and α-ketoglutaric acid accumulate in about equal amounts. A mixture of succinate and acetate does not replace protogen. A function other than the oxidation of pyruvic and α-ketoglutaric acids in the metabolism of *Tetrahymena* is postulated.

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Effect of Hyaluronidase on Edema Following Experimental Cold Injury.* (19605)

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The formation of edema following experimental frostbite is accompanied by rising interstitial fluid pressure and distortion of tissues which may cause obstruction of capillaries(1,3,5). The resulting stasis with limited exchange of metabolites would suggest that the accumulating edema is a contributing factor in the resulting tissue loss(3,5). Several attempts therefore have been made to control the fluid loss from the blood stream into the injured tissues(2,3,8,10,11,12). In our experiments we attempted to achieve a similar objective by use of hyaluronidase. This enzyme reduces the resistance of tissues to fluid absorption and increases tissue permeability(4,7). We have investigated whether facilitation of absorption of edema fluid with corresponding decrease in swelling would influence the appearance and extent of the resulting gangrene.

Material and methods. Six adult albino rabbits weighing 2450 to 2850 g were used. All animals were anesthetized with pentobarbital sodium intraperitoneally. Both depilated hind legs were immersed for 4 minutes in a medium consisting of ethylene glycol, alcohol and water, previously cooled to a temperature of —35°C to —37°C. The exposed extremities were allowed to thaw in air at room temperature. Hyaluronidase (Schering) was injected subcutaneously into the edematous tissues of one of the injured extremities using aseptic technic. The other extremity served as a control. One hundred and fifty turbidity-reducing units (TRU) of the enzyme, dissolved in 1 cc of distilled water, were injected in 3 different places on both the medial and lateral surfaces of the extremity. Thus a total dose of 300 TRU was injected into one extremity in 24 hours. The first injection was made 3 hours after exposure to cold, the other 24 hours later. The changes in volume of both extremities representing actual alterations in edema formation were determined by measuring the amount of water displaced during immersion of each extremity to a standard

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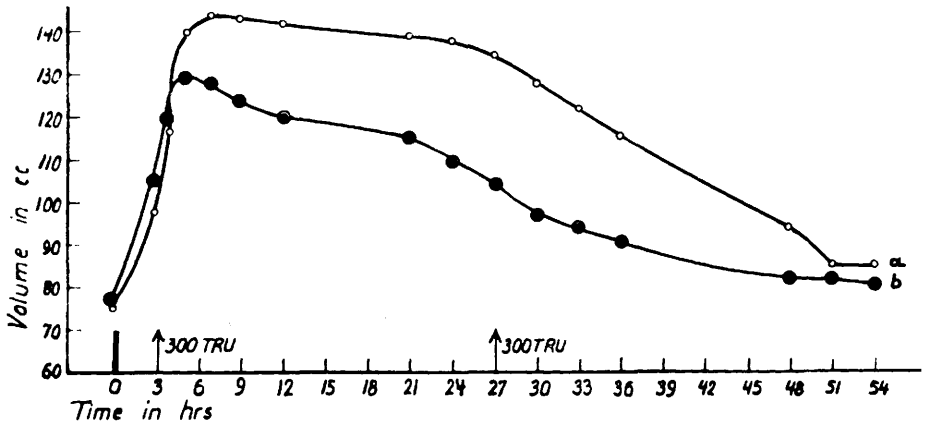

FIG. 1. Comparison of volume changes in rabbit legs after exposure to severe cold (I). The lines indicate the mean values in 6 extremities treated with hyaluronidase (b) and in the controls (a).

mark. The measurements were taken prior to exposure to cold, before each injection of hyaluronidase and at intervals of 3 hours.

Observations. The changes in volumes of both exposed extremities indicating the actual alterations in edema formations during the experimental period are presented in Fig. 1. The lines demonstrate the mean values of measured leg volumes of all rabbits. The upper line a) shows the mean values of control frozen extremities, while the lower b) that of frozen legs treated with hyaluronidase.

The thawing of the extremities was followed by rapid and extensive swelling expressed by increase in volume by about 53%, 3 hours after injury. Two hours after injection of hyaluronidase there is no further swelling in the treated extremities b), while the controls show a progressive augmentation in volume by about 87% a). During the following 22 hours the volume of treated extremities declines progressively b), whereas that of injured but not injected legs remains constant a). Further diminution in volume of treated extremities can be observed in the 24 following hours after the second injection of the enzyme while the controls showed a lesser decrease in volume. Fifty-four hours after injury there are no significant differences in the volumes of treated and control extremities.

Wet gangrene developed in all animals about 50 hours after injury in both treated and control extremities simultaneously. There were no differences in the extent of the gangrene in both legs.

The routine histological examinations of the post mortem specimens taken from treated and untreated extremities showed an extensive necrosis of injured tissues. There was less edema in subcutaneous tissue and interstitial spaces in specimens taken from hyaluronidase treated extremities.

Discussion. The incorporation of hyaluronidase into the edematous tissues previously injured by cold prevented further swelling of the treated extremity and caused a progressive and significant decline in volume as compared to controls. The cessation in edema formation and the evacuation of accumulated extracellular fluid may be due to reduction of the viscosity of the hyaluronic acid gel with a resultant lessening in resistance to fluid diffusion (6,7,9). The accelerated reabsorption of accumulated edema fluid results in a marked decrease in volume of the swollen extremity.

The removal of edema did not prevent the development of gangrene which appeared simultaneously in both the treated and control extremities in all animals. There were no differences in the extent of final tissue loss nor in the microscopical appearance of the cells damaged by cold. The failure to prevent or to restrict the formation of gangrene by the use of hyaluronidase in our experiments is in contrast to the results obtained by mechanical control of edema. Crismon and Fuhrman(3)

reported that application of casts and elastic pressure dressings before and after thawing restricted significantly the amount of gangrene. The most beneficial effects were obtained by using these methods combined with rapid rewarming. These results were not confirmed by other investigators. Lange and Boyd(8), using pressure dressings with and without heparinization, did not observe any beneficial effect. Quintanilla *et al.*(10) stated that pressure dressings and plaster casts were ineffective. Shumacker and his associates (11) demonstrated, in experiments conducted with precise controls, that plaster casts left in place for 10 days, as recommended by Crismon and Fuhrman, were without benefit.

The problem whether the accumulating edema following cold injury is a major contributing factor to tissue loss, cannot be definitely determined on the basis of these experiments. It appears, however, from our experiments and that of the others, that the role of edema *per se* is not important in the pathogenesis of final tissue loss.

Finally, it should be pointed out that theoretically, the increased rate of absorption of edema fluid will simultaneously increase the absorption rate of any substances released from injured cells. These may, by their presence or concentration in general circulation, have toxic effects.

Summary and conclusions. 1. Considering that the edema formation following experimental cold injury might be a factor contributing to tissue loss, the prevention of gangrene was attempted by controlling the edema by use of the enzyme hyaluronidase. 2. Tissues edematous after cold injury showed no further swelling and a significant decline in volume after local injection of the enzyme, presumably due to an increased absorption rate of the accumulated fluid. 3. The evacuation of edema did not influence the gangrene as it was observed to develop simultaneously and equally in treated and control extremities. It seems, therefore, that tissue necrosis is not primarily related to accumulation of edema in the region of injury.

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Role of Protogen in the Oxidation of Pyruvic Acid.* (19606)

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Protogen, an essential growth factor for the ciliate *Tetrahymena geleii*(1), which is identical with the acetate replacing factor(2), and the pyruvic oxidation factor(3), has recently been highly purified(4,5), crystallized(6), and tentatively renamed lipoic acid(6). As was expected, lipoic acid was found to be capable of replacing protogen in the growth of *Tetrahymena*(7,8). The initial reaction of pyruvate oxidation may be considered as involving 2 components (as shown in reaction 1): oxi-

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