However, where the lysosomes were treated with both enzyme and prednisolone for 2 hr or by 5×10^{-4} M prednisolone for 60 min followed by PLC for 60 min the steroid has competed successfully against the enzyme for the phospholipid sites as is shown by lower levels of enzyme in the supernatant.

In a parallel set of experiments lysosomal suspensions were pretreated separately for 60 min at 37° with cortisol, cortisone, dexamethasone, prednisone, prednisolone and triamcinolone at a final concentration of 5×10^{-4} M. Then TNBS was added to a final concentration of 1 mM and incubation continued for a further 60 min. When compared to controls no stabilization against the lytic action of TNBS was found with any steroid tested.

Both PLC and TNBS caused the release of enzymes from lysosomes. Since anti-inflammatory steroids were effective in blocking the action of PLC on lysosomes, but not that of TNBS, then it appears that the stabilizing action of anti-inflammatory steroids on lysosomes is due to steroid phospholipid interactions rather than steroid-protein interactions, and that anti-inflammatory steroids will protect lysosomal membranes against lipase action.

Acknowledgement—One of us (A.M.S.) wishes to thank the Medical Research Council for a postgraduate training award during the tenure of which this work was done at the University of Bath.

Department of Pharmacy,
University of Aston in Birmingham
Gosta Green,
Birmingham
DAVID A. LEWIS

Ministry of Agriculture,
Fisheries and Food,
Central Veterinary Laboratories,
New Haw,
Weybridge, Surrey
ANDREW M. SYMONS

School of Pharmacy,
University of Bath,
Bath,
Somerset, England
RONALD J. ANCILL

REFERENCES

1. A. D. BANGHAM, M. M. STANDISH and J. C. WATKINS, *J. Molec. Biol.* **13,** 238 (1965).
2. D. A. LEWIS, A. M. SYMONS and R. J. ANCILL, *J. Pharm. Pharmac.* **22,** 902 (1970).

Biochemical Pharmacology, Vol. 23, pp. 470–472. Pergamon Press, 1974. Printed in Great Britain.

Inhibition of mitochondrial protein synthesis by nitrofurantoin in rat and goat liver

(*Received* 5 *June* 1973; *accepted* 30 *July* 1973)

THE MECHANISM of action of nitrofurantoin has not been investigated thoroughly although this and related compounds have been known for almost 43 yr.[1] Roschenthaler *et al.* examined the effect of nitrofurantoin upon RNA and protein synthesis in *E. coli* K_{12} by using the β-galactosidase system and concluded that the inhibition of the enzyme synthesis could be due either (i) to a decreased pool of nucleotide triphosphates by interference with the energy metabolism, or (ii) to disturbances of the cell membrane or (iii) to a more direct action on the protein synthesis apparatus.[2]

It is now well established that mitochondria possess their own protein synthesizing machinery[3–5] and some features of the mitochondrial protein synthesizing system resemble those of the bacterial system.[6,7] We present the results of a study of the effect of nitrofurantoin on the incorporation of ^{14}C-valine into proteins by intact mitochondria isolated from rat and goat liver.

TABLE 1. EFFECT OF NITROFURANTOIN AND OTHER INHIBITORS ON THE INCORPORATION OF L-VALINE-U-^{14}C INTO PROTEINS BY ISOLATED MITOCHONDRIA FROM RAT AND GOAT LIVER

System	Incorporation (counts/min per mg protein)	
	Goat liver	Rat liver
Complete	4250 ± 420	4800 ± 460
Complete − ATP and its generating system	4100 ± 390	4760 ± 430
Complete + RNase (20 μg)	4260 ± 400	4780 ± 420
Complete + cycloheximide (75 μg)	4090 ± 380	4700 ± 410
Complete + chloramphenicol (50 μg)	850 ± 75	990 ± 70
Complete + NFT (10 μg)	2200 ± 160	2480 ± 240
Complete + NFT (30 μg)	380 ± 30	500 ± 50
Complete − ATP and its generating system + NFT (10 μg)	2180 ± 130	2410 ± 2110

NFT: Stands for nitrofurantoin.
Results are the averages of five experiments with ± S.D.

Mitochondria from goat and rat liver were prepared according to the method described by Dube *et al.*[8] Liver from both the sources was homogenized (separately) with Potter-Elvehjem homogenizer in 8 vol. of ice-cold medium A (0·25 M sucrose, 0·05 M Tris–HCl buffer, pH 7·4 and 0·025 M potassium phosphate buffer, pH 7·4). The homogenate was centrifuged at 1000 *g* for 10 min at 0°. The supernatant fluid was finally centrifuged at 10,000 *g* to obtain the mitochondrial fraction. The 10,000 *g* pellet thus separated was washed twice with cold medium A. Isolated mitochondria were further purified on a discontinuous sucrose gradient according to the method of Richter and Lipmann[9] as described elsewhere.[10]

The complete incubation system contained 1 μmole of ATP, 3 μmoles phosphoenolpyruvate, 5 μmoles of $MgCl_2$, 20 μmoles of sucrose, 50 μmoles of Tris–HCl buffer (pH 7·4), 20 μmoles of potassium phosphate buffer (pH 7·4), L-Valine-U-^{14}C (counts/min $1{\cdot}10 \times 10^5$), specific radioactivity (30 mCi/m-moles) and 4–4·5 mg of mitochondrial protein. The total volume of the incubation mixture was 1 ml. The incubation was carried out aerobically for 2 hr at 37° with constant shaking. The incubation was stopped by the addition of 0·3 ml of 30% TCA.

For the measurement of radioactivity, protein was processed according to the method of Stachiewicz and Quastel as described by Dube *et al.*[11] Protein content of the mitochondrial fraction was measured according to the method of Lowry *et al.*[12]

The data given in Table 1 indicate that mitochondria from goat and rat liver can incorporate labelled valine into proteins. The incorporation is resistant to RNase which clearly rules out the doubt about the intactness of mitochondria.[13] Again, the incorporation is actively taking place even in the absence of externally added ATP and its generating system indicating the presence of sizable nucleotide triphosphate pool within the mitochondria. Cycloheximide, an inhibitor of eukaryotic protein synthesis, has no effect on mitochondrial protein synthesis in the present system indicating the absence of microsomal contamination, but chloramphenicol inhibits the incorporation of valine to a great extent.[3,14] Nitrofurantoin inhibits labelled valine incorporation even at a concentration as low as 10 μg/ml and a 90 per cent inhibition takes place at a concentration of 30 μg/ml. The inhibitory effect of nitrofurantoin in both the systems has been found even in the absence of externally added ATP and its generating system.

From the present study it may be concluded that the inhibition of incorporation of ^{14}C-amino acids into mitochondrial proteins is due (i) to a direct action on the protein synthetic apparatus or, (ii) to a disturbance of mitochondrial membrane, but not due to a decreased nucleotide triphosphates pool by the interference with energy metabolism. Further studies are required to reveal the actual locii where nitrofurantoin acts.

Acknowledgement—Thanks are due to University Grants Commission, New Delhi, India for financial assistance.

Department of Biochemistry,
Calcutta University,
35 *Ballygunge Circular Road,*
Calcutta 700019,
India

BISWENDU B. GOSWAMI
SYAMALIMA CHAKRABARTI
PRABIR BHATTACHARYYA
SANDIP N. SINHA
BIMAL K. ROY
PURNIMA RAYCHOUDHURI
DIPAK K. DUBE

REFERENCES

1. K. MIURA and H. K. RECKENDORF, *Progr. med. chem.* **5**, 320 (1967).
2. K. ROSCHEMTHALER, P. KINDLER, P. HERRLICH and J. IGBOKWE, *Zbl. Bakt. I. Abt. Org.* **215**, 203 (1970).
3. M. ASHWELL and J. S. WORK, *Ann. Rev. Biochem.* **39**, 251 (1970).
4. W. E. BARNETT, D. H. BROWN and J. L. EPLEN, *Proc. natn. Acad. Sci. U.S.A.* **57**, 1775 (1967).
5. H. K. DAS, S. K. CHATTERJEE and S. C. ROY, *J. biol. Chem.* **243**, 4089 (1964).
6. J. P. LEIS and E. B. KELLER, *Biochem. Biophys. Res. Commun.* **40**, 416 (1970).
7. J. LUCAS-LENARD and F. LIPMANN, *Ann. Rev. Biochem.* **40**, 434 (1971).
8. D. K. DUBE, S. CHAKRABARTI, P. BHATTACHARYA, B. B. GOSWAMI and S. C. ROY, *Biochem. Pharmac.* **22**, 659 (1973).
9. D. RICHTER and F. LIPMANN, *Biochemistry* **9**, 5065 (1970).
10. B. B. GOSWAMI, S. CHAKRABARTI, D. K. DUBE and S. C. ROY, *Biochem. J.*, **134**, 815 (1973).
11. D. K. DUBE, S. CHAKRABARTI and S. C. ROY, *Cancer* **29**, 1575 (1972).
12. O. H. LOWRY, N. J. ROSEBROUGH, A. I. FARR and R. J. RANDALL, *J. biol. Chem.* **193**, 265 (1951).
13. R. F. SWANSON, *Nature, Lond.* **231**, 31 (1971).
14. S. CHAKRABARTI, D. K. DUBE and S. C. ROY, *Biochem. J.* **128**, 461 (1972).

Biochemical Pharmacology, Vol. 23, pp. 472–474. Pergamon Press, 1974. Printed in Great Britain.

The passage of δ-aminolaevulinic acid across the blood brain barrier of the rat: effect of ethanol

(*Received* 28 *June* 1973; *accepted* 30 *July* 1973)

ONE OF the distinctive features of acute intermittent porphyria (AIP) is the excessive urinary excretion of certain porphyrins and their precursors, δ-aminolaevulinic acid (ALA) and porphobilinogen (PBG). This is a result of the genetically mediated increase in porphyrin biosynthesis characteristic of the disease. One result of this elevated biosynthesis is that there is an associated increase in the plasma concentration of ALA. ALA is virtually undetectable in the plasma of normal subjects, but a level of 24 μg/ml has been reported in an acute porphyric attack. Similarly, normal cerebrospinal fluid (CSF) contains no ALA, yet ALA can be detected in patients with AIP.[1] These observations suggest that at elevated plasma concentrations ALA can pass the blood–brain barrier (BBB), thereby entering the brain tissue and CSF. There is, however, conflicting evidence on this possibility. Thus, although Kramer[2] has indicated that ALA is readily taken up by brain tissue, Musyka[3] has reported that the BBB is impermeable to ALA.

The resolution of these conflicting reports is of importance since there is increasing evidence that ALA may be an aetiological factor in the production of the clinical manifestations of the disease. Although the purified porphyrins and porphobilinogen are pharamcologically inactive,[4] ALA has been shown to inhibit brain ATPase activity[2] and membrane sodium transport *in vitro*.[5] Further, ALA causes behavioural changes in mice[6] and has a hypotensive action in anaesthetised rats.[7] Such actions of ALA could bear some relationship to the fact that some 55 per cent of patients with AIP exhibit psychological symptoms; and post-mortem examination of porphyric patients has revealed central neuropathy.[8] There is also convincing evidence that ALA can increase the susceptibility of experimental animals to drug induced convulsions.[9]

Certain porphyrinogenic drugs are known to provoke attacks in subjects with latent AIP.[10] These drugs, which include ethanol, increase the activity of hepatic ALA synthetase, the enzyme which produces ALA from glycine and succinyl-CoA. There is the possibility, therefore, that they precipitate porphyric attack by increasing the circulating level of ALA and thus, if ALA can pass the BBB, the brain tissue level of ALA. Alternatively, they could cause the BBB to become permeable or more permeable to ALA. Acute attacks of porphyria have been provoked by an episode of over-indulgence in ethanol.[11] This experiment was designed therefore, to confirm whether or not ALA could pass the BBB in normal animals and in animals under acute ethanol intoxication.