exchangeable with added free amino acid would explain the present observations. Current work is directed to elucidating the site and nature of the precursor.

Other indications for precursors between free amino acid and protein have been published recently[2,3].

RICHARD W. HENDLER

Laboratory of Cellular Physiology and Metabolism, National Heart Institute, Bethesda, Md. (U.S.A.)

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The role of cytidine nucleotides in the formation of inositol-containing lipid

It has been reported that tissue slices incubated with inorganic ^{32}P incorporate radioactivity into the inositol phosphatide fractions at a greater rate than into the other phospholipids[1,2]. Recently, it has been shown that the inositol moiety of the phosphatide exchanges with free inositol[3,4]. We wish to report preliminary observations on the enzymic route of inositol incorporation into inositol lipid. Preparations of guinea pig kidney were used, since previous studies indicated active inositol utilization[5] and incorporation into lipid[3] by this tissue. *myo*Inositol-2-^3H was prepared from *scyllo*inosose and ^3HOH by a method analogous to that described for deuteration[6]. The radioactive inositol was counted in a proportional flow counter. The specific activity was $1.4 \cdot 10^6$ c.p.m./μmole.

Guinea pig kidney tissue was homogenized in 4 vol. of a solution containing KCl (0.13 *M*), $MgSO_4$ (0.03 *M*), and potassium phosphate buffer, pH 7.4 (0.012 *M*). The cell-free preparation was centrifuged at 600 × *g* for 1 min to remove cellular debris, and the overlying suspension was centrifuged at 7500 × *g* for 20 min. The resulting supernatant suspension was decanted and further centrifuged at 100,000 × *g* for 30 min. The clear supernatant solution was poured off,

TABLE I

INCORPORATION OF INOSITOL INTO INOSITOL LIPID

1 ml samples were incubated with 1 μmole labeled inositol at 37° C in 95% O_2–5% CO_2 with constant shaking. At the end of 2 h, 2 ml of cold TCA* were added and each precipitate was filtered with suction, washed with 50 ml 10% TCA, 50 ml H_2O, and dried *in vacuo*. The lipid fraction was then recovered by Soxhlet extraction with 1:1 methanol-chloroform[1].

Experiment	*Preparation*	*c.p.m. incorporated into lipid*
1	Whole homogenate	10,400
	Supernatant + microsomes	2,128
	Supernatant + mitochondria	6,440
	Supernatant	504
	Microsomes	1,560
	Mitochondria	260
2	Supernatant + heated mitochondria	715
	Heated supernatant + mitochondria	5,500
3	Mitochondria + 1 μmole ATP + 1 μmole CDP + 10 μmoles α-KG	13,670
	Mitochondria + 1 μmole ATP + 10 μmoles α-KG	2,575
	Mitochondria + 1 μmole ATP + 1 μmole CDP	3,520
	Mitochondria + 1 μmole CDP + 10 μmoles α-KG	4,900

* Abbreviations used are: ATP, adenosine triphosphate; ADP, adenosine diphosphate; CTP, cytidine triphosphate; CDP, cytidine diphosphate; CMP, cytidine monophosphate; GDP, guanosine diphosphate; IDP, inosine diphosphate; UDP, uridine diphosphate; α-KG, α-ketoglutarate; TCA, trichloroacetic acid; c.p.m., counts/min.

and the microsomal residue was resuspended in either the buffer or the supernatant solution. The mitochondrial residue obtained at 7500 × *g* was resuspended and washed twice in the buffer solution. All of the preceding operations were performed at 2° C.

For the experiments reported in Table I, the mitochondria were reconstituted with the buffer or supernatant solution in a final volume of 1 ml in order to approximate the concentration in the whole homogenate. The microsomes were about 3 times more concentrated than in the homogenate. It can be seen from Table I that washed mitochondria and heat-stable factors from the supernatant solution were necessary for the incorporation of inositol into lipid. Hydrolysis of the lipid[7] resulted in recovery of the radioactivity as inositol. It is also seen that the combination of ATP, CDP, and α-KG can fully replace the supernatant factors.

Subsequent investigations revealed that the insoluble residue of lyophilized kidney mitochondria catalyzed the incorporation of inositol into lipid in the presence of Mg^{++} and cytidine nucleotide under anaerobic conditions. Experiments demonstrating the specificity for this nucleotide are shown in Table II. Other cytidine nucleotides were later found to be even more effective than CDP.

TABLE II

NUCLEOTIDE REQUIREMENT FOR THE INCORPORATION OF INOSITOL INTO LIPID

Washed mitochondria were lyophilized, triturated, and the residue was washed and suspended in 100 vol. of the KCl-K phosphate-$MgSO_4$ solution. 0.2 μmole labeled inositol was incubated with 0.3 ml of the suspended residue under N_2 at 37° C. At the end of 2 h, 1 ml 1% serum albumin was added and the lipid was recovered by the extraction procedure outlined in Table I.

Nucleotide added (1 μmole)	*c.p.m. incorporated into lipid*
CDP	2,416
GDP	26
IDP	282
ADP	260
UDP	202
none	73

The role of the cytidine nucleotides in the incorporation of phosphorylcholine into lecithin has been well established by KENNEDY AND WEISS[8]. An analogous series of reactions with inositol in the system described in Table II seems improbable since there would appear to be little possibility for the phosphorylation of inositol. Preincubation with hexokinase and glucose did not depress the incorporation of inositol. Furthermore, all of the cytidine nucleotides catalyzed the incorporation of inositol into lipid, in the following order of decreasing effectiveness at limiting concentrations: CDP-choline > CMP > CDP > CTP. Cytidine was ineffective. Since CTP and CDP might give rise to CMP which could react with lecithin in the residue by the action of "PC glyceride transferase"[8] to form CDP-choline, this latter substance might be the true reactant in the inositol–inositol lipid exchange. Insufficient evidence exists at present to postulate the exact nature of inositol activation. However, free choline has been found to depress the incorporation of inositol in the presence of CMP, further implicating a dynamic interrelationship between the choline and inositol lipids.

BERNARD W. AGRANOFF
ROY M. BRADLEY
ROSCOE O. BRADY

National Institute of Neurological Diseases and Blindness, National Institutes of Health, Public Health Service, Department of Health, Education, and Welfare, Bethesda, Md. (U.S.A.)

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