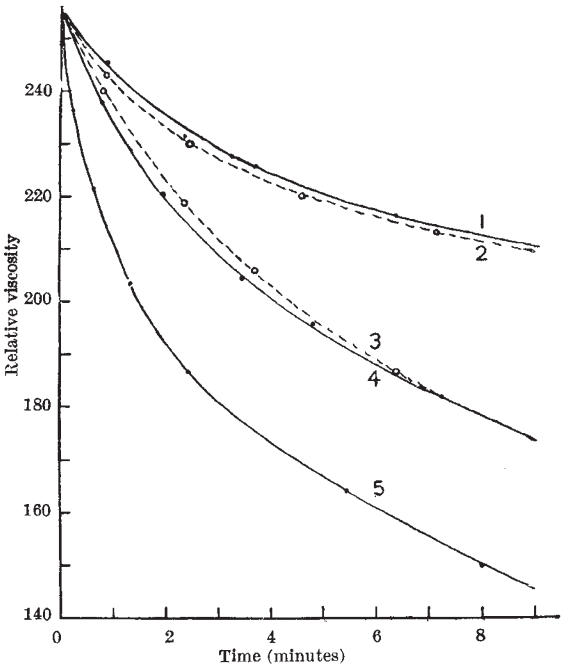
Effect of Histamine on the Activity of Hyaluronidase

M. Fabinyi and J. Szebehelyi[1] have reported that histamine neutralizes the inhibition of hyaluronidase with heparin. We had already dealt with the problem of the influence of histamine on the system hyaluronic acid – hyaluronidase – hyaluronidase-inhibiting agents. Since we had not observed any effect of histamine at that time, we decided to repeat our investigations. Unfortunately, the communication by Fabinyi and Szebehelyi gives no information as to the source of their hyaluronic acid and hyaluronidase, or the concentrations used in their investigations; and we have received no reply to a request for further information.

In our latest investigations we used as substrate highly purified hyaluronic acid (from umbilical cords) as well as a fresh, watery extract from umbilical cords. A hyaluronidase preparation by Armour Laboratories, Chicago, and a testicular extract from guinea pigs were used as enzymes. As inhibitory agents we used partly heparin (Hoffmann–La Roche, Basle) and partly fresh human serum. Viscosity measurements were carried out with a viscosimeter of the Ostwald type at a temperature of 37° C. McIlvain's citrate buffer, together with sodium chloride, *p*H 7·0, were used as solvents. The concentrations of heparin and serum were chosen with the view of producing a distinct, but not complete, inhibition of the hyaluronidase.

We find that the time-curves for the reduction of hyaluronic acid in the presence of heparin and histamine (the latter up to 20 mgm. per cent) coincide with the control without histamine. Histamine *in vitro* has therefore no marked influence on the system hyaluronic acid – hyaluronidase – heparin.

According to Swyer[2], there is also *in vivo* merely an additional effect but no reciprocal action between hyaluronidase and histamine.



Decrease of viscosity of a solution of hyaluronic acid by means of hyaluronidase. Curve 1, with heparin (8·5 mgm. per cent); curve 2, with heparin (8·5 mgm. per cent) and histamine (14·7 mgm. per cent); curve 3, with heparin (2·1 mgm. per cent) and histamine (14·7 mgm. per cent); curve 4, with heparin (2·1 mgm. per cent); curve 5, without heparin and histamine

We conclude that Fabinyi and Szebehelyi's view that histamine causes an enzymatic depolymerization of tissue mucoids by favouring the action of the hyaluronidase, inhibited by heparin, is untenable.

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[1] Fabinyi, M., and Szebehelyi, J., *Nature*, **163**, 533 (1949).
[2] Swyer, G. I. M., *Biochem. J.*, **42**, 28 (1948).

Response of the Isolated Rat Diaphragm to Repeated Stimulation

Gans and Miley[1] showed that skeletal muscle of the adrenalectomized animal is easily fatigued *in situ*. Ingle[2] used this property of the adrenalectomized rat as a basis for the assay of cortical hormones, and he has more recently shown[3] that cortisone also increases its resistance to fatigue. Schweitzer[4] attributed this readiness to fatigue to a fall in blood pressure due to the loss of the adrenals. The fatigue has also been attributed in part to impaired neuromuscular transmission[5], but Walker[6] opposes this view.

The response of the isolated rat diaphragm to frequent stimulation through the phrenic nerve, and the effect of cortisone and of deoxycorticosterone acetate on this response have been investigated. Normal and salt-treated adrenalectomized rats of about 150 gm. were used.

Phrenic nerve-diaphragm preparations, suspended in Krebs's solution (with 5 mgm. calcium ions per 100 ml.) at 38° C. through which a gas mixture of 95 per cent oxygen and 5 per cent carbon dioxide was bubbled, were stimulated through the nerve to give a maximal response with a current duration of 200 μsec. at a frequency of 12/min. After a steady height of contraction had been reached (1–2 hr.), the rate of stimulation was increased to 2/sec. for 5 min., and then restored to 12/min.

In the preparation from normal rats, the twitch tension immediately after the 'fatiguing' stimuli was usually about 68 per cent of what it was immediately before. Experiments carried out throughout the day at intervals gave similar results. After a period of rapid stimulation, the twitch tension never recovered its original height unless the fluid in the bath was replaced; even then recovery was usually incomplete. Similar preparations from adrenalectomized animals gave similar results (see table; cf. Ramey *et al.*[7]).

The effect of adding cortisone or of deoxycorticosterone acetate to the bath fluid was tried. Four separate experiments, as described above, were carried out on each preparation, the periods of rapid stimulation (2/sec.) being separated by an interval of 1 hr. The bath fluid was changed after each experiment.

PER CENT DECREASE IN TWITCH TENSION DUE TO RAPID STIMULATION (2/sec. for 5 min.)

	Exp. 1 control	Exp. 2 with drug	Exp. 3 control	Exp. 4 with drug
Normal	32	34 *C*	31	44 *C*
Adrenalectomized	35	35 *C*	37	34 *C*
Normal	26	24 *D*	14	25 *D*
Adrenalectomized	35	38 *D*	31	29 *D*

C = Cortisone. *D* = Deoxycorticosterone acetate