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.

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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] $p < 0.01$.

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normotensive Wistar rats. The increases in the enzyme activities of both adrenals were about 40% and 30%, respectively. In contrast, the activities of monoamine oxidase and catechol *O*-methyltransferase in the adrenals of SH-rats did not change significantly as compared with those of normotensive Wistar rats (Table II).

These results showed that, among the enzymes involved in the biosynthesis of catecholamines, enzyme activities of DOPA decarboxylase and phenylethanolamine N-methyltransferase were only slightly increased, as compared with those of tyrosine hydroxylase and dopamine β-hydroxylase, which had been shown to increase about 1.9-fold and 1.8-fold, respectively[1]. The enzyme activities of monoamine oxidase and catechol *O*-methyltransferase did not change.

The results indicate that the increase in the activities of tyrosine hydroxylase and dopamine β-hydroxylase in the adrenal glands of SH-rats are specific, and that both enzymes may be easily induced[8].

Table II. The activities of monoamine oxidase and catechol *O*-methyltransferase in the adrenals of spontaneously hypertensive rats

Enzyme activity	Normotensive Wistar rats	Spontaneously hypertensive rats
Monoamine oxidase		
nmoles/min/both adrenals $\pm$ S.E.M.	1.51 ± 0.07	1.48 ± 0.10
nmoles/min/mg protein $\pm$ S.E.M.	0.25 ± 0.01	0.22 ± 0.01 [a]
Catechol *O*-methyltransferase		
pmoles/min/both adrenals $\pm$ S.E.M.	42.6 ± 2.2	40.0 ± 3.5
pmoles/min/mg protein $\pm$ S.E.M.	6.99 ± 0.40	5.99 ± 0.32 [b]

The result is the average for 6 animals. [a] $p < 0.20$. [b] $p < 0.10$.

Zusammenfassung. Es wird gezeigt, dass Dopa-Decarboxylase und Phenylethanolamin-N-methyl-transferase bei spontan hypertonischen Ratten im Vergleich zu normotonen Kontrollen leicht erhöht sind; hingegen wurde keine Veränderung der Catecholamin-abbauenden Enzyme festgestellt.

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On the Nature of Biological Clocks?

There has been much speculation on the fundamental cellular mechanism underlying Biological Clocks. Various mathematical models have been proposed[1,2] and physiological oscillations have been cited to explain their working[3,4].

Light, dark cold (and even pressure) are all agencies that readily phase Biological Clocks[5]. Moreover, rhythms may be extinguished under continuous application of the agency, to be reinstated in a fresh phase when it is removed[6,7]. A notable general observation made by BUNNING[5] is that the physiological effect of cold during a light period coincides with that produced by dark. Current theories of Circadian rhythms do not however, admit a resolution of this paradox. A possible general explanation is however implicit in some recent work of the present author, who has shown that in a wide variety of cells and tissues, ATP levels vary inversely with temperature[8–12], and decrease under illumination[10–12]. Both such effects have been demonstrated in the leaves of *Chenopodium rubrum* and *Phaseolus vulgaris*[10], in *Dictyostelium* myxamoebae[11] and also in chick embryos in ovo[12].

All the phasing agencies described have the common effect of altering cytoplasmic viscosity[13]; and reference to the literature[13,14] establishes that the increases in ATP level described above in dark or cold correspond to decrease of cytoplasmic viscosity, a predictable correlation knowing the effect of microinjected ATP[15].

On this basis therefore, the paradoxical common effect of dark and cold applied during illumination in phasing rhythmic phenomena is readily explicable, for whereas the effect of cold is to raise ATP, its level in the dark is also higher than that under illumination. The effect of pressure in phasing clocks[5,6] may be on this basis also, for LANDAU[16] has also described pressure-induced increases in ATP level.

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