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.

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

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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.

troscopic evidence for the existence of such a complex is lacking except for the heme containing enzymes, catalase (for discussion *cf.* Green[7]) and peroxidase[8] where the addition of substrate to the enzyme causes a shift of absorption bands in the visible region of the spectrum. The present work presents a study of the ultraviolet absorption spectrum of bovine phosphatase, and the effects of its substrate, sodium β-glycerophosphate, upon this spectrum.

Methods. The absorption spectra were taken with a Hilger quartz spectrograph (Model E484) equipped with a Spekker photometer using a tungsten spark. The photographic plates were projected and the match points at the different wave-lengths read by eye. Several bovine phosphatase preparations of different degrees of purity were used. The intestinal phosphatase (prepared by Dr. Gerhardt Schmidt by tryptic digestion of the intestinal mucosa, followed by extraction of lipids and differential salt precipitation of the protein components) was highly purified and contained only the alkaline phosphatase, while the kidney phosphatase, prepared according to Albers,[9] contained both alkaline and acid phosphatase. The enzyme was dissolved in glass distilled water and placed in a 2 cm quartz cell. The duplicate cell contained only the solvent. The pH of the solution was measured with the glass electrode at room temperature immediately after the absorption spectrum had been taken.

Results. From the ultraviolet absorption spectrum it is apparent that intestinal phosphatase does not absorb in the visible region,[10] but absorbs strongly in the ultraviolet with a sharp maximum at 278 mμ, a minimum at 253 mμ, and "end absorption" below 236 mμ (Fig. 1, curve A). The absorption spectrum is the same before and after purification of the phosphatase by treatment with $Al(OH)_3$. Essentially similar curves were obtained with several preparations of kidney phosphatase of different degrees of purity, with an average maximum at 275 mμ and minimum at 257 mμ. The small differences in the values for the intestinal and kidney phosphatase can probably be ascribed to the presence of impurities in the kidney phosphatase.

Different proteins have characteristic ultraviolet absorption spectra,[11] and of these the absorption spectrum of phosphatase

7 Green, D. E., *Mechanisms of Biological Oxidations*, Cambridge, 1940.

8 Chance, B., *J. Biol. Chem.*, 1941, **140**, XXIV.

9 Albers, D., *Biochem. Z.*, 1940, **306**, 143.

10 Kraemer, E. O., Weil, L., Lanigar, E. B., and Allen, M. T., *J. Frank. Inst.*, 1941, **232**, 587.

11 Ellinger, F., *Absorptions Spektroskopie im Ultraviolett*, The Hague, 1927.

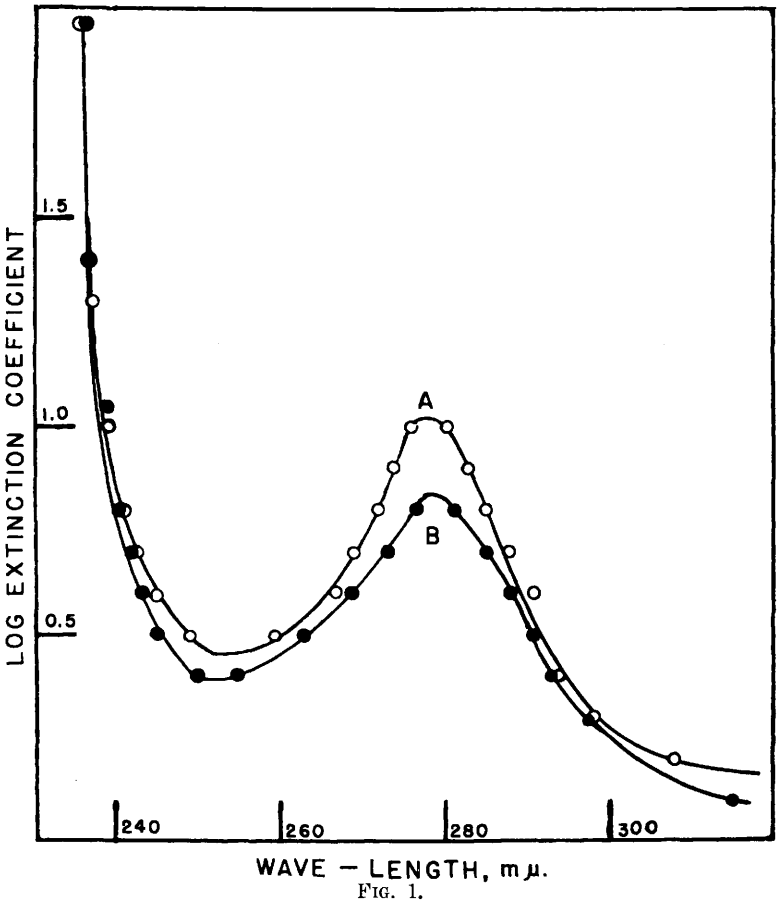

FIG. 1.

Ultraviolet absorption spectrum of 0.009% bovine intestinal phosphatase.
Curve A. No added substrate, pH 4.97.
Curve B. Solution contains 0.1% sodium β glycerophosphate, pH 7.72.

closely resembles that of typical globulins. The suggestion that this enzyme may be a globulin is confirmed by chemical and physical investigations of other workers.[9, 10, 12] An attempt was also made to relate the ultraviolet absorption spectrum to the spectrum of amino acids which might be present in the phosphatase molecule. Of the various amino acids which absorb strongly in the ultraviolet it was found that tyrosine (aqueous solution, 0.05 mg/ml, pH 5.63) has a maximum absorption at 276 mμ and a minimum at 245 mμ, while tryptophane (aqueous solution, 0.015 mg/ml, pH 5.21) has a maximum absorption at 280 mμ and a minimum at 242 mμ. These values agree well with those reported previously[13] and suggest that

[12] Perlmann, G. E., and Ferry, R. M., *J. Biol. Chem.*, 1942, **142**, 513.
[13] Loofbourow, J. R., *Rev. Mod. Physics*, 1940, **12**, 267.

the ultraviolet absorption spectrum of these 2 amino acids largely accounts for the characteristic absorption spectrum of phosphatase.

A change in ultraviolet absorption would occur on the addition of substrate to enzyme, if a complex is formed of which the absorbing characteristics (arising from increased or decreased saturation, substitution of unsaturated linkages, etc.) are different from the original enzyme and substrate. The effect of substrate on the ultraviolet absorption spectrum of phosphatase was studied by dissolving the enzyme in 0.1% sodium β-glycerophosphate (Merck). The quartz cell for solvent contained the same concentration of substrate but no enzyme. The absorption spectrum is shown in Fig. 1, curve B. It is apparent from a comparison of the two curves that although the total absorption is less in curve B (possibly due to a difference in pH or concentration of the two solutions), there is no shift in the absorption curve indicative of the formation of an enzyme-substrate complex. As a very sensitive method of detecting any spectral shift of the enzyme caused by the substrate, one beam from the tungsten spark is passed through a solution containing phosphatase plus sodium β-glycerophosphate in the same quartz cell, while the other beam is passed through two cells, one containing enzyme, the other substrate. Any difference in the absorption of light by these 2 systems must be caused by the formation of an enzyme-substrate complex in the cell containing both enzyme and substrate. Experiments of this type were performed, using both intestinal and kidney phosphatase, but revealed no evidence of an enzyme-substrate complex. The lack of shift in spectrum does not eliminate the possibility that an enzyme substrate complex is formed, but if such a complex exists the manner of combination must be such as to have no appreciable influence on the ultraviolet absorption.

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Summary. The ultraviolet absorption spectrum of bovine intestinal and kidney phosphatase indicates a maximum at 278 mμ, a minimum at 253 mμ and "end absorption" below 236 mμ. The absorption curve resembles that of typical proteins and suggests that phosphatase is a globulin. Tyrosin and tryptophane in the phosphatase may largely account for its characteristic absorption. No spectroscopic evidence was found for the formation of an enzyme-substrate complex when sodium β-glycerophosphate was added to a phosphatase solution.