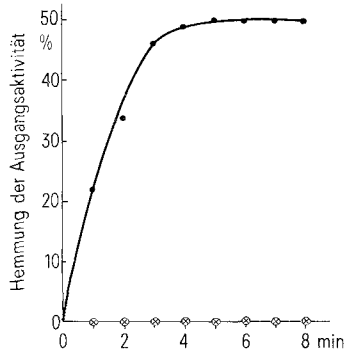
dem Zusatzgerät SP 820 zur Registrierung bei fester Wellenlänge (Fa. Unicam Instruments LTD, Cambridge/England) unter geringfügiger Modifizierung des von ORR[4] abgewandelten Verfahrens von CHANCE[7] registriert. Die Vergleichsküvette enthielt den bei den Experimenten verwendeten Phosphatpuffer von pH 7,0. Die Wasserstoffperoxidkonzentration wurde auf ungefähr 18 mM eingestellt, was bei pH 7,0 einer Extinktion von um 0,8 entspricht. Gestartet wurde die Reaktion durch Zugabe von einer jeweils frisch bereiteten wässrigen Lösung des zu zersetzenden Wasserstoffperoxids.

Ergebnisse. 1. pH-Optimum: Man findet ein breites pH-Optimum zwischen pH = 6,0 und pH = 7,0.

2. Kinetik der Hemmung bei pH 7,0: Inkubiert man Katalase anaerob in Gegenwart von Monodehydro-L(+)-ascorbat, so wird sie bei gleicher Molarität von L(+)-Ascorbat und Didehydro-L(+)-ascorbat im Ansatz innerhalb weniger Minuten zu 50% gehemmt. Inkubiert man Katalase unter den gleichen Bedingungen mit L(+)-Ascorbat, so wird sie selbst nach 3 h nicht inaktiviert. Didehydro-L(+)-ascorbat allein vermag ebenfalls Katalase nicht zu inaktivieren. Die Figur zeigt die Kinetik der Hemmung über 8 min.



Kinetik der Hemmung von Katalase durch Monodehydro-L(+)-ascorbat (●●●), sowie Einfluss von L(+)-Ascorbat (○ ○ ○) und Didehydro-L(+)-ascorbat (× × ×) auf die katalatische Aktivität der Katalase. Katalase 8 µg in 50 ml, L(+)-Ascorbat und Didehydro-L(+)-ascorbat jeweils 10^{-4} M, 0,01 M Phosphatpuffer, pH 7,0, 25 °C. Messvolumen 2,5 ml, davon 2,45 ml des Inkubationsansatzes 0,392 µg Katalase und 0,05 ml einer 0,45 M Wasserstoffperoxidlösung enthaltend. Mittelwerte aus 8–12 Messungen, maximale Abweichung ± 2%.

3. Irreversibilität der Hemmung: Die Hemmreaktion wird nicht mehr beobachtet bei Konzentrationsverhältnissen von L(+)-Ascorbat: Didehydro-L(+)-ascorbat von jenseits 40 und 0,025. Maximale Hemmung der Katalase findet man beim Verhältnis von L(+)-Ascorbat: Didehydro-L(+)-ascorbat von 1. Dies wird auch bei von den in der Legende zur Figur unterschiedlichen Konzentrationen gefunden. Hemmt man die Katalase maximal und stellt anschliessend durch Zugabe von fester L(+)-Ascorbinsäure oder fester Didehydro-L(+)-ascorbinsäure ein anderes Konzentrationsverhältnis ein, so bleibt die Hemmung selbst nach zweistündiger Inkubation bei 25 °C maximal.

Diskussion. Durch die mitgeteilte Reaktion ist erstmals gezeigt, dass eine Enzymaktivität durch das Radikalanion Monodehydro-L(+)-ascorbat verändert wird. Es ist zu erwarten, dass die Aktivitäten weiterer Enzyme durch Monodehydro-L(+)-ascorbat beeinflusst werden. Der Mechanismus der hier beschriebenen Reaktion bedarf der Klärung.

[1] C. W. M. ORR, Biochem. biophys. Res. Commun. *23*, 854 (1966).
[2] C. W. M. ORR, Biochemistry *6*, 2995 (1967).
[3] C. W. M. ORR, Biochemistry *6*, 3000 (1967).
[4] C. W. M. ORR, in *Methods in Enzymology* (Eds. S. P. COLOWICK und N. O. KAPLAN; Academic Press, New York und London 1970), Vol. 18, p. 59.
[5] HJ. STAUDINGER und W. WEIS, Hoppe-Seyler's Z. physiol. Chem. *337*, 284 (1964).
[6] G. v. FOERSTER, W. WEIS und HJ. STAUDINGER, Justus Liebigs Annln Chem. *690*, 166 (1965).
[7] B. CHANCE, in *Methods of Biochemical Analysis* (Ed. D. GLICK; Interscience Publishers, Inc., New York, Interscience Publishers LTD., London 1957), Vol. 1, p. 412.

The Relative Distribution of Soluble and Insoluble Cholinesterases in Rat Excitable Tissues

J. M. VARELA and P. MANDEL

Centre de Neurochimie, Faculté de Médecine, 11, rue Human, F–67085 Strasbourg Cédex (France), 24 February 1976.

Summary. Three ratios were studied here: bound to free AChE (R_1), bound to free BChE (R_2), and the ratios between these two (R_3). The first one proved relevant in that it contributed to the division of the cholinergic tissues into 3 classes: high values (nicotinic tissues: skeletal muscle), low values (muscarinic tissues: small intestine, uterus, heart), and middle values (mixed, nicotinic and muscarinic cholinergic innervation: brain). The third ratio (R_3) showed different values in the muscarinic tissues studied; no significant differences could, however, be found between the ratios of brain and skeletal muscle. Further exploration of this ratio should indicate whether it is of some importance for the characterization of excitable tissues.

Cholinesterases, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) occur in two native (free and bound) states in mammalian tissues[1–4], but it is still not clear whether this is also true for other species. It has been reported that the enzyme is solubilized almost completely by means of saline media in some electrical fishes, for example (see discussion in [2]). Recently, however, it was found that even in the electroplax of *Electrophorus*, when Triton X-100 is added to the current aqueous or saline media, more enzyme is released than with these extraction solutions alone[5]. One may ask whether the fraction of the enzyme, which seems to be linked to the

[1] J. M. VARELA, Experientia *29*, 1437 (1973).
[2] J. M. VARELA in *Isozymes II-Physiological Function* (Ed. C. MARKERT; Academic Press, New York 1975), p. 315.
[3] C. H. S. MCINTOSH and D. T. PLUMMER, Biochem. J. *133*, 655 (1973).
[4] J. S. KANGIEL-KRAMSKA and S. NIEMIERKO, J. Neurochem. *24*, 1135 (1975).
[5] Y. DUDAI and I. SILMAN, J. Neurochem. *23*, 1177 (1974).

Table I. Distribution of AChE and BChE

	Free AChE	Bound AChE	Free BChE	Bound BChE
Brain (7)	1236 ± 333 [a]	20,197 ± 4,694	115 ± 33	460 ± 104
	324 ± 93 [b]	3,929 ± 883	35 ± 20	89 ± 19
Skeletal muscle (6)	168 ± 21	1,134 ± 113	56 ± 15	71 ± 28
	17 ± 4	526 ± 50	5 ± 1	33 ± 12
Heart (5)	1829 ± 106	2,392 ± 745	876 ± 104	911 ± 387
	199 ± 50	600 ± 36	97 ± 33	221 ± 42
Uterus (5)	938 ± 281	528 ± 189	568 ± 161	149 ± 70
	101 ± 22	508 ± 140	62 ± 13	136 ± 58
Small intestine (5)	4135 ± 742	4,332 ± 945	2319 ± 543	1676 ± 420
	573 ± 82	1,810 ± 249	317 ± 52	702 ± 148
Nerve (4)	555 ± 118	1,158 ± 386	139 ± 29	86 ± 33
	248 ± 41	1,920 ± 267	63 ± 17	119 ± 45

[a] AChE activities in nmoles ATC hydrolyzed per min per g wet wt. tissue. BChE activities in nmoles BTC hydrolyzed per min per g wet wt. tissue.
[b] AChE activities in nmoles ATC hydrolyzed per min per mg protein. BChE activities in nmoles BTC hydrolyzed per min per mg protein.
Mean ± SD, number of experiments in parentheses.

Table II. Ratios of bound to free AChE and BChE activities of Table I

	Bound/free AChE (R_1)	Bound/free BChE (R_2)	R_3 (R_1/R_2)
Brain	12.33±1.30	3.03±1.22	4.62±1.77
Skeletal muscle	33.90±6.86	6.10±1.68	5.86±1.83
Heart	3.14±0.70	2.40±0.76	1.34±0.21
Uterus	4.94±0.40	2.10±0.58	2.00±1.08
Small intestine	3.14±0.58	2.20±0.50	1.45±0.15
Nerve	7.78±0.79	2.45±0.60	3.43±1.40

membranes, is more or less tightly bound according to the species considered, a fact which could be related to different affinity or specificity in composition and/or organization of these biological barriers, and which could explain the interspecies differences in yields of solubilized enzyme referred to.

Another point which remains to be understood is the actual functional significance of the occurrence of both esterases in these two forms. More knowledge on the distribution of the two states of the enzyme in excitable tissues may perhaps help in such a task.

Materials and methods. Brain, skeletal muscle, heart, uterus, small intestine and peripheral nerves were taken from decapitated adult rats and homogenized with Ultra Turrax. Prior to homogenization, the heart was perfused with chilled 0.9% NaCl to remove the blood, and the brain was stripped of membranes and external vessels and washed carefully, as were also the other organs. 1:10 saline (0.9% NaCl) homogenates were prepared and centrifuged at 105,000 *g* for 60 min at 4 °C. After the first centrifugation with 0.9% NaCl to obtain the soluble (or free) enzyme, the pellets were suspended in the same volume of 0.9% NaCl, homogenized and centrifuged. The supernatant fluids were then discarded and the pellets resuspended in 1% Triton X-100 buffered to pH 7.0 in 0.1 *M Tris*-HCl, and centrifuged to release the membrane bound (or insoluble) enzyme. The same speed, time, temperature and volume of the eluent (see above) were employed in the 3 centrifugations.

AChE and BChE were determined using acetylthiocholine iodide (ATC) (Fluka) and butyrylthiocholine iodide (BTC) (Fluka) as substrates. 284C51 (Wellcome) and iso-OMPA (Koch Light) were the inhibitors employed. Both enzymes were assayed at 25 °C with the method of ELLMAN et al.[6], but pH 7.7 phosphate buffer was used instead of 8.0, since, as described previously[1], the former is the optimal pH for AChE of rat in our working conditions. Proteins were estimated according to LOWRY et al.[7] with bovine serum albumin as standard. Dithio-*bis*-2 nitrobenzoic acid and Triton X-100 were purchased from Fluka and Carl Roth (Karlsruhe), respectively.

Results. As can be seen in Table I, the relative amounts of soluble and insoluble enzymes (both AChE and BChE) differ characteristically in the tissues studied. The tissues which possess muscarinic innervation (small intestine, heart, uterus) have conspicuous activities of both soluble and insoluble BChE; the skeletal muscle, whose receptor is nicotinic in character, shows the lowest content; and the brain, which is endowed with both muscarinic and nicotinic cholinergic synapses, displays middle levels of enzymic activities.

As for AChE, it was found that, again, the tissues could be divided into 3 classes according to their enzymatic content. The muscarinic tissue showed the lowest ratios of bound to free AChE activities (3.14 ±0.58–4.94 ±0.40), the nicotinic has the highest (33.9 ±6.86), and the brain displays middle values (12.33 ±1.30). The soluble enzyme amounted to more than 30% of the total cholinesterasic activity in the muscarinic systems (Table II). It should be stressed that AChE in both the brain and skeletal muscle was found to be inhibited by 284C51, whereas in the muscarinic organs studied it was inhibited by iso-OMPA. This very interesting finding will be reported in detail elsewhere.

Discussion. The finding that the ratio of bound to free AChE differ so characteristically in tissues with nicotinic, muscarinic and mixed innervation raises the question as to whether such a ratio could be specifically related to the

[6] G. L. ELLMAN, K. D. COURTNEY, V. ANDRES jr. and R. M. FEATHERSTONE, Biochem. Pharmac. 7, 88 (1961).
[7] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).

synaptic events, and, therefore, somehow constitute one of the physiological parameters of bioelectrogenesis in excitable tissues.

If the two (cytosolic and membrane) stores of the enzyme are exchangeable, a view not too far-fetched (see discussion in [2]), a rate of the replenishment of them could exist in the excitable cells and be paced in a scheduled fashion. Should this prove to be true, one would like to know whether the ratio of bound to free AChE could reflect the dynamic of interconversion in excitable cells of the two native states of the enzyme.

One may further wonder whether during nerve activity such an interconversion takes place; the phenomenon could be strictly regulated and contribute to some features of the biopotentials, such as the differences noted in nicotinic as compared to muscarinic conduction and transmission. In the pace of such interconversion, the second cholinesterasic ratio (R_2) could be involved. Hypotheses concerning a possible role of pseudocholinesterases, viz. BChE, in nerve activity have been successively advanced and rejected, and it should be recognized that the function of this enzyme remains unknown. We shall try to test the above assumption.

The third ratio (R_3) was found to have different values ($p<0.001$) in the muscarinic tissues studied, but no significant differences between brain and muscle ratios were observed (Table II). Remembering that all these muscarinic tissues differ in their cholinergic innervation[8], parasympathetic of inhibitory (heart) and excitatory type (intestine) or sympathetic of excitatory type (uterus), such differences could, if anything, be expected, but the lack of significance between the values of brain and muscle is surprizing.

The observation that a significant (bound to free) AChE ratio also occurs in excitable tissues of the chick, and that it seems to be attained at a specific period of development (VARELA and MANDEL, unpublished) renders the foregoing views worthy of consideration.

Lastly, the enzymic values found in nerve reminds one of the debate about the participation of AChE in conduction besides its role in synaptic transmission[9].

[8] M. R. BENNETT, *Autonomic Neuromuscular Transmission* (Cambridge University Press, Cambridge 1972).

[9] D. NACHMANSOHN and B. NEUMANN, *Chemical and Molecular Basis of Nerve Activity* (Academic Press, New York 1975), p. 297.

Genotype Dependence of Monoamine Oxidase in Inbred Strains of Mice[1]

A. D. MACPIKE and H. MEIER

The Jackson Laboratory, Bar Harbor (Maine 04609, USA), 13 January 1976.

Summary. MAO activity was found to be influenced by the genotype or strain of mouse up to 20 days of age. The strain differences observed may derive from different rates of brain development. A number of neurological mutations comprizing three pathological classes had no effect on MAO.

Monoamine oxidase (E.C. 1.4. 3.4.; MAO) is a flavoprotein-linked deaminating enzyme of biogenic amines[2–4]; it is present in mitochondria of mammalian liver, kidneys, intestines and brain. In the brain it occurs in both glial cells and synaptosomes[5,6]. Apparently, multiple forms of MAO exist in the brain although there is still controversy as to the existence of true isozymes or binding of a single enzyme protein to different combinations of phospholipids[7–10]. The probable existence of multiple enzymic forms was suggested by studies, mostly in rats, on the role of MAO in brain maturation. In rats[11,12] and mice[13–15] MAO activity is clearly age-dependent; it increases to about 18 days of age but changes little thereafter in rats, but continues to fluctuate in mice until 6 weeks of age. Differences in MAO activity as related to brain regions and estrus cycle have been noted in the rat[16]; regional changes have also been reported in mice following fighting[17].

In our continuing studies of neurological mutations of mice we determined brain and liver MAO of a number of mutants belonging to 3 different clinico-pathological classes. These were mutants with a) cerebellar malformations and ataxia, b) cerebellar malformations, ataxia and epileptiform seizures, and c) tremors and fatal seizures without anatomical changes of the cerebellum.

Material and methods. The following mutations were studied, leaner (C57BL/6J – tg^{la}/tg^{la}) and reeler (C57BL/6J – *rl/rl*) belonging to class (a), weaver ($B6CBAF_1$ – *wv/wv*) of class (b), and jimpy ($B6CBAF_1$ – jp^{msd}/y) and wobbler-lethal (WLHR/J – *wl/wl*) of class (c). They were compared with their respective normal (+/–) littermate controls. Since several of these mutations are maintained on different genetic backgrounds we also analyzed the effect of the genotype or inbred strain on MAO. Due to the shortened life span of the mutants, they and their controls were generally 18 to 20 days old but we also studied the different inbred strains at 6 weeks of age.

[1] This work was supported in part by NIH grant No. NS06448 from the National Institute of Neurological Disease and Stroke.

[2] D. GLICK, *Analysis of Biogenic Amines and Their Related Enzymes* (John Wiley and Sons, Inc., New York 1971).

[3] V. Z. GORKIN, Adv. Pharmac. Chemother. *11*, 1 (1973).

[4] K. F. TIPTON, Br. med. Bull. *29*, 116 (1973).

[5] RODRIGUEZ DE LOREZ ARNAIZ and DE ROBERTIS, J. Neurochem. *19*, 503 (1962).

[6] S. D. SILBERSTEIN, H. M. SHEIN and K. R. BERV, Brain Res. *41*, 245 (1972).

[7] M. SANDLER and M. B. H. YOUDIM, Pharmac. Rev. *24*, 2549 (1971).

[8] J. H. C. SHIH and S. EIDUSON, J. Neurochem. *18*, 1221 (1971).

[9] J. H. C. SHIH and S. EIDUSON, J. Neurochem. *21*, 41 (1973).

[10] K. F. TIPTON, M. D. HOUSLAY and N. J. GARRETT, Nature New Biol. *246*, 213 (1973).

[11] V. T. NACHMIAS, J. Neurochem. *6*, 99 (1960).

[12] A. VACCARI, M. MAURA, M. MARCHI and F. CUGURRA, J. Neurochem. *19*, 2453 (1962).

[13] P. C. BAKER and K. M. HOFF, J. Neurochem. *19*, 2011 (1972).

[14] P. C. BAKER, K. M. HOFF and M. D. SMITH, Brain Res. *65*, 255 (1974).

[15] F. JOURDIKIAN, B. TABAKOFF and S. G. A. ALIVISATOS, Brain Res. *93*, 301 (1975).

[16] K. W. BOEHLKE and B. E. ELEFTHERIOU, Nature, Lond. *213*, 739 (1967).

[17] A. J. ZOLOVICK, R. PEARJE, K. W. BOEHLKE and B. E. ELEFTHERIOU, Science *154*, 649 (1966).