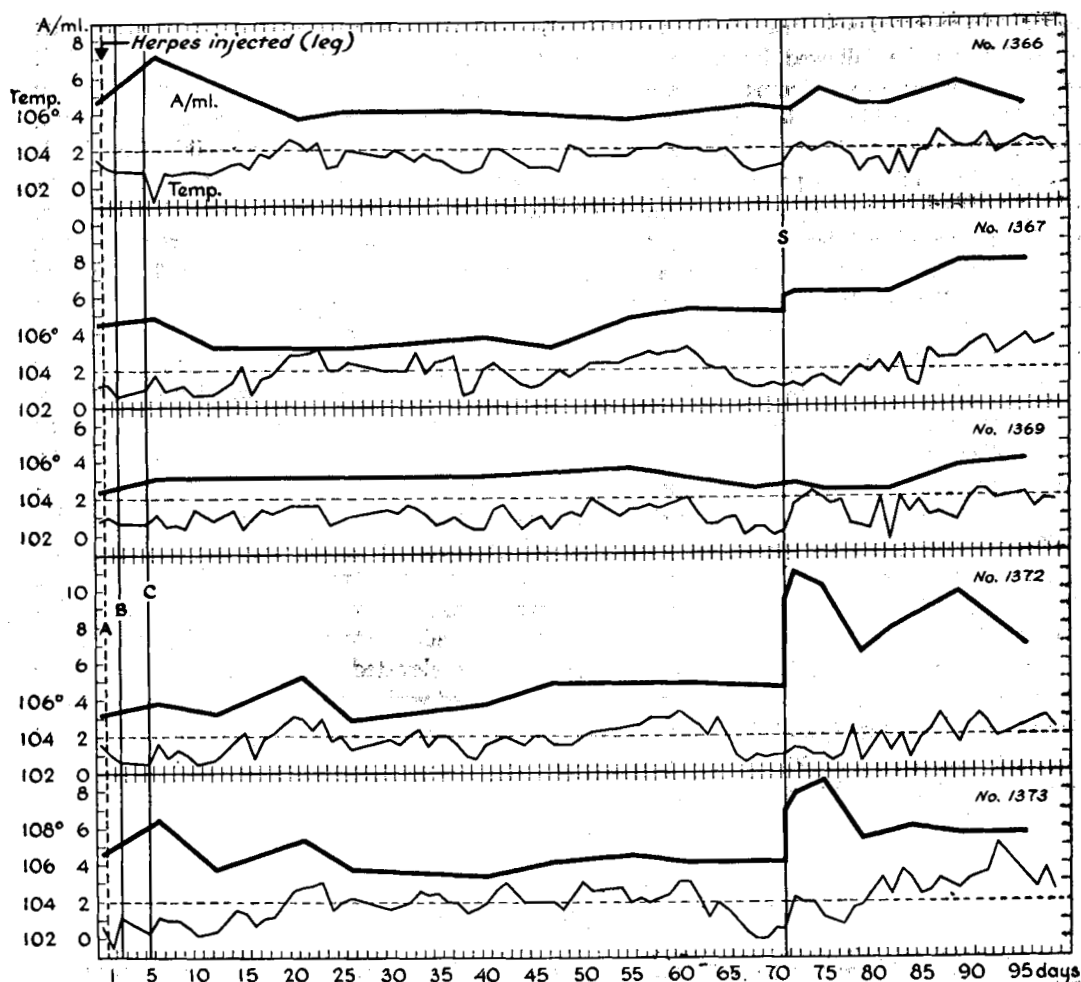


FIG. 4.

Effect of herpetic infection via the leg and subsequent anaphylactic shock on the level of hyaluronidase inhibitor in rabbit serum. A, B, C represent the time of sensitizing injections and S the time of the shock injection.

In the procedure employed, 0.05 cc of serum was required for each determination. The anaphylaxis was effected by sensitizing the rabbits to injections as follows: 1 cc i.v., first day; 0.5 cc i.v., third day; 1 cc i.m., fifth day; and shocking with 0.6 cc i.v. Infections were induced through both the corneal and leg routes. The former were effected by flooding the scarified cornea with a 10% suspension of herpetic mouse brain in physiological saline solution, and the latter by injection of 0.5 cc of the brain suspension into the quadriceps muscle of the right leg.

Results and discussion. The effect of anaphylaxis *per se* on the hyaluronidase inhibitor

in blood serum is shown in Fig. 1. The variations which occurred before and during the period of sensitization to the egg white followed no consistent pattern and were of small magnitude. Shock killed 2 of the animals, and in the two that survived, a small elevation in the inhibitor concentration was produced which cannot be considered significant. No consistent effect was observed as a consequence of shocking 6 additional animals. Fig. 2. A small depression was observed the second day after shock, but the magnitude of this effect was slight, and the subsequent changes were widely variable.

Infection through the corneal route pro-

duces an acute systemic reaction in the rabbit which can be followed by means of the animal's temperature. Temperatures exceeding 104° are considered definitely pathognomonic. In animals which survive the disease it is usually difficult to precipitate it again, or as severely, by shock, compared to those which survive after infection through the leg route. On the other hand, the latter group do not usually develop the acute symptoms and high fever that characterize the former during the initial sickness.

These influences are reflected in the changes in the inhibitor concentration. Thus, in Fig. 3, the effect of the corneal infection can be seen to produce a consistent elevation which has a characteristic double peak during the period of acute illness. After recovery, sensitization and shock failed to cause important changes in either the infective state or the inhibitor level.

When the virus was injected into the leg, no large increases in the inhibitor concentration were found, Fig. 4, and the animals were not acutely ill. The sensitization was begun on the same day that the rabbits were infected

in order to save time, as these experiments continue for months. From Figs. 1 and 3, it is apparent that sensitization *per se* has no appreciable influence on the inhibitor level. A prompt elevation of the inhibitor level followed shock in those animals in which precipitation of the disease occurred. Animals No. 1372, 1373 showed the most severe symptoms, No. 1366 little effect, and No. 1369 none. The changes in the inhibitor show a correlation with these symptomatic effects.

Summary. Hyaluronidase inhibitor in the blood serum of the rabbit was found to undergo no consistent change in concentration as the result of anaphylaxis induced by egg white. An elevation of the inhibitor level was observed during the acute phase of infection by *Herpes simplex* virus administered via the corneal route. Anaphylactic shock in rabbits which had recovered from the herpetic infection, administered via the leg route, resulted in elevated inhibitor values which were correlated with the severity of the disease thus precipitated from the latent state by the shock.

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Effects of Heterologous Seminal Plasma and Sperm Cells on Fertilizing Capacity of Rabbit Spermatozoa.

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In a previous experiment,¹ the beneficial effects of rabbit seminal plasma on the fertilizing capacity of rabbit spermatozoa was demonstrated. This paper reports the effects of human, bull, and rabbit seminal plasma, as well as of dead sperm of these species, on the fertilizing capacity of rabbit spermatozoa.

Methods: Human semen was collected into a sterile tube and stored at 2°C no more than 12 hours. Bull semen and vasectomized

rabbit semen were obtained by means of an artificial vagina^{2,3} about 1 to 2 hours before use. Human and bull samples were centrifuged at 2,000 R.P.M. for about 30 to 40 minutes and the supernatant fluid was used as seminal plasma.

The semen of a single rabbit, collected at

¹ Chang, M. C., *Proc. Soc. Exp. Biol. and Med.*, 1947, **66**, 51.

² Walton, A., *Notes on Artificial Insemination of Sheep, Cattle and Horses*. 1942, London: Holborn Instrument Co.

³ Macirone, C., and Walton, A., *J. Agri. Sci.*, 1938, **28**, 122.