The effect of fatty acids on amino acid incorporation

It has been observed that brain ribosomes are considerably more active in the incorporation of amino acids *in vitro* than the microsomes from which they are derived[1]. Since deoxycholate solubilizes the lipid-rich membranes of the endoplasmic reticulum, it was of interest to determine whether or not various lipids could inhibit the incorporation of amino acids into brain ribosomes. Lecithin, cephalin and neutral fats had little or no effect; however, in the presence of free fatty acids, a marked inhibition of amino acid incorporation was found (Table I, Fig. 1).

Although lauric acid appeared to be the most effective inhibitor, correlation between chain length and inhibition was made difficult by the insolubility of the fatty acids at magnesium concentrations necessary for optimal incorporation. Increasing the magnesium concentration from 5 to 10 m*M* did not reduce the inhibition caused by 1 m*M* lauric acid, a fact which indicates that the fatty acids do not act by removing magnesium from the medium.

Brain microsomes when assayed by the method of VAN DER VIES[2] were found to contain 50 mμmoles of free fatty acids per mg protein, a value which increased

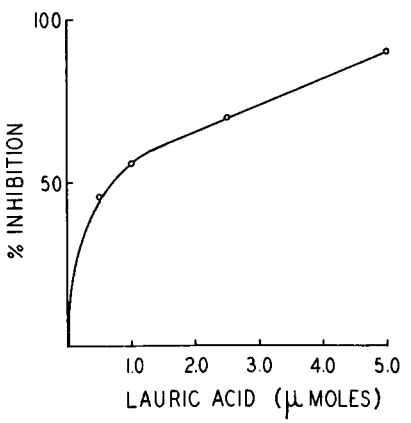

Fig. 1. The effect of lauric acid concentration on [^{14}C]leucine incorporation into brain ribosomes. Conditions as in the legend to Table I. Maximal incorporation (in the absence of lauric acid) was 250 counts/min/mg protein.

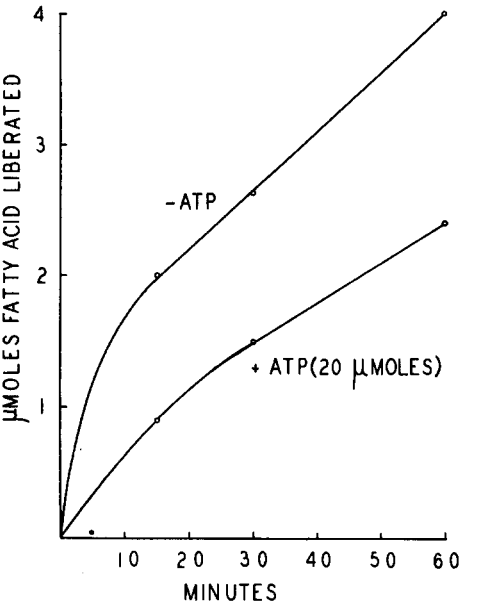

Fig. 2. The effect of ATP on fatty acid liberation from liver microsomes. Each tube contained: Tris buffer (pH 7.6), 100 μmoles; $MgCl_2$, 10 μmoles; microsomes, 50 mg protein. Final volume, 2 ml. Fatty acids were determined according to VAN DER VIES[2].

to 120 mμmoles upon incubating for 15 min at 37°, in the presence of Tris buffer and magnesium. It seems probable, therefore, that the discrepancy in amino acid incorporation between brain ribosomes and microsomes may be explained by the inhibitory effect of free fatty acids.

Since the initial rate of amino acid incorporation into liver ribosomes is the same as that into the corresponding microsomes[3,1], it is significant that no free fatty acids could be detected in the latter particles, even when 50 mg of protein were taken for assay. Upon incubation of liver microsomes, however, we observed a liberation of free fatty acids, which could be partially prevented by the presence of ATP (Fig. 2). It may be suggested, that the accumulation of these inhibitory substances in liver microsomes, during incubation, could contribute to the rapid decrease in the rate of amino acid incorporation observed with these particles. Furthermore,

TABLE I

THE EFFECT OF LIPIDS ON AMINO ACID INCORPORATION INTO RIBOSOMES

Complete system: Tris buffer (pH 7.6), 50 μmoles; $MgCl_2$, 5 μmoles; ATP, 2 μmoles; phosphoenolpyruvate, 10 μmoles; pyruvate kinase, 0.1 mg; GTP, 0.2 μmole; L-[^{14}C]leucine (6.1 μC/μmole), 0.1 μmole (360000 counts); guinea-pig liver supernatant (100000 $\times$ *g*), 1 mg protein; brain ribosomes, 1.5–2.0 mg protein; lipid, 1 μmole. Final volume, 1 ml. Incubations were for 30 min at 37°.

Additions	% *inhibition*
Octanoic acid	0
Capric acid	19
Lauric acid	56
Palmitic acid*	27
Stearic acid*	21
Lecithin*	0
Cephalin*	0
Triolein*	0

* These compounds were tested in the presence of purified[4] Tween-20 (5 mg). Lecithin and cephalin were commercial preparations obtained from the Sigma Chemical Corp.

ATP, besides yielding the necessary energy for the activation of amino acids, may affect the incorporating system, indirectly, by controlling the accumulation of inhibitory substances.

These *in vitro* findings may indicate one of the mechanisms involved in the regulation of protein formation.

We are indebted to Dr. H. WAELSCH for his support and counsel during the course of this work and to Dr. E. P. KENNEDY for valuable discussion.

This investigation was supported in part by grant B557 from the National Institute of Neurological Disease and Blindness, National Institutes of Health, U.S. Public Health Service.

Department of Biochemistry,
College of Physicians and Surgeons,
Columbia University and
the New York State Psychiatric Institute,
New York, N.Y. (U.S.A.)

GEORGE ACS
AMOS NEIDLE
NATALIE SCHNEIDERMAN

[1] G. ACS, A. NEIDLE AND H. WAELSCH, *Biochim. Biophys. Acta*, 50 (1961) 403.
[2] J. VAN DER VIES, *Biochem. J.*, 60 (1955) 627.
[3] J. F. KIRSCH, P. SIEKEVITZ AND G. E. PALADE, *J. Biol. Chem.*, 235 (1960) 1419.
[4] M. BIER, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. 1, Academic Press, Inc., New York, 1955, p. 627.

Received August 14th, 1961