frequent removal of 7 to 15 percent of their blood volume. This experimental model closely mimicks conditions in homozygous Hb S disease in vivo, in which there is expanded erythropoiesis and a continuous loss of mature erythrocytes by hemolysis.
8. T. Papayannopoulou, E. Vichinsky, G. Stamatoyannopoulos, *Br. J. Haematol.* **44**, 535 (1980). Since a portion of the animals' blood volume was removed by phlebotomy, the Hb F response after treatment with Ara-C is best measured by the counts of F reticulocytes and the measurements of γ-globin biosynthesis.
9. Burst-forming units erythroid are the earliest cells committed to erythroid lineage. Their progeny are CFUe. Erythroid cluster-forming cells are the most mature progenitors in baboon erythropoiesis. These progenitors were assayed in plasma clot cultures [D. L. McLeod, M. M. Shreeve, A. A. Axelrad, *Blood* **44**, 517 (1974)] or in methylcellulose cultures [N. N. Iscove and F. Sieber, *Exp. Hematol. (Oak Ridge, Tenn.)* **3**, 32 (1975)].
10. N. N. Iscove and L. G. Guilbert, *In Vitro Aspects of Erythropoiesis* (Springer-Verlag, New York, 1978), pp. 3–7; F. C. Monette *et al.*, *Exp. Hematol. (Oak Ridge, Tenn.)* **8**, 484 (1980); A. A. Fauser and H. A. Messner, *Blood* **54**, 1197 (1979).
11. T. Papayannopoulou, M. Brice, G. Stamatoyannopoulos, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 2923 (1977); Th. Papayanopoulou *et al.*, *Science* **199**, 1349 (1978); G. Stamatoyannopoulos and T. Papayannopoulou, *Cellular and Molecular Regulation of Hemoglobin Switching* (Grune & Stratton, New York, 1979), pp. 323–349.
12. The striking increase in the number of late progenitor cells after treatment reflects the animal's attempt to rebuild the late erythroid progenitor cell pools.
13. Another possibility is that Ara-C and 5-azacytidine stimulate F-cell production by directly stimulating early progenitors to downstream differentiation and enhancing the erythroid maturation process. A similar mechanism has been proposed to explain induction of cell differentiation in leukemia patients treated with Ara-C [S. J. Wisch *et al.*, *N. Engl. J. Med.* **309**, 1599 (1983); J. F. Desforges, *ibid.*, p. 1637].
14. Hydroxyurea or methotrexate are considered to have low carcinogenic potential. We have treated baboons with hydroxyurea and found that this compound perturbs the erythroid progenitor cell pools and stimulates Hb F synthesis.
15. Supported by NIH grants HL-20899, GM 15253, HL-07093, and RR00166.

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Isolated Chromaffin Cells from Adrenal Medulla Contain Primarily Monoamine Oxidase B

Abstract. *Cultured chromaffin cells from bovine adrenal medulla were found to contain primarily the B form of monoamine oxidase. This monoamine oxidase B enzyme was somewhat distinct from B enzymes from other sources, in that noradrenaline was a much poorer substrate than serotonin. Nonetheless, studies with selective inhibitors of the A form (clorgyline) and the B form [(−)-deprenyl] confirmed that chromaffin cell monoamine oxidase was the B form. The observation that chromaffin cell monoamine oxidase has poor affinity for catecholamines is consistent with physiological needs that require the cell to synthesize and store large amounts of catecholamines.*

Chromaffin cells have the highest concentrations of catecholamines in the body, and an elaborate enzymatic system exists to achieve this end (*1*). Tyrosine hydroxylase in the cytoplasm converts tyrosine to dopa (*2*), which is rapidly converted to dopamine by nonspecific aromatic amino acid decarboxylase (*3*). Dopamine, still in the cytoplasm, is then taken up into the chromaffin granule by an adenosine triphosphate–dependent mechanism (*4*), where it is converted to noradrenaline by dopamine-β-hydroxylase (*5*). Subsequently, noradrenaline returns to the cytoplasm where it is methylated to adrenaline by phenylethanolamine *N*-methyl transferase (*6*) before final uptake into the chromaffin granule for storage.

In spite of the profound flux of catecholamines through the cytosolic portion of the cell, one can detect relatively little evidence of catecholamine deamination by the otherwise quite active adrenal medullary monoamine oxidase (MAO) (*7*). Indeed, we had noted in preliminary studies (*8*) that inclusion of a nonselective monoamine oxidase inhibitor such as tranylcypromine had no apparent influence on measured noradrenaline accumulation either in partially purified chromaffin granules or in isolated chromaffin cells. Pargyline, a nonspecific MAO inhibitor, apparently does not have any profound effects on cytosolic catecholamine levels in pheochromocytoma cells, since neither synthesis nor secretion of catecholamines was affected (*9*). Such a result could be accounted for either by the chromaffin cells having relatively low MAO activity or by the cells having a form of MAO that had a selectively poor affinity for catecholamines.

Monoamine oxidase is found in two forms, A and B, which differ in substrate specificity and inhibitor sensitivity (*10*). For example, MAO-A preferentially deaminates serotonin and noradrenaline (*10, 11*) and is selectively inhibited by clorgyline (*11*). By contrast, MAO-B has a high affinity for phenylethylamine and benzylamine (*10*) and is selectively inhibited by (−)-deprenyl (*12*). Tyramine and dopamine are substrates for both enzyme forms (*11*).

To examine the role of MAO directly, we prepared purified chromaffin cells by collagenase digestion using our modification (*13*) of the method of Livett *et al.* (*14*). Chromaffin cells were separated from contaminating fibroblasts by differential plating (*15*). These cells were frozen and thawed twice at −70°C, then centrifuged at 14,000*g* for 20 minutes at 4°C, and the pellet was washed once in 0.3*M* sucrose. The pellet was finally suspended in 0.1*M* Na-K-PO_4 buffer, *p*H 7.4, and used as a source of MAO enzyme. MAO activity was measured with various substrates by the method of Tipton and Youdim (*16*). As shown in Table 1, chromaffin cell MAO can deaminate monoamines with different efficiencies and relative values of maximum velocity (V_{max}). Nonhydroxylated amines such as phenylethylamine and kynuramine had significantly lower values of the Michaelis constant (K_m) than hydroxylated amines such as tyramine, dopamine, serotonin, and noradrenaline. The K_m of MAO for noradrenaline was 1100 μ*M*, nearly three times that of serotonin and 50 times that of phenylethylamine. Furthermore, the relative V_{max} for noradrenaline, as compared to tyramine, phenylethylamine, and dopamine, was significantly lower. These observations suggested that the MAO in the chromaffin cell was somewhat unusual and was perhaps the B type.

We examined the inhibitor specificity of chromaffin cell MAO with selective inhibitors of MAO-A and MAO-B (*11, 12*). Clorgyline, the selective inhibitor of MAO-A, was a poor inactivator of chromaffin cell MAO when tyramine, phenylethylamine, and serotonin were used as substrates (Fig. 1A). The median inhibitory concentration (IC_{50}) ranged from 10^{-7} to 5×10^{-6}*M*. In addition, with all the three substrates, clorgyline exhibited a simple sigmoid curve, indicating one type of enzyme (*11*).

(−)-Deprenyl also showed a single inhibitory curve, but in contrast to clorgyline, it was a much more potent inhibitor of tyramine, phenylethylamine, and serotonin deamination. The IC_{50} values for (−)-deprenyl ranged from 5×10^{-9}*M* to 5×10^{-8}*M*. These results are similar to the inhibitory potencies of (−)-deprenyl for MAO-B in other tissues such as liver (*17*), human platelet (*18*), brain (*19*), and lung (*20*).

These data thus indicate that the MAO in the chromaffin cell is primarily in the B form of the enzyme. This conclusion is primarily substantiated by the observation that the tyramine inhibition curves with clorgyline and (−)-deprenyl were of simple single form and that (−)-deprenyl was a much better inhibitor of chromaffin cell MAO than was clorgyline. In addition, the relative V_{max} (Table 1) was higher for the type B substrate phenylethylamine than for the MAO-A substrates serotonin and noradrenaline. Ear-

lier findings (*21, 22*) that adrenal gland tissue had equal parts of MAO-A and -B resulted from the contribution of MAO-A from nonchromaffin cells. These cells have been isolated as a pure line culture and identified as capillary endothelial cells, which contain only MAO-A activity (*23*).

The primary presence of MAO-B in chromaffin cells is consistent with our understanding of the physiology of these cells. As indicated above, chromaffin cells synthesize catecholamines by means of various enzymes in the cytosol. Most investigators had presumed that MAO was probably not particularly important in the process of noradrenaline metabolism in chromaffin cells, since little metabolism occurred (*9*). A better explanation now is that the MAO present in the chromaffin cell is of type B, and, for this reason, little deamination of noradrenaline occurs.

An analogous situation exists in the human platelet, which can store the highest concentration of serotonin in the body without that compound being deaminated (*24*). In this case, the MAO in the platelet is entirely type B (*18, 25*). However, there is a curious and telling difference between the B-type MAO found in chromaffin cells and that found in platelets. In the platelet, Garrick and Murphy (*26*) showed that the MAO-B has a K_m for noradrenaline of 380 μM, but a K_m for serotonin of 1000 μM. This is in contrast to what we now report for the chromaffin cell, in which the relative K_m values for serotonin and noradrenaline are reversed. Thus, although both chromaffin cells and platelets contain MAO-B, the kinetic and substrate specificity studies indicate that the two cells may contain different variants of the type B enzyme. The physiological importance of the existence of these variants has a basis in the fact that the platelet must preserve serotonin, whereas chromaffin cells must conserve noradrenaline. We previously reported a similar high K_m for noradrenaline and adrenaline in beef adrenal medulla homogenates without being able to devise a molecular explanation (*7*).

Table 1. Michaelis constants and relative V_{max} of MAO in frozen and thawed chromaffin cells for various monoamines. Maximum velocity is expressed relative to the V_{max} of tyramine. The K_m and V_{max} values were calculated from the mean of four separate experiments.

Substrate	K_m (μM)	V_{max}
Tyramine	280	100
Serotonin	385	35
Phenylethylamine	25	60
Noradrenaline	1100	20
Dopamine	400	65

Our present results are in sharp contrast to the situation in the adrenergic and serotonergic nerve terminals and in a number of cell types of neural origin grown in tissue culture (*27, 28*). In these cases the major form of MAO is type A, and the selective inhibition of the type A enzyme by clorgyline results in an increase in noradrenaline and serotonin and a decrease in their metabolites (*28, 29*). To date, however, no tissue of neural origin in culture having predominantly type B activity has been described, except the present findings. Thus it would appear that in nerve endings, noradrenaline and serotonin deamination by MAO-A is needed for regulation of neurotransmission (*22*). By contrast, in the chromaffin cell, the influence of MAO activity on catecholamine metabolism is limited, thus enhancing the capability of significant catecholamine synthesis and storage for emergency peripheral use. This hypothesis is further supported by the observation that tyramine and phenylethylamine are relatively good substrates for the chromaffin cell MAO-B. Indeed, this physiological situation is required, since both substrates are indirectly acting sympathomimetic amines, which can cause release of catecholamines from their binding sites (*22*) with profound systemic consequences if they acted unhindered in the adrenal medulla.

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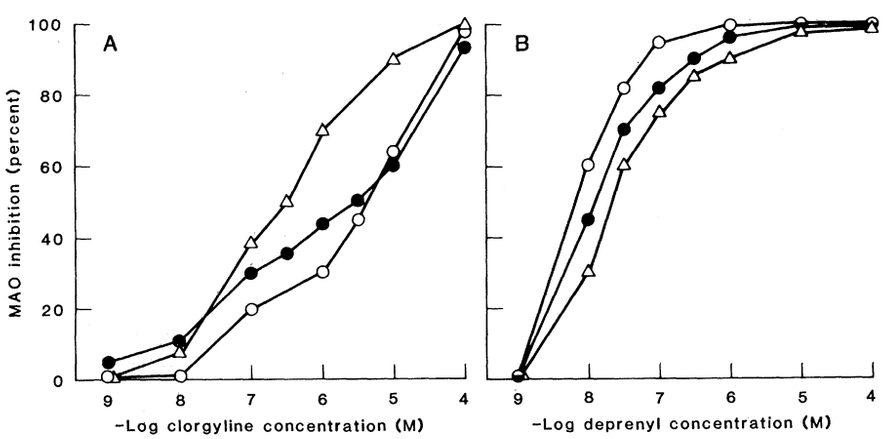

Fig. 1. (A) Clorgyline and (B) (−)-deprenyl inhibition of MAO activity in frozen and thawed isolated chromaffin cells using (△) $10^{-3}M$ serotonin, (●) $10^{-3}M$ tyramine, and (○) $2 \times 10^{-5}M$ β-phenylethylamine as substrates (*16*). The enzyme preparations were incubated with the inhibitors for 20 minutes before the substrates were added. Curves represent the mean of four separate experiments for each substrate. Standard errors of the mean are excluded for the sake of clarity and are no more than 10 to 15 percent.

References and Notes

1. N. Kirshner, in *Handbook of Physiology*, section 7, *Endocrinology*, H. Blaschko, Ed. (American Physiological Society, Bethesda, Md., 1974), vol. 6, p. 341.
2. T. Nagatsu, M. Levitt, S. Udenfriend, *J. Biol. Chem.* **239**, 2910 (1964).
3. W. Lovenberg, H. Weissbach, S. Udenfriend, *ibid.* **237**, 89 (1962).
4. N. Kirshner, *ibid.*, p. 2311.
5. ———, *ibid.* **226**, 821 (1957).
6. N. Weiner, in *Handbook of Physiology*, section 7, *Endocrinology*, H. Blaschko, Ed. (American Physiological Society, Bethesda, Md., 1974), vol. 6, p. 357; A. Ungar and J. H. Phillips, *Physiol. Rev.* **63**, 787 (1983); A. Ramu, C. J. Pazoles, C. E. Creutz, H. B. Pollard, *J. Biol. Chem.* **256**, 1229 (1981).
7. K. F. Tipton, M. B. H. Youdim, I. P. C. Spires, *Biochem. Pharmacol.* **21**, 2197 (1972); M. B. H. Youdim and M. Holzbauer, in *Monoamine Oxidase and Its Inhibition*, G. E. W. Wolstenholme and J. Knight, Eds. (Elsevier, Amsterdam, 1976), p. 105; O. Zinder, P. G. Hoffman, W. M. Bonner, H. B. Pollard, *Cell Tissue Res.* **188**, 153 (1978).
8. M. B. H. Youdim, D. K. Banerjee, H. B. Pollard, unpublished data.
9. M. Chalfie and R. L. Perlman, *J. Pharmacol. Exp. Ther.* **200**, 588 (1977).
10. M. B. H. Youdim and J. P. Finberg, in *Psychopharmacology*, D. G. Grahame-Smith, Ed. (Excerpt Medica, Amsterdam, 1982), p. 38.
11. J. P. Johnston, *Biochem. Pharmacol.* **17**, 1285 (1968).
12. J. Knoll and K. Magyar, *Adv. Biochem. Psychopharmacol.* **5**, 393 (1972).
13. H. B. Pollard, C. J. Pazoles, C. E. Creutz, J. H. Scott, O. Zinder, A. Hotchkiss, *J. Biol. Chem.*, in press.
14. B. G. Livett, D. M. Dean, L. G. Whelan, S. Udenfriend, J. Rosier, *Nature (London)* **289**, 317 (1981).
15. M. Levine and H. B. Pollard, *FEBS Lett.* **158**, 134 (1983).
16. K. F. Tipton and M. B. H. Youdim, in *Monoamine Oxidase and Its Inhibition*, G. E. W. Wolstenholme and J. Knight, Eds. (Elsevier, Amsterdam, 1976), p. 393.
17. A. Kalir, A. Sabbagh, M. B. H. Youdim, *Br. J. Pharmacol.* **72**, 55 (1981).
18. D. L. Murphy and C. H. Donnelly, *Adv. Biochem. Psychopharmacol.* **12**, 73 (1974).
19. K. F. Tipton, M. D. Houslay, N. J. Garratt, *Nature (London)* **246**, 213 (1973).
20. Y. Bakhle and M. B. H. Youdim, *Br. J. Pharmacol.* **65**, 147 (1979).
21. K. F. Tipton, M. D. Houslay, T. J. Mantle, in *Monoamine Oxidase and Its Inhibition*, G. E. W. Wolstenholme and J. Knight, Eds. (Elsevier, Amsterdam, 1976), p. 5.
22. U. Trendelenburg, P. R. Draskoczy, K. H. Graefe, *Adv. Biochem. Psychopharmacol.* **5**, 371 (1972); A. R. Green and M. B. H. Youdim, *Br. J. Pharmacol.* **55**, 415 (1975); M. B. H. Youdim, J. P. M. Finberg, K. F. Tipton, in *Handbook of Experimental Pharmacology, Cat-*

echolamines II, N. Weiner and U. Trendelenburg, Eds. (Springer-Verlag, Berlin, in press); I. W. Chubb, W. P. DePotter, A. F. DeSchaepdryer, *Naunyn-Schmiedebergs Arch. Pharmacol.* **274**, 281 (1972); J. P. M. Finberg and M. Tenne, *Br. J. Pharmacol.* **77**, 13 (1982); L. Simpson, *J. Pharmacol. Exp. Ther.* **205**, 392 (1978); B. Blackwell, *Lancet* **1963-II**, 849 (1963); C. Braestrup, H. Anderson, A. Randrup, *Eur. J. Pharmacol.* **34**, 181 (1975); G. B. Baker, M. Raiteri, A. Bertollini, R. Del Carmine, P. E. Keane, I. L. Martin, *J. Pharm. Pharmacol.* **38**, 456 (1976); S. R. Philips, in *Non-catecholic Phenylethylamines*, A. D. Mosnaim and M. E. Wolf, Eds. (Dekker, New York, 1978), p. 113. R. Ashkenazi, in *Enzymes and Neurotransmitters in Mental Disease*, E. Usdin, T. L. Sourkes, M. B. H. Youdim, Eds. (Wiley, Chichester, 1980), p. 445; L. F. Tseng, M. K. Menon, H. H. Loh, *J. Pharmacol. Exp. Ther.* **197**, 263 (1976).

23. D. K. Banerjee, M. B. H. Youdim, H. B. Pollard, abstract of paper presented at the 9th International Congress of Pharmacology, London (1984); M. B. H. Youdim, D. K. Banerjee, H. B. Pollard, E. Heldman, abstract of paper presented at the international symposium on Molecular Biology of Peripheral Catecholamine-Storing Tissues, Colmar, France (1984).
24. A. Pletscher, in *Essays in Neurochemistry and Neuropharmacology*, M. B. H. Youdim, W. Lovenberg, D. F. Sharman, J. Lagnado, Eds. (Wiley, Chichester, 1978), vol. 3, p. 49.
25. M. B. H. Youdim, H. F. Woods, B. Mitchell, D. G. Grahame-Smith, S. Callender, *Clin. Sci. Mol. Med.* **48**, 289 (1975).
26. N. A. Garrick and D. L. Murphy, *Biochem. Pharmacol.* **24**, 4061 (1982).
27. C. Goridis and N. H. Neff, *Neuropharmacology* **10**, 557 (1971).
28. R. Ashkenazi, J. P. M. Finberg, M. B. H. Youdim, *Br. J. Pharmacol.* **79**, 765 (1983); M. B. H. Youdim and J. P. M. Finberg, in *Handbook of Neurochemistry*, A. Lajath, Ed. (Plenum, New York, 1983), vol. 4, p. 293.
29. M. B. H. Youdim, *Br. J. Pharmacol.* **79**, 477 (1983).

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Reduction of a Larval Herring Population by Jellyfish Predator

Abstract. *The scyphomedusa* Aurelia aurita *consumes large amounts of yolk-sac herring larvae in Kiel Fjord. The decline of the larval herring population in late spring coincides with a major population growth of the jellyfish. The size of the larval herring population seems to be more significantly affected by the size of the predator stock than by the size of the parental herring stock.*

The mass occurrence of jellyfish is a nuisance in various coastal areas, causing clogged fishing nets and power plant intake systems and injury to bathers and fishermen. *Aurelia aurita* causes such problems in Japanese waters and in the Baltic Sea, *Cyanea capillata* in the Belt Sea, *Chrysaora plocamia* in Peruvian waters, and *Pelagia noctiluca* in the Mediterranean Sea (*1, 2*).

The mass occurrence of ctenophores and scyphomedusae has been found to reduce local stocks of copepods (*3–6*). These organisms therefore are severe competitors for fish larvae and planktivorous adult species. Five species of ctenophores and 34 species of medusae have been identified that feed on fish larvae in the field or under laboratory conditions (*7*).

In Kiel Fjord, young *Aurelia aurita* prey heavily on herring larvae but nearly exclusively on herring yolk-sac stages (5 to 7 mm long). During three periods of investigation in May 1979 and May 1980, the stomach contents of 5800 young medusae (6 to 50 mm in bell diameter) were examined. On the average, 0.4, 0.2, and 4.4 larvae were found per medusa. The number of larvae per *Aurelia* increased with increasing medusa size: the smallest *Aurelia* that had caught a herring larva measured 6 mm in bell diameter; as many as ten larvae were found in a medusa 12 mm in bell diameter; and up to 68 larvae were discovered in a medusa 42 mm in bell diameter (*2*).

Kiel Fjord is one of the spawning grounds for herring (*Clupea harengus*) in the Western Baltic Sea. Large shoals invade the fjord in March and April and deposit their eggs on gravel beds, mussel banks, and algae along the western shore of the fjord. The first hatch of yolk-sac larvae (5 mm in length) can be observed between mid-April and mid-May, depending on water temperature. Upon hatching, herring larvae are exposed to swarms of jellyfish because Kiel Fjord is also a major area of *Aurelia aurita* production. Most of the ephyra-stage jellyfish are released from the polyps in April, and an increase in their biomass takes place in May and June (*8*).

A 4-year survey was carried out at 26 stations in the fjord to determine the impact of jellyfish predation on the development of the local larval herring population. Double vertical hauls were taken weekly with a CalCOFi plankton net 1 m in diameter and 0.5 mm in mesh size. Herring larvae were counted, and the abundance of jellyfish was expressed as volume (milliliters) per 100 m^3. The recorded fluctuations in both populations included only those surveys in which more than one herring larva per 100 m^3 was caught (Fig. 1).

The impact of jellyfish predation should be demonstrable in three contexts. Relatively low numbers of herring larvae should be found (i) at stations with a relatively large *Aurelia* biomass, (ii) during days when the *Aurelia* biomass averaged from 26 stations was relatively large, and (iii) during years when the number of jellyfish averaged from all surveys was relatively large. Negative rank correlations were calculated on comparing the abundances of both species at stations where single surveys were conducted. Confidence levels

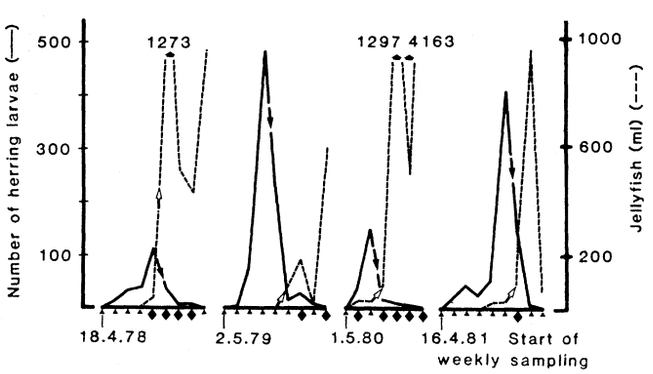

Fig. 1 (left). Average abundance of yolk-sac herring larvae (number per 100 m^3) and of *Aurelia aurita* (milliliters per 100 m^3) from 26 stations in Kiel Fjord sampled at weekly intervals (▲). The start of a major population growth of the jellyfish (↑) coincides with the rapid decline (↓) of the larval herring population. Negative correlations between the sizes of both populations were found during 12 surveys (◆). Fig. 2 (right). Drawings symbolize the relative abundances (100 = value in the year of the maximum abundance) of *Aurelia aurita*, yolk-sac herring larvae, and adult spawning herring during 4 years in Kiel Fjord. *Aurelia* and herring larvae: average abundance during eight weekly surveys after the first emergence of herring larvae; adult herring: catches of spawners from Kiel Fjord in April of the year indicated.