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REFERENCES

1. G. B. ANSELL and J. N. HAWTHORNE, in *Phospholipids* (BBA Library), Vol. 3, p. 378. Elvesier, Amsterdam (1964).
2. L. E. HOKIN, *Ann. N.Y. Acad. Sci.* **165,** 695 (1969).
3. J. A. KAPPERS, *Zell. mikrosk. anat. Forsch.* **52,** 163 (1960).
4. G. R. BERG, D. C. KLEIN and W. H. GLINSMANN, *Fedn Proc.* **30,** 364 (1971).
5. R. E. WUTHIER, *J. Lipid Res.* **7,** 544 (1966).
6. G. HÜBSCHER and B. CLARK, *Biochem. biophys. Acta* **41,** 45 (1960).
7. M. FANG and G. V. MARINETTI, in *Methods in enzymology* (Ed. J. M. LOWENSTEIN), Vol. 14, p. 605. Academic Press, New York (1969).
8. T. YOSIOKA, K. AKATSUKA, A. YAMAGUMI and Y. KANEMASA, *Acta Med. Okayama* **22,** 147 (1968).
9. E. J. MASORO, in *Physiological chemistry of lipids in mammals*, p. 135. Saunders, Philadelphia (1968).

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Effect of cycloheximide and emitine on ^{14}C-amino acids incorporation by different subcellular fractions from rat liver

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EMETINE, an alkaloid having some antitumor and antiviral activities has been reported to inhibit protein synthesis by the HeLa cells.[1,2] Cycloheximide, a glutarimide antibiotic and a known inhibitor of protein synthesis, has no effect on mitochondrial protein synthesis.[3] It has been reported that emetine, in its mode of action, resembles cycloheximide and related glutarimide antibiotics.[4] This communication is mainly concerned with the effects of emetine and cycloheximide on the incorporation of ^{14}C-amino acids by different subcellular fractions when cell-free extracts of rat liver were incubated with ^{14}C-amino acids.

Rat liver was homogenized with Potter–Elvehjem homogenizer in 8 vol. of ice-cold medium (0·25 M, sucrose; 0·05 M, Tris–HCl buffer, pH 7·4; 0·025 M, potassium phosphate buffer, pH 7·4 and 0·025 M, KCl). Homogenate thus obtained was centrifuged to a cell-free extract at 1000 *g* for 10 min at 0°.

Complete incubation system contained 50 μmoles ATP; 25 μmoles Mg^{2+}; 625 μmoles Tris–HCl buffer (pH 7·4); 625 μmoles sucrose; ^{14}C-algal protein hydrolysate (having total count/min: $1{\cdot}25 \times 10^6$); 30–32 mg protein of cell-free extract. Total volume of the incubation mixture was 5 ml. The incubation was carried out for 120 min at 37°, with constant shaking and was stopped by the addition of 0·3 ml of casein hydrolysate (5 mg/ml). The incubation mixture was then fractionated into different subcellular constituents and processed according to Schneider and Hogeboom.[5] The radioactivity in different subcellular fractions was determined by processing the protein according to the method of Stachiewicz and Quastel,[6] as described by Banerjee *et al.*[7]. Radioactive counts were taken in a gas-flow counter (Nuclear-Chicago). Protein content of the tissue extract was determined by the biuret method.[8]

The results given in Table 1 indicate that different subcellular fractions *viz.* mitochondria, microsomes and soluble supernatants from rat liver can incorporate amino acids when cell-free extracts

TABLE 1. EFFECT OF EMETINE AND CYCLOHEXIMIDE ON THE INCORPORATION OF ^{14}C-AMINO ACIDS BY DIFFERENT SUBCELLULAR FRACTIONS WHEN CELL-FREE EXTRACT OF RAT LIVER WAS INCUBATED WITH ^{14}C-ALGAL PROTEIN HYDROLYSATE

System	Incorporation: cpm/mg protein		
	Mitochondria	Microsome	Soluble supernatant
Complete	2113 ± 153	2749 ± 169	672 ± 73
Complete + emetine (10^{-5} M)	842 ± 69	761 ± 70	195 ± 82
Complete + emetine (10^{-4} M)	450 ± 81	236 ± 58	84 ± 70
Complete + cycloheximide (50 μg/ml)	1948 ± 158	718 ± 43	254 ± 86
Complete + cycloheximide (75 μg/ml)	1863 ± 168	301 ± 32	131 ± 95

Results are expressed as cpm/mg protein and the averages of five experiments with ±S.D.

were incubated in presence of ^{14}C-amino acids. Protein in soluble supernatant may include some proteins released after their formation on the microsomes. Protein synthesized in mitochondria is not so easily released.[9]

The data given in Table 1, also indicate that emetine and cycloheximide inhibit microsomal protein synthesis in rat liver, but unlike cycloheximide emetine, strongly inhibits mitochondrial synthesis when cell-free extract of rat liver was incubated with ^{14}C-amino acids. It has previously been reported that cycloheximide inhibits protein synthesis in the cytoribosome-cell sap, but has no effect on protein synthesis by isolated mitochondria.[3] Our findings are consistent with those previously reported.[3] Hence, it may be concluded that emetine is more potent inhibitor for protein synthesis than cycloheximide in living system.

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REFERENCES

1. A. P. GROLLMAN, *Proc. Natn. Acad. Sci. U.S.A.* **56,** 1867 (1966).
2. A. P. GROLLMAN, *J. biol. Chem.* **243,** 4089 (1968).
3. M. A. ASHWELL and T. S. WORK, *Ann. Rev. Biochem.* **39,** 251 (1970).
4. H. D. SISLER and M. R. SIEGEL, *Antibiotics* **1,** 283 (1967).
5. W. C. SCHNEIDER and G. H. HOGEBOOM, *J. biol. Chen* **183,** 123 (1950).
6. E. STACEIWICS and J. H. QUASTEL, *Can. J. Biochem. Physiol.* **37,** 687 (1959).
7. A. K. BANERJEE, SIPRA BANERJEE and S. C. ROY, *Ind. J. Biochem.* **2,** 109 (1965).
8. A. G. GORNALL, C. J. BARDAWILL and M. M. DAVID, *J. biol. Chem.* **177,** 751 (1949).
9. H. K. DAS, S. K. CHATTERJEE and S. C. ROY, *J. biol. Chem.* **239,** 1126 (1964).

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Damage effect of chronic intoxication by CCl_4 on structural organization of liver microsomes and cytochromes $(b)_5$ and P-450

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IT IS now widely known that carbon tetrachloride is metabolized in the liver and can lead to centrolobular necrosis, accumulation of neutral lipids[1] and decreased activity of microsomal mixed-function oxidases;[2,3] which are involved in the processes of drug biotransformation. However, other investigators have succeeded in proving that lipoperoxidation of microsomal lipids is an important factor,