were studied by subjecting them to a further partial hydrolysis.

3. A dipeptide Ala-SerP was identified and shown to be derived from the inversion of SerP-Ala present in the original protein (SerP refers to serine phosphate).

4. The presence of Asp-SerP dipeptides in three forms was detected. A diagonal ionophoretic technique was used to study the interconversion of the aspartate-containing peptides.

5. On the bases of ionophoretic mobilities and periodate oxidation of the radioactive peptides it was concluded that peptides accounting for the sequence (Thr or Ser)-Asp-SerP-Ala-neutral amino acid were present in the partial acid hydrolysate.

6. The hydrolysis with dilute acid gave a preferential splitting at the aspartate residue, but phosphate was also produced in high yields. Splitting at sites other than aspartate residues was also detected.

7. The results are discussed in connexion with the use of tracer techniques in determinations of amino acid sequences. The similarity between the sequence described and those of other hydrolytic and proteolytic enzymes is also discussed.

I am grateful to Mrs M. F. de Strasnoy for her assistance at different stages of this work, and to the Consejo Nacional de Investigaciones Cientificas y Tecnicas (Republica Argentina) for financial support during part of this work.

REFERENCES

Anderson, L. & Jollès, G. R. (1957). *Arch. Biochem. Biophys.* **70**, 121.
Engström, L. (1961*a*). *Biochim. biophys. Acta*, **52**, 49.
Engström, L. (1961*b*). *Biochim. biophys. Acta*, **54**, 179.
Engström, L. (1962*a*). *Biochim. biophys. Acta*, **56**, 606.
Engström, L. (1962*b*). *Ark. Kemi*, **19**, 129.
Fölsh, G., Mellander, O. & Strid, L. (1960). *Acta chem. scand.* **14**, 625.
Garen, A. & Levinthal, C. (1960). *Biochim. biophys. Acta*, **38**, 396.
Grazi, E., Rowley, P. T., Cheng, T., Tchola, O. & Horecker, B. L. (1962). *Biochim. biophys. Res. Commun.* **9**, 38.
Hirs, C. H. W. (1956). *J. biol. Chem.* **219**, 611.
Michl, H. (1951). *Mh. Chem.* **82**, 489.
Milstein, C. (1961). *Biochem. J.* **79**, 26*P*.
Milstein, C. (1963). *Biochim. biophys. Acta*, **67**, 171.
Milstein, C. & Sanger, F. (1960). *Biochim. biophys. Acta*, **42**, 173.
Milstein, C. & Sanger, F. (1961). *Biochem. J.* **79**, 456.
Naughton, M. A., Sanger, F., Hartley, B. S. & Shaw, D. C. (1960). *Biochem. J.* **77**, 149.
Park, J. H., Meriwether, B. P., Clodfelder, P. & Cunningham, L. W. (1961). *J. biol. Chem.* **236**, 136.
Pigretti, M. E. & Milstein, C. (1964). *Biochem. J.* (in the Press).
Sanger, F. (1963). *Proc. chem. Soc.* p. 76.
Sanger, F. & Shaw, D. C. (1960). *Nature, Lond.*, **187**, 872.
Schwartz, J. H., Crestfield, A. M. & Lipmann, F. (1963). *Proc. nat. Acad. Sci., Wash.*, **49**, 122.
Schwartz, J. H. & Lipmann, F. (1961). *Proc. nat. Acad. Sci., Wash.*, **47**, 1996.
Witkop, B. (1961). *Advanc. Protein Chem.* **16**, 221.

APPENDIX

The Amino Acid Sequence Around the Reactive Serine Residue in Alkaline Phosphatase of *Serratia marcescens*

BY N. ZWAIG* AND C. MILSTEIN†
Division de Biologia Molecular, Instituto Nacional de Microbiologia, Buenos Aires, Argentina

(*Received* 3 *December* 1963)

A comparative study of the amino acid sequence around the reactive serine residue in alkaline phosphatases of *Serratia marcescens* and *Escherichia coli* was made by using the technique of matching radioactive peptides. Alkaline phosphatase from *Serratia* has been studied by Signer, Torriani & Levinthal (1961) and found to be similar in many respects to the *E. coli* enzyme. Significant differences were, however, detected, mainly in the isoelectric point and the 'fingerprints' of both proteins.

* Present address: Departamento de Microbiologia, Facultad de Ciencias Exactas, Universidad de Buenos Aires, Argentina.

† Present address: M.R.C. Laboratory of Molecular Biology, Postgraduate Medical School, Cambridge.

The enzyme from *Serratia* was purified by the same procedure as used for the enzyme of *E. coli* by Milstein (1964). Two preparations containing about 0·5 mg. of enzyme, one of *E. coli* and one of *Serratia*, were labelled at the same time by the general procedure described by Milstein (1964), except that 1 mg. of carrier γ-globulin was added after the incubation with [^{32}P]phosphate. The precipitated protein in acid acetone was suspended in distilled water and dialysed overnight against distilled water. The insoluble γ-globulin was centrifuged down and the soluble radioactive protein was used, after drying over sodium hydroxide. The radioactive residues were partially acid hydrolysed with constant-boiling hydrochloric acid for 30 min. in a boiling-water bath. After complete elimination

of the hydrochloric acid, the two preparations (about 20 μmc of each) were run side by side by ionophoresis at pH 3·5. Both preparations gave very similar patterns (Fig. 1), the only difference being the presence of a few extra positively charged bands in the *Serratia* ionogram.

The main bands of both preparations were cut out and subjected to ionophoresis in a second dimension at pH 6·5. This permitted a check of the matching of the individual peptides by ionophoresis at two different pH values. The dipeptides and tripeptides matched very closely in both proteins. Also, the yields were apparently very similar, except for some components of bands 15 and 16. The tripeptides present in 11, 4 and 7, closely matching in both enzymes, include five amino acids around the reactive serine residue (Milstein, 1964). We conclude that the sequence (Thr or Ser)-Asp-SerP-Ala-neutral residue is probably common to both proteins.

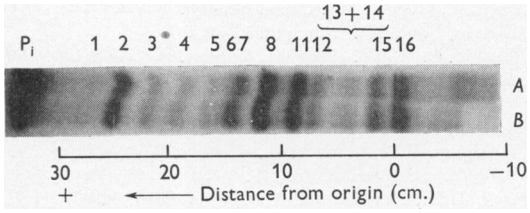

Fig. 1. Radioautograph obtained after ionophoresis at pH 3·5 (at 35 v/cm. for 2·5 hr. on Whatman no. 52 paper) of partial acid hydrolysates of ^{32}P-labelled alkaline phosphatases of: *A*, *S. marcescens*; *B*, *E. coli*.

The extra radioactive peptides present in the partial acid hydrolysate of the *Serratia* enzyme as compared with the *E. coli* enzyme (Fig. 1) suggest a difference in the amino acid sequence near the common pentapeptide described above. Close similarity in the amino acid sequences at the active site in the same enzyme of different origins has been found in other cases (Milstein, 1961; Perham & Harris, 1963) and indicates the importance of those amino acids for the normal function of the enzyme.

REFERENCES

Milstein, C. (1961). *Biochem. J.* **79**, 26P.
Milstein, C. (1964). *Biochem. J.* **92**, 410.
Perham, R. N. & Harris, J. I. (1963). *J. molec. Biol.* **7**, 316.
Signer, E. R., Torriani, A. & Levinthal, C. (1961). *Cold Spr. Harb. Symp. quant. Biol.* **26**, 31.

Biochem. J. (1964), **92**, 422

Sequential Changes in Liver and Heart Lipids after Giving Linoleate or Linoleate plus Pyridoxine to Rats Depleted of Fat and Pyridoxine

BY GENEVIEVE E. SCHEIER* AND MARY A. WILLIAMS
Department of Nutritional Sciences, University of California, Berkeley, Calif., U.S.A.

(*Received* 23 *September* 1963)

Despite considerable investigation, the role of pyridoxine in the metabolism of linoleic acid and arachidonic acid is still not clear (Mueller & Iacono, 1963). In general, studies of the effect of pyridoxine deficiency on the utilization of dietary linoleate have been of two types. The first has been concerned with the effects of pyridoxine deficiency on the utilization of linoleate by fat-deficient animals. Witten & Holman (1952), in a study of this type, observed that total body arachidonate (mg./rat) was greater in rats supplemented with linoleate and pyridoxine for 8 weeks than in rats given only linoleate. Before supplementation, the rats had been depleted of both fat and pyridoxine by giving a fat-free pyridoxine-free diet. From these observations it was suggested that 'pyridoxine is involved in the conversion of linoleate to arachidonate'. Kirschman & Coniglio (1961) also used this type of approach and likewise found more arachidonate in the carcass fat of rats supplemented with linoleate and pyridoxine for 3 weeks than in that of rats given only linoleate. The ratios of arachidonate to fat-free carcass weight, however, were similar in both groups of rats, and it was concluded that the increase in arachidonate in the rats given linoleate plus pyridoxine was the result of tissue growth and not a specific effect of pyridoxine in the conversion of linoleate into arachidonate.

The second type of approach has involved the effects of pyridoxine deficiency on linoleate utilization, uncomplicated by recovery from dietary fat deficiency. Swell, Law, Schools & Treadwell (1961) found that the development of pyridoxine defi-

* Present address: San Diego State College, Department of Home Economics, San Diego 15, Calif., U.S.A.