able, is to use several sequence plots as in Fig. 4, each for a selected discrete value of that parameter. The results of models with high dimensionality are inherently difficult to display by any technique, however, and careful thought and innovation are crucial to success.

General conclusions. It seems possible to draw several general conclusions from this work. First, scientific computer models often benefit from utilizing displays of results that present the full dimensionality or the maximum presentable dimensionality of the calculation. Second, routine plotting of results should be made as automatic as possible. If these two principles are followed, the analyst will often discover features of the results that would otherwise have gone unnoticed. Finally, color is very effective as a medium of information transfer and should be used freely in the displays, provided it is used with sufficient discretion that the viewer is not overloaded with information. Color graphics for the display of scientific model results, it would seem, has come of age.

References and Notes

1. W. H. Duewer, D. J. Wuebbles, H. W. Elsaesser, J. S. Chang. *J. Geophys. Res.* **82**, 935 (1977); J. A. Logan, M. J. Prather, S. C. Wofsy, M. B. McElroy, *Philos. Trans. R. Soc. London Ser. A* **290**, 187 (1978).
2. T. E. Graedel and L. A. Farrow, *J. Geophys. Res.* **82**, 4943 (1977).
3. M. Bishop, J. Roth, H. L. Frisch, *J. Phys. Chem.* **81**, 2500 (1977).
4. J. S. Turner, *ibid.*, p. 2379
5. R. G. Pearson, *ibid.*, p. 2323; D. F. Strobel, *Geophys. Res. Lett.* **4**, 424 (1977).
6. J. W. Bottenheim, S. E. Braslavsky, O. P. Strausz, *Environ. Sci. Technol.* **11**, 801 (1977).
7. H. Rishbeth, *Space Sci. Rev.* **8**, 543 (1968); M. B. McElroy, S. C. Wofsy, J. E. Penner, J. C. McConnell, *J. Atmos. Sci.* **31**, 287 (1974).
8. D. R. Bates, *Contemp. Phys.* **11**, 105 (1970); M. Nicolet, *Planet. Space Sci.* **20**, 1671 (1972); T. M. Donahue, R. J. Cicerone, S. C. Liu, W. L. Chameides, *Geophys. Res. Lett.* **3**, 105 (1976).
9. J. A. Miller and R. J. Kee, *J. Phys. Chem.* **81**, 2534 (1977).
10. D. M. Hunten, *Geophys. Res. Lett.* **2**, 26 (1975); S. C. Wofsy, *Annu. Rev. Earth Planet. Sci.* **4**, 441 (1976).
11. M. B. McElroy and T. Y. Kong, *Geophys. Res. Lett.* **3**, 569 (1976).
12. I. S. A. Isaksen and O. Hov, in *Symposium on Boundary Layer Physics* (World Meteorological Organization, Geneva, 1978), p. 211.
13. P. J. Crutzen, *Ambio* **3**, 201 (1974); K. A. Goettel and J. S. Lewis, *J. Atmos. Sci.* **31**, 828 (1974); T. A. Hecht, J. A. Seinfeld, M. C. Dodge, *Environ. Sci. Technol.* **8**, 327 (1974); W. L. Chameides and J. C. G. Walker, *Science* **190**, 1294 (1975); J. E. Frederick, *J. Geophys. Res.* **81**, 3179 (1976); M. B. McElroy and T. Y. Kong, *Geophys. Res. Lett.* **3**, 567 (1976); D. L. Allara and D. Edelson, *J. Phys. Chem.* **81**, 2443 (1977); D. T. Gillespie, *ibid.*, p. 2340; E. Hesstvedt, O. Hov, I. S. A. Isaksen, *Geophys. Norv.* **31**, 27 (1977); D. H. Ehhalt, J. S. Chang, D. M. Butler, *J. Geophys. Res.* **84**, 7889 (1979); R. P. Turco, R. C. Whitten, O. B. Toon, J. B. Pollack, P. Hamill, *Nature (London)* **283**, 283 (1980).
14. J. T. Hwang, E. P. Dougherty, S. Rabitz, H. Rabitz, *J. Chem. Phys.* **69**, 5180 (1978).
15. M. Oppenheimer, A. Dalgarno, H. C. Brinton, *J. Geophys. Res.* **81**, 3762 (1976); H. S. Johnston and H. Nelson, *ibid.* **82**, 2593 (1977).
16. D. B. Olson and W. C. Gardiner, Jr., *J. Phys. Chem.* **81**, 2514 (1977).
17. J. A. Schiavone and T. E. Graedel, *Atmos. Environ.* **15**, 163 (1981); T. E. Graedel and J. A. Schiavone, *ibid.*, p. 353.
18. D. Cunnold, F. Alyea, N. Phillips, R. Prinn, *J. Atmos. Sci.* **32**, 170 (1975); E. S. Oran. P. S. Julienne, D. F. Strobel, *J. Geophys. Res.* **80**, 3068 (1975); J. D. Mahlman, H. Levy II, W. J. Moxim, *J. Atmos. Sci.* **37**, 655 (1980).
19. L. N. Myrabo, P. Schleifer, K. R. Wilson, *Calif. Air Environ.* **4** (No. 3), 3 (1974); T. E. Graedel, M. A. Frerking, W. D. Langer, *Astrophys. J.*, in press.
20. G. H. Gilmer, *Science* **208**, 355 (1980).
21. C. K. Johnson, *ORTEP: A Fortran Thermal-Ellipsoid Plot Program for Crystal Structure Illustrations* (ORNL-3794, Oak Ridge National Laboratory, Oak Ridge, Tenn., 1965).
22. R. Langridge, T. E. Ferrin, I. D. Kuntz, M. L. Connolly, *Science* **211**, 661 (1981).
23. T. A. Weber and E. Helfand, *J. Chem. Phys.* **71**, 4760 (1979).
24. D. T. Gillespie, *J. Phys. Chem.* **81**, 2340 (1977).
25. T. E. Graedel, L. A. Farrow, T. A. Weber, *Atmos. Environ.* **10**, 1095 (1976); J. R. Creighton, *J. Phys. Chem.* **81**, 2520 (1977); H. E. Dove *et al.*, *ibid.*, p. 2564.
26. D. Edelson and J. M. Chambers, paper COMP-4, presented at the 169th National Meeting, American Chemical Society, Philadelphia, April 1975.
27. S. E. Fienberg, *Am. Stat.* **33**, 165 (1979).
28. H. Wainer and C. M. Francolini, *ibid.* **34**, 81 (1980).
29. R. McGill and T. E. Graedel, in preparation.
30. A variety of color graphics hardware is commercially available, or can be fashioned from appropriate components. The color displays in this article were produced on a Bell Laboratories PRISM printer—a computer-driven Xerox 6500 color copier. Black, white, and the six additive and subtractive primary colors are available. The resolution is 128 pixels per inch; the speed is about three plots per minute (depending on complexity). The hardware cost is about $50,000. Similar displays can be produced with other equipment and techniques. The least expensive and slowest option is a multipen flatbed plotter. More utilitarian are the raster color terminals with photographic output recorders that are in common use [for instance, see Langridge *et al.* (*22*)]. Other alternatives include color ink jet plotters and high-resolution color line printers operating under computer control.
31. J. Bertin, *Semiologie Graphique* (Mouton, Paris, ed. 2, 1973), p. 325.
32. W. S. Cleveland and R. McGill, in *Proceedings, International Conference for Users of Computer Mapping and Management Graphics* (Harvard Laboratory for Computer Graphics, Cambridge, Mass., 1980).

33. We thank C. J. Harris, P. E. Rosenfeld, and V. B. Turner for assistance in the development of the chromatic displays and D. Edelson and G. H. Gilmer for helpful discussions.

Stages of Neurotransmitter Development in Autonomic Neurons

Ira B. Black

The evolution of multicellular forms from unicellular progenitors represented a new strategy for selective advantage, and foreshadowed cellular diversification and specialization within a single organism. Cellular specialization, in turn, required the evolution of mechanisms to govern intracellular differentiation, and necessitated intercellular synchronization during development and maturity. The processes of differentiation and cellular synchronization reach a remarkable level of complexity in the nervous system: different neurons develop the capacity to synthesize and use different specific chemical signals, the neurotransmitters. Moreover, billions of individual neurons form thousands of highly specific interconnections, allowing the different transmitter signals to function in physiologically appropriate circuits. A central problem in neurobiology concerns the mechanisms generating such specificity.

One approach to this problem has involved study of the relatively simple, peripheral autonomic nervous system. Autonomic neurons use a number of well-characterized transmitters, including norepinephrine and acetylcholine. The availability of sensitive techniques to measure noradrenergic and cholinergic gene products, such as their biosynthetic enzymes, has allowed examination of individual transmitter phenotypic characters. Analysis of individual phenotypic characters is critical for elucidation of mechanisms governing transmitter ontogeny, since expression of any given transmitter depends on development of multiple individual characters (such as the foregoing enzymes).

Emerging evidence suggests that autonomic neurons pass through a sequence of distinct stages during neurotransmitter differentiation. The stages may be distinguished by the specific transmitter gene products expressed, and by the mechanisms influencing phenotypic expression of these transmitter charac-

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ters (Fig. 1). This article represents an effort to delineate these developmental stages in the form of testable hypotheses. It is proposed that embryonic autonomic neuroblasts initially express transmitter characters in a highly mutable fashion and that this expression becomes progressively more restricted with age. It is further proposed, however, that mutability persists into adulthood, and perhaps for the life of the neuron. The apparent restriction of mutability during maturity is largely due to ongoing stabilizing influences of extraneuronal factors, including afferent and efferent connections, association with nonneuronal cells, and exposure to humoral agents, which obscure intrinsic mutability of the mature neuron. Discrete developmental events, and stages, are superimposed on the gradual process of restriction of mutability. Transmitter development, therefore, may consist of the successive stages of (i) early expression, (ii) definitive expression, (iii) modulation, and (iv) regulation, extending from embryonic life through maturity, and paralleling the progressive restriction of phenotypic mutability (Fig. 1). During the embryonic period of maximal mutability, the primitive neuron may qualitatively change the transmitter phenotypic characters that are expressed, depending on the sequence of extracellular signals. During this embryonic stage of early expression, the transmitter characters may be entirely different from those expressed by the mature neuron. Subsequently, the stage (process) of definitive expression consists of appropriate "choice" of the adult transmitter phenotype. The process of modulation results in expression of transmitter characters in "correct" quantities. The processes of definitive expression and modulation culminate in stabilization of the adult phenotypic characters, which are then subject to ongoing biochemical regulation during maturity. Although the discussion in this article focuses on transmitter development exclusively, the molecular mechanisms involved may be relevant to neuronal and cellular ontogenetic processes in general.

Definition of these developmental stages has been made possible by biochemical and immunocytochemical methodologic advances that permit demonstration of transmitter enzyme gene products. Tyrosine hydroxylase, the rate-limiting enzyme in catecholamine biosynthesis (*1*), dopamine β-hydroxylase, which converts dopamine to norepinephrine (*2*), and phenylethanolamine *N*-methyltransferase (PNMT), which converts norepinephrine to epinephrine (*3*), have been used to monitor noradrenergic and adrenergic expression. Choline acetyltransferase, which catalyzes the synthesis of acetylcholine (*4*), has been used as an index of cholinergic expression. In addition to these transmitter enzyme characters, it has been possible to define expression of more complex traits, such as the specific, high-affinity neuronal uptake mechanism for norepinephrine. Finally, the presence of catecholamine transmitters themselves has been demonstrated by histofluorescence techniques.

Autonomic Embryogenesis

The neural crest is a transient embryonic structure which is the progenitor of the autonomic system. In addition, the crest gives rise to chromaffin cells, sensory neurons, nonneuronal cells of the peripheral nervous system, calcitonin-producing cells, and mesenchymal derivatives of the cephalic region (*5*).

Early Expression

Various neuronal derivatives of the crest express noradrenergic phenotypic characters early in embryonic development, regardless of the transmitter phenotype that is expressed during maturity. Noradrenergic traits are expressed (i) in sympathetic ganglion primordia that remain noradrenergic in vivo (*10*); (ii) in caudal thoracic populations that are destined to become adrenergic adrenomedullary elements (*9, 11*); (iii) in primitive gut populations that lose most noradrenergic characters after several days (*10, 12*) (Fig. 1); and (iv) in rat eccrine sweat gland fibers that appear to become cholinergic (*13*).

Although recent work has suggested that the embryonic microenvironment influences initial expression, precise interactions between environment and neuron remain undefined. In avian embryos, the initial appearance of catecholamine histofluorescence in neurons is influenced by interactions with somitic

Summary. The ontogeny of neurotransmitters in autonomic neurons proceeds through the successive stages of early expression, definitive expression, modulation, and regulation, extending from embryonic life to maturity. Although different extracellular signals influence development at different stages, a number of signals that influence development continue to govern transmitter function during maturity. The sequential ontogenetic stages parallel the progressive restriction of mutability of phenotypic expression; however, some degree of neuronal mutability appears to persist through maturity.

The crest, lying on either side of the neural plate, assumes a dorsal midline position as the plate closes to form the neural tube (*6*). From this location, crest cells migrate dorsolaterally within the ectoderm and ventrolaterally within the mesoderm (*5*). The ventrolaterally migrating cells assume positions lateral to the aorta to form sympathetic ganglion primordia, and these cells also populate the gut to form precursors of at least some of the enteric ganglia (*7*). At the caudal thoracic level, cells subsequently migrate to form adrenomedullary chromaffin cells (*8, 9*). Analysis of these three populations—the ganglion primordia, adrenomedullary chromaffin cells, and cells of the gut—makes it possible to identify the foregoing stages, and address a number of critical questions. When are transmitter phenotypic characters initially expressed? What factors govern early expression? What is the relationship of an early expression to subsequent stages? Is definitive expression irreversible? How is regulation in the adult related to developmental mechanisms?

mesenchyme, and therefore may require ventral crest migration (*14*). Moreover, ablation of ventral neural tube reduces the quantity of nervous tissue formed, suggesting that neural tube, somitic mesenchyme, and neurons undergo critical interactions (*14*). These observations are supported by in vitro studies showing that crest must be contiguous with somite for catecholaminergic expression, and that ventral neural tube may act across a Millipore filter to induce appropriate changes in somite (*15*). In contrast, more recent studies (*16*) indicate that crest neuroblasts in culture can express noradrenergic traits in the absence of the normal embryonic microenvironment. Different cells derived from a single clone in the same cell culture initially express catecholamine fluorescence or melanin. Consequently, at least certain noradrenergic traits can be expressed in artificial environments of undefined composition. Thus, while the crest neuroblast is neither wholly predetermined regarding initial transmitter expression, nor a true tabula rasa, simply reflecting environmental signals, mechanisms reg-

ulating early expression remain to be defined.

In the foregoing in vivo studies, it is unclear whether environmental signals directly influence neuronal phenotypic fate, or select for predetermined neuronal populations. The question of mutability at the cellular level versus selection of populations is difficult to approach through in vivo studies alone, and constitutes one outstanding issue in developmental neurobiology.

Despite these ambiguities, the phenomenon of early expression of some neuronal traits can be defined in greater detail. It appears that early expression (i) is reversible and does not assure persistence (definitive expression) of the phenotype expressed initially; (ii) does not require arrival at the definitive site in the body; (iii) antedates completion of afferent and efferent innervation and therefore is largely independent of these influences; (iv) involves the simultaneous expression of a number of transmitter phenotypic characters; (v) does not preclude subsequent DNA synthesis; and (vi) does not preclude subsequent migration.

The fact that cells of diverse transmitter fates—in ganglia, adrenal, and gut—express noradrenergic characters early in development suggests that early expression may be different from the subsequent process of definitive expression. For example, a population of cells in the embryonic gut transiently expresses a number of noradrenergic phenotypic characters. At 11.5 days of gestation in the rat (27 to 30 somites) these cells exhibit tyrosine hydroxylase, dopamine β-hydroxylase, and catecholamines (*10*). Over the ensuing 24 hours the number of cells increases, but by 13.5 days, virtually no endogenous noradrenergic characters are present in the gut. However, these cells apparently do not die, since the population can take up exogenous norepinephrine, thereby permitting identification (*12*). Consequently, the cells appear to persist, selectively retaining the high-affinity uptake system for norepinephrine (*12*). The ultimate transmitter fate of this population is not defined, since it is not yet clear whether these cells acquire new transmitter characters. In work with another developing population,the sympathetic innervation of rat eccrine sweat glands, accumulating evidence suggests that fibers do convert from the noradrenergic to cholinergic phenotypes (*13*). These observations suggest that early expression does not result in irrevocable commitment to that phenotype. The foregoing in vivo studies are complemented by extensive in vitro experiments indicating that noradrenergic sympathetic neurons may acquire cholinergic characteristics under appropriate conditions (*17–19*). Additional work in vivo and in culture is now required to determine whether early expression represents a stage common to transmitter differentiation of other neuronal populations, and whether analogous stages characterize differentiation of nonneuronal cells.

It is unlikely that early expression is evoked solely by unique properties of the immediate embryonic environment in which it occurs, since the noradrenergic phenotype is expressed in paraaortic ganglia where it will persist, as well as in cells of the gut, where it rapidly disappears (*10, 12*). It is possible, however, that noradrenergic expression is triggered by prior passage through the microenvironment of the dorsal embryo during early crest cell migration. Nevertheless, environmental cues may not be required for initial expression, but may simply amplify or influence preprogrammed, intracellular events. In addition, of course, it is probable that environmental cues change throughout development. Finally, it is quite clear that early expression does not require arrival at the definitive site, since noradrenergic characters are expressed in primordial ganglia by neuroblasts that later migrate to the adrenal medulla or secondary ganglia (*9, 14*).

Although this discussion has focused on early expression of noradrenergic characters, recent studies suggest that some crest populations may actually exhibit cholinergic properties even earlier in development [for review, see (*20*)]. While a great deal of additional work is required to delineate the sequence of appearance of different transmitter characters, the appearance of cholinergic or noradrenergic traits in populations of diverse transmitter fates supports the contention that early expression represents a discrete developmental stage.

What is the manner in which cells initially express noradrenergic phenotypic characters? Each phenotypic character could be expressed individually at a characteristic time, or, alternatively, groups of characters could be expressed simultaneously. Different patterns may imply different underlying mechanisms (*21*). In fact, available evidence suggests that a constellation of phenotypic characters is expressed simultaneously in the populations under discussion. In these

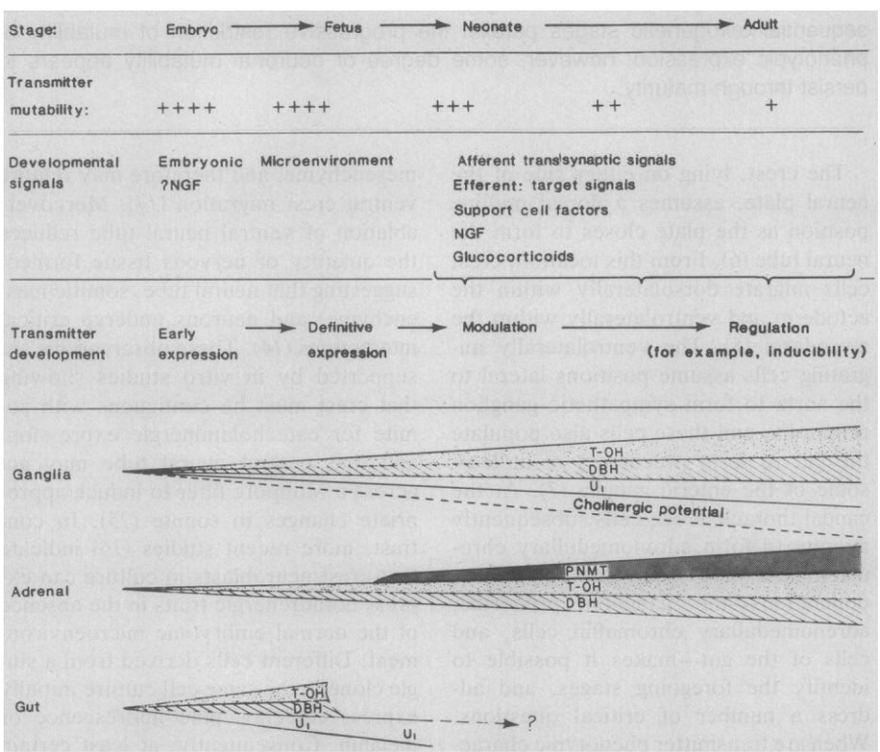

Fig. 1. Schematic representation of transmitter development. Developmental age proceeds from left to right in each panel, and vertical alignment among the entries in different panels approximates simultaneous processes or events. Lower segment of schema represents appearance and development of individual transmitter characters for sympathetic ganglia, adrenal chromaffin cells, and the transiently noradrenergic cells of the gut. Phenylethanolamine *N*-methyltransferase (*PNMT*) appears during definitive expression in the adrenal and tyrosine hydroxylase (*T-OH*) and dopamine β-hydroxylase (*DBH*) disappear in the gut cells, while high-affinity uptake system for norepinephrine (U_1) persists. *NGF* denotes nerve growth factor.

cells, the tyrosine hydroxylase and dopamine β-hydroxylase gene products appear simultaneously (*10*) (within the limits of detection), suggesting that either (i) a single stimulus affects the synthesis (or degradation) of both molecules, (ii) expression of the two enzymes is regulated by two or more signals which appear simultaneously, or (iii) the two enzymes are expressed by a single intracellular mechanism, analogous to coordinate regulation. While no conclusion is warranted at present, it is of interest that processes which biochemically induce sympathoadrenal tyrosine hydroxylase in the adult, such as pharmacologic stress and transsynaptic stimulation, also induce dopamine β-hydroxylase (*22*), and no known procedure induces only one of these enzymes. While both enzymes are expressed simultaneously and develop in concert in ganglia, adrenal, and gut, this is not necessarily universal; in contrast to noradrenergic and adrenergic cells, dopaminergic neurons never express dopamine β-hydroxylase. Consequently, regulatory mechanisms appear to be population-specific.

In addition to appearing simultaneously, some traits also disappear simultaneously. Thus, the gut cells lose both tyrosine hydroxylase and dopamine β-hydroxylase at 13.5 days of gestation. However, the high-affinity uptake system for norepinephrine persists (*10, 12*). Furthermore, previous investigations have indicated that transmitter uptake mechanisms are expressed before endogenous catecholamine histofluorescence in chick ganglia (*23*), terminals of the chick spinal cord (*24*), and rat central noradrenergic neurons (*25*). In summary, available data indicate that early expression involves a number of noradrenergic characters, and not single traits, suggesting that the units of phenotypic organization in this system are groups of noradrenergic characters.

Conversely, it is apparent that some noradrenergic and adrenergic characters are expressed differently from others, since expression of the high-affinity uptake system is regulated differently from expression of tyrosine hydroxylase and dopamine β-hydroxylase. Consequently, it is clearly inadequate, at the molecular level, to refer to development of the "noradrenergic" or "adrenergic" phenotypes as if their component traits are all similarly expressed and regulated. In fact, the autonomic system, with well-defined transmitter characters, may be ideal for detailed analysis of mechanisms synchronizing expression of different groups of traits for each transmitter.

Finally, it is apparent that early expression does not depend on formation of the great bulk of neuronal connections (*26–28*). Noradrenergic characters are expressed long before afferent or efferent synaptogenesis is completed. Although synaptic interactions play a role during later stages, they appear to be less important in early expression.

Early expression does not appear to bear an obligatory relationship to the processes of migration or mitosis. In the case of the sympathetic ganglia, early expression of noradrenergic characters occurs after initial migration from crest to ganglion primordium, but before and during secondary migration to the definitive ganglia (*14*). Similarly, adrenomedullary precursors express noradrenergic characters before and during migration from the primitive ganglia to the adrenal anlage (*9*). While exposure to the appropriate microenvironment during initial migration may elicit noradrenergic expression (see above), migratory mechanisms per se are not necessary for, and do not produce, initial expression. Similarly, DNA synthesis occurs after neuroblasts already exhibit catecholamine fluorescence (*14, 23*), suggesting that transmitter differentiation in autonomic neurons is not simply a postmitotic event. In more general terms, transmitter development and such cellular processes as neurite elongation and target innervation appear to proceed independently.

At present it is not clear whether early expression itself plays a functional role during development. Do catecholamines or acetylcholine subserve an intracellular or extracellular ontogenetic function? While the role of putative transmitters during early neuronal development has been the focus of recent interest (*29*), relevance to neural crest derivatives remains to be defined. Future studies, presumably, will indicate whether the early appearance of catecholamines serves a developmental function, or represents a vestige of mechanisms restricted to protochordate ancestors (*30*).

Definitive Expression and Modulation

While the role of early expression in the economy of development remains obscure, the subsequent stages of definitive expression and modulation constitute the critical qualitative and quantitative processes underlying normal maturation (Fig. 1). During definitive expression, the neuron "chooses" the appropriate transmitter characters qualitatively. Modulation consists of the processes through which these gene products are expressed in a quantitatively appropriate fashion, assuring normal mature function.

The discrete nature of the stage of definitive expression can be appreciated by examining adrenomedullary precursors, innervation of eccrine sweat glands, cells of the gut, and, finally, sympathetic ganglia. Adrenomedullary precursors add new characters during definitive expression, resulting in conversion from the noradrenergic to adrenergic phenotype (Fig. 1). In addition, recent studies have suggested that sympathetic fibers innervating rat eccrine sweat glands may convert from the noradrenergic to cholinergic phenotype during definitive expression (*13*). In the transiently noradrenergic gut cells, some of the original characters may disappear entirely during definitive expression, but cellular fate is undetermined. In contrast, in ganglion primordia the characters initially expressed simply increase in concentration to normal adult levels in vivo. Nevertheless, in vitro studies have indicated that sympathetic ganglion neurons are capable of expressing cholinergic as well as noradrenergic characters (*17*). The precise factors governing transmitter fate in sympathetic neurons in vivo have yet to be defined. Another population in sympathetic ganglia, the small intensely fluorescent cells, have recently become the focus of studies in vivo (*31*) and in vitro (*32*). It is already apparent that this population may elaborate neurites in culture in response to nerve growth factor (NGF) (*32*), and responds to glucocorticoids by increasing PNMT activity in vivo and in culture (*31, 32*). A great deal of additional work is required to identify discrete stages of development in this population. Regardless of outcome, definitive expression involves commitment of the cell to a subset of transmitter gene products. In the case of the noradrenergic sympathetic neuron, the products include tyrosine hydroxylase, dopamine β-hydroxylase, proteins associated with the uptake system, vesicular storage mechanisms, and so forth.

The relation of mechanisms underlying qualitative transmitter "choice" to those underlying elaboration of appropriate numbers of the chosen molecules is not yet defined. However, recent work suggests that the control of transmitter choice, a qualitative decision, differs from regulation of the subsequent quantitative increase in transmitter characters. In adrenomedullary cells, for example, initial expression of the adrenergic enzyme, PNMT, at 17 days of gestation appears to occur independent of glucocorticoid influences, while the subse-

quent developmental increase in PNMT is dependent on steroid hormones and the pituitary-adrenal axis (*9*). In another example, the trophic molecule NGF, increases whatever transmitter traits have been expressed, whether cholinergic (*33*), noradrenergic (*34*), or peptidergic (*35*), but does not appear to affect the qualitative choice itself (*33, 34*). These observations suggest that qualitative and quantitative mechanisms may differ in the cell. Consequently, in experimental animals, and perhaps in disease states as well, quantitative aspects of expression may be abnormal though appropriate qualitative mechanisms are present.

Definitive expression and modulation occur during the "critical period" of developmental plasticity when transmitter fate is determined. But how do developmental mechanisms produce mature neurons which express "correct" transmitter characters in a quantitatively appropriate manner? Posed slightly differently, what specific information, incorporated in the process of modulation, yields neurons capable of normal adult function? A parsimonious mechanism is responsible. The close relationship between quantitative modulatory and adult regulatory mechanisms is certainly not fortuitous, and is of obvious functional and survival value. It appears that the very same factors to which the neuron must respond to function normally during adulthood also influence the quantitative expression of transmitter phenotypic characters during development. Consequently, a neuron which "successfully" negotiates modulation is ipso facto prepared for normal mature function. Orthograde transsynaptic stimulation, NGF, and glucocorticoids, for example, regulate tyrosine hydroxylase in adult sympathetic ganglia (*22, 36, 37*) and also govern the developmental increase of its activity (*26, 38, 39*) (Fig. 2).

To epitomize, the commonality of quantitative developmental and adult regulatory mechanisms efficiently assures that normal development yields normal adult function. Moreover, this implies that a number of developmental "growth factors" may also serve as neuronal "maintenance" factors during adulthood. The factors that govern transmitter choice in vivo remain to be defined. It is not yet clear, then, whether such factors also play a role during adult regulation.

Detailed examination of a single common developmental regulatory mechanism, transsynaptic stimulation, illustrates that developmental and adult regulatory processes may involve similar mechanisms. In the adult, transsynaptic

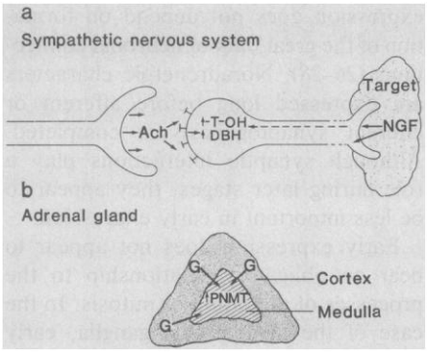

Fig. 2. Common factors regulate the quantity of catecholaminergic characters during development and maturity. (a) In the sympathetic nervous system transsynaptic acetylcholine (*ACh*) interacts with postsynaptic receptors, leading to developmental increases in tyrosine hydroxylase (*T-OH*) and dopamine β-hydroxylase (*DBH*) in neonates and enzyme induction in adults. Nerve growth factor (*NGF*), presumably of target origin, is transported in a retrograde fashion, and also increases tyrosine hydroxylase and dopamine β-hydroxylase during development and maturity. (b) In another example, glucocorticoids (*G*) elaborated by the adrenal cortex, are necessary for the developmental increase in medullary PNMT, and maintenance of normal adult levels of the enzyme.

stimulation of sympathetic noradrenergic neurons results in the biochemical induction of tyrosine hydroxylase and dopamine β-hydrolase through the mediation of acetylcholine and stimulation of postsynaptic nicotinic receptors (*22*). Transsynaptic modulation of tyrosine hydroxylase during development, which results in a six- to tenfold rise in enzyme molecule numbers to adult plateau values, utilizes similar cellular mechanisms; cholinergic stimulation of nicotinic receptors results in elevated numbers of enzyme molecules (*26, 39*). Recent work with neonatal rat sympathetic neurons in vitro indicates that depolarizing stimuli foster noradrenergic development (*40*). It may be inferred that transsynaptic influences during definitive expression in vivo play a role in stabilization of transmitter choice (*40*) as well as acquisition of the normal complement of noradrenergic characters (*39*).

While depolarization is a noradrenergic stimulus, diffusible factor or factors produced by nonneuronal cells induce cholinergicity (*17*). Moreover, cholinergic-noradrenergic "dual function" cells have been identified in culture (*18*), indicating that these choices are not mutually exclusive. Consequently, there is apparently no cellular mechanism that a priori prevents simultaneous expression of more than one transmitter phenotype.

The flexibility of phenotypic expression during development, and the distinction between early expression and definitive expression, are further illustrated by recent work performed in avian embryos. Normally, the trunk neural crest region gives rise to catecholaminergic sympathetic ganglion and adrenomedullary cells, whereas the "vagal" region gives rise to cholinergic enteric ganglion cells. However, if trunk crest is grafted to the vagal region, cells colonize the gut to form cholinergic enteric ganglion populations. On the other hand, cephalic crest transplanted to the trunk region gives rise to catecholaminergic adrenomedullary chromaffin tissue (*41*). Moreover, this flexibility persists even after definitive ganglia have formed. Cells of the cholinergic ganglion of Remak and ciliary ganglion resume migration when transplanted to the trunk region, and some of the cells exhibit catecholamine histofluorescence in sympathetic ganglia (*42*). Finally, recent work has suggested that cellular elements of sensory ganglia may express autonomic characters (as judged by catecholamine fluorescence) after appropriate transplantation (*43*). These studies, viewed in aggregate, suggest that the environment encountered during migration, and at the definitive site may play a role in definitive expression. Moreover, at the time of definitive expression neurons may convert from one phenotype to another, indicating that early expression and definitive expression are distinct stages. Nevertheless, the possibility remains that the environment of the definitive site simply selects for other populations of cells in these in vivo studies.

It is not clear at present to what extent phenotypic flexibility persists beyond the normal developmental period into adulthood. Recent studies suggest, however, that autonomic neurons in adults may retain the ability to alter phenotypic expression.

Phenotypic Expression and Plasticity During Maturity

Adult phenotypic characters are expressed within precise, quantitative limits, resulting in stable baseline values for any single trait. After the definitive phenotype is first expressed, mechanisms presumably increase the rate of synthesis of each trait (*26, 39*) so that molecule numbers rise to a new (adult) steady-state level. Although it is apparent that adult plateau levels of transmitter characters reflect stable rates of synthesis and degradation, the mechanisms underlying such stability are largely unknown. It may be relevant, however, that mature

expression, quantitatively as well as qualitatively, occurs in close temporal proximity to completion of afferent and efferent synaptogenesis (*26, 39*). There is evidence, in fact, that orthograde transsynaptic factors and peripheral target structures contribute to the maintenance of stable adult levels of transmitter traits in sympathetic neurons (*36, 44*) (Fig. 2).

Denervation (decentralization) of adult sympathetic ganglia, for example, results in reduction of tyrosine hydroxylase activity (*44*). Conversely, postganglionic axotomy or placement of a postganglionic colchicine cuff, which inhibits axonal transport, also reduces tyrosine hydroxylase activity (*36, 45*). Consequently, functional separation of sympathetic neurons from afferent input or targets reduces transmitter traits below normal adult levels. In the case of transsynaptic stimulation, the active process involves acetylcholine and postsynaptic depolarization. The target maintenance role may be mediated, at least in part, by NGF, since treatment with the protein reverses the effects of axotomy or colchicine (*36, 45*), and since antiserum to NGF mimics some of the effects of the axotomy (*46*). It may be concluded that maintenance of normal transmitter character levels during maturity is an active process, dependent on continued exposure to extracellular factors. Mature expression, then, is not simply the passive end product of earlier, modulatory mechanisms.

Since dissociated adult noradrenergic sympathetic neurons in culture may acquire cholinergic characters (*47*), these neurons may remain intrinsically mutable throughout life, while afferent, efferent, support cell and humoral factors impose apparent phenotypic stability during adulthood. This contention is consistent with the observation that adult sympathetic populations may exhibit cholinergic characteristics in vivo and in vitro, if their axons are directed toward cholinergic targets (*48*). Moreover, recent studies indicate that cholinergic sympathetic fibers which innervate eccrine sweat glands are capable of taking up α-methylnorepinephrine (*13*), a process characteristic of noradrenergic neurons. In another example, chromaffin cells from postnatal rats or adult bovine adrenal may extend neurites in culture under appropriate conditions (*49*). Therefore, sympathoadrenal cells may retain the potential for phenotypic plasticity through adulthood. This contention is supported by the recent observation that decentralization of neonatal or adult sympathetic ganglia markedly increased levels of the putative peptide transmitter substance P, in principal ganglion neurons (*50*). Consequently, phenotypic changes may emerge when neurons are separated from input or targets, or exposed to new environmental stimuli. Indeed, the chromatolytic process itself may represent one instance of reemergence of plasticity in the adult neuron. Clearly, additional studies are required to characterize phenotypic mutability in organisms of different ages.

Regulation

As mature expression is established, regulation of the adult phenotypic characters commences (Fig. 1). In the broadest sense, regulation refers to mechanisms governing the activity and amount of phenotypic characters in the mature neuron. Although the factors governing phenotypic expression during development and maturity are frequently identical, temporal profiles of neuronal responses may differ. During the developmental period, the presence of appropriate factors, such as transsynaptic stimulation or NGF, assures normal sympathetic maturation and results in long-term increases in phenotypic trait concentrations. In contrast, increased transsynaptic stimulation during maturity results in a well-circumscribed, transient increase in the concentration of characters such as tyrosine hydroxylase and dopamine β-hydroxylase (*22*).

Recent methodologic advances may allow approaches to various related problems: Are the same molecular mechanisms involved in elevated synthesis during development and maturity? Are the same processes involved in modulation and regulation of transmitter characters, or do fundamentally different genomic mechanisms operate during development and maturity? Do modulatory factors directly or indirectly remove inhibitors of character synthesis (at the transcriptional or translational level) or activate synthesis through positive mechanisms? Are there fundamental differences in translational and posttranslational mechanisms between modulation and regulation?

Transmitter Development and Morphogenesis

Available evidence suggests that there is no obligatory relationship between development of transmitter characters and such morphogenetic processes as cellular migration, mitosis, and neurite elongation. Catecholamine phenotypic characters appear in sympathetic neuroblasts prior to and during DNA synthesis (see above). Moreover, the catecholaminergic phenotypic characters, tyrosine hydroxylase and dopamine β-hydroxylase, are present in these cells before and during migration (*9, 10*). Finally, in vivo and in vitro studies have indicated that normal transmitter mechanisms are not required for neurite elongation. Sympathetic ganglia transplanted to the anterior chamber of the eye form neurites and innervate the host iris after treatments which markedly alter levels of norepinephrine (*51*). In culture, embryonic sympathetic ganglia elaborate neurites with morphologically normal growth cones, while tyrosine hydroxylase fails to develop, when RNA and protein synthesis are inhibited (*52*). Consequently, the normal development of transmitter phenotypic characters is not a necessary condition for maturation of all other neuronal traits. In a more general sense, then, different aspects of neuronal maturation appear to proceed independently, suggesting that separate and distinct underlying mechanisms are involved. Consequently, mitosis, migration, transmitter expression, and target innervation do not appear to be interdependent, locked to a rigid developmental algorithm. Rather, the neuron is endowed with the flexibility to develop certain traits normally, although development of others may be abnormal. This constitutes an efficient, developmental mechanism in which temporal aberration of a single external signal does not derange all aspects of development of a neuron.

Conversely, highly circumscribed deficits of neuronal development may occur in response to abnormalities of distinct ontogenetic mechanisms. One might anticipate that diseases of neuronal development may result from primary deficits in migratory, mitotic, neuritic, or transmitter expression mechanisms, for example, without necessarily affecting other aspects of ontogeny.

References and Notes

1. M. Levitt, S. Spector, A. Sjoerdsma, S. Udenfriend, *J. Pharmacol. Exp. Ther.* **148**, 1 (1965).
2. N. Kirshner, *Pharmacol. Rev.* **11**, 350 (1959).
3. E. Bulbring, *Br. J. Pharmacol.* **4**, 234 (1949); E. B. Keller, R. A. Boissonnas, V. DuVigneaud, *J. Biol. Chem.* **183**, 627 (1950); N. Kirshner and M. Goodall, *Biochim. Biophys. Acta* **24**, 658 (1957); R. J. Wurtman and J. Axelrod, *J. Biol. Chem.* **241**, 2301 (1966); R. D. Ciaranello, *Mol. Pharmacol.* **14**, 478 (1978); _____, D. Jacobowitz, J. Axelrod, *J. Neurochem.* **20**, 799 (1973).
4. F. Fonnum, *Norwegian Defense Research Establishment Report No. 58* (1970).
5. A. J. Coulombre, M. C. Johnston, J. A. Weston, *Dev. Biol.* **36**, f1 (1974); S. Horstadius, *The Neural Crest* (Oxford Univ. Press, London, 1950); J. A. Weston, *Adv. Morphog.* **8**, 41 (1970); _____ and S. L. Butler, *Dev. Biol.* **14**, 246 (1966).
6. L. Saxen and S. Toivonen, *Primary Embryonic Induction*, (Prentice-Hall, Englewood Cliffs, N.J., 1962).

7. A. Andrew, *J. Anat.* **105**, 89 (1969); *ibid.* **107**, 327 (1970); C. L. Yntema and W. S. Hammond, *J. Exp. Zool.* **100**, 237 (1945); N. LeDouarin and M. A. Teillet, *J. Embryol. Exp. Morphol.* **30**, 31 (1973); *Dev. Biol.* **41**, 162 (1974).
8. W. S. Hammond and C. L. Yntema, *J. Comp. Neurol.* **85**, 237 (1947); C. L. Yntema and W. S. Hammond, *ibid.* **101**, 515 (1954).
9. M. C. Bohn, M. Goldstein, I. B. Black, *Dev. Biol.* **82**, 1 (1981).
10. P. Cochard, M. Goldstein, I. B. Black, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 2986 (1978); *Dev. Biol.* **71**, 100 (1979).
11. G. Teitelman, H. Baker, T. H. Joh, D. J. Reis, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 509 (1979).
12. G. M. Jonakait, J. Wolf, P. Cochard, M. Goldstein, I. B. Black, *ibid.*, p. 4683.
13. S. C. Landis and D. Keefe, *Soc. Neurosci. Abstr.* **6**, 379 (1980).
14. A. L. Cohen, *J. Exp. Zool.* **179**, 167 (1972); in *Dynamics of Degeneration and Growth in Neurons*, K. Fuxe, L. Olson, Y. Zotterman, Eds. (Pergamon, New York, 1974).
15. S. C. Norr, *Dev. Biol.* **34**, 16 (1973).
16. M. Sieber-Blum and A. L. Cohen, *J. Cell Biol.* **79**, 31a (1978).
17. P. H. Patterson and L. L. Y. Chun, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3607 (1974); *Dev. Biol.* **56**, 263 (1977).
18. E. J. Furshpan, P. R. MacLeish, P. H. O'Lague, D. D. Potter, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 4225 (1976).
19. C. P. Ko, H. Burton, M. I. Johnson, R. P. Bunge, *Brain Res.* **117**, 461 (1976).
20. N. M. LeDouarin, *Nature (London)* **286**, 663 (1980).
21. E. H. Davidson and R. J. Britten, *Science* **204**, 1052 (1979).
22. P. B. Molinoff, S. Brimijoin, R. Weinshilboum, J. Axelrod, *Proc. Natl. Acad. Sci. U.S.A.* **66**, 453 (1970); U. Otten, U. Paravicini, F. Oesch, H. Thoenen, *Naunyn-Schmiedeberg's Arch. Pharmacol.* **280**, 117 (1973); H. Thoenen, R. Kettler, W. Burkard, A. Saner, *ibid.* **270**, 146 (1971).
23. T. P. Rothman, M. D. Gershon, H. Holtzer, *Dev. Biol.* **65**, 322 (1978).
24. H. S. Singer, J. T. Coyle, N. Vernon, C. H. Kallman, D. L. Price, *Brain Res.* **191**, 417 (1980).
25. J. T. Coyle and J. Axelrod, *J. Neurochem.* **18**, 2061 (1971).
26. I. B. Black, I. A. Hendry, L. L. Iversen, *Brain Res.* **34**, 229 (1971).
27. A. Smolen and G. Raisman, *ibid.* **181**, 315 (1980).
28. I. B. Black and C. Mytilineou, *ibid.* **101**, 503 (1976).
29. J. M. Lauder and H. Krebs, *Dev. Neurosci.* **1**, 15 (1978).
30. H. B. Sarnat and M. G. Netsky, *Evolution of the Nervous System* (Oxford Univ. Press, New York, 1974).
31. I. B. Black, M. C. Bohn, E. M. Bloom, M. Goldstein, *Soc. Neurosci. Abstr.* **6**, 408 (1980).
32. A. J. Doupe, P. H. Patterson, S. C. Landis, *ibid.*, p. 409.
33. L. L. Y. Chun and P. Patterson, *J. Cell Biol.* **75**, 712 (1977); M. D. Coughlin, M. D. Dibner, D. M. Boyer, I. B. Black, *Dev. Biol.* **66**, 513 (1978).
34. L. L. Y. Chun and P. Patterson, *J. Cell Biol.* **75**, 694 (1977); I. B. Black, I. A. Hendry, L. L. Iversen, *J. Neurochem.* **19**, 1367 (1972).
35. J. A. Kessler and I. B. Black, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 649 (1980).
36. I. A. Hendry and H. Thoenen, *J. Neurochem.* **22**, 999 (1974); J. A. Kessler and I. B. Black, *Brain Res.* **171**, 415 (1979).
37. I. Hanbauer, A. Guidotti, E. Costa, *Brain Res.* **85**, 527 (1975); U. Otten and H. Thoenen, *ibid.* **111**, 438 (1976).
38. For review, see L. A. Greene and E. Shooter, *Annu. Rev. Neurosci.* **3**, 353 (1980); I. S. McLennan, C. E. Hill, I. A. Hendry, *Nature (London)* **283**, 206 (1980); D. Sandquist, A. C. Black, Jr., S. K. Sahu, L. Williams, T. H. Williams, *Exp. Cell Res.* **123**, 417 (1979); D. H. Edgar and H. Thoenen, *Brain Res.* **154**, 186 (1978).
39. I. B. Black and S. C. Geen, *Brain Res.* **63**, 291 (1973); I. B. Black, T. H. Joh, D. J. Reis, *ibid.* **75**, 133 (1974).
40. P. A. Walicke, P. B. Campenot, P. H. Patterson, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5767 (1977).
41. N. M. LeDouarin, D. Renaud, M. A. Teillet, G. H. LeDouarin, *ibid.* **72**, 728 (1975); N. M. LeDouarin, M. A. Teillet, C. LeLievre, in *Cell and Tissue Interactions*, J. W. Lash and M. M. Burger, Eds. (Raven Press, New York, 1977), pp. 11–27.
42. N. M. LeDouarin, M. A. Teillet, C. Ziller, J. Smith, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 2030 (1978).
43. C. S. LeLievre, G. G. Schweizer, C. M. Ziller, N. M. LeDouarin, *Dev. Biol.* **77**, 362 (1980).
44. I. A. Hendry, L. L. Iversen, I. B. Black, *J. Neurochem.* **20**, 1683 (1973).
45. I. A. Hendry, *Brain Res.* **94**, 87 (1975).
46. B. Bjerre, L. Wiklund, D. C. Edwards, *ibid.* **92**, 257 (1975); P. D. Gorin and E. M. Johnson, *Soc. Neurosci. Abstr.* **6**, 100 (1980).
47. E. Wakshull, M. I. Johnson, H. Burton, *J. Cell Biol.* **79**, 121 (1978); M. I. Johnson and R. P. Bunge, in *Proceedings of the First International Spinal Cord Reconstruction*, R. P. Bunge and T. Reier, Eds. (Raven Press, New York, in press).
48. S. K. Kemplay and J. R. Garrett, *Cell Tissue Res.* **167**, 197 (1976); R. D. Purves, C. E. Hill, J. Chamley, G. E. Mark, D. M. Fry, G. Burnstock, *Pfluegers Arch. Gesamite Physiol. Menschen Tiere* **350**, 1 (1974).
49. K. Unsicker, B. Krisch, U. Otten, H. Thoenen, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 3498 (1978); B. G. Livett, D. M. Dean, G. M. Bray, *Soc. Neurosci. Abstr.* **4**, 592 (1978); K. Unsicker, G.-H. Griesser, R. Lindmar, K. Loffelholz, U. Wolf, *Neuroscience* **5**, 1445 (1980).
50. J. A. Kessler, J. E. Adler, M. C. Bohn, I. B. Black, *Science* **214**, 335 (1981).
51. L. Olson and T. Malmfors, *Acta Physiol. Scand. Suppl.* **348**, 1 (1970).
52. E. M. Bloom and I. B. Black, *Dev. Biol.* **68**, 568 (1979).
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Technology, Enterprise, and American Economic Growth

Jordan D. Lewis

The success of the space shuttle and American-led advances in biotechnology, artificial intelligence, exotic materials, and other dramatic developments, have given hope to many that the United States is returning to a path of vigorous economic growth (*1*). This view reflects a misunderstanding of technology and how it contributes to a nation's economic vitality; the significant decline of American technological prowess since the 1960's, when our technological might was extolled (*2*), is beyond dispute. American industry was once the world leader in the production of consumer electronics and photographic equipment, ships, machine tools, office copiers, textile machinery, industrial chemicals, computers and semiconductor electronics, farm equipment, jet aircraft, automobiles, and steel. But today U.S. firms in these and other technology-based industries have been outpaced by or face serious challenges from foreign rivals for domestic and world markets (*3–5*).

The American descent from technological preeminence has been partially unavoidable. Our nation was technologically and economically superior to others in the decades following World War II because our allies and adversaries were recovering from massive destruction, not because we were smarter, better organized, or worked harder. What tends to be forgotten is that we were not superior to other nations in many fields before World War II. Then, for example, German chemical engineering was preeminent and European science excelled. In fact, many of the scientists who built America's postwar technology base were refugees. We are not likely to benefit from a European brain drain again. Since the end of World War II, the former combatants have rebuilt their industries, and the United States is watching other nations pass it by. The strength of the European and Japanese economies can no longer be attributed to lower wage rates or to these countries skimming the cream off our technology base. Much of it is due to greater technological vitality.

Recent explanations of this new American dilemma tend to focus on a variety of factors—too much regulation, too

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