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.

1. Bigelow, W. G., *Canad. M. A. J.*, 1942, v47, 529.

2. Bundschuh, E., *Muenchn. Med. Wschr.*, 1915, v62, 416.

3. Crismon, J. M., and Fuhrman, F. A., *J. Clin. Invest.*, 1947, v26, 486.

4. Duran-Reynals, F., *Bact. Rev.*, 1942, v6, 197.

5. Fuhrman, F. A., and Crismon, J. M., *J. Clin. Invest.*, 1947, v26, 245.

6. Hechter, O., Dopkeen, S. K., and Yudell, M. H., *J. Pediat.*, 1947, v30, 645.

7. Jaworski, A. A., and Farley, J. E., *Am. J. Dis. Child.*, 1950, v79, 59.

8. Lange, K., and Boyd, L. J., *Surg., Gynec. and Obst.*, 1945, v80, 346.

9. Meyer, K., *Physiol. Rev.*, 1947, v27, 335.

10. Quintanilla, R., Krusen, F. H., and Essex, H. E., *Am. J. Physiol.*, 1947, v149, 149.

11. Shumacker, H. B., Jr., Radigan, L. R., Ziperman, H. H., and Hughes, R. R., *Angiology*, 1951, v2, 100.

12. Wittek, A., *Muenchn. Med. Wschr.*, 1915, v62, 416.

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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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dative decarboxylation, which involves a hydrogen transfer to diphosphopyridine nucleotide (DPN), and secondly, a transfer of the acetyl group to coenzyme A (Co A).

1. pyruvate + Co A + $DPN_{oxid.}$ ⇌
acetyl–Co A + $DPN_{red.}$ + CO_2

Kidder and Dewey have(9) suggested that protogen is necessary for the hydrogen transfer mechanism, whereas Gunsalus(5) holds that the factor is probably concerned with the acetylation stage of the reaction. When pure preparations of the factor recently became available† it was possible to initiate investigations as to the exact enzymatic role of the factor in the oxidation of pyruvate.

Methods. Pyruvic oxidase prepared by precipitation at pH 5.4(10) of a washed extract of *Tetrahymena geleii* S. was used for the study of the oxidation reaction. Assays for protogen indicated the presence of significant quantities of the growth factor in the preparation, which however could be readily removed by shaking with alumina(5). Oxidative activity was measured by spectrophotometrically following the rate of reduction of the dye, 2,6-dichlorophenol indophenol(10). The acetylation activity of the preparation was assayed by following the rate of acetyl formation by the hydroxylamine reaction(11). When the acetylation reaction was studied, the enzyme was first heated to 54°C for 10 minutes to destroy transacetylase activity(12).

Results. Removal of protogen from the pyruvic oxidase by treatment with alumina did not decrease the oxidative activity of the preparation as measured by the rate of dye reduction. In like manner, the addition of protogen to alumina treated preparations (in amounts up to 25 units/ml) had no effect. On the other hand, when the acetylation reaction was measured, protogen was found to be necessary for activity. As shown in Fig. 1, alumina treated enzyme is completely inactive in the pyruvic oxidase acetylation step. The addition of protogen to the mixture containing

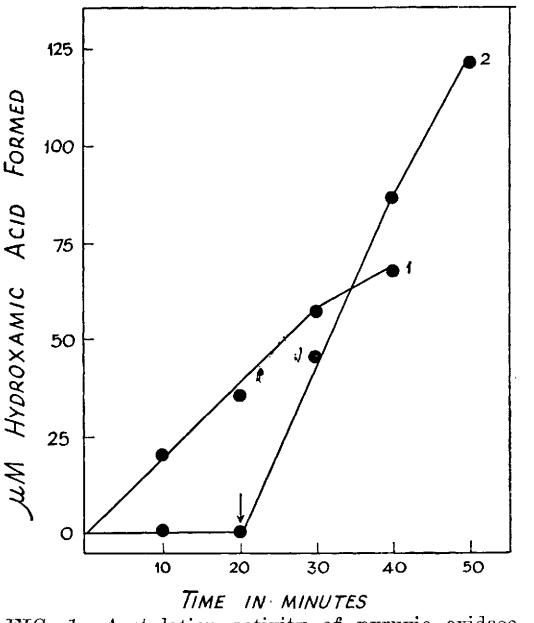

FIG. 1. Acetylation activity of pyruvic oxidase expressed as micromoles of acethydroxamic acid formed. Incubation mixture contained .2 ml of enzyme, 35 units of coenzyme A,‡ and the following in micromoles: Sodium pyruvate, 200; cocarboxylase, .4; diphosphopyridine nucleotide, .9; $MgCl_2$, .4; $NaHCO_3$, 10; cysteine, 8.4 pH, 6.8. The final volume of the incubation mixture was 1 ml. Temp., 25°C. Curve 1, untreated enzyme. Curve 2, enzyme treated with alumina, 25 units of protogen added at time indicated by arrow.

alumina treated enzyme restores the activity to a rate greater than obtained with untreated enzyme. It is thus apparent that the factor is necessary for the acetylation, and not the oxidative activity of the pyruvic enzyme. In the purification of the enzyme, protogen was not entirely removed, as evidenced by acetylation activity of the untreated enzyme in the absence of added protogen. The amount of the factor remaining in the preparation however, is not optimal since greater activity was obtained when excess amounts (25 units) were added to alumina treated enzyme.

Summary. Protogen has been found to be essential for the acetylation reaction, but not for the oxidative phase, of pyruvate oxidation, when assayed in a purified pyruvic oxidase preparation from the ciliate *Tetrahymena geleii* S.

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1. Stokstad, E. L. R., Hoffman, C. E., Regan, M. A., Fordham, D., and Jukes, T. H., *Arch.*

Biochem., 1949, v20, 75.

2. Guirard, B. M., Snell, E. E., and Williams, R. J., *Arch. Biochem.*, 1946, v9, 381.

3. O'Kane, D. J., and Gunsalus, I. C., *J. Bact.*, 1948, v56, 499.

4. Reed, L. J., DeBusk, B. G., Johnston, P. M., and Getzendaner, M. E., *J. Biol. Chem.*, 1951, v192, 859.

5. Gunsalus, I. C., Struglia, L., and O'Kane, D. J., *J. Biol. Chem.*, 1952, v194, 859.

6. Reed, L. J., DeBusk, B. G., Gunsalus, I. C., and Hornberger, C. S., *Science*, 1951, v114, 93.

7. Seaman, G. R., PROC. SOC. EXP. BIOL. AND MED., 1952, v79, 158.

8. Slater, J. V., *Science*, 1952, v115, 376.

9. Kidder, G. W., and Dewey, V. C., in Lwoff, A., *Biochemistry and Physiology of Protozoa*, New York, 1951.

10. Sanadi, D. R., and Littlefield, J. W., *J. Biol. Chem.*, 1951, v193, 683.

11. Lipmann, F., and Tuttle, C., *J. Biol. Chem.*, 1945, v159, 21.

12. Korkes, S., del Campillo, A., Gunsalus, I. C., and Ochoa, S., *J. Biol. Chem.*, 1951, v193, 721.

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Effect of Nitrogen Mustard on Virus of Serum Hepatitis in Whole Blood.* (19607)

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The increasing use of blood and blood products in civilian and military medicine has brought to the fore, as one of the most urgent problems, the control of serum hepatitis (SH). The natural history of this virus disease is still obscure, but it has been definitely established that a carrier state may exist in individuals without a known history of jaundice and that such persons may harbor the virus in their blood in the absence of abnormal liver function tests(1). This situation obviously prevents the proper selection of safe donors. It is essential, therefore, to devise methods to sterilize blood and blood products. As far as plasma and sera are concerned an effective method appears to be at hand. Ultraviolet irradiation of these products has decreased the subsequent incidence of SH although the full extent of its efficacy has not as yet been critically evaluated. On the other hand, sterilization of whole blood has not been accomplished at present. In view of the relatively high incidence of the disease following transfusions the need for a satisfactory method of inactivating SH virus in whole blood is most urgent. The experience gained with nitrogen and sulfur mustards in the inactivation of various viruses seemed to offer an approach to this problem. It was first noted by Bawden and Pirie that mustards abolished the infectivity of certain plant viruses, the agents causing tobacco mosaic and bushy stunt disease, as cited by Gilman and Phillips(2). Later it was reported that sulfur mustard inactivated the viruses of rabies, Eastern equine encephalomyelitis, and hog cholera(3), and that both sulfur and nitrogen mustards rendered influenza virus non-infective(4). Herriott(5) studied the rates of inactivation of 6 animal, one plant, and 2 bacterial viruses by mustard gas and noted that those containing desoxyribose nucleic acid were more rapidly affected than the viruses containing ribose nucleic acid. Hartman *et al.*(6,7) demonstrated that the nitrogen mustard salt (methyl-bis- (beta-chloro-ethyl) amine hydrochloride) was an effective virucidal and bactericidal agent when added to plasma or whole blood in a concentration of 500 mg per liter. Plasma or blood thus treated, and subsequently neutral-

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