not reported. Fox and Keston (*4*) observed in mice injured by heat or by trauma that sodium accumulated in the injured tissues, and that the gain in sodium exceeded the gain in water. In rats, both hemorrhagic shock and hepatic anoxia, induced by occlusion of the hepatic artery, were accompanied by increase in liver sodium (*3*).

The results reported here, together with those obtained after the various other types of injury mentioned above, suggest that redistribution of sodium is a nonspecific event which follows severe tissue injury, regardless of the means by which the injury was produced.

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Mechanism of Hyaluronidase Action in Skin[1]

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The mechanism whereby spreading factors (S.F.) from bacteria, testis, venoms, etc. increase the permeability of skin remained obscure until Chain and Duthrie (*1*) suggested that S.F. are enzymes (hyaluronidases) which lower the viscosity of the mucoid ground substance of the connective tissues (hyaluronic acid). This enzymatic theory has been supported and extended by some workers, but the explanation of the entire mechanism of spreading on the basis of the hyaluronidase activity of S.F. has met with opposition (cf. the review of Duran-Reynals, *2*). It is the purpose of this report to point out that the spreading produced by hyaluronidase is not only dependent upon enzyme concentration but is also directly related to the increase of interstitial pressure produced by the injected fluid.

Spreading in shaved rabbit abdominal skin was measured using methemoglobin (obtained from twice-recrystallized bovine hemoglobin) as indicator. It was observed that the induction of spreading by purified bovine testis hyaluronidase[2] is limited to the first 10 minutes following intradermal injection, and that thereafter the rates of spreading in hyaluronidase-treated and control areas are identical. Table 1

TABLE 1

Enzyme concentration (μg./cc.)	Area increase over control at 10 min. (cm.²)
0.16	0.24
0.33	0.62
0.67	0.96
1.33	2.28
1.67	2.42
6.67	3.56
33.33	3.49
66.67	3.60

shows the effect of hyaluronidase concentration (administered intradermally in a constant volume of 0.2 cc.) upon the increase in the area of spread after 10 minutes. It will be seen that there is a quantitative relationship between enzyme concentration and spreading in the low-dosage range. With higher doses, maximal effects are obtained with a particular enzyme concentration, so that increasing the enzyme concentration 10 times does not demonstrably increase the spread. This lack of correspondence between high dosages and spread has been noted previously (*3*) and has been ascribed to the presence in skin of an active mechanism for S.F. inactivation. Search for such a possible mechanism was undertaken along the following lines:

(a) *Inhibitor in skin*: *in vitro* incubation of hyaluronidase with extracts of whole skin or dermis; or the insoluble residues from these extracts; or skin or dermal breis.

(b) *Inhibitor formed during the reaction between enzyme and hyaluronic acid*: *in vitro* incubation of hyaluronidase with hyaluronic acid from umbilical cord at pH 7.0.

(c) *Inhibitor in blood*: comparison of hyaluronidase spreading activity in normal, hyperemic (xylol), ischemic (hemorrhagic shock), and dead skin.

Evidence for hyaluronidase inhibition sufficient to account for the lack of correspondence between high doses and spreading was not obtainable from the experiments listed in (a), (b), or (c).

These negative findings, coupled with an observed quantitative correspondence of hyaluronidase action in living and dead skin; suggested that the S.F. effect might be due in part to simple mechanical action. The question arose as to whether hyaluronidase might simply reduce the resistance of skin to fluid passage. However, this effect could be evident only when accompanied by a localized increase of interstitial pressure and volume in the skin. On this basis, once the bleb injected with maximal amounts of hyaluronidase had spread (and had correspondingly decreased the interstitial pressure), the presence or absence of ex-

[1] Aided by a grant from G. D. Searle and Company.
[2] Obtained through the courtesy of E. Schwenk, of the Schering Corporation. The preparation used was purified by the method of Madinaveitia (*4*).

cess hyaluronidase should have no further significance.

To test this hypothesis, highly concentrated hyaluronidase solutions (3.3–333 μg./cc.) plus indicator were placed on superficial epidermal incisions for as long as 90 minutes and their spread compared to indicator solutions not containing the enzyme. No significant spreading effect of hyaluronidase was evident in these experiments wherein fluid was administered under zero pressure. In the next experiments, varying skin interstitial pressures were obtained by varying the volume of hyaluronidase administered intradermally (the enzyme concentration, 3.33 μg./cc., was kept constant).

Table 2 shows these results. The value "T" is calculated from T = volume administered/area spread

TABLE 2

Vol. (cm.³)	Initial T (mm.)	Initial rate (cm.²/min.)	Area increase over control at 10 min. (cm.²)	T at 10 min. (mm.)
1.0	3.3	7.41	9.86	0.64
0.5	3.2	6.95	8.37	0.43
0.25	1.9	3.26	3.06	0.45
0.10	1.3	1.39	1.01	0.38

(assuming that there is neither gain nor loss of fluid from the injected fluid volume). "T" is thus an index of the average "thickness" of the injected bleb and is regarded as a value proportional to the increased interstitial pressure produced by intradermal fluid administration. Table 2 demonstrates that:

(1) The initial rate and final areas of spread of hyaluronidase solutions are directly related to volume from 0.1 to 0.5 cc. and thereafter level off.

(2) The initial rate is directly proportional to "T," the thickness of the bleb at that time.

(3) The "T" values at 10 minutes, independent of the volume injected and initial "T" values, are approximately the same.

TABLE 3

Vol. (cm.³)	Enzyme concentration (μg./cc.)	Initial rate (cm.²/min.)	Area increase over control at 10 min. (cm.²)
0.25	13.4	2.97	3.04
0.10	33.3	1.53	1.03

Another experiment, the results of which are shown in Table 3, answers possible objections that the results in Table 2 are due to differences in the total amounts of hyaluronidase administered. Here the total amounts of enzyme were kept constant (3.3 μg.), but the volumes were varied. As will be seen, the solution administered in largest volume (but lowest concentration) spread to a greater extent than did the solution injected in smaller volume and highest concentration.

The finding that hyaluronidase induces spreading only when local interstitial pressure is increased by fluid administration, coupled with the demonstrated correspondence between spreading and interstitial pressure-volume relationships, helps to elucidate many obscure points of S.F. action. Space does not permit adequate treatment of this material, which will be discussed in subsequent reports. However, the significance of these findings as regards the relation of S.F. to bacterial invasiveness through skin might briefly be mentioned. Bacteria usually penetrate the skin through abrasions with only minimal amounts of fluid accompanying the invading organism. Thus, the spread of organisms through the interstitial spaces will depend as much upon the ability of the bacteria to stimulate the production of edema-inducing "leukotaxin-like" substances (*5*) as it does upon S.F. production.

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Grafts of Free Muscle Transplants Upon the Myocardium

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Within recent years the introduction of nerve-muscle transplants and pedicle-muscle grafts has made possible remarkable advances in many branches of clinical surgery. It is now becoming feasible to replace necrotized and nonfunctioning tissue destroyed by trauma and infection with free muscle grafts.

Experiments of Beck (*1*) and O'Shaughnessy (*4*) have demonstrated that pedicle-muscle grafts onto the heart resulted in the creation of a significant vascular anastomosis between the two tissues, particularly if the myocardium were rendered ischemic. Such grafts not only brought extra cardiac blood to the heart but also served as an anastomotic channel for the transport of blood between healthy and diseased coronary beds. Although this method gave great experimental promise, the clinical application of large pedicle-muscle grafts was necessarily limited because of the extensive surgical manipulation involved in its adaptation to human cardiac surgery. Therefore, simpler methods obviating past difficulties have been sought.