Solution B yielded a typical precipitate with 2.3 M phosphate buffer, whereas solution A gave none. The irreversibility of this degradation of collagen was shown by the fact that upon dialysis solution B formed a firm gel, whereas solution A exhibited further decrease in relative viscosity to 1.7.

Thymonucleohistone. Minced thymus gland was extracted twice with 10% sodium chloride at 4° for 2 days. Following centrifugation the viscous solution was precipitated by pouring into 3 volumes of 1% acetic acid. The resulting fibrous precipitate was redissolved in M/5 phosphate buffer, pH 6.2, containing 5% NaCl.

The addition of 2 ml of M/5 phosphate buffer containing 20 mg of ascorbic acid and 1 ml of 5% hydrogen peroxide to 25 ml of this solution caused a breakdown of the nucleohistone within one hour as indicated by the flocculent precipitate obtained in 1% acetic acid. The control solution, lacking ascorbic acid, yielded a fibrous, ropey precipitate even at the end of 24 hours.‡ The relative viscosity of the degraded nucleohistone after one hour was 1.28 as compared with 4.51 for the control solution.

Summary. Ascorbic acid and hydrogen peroxide cause an irreversible reduction of viscosity of solutions of collagen and thymonucleohistone.

13674

An Unsuccessful Search for Dopa in Protein Hydrolysates.

A. M. SBOROV, LAWRENCE PETERS AND L. EARLE ARNOW.

From the Laboratory of Physiological Chemistry, University of Minnesota Medical School, Minneapolis.

Dopa (*l*(—)-3,4-dihydroxyphenylalanine) occurs in free form in the broad bean *(Vicia faba)*[1] in the Georgia velvet bean *(Stizolobium deeringianum)*,[2] and in certain insects.[3] It has not been

‡ The change in the precipitate was similar to that observed in the case of synovial fluid mucin, which was illustrated in a previous publication.[4]

[4] Robertson, W. v. B., Ropes, M. W., and Bauer, W., *J. Biol. Chem.*, 1940, **133**, 261.

[1] Torquati, T., *Arch. farmocol. sper.*, 1913, **15**, 213, 308; Guggenheim, M., *Z. physiol. Chem.*, 1913, **88**, 276.

[2] Miller, E. R., *J. Biol. Chem.*, 1920, **44**, 481.

[3] Schmalfuss, H., and Muller, A., *Biochem. Z.*, 1927, **183**, 362.

isolated from protein hydrolysates. Dunn[4] has suggested that its ease of oxidation might account for its apparent absence from hydrolysates.

In an orienting experiment, 45 mg of dopa were boiled under reflux with constant boiling HCl solution. At the end of 20 hours, 75% of the dopa still was present; at the end of 238 hours, 27% remained. The analytical method employed has been described elsewhere.[5]

Several proteins (purified egg albumin, casein, edestin, fibrin) were hydrolyzed with sodium hydroxide, sulfuric acid, or hydrochloric acid. No dopa could be detected in any of the hydrolysates. When dopa equivalent to 1% of the protein was added prior to hydrolysis, no dopa could be found in the alkaline hydrolysates, but 70 to 90% of the added material was found in the acid hydrolysates. Since a part of the loss resulted from adsorption of the dopa on the charcoal used for clarification of the hydrolysates, it is probable that only slight destruction occurs when the compound is boiled with acid in the presence of protein.

Since dopa is known to occur in the Georgia velvet bean, the protein of this bean was investigated. The beans were powdered in a drug mill, and the powder was thoroughly extracted with 20% trichloracetic acid until the washings no longer gave a test for dopa. The insoluble residue then was washed with 80% alcohol until the washings no longer were acid to litmus. The dopa-free protein was hydrolyzed with HCl. No dopa could be detected in the hydrolysate, although positive tests were obtained when dopa was added to the protein prior to hydrolysis.

Since some samples of charcoal were found to adsorb dopa quantitatively, hydrolysates of the velvet bean protein were clarified with cuprous oxide.[6] Again, tests for dopa were negative unless the substance had been added to the mixture either before or after hydrolysis.

When the velvet bean protein hydrolysate was adjusted to neutrality and extracted with butyl alcohol,[7] any dopa present was found to be in the proline fraction—*i. e.*, in solution in the butyl alcohol-water mixture present in the flask from which the butyl alcohol was distilled. No dopa was found in this fraction unless it had been added to the protein or to the hydrolysate.

[4] Dunn, M. S., in Schmidt, C. L. A., *Chemistry of the Amino Acids and Proteins*, Charles C. Thomas, Springfield, 1938.

[5] Arnow, L. E., *J. Biol. Chem.*, 1937, **118**, 531.

[6] Abderhalden, E., and Fuchs, D., *Z. physiol. Chem.*, 1908, **57**, 339.

[7] Dakin, H. D., *Biochem. J.*, 1918, **12**, 1290.

Comment. The evidence here presented can be interpreted to indicate that: (1) Dopa is not a constituent of any of the proteins examined. (2) Or, that dopa in combined form is more readily destroyed than is free dopa. (3) Or, that linkages involving dopa are not cleaved by the procedures used for hydrolysis. This interpretation involves the unlikely assumption that combined dopa does not yield a color with the nitrite-molybdate reagent.

Summary. Dopa is destroyed rapidly in boiling alkaline solutions, and much more slowly in boiling acid solutions. When dopa was added to proteins (egg albumin, casein, edestin, fibrin, velvet bean protein) prior to acid hydrolysis, most of it could be detected in the hydrolysates. However, no dopa was found in any of the hydrolysates prepared without previous addition of this compound.

13675

Ultraviolet Absorption Spectrum of Bovine Phosphatase in Presence and Absence of Added Substrate.

IRWIN W. SIZER. (Introduced by F. O. Schmitt.)

From the Department of Biology and Biological Engineering, Massachusetts Institute of Technology, Cambridge, Mass.

The ultraviolet absorption spectra of enzymes which have been studied (pepsin,[1] papain,[2] urease,[3] yellow enzymes,[4] milk flavoprotein,[5] ribonuclease[6]) resemble closely those which have been reported for proteins. The absorption maximum at 270-280 mμ and minimum at 240-250 mμ have been attributed to the presence of tyrosine, tryptophane and, to a lesser degree, phenylalanine in the protein molecule. The so-called "end absorption" is ascribed to the presence in the protein of many different amino acids which strongly absorb ultraviolet below a wavelength of about 240 mμ.

The most generally accepted theory of enzyme catalysis postulates the formation of an intermediary enzyme-substrate complex. Spec-

1 Gates, F. L., *J. Gen. Physiol.*, 1934, **18**, 265.

2 Darby, H. H., *J. Biol. Chem.*, 1941, **139**, 721.

3 Kubowitz, F., and Haas, E., *Biochem. Z.*, 1933, **257**, 337.

4 Theorell, H., *Biochem. Z.*, 1935, **278**, 279.

5 Corran, H. S., Dewan, J. G., Gordon, A. H., and Green, D. E., *Biochem. J.*, 1939, **33**, 1694.

6 Uber, F. M., and Ells, V. R., *J. Biol. Chem.*, 1941, **141**, 229.