tions of their psychotic complaints and showed distinct changes in attitudes toward their environment.

(5) Marked individual differences in response to the diets were observed in both old and young groups, not only in time of onset of symptoms but also in the degree of resulting abnormality. The subjects who gave evidence of vitamin deficiency early manifested abnormalities which were greater in degree than those observed in others who were affected more slowly. Especially was this true with respect to the cardiovascular system. In general, the older persons were affected earlier and more severely than were the younger.

Recovery when yeast extract providing 6 mg. of supplementary thiamine was added to the diet was dramatic, especially in the subjective fields of appetite, general feeling tone, pain, and paresthesia. The levels of lactic and pyruvic acids after the standardized conditions of exercise and glucose rapidly returned to their predeficiency levels. Likewise, patellar reflexes which had been lost commenced to return soon after the yeast extract was added to the diet. On the other hand, return of the Achilles tendon reflex was very slow.

Technical Papers

Distribution of Sodium and Water in Muscle Following Severe Cold Injury[1]

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In a recent investigation of experimental cold injury (frostbite) it became evident that the physiological and chemical changes occurring in the injured regions after thawing resembled those found after severe injury produced by other means. The similarity in the natural history of the pathological processes in burns and frostbite has already been noted by Harkins (*5*). Our complete data, to be published later, demonstrate further points of similarity. We wish here to report the distribution of sodium and water in normal muscle and in muscle removed from rabbits' legs subjected to severe cold injury.

Six rabbits were anesthetized with dial, the hair removed from one hind limb as far as the lower end of the femur, and the leg immersed for 3 minutes in a water-alcohol-ethylene glycol mixture cooled to −55° C. Blood samples from the marginal ear vein and muscle samples from the tibialis anticus of the normal and injured leg were taken 166 to 255 minutes after injury. Determinations of water and sodium by the method of Butler and Tuthill (*1*), as modified by Consolazio and Dill (*2*), were made on samples of plasma and muscle.

The results of the analyses and derived values obtained by the method of Harrison, Darrow, and Yannet (*6*) are presented in Table 1. Since the experiments involved comparisons between muscles in the same animal and since only brief intervals were allowed to elapse between injury and sampling, it was considered to be unnecessary to determine and correct for fat content of the muscle.

TABLE 1

CHANGES IN WATER AND SODIUM IN TIBIALIS ANTICUS MUSCLES AND PLASMA OF RABBITS AFTER IMMERSION-FREEZING

Animal Nos.	31	64	66	67	75	80
Plasma						
$[Na]_e$ m. eq./l. plasma ultrafiltrate	138.1		145.8		141.9	
Control Muscle						
Total H_2O*	313.5	321.2	318.0	315.0	328.5	360.0
Total Na†	7.56	7.32	6.76	7.44	8.26	8.58
Extracellular H_2O	54.7		46.4		53.2	
Frostbitten Muscle						
Total H_2O	328.5	399.0	440.0	507.0	500.0	530.0
Total Na	9.84	24.65	34.63	40.05	53.90	68.40
Extracellular H_2O	71.2		237.4		380.0	
Gain of water (%)	4.8	24.4	38.4	61.0	52.4	47.2
Gain of sodium (%)	30.2	237.0	412.3	438.0	553.0	697.2

* H_2O expressed in grams/100 grams dry tissue.
† Na expressed in m. eq./100 grams dry tissue.

The data show that the gain of water by muscle following severe injury by cold ranged from 5 to 60 per cent, while the increase in sodium was proportionately much larger: 30 per cent in the case of the smallest increase and 237–697 per cent in the remaining five animals studied. Such large disproportion between the gain of water and that of sodium could occur only if large quantities of sodium penetrated the intracellular phase or in some other way became excluded from free equilibrium with the remainder of the sodium in the extracellular phase.

A fall in the level of serum sodium in man (*8*) and animals (*7*) after severe burns led Lowdon, *et al.* (*7*) to suggest that sodium was being lost into the injured tissues. Sodium in venous blood was found to be lower than in arterial blood, but tissue analyses were

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Stanford University.

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*).