Lyndiol treatment for 90 days, resulted in 132.5% increase in the cholinesterase activity of the perieto-occipital cortex of treated animals. Increase in the brain cholinesterase activity eventually may cause decrease of free acetyl choline. As it has been shown earlier in experimental animals, the increase in the free acetyl choline content of brain leads to somnolence[11], it may be presumed that decrease of the same in the perieto-occipital cortex might have excited the cortical neurones leading to mild desynchronization in the present experiment. However, nothing conclusive can be stated from the present study as we have not measured the free acetyl choline simultaneously..

The enormous increase in the cholinesterase enzyme may be ascribed to be due to the anabolic effect of the steroid hormone content of Lyndiol, which may be substantiated by the fact that the treated group of animals showed 68% increase in body weight at the end of the treatment period, whereas the control group showed only 40% increase.

Toman and Davis[13] have summarized the effects of barbiturates on the EEG of experimental animal and they are of opinion that barbiturate anaesthesia induce cortical LVFA in general. If any such effect is apparent in the present experiment, it might have influenced the control and treated animals uniformly. Thus the EEG change in the treated group appears to be due to Lyndiol treatment only. But one thing which is to be considered here is that the dose of the drug selected for the present experiment was ten times the dose used for a human being. The criteria of selection of such a high dose for smaller animals are that these animals are far more resistant than human beings. However, it is not unlikely that the change in the EEG and brain cholinesterase were the outcome of cumulative effect of Lyndiol.

1. Rock, J., Pincus, G. and Garcia, C. R., *Science*, 1956, **124**, 891.
2. Inman, W. H. W. and Vessey, M. P., *Br. Med. J.*, 1968, **2**, 651.
3. Vessey, M. P., Mann, J. I., Thorogood, M. and Doll, R., *Br. Med. J.*, 1975, **2**, 41.
4. Mann, J. I. and Inman, W. H. W., *Br. Med. J.*, 1975, **2**, 245.
5. Mukherjee Chitralekha, Dey, C. D. and Deb, C., *Curr. Sci.*, 1978, **47**, 614.
6. Ferin, J., *Acta Endocrinol. Suppl.*, 1960, **50**, 149.
7. Ferin, J., *Acta Endocrinol.*, 1962, **39**, 47.
8. Overbeek, G. A., Madjerik, Z. and de Visser, J., *Acta Endocrinol.*, 1962, **41**, 351.
9. Helen, K., Onderka and Kirksey Avanelle, *J. Nutrition*, 1975, **105**, 1269.
10. Hestrin, S., *J. Biol. Chem.*, 1949, **180**, 249.
11. Davis, P. A., *J. Neurophysiol.*, 1941, **4**, 92.
12. Ganong, W. F., *Rev. Med. Physiol.*, Kothari Book Depot, Parel, Bombay, India. p. 139, 1969.
13. Toman, J. E. P. and Davis, *J. Pharmacol. Rev.*, 1949, **1**, 425.

EFFECT OF PATULIN ON INTESTINAL AMINO ACID UPTAKE

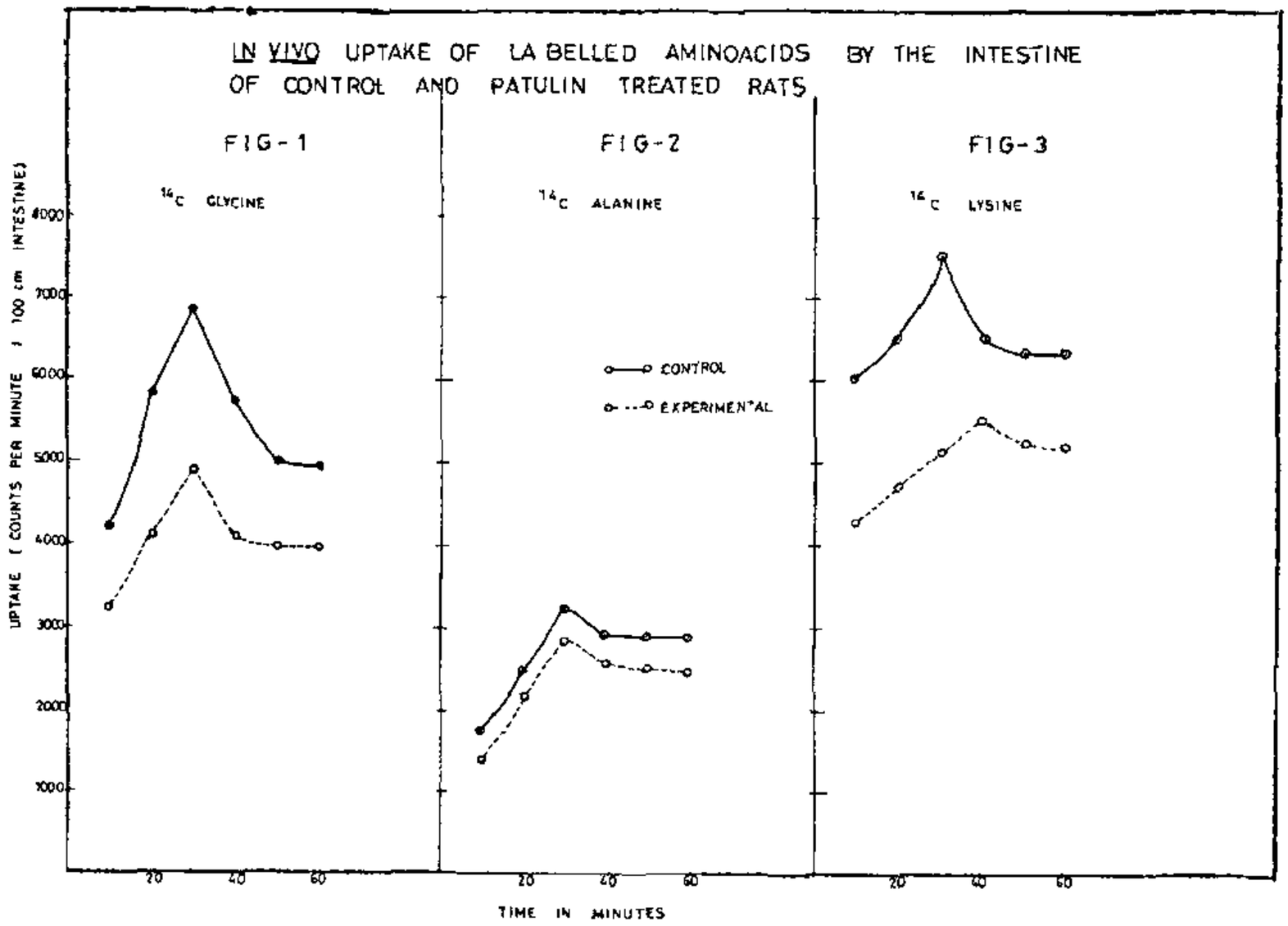
H. DEVARAJ, K. RADHA SHANMUGASUNDARAM* AND
E. R. B. SHANMUGASUNDARAM
University Biochemical Laboratories, University of Madras, Guindy Campus, Madras 600 025, India.
*Department of Biochemistry, Post-Graduate Institute of Basic Medical Sciences, University of Madras, Madras 600 042, India.

Abstract

The function of membrane bound enzymes like total ATPase, Na^+-K^+ dependent ATPase and alkaline phosphatase in amino acid transport across the intestinal membrane during patulin toxicoses was studied along with intestinal uptake of radioactive amino acids. A lowering in the level of these enzymes during toxicoses indicates that transport of amino acid is inhibited during toxicoses.

Introduction

AGAR *et al.*[1] defined the absorption of amino acids as the removal of amino acids from the lumen of the gut *in vivo*. The removal of amino acids from the lumen and their accumulation in the segment of the intestine is also termed as amino acid uptake. Ueno *et al.*[2] have reported that patulin inhibits the sodium-dependent glycine transport in rabbit reticulocytes. To study the action of patulin toxicity in membrane transport, investigations have been made on the intestinal uptake of ^{14}C labelled glycine, alanine



and lysine in control and patulin administered rats by perfusion technique[3].

Materials and Methods

Patulin, a potent mycotoxin, was isolated from the culture filtrate of *Penicillium patulum* according to the method of Scott and Kennedy[4]. A group of forty weanling albino rats of either sex weighing about 25 g received 100 mcg of patulin in propyleneglycol intraperitoneally on alternate days for 30 days. Control group of the same number received propyleneglycol alone and the animals were used for perfusion studies.

Another set of twenty rats was divided into two groups. The experimental group received 100 mcg patulin in propyleneglycol intraperitoneally on alternate days. At the end of the 30th day the rats were sacrificed and the intestines removed immediately, washed with ice cold saline, and used for the assay of membrane bound enzymes like alkaline phosphatase by the method described by King[5] and total ATPase and Na^+-K^+ dependent ATPase by the method of Evans[6].

Results and Discussion

Table 1 shows the activities of total ATPase, Na^+-K^+ dependent ATPase and alkaline phosphatase while figures 1-3 give the uptake of ^{14}C glycine, ^{14}C alanine and ^{14}C lysine. The total ATPases decreased in the experimental rat intestine (39%, $p < 0.001$). Marked inhibition of Na^+-K^+ dependent ATPase (34%, $p < 0.001$) and alkaline phosphatase (41%, $p < 0.001$) in intestine was observed in experimental rats.

The total uptake of these three amino acids is significantly lowered in the experimental rat intestine and a maximum uptake of glycine and alanine is observed at the end of 30 minutes whereas lysine shows delayed uptake.

A significant decrease in the uptake of labelled glycine, alanine and lysine is seen (figures 1-3) suggesting an interference of patulin with amino acid uptake. This could be discussed in terms of membrane damage, damage to the carrier systems or impairment of reactions which provide energy for active transport of amino acids.

Uptake of glycine, proline and hydorxyproline is mediated through a common transport protein. The transport system in iminoglycinuric patients has earlier been studied[7-9]. Our experiments show that patulin may interact with this transport mediator by altering the affinity of this carrier for glycine. Similarly, the reduction in the uptake of alanine and the basic amino acid lysine suggests that patulin may interact with the two transport mediators of neutral as well as the basic amino acids.

The Na^+-K^+ dependent ATPase catalyses the outward flux of sodium ion across the membrane and the inward flux of potassium ion and this is responsible for the establishment of sodium gradient which drives amino acid transport[10,11]. Involvement of Na^+-K^+ dependent ATPase, in the active translocation of amino acids, across cell membranes has been well established[12,13]. The inhibition of this important enzyme by patulin in the present investigation may thus indirectly affect the uptake of amino acids.

Govindasamy and Shanmugasundaram[14] have investigated the uptake of histidine and tryptophan in normal and patulin treated rat intestinal segments *in*

vitro. They have observed that the uptake of both the amino acids is comparatively low in the experimental animal. Observations on amino acid transport in normal animals have suggested that the inhibition of Na^+-K^+ transport in erythrocytes[15], muscle[16] and kidney[17] as well as the inhibition of the erythrocyte Na^+-K^+ dependent ATPase[18] require the presence of lactone ring in the structure of inhibitors.

The cardiac glycoside, ouabain, which also possesses a lactone ring has been established to be a specific and sensitive inhibitor of the Na^+ -K^+ transport system in many tissues[19-21]. It is interesting to note that patulin has a lactone ring which is known for its toxicity[22,23]. The presence of lactone ring in the patulin may be responsible for the inhibition of Na^+ -K^+ dependent ATPase which inturn inhibits amino acid uptake in small intestine (table 1).

TABLE 1

Activities of total ATPase, Na^+-K^+dependent ATPase and alkaline phosphatase in intestinal tissue from control and patulin administered rats.

Enzyme	Control	Experimental
Total ATPase	3.99 ± 0.21	2.43 ± 0.16*
Na^+-K^+ dependent ATPase	1.85 ± 0.20	1.25 ± 0.11*
Alkaline phosphatase	1.84 ± 0.14	1.09 ± 0.09*

*$p < 0.001$

Enzyme activities are expressed as micromoles of product liberated/mg protein under incubation conditions (mean ± S.D).

Ueno *et al*[2] studied the inhibitory effect of patulin on the Na^+ -dependent transport of glycine in rabbit reticulocytes. They concluded that patulin irreversibly binds to the −SH groups of the cell membrane and thus by masking the −SH groups, a favourable Na^+ gradiant which is necessary for the amino acid transport across the cell membrane is not maintained. Alkaline phosphatase, another membrane bound enzyme also shows drastic reduction in the intestine of patulin-treated rats (table 1) and Moog[24] had observed that the intestinal alkaline phosphatase is involved in the transport process.

1. Agar, W. T., Hird, F. J. R. and Sidhu, G. S., *Biochim. Biophys. Acta.*, 1956, **22,** 21.
2. Ueno, Y., Matsumoto, H., Ishii, K. and Kukita, K., *Biochem. Pharmacol.*, 1976, **25,** 2091.
3. Rosenberg, L. E. and Scriver, C. R. In *Amino acid metabolism and its disorders* (eds C. R. Scriver and L. E. Rosenberg), Saunders and Co., Philadelphia, 1973, p. 143.
4. Scott, P. M. and Kennedy, B., *J. Assoc. Off. Anal. Chem.*, 1973, **56,** 813.
5. King, J., In *Practical clinical enzymology*, D. Van Nostrand Company Ltd., London, 1965, p. 106.
6. Evans, D. J. Jr., *J. Bacteriol.*, 1969, **100,** 914.
7. Scriver, C. R., Pueschel, S. and Davies, E., *New Engl. J. Med.*, 1966, **274,** 636.
8. Tada, K., Morikawa, T., Ando, T., Yoshida, T. and Minagawa, A., *Tohoku. J. Exp. Med.*, 1965, **87,** 133.
9. Goodman, S. I., McIntyre, C. A. and O'Brien, D., *J. Pediat.*, 1967, **71,** 246.
10. Katz, B., In *Muscle and Synapse*, McGraw-Hill Inc., New York, 1966.
11. Rosenberg, L. E. and Scriver, C. R., In *Duncan's disease of metabolism*, 7th Edn. (Eds. P. K. Bondy and L. E. Rosenberg), W. B. Saunders Co., Tokyo, Japan, 1974, p. 465.
12. Bonting, S. L., Caravaggio, L. L. and Hawkins, N. M., *Arch. Biochem. Biophys.*, 1962, **98,** 413.
13. Curran, P. E., Schultz, S. G., Chez, R. A. and Fuisz, R. E., *J. Gen. Physiol.*, 1967, **50,** 1261.
14. Govindasamy, S. and Shanmugasundaram, E. R. B., *J. Madras Univ.*, B 1981, (In press).
15. Glynn, I. M., *J. Physiol.*, 1957, **136,** 148.
16. Johnson, J. A., *Am. J. Physiol.*, 1956, **187,** 328.
17. Strickler, J. C., Kessler, R. H. and Knutson, B. A., *J. Clin. Invest.*, 1961, **40,** 311.
18. Dunham, E. T. and Glynn, J. M., *J. Physiol.*, 1961, **156,** 274.
19. Hajdu, S., *Am. J. Physiol.*, 1953, **174,** 371.
20. Holland, W. C., *Am. J. Physiol.*, 1960, **198,** 1223.
21. Orloff, J. and Burg, M., *Am. J. Physiol.*, 1960, **199,** 49.
22. Dickens, C., in *Potential carcinogenic hazard from drugs*, (ed.) R. Truhant, Springer, Berlin, 1967, p. 144.
23. Ciegler, A., Detroy, R. W. and Lillehoj, E. B., in *Microbial Toxins*, Vol. VI, (eds A. Ciegler, S. Kadis, and S. J. Ajl), Academic Press, New York and London, 1971, p. 407.
24. Moog, F., *J. Exp. Zool.*, 1951, **118,** 187.