react readily with both proteins[21] and nucleic acids[22,23], to possess antiviral activity[24], to inhibit protein synthesis[25] and cell division[26], and have been postulated to be involved in the regulation of these processes[27]. The role of the glyoxalases in preventing an accumulation of hydroxymethylglyoxal phosphate would therefore seem to be essential for normal cell function. The oxidation of enzyme-substrate carbanions by H_2O_2 might also be considered as a metabolic feature mediating the effect of an increased intracellular H_2O_2 concentration in biological processes such as oxygen poisoning[28], radiation damage[29], or phagocytosis by leukocytes[18,19].

Zusammenfassung. H_2O_2 oxydiert eine Enzym-Substrat-Zwischenverbindung der Fructose-1,6-diphosphat-Aldolase. Aus Dihydroxyacetonphosphat wird dabei Hydroxymethylglyoxalphosphat gebildet. Dieser Ketoaldehyd dürfte eines der lange gesuchten Substrate für das Glyoxalase-System sein.

P. Christen, T. K. Anderson, and M. J. Healy

Biochemisches Institut der Universität Zürich, Zürichbergstrasse 4, CH-8032 Zürich (Switzerland), 21 December 1973.

[21] E. Racker, in *Glutathione* (Eds. S. Colowick, A. Lazarow, E. Racker, D. R. Schwartz, E. Stadtman and H. Waelch; Academic Press, New York 1954), p. 171.
[22] M. Staehelin, Biochim. biophys. Acta *31*, 448 (1959).
[23] N. Krymkiewicz, FEBS Lett. *29*, 51 (1973).
[24] B. D. Tiffany, J. B. Wright, R. B. Moffett, R. V. Heinzelman, R. E. Strube, B. D. Aspergren, E. H. Lincoln and J. L. White, J. Am. chem. Soc. *79*, 1682 (1957).
[25] L. G. Együd and A. Szent-Györgyi, Proc. natn. Acad. Sci. USA *56*, 203 (1966).
[26] L. G. Együd and A. Szent-Györgyi, Proc. natn. Acad. Sci. USA 55, 388 (1966).
[27] A. Szent-Györgyi, *The Living State. With Observations on Cancer* (Academic Press, New York 1972), p. 88.
[28] R. Gerschman, in *Oxygen in the Animal Organism* (Eds. F. Dickens and E. Neil; Pergamon Press, Oxford 1964), p. 475.
[29] D. B. Menzel, A. Rev. Pharmac. *10*, 379 (1970).

A 24-Hour Rhythm in Human Serum Dopamine-β-Hydroxylase Activity

Human serum contains dopamine-β-hydroxylase (DBH), which is released from the sympathetic nerve endings and the adrenal medulla[1]. The enzyme activity in serum can be measured either by enzymatic radioassays using phenylethanolamine-N-methyltransferase[2,3], or by a micro-spectrophotometric assay[4].

The levels of DBH activity in human serum are especially high compared with various animal species[4], they showed a marked individual variation (1-100 U, μmoles/min, per 1 serum), but were very constant on successive days for a given individual[2-4]. The high enzyme activity in human serum is assumed to be caused by the increased sympathetic nerve activity due to the erect posture. Since the enzyme is secreted from the sympathetic nerves and the adrenal medulla together with norepinephrine and epinephrine, the enzyme activity in serum may be expected to have a 24-h rhythm caused by the changes in peripheral sympathetic activity. However, there has been no report on the 24-h rhythm of serum DBH activity in humans. If there is such a rhythm, it may be an important basic information for the clinical study of the enzyme activity in human serum. We have, therefore, examined changes in the enzyme activity in human serum during 24-h.

Dopamine-β-hydroxylase activity in serum was assayed by a micro-photometric method by Nagatsu and Udenfriend[4] using tyramine as substrate. 10 and 20 μl of serum were used for the duplicate assays. This assay method is highly reproducible, and the maximum velocity can be obtained under the saturated substrate

[1] J. Axelrod, Pharmac. Rev. *24*, 233 (1972).
[2] R. Weinshilboum and J. Axelrod, Circulation Res. *28*, 307 (1971).
[3] M. Goldstein, L. S. Freedman and M. Bonnay, Experientia *27*, 632 (1971).
[4] T. Nagatsu and S. Udenfriend, Clin. Chem. *18*, 980 (1972).

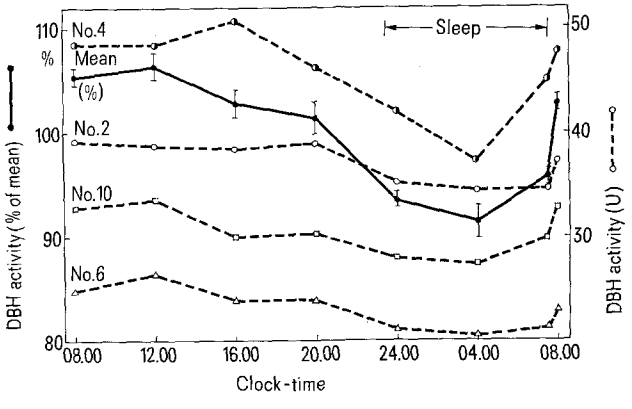


Fig. 1. Dopamine-β-hydroxylase (DBH) activities in human sera. —, Mean values (± SEM) of 10 female volunteers at various times during 24 h are expressed as the percentage of 24-h mean activity of each individual. ---, Actual DBH activities of 4 cases are shown in terms of international unit (U, μmoles/min, per 1 serum). Experiments were started at 08.00 h.

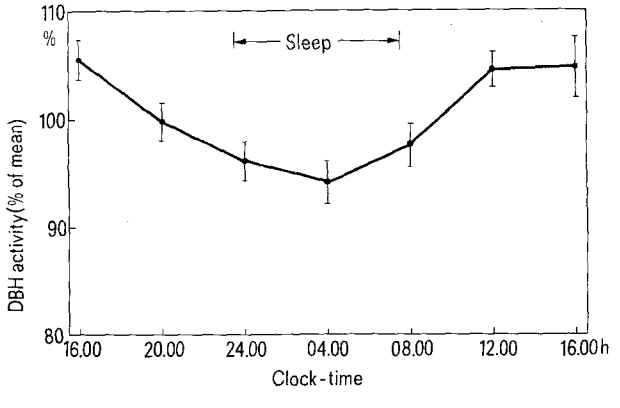


Fig. 2. DBH activities in human sera. Mean values (± SEM) of 13 male volunteers at various times during 24 h are expressed as the percentage of 24-h mean activity of each individual. Experiments were started at 16.00 h.

concentration (20 mM) and optimum pH (5.0)[4]. The reproducibility of the assay with replicates of the same sample was 100 ± 1.8% (S. D.).

Incubation mixture contained (total volume 1.0 ml): diluted human serum (10 or 20 µl of human serum diluted with water), 400 µl; 1 M acetate buffer, pH 5.0, 200 µl; 0.2 M N-ethylmaleimide, 150 µl; 0.2 M sodium fumarate, 50 µl; 0.02 M pargyline, 50 µl; 0.2 M ascorbic acid, 50 µl; catalase (1 mg/ml), 50 µl containing 1500 units; and 0.4 M tyramine HCl, 50 µl. Boiled diluted serum (95 °C for 5 min) served for the blank. Incubation was carried out for 60 min at 37 °C in air with constant shaking.

Experiments were carried out on healthy 13 male and 10 female volunteers aged 18-26 years. They were all students. They came to the laboratory at 08.00 h or 16.00 h, and the initial blood sample (about 1 ml) was drawn from an anticubital vein. Blood samples were taken every 4 h during 24 h. During daytime, they had lectures at the school.

The 24-h change of serum DBH activity with 10 females is shown in Figure 1. The enzyme activities at various times during 24 h were expressed as the mean value (± SEM) of the percentage of 24-h mean activity of each individual. The 24-h mean activities of the subjects in terms of international unit (U/l serum) ranged from 21.6 ± 1.6 to 49.8 ± 2.9. Actual activities of 4 cases with low, middle, and high activities (No. 2, 4, 6 and 10) are also shown in Figure 1. A small but significant 24-h rhythm of serum DBH activity was observed with all the subjects. The enzyme activity was significantly higher during the daytime, and decreased significantly at night. The maximum difference during 24 h was about 15%.

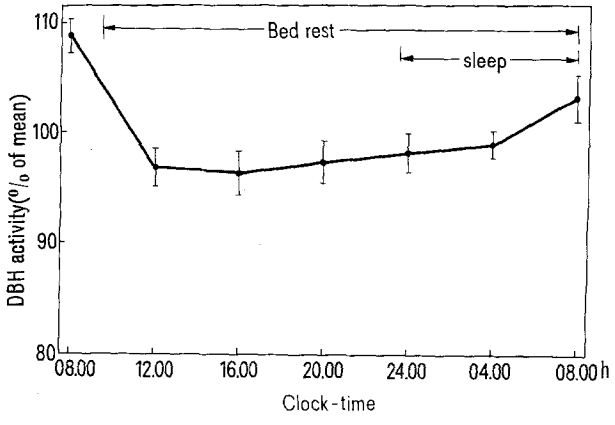


Fig. 3. DBH activities in human sera. Mean values (± SEM) of 2 female and 8 male volunteers at various times during 24 h are expressed as the percentage of 24-h mean activity of each individual. A recumbent posture in bed was kept from 09.30 h to 08.00 h next morning with each subject. All the subjects slept from 23.30 h to 08.00 h.

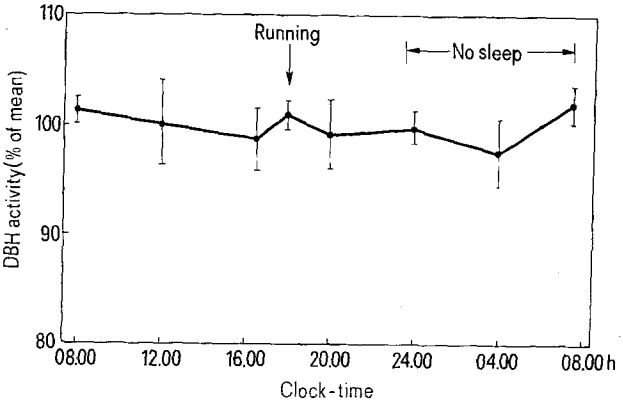


Fig. 4. DBH activities in human sera from 5 female and 5 male volunteers. The experimental conditions were the same as in Figure 3, except that the ordinary physical activities and an erect posture (sitting or standing) were continued for 24 h, in the class-room during the daytime, and in the dormitory at night with each subject without sleep.

Essentially similar results were obtained with 13 male volunteers as shown in Figure 2. The 24 h mean activities of the subjects ranged from 7.3 ± 0.8 to 53.3 ± 0.7 U/l serum. Experiments were started from 16.00 h in this case, and continued until 16.00 h next day.

In order to see whether or not the 24-h rhythm in human serum DBH activity is due to the effect of physical activity (sitting or standing during the daytime), a second experiment was carried out. 10 volunteers (8 males and 2 females) took bed rest for 24 h. At 08.00 h the initial blood samples were drawn, and the subjects started taking bed rest in a recumbent position. Physical activities were kept minimal except for taking lunch and supper for 30 min in bed. As shown in Figure 3, serum DBH levels decreased slightly but significantly (−12%) after taking bed rest in the morning, and this lowered levels continued for 24 h. Sleeping at night did not further lower the serum DBH level. The results indicate that the low enzyme activity at night in the first experiment may be due to the decrease in the physical activity and the change in the posture from an erect posture to a recumbent posture.

To see the effect of sleep at night on the serum DBH activity, a third experiment was carried out. 5 male and 5 female volunteeres were deprivated of sleep, and they spent all night sitting on a chair and reading just as in the class-room. As shown in Figure 4, the 24-h rhythm was not observed, and the level of DBH activity did not decrease significantly at night. Running for 8 min was carried out at 18.00 h just before drawing a blood sample, and a very small elevation in serum DBH levels was observed with all cases. WOOTEN and CARDON[5] and FREWIN, DOWNEY and LEVITT[6] also reported a small but significant elevation in serum DBH levels after physical exercise.

In order to see whether or not these changes in human serum DBH activity are specific for DBH which is secreted mainly from the sympathetic nerve endings, the enzyme activities of leucyl β-naphthylamidase, which is assumed to be derived mainly from the liver, were examined. Leucyl β-naphthylamidase activity was assayed photometrically as described before[7]. Incubation mixture for serum leucyl β-naphthylamidase activity contained (total volume, 0.90 ml): 0.2 M *tris*-maleate buffer, pH 7.0, 0.45 ml; 3 mM L-leucyl β-naphthylamide HCl, 0.15 ml, and 10 µl of human serum. Incubation was carried out at 37 °C for 30 min. Mean enzyme activity of serum leucyl β-naphthylamidase of 5 male volunteers in the first experiment at 04.00 h was 37.8 ± 2.0 (SEM) U/l, whereas the mean enzyme activity at 12.00 h was 37.2 ± 2.4 (SEM) U/l. The results indicate that there is no significant change during 24 h with leucyl β-naphthylamidase activi-

[5] G. F. WOOTEN and P. V. CARDON, Arch. Neurol. *28*, 103 (1973).
[6] D. B. FREWIN, J. A. DOWNEY and M. LEVITT, Can. J. Physiol. Pharmac. *51*, 986. (1973).
[7] I. NAGATSU, T. NAGATSU and G. G. GLENNER, Enzymologia *34*, 73 (1968).

ty. Therefore, it is concluded that the 24-h change observed in DBH activity in human serum may be specific for the enzyme derived from the sympathetic nerves.

In conclusion, DBH activity in human serum has a small but significant 24-h rhythm, being higher at daytime and lower at night. This 24-h rhythm may be due to an increased physical activity or due to an erect posture during daytime, and sleep itself may have little effect on the enzyme activity.

Zusammenfassung. Nachweis, dass die Dopamin-β-Hydroxylase im menschlichen Serum einen Tag-Nacht-Rhythmus aufweist, wobei die Nachtaktivität im Verhältnis zu derjenigen des Tages reduziert ist.

T. Okada, T. Fujita, T. Ohta, T. Kato, K. Ikuta and T. Nagatsu

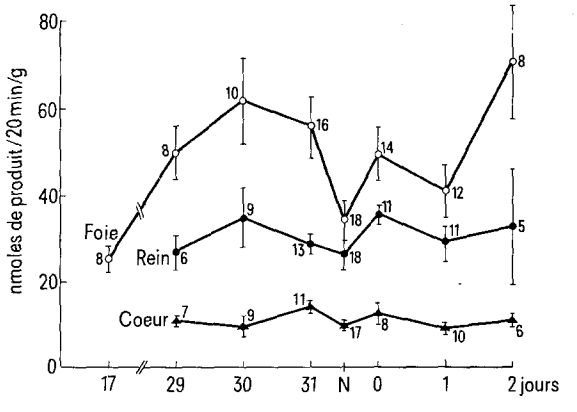
Department of Neuropsychiatry, Faculty of Medicine, University of Nagoya, Nagoya (Japan), and Department of Biochemistry, School of Dentistry, Aichi-Gakuin University, Nagoya 464 (Japan),
14 December 1973.

Activité de la monoamine-oxydase au cours de la période peri-natale chez le Lapin

De nombreuses publications décrivent les changements de l'activité de la monoamine-oxydase (MAO; EC. 1.4.3.4.) au cours de la croissance. Certaines d'entre elles montrent que la distribution des différentes formes de l'enzyme, qui semble être particulière à chaque organe, varie avec l'âge de l'animal[1]. Très souvent les dosages ne concernent que le développement post-natal, ce qui délaisse deux périodes que l'on sait être importantes dans l'évolution de nombreuses activités enzymatiques[2]: la fin de la période fœtale et la période néonatale. D'autre part, le lapin a été très peu étudié de ce point de vue; toutefois, l'évolution de l'activité MAO a été étudiée dans plusieurs régions du cerveau, à partir du stade de 3 jours[3]: à cet âge, l'activité enzymatique est déjà sensiblement égale ou supérieure à celle de l'adulte, selon la région étudiée.

La présente étude a été entreprise pour établir l'évolution de l'activité MAO dans trois organes du Lapin pendant la fin de la vie fœtale, la naissance et le début de la vie post-natale.

Matériel et méthodes. Des lapins Néo-Zélandais ont été utilisés chez lesquels la durée de la gestation est de 31 jours. Les nouveau-nés (N) correspondent à des animaux nés depuis 1 min, 1 h et 2 h et le stade de 0 jour correspond à des animaux âgés de 12 ± 3 h. Les fœtus sont prélevés dans l'utérus, après anesthésie de la mère au pentobarbital (0,5 ml/kg d'une solution à 6%). Fœtus et nouveau-nés sont tués par décapitation et les organes sont immédiatement prélevés et placés dans du KCl 0,14 *M* à 4 °C. Les échantillons sont conservés à −20 °C. L'activité MAO est dosée par la méthode de Wurtman et Axelrod[4] utilisant de la tryptamine-bisuccinate comme substrat. Les résultats sont exprimés en nmoles de produit formé (acide indole-acétique) par 20 min et par g de tissu frais ± l'intervalle de confiance calculé pour P 0,05. Pour chaque stade, les fœtus ou nouveau-nés proviennent d'au moins trois portées différentes (6 portées pour le stade Naissance).



Activité MAO au cours de la période peri-natale exprimée en nmoles de produit formé en 20 min et par g de tissu frais ± intervalle de confiance calculé pour P 0,05. N, naissance. Les nombres situés à côté des points indiquent le nombre de cas. Différences statistiquement significatives: Foie, 31 j et N: $P < 0{,}001$; 0 j et 1 j: $P < 0{,}05$. Rein, 30 j et N: $P < 0{,}02$; 0 j et 1 j: $P < 0{,}01$. Cœur, 31 j et N: $P < 0{,}001$; 0 j et 1 j : $P < 0{,}001$.

Résultats. La Figure présente l'évolution de l'activité MAO de 29 j de vie fœtale à 2 j de vie néo-natale. Pour le foie, l'activité de l'enzyme a été dosée également chez des fœtus de 17 j.

L'activité du foie double entre 17 et 29 j; le taux d'accroissement global de l'activité enzymatique pendant ces 12 jours est nettement inférieur à celui de la période 29–30 j. A 30 j., l'activité du foie fœtal a atteint celle de l'adulte qui est de 68,5 ± 8 nmoles/20 min/g. A 2 j de vie post-natale, l'activité du rein est encore très inférieure à celle de l'adulte (88 ± 25 nmoles/20 min/g pour le cortex et 164,6 ± 67,7 nmoles/20 min/g pour la medulla).

Tout au long de la période étudiée, les activités du foie et du rein évoluent de façon très comparable: elles augmentent entre 29 et 30 j, entre la naissance et 0 j (les activités mesurées dans les 3 organes restent constantes après 1 min, 1 h et 2 h de vie post-natale) et entre 1 et 2 j. Elles diminuent entre 31 j et la naissance et entre 0 et 1 j.

A 29 j, l'activité enzymatique du cœur a atteint le niveau de l'adulte (10,5 ± 1,3 nmoles/20 min/g). A partir de 31 j elle suit la même évolution que celles du foie et du rein, mais l'amplitude des variations observées est très faible.

Discussion. Les variations de l'activité enzymatique des 3 organes présentent des analogies telles que l'on peut penser que les causes qui les déterminent sont les mêmes. Par contre, les niveaux d'activité atteints en fin de vie fœtale suggèrent une distribution hétérogène dans ces 3 organes au cours du développement de la forme ou des formes de la MAO dosées par la méthode utilisée c'est à dire celles qui dégradent à une vitesse appréciable la tryptamine.

[1] S. Eiduson, in *Monoamine Oxidase New Vistas* (Eds. E. Costa et M. Sandler; Raven Press, New York 1972), p. 271.
[2] O. Greengard, in *Biochemical Actions of Hormones* (Ed. G. Litwack; Academic Press, New York 1970), p. 53.
[3] R. E. McCaman et M. H. Aprison, Progr. Brain Res. *9*, 220 (1964).
[4] R. J. Wurtman et J. Axelrod, Biochem. Pharmac. *12*, 1439 (1963).