from glucose than on its formation from glutamic acid. It has also been found that the incorporation of radioactivity from glutamic acid proceeds also during germination in complete darkness. A marked accumulation of free ammonia during neopyrithiamine treatment was also noticed, possibly due to a lack of adequate production of oxalacetic acid to fix it as asparagine. Full details will be published elsewhere.

We wish to thank Dr. KARL FOLKERS of Merck & Co., Inc., Rahway, N.J. (U.S.A.) for a generous gift of neopyrithiamine hydrobromide used in this investigation.

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Received June 12th, 1954

PROTEIN-BOUND COMPONENTS OF LIVER AND BRAIN

by

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In 1951 DAVIDSON, FRAZER AND HUTCHISON[1] pointed out that when liver tissue is submitted to the procedure of SCHMIDT AND THANNHAUSER[2] for the separation of ribonucleic acid and deoxyribonucleic acid, the mixture of ribonucleotides obtained by alkaline hydrolysis and subsequent acidification (fraction A_2S) is insufficient to account for all the phosphorus in the fraction. DAVIDSON AND SMELLIE[3] showed that this fraction contained, in addition to the ribonucleotides which accounted for some 75 to 80% of its phosphorus, at least six phosphorus compounds, one of which was inorganic phosphate. These compounds could be separated from each other and from the ribonucleotides by ionophoresis on paper and were provisionally designated, in descending order of mobility, concomitants A, B, C, D, E and F. When nervous tissue was submitted to the procedure of SCHMIDT AND THANNHAUSER by LOGAN, MANNELL AND ROSSITER[4] only about 25 to 50% of the phosphorus of the ribonucleotide fraction was found to be derived from ribonucleotides. The remainder consisted of a small amount of inorganic phosphate and a large amount of organic phosphate which LOGAN *et al.* designated "inositide P" since FOLCH AND LE BARON[5] and FOLCH[6] had claimed that neurokeratin after extraction of lipids still contains protein-bound inositol diphosphate which is released by warm alkali.

We have recently submitted the A_2S fraction[1] obtained from rat liver tissue by the method of SCHMIDT AND THANNHAUSER[2] as modified by DAVIDSON AND SMELLIE[3] not only to ionophoresis on paper but also to chromatography on columns of Dowex ion exchange resins. A preliminary removal of adenylic, guanylic and cytidylic acids was carried out by passage through a Dowex 50 column. The uridylic acid fraction containing the concomitants was adsorbed on a Dowex 1 column and the column eluted successively with water and increasing concentrations of formic acid and ammonium formate. By using material from rats injected with radioactive inorganic phosphate, it was possible to follow the elution of the phosphorus derivatives by allowing the eluate to pass through a flow counter attached to a ratemeter and a recording milliameter. In this manner some fifteen major activity peaks were detected. The multiplicity of phosphate esters in the A_2S fraction is also revealed by ion-exchange chromatography of the concomitants A to F respectively.

The material which passed through the Dowex 1 column without exchanging (eluate I) was ninhydrin positive and could be subdivided into a dialysable peptide fraction containing about 75% to the total phosphorus and a non-dialysable peptide fraction containing about 25% of the total phosphorus. On hydrolysis both fractions yielded phosphoserine which was identified by chromatography. The major part of this material appeared to have the same ionophoretic mobility as concomitant F.

In view of the claim of FOLCH[6] that inositol diphosphate is released by alkaline incubation of neurokeratin, the fractions obtained by column and ionophoretic separations were hydrolysed

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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Received June 17th, 1954

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.