THE INHIBITION BY NEOPYRITHIAMINE OF ASPARAGINE SYNTHESIS FROM GLUTAMIC ACID AND GLUCOSE

by

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Our previous investigations on the influence of neopyrithiamine and γ-(3,4-ureylenecyclohexyl)-butyric acid on amino acid changes during germination of *Phaeseolus radiatus* seeds[1] have provided strong presumptive evidence for a conversion of glutamic acid to aspartic acid** during germination. Normally there is a marked fall in glutamic acid and considerable rise in aspartic acid as germination proceeds. But the addition of increasing amounts of these antivitamins to the growth medium produces a progressive accumulation of glutamic acid and a corresponding fall in the aspartic acid synthesized. These observations are explained on the basis that glutamic acid is converted into aspartic acid and asparagine during germination and that the two antivitamins inhibit this conversion. Also, since the fall in aspartic acid, expressed in terms of gramme molecules, far exceeded the rise in glutamic acid at several concentrations of the antivitamins, it was also postulated that glutamic acid is not the sole source for aspartic acid, and that the formation of aspartic acid from other source or sources is also inhibited by these antivitamins producing the excessive fall observed in aspartic acid. With the use of uniformly ^{14}C-labelled glutamic acid and glucose, we have now obtained direct evidence for the formation of asparagine from these compounds and for the inhibition by neopyrithiamine of these conversions. Glucose thus constitutes the other important source for asparagine biosynthesis.

The experiment consists essentially in germinating the seeds (*Phaeseolus radiatus*) with the labelled compound (either glutamic acid or glucose as the case may be) in the medium, isolating the asparagine in a pure crystalline state from the seedlings after 72 h germination carried out in diffused light, and measuring the radioactivity of the sample in a flow-gas counter using an autoscaler. For the isolation of asparagine, the seedlings were extracted with warm water, and the extract deproteinised by heat coagulation at pH 5.0–5.5. An aliquot of the deproteinised extract was used for asparagine estimation[2], while the rest, after the addition of carrier asparagine and adjustment of pH to 7.0, was concentrated *in vacuo*, clarified with basic lead acetate and after removal of lead as sulphide, concentrated to crystallisation.

To study the inhibitory effect of neopyrithiamine, neopyrithiamine hydrobromide was added to the medium (1.6 mg per 24 ml) and the germination carried out simultaneously with the Controls (*i.e.* without neopyrithiamine). Results obtained are presented in Table I.

TABLE I

INCORPORATION OF RADIOACTIVITY FROM ^{14}C-GLUTAMIC ACID AND ^{14}C-GLUCOSE INTO ASPARAGINE

	^{14}C-Glutamic acid		*^{14}C-Glucose*	
	Control	*Neopyrithiamine added*	*Control*	*Neopyrithiamine added*
Activity supplied in c.p.m.	$2.852 \cdot 10^6$	$2.852 \cdot 10^6$	$5.906 \cdot 10^6$	$5.906 \cdot 10^6$
Asparagine formed in mg	283.4	105.8	139.5	28.87
Activity incorporated in asparagine in c.p.m.	20,633	6996	21,994	1,913
Percent activity incorporated in asparagine	0.7235	0.2453	0.3723	0.0324

The percentage of activity incorporated from both the sources are of the same order though different in magnitude. Thus, the amide is synthesized by the degradation of both proteins and carbohydrates. Neopyrithiamine exerts a greater inhibitory effect on the formation of asparagine

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** The term "Aspartic acid" used here actually refers to both aspartic acid and asparagine, since the method of estimation employed does not distinguish between the two, but gives the sum of both.

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