with HCl and tested for their ability to support the growth of the inositol-less mutant 37401 of *Neurospora crassa* (BEADLE[7]). A positive growth response was obtained from concomitants F and D, and from eluate I.

When the A_2S fraction obtained from brain tissue was submitted to ionophoresis the pattern of concomitants was similar to that obtained from liver. Microbiological tests for inositol yielded positive growth responses from concomitants F and D and from a small amount of non-ultraviolet absorbing material which moves more rapidly than A and may be inositol diphosphate itself. Since these concomitants account for only about 30% of the non-nucleotide phosphorus, it seems that by no means all the non-nucleotide phosphorus in the A_2S fraction from brain is "inositide P".

Positive inositol growth tests have been obtained from the A_2S fraction of calf thymus nuclei, of rat liver mitochondria, microsomes, and cell sap (prepared by Dr. R. M. S. SMELLIE), of bull sperm and of sea urchin sperm. Positive responses have also been obtained from the material obtained by alkaline incubation of the protein residue remaining after liver tissue is submitted to the procedure of SCHNEIDER[8] for the removal of nucleic acids (the fraction A_3S of DAVIDSON, FRAZER AND HUTCHISON[1]).

Full details of these experiments will be published later. The work was aided by a grant from the Medical Research Council of Great Britain to one of us (J.N.D.).

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AMINO ACID SEQUENCES IN LYSOZYME

by

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The N-terminal sequence of lysozyme has been shown to be Lys. Val. Phe. Gly.[1], and FROMAGEOT[2] has obtained evidence that the next residue is arginine. This is in conflict with the evidence of LANDMANN *et al.*[3] that serine is the fifth residue. If it is indeed arginine then presumably the serine arises as an artefact of the thiohydantoin method used by LANDMANN (cf. THOMPSON[4]). On the basis of peptides isolated from acid hydrolysates, ACHER *et al.*[5] have proposed the sequence Arg. His. Lys. They have identified the two peptides Tyr. Gly. and Gly. Tyr., and ACHER *et al.*[6], Phe. Glu. and Phe. Asp.

From studies over the past three years on acid hydrolysates of lysozyme a large number of peptides have been isolated and identified. The chief problem has been to find a method of high resolving power for separating the extraordinary complex mixture of peptides produced when lysozyme is hydrolysed in 12 *N* HCl (at 37° C for 4 days). Displacement chromatography on ion exchange resins was attempted following the success achieved by PARTRIDGE and his co-workers[7] in the separation of amino acids by this method and bearing in mind the high capacity of such columns. It was found, however, that the resolution for peptides was inferior to that for amino acids owing to the increased importance of Van der Waals forces with these larger molecules.

MOORE AND STEIN[8] have shown the high resolving power of elution chromatography on ion exchange resins for amino acids and DOWMONT AND FRUTON[9] have successfully chromatographed some simple peptides in a similar way. This method was applied to the problem of separating the peptides in a partial acid hydrolysate of lysozyme.

Gradient elution was used with ammonium formate and acetate buffers from pH 3.4 (0.2 *M*) to pH 6.8 (0.6 *M*) on Dowex 50 (4% cross-linked, 200–400 mesh). Ammonia was removed from samples of eluate before determining the colour produced by ninhydrin and in this way an elution curve was plotted. The peaks did not correspond, in general, to pure amino acids and peptides but to a fairly simple mixture of up to eight peptides which could be readily resolved on a two dimensional paper chromatogram. The procedures of SANGER and co-workers[10] were followed in determining the structure of these peptides. In certain cases carboxypeptidase was used to determine the C-terminal residue of peptides where only one N-terminal residue had been obtained, suggesting a pure compound. In the case of tripeptides, this established the tripeptide sequence.

From the large number of peptides identified in these experiments, the following sequences have been derived:

Ser. Asp. Gly. $MetO_2$. Asp.	Leu. Thr. Ala.
Thr. Asp. Val. Glu. Ala.	Ala. $MetO_2$. Lys. $CySO_3H$. Arg.
Ileu. Glu. Leu. Ala. Leu.	Gly. Phe. Glu. Asp. Ileu.
Thr. Glu. Ala.	Arg. $CySO_3H$. Glu. Ala.
Asp. Glu. Ala.	Thr. Pro. Gly.

In addition, a number of dipeptides which represent sequences in the molecule have been identified.

Ala. Ala.	$CySO_3H$. Ala.	Ileu. Asp.	Ser. Ala.
Ala. Lys.	$CySO_3H$. Asp.	Ileu. Arg.	Ser. Arg.
Asp. Ala.	$CySO_3H$. Lys.	Ileu. Val.	Ser. Leu.
Asp. Arg.	Gly. Leu.	Leu. Leu.	Ser. Val.
Asp. Leu.	Gly. Lys.	Phe. Asp.	Thr. Gly.
Arg. Asp.			
Arg. Leu.			

The composition and N-terminal residues of a considerable number of other peptides has been established but as yet, insufficient evidence has been obtained to establish the sequences of which they form part. Evidence for these peptides and the sequences listed above will be presented in detail in subsequent communications.

The first part of this work was carried out at the Low Temperature Research Station, University of Cambridge and forms part of the programme of the Food Investigation Organization of the Department of Scientific and Industrial Research. The results were reported briefly at the 2nd International Congress of Biochemistry in Paris, 1950. Later stages of the investigations were undertaken at the College of Medicine, University of Utah, Salt Lake City, Utah, U.S.A. and I am grateful to Professor EMIL L. SMITH for his hospitality and interest in this work. It is a pleasure to acknowledge several helpful discussions with Dr. S. MOORE and Dr. W. H. STEIN of the Rockefeller Institute, New York on aspects of elution chromatography. The investigations were continued at the Biochemistry Unit, Wool Textile Research Laboratories, C.S.I.R.O., Melbourne, Australia.

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