

reaction, although of enzymatic nature, is slow and it has not been possible yet to prepare pure A_2 or P_2 . Chemical analyses of the reaction mixtures show that A_2 and P_2 do not contain phosphorus. This fact, and the failure of the prostate enzyme to remove the second phosphorus of A_1 and P_1 , indicate a difference in the chemical nature of the two phosphorus-containing groups of these proteins.

In conclusion, it should be pointed out that only three properties, namely, electrophoretic mobility, crystal form and phosphorus content, have been used to identify the products of mild enzymatic degradation of ovalbumin. Although chemical differences other than the phosphorus content may exist between A_1 , A_2 and A_3 and between P_1 , P_2 and P_3 , it is probable that the mobility increments are largely due to the loss of the charged phosphate groups. Moreover, additional confirmation that the P_2 obtained by dephosphorylation of P_1 is identical with that obtained by proteolysis of A_2 would be of interest in this connexion.

I wish to express my thanks to Dr. L. G. Longsworth for help in preparing this manuscript.

GERTRUDE E. PERLMANN

Rockefeller Institute for Medical Research,
New York. June 24.

¹ Linderström-Lang, K., and Ottesen, M., *C.R. Lab. Carlsberg*, **26**, No. 16 (1949).

² Longworth, L. G., *J. Amer. Chem. Soc.*, **61**, 529 (1939).

³ Linderström-Lang, K., and Ottesen, M., *Nature*, **159**, 807 (1947).

⁴ Eeg-Larsen, N., Linderström-Lang, K., and Ottesen, M., *Arch. Biochem.*, **19**, 340 (1948).

⁵ Perlmann, G. E., *J. Amer. Chem. Soc.*, **71**, 1146 (1949).

⁶ Perlmann, G. E., *Nature*, **164**, 961 (1949).

Protein Activation of Streptolysin 'O'

MANY studies have been made of streptococcal hæmolysins. Todd¹ demonstrated essential differences between the 'O' and 'S' forms of streptolysin. Herbert and Todd² made a more exhaustive study of streptolysin 'O' and characterized it as a protein capable of hæmolysing red cells, only, however, after activation with —SH-containing reducing agents.

During experiments involving the use of certain streptococcal preparations and albumen and globulin fractions of serum, it was observed that rabbit red-cell suspensions were hæmolysed in the presence of mixtures of the streptococcal preparation and serum albumin.

Streptolysin 'O' prepared in Todd-Hewitt broth³ and partially purified by Herbert and Todd's² method was found to be activated not only by —SH-containing reducing agents such as thioglycolic acid, cysteine, etc., but also by serum albumin.

Albumin fractions separated from serum by 50 per cent saturation with ammonium sulphate consistently showed an activating effect when allowed to stand at room temperature with inactive streptolysin 'O'. The fractions were dialysed before testing, to remove ammonium sulphate, and were made isotonic by the addition of sodium chloride, their volumes being corrected to that of the original volume of serum used for precipitation. The effect of these samples upon inactive streptolysin 'O' was determined by mixing 0.5-ml. volumes of the fraction under test with varying doses of previously standardized inactive lysin in equal volumes. The mixtures were allowed to stand five to ten minutes at room temperature before the addition of 0.5 ml. of 3 per cent washed rabbit red-cell suspension. Buffer-saline mixture at pH 6.6

was used as diluent. Controls of inactivated lysin and lysin activated with sodium thioglycollate were included with each series of tests. Hæmolysis was observed after one hour in the water-bath at 37° C.

Albumin fractions prepared from human, bovine (bovine crystalline albumin: Armour and Co.), horse and rabbit serum all showed varying though definite activation of streptolysin 'O'. The intact sera and the globulins of these species, with the exception of one sample of rabbit globulin, were without effect. Two samples of ovalbumin and a muscle protein solution were also without effect. If iodoacetate was added to the albumin-streptolysin mixtures, activation was either abolished or markedly reduced.

Herbert and Todd² suggest that streptolysin 'O' contains disulphide linkages which can be reversibly reduced to sulphhydryl groups, the —S—S— form being hæmolysically inactive whereas the —SH form is active. An interpretation of the activation of streptolysin 'O' by reducing agents having free —SH groups has been made on this basis².

The activation of streptolysin 'O' by serum albumin is most probably due to readily available —SH groups in the latter, since such activation was prevented by iodoacetate. Anson⁴ and Olcott and Fraenkel-Conrat⁵, in reviews of properties of protein groups, point out that the reactivity of —SH groups of proteins varies with the protein and upon the amount of denaturation it has undergone. The inability of whole serum and the globulin fractions to activate streptolysin 'O' might be explained on this basis; however, the presence of small amounts of anti-streptolysin 'O' found in the globulin of even 'normal' sera provides an alternative explanation.

G. S. TURNER

Northwestern University,
Rheumatic Fever Research Institute,
Chicago, Illinois. June 15.

¹ Todd, E. W., *J. Path. Bact.*, **47**, 423 (1938).

² Herbert, D., and Todd, E. W., *Biochem. J.*, **35**, 1124 (1941).

³ Todd, E. W., and Hewitt, L. F., *J. Path. Bact.*, **35**, 973 (1932).

⁴ Anson, M. L., "Advances in Protein Chemistry", **2**, 361 (1945).

⁵ Olcott, H. C., and Fraenkel-Conrat, H., *Chem. Rev.*, **41**, 1, 151 (1947).

Biological Oxidation of Iron in Soil

Lees and Quastel¹ described a soil perfusion apparatus in which a liquid could be passed continuously through a column of soil under conditions of maximum aeration. The apparatus as modified by Audus² has been used in the present investigations on the biological oxidation of iron in soil.

Biological oxidation of divalent iron in soil. 20-gm. columns of air-dried, crumbed soil maintained at pH 3.0 were perfused with solutions of ferrous sulphate. Daily analyses showed that apart from a small initial fixation (probably caused by the formation of ferrous phosphate) the concentration of ferrous ions in the perfusate steadily fell. As divalent iron salts are not oxidized in air at a pH below 5.0, it would appear that the recorded effect must be attributed to biological action of the soil flora.

This biological action does not occur in the perfusate itself, but is limited to the soil. It is probably bound up with the favourable environment presented to the micro-organisms by the optimum diffusion of oxygen in the film of fluid coating the soil particles.

In some soils it has been found that the rate of oxidation is initially constant, probably due to a high incidence of the specific iron-oxidizing micro-organ-