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Biosynthesis of Lysophosphatidic Acid from ATP and 1-Monoolein by Subcellular Particles of Intestinal Mucosa* (33878)

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(Introduced by J. F. Mead)

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In a previous report (1), we presented evidence for the biosynthesis of lysophosphatidic acid by rat intestinal mucosa during absorption of a mixture of doubly labeled 1-monopalmitin (^{14}C glycerol and ^{3}H palmitic acid) and free fatty acids. Isotopic ratios of both phosphatidic and lysophosphatidic acids indicated that direct phosphorylation of the 1-monopalmitin occurred, followed by acylation to produce phosphatidic acid. This result was obtained under *in vivo* conditions and requires additional *in vitro* evidence. For this purpose, we have adopted an experimental procedure described by Pieringer and Hokin (2) for brain and liver homogenates, modified as follows: 98% pure ATP$\gamma^{32}P$ (C.E.A., Saclay, France) is substituted for the ATP generating system and incubations are carried out with intact subcellular particles (i.e., microsomes and mitochondria) instead of their deoxycholate extracts.

Materials and Methods. Subcellular fractions were obtained by fractional sedimentation of homogenates of the intestinal mucosa of 6 rats by the method of Hübscher *et al.* (3). Homogenates were prepared from mucosal scrapings and 0.3 *M* sucrose, pH 7.4 (1/9, w/v).

Conditions of incubation are described in Table I. The reaction was stopped by addition of 16 ml of methanol followed by 64 ml of methylal. After 2 hr the mixture was filtered, dried over Na_2SO_4 and concentrated under vacuum at a temperature not exceeding 50°. Total ^{32}P radioactivity of the extract was determined by liquid scintillation counting of an aliquot.

Fractionation of the total extract into different phospholipid classes was carried out by thin-layer chromatography on Kieselgel G using chloroform–methanol–water (65/25/4, v/v/v) as the developing solvent. The different phospholipids were identified by comparison with known compounds [phosphatidic acid, cephalins and lecithins extracted from rat liver and lysophosphatidic acid synthesized according to the method of Kabashima (4)].

Results and Discussion. The data in Table I clearly show that incorporation of labeled phosphate is greater with 1-monoolein than with 1, 3-diolein as acceptor. From this observation, one can conclude directly that monoglyceride phosphokinase is present in the mucosa of the small intestine of the rat and that its activity is higher than that of diglyceride phosphokinase.

The distribution of the ^{32}P radioactivity on the different phospholipids separated by thin-layer chromatography (Expt. 1) clearly indicate that lysophosphatidic acid is the main product (83 and 71% with mitochondria and 76 and 68% with microsomes). This observation serves as additional proof for direct

TABLE I. Distribution of ^{32}P Phosphate on the Different Phospholipids at the End of Incubation with Mitochondria and Microsomes.[a]

No. of expt.	Added lipids	Mitochondria (^{32}P, cpm × 10^{-2}) PA[c]	LPA[c]	Other PL[c]	Microsomes (^{32}P, cpm × 10^{-2}) PA	LPA	Other PL	Without subcellular fractions (^{32}P, cpm × 10^{-2}) PA, LPA, and other PL
1	1-Monoolein + oleic acid[b]	29	110	15	122	514	41	1.7
		33	235	11	29	218	74	1.8
2	1-3 Diolein[b]		4.6			3.1		0
			7.8			3.2		0
3	Glycerol + oleic acid[b]		5.3			2.2		0
						2.8		0
4	None		4.7			5.8		—

[a] Conditions of incubation: A 4-ml aliquot of fresh mitochondria or microsomes in phosphate buffer, pH 7.0 (1/9 w/v) was added to 7 ml of the incubation mixture to give the following final concentrations: 0.043 *M* sucrose, 0.003 *M* sodium deoxycholate, 0.002 *M* $MgSO_4$, 0.01 *M* NaF, 0.0001 *M* coenzyme A, 0.005 *M* glutathione, 0.00048 *M* γ ^{32}P ATP (70×10^6 cpm in each flask). The substrate concentrations were 0.002 *M* 1-monoolein and 0.0002 *M* oleic acid in Expt. 1; 0.002 *M* diolein in Expt. 2, and 0.002 *M* glycerol and 0.001 *M* oleic acid in Expt. 3. Incubations were carried out for 1 hr at 37° with shaking.

[b] Results of two similar experiments. In Expt. 3 (with mitochondria) and Expt. 4, only one assay was performed.

[c] PA = phosphatidic acid; LPA = lysophosphatidic acid; PL = phospholipids.

phosphorylation of monoolein since this biosynthesis does not occur from free glycerol as we shall see later. Labeled phosphatidic acid may be considered as the product of the acylation of a fraction of the newly synthesized lysophosphatidic acid as previously shown by Pieringer and Hokin (5) and Lands and Hart (6). It is not so easy to explain the presence of labeled phosphate in the fraction called "other phospholipids" which includes lecithins, cephalins, and minor compounds. However, it is reasonnable to suppose that this radioactivity is due to phosphatidic and lysophosphatidic acids since these compounds are known to move as elongated spots.

It could be argued alternatively that lysophosphatidic acid biosynthesis proceeds by another pathway involving hydrolysis of monoolein by intestinal monoglyceride lipase located in the microsomal fraction (7, 8), followed by phosphorylation of the free glycerol (9) and esterification by fatty acids (10). As a test of the efficiency of this three-step pathway, the experimental results with free glycerol suggest that this mechanism is at least incomplete (Table I). It is possible that the first two steps occur but we have not isolated the α-glycerophosphate which could be formed during the incubation as previously shown by Haessler and Isselbacher (9).

These results corroborate our hypothesis according to which monolein is directly phosphorylated to lysophosphatidic acid. Yields of the reaction in the system involving moolein have the same values as those that can be calculated from the data of Pieringer and Hokin (2). However, it should be noted that our results are somewhat different from those of these authors who demonstrated that diglycerides and monoglycerides are phosphorylated with the same yields by the microsomal fraction of brain and liver. From our results it can be concluded that the intestinal mucosa does not possess diglyceride phosphokinase since lipids are preferentially absorbed as monoglycerides and free fatty acids and not or very little as diglycerides.

Summary. Evidence is presented that incubation of monoolein with ATP, fatty acids, and other appropriate additions in the presence of subcellular fractions from intest-

inal mucosal cells results in the formation of lysophosphatidic acid (LPA). These results indicate that LPA is an intermediate in the formation of phosphoglycerides in the intestinal mucosa during fat absorption.

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Effect of Inhibitors of Protein Synthesis on Renal Pressor Factors* (33879)

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The influence of vasoactive proteins on hemodynamics was approached from a fresh viewpoint with the recent demonstration in this laboratory that several inhibitors of proteins synthesis, actinomycin D, acetoxycycloheximide, and chloramphenicol promptly and markedly reduce blood pressure of normotensive and hypertensive rats (1, 2). From the available evidence it appears that this depressor effect may be mediated through specific derangements of, as yet unknown, proteins or related compounds acting on vascular tissues rather than through nonspecific actions such as pharmacological toxicity, malnutrition, or cachexia following suppression of adrenal cortex function. In a further effort to clarify the mechanism of the depressor effect, the integrity of arterial smooth muscle during the hypotensive state was studied to determine whether interference with phasic contractile protein function might be responsible. It was shown, however, that while blood pressure was appreciably reduced within several hours after initiation of antimetabolite administration, no decrease in phasic contractility was observed until after 14 days of such treatment (3). It appears therefore that more labile vasoactive proteins than those responsible for smooth muscle contraction are involved in the depressor effect induced by inhibitors of protein synthesis.

In this regard, it was considered likely that the protein metabolic inhibitors might deter the synthesis of renin in the kidney, thus removing support for the maintenance of vascular resistance, resulting in a decline in blood pressure. A similar hemodynamic effect might also stem from a situation in which the inhibitors do not interfere with renin synthesis but diminish its vasoconstricting effectiveness. The present experiments were designed to study these two questions.

The effect of antimetabolites on renin formation was tested physiologically by comparing the pressor effect of kidney extracts from normal rats to similar extracts from rats treated with antinomycin D, an inhibitor of DNA-dependent protein synthesis. The second experiment consisted of an examination of the pressor effects of graded doses of angiotensin on rats during the hypotensive state induced by actinomycin D and by acetoxycycloheximide, an inhibitor of RNA-dependent protein synthesis, to determine

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