and $H_2[Co(C_{17}H_{19}N_2O)_2\ Br_2]$ three bands with peaks at 9000 cm^{-1}, 8203 cm^{-1} (γ_1); 20000 cm^{-1}, 17,310 cm^{-1} (γ_2) and 25,000 cm^{-1}, 21,012 cm^{-1} (γ_3) were observed which are assigned to transition $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$, $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ and $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$. From these transitions octahedral coordination[10,12] for cobalt(II) complex is suggested. The ratio γ_2/γ_1 which lies between 1·90/2·4 also strengthens the above co-ordination[11,12].

Infrared Spectral Studies

In the i.r. spectra of metal chelates the stretching frequencies of azomethine $CH_3\ C=N$ (1600 cm^{-1}) and phenolic –OH (3400 cm^{-1}) groups are lowered and bands around 1490–1550 cm^{-1} and 3350–3400 cm^{-1} appear, showing thereby complex formation. This view is further supported by the formation of M–N (490–515 cm^{-1}) and M–O (460–478 cm^{-1}) bonds. In all these complexes metal to halogen bonds also formed as revealed by the appearance of bands around 350–375 cm^{-1}.

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1. Yamada, S., Kuge, Y. and Yamananouchi, K., *Bull. Chem. Soc.,* 1967, 40, 1864.
2. —, — and —, *Co-ordin. Chem. Review,* 1966, 1, 415.
3. Ghosh, S. P. and Bhattacharji, P., *J. Ind. Chem. Soc.,* 1974, 51, 308.
4. Ozha, D. D. and Kaul, K. N., *Curr. Sci.,* 1974, 43, 344.
5. Rastogi, D. K., Srivastava, A. K. and Jain, P. C., *J. Inorg. Nucl. Chem.,* 1970, 32, 3949.
6. Carlin, R. L., *Transition Metal Chemistry,* Marcel Dekker, Inc., N.Y., Vol. 4, 1968.
7. Tanka, T., *J. Am. Chem. Soc.,* 1958, 80, 4108.
8. Sheat, S. V. and Waters, T. N., *J. Inorg. Nucl. Chem.,* 1961, 19, 73.
9. Ballhausen, C. J., *Introduction to Ligand Field Theory,* McGraw-Hill, Book Co., N.Y., 1962.
10. Figgis, B. N., *Introduction to Ligand Fields,* John Wiley and Sons. Inc., N.Y., 1967.
11. Holms, O. G. and Meclure, D. S., *J. Chem. Phys.,* 1957, 26, 1686.
12. Singh, P., Singh, V., Singh, B. P. and Mahesh, R. P., *Ind. J. Chem.,* 1975, 13, 734.

EFFECTS OF RIFAMPICIN AND NITROFURANTOIN ON ^{14}C AMINO ACIDS INCORPORATION BY DIFFERENT SUBCELLURAR FRACTIONS FROM MOUSE SKELETAL MUSCLE AND FIBROSARCOMA

Rifampicin and Nitrofurantoin are two potent inhibitors of macromolecule synthesis in different subcellular fractions. While the mode of action of nitrofurantoin is obscure, that of rifampicin is much debated. Rifamycins are known to inhibit bacterial[1], as well as nucleolar and nucleoplasmic[2] and mitochondrial[3] RNA polymerases. In addition rifamycins cause inhibition of polyphenylalanine synthesis in cell-free systems from bacteria[4] animal cells[2]. It has been reported from this laboratory that rifampicin inhibits polyphenylalanine synthesis by intact mitochondria and that this inhibition cannot be released by poly (U)[5]. Similar results have also been reported by others[6]. On the other hand it has also been reported that poly (U) can reverse the inhibition of polyphenylalanine synthesis in a cell-free system consisting of mitochondrial ribosomes and supernatant proteins[7]. The mode of action of nitrofurantoin has not been studied in detail. Röschenthaler *et al.*[8], suggested that the inhibition of growth and IPTG induced β-galactosidase synthesis in *E. coli* by nitrofurantoin could be due (*i*) to interference with energy metabolism or (*ii*) to a disturbance of the cell membrane or (*iii*) to a more direct action on the protein synthetic machinary. But we have previously reported that in mitochondria the inhibition of protein synthesis is not due to an interference with the energy metabolism[9].

Materials and Methods

L[U-^{14}C] lysine (specific radioactivity, 114 mCi/mmol) was obtained from Bhabha Atomic Research Centre, Bombay, India. Rifampicin was a gift from CIBA, Ltd., Basel, Switzerland and Cycloheximide, Chloramphenicol, Nitrofurantoin, poly (A), ATP, GTP, Phosphoenol pyruvate, Pyruvate kinase were purchased from Sigma Chemicals, Mo., U.S.A. All other chemicals were of analar grade.

Mitochondria were isolated according to the method of Chakrabarti *et al.*[10], and further purified by washing at 7000 g as described by O'Brien and Kalf[11]. Microsomes and pH 5 enzymes were prepared following the method of Dube *et al.*[12]. Mitochondria and microsomes were incubated in mediums identical to those described by Dube *et al.*[12]. The reaction was terminated by adding TCA containing sodium tungstate to a final concentration of 5% TCA and 0·25 *M* sodium tungstate and the radioactivity was determined as described elsewhere[12].

TABLE I

Effects of rifampicin, nitrofurantoin and poly (A) on the incorporation of ^{14}C-amino acid by different subcellular fractions from mouse skeletal muscle and fibrasarcoma

(Results are expressed as counts/min/mg. of protein and represents values for 6 different experiments with ± S.D.)

Incubation system		Mitochondria		Microsomes	
		Normal	Malignant	Normal	Malignant
Complete		1463±65	2172±100	2108±109	3843±215
,,	— pH 5 enzymes	..	..	420± 22	1924± 71
,,	+ Cycloheximide (100 μg)	1297±59	2156±101	1024± 60	1565± 66
,,	+ Chloramphenicol (100 μg)	507±26	1060± 58	1911± 67	3904±200
,,	+ Poly (A) (100 μg)*	1970±73	3208±120	2810±119	4476±189
,,	+ Rif. 10 μg *	660±28	1040± 56	722± 32	1924± 69
,,	+ Poly (A) (100 μg) + Rif (10 μg) *	710±30	1654± 70	98± 36	3236±240
,,	+ Poly (A) (100 μg * + Rif (10 μg)	750±33	1908± 75	815± 40	3560±210
,,	+ NFT (20 μg) *	433±22	1117± 80	1052± 55	2304±100
,	+ Poly (A) (100 μg)* + NFT (20 μg)	620±27	1535± 65	890± 50	2850± 99
,,	+ Poly (A) (100 μg) + NFT (20 μg)*	706±30	1500± 63	923± 56	2696±105

* Different subcellular fractions were pre incubated with either poly (A) or rifampicin (Rif) or nitrofurantoin (NFT) for 15 min at 37° C.

From Table I it will appear that cycloheximide and chloramphenicol selectively inhibit microsomal and mitochondrial protein synthesis respectively. This is in accordance with previous findings[13,14]. With poly (A) there is significant increase of lysine incorporation into all subcellular fractions studied. Rifampicin inhibits lysine incorporation into all subcellular fractions, but a reversal of this inhibition by poly (A) to any significant extent is observed only in the case of mitochondria and microsomes isolated from malignant tissues. But a careful consideration of these and previously reported data[4-7] indicates that in either case rifampicin must affect protein synthesis at the level of translation.

Table I also shows that nitrofurantoin inhibits lysine incorporation by all subcellular fractions. Since we have already reported that nitrofurantoin induced inhibition is not due to an interference with energy metabolism[10] and since the question of disturbance of membrane does not arise in case of a cell-free system consisting of microsomes and pH 5 enzymes it appears that nitrofurantoin directly affects the protein synthesis apparatus at least in the microsomal system. The slight increase of incorporation when malignant mitochondria or microsomes are pre-incubated with either nitrofurantoin or poly (A) seems to be the effect of pre-incubation on the incorporation rather than an effect of poly (A).

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1. Wehrli, W. and Staehelin, M., *Bact. Rev.*, 1971; 35, 290.
2. Busiello, E., Girolamo, A. D., Girolamo; M. D.; Fantuzzi, L. F. and Vesco, C., *Eur. J. Biochem.*, 1973, 35, 251.

3. Wu, G. J. and Dawid, I. B., *J. Biol. Chem.*, 1974, 249, 4412.
4. Calvori, C., Frontali, L., Leoni; L. and Tecce, G., *Nature,* 1965, 207, 417.
5. Duhe, D. K., Chakrabarti, S., Sarkar, A., Bhattacharya, P., Goswami, B. B. and Roy, S. C., *Biochem. Pharmacol.,* 1973, 22, 659.
6. Grant, W. D. and Poulter, R. T. M., *J. Mol. Biol.*, 1973, 73, 439.
7. Goswami, B. B., Chakrabarti, S., Dube, D. K. and Roy, S. C., *Physiol. Plantarum,* 1974, 32, 291.
8. Röschenthaler, R., Kindler, P., Herrlich, P. and Igbokwe, J., *Zbl. Bakt. I. Abt. Orig.,* 1970, 215, 203.
9. Goswami, B. B., Chakrabarti, S., Bhattacharya, P., Sinha, S. N., Roy, B. K., Roychowdhuri, P. and Dube, D. K., *Biochemi. Pharmacol.,* 1974, 23, 470.
10. Chakrabarti, S., Dube, D. K. and Roy, S. C., *Biochem. J.*, 1972, 128, 461.
11. O'Brien, J. W. and Kalf, G., *J. Biol. Chem.,* 1967, 247, 2172.
12. Dube, D. K., Chakrabarti, S. and Roy, S. C., *Cancer,* 1972, 29, 1575.
13. Sisler, H. D. and Siegel, M. R., *Antibiotics,* 1967, 1, 283.
14. Ashwell, M. A. and Work, T. S., *Ann. Rev. Biochem.,* 1970, 39, 251.

ON THE SIGNIFICANCE OF CALCITE CEMENTATION IN GARUDAMANGALAM LIMESTONE, TRICHINOPOLY UPPER CRETACEOUS, SOUTH INDIA

GARUDAMANGALAM Limestone is a thin elongated, impersistent, shell bearing calcareous sandstone occurring at the base of Trichinopoly Group of Blanford (1855) or the Garudamangalam Group of Rama Rao (1956) in the exposed upper cretaceous sequence of South India. Petrographically, Garudamangalam Limestone shows a floating framework of elongated but subrounded quartz and feldspar grains, heavy minerals and shell fragments held in a medium of coarse, blocky, sparry calcite cement. Modally calcite cement constitutes about 48 to 58% of the rock. Much of quartz and feldspar grains are corroded by blocky calcite cement more along the longer sides of the grains than at their terminal ends. The form, nature, size and disposition of calcite cement suggest that it was deposited in the abundant pore space of the original quartz, feldspar, shell framework by slow percolating solution (Dapples, 1971).

Numerous field and petrographic evidences are presented by Rao and Siddiqui (1974) to show that Garudamangalam limestone is an ancient analog of a modern sandy beach rock. Folk (1974) has recently discussed the natural history of calcium carbonate and has shown that modern beach rocks represent high Mg^{++} and high Na^{+} environment and therefore beach rock cementation is either by micritic or fibrous aragonite, or micritic and bladed or fibrous high Mg–calcite. Low–Mg sparry calcite cement is rare in such an environment. If this is true, then Garudamangalam limestone shows sparry calcite cement and therefore may not be a beach rock, whereas other field and petrographic evidences reveal that Garudamangalam limestone is indeed a beach sediment. In order to clear the doubt whether the sparry calcite cement in Garudamangalam limestone is indeed a low-Mg calcite, X-ray diffractograms were taken from the cement portion of Garudamangalam limestone by a General Electric, XRD–6 X-ray diffractometer ($K\alpha$ radiation) with 2° 2 θ rotation per minute over the range of 25° to 32°, 2 θ angle (Fig. 1). Invariably it turned out to be low-Mg calcite (3·029 Å d); then here is a problem where an ancient beach rock shows a low-Mg, sparry calcite cement.

TEXT FIG.-1

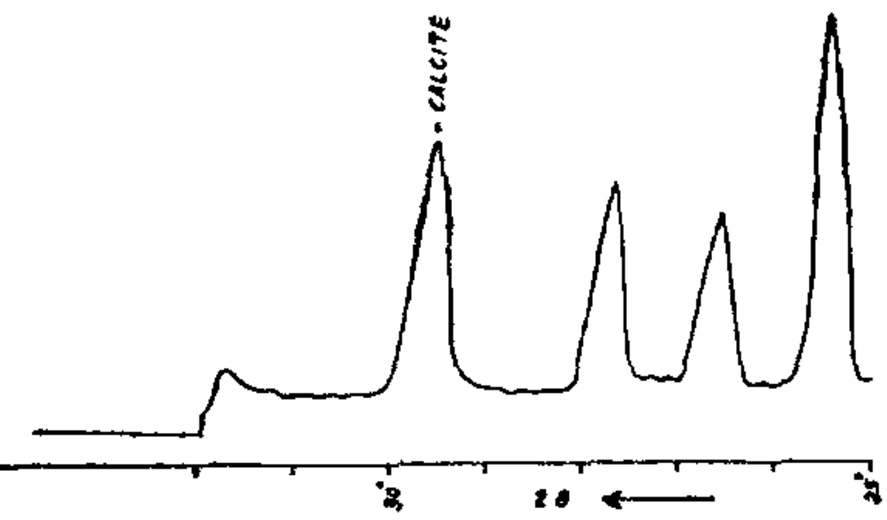

FIG. 1. X-ray diffractogram of sparry calcite in Garudamangalam Limestone, Trichinopoly cement Upper Cretaceous, S. India. Note the prominent peak for calcite corresponds to Mg-poor calcite.

Folk (1974) discusses this issue and suggests two alternatives for low-Mg calcite cementation in ancient beach rocks : (1) exposure to surface conditions where the beach comes into contact with the surface fresh waters low in Mg^{++} or (2) deep burial where the beach sediment comes into contact with Mg^{++} depleted connate or meteroic water diluted subsurface brines. The Mg^{++} is removed at the shallower levels, either by clay minerals or by capture by replacement dolomite. The option with reference to Garudamangalam limestone is for the second alternative for, the sparry calcite cement here is blocky, absence of solution deposition babrics in the associated shell debris and the absence of micritic or fibrous calcite crust to open spaces giving place centropetally to coarse mosaic spar.

There is also a suggestion (Folk *op. cit.*) that modern seas are rich in Mg^{++} and Na^{+} and poor in Ca^{++} as most of Ca^{++} is extracted by marine organisms to build their calcareous skeletons. Mg^{++} concentration in marine waters has progressively