Liver Monoamine Oxidase in Normal and Regenerating *Diemictylus viridescens*

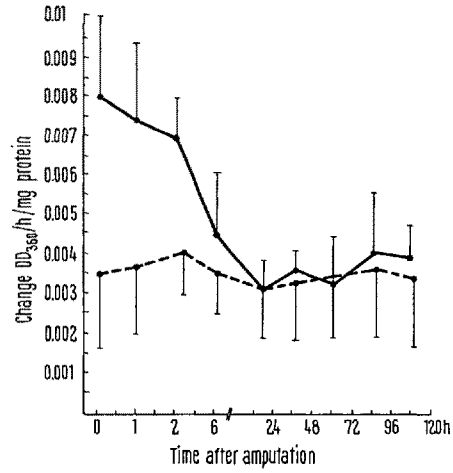
The amputation of limbs in adult urodeles initiates a series of local and systemic reactions leading to the regeneration and restitution of a functional limb. Much attention has been focused on the histo-chemistry and molecular ecology of the wound site (HAY[1], SCHMIDT[2]). Recently WOLFE and COHEN[3] have demonstrated that amputation causes a temporary increase in α-glucan phosphorylase activity in injured muscles and a gradual increase in muscle glucose 6-PO_4 dehydrogenase during dedifferentiation in urodele limbs. SCHMIDT[4] has shown that increased enzyme activity is reflected in a rapid decrease in limb stump glycogen. However, reports of systemic metabolic alterations in response to amputation are scanty. SCHMIDT[5] has shown that liver aldolase activity increases after limb amputation. Recently histochemical investigations have indicated that liver α-glucan phosphorylase and glucose 6-PO_4 dehydrogenase activities significantly increase after limb amputation[6].

SCHOTTÉ[7] has suggested that the physiological stress resulting from amputation initiates a series of hormonal reactions, particularly increased ACTH and cortical hormone levels, which are necessary for wound healing and limb regeneration. It is possible that catecholamines produced by adrenergic neurons and/or adrenal chromaffin may be the stress affectors[8]. WEBER et al.[9] have shown that catecholamines alter pentose pathway metabolism in the livers of injured mammals. Significantly, KOPIN[10] demonstrated that in mammals the liver is the major site of catecholamine metabolism by S-adenosylmethionine and monamine oxidase (MAO). It was of interest in this study to evaluate possible catecholamine involvement in limb regeneration by determining the presence and activity of liver MAO in normal and forelimb regenerating *D. viridescens*.

Materials and methods. Adult *D. viridescens* were bilaterally amputated proximal to the humeral condyles with a single edged razor. Amputated and control animals were maintained in individual finger bowls provided with pond water treated with Fungistop to prevent infection, and fed raw beef liver daily. Monamine oxidase activity was monitored in 12 unoperated animals (controls) and in 12 amputated animals at eight different times after forelimb amputation: 0, 1, 2, 24, 48, 72, 96 and 120 h. Both groups of animals were maintained under identical conditions. At the times specified livers were quickly removed, weighed in 5 ml cold 0.25 *M* sucrose, pH 7.8, and homogenized for 2 min in 4 ml cold sucrose (0.25 *M*) in a cold glass homogenizer. For convenience, homogenates were frozen at −20 °C until use. MAO activity was determined by the method of WEISSBACH et al.[11]. One ml of crude tissue homogenate at room temperature was introduced into a 3 ml silica cuvette, already containing 0.3 μmole Kynuramine (Regis Chemical Co., Chicago), 0.3 ml phosphate buffer, pH 7.4, and water to a volume of 3 ml. Kynuramine is rapidly oxidized by MAO present and the rate of kynuramine disappearance can be followed as a change in optical density. Spectrophotometric readings at 360 nm were performed for 1 h at 37 °C in a Beckman D.B. recording spectrophotometer equipped with a Haake constant temperature apparatus. Enzyme activity was expressed as the change in absorbancy at 360 nm/h. Tissue protein content was determined by the Biuret test[11]. The *t*-statistic was calculated on an IBM/360 digital computer, using the IBM scientific subroutine for *t*-test. Means and standard deviations were calculated simultaneously.

Results and discussion. The evidence clearly indicates that MAO is present in an active form in the adult urodele liver. It appears that MAO activity is independent of sex. The results also indicate (Figure) that there is a significant increase in MAO activity (0.01 confidence level) immediately after forelimb amputation. In mammals the response to stress is nearly immediate, occurring within seconds of the stimulus[12]. EXTON and PARKS[13] noted that stress-induced breakdown of hepatic and muscle glycogen in mammals occurs as a result of catecholamine mediated activation of α-glucan phosphorylase. In urodeles, SCHMIDT[4] demonstrated a qualitative glycogen loss from injured portions of striated muscle fibers within 24 h after amputation. Recent evidence[6] indicates that a 50% decrease in glycogen occurs in the urodele liver 2–4 h after limb amputation.

While stress response intervals are not known for lower vertebrates, it is reasonable to assume that those responses mediated by the catecholamines would occur instantaneously[9]. Since the catecholamines are metabolized very rapidly (LEHNINGER[14]) an immediate rise in



Liver MAO activity in amputated animals (—) expressed as changes in optical density at 360 nm/h/mg protein. Control (unamputated) values (---) varied between 0.0040 ± 0.0010 and 0.0032 ± 0.0014 during the 120 h. A significant change in MAO activity (0.01 confidence level) occurred at 0 through 2 h after amputation.

[1] E. D. HAY, *Regeneration* (Holt, Rinehart and Winston, New York 1966).
[2] A. J. SCHMIDT, *The Cellular Biology of Vertebrate Regeneration and Repair* (University of Chicago Press, Chicago 1968).
[3] H. J. WOLFE and R. B. COHEN, Devl. Biol. *8*, 48 (1963).
[4] A. J. SCHMIDT, J. exp. Zool. *149*, 171 (1962).
[5] A. J. SCHMIDT, *The Molecular Basis of Regeneration: Enzymes* (University of Illinois Press, Chicago 1966).
[6] D. J. PROCACCINI and C. M. DOYLE, in press (1971).
[7] O. E. SCHOTTÉ, in *Molecular and Cellular Synthesis* (Ed. D. RHUDNICK; Ronald Press, New York 1961), p. 161.
[8] O. E. SCHOTTÉ and A. DORIN, Rev. suisse Zool. *72*, 205 (1965).
[9] G. WEBER, R. R. SINGHALL, N. B. STAMM, E. A. FISHER and M. A. MENTERDIEK, Adv. Enzyme Reg. *2*, 1 (1964).
[10] I. J. KOPIN, Pharm. Rev. *16*, 179 (1964).
[11] H. WEISSBACH, T. E. SMITH, J. W. DALY, B. WITCOP and S. UDENFRIEND, J. biol. Chem. *235*, 660 (1960).
[12] C. D. TURNER, *General Endocrinology* (W. B. Saunders, Philadelphia 1966).
[13] J. H. EXTON and C. R. PARKS, Pharm. Rev. *18*, 181 (1966).
[14] A. L. LEHNINGER, *Biochemistry* (Worth Publishing Co., New York 1970).

liver MAO activity after limb amputation is consistent with the notion that amputation increases circulating catecholamine levels, even though there is no conclusive proof that MAO is acting exclusively on catecholamines. In view of the results, it is interesting to speculate that the significant and rapid increase in MAO activity after amputation is probably due to activation of MAO precursors present in the liver rather than de novo induction of the enzyme.

Résumé. L'oxydase monoamine se trouve sous une forme active dans le foie d'un urodèle adulte capable d'activité normale et régénératrice. Au cours des deux premières heures qui suivent l'amputation d'un membre antérieur, on peut noter une augmentation appréciable de l'activité de l'oxydase monoamine. Cette augmentation indique que la tension physiologique produite par l'amputation rend actifs dans le foie les précurseurs de l'oxydase monoamine qui peuvent, à leur tour, stimuler un métabolisme plus rapide et augmenter la quantité des catécholamines qui circulent.

D. J. PROCACCINI, R. L. PROCACCINI and CATHERINE M. DOYLE

Department of Biology, Emmanuel College, 400 The Fenway, Boston (Massachusetts 02115, USA), and Department of Pharmacology, University of Rhode Island, Kingston (R.I., USA), 8 March 1971.

Adrenal Enzymes of Catecholamine Biosynthesis and Metabolism in Spontaneously Hypertensive Rats

It was reported in our previous paper[1] that the activities of tyrosine hydroxylase and dopamine β-hydroxylase in the adrenal glands of spontaneously hypertensive (SH) rats (a strain of Wistar rats, which had been developed by OKAMOTO and AOKI[2]) are increased about 2-fold as compared with those of the normotensive Wistar rats. The marked hypotensive effect of the inhibitors of tyrosine hydroxylase and dopamine β-hydroxylase on SH-rats was also noted[1]. This finding prompted us to investigate the changes of other enzymes involved in the catecholamine biosynthesis and metabolism in the adrenals of SH-rats. This communication describes the activities of DOPA decarboxylase, phenylethanolamine N-methyltransferase, monoamine oxidase and catechol *O*-methyltransferase in the adrenals of SH-rats.

SH-rats used were 4 months old. Their blood pressures were between 160 and 200 mm Hg. Normotensive Wistar rats, of the same age and raised in the same conditions, were used as controls. The rats were sacrificed by decapitation. The adrenals were quickly removed and stored frozen on dry ice. The frozen adrenals were homogenized in 2 ml of 0.1 *M* potassium phosphate buffer (pH 7.5).

DOPA decarboxylase activity was measured by the method of LOVENBERG et al.[3]. The incubation mixture contained 100 μmoles of potassium phosphate buffer (pH 7.0), 0.3 μmole of harmaline, 0.07 μmole of pyridoxal phosphate, 1.0 μmole of L-DOPA, 0.2 ml of the homogenate, and water to 1.0 ml. Incubation was carried out at 37 °C for 20 min in air under shaking. As blank, L-DOPA was omitted during the incubation and added after the incubation. Phenylethanolamine N-methyltransferase was determined by the method of CONNETT and KIRSHNER[4]. The incubation mixture contained 25 μmoles of potassium phosphate buffer (pH 7.9), 115 nmoles of DL-normetanephrine, 1.8 nmoles of S-adenosyl-L-methionine containing 0.1 μCi of S-adenosyl-L-methionine (methyl-^{14}C), 200 μl of the homogenate and water to 300 μl. Incubation was carried out at 37 °C for 60 min in air under shaking. As blank, S-adenosyl-L-methionine (methyl-^{14}C) was omitted during the incubation and added after the incubation. Monoamine oxidase activity was measured fluorometrically by the formation of 4-hydroxyquinoline from kynuramine[5]. The incubation mixture contained 75 μmoles of potassium phosphate buffer (pH 7.4), 0.25 μmole of kynuramine and 0.05 ml of the homogenate in a total volume of 1.5 ml. Catechol *O*-methyltransferase was measured by the method of AXELROD and TOMCHICK[6]. The incubation mixture (50 μl) contained 5 μmoles of potassium phosphate buffer (pH 7.6), 0.5 μmole of magnesium chloride, 15 nmoles of epinephrine, 1.8 nmoles of S-adenosyl-L-methionine containing 0.1 μCi of S-adenosyl-L-methionine (methyl-^{14}C) and 25 μl of the homogenate. Incubation was carried out at 37 °C for 20 min in air under shaking. As blank, S-adenosyl-L-methionine (methyl-^{14}C) was omitted during the incubation and added after the incubation. The enzyme activities are expressed in the amount of the product (nmoles or pmoles)/min per both adrenals or per mg protein. Protein was measured by the method of LOWRY et al.[7].

As shown in Table I, DOPA decarboxylase and phenylethanolamine N-methyltransferase activities in the adrenal glands of SH-rats were slightly higher than those of

Table I. The activities of DOPA decarboxylase and phenylethanolamine N-methyltransferase in the adrenals of spontaneously hypertensive rats

Enzyme activity	Normotensive Wistar rats	Spontaneously hypertensive rats
DOPA decarboxylase		
nmoles/min/both adrenals ± S.E.M.	11.9 ± 1.2	16.9 ± 1.4[a]
nmoles/min/mg protein ± S.E.M.	1.95 ± 0.17	2.53 ± 0.12[b]
Phenylethanolamine N-methyltransferase		
pmoles/min/both adrenals ± S.E.M.	16.3 ± 0.8	21.1 ± 1.3[c]
pmoles/min/mg protein ± S.E.M.	2.67 ± 0.10	3.15 ± 0.08[d]

The result is the average for 6 animals. [a] $p < 0.02$. [b] $p < 0.05$. [c] $p < 0.02$. [d] $p < 0.01$.

[1] I. NAGATSU, T. NAGATSU, K. MIZUTANI, H. UMEZAWA, M. MATSUZAKI and T. TAKEUCHI, Nature, Lond. *230*, 381 (1971).
[2] K. OKAMOTO and K. AOKI, Jap. Circ. J. *27*, 282 (1963).
[3] W. LOVENBERG, H. WEISSBACH and S. UDENFRIEND, J. biol. Chem. *237*, 89 (1962).
[4] R. J. CONNETT and N. KIRSHNER, J. biol. Chem. *245*, 329 (1970).
[5] M. KRAML, Biochem. Pharmac. *14*, 1684 (1965).
[6] J. AXELROD and R. TOMCHICK, J. biol. Chem. *233*, 702 (1958).
[7] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).