Evidence for an intermediate stage in the process of amino acid incorporation into hen oviduct proteins

A mince of hen oviduct will incorporate radioactive amino acids into its proteins, but when the tissue is homogenized under conditions previously described[1] this ability is almost completely lost (Table I).

TABLE I

	Total cts/min incorporated into protein
Whole mince in buffer	7290
Whole homogenate in fortified buffer*	39

Buffer contains HCO_3^-, K^+, Na^+, Cl^-, glucose, and glycine-1,2-^{14}C[1].

* ATP, Mg^{++}, yeast extract and $HPO_4^=$ added. When these additions were omitted, no detectable incorporation was observed.

A mince was first incubated with radioactive amino acid (alanine in this experiment), then homogenized and the cell-debris obtained and washed[1]. This fraction contained radioactive precursors, which upon subsequent incubation with an unlabelled cell-supernatant, became incorporated into the proteins of the homogenate. Under the same conditions with the same tissue, free amino acid at more than 100 times the radioactivity that was present in the pre-incubated cell-debris fraction was not appreciably incorporated (Fig. 1).

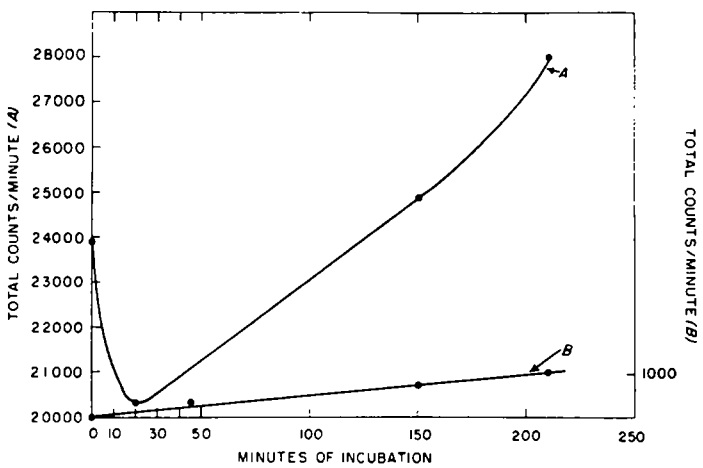

Fig. 1. Increase of radioactivity of total proteins of a hen oviduct homogenate with either a pre-labelled cell-debris (A; ordinate at left) or free amino acid (B; ordinate at right) as the source of radioactivity.

Curve A shows the radioactivity in the total proteins from an incubation of pre-labelled cell-debris with unlabelled cell-supernatant. After an initial loss of radioactivity which might be explained by hydrolysis of labile protein or loss of active precursor material, there is a steady increase of incorporated radioactivity. In other experiments an increase in total radioactivity was observed without this preliminary loss. Curve B shows the radioactivity in the total proteins from an incubation of unlabelled cell-debris from a preincubated mince, unlabelled cell-supernatant and radioactive alanine at about 100 times the total radioactivity present under the conditions of Curve A. It is seen that the radioactive precursor for the proteins represented by Curve A cannot be the free alanine in the medium.

All the protein fractions were boiled in trichloroacetic acid and alcohol-ether before counting. Therefore, the radioactive precursor is released from the cell-debris by the above treatment. Either a chemically combined or spatially confined amino acid, such that it would not be readily

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].

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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.