Biochem. J. (1972) **128**, 461–462
Printed in Great Britain

Effects of Emetine and Cycloheximide on Mitochondrial Protein Synthesis in Different Systems

By SYAMALIMA CHAKRABARTI, D. K. DUBE and S. C. ROY
Department of Biochemistry, University College of Science, 35 Ballygunge Circular Road, Calcutta-19, India

(*Received* 29 *March* 1972)

Protein synthesis in mitochondria isolated from various sources has been studied widely (Ashwell & Work, 1968; Barnett *et al.*, 1967; Das *et al.*, 1964). Cycloheximide, a glutarimide antibiotic (Sisler & Siegel, 1967), and emetine, an alkaloid having some antitumour and antiviral activities, have been found to inhibit microsomal protein synthesis in HeLa cells (Grollman, 1966, 1968). Grollman (1968) has demonstrated that emetine, in its mode of action, resembles cycloheximide and other glutarimide antibiotics and acts at the aminoacyl-transfer level. But, so far as mitochondrial protein synthesis is concerned, cycloheximide fails to exhibit any inhibitory effect (Ashwell & Work, 1968; Loeb & Hubley, 1968). Unlike cycloheximide, emetine has been found to inhibit protein synthesis by isolated mitochondria from different sources (as given in Table 1). The extent of inhibition is practically the same in all the cases.

Methods

Human malignant cervical tissues from surgical cases and comparable normal tissue from abdominal total hysterectomy were obtained from the local hospitals. All tissues were subjected to parallel histological examinations before they were used for the preparation of cell-free extract and mitochondrial fractions.

The tissues (rat liver also) were ground with quartz powder, and homogenates were prepared with 2.5vol. of ice-cold medium A [0.25M-sucrose–50mM-tris–HCl buffer (pH7.4)–25mM-potassium phosphate buffer (pH7.4)–25mM-KCl]. The homogenate was centrifuged at 1000*g* for 10min at 0°C. The supernatant fluid was centrifuged at 10000*g* for 40min, and the sediment was suspended in medium A and then centrifuged at 1000*g* for 10min at 0°C. The supernatant fluid was finally centrifuged at 10000*g* to obtain the mitochondrial fraction.

The mitochondrial preparations were practically devoid of glucose 6-phosphatase activity, and there was no degradation of their RNA content on the addition of ribonuclease, indicating that there was no significant contamination by the microsomal fraction (Webster, 1955). Phase-contrast microscopy was used to check that the preparation essentially contained only intact mitochondria.

For the measurement of radioactivity, protein was processed by the method of Stachiewicz & Quastel (1959), as reported by Das *et al.* (1964). The radioactivity was determined in a gas-flow counter (Nuclear–Chicago).

Viable bacteria were counted by the plate-and-dilution method in Euganagar (a trypticase soy agar obtained from the Baltimore Biological Laboratory, Baltimore, Md., U.S.A.) medium.

Results and discussion

The results in Table 1 show that L-[U-^{14}C]lysine is actively incorporated into protein by mitochondria from human normal and malignant cervical tissues, but the incorporation is much greater with the latter. It is very likely that synthetic activities of tumour tissues are oriented more towards the construction of molecules such as proteins that are immediately used for cell growth than towards the storage of reserve substances such as glycogen etc. (Zamecnik *et al.*, 1951). The incorporation of L-lysine by mitochondrial preparations from human normal and malignant cervical tissues does not require externally added ATP and its generating system and is unaffected by the addition of ribonuclease. It is now established that mitochondrial systems are self-sufficient with regard to the supply of ATP, mRNA etc., and the incorporation of amino acids by intact mitochondria is unaffected by the addition of ribonuclease (Ashwell & Work, 1970).

The results given in Table 1 also indicate that cycloheximide fails to inhibit the incorporation of L-lysine by mitochondrial preparations from all the sources, whereas emetine exhibits a strong inhibitory effect. The extent of inhibition is more or less the same in each case. It has already been reported that cycloheximide inhibits protein synthesis in the cytoribosome–cell-sap system (Sisler & Siegel, 1967) but has no effect on protein synthesis by isolated mitochondria even at high concentration (Ashwell & Work, 1968), and this finding is consistent with the present observation. In contrast, emetine, a potent inhibitor of microsomal protein synthesis (Grollman, 1968), inhibits also protein synthesis by isolated mitochondria from widely different sources. Hence further analysis of the effects of the two inhibitors should allow a better understanding of the two

Table 1. *Characteristics of the incorporation of* L-[*U*-^{14}*C*]*lysine into proteins by mitochondria from human normal and malignant cervical tissue and from rat liver and the effects of emetine and cycloheximide on the process*

Results are expressed as means ± S.D. of five experiments. The complete incubation system contained 1 μmol of ATP, 3 μmol of phosphoenolpyruvate, 10 μg of pyruvate kinase, 5 μmol of $MgCl_2$, 250 μmol of sucrose, 50 μmol of tris–HCl buffer, pH 7.4, 20 μmol of potassium phosphate buffer, pH 7.4, L-[U-^{14}C]lysine (2.25×10^4 c.p.m.; specific radioactivity 30 Ci/mol) and 4–4.5 mg of mitochondrial protein. The total volume of the incubation mixture was 1 ml. The incubation was carried out for 2 h at 37°C with constant shaking, the gas phase being air. The incubation was stopped by the addition of 0.3 ml of 30% (w/v) trichloroacetic acid.

System	Incorporation (c.p.m./mg of protein)		
	Normal cervical tissue	Malignant cervical tissue	Rat liver
Complete	1134 ± 74	3245 ± 120	3829 ± 141
Minus ATP and its generating system	1102 ± 65	3219 ± 125	—
Plus ribonuclease (10 μg)	1128 ± 72	3240 ± 116	3792 ± 130
Plus emetine (1 μM)	654 ± 40	1728 ± 76	2054 ± 102
Plus emetine (10 μM)	321 ± 35	704 ± 65	1109 ± 114
Plus emetine (0.1 mM)	95 ± 38	200 ± 42	417 ± 59
Plus emetine (1 mM)	89 ± 36	165 ± 45	205 ± 72
Plus cycloheximide (50 μg)	1098 ± 65	3241 ± 112	3682 ± 165
Plus cycloheximide (75 μg)	1120 ± 70	3204 ± 125	3640 ± 141

systems (microsomal and mitochondrial) for protein synthesis present in eukaryotic cells.

The authors are grateful to Dr. B. N. Chakrabarti, Nil Ratan Sircar Medical College and Hospital, Calcutta, India, for supplying the tissue materials. Grateful acknowledgement is also due to University Grants Commission, New Delhi, India, for sponsoring the work.

Ashwell, M. A. & Work, T. S. (1968) *Biochem. Biophys. Res. Commun.* **32**, 1006
Ashwell, M. A. & Work, T. S. (1970) *Annu. Rev. Biochem.* **39**, 251
Barnett, W. E., Brown, D. H. & Eplen, J. L. (1967) *Proc. Nat. Acad. Sci. U.S.* **57**, 1775
Das, H. K., Chatterjee, S. K. & Roy, S. C. (1964) *J. Biol. Chem.* **239**, 1126
Grollman, A. P. (1966) *Proc. Nat. Acad. Sci. U.S.* **56**, 1867
Grollman, A. P. (1968) *J. Biol. Chem.* **243**, 4089
Loeb, J. N. & Hubley, B. G. (1968) *Biochim. Biophys. Acta* **166**, 745
Sisler, H. D. & Siegel, M. R. (1967) *Antibiotics* **1**, 283
Stachiewicz, E. & Quastel, J. H. (1959) *Can. J. Biochem. Physiol.* **37**, 687
Webster, G. C. (1955) *Plant Physiol.* **30**, 351
Zamecnik, P. C., Loftfield, R. B., Stephenson, M. L. & Steele, J. M. (1951) *Cancer Res.* **11**, 592