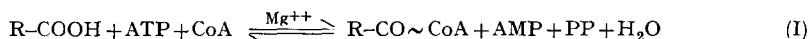


Activation of long-chain fatty acids by rat-gut mucosa

The esterification of long-chain fatty acids has been shown to be an important phase of intestinal fat absorption. Demonstration that the incorporation of free fatty acids into glycerides by homogenates of human- and rat-gut mucosa¹ is dependent on CoA, ATP and Mg^{++} suggested that the initial step in the process might be activation of the fatty acids to acyl-CoA thioesters. Such a step occurs in the liver in formation of phosphatidic acid intermediates² from which triglycerides and phospholipids are thought to be derived³.

Although it is possible that to some extent monoglycerides, at least, may be absorbed intact without hydrolysis^{4,5}, current evidence indicates that the major pathway of intestinal fat absorption involves the mucosal transport of free fatty acids, with subsequent esterification into chylomicron triglyceride⁶. Therefore, the presence in the gut of a system capable of synthesizing CoA derivatives of long-chain fatty acids was not unexpected.

Studies on homogenates of rat proximal jejunal mucosa and subcellular particles obtained therefrom have shown a very active long-chain fatty acid thiokinase requiring CoA, ATP and Mg^{++} , similar to that found in liver² and adipose tissue⁷ (Reaction I).



Assay of this thiokinase was carried out using NH_2OH as a trapping agent for the acyl-CoA produced (Reaction II) and spectrophotometric estimation of the ferric-hydroxamic acid complexes by absorption at $520\text{ m}\mu$ ($\epsilon_M = 1.28 \cdot 10^3$). Alternatively, using [$1-^{14}C$]palmitate as a substrate, the resultant labeled palmithydroxamic acid was separated on anion-exchange resin paper (IRA-400) and counted directly in a liquid scintillation spectrometer.

Maximal palmitate thiokinase activity appeared to be localized in the microsomal fraction (Table I), although mitochondria from neutral-isotonic-KCl homogenates also showed considerable activity. The latter particles were rather heavily contaminated with microsomes, however, as shown by comparative alkaline phosphatase activities⁸. The microsomes, on the other hand, seemed almost free of mitochondria by phase microscopy and cytochrome oxidase⁹ estimations. Virtually no activity was found in the supernatant fraction, in contrast to the findings of KORNBERG AND PRICER in guinea-pig liver². Sucrose (0.25 *M*) homogenates of gut mucosa, although providing more cleanly separated subcellular particles, were considerably less active and the diminished particulate activities were not enhanced by washing and resuspension in isotonic KCl or Ringer's phosphate solution. Additional media and conditions are being investigated.

The specific activity of the rat-gut mucosal thiokinase was several-fold greater in the duodenum and proximal jejunum than in the distal intestine and was not impaired by 48 h of fasting. Although the specific activity of the microsomal preparations varied somewhat, it was in general of the same order of magnitude as reported for guinea-pig liver microsomes², namely, 0.2 to 1.0 $\mu\text{moles/h/mg}$ protein. Homogenates

Abbreviations: CoA, coenzyme A; ATP, adenosine triphosphate; AMP, adenosine monophosphate; PP, pyrophosphate; GSH, glutathione; Tris, tris(hydroxymethyl)aminomethane.

TABLE I

DISTRIBUTION OF A FATTY ACID THIOKINASE ACTIVITY IN RAT-GUT MUCOSA

The reaction system contained 0.5 ml suspended enzyme preparation (2–4 mg protein/ml), 2.0 μ moles palmitate (or [1^{14}C] palmitate, 140,000 counts/min/ μ mole) in 5% bovine albumin, 20 μ moles ATP, 20 μ moles MgCl_2 , 50 μ moles KF, 1 mg CoA (approximately 70% pure), 20 μ moles GSH, 1 mmole NH_2OH and 125 μ moles Tris-HCl, pH 7.4, in a total volume of 2.5 ml. Incubation was at 37° in air, for 30 min, and was stopped by chilling in ice and adding 0.2 ml 21% HClO_4 . Lipids were extracted with $\text{CHCl}_3\text{-CH}_3\text{OH}$ (2:1), separated from water-soluble material¹⁰, and isolated for assay by evaporation of solvent with air or N_2 at 40°.

Fraction	Protein		Palmitate thiokinase
	mg/g wet mucosa	% of total	μ moles hydroxamate/h/mg protein
Whole homogenate, 5% (w/v)	44	100	0.082
Nuclei			
10 min at 2,000 \times g, twice	16	36	0.075
Mitochondria			
20 min at 7,500 \times g, twice	4	9	0.150
Microsomes			
30 min at 105,000 \times g,	5	11	0.287
Supernatant	18	41	0.001

* Homogenates and fractions were prepared at 0–4°, washed and resuspended in 0.154 M KCl (pH 7.4, using 0.1 N KOH) or 0.25 M sucrose.

** Protein was measured by the biuret or Folin-Ciocalteu method¹¹.

and particles were capable of activating fatty acids other than palmitic, with comparable effectiveness, including stearic, oleic, myristic, and to a lesser extent, lauric and shorter-chain acids. A rather broad pH optimum between 6.8–7.6 was found. Taurocholate ($5 \cdot 10^{-3}$ M) almost completely abolished microsomal activity.

The demonstration in rat-gut mucosa of a long-chain fatty acid thiokinase, with greatest activity in the microsomal fraction may be relevant to the electron-microscopic observations of PALAY AND KARLIN¹² that pinocytosis is involved in intestinal fat absorption. Since in this process transported lipid is surrounded by endoplasmic reticulum (microsomes), fatty acid activation and possibly further reactions in glyceride synthesis may occur while the lipid is within the pinocytotic vesicle.

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