carcinoma extracts lose their hemolytic potency, when incubated at 37°C, after 5 hours. On the other hand, the hemolytic potency of the tumor extracts is either moderately, or substantially increased following incubation in presence of tumor cells, at 37°C, for either 5 or 24 hours.

Fresh extracts prepared from normal mouse cells have in most instances no hemolytic potency, except for those made from active mouse mammary gland.[2] If the normal mouse cell suspensions (liver, lungs, muscle, or pooled organs), however, are incubated at 37°C for 5, or better for 24 hours, some of them may show a slight hemolytic potency, though to a lesser degree than that observed in the case of pre-incubated tumor cell suspensions.

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Reconstitution of the Dermal Barrier to Fluid Diffusion Following Administration of Hyaluronidase.*

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It is now generally accepted that hyaluronidase owes its spreading activity to the action of the enzyme upon a viscous hyaluronic acid gel in connective tissues[1,2] which is one of the components of the tissue barrier to interstitial fluid diffusion.[3] The effect of hyaluronidase in facilitating the spreading in skin remains evident in the locally treated area for as long as 24 hours after administration of the enzyme, and thereafter is absent.[4] This demonstrates that after a certain latent period, the organism replaces the component of the skin barrier removed by the hyaluronidase.

It appeared likely that a quantitative study of the rate at which the dermal barrier to spreading of fluids was restored, after administration of hyaluronidase, might provide information concerning the formation of new dermal hyaluronic acid *in vivo*. Accordingly, such a study was undertaken in adult humans.

The hyaluronidase used was a purified bovine testis preparation (Schering) which assayed 20 turbidity-reducing units (TRU) per mg as evaluated by the method of Kass and Seastone.[5] The enzyme was used directly after dissolution in 0.85% sodium chloride. Three men and 3 women received intradermal injections of 0.20 cc of enzyme solution containing 20, 2, 0.2, 0.02, 0.002, and 0.0 TRU per cc into separate areas of the 2 arms. After the injection of the enzyme solutions, the local areas were marked with tincture of merthiolate. The time of wheal disappearance following intradermal injection served as a measure of hyaluronidase spreading activity. The local area was examined continuously for 10 minutes after the injections, then at 5-minute intervals until the wheal has disappeared, *i.e.*, until the injected local area appeared completely flat as viewed from a 90° angle. At 24, and then again at 48, hours after the initial injections of enzyme 0.20 cc of an 0.85% solution of NaCl was injected into each previously treated area, and the time of wheal disappearance determined.

Table I illustrates the interrelationships between the time required for wheal disappearance and the concentration of enzyme injected. On the assumption that the time of wheal disappearance is a measure of the barrier efficiency of the dermal hyaluronic acid gel associated with the tissue barrier to

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2 Meyer, K., *Physiol. Rev.*, 1947, **27**, 335.

3 Hechter, O., *Fed. Proc.*, 1947, **6**, 126.

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TABLE I.
The Wheal Disappearance Time of Intradermally Administered Saline, Injected into Areas Treated with Hyaluronidase.

No. of subjects	Amt of hyaluronidase,* T.R.U.	Time of saline injection after hyaluronidase: 0 hr W.D.† min.	24 hr W.D. min.	24 hr B§ %	48 hr W.D.† min.	48 hr B§ %
6	4	0.20 ± 0.06‡	6.50 ± 1.8	11	62.5 ± 6.6	100
6	4×10^{-1}	0.78 ± 0.17	13.7 ± 2.8	23	61.7 ± 7.2	100
6	4×10^{-2}	3.0 ± 0.70	24.7 ± 4.3	41	61.7 ± 8.5	100
6	4×10^{-3}	6.1 ± 1.2	36.7 ± 4.7	61	67.5 ± 5.9	100
6	4×10^{-4}	15.0 ± 5.6	46.7 ± 6.2	78	63.3 ± 6.6	100
6	0	65.0 ± 8.4	58.0 ± 9.0	—	61.1 ± 7.8	—

* Intradermal administration in a constant volume of 0.20 cc.
† W.D. is the mean time of wheal disappearance.
‡ Is the standard error of the mean.
§ B is the percentage of the normal effective barrier present after enzyme administration assuming (a) a linear relationship between effective barrier and spreading and (b) that a normal barrier has a wheal disappearance time of 60 minutes.

diffusion, the percentage restoration of dermal hyaluronic acid 24 and 48 hours after the administration of enzyme has been calculated and these values recorded in Table I.

Twenty-four hours after the administration of hyaluronidase in amounts ranging from 4 x 4 x 10^{-4} TRU (in a volume of 0.2 cc), the dermal barrier of the local area of injection exhibited a permeability which was directly relateed to the dose of the enzyme; after 48 hours, the permeability of the barrier in all treated areas was decreased to normal.

In skin, purified testis hyaluronidase appears to act specifically upon hyaluronic acid[2] and does not affect other mucopolysaccharides, such as chondroitin sulfate, which is present in a concentration equivalent to that of hyaluronate.[2] This does not hold for other tissues, purified testis hyaluronidase having been reported to hydrolyze the monosulfuric acid ester of hyaluronic acid obtained from cornea[6] and the chondroitin sulfate of hyaline cartilage.[7,8] We have found that injection of hyaluronidase into areas which have "recovered" from a previous dose of enzyme produces its usual spreading reaction, thus strongly suggesting that new dermal hyaluronate, and not another mucopolysaccharide, insensitive to hyaluronidase, is involved in the reconstitution of the barrier.

Summary. The reconstitution of the dermal barrier removed by intradermal injection of various doses of hyaluronidase has been studied 24 and 48 hours after enzyme injection in adult humans. At 24 hours the restoration of the barrier is incomplete and inversely related to the dosage of enzyme; at 48 hours the barrier is completely restored in all enzyme-treated areas.

6 Meyer, K., and Chaffee, E., *Am. J. Ophth.*, 1940, **23**, 1320.

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8 Madinaveitia, J., and Stacey, M., *Biochem. J.*, 1941, **38**, 413.