CAPILLARY PERMEABILITY-INCREASING PROPERTY OF HYALURONIDASE IN RAT

By

M. N. GHOSH[1], R. M. BANERJIE AND S. K. MUKHERJI

Defence Research Laboratory, Kanpur, India

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The swelling produced by the subcutaneous injection of a small volume of fluid in rat's foot was measured plethysmometrically every 5 min over a period of 30 min. Following the injection of normal saline (control), the swelling decreased in volume slowly. The swelling produced by the injection of hyaluronidase, on the other hand, increased in size at first, reaching maximum in 10 to 15 min after which it gradually decreased but not to its original size. The duration-action response was graded depending on the concentration of hyaluronidase. There was marked inhibition of the response when the enzyme was inactivated by heating at 100°C for 15 min at pH 10.

It is concluded that the oedema produced by hyaluronidase is due to increase in the capillary permeability. Furthermore, the effect is specific and is not due to contaminants like histamine or other permeability factors.

Whether hyaluronidase increases the permeability of capillaries is still a controversial point. A group of workers have shown that the extravasation of Evans blue from the blood was accelerated by addition of a crude extract from testes (Duran—Reynals, 1939; Aylward, 1942)); while others have failed to confirm this (Rocha e Silva and Dragstedt, 1941; Zweifach and Chambers, 1950). Benditt *et al.* (1951) found a decrease in the permeability-inducing action with increase in the purity of the preparations of hyaluronidase. This latter finding suggests that the capillary permeability-increasing action of crude hyaluronidase demonstrated by some workers may be due to the presence of impurities such as histamine or other permeability factors.

The present work, which was taken up with the idea of developing an *in vivo* bioassay method for hyaluronidase in rat, however, lent some evidence towards the capillary permeability-increasing action of hyaluronidase. This has been presented here.

METHODS

Rats, 100-180 g were lightly anaesthetised by intraperitoneal injection of pentobarbitone sodium 5 mg/100 g. The degree of swelling produced

1 Present address: Department of Pharmacology, Medical College, Pondicherry, S. India.

by a constant volume of fluid injected subcutaneously in rat's foot was measured plethysmometrically by the method described by Buttle *et al.* (1957), with the modifications that sodium pentobarbital was used in place of sodium amytal; only the upper mark A was graduated on the wide mouth of the burette and a mark put below the knee of the rat with some water-proof ink and this was made to coincide with the mark A. The rat was allowed to lie on a wooden platform just over the burette and through a hole in it the leg was introduced and dipped in the water in the burette.

The volume of the foot was first measured before the injection. Normal saline 0.4 ml with or without hyaluronidase, was then injected subcutaneously in the dorsum of the foot and the volume of the foot measured again and then every 5 min for a period of 30 min. Difference in the volume at any given time from the initial normal volume gave the volume of the injected fluid still remaining unabsorbed in the foot.

The concentrations of hyaluronidase used were 400, 500, 600, 700 and 800 i.u./ml. The different concentrations of hyaluronidase as well as the control saline were randomly distributed amongst a total of 14 rats so that each rat had either control and hyaluronidase, or two different concentrations, or same concentrations of hyaluronidase on the two feet. For each concentration as well as for the control 3-4 replications were obtained.

Later, in a separate series of experiments, the effect of heating the enzyme preliminary to injection was observed. In one foot of each of 3 rats 700 i.u./ml of hyalurohidase was injected, while in the other foot same was injected after preliminary heating on a water bath at 100°C for 15 min at a pH adjusted to 10.

The hyaluronidase used in these experiments was highly purified preparation Rondase (Evans Medical Supplies, Ltd.) available in ampoules of 1,500 i.u. and Kinaden (Schering A. G. Berlin) available in ampoules of 350 i.u.

RESULTS

The average time courses of action following varying concentrations of hyaluronidase have been presented in Fig. 1. With hyaluronidase, between 400-800 i.u./ml., the swelling of the foot increased as evidenced by the upward rise of the graphs from the initial level, reaching maximum in about 10-15 min. Within 30 min period of observation, none of the swellings excepting that caused by the lowest concentration (i.e., 400 i.u.) returned to the initial normal volume. Only in this lowest concentration group, there

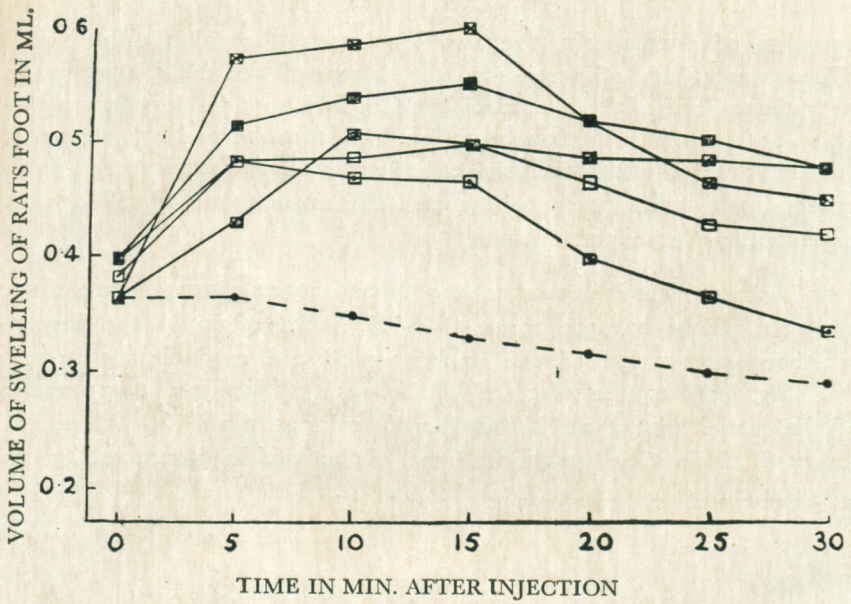

Fig. 1. Mean duration-action curve of oedema produced by different concentrations hyaluronidase injected subcutaneously in rat's paw. .———. control, normal saline (4); ▪——▪ Hyaluronidase 400 i. u./ml. (3); •———• 500 i. u./ml. (4); ▸———◂ 600 i. u./ml. (4); □———□ 700 i. u./ml. (3); △———△ 800 i. u./ml. (4). *The figures in parenthises indicate the number of observa ons.*

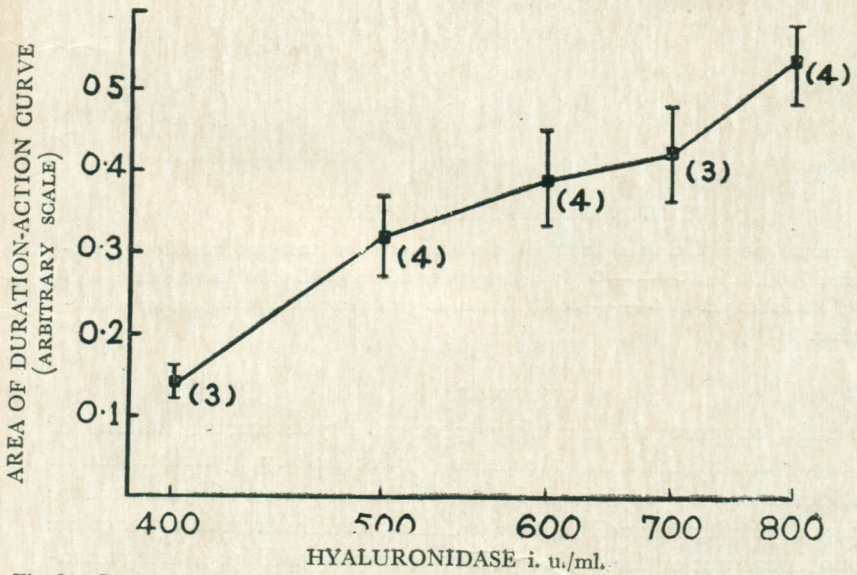

Fig. 2. Concentration-action curve of hyaluronidase. Abscissa: concentrations of hyaluronidase plotted on a log scale. Ordinate: area of the duration-action curve measured with a planimeter. Vertical lines represent standard errors of the means. Figures in parentheses indicate the number of observations.

was some sign of decrease in the swelling beyond the initial size after 20 min. There was no sign of increase in the volume of the control foot while increase in the volume of the hyaluronidase foot had been produced in a graded fashion depending on the concentration. This has been clearly shown in Fig. 2, where area of each duration-action curve above the respective base lines was measured with a planimeter and the mean values plotted against the corresponding log-concentrations.

Since hyaluronidase is known to lose its activity at a temperature above 57°C and at a pH beyond the range of 4-7.5, it was of interest to investigate whether inactivation of the enzyme produced any change in the response. Fig. 3 shows the effect of 700 i.u. of hyaluronidase compared to that of the same heated at 100°C for 15 min at a pH 10. There had been marked diminution in the volume of the swelling produced by the heated hyaluronidase as compared to the control.

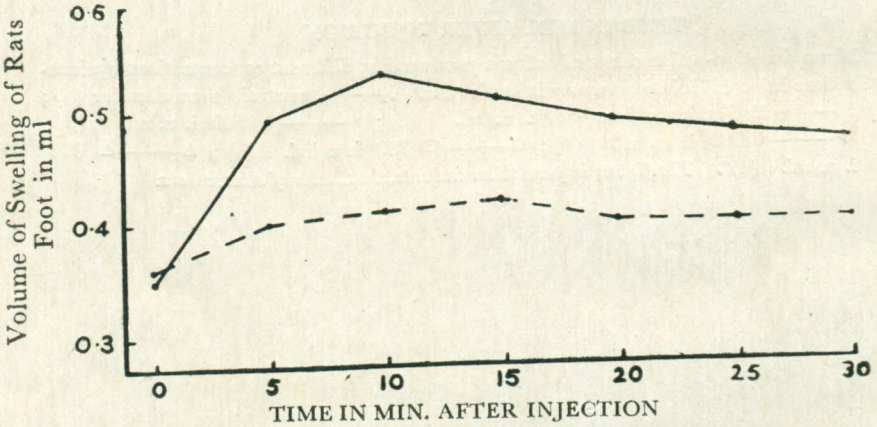

Fig. 3. A comparison of the mean duration-action curves of oedema produced by hyaluronidase 700 i. u./ml ○———○ (3) and hyaluronidase 700 i. u./ml heated at 100°C for 15 min. at pH 10 △———△ (3) *Figures in parentheses indicate the number of observations.*

DISCUSSION

The results support the capillary permeability-increasing action of hyaluronidase. The mechanism of oedema production by hyaluronidase in the present series can be explained in the following lines. Hyaluronidase depolymerises the hyaluronic acid not only of the intercellular substances of the subcutaneous tissues but of the capillary endothelium as well, producing an increase in the capillary permeability. As the total volume of fluid injected was very small (i.e., 0.4 ml), the hydrostatic pressure exerted by the intracapillary blood was much higher than the surrounding fluid. As a consequence, the

fluid came out of the capillaries into the surrounding tissues giving rise to oedema. The work of Elster, Freeman and Dorfman (1949) producing oedema with subcutaneous injection of hyaluronidase also points to a capillary permeability-increasing action. The argument that the said action is probably due to impurities like histamine, etc., does not apply at least to the present series where a comparatively pure substance was used. The demonstration that inactivation of the enzyme markedly decreased the amount of swelling, confirms that the increase in the capillary permeability is due to the enzyme itself and not due to any impurities.

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REFERENCES

Aylward, F. X. (1942). *Proc. Soc. Exp. Biol.* (*N. Y.*), **49**, 342.

Benditt, E. P., Schiller, S., Mathews, M. B. and Dorfman, A. (1951). *Proc. Soc. Exp. Biol.* (*N. Y.*), **77**, 643.

Buttle, G. A. H., D'Arcy, P. F., Howard, E. M. and Kellett, D. N. (1957). *Nature*, **179**, 629.

Duran-Reynals, F. (1939). *Yale J. Biol. Med.*, **11**, 601.

Elster, S., Freeman, M. and Dorfman, A. (1949). *Amer. J. Physiol.*, **156**, 429.

Rocha e Silva, M. and Dragstedt, C. A. (1941). *J. Pharmacol.* **73**, 405.

Zeifach. B. and Chambers, R. (1950). *Ann. N. Y. Acad. Sci.*, **52**, 1047.