References and Notes

1. For a derivation of the hydrostatic balance equation and a useful survey of atmospheric physics, see R. A. Craig, *The Upper Atmosphere: Meteorology and Physics* (Academic Press, New York, 1965). For a less technical review see J. C. G. Walker, in *The Encyclopedia of Atmospheric Sciences and Astrogeology*, R. W. Fairbridge, Ed. (Reinhold, New York, 1967), pp. 1064–1073.
2. L. Spitzer, in *The Atmospheres of the Earth and Planets*, G. P. Kuiper, Ed. (Univ. of Chicago Press, Chicago, 1949), pp. 211–247.
3. F. S. Johnson, *Ann. Geophys.* **14**, 94 (1958); D. C. Hunt and T. E. VanZandt, *J. Geophys. Res.* **66**, 1673 (1961); M. Nicolet, *Planetary Space Sci.* **5**, 1 (1961); I. Harris and W. Priester, *J. Atmospheric Sci.* **19**, 286 (1962).
4. S. Chapman and E. A. Milne, *Quart. J. Roy. Meteorol. Soc.* **46**, 357 (1920); ———, *Proc. Phys. Soc. London*, **43**, 26 (1931); K. Watanabe and H. E. Hinteregger, *J. Geophys. Res.* **67**, 999 (1962).
5. E. E. Ferguson, *Rev. Geophys.* **5**, 305 (1967).
6. E. O. Hulburt, *Phys. Rev.* **31**, 1018 (1928); V. C. A. Ferraro, *Terrest. Magnetism Atmospheric Elect.* **50**, 215 (1945); H. Rishbeth, *Proc. I.E.E.E. (Inst. Elec. Electron. Engrs.)* **55**, 16 (1967); ———, *Rev. Geophys.* **6**, 33 (1968).
7. D. R. Bates, *Monthly Notices Roy. Astron. Soc.* **109**, 215 (1949).
8. ——— and M. R. C. McDowell, *J. Atmospheric Terrest. Phys.* **11**, 200 (1957); ———, *ibid.* **16**, 393 (1959); M. Nicolet, *Ann. Geophys.* **13**, 1 (1957); G. J. F. MacDonald, *Rev. Geophys.* **1**, 305 (1963); E. E. Ferguson, F. C. Fehsenfeld, A. L. Schmeltekopf, *Planetary Space Sci.* **13**, 925 (1965); W. I. Axford, *J. Geophys. Res.* **73**, 6855 (1968).
9. D. M. Hunten, *Ann. Geophys.* **17**, 249 (1961).
10. See, however, J. H. Brandy, *Can. J. Phys.* **43**, 1697 (1965).
11. D. G. King-Hele, *Theory of Satellite Orbits in an Atmosphere* (Butterworths, London, 1964); *Ann. Geophys.* **22**, 40 (1966).
12. ——— and E. Quinn, *J. Atmospheric Terrest. Phys.* **27**, 197 (1965); L. G. Jacchia, *Rev. Mod. Phys.* **35**, 973 (1963); ———, *Smithsonian Inst. Astrophys. Obs. Spec. Rep. No. 170* (1964); ———, *Smithsonian Inst. Astrophys. Obs. Spec. Rep. No. 184* (1965); ———, *Smithsonian Inst. Astrophys. Obs. Spec. Rep. No. 245* (1967).
13. J. A. Stein and J. C. G. Walker, *J. Atmospheric Sci.* **22**, 11 (1965).
14. D. G. King-Hele and E. Quinn, *Planetary Space Sci.* **14**, 1023 (1966); ——— and J. Hingston, *ibid.* **16**, 675 (1968).
15. N. W. Spencer, L. H. Brace, G. R. Carignan, D. R. Taeusch, H. Niemann, *J. Geophys. Res.* **70**, 2665 (1965); N. W. Spencer, D. R. Taeusch, G. R. Carignan, *Ann. Geophys.* **22**, 151 (1966); N. W. Spencer, G. R. Carignan, D. R. Taeusch, *Meteorol. Monograph*, in press.
16. A. Reifman and W. G. Dow, *Phys. Rev.* **76**, 987 (1949); G. Hok, N. W. Spencer, W. G. Dow, *J. Geophys. Res.* **58**, 235 (1953).
17. H. M. Mott-Smith and I. Langmuir, *Phys. Rev.* **28**, 727 (1926).
18. R. L. Boggess, L. H. Brace, N. W. Spencer, *J. Geophys. Res.* **64**, 1627 (1959).
19. N. W. Spencer, L. H. Brace, G. R. Carignan, *ibid.* **67**, 157 (1962); L. H. Brace, N. W. Spencer, G. R. Carignan, *ibid.* **68**, 5397 (1963).
20. W. B. Hanson and F. S. Johnson, *Mem. Soc. Roy. Sci. Liege* **4**, 390 (1961); A. Dalgarno, M. B. McElroy, R. J. Moffet, *Planetary Space Sci.* **11**, 463 (1963).
21. W. B. Hanson, *Proc. Intern. Space Sci. Symp. 3rd Washington, D.C., 1962* (1963), p. 282.
22. G. Drukarev, *J. Phys. (USSR)* **10**, 81 (1946).
23. The case for equal electron and gas temperatures at high altitudes was stated most forcefully by S. J. Bauer and R. E. Bourdeau, *J. Atmospheric Sci.* **19**, 218 (1962), and by R. E. Bourdeau, *Space Sci. Rev.* **1**, 683 (1963).
24. G. P. Serbu, R. E. Bourdeau, J. L. Donley, *J. Geophys. Res.* **66**, 4313 (1961).
25. J. V. Evans, *ibid.* **67**, 4914 (1962); V. C. Pineo and D. P. Hynek, *ibid.*, p. 5119; K. L. Bowles, G. R. Ochs, J. L. Green, *J. Res. Nat. Bur. Std. D* **66**, 395 (1962).
26. For a recent review and references to theoretical papers, see W. E. Gordon, *Rev. Geophys.* **5**, 191 (1967).
27. J. V. Evans, *J. Geophys. Res.* **69**, 1436 (1964); see also A. F. Nagy, L. H. Brace, G. R. Carignan, M. Kanal, *ibid.* **68**, 6401 (1963).
28. P. J. Bowen, R. L. F. Boyd, C. L. Henderson, A. P. Willmore, *Proc. Roy. Soc. London Ser. A* **281**, 526 (1964); L. H. Brace, N. W. Spencer, A. Dalgarno, *Planetary Space Sci.* **13**, 647 (1965); J. V. Evans, *ibid.*, p. 1031; ——— and M. Loewenthal, *ibid.* **12**, 915 (1964).
29. A. Dalgarno, M. B. McElroy, J. C. G. Walker, *Planetary Space Sci.* **15**, 331 (1967).
30. A. Dalgarno, M. B. McElroy, M. H. Rees, J. C. G. Walker, *ibid.* in press.
31. J. V. Evans, *J. Geophys. Res.* **72**, 3343 (1967); H. Carru, M. Petit, P. Waldteufel, *J. Atmospheric Terrest. Phys.* **29**, 351 (1967); L. G. Smith, C. A. Accardo, L. H. Weeks, P. J. McKinnon, *ibid.* **27**, 803 (1965); L. G. Smith, L. H. Weeks, P. J. McKinnon, *ibid.* **30**, 1301 (1968).
32. J. C. G. Walker, *Planetary Space Sci.* **16**, 321 (1968).
33. *Solar-Geophysical Data* (Institute for Telecommunication Sciences and Aeronomy, Environmental Sciences Services Administration, Boulder, Colorado, 1967).
34. J. V. Evans, *Planetary Space Sci.* **15**, 1557 (1967).
35. L. H. Brace, B. M. Reddy, H. G. Mayr, *J. Geophys. Res.* **72**, 265 (1967).

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Psychochemical Research Studies in Man

Research approaches to the chemistry of the mind of man, although promising, are difficult to interpret.

Arnold J. Mandell and Charles E. Spooner

Unlike many previous sociologists and historians of science, recent examiners of the scientific process have been struck by the influence of factors within the game of science that appeared to significantly influence trends in research strategies in ways which seemed prepotent over substantive "discoveries" in an area. Kuhn, in his classic essay "The Structure of Scientific Revolutions" (*1*), using the history of some areas of physics as his models, developed a theory of the progression of what he calls "normal science" as the serial emergence and decline of paradigms and, along with them, their practitioners. A paradigm is defined as a shared and consensually agreed upon system of assumptions, acceptable operations, standards for evidence, and rules of conduct for a scientific endeavor that are dominant at a particular time in a field of investigation and is expressed in the form of model problems and solutions. Kuhn would probably regard the subject of this paper as "preparadigmatic"; this status would be earned by both the insufficiency and inchoateness of our information. He pointed out, however, that even in primitive areas such as psychochemistry, rather sizable groups of scientists may often reach temporary agreement about what constitutes good research methodology and acceptable results. Kuhn would predict, however, that consensus in a preparadigmatic field would eventually disappear, and another preparadigmatic school would emerge. The shift, by the biologically oriented student of behavior in man, from electricity to juices in the past decade seems to be a good example of such a preparadigmatic transition. Drawing much of our scientific aura from the basic neurophysiologists, who studied electrical potentials from cells and brain systems in animals in the 1940's and 1950's, we grabbed at any evidence of direct or transduced electricity we could get from the entire surface of man. Taking our cue from such representations of what was *au courant* as Fulton's 1955 *Textbook of Physiology* (*2*) which allowed only 13 pages of talk about neurohumors in 502 pages of brain circuitry, we focused on electricity. The massive number of intervening variables between thought and scalp or palm was

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acknowledged, but we could not get to man's brain directly, and we were doing what we could. Physiological correlates of central states in man became "psychophysiology," and psychophysiological parameters began to invade the studies of man in psychiatric and psychological laboratories. In the early and middle 1950's, behavioral-oriented neurochemists and neuropharmacologists came forth with new findings about the brain of animals. Interesting compounds apparently related to excitability of the nervous system became elucidated; drugs affecting these systems changed behavior in man. Genetic metabolic defects manifested by changes in brain function and behavior were elucidated. Hallucinogens reproduced aspects of psychopathological states. Juices were becoming more popular among basic brain researchers. Biochemical variables, when studied in relation to central states, which were reflected in changes in behavior became "psychochemistry." Instead of skin, the psychochemist's surface in man was body fluids. All, of course, are several membranes and transport systems away from the internal milieu of the brain. The palm and the scalp gave way to cerebrospinal fluid, blood, and urine. Again, a body of empirical relations between biological variables and behavior began to emerge. In addition, various animal experimental models came into existence for comparison with human psychological states. A behavioral depression induced by reserpine in an animal was used for neurochemical and pharmacological study thought to be pertinent to human depression (*3*). Changes in excitability of peripheral autonomic synapses as a result of chemical manipulation (with natural substrates or drugs) were used as models for generalization to the central synapses of animals and man (*4*). On the basis of numerous studies of the peripheral nervous system, patterns of metabolism of compounds were considered differentially reflective of presynaptic or postsynaptic activity in the central nervous system. Electrical patterns of central nervous system activity in animals, patterns which are similar to those seen in man, were used as dependent variables in chemical studies leading to deductions about the biochemical substrates of these electrical states in man (*5*).

In this article we focus on four examples of research strategies for making deductions about biochemical mechanisms associated with behavioral phenomena in man. These representative approaches were chosen because of our personal familiarity with them (*6*).

The four strategies discussed include: (i) the neuropharmacological manipulation of neurochemistry of animal brain with agents having established behavioral effects in man; (ii) the administration to man, by the "load" technique, of precursors of metabolic substances hypothesized to be active in the central nervous system with behavioral ramifications; (iii) the use of sleep stage as an electrophysiological state that is relatively stereotyped across species so that various chemically induced changes in this parameter can be studied in both animals and man; (iv) the search for metabolic errors reflected in changes in both body chemistry and behavior, so that either absences of normal metabolites or the surfeit of usually insignificant substances may be correlated with behavioral phenomena and suggest underlying chemical mechanisms.

An important general assumption is made in all these strategies, which bears some discussion. This concerns our ability to label certain classes or specific compounds as "transmitters" or "modulators of excitability" in the central nervous system.

Bases for Regarding Substances as Neurotransmitters

There is remarkably little evidence for the physiological role of even the most accepted of substances present in the brain. The study of the transmitter role of substances has been best worked out in the peripheral nervous system. The role of acetylcholine in the neuromuscular junction is perhaps the best mammalian model problem and set of solutions in the area of the establishment of neurotransmitter function of a chemical substance. Dale (*7*) and Katz (*8*) and their followers showed that the following events take place at the neuromuscular junction. (i) Acetylcholine is released in a suitable amount to produce the critical physiological effect (*9*). (ii) Preganglionic autonomic nerves and motor nerves to muscle can synthesize acetylcholine at a high rate (*10*). (iii) Quantal release of this transmitter produced mathematically equivalent responses of postsynaptic end-plate potentials (*11*). (iv) Some data evolving from studies of subcellular fractionation indicate that acetylcholine can be stored in readiness for release in vesicles in the presynaptic ending (*12*). (v) The degradative enzyme, acetylcholinesterase, is available on the surface of the postsynaptic membrane to inactivate the transmitter (*13*). (vi) In addition to the site for degradative hydrolysis, pharmacological work has suggested a separate and specific postsynaptic receptor site which activates the membrane (*14*). (vii) Regulatory mechanisms appear to be present for the modulation of release and synthesis in this system (*15*). Many similar findings have been either suggested or established in studies of the peripheral adrenergic neuron by the use of such preparations as the splenic nerve, the sympathetic nerve supply to the vas deferens, the adrenal medulla, the rat iris, and the sympathetic nerve supply to the heart (*16*).

The technical problems involved with any attempt to use analogously systematic research approaches to a potential transmitter in the central nervous system are great. Peripheral synapses can be isolated by microdissection, they can remain functional in an isolated profusion experimental situation for many hours, and their activation can be manifested by clearly defined and measurable phenomena (such as the miniature end-plate potential or the contraction of smooth muscle). The central nervous system has little in the way of focal synaptic regions. The dendrites and the cell bodies of central neurons are densely covered with synapses, many of which may be of a chemically heterogenous nature (*17*). In addition, the extraneuronal space is packed with a tangle of glia, closely approximating the membranous surfaces of nerve cells and possibly intrinsically important to their functioning (*18*). This makes the isolated, chemical manipulation of a central synapse extremely difficult. When the data associated with the study of the potential transmitter or modulator role of a compound such as norepinephrine in the central nervous system is compared with the studies of acetylcholine at the peripheral snyapse, the evidence seems relatively indirect. This evidence includes (i) the existence of regional brain differences in the distribution of norepinephrine, which appear to be related to some neurophysiological organizations of the nervous system (*19*); (ii) a regional distribution of synthesizing and degradative enzymes that appears, in most cases, to follow the distribution of the amine (*19*); (iii) high levels of norepinephrine and enzymes related to the synthesis of norepinephrine in the subcellular brain fraction associated with structures that look like synaptic vesicles (*20*); (iv)

the fluorescent histochemical localization deemed characteristic of catecholamines in central neurons (*21*); (v) the subcellular localization of degradative enzymes in the fraction associated with synaptic structures ("synaptosomes") (*20*); (vi) pharmacological studies demonstrating the effects of intravenous, intraarterial, or intraventricular administration of norepinephrine or drugs assumed to influence mobility of norepinephrine and metabolism on spontaneous and evoked dependent variables such as unit activity, electroencephalogram, or behavior (*22–26*); (vii) studies demonstrating effects of induced neural activity on brain concentrations of norepinephrine both in brain slices and in vivo (*27*); (viii) studies of the effects on spontaneous or evoked firing rates of cells to which norepinephrine has been applied by "microiontophoresis" (*28*). Even with the work associated with this last technique, which eliminates the problems of the blood-brain barrier and diffuseness of drug application, there are many problems.

The effect of the iontophoretic application of norepinephrine outside the membrane of nerve cells (which most frequently results in depression of activity) does not resemble the effects of stabilization of membrane usually thought to be associated with nervous inhibition for three reasons. (i) The cells still respond to electrical or chemical stimulation; (ii) the latency of onset of action of norepinephrine is quite long (up to 30 seconds) when compared with the effects of acetylcholine on some spinal cord cells (less than 1 second); (iii) the duration of the effect of norepinephrine seems remarkably long (up to 5 minutes), when one considers that a full complement of potential inactivating mechanisms for norepinephrine are present in the nervous system. Many of the kinds of evidence for norepinephrine as a neurotransmitter or modulator in the central nervous system are not unique to this compound but apply to such varied substances as γ-aminobutyric acid, glutamic acid, glycine, dopamine, and serotonin (*29*). When one reviews such work as Perry's on the amine content of the human brain (*30*) and notes the large number of both identified and unidentified compounds present, one is struck with the possibility that we are entering an era when various "transmitters" will come in and out of fashion for a long time. The nonspecificity of the synthetic, binding, release, receptor, and degradative mechanisms that have been demonstrated for norepinephrine [which Day and Rand, Kopin, and others (*31*) defined out of this status by use of the concept of "false transmitter" when the substance is not norepinephrine] underlies further the nebulosity of our findings relative to noreprinephrine as a neural transmitter. Even on a neurochemical level, we must talk about empirical correlates rather than determinants of behavioral states. Rigorous establishment of the transmitter or modulator role of brain substances appears to remain for the future.

Strategies

1) *The neuropharmacological manipulation of animal brain neurochemistry with agents having established behavioral effects in man.* In this approach, neurochemical correlates of drug action in animals are studied with drugs shown to be effective in producing or changing behavioral states in man. A summary of the evidence for one of the theories generated principally by this kind of strategy has been reviewed by Schildkraut and Kety (*4*). This neurochemical model for a human behavioral state has been called the "catecholamine theory of mood" which posits that clinical depression is associated with a functional deficiency of central norepinephrine and, perhaps, the inverse in states of mania. The model, around which most of this kind of psychochemical hypothesis revolves, is a theoretical noradrenergic synapse synthesized from data obtained from studies of peripheral sympathetic synapses in isolated tissues and of the patterns of metabolites when drugs and isotopically labeled norepinephrine are allowed to interact after intraventricular administration of the postulated neurotransmitter. From these kinds of data, it has been suggested that increases in "synaptically active" norepinephrine can theoretically result from short- or long-term increases in synthesis, decreases in binding, increases in release into the synaptic cleft, decreases

BEHAVIORAL EFFECTS OF THE DIRECT CNS ACTION OF EPINEPHRINE AND NOREPINEPHRINE

DATE	INVESTIGATOR	ANIMAL	COMPOUND	BEHAVIORAL EFFECT
1914	Bass	Dog	Epinephrine	Sedation and sleep
1929	Marinesco et al.	Cat	Epinephrine	Lethargy and sleep
1947-50	Leimdorfer et al.	Dog and man	Epinephrine	Behavioral depression, deep anesthesia
1947	Seifter et al.	Chick	Epinephrine; norepinephrine	Lethargy and behavioral depression
1954-63	Feldberg	Cat	Norepinephrine; epinephrine	Stupor, sleep anesthesia
1955	Sherwood	Man	Epinephrine	Lethargy and drowsiness
1957	Reitter	Dog	Epinephrine	Deep anesthesia
1957	Haley et al.	Rat	Epinephrine	Lethargy and stupor
1958	Rothballer	Cat	Epinephrine	Soporific action
1959	Palmer	Sheep	Epinephrine	Sedation, sleepiness
1962-63	Marley and Key	Chick, kitten	Norepinephrine; epinephrine	Sedation and sleep
1963	Milhaud and Glowinski	Rat	Norepinephrine	Unconsciousness
1964	Myers	Cat	Epinephrine	Drowsiness
1964-68	Spooner and Winters	Chick	Norepinephrine Antagonism of amphetamine	Behavioral depression, sedation, sleep
1965	Maas	Dog	Norepinephrine	Lethargy and somnulence
1966	Abbuzzahab	Chick	Norepinephrine	Sedation
1966	Havlicek et al.	Rat	DOPS→(Norepinephrine)	Behavioral depression
1966	Brittain	Mouse	Norepinephrine; imipramine and norepinephrine	Sedation and imipramine antagonism
1968	Mandell, Spooner et al.	Chick	Norepinephrine; imipramine and norepinephrine	Behavioral depression and imipramine antagonism
1968	Creveling et al.	Rat	DOPS failed to reverse reserpine depression - DOPA did	
1968	Findley and Thompson	Ox	Norepinephrine	Sedation

Fig. 1. A summary of the results of research by a number of investigators reporting monotonic, dose-related behavioral effects of norepinephrine and epinephrine administered in order to cross the blood-brain barrier (intracisternally, intraventricularly, or intravenously into animals with immature blood-brain barriers). Though the dose of catecholamine was invariably much larger than the amount normally found in brain, in those studies when graded smaller amounts were used, evidence of excitement was not reported. Either there was no effect or behavioral depression (*30*).

in reuptake binding, and decreases in inactivation by *O*-methylation (*32*). For example, a relative increase in brain *O*-methylated metabolites is thought to result from either an increase in synaptic release or a decrease in synaptic reuptake of norepinephrine (greater availability to the synaptic or postsynaptic catechol-*O*-methyltransferase). A relative increase in acid metabolites is supposed to reflect nonsynaptic release from storage sites and oxidation by mitochondrial monoamine oxidase. Practically all the drugs that influence mood and motility in man have been tied to this model (*33*), in spite of the fact that, without exception, no drug thus explained acts on norepinephrine alone. For example, reserpine in man produces syndromes resembling "endogenous" depression and depletes brain norepinephrine. But this substance also depletes serotonin, dopamine, and perhaps other amines (*16*). The mechanisms of action of many other drugs in common clinical use, which influence mood or motility in normal and psychopathological states in man, have been explained by this norepinephrine theory (*33*). They include cocaine, amphetamines, monoamine oxidase inhibitors, lithium [which appears to act on norepinephrine stores much like reserpine and yet has been shown to prevent recurring depressions (*34*)], and the phenothiazines (*33*). Experimentally, metabolic inhibitors have been used to test the norepinephrine hypothesis. For example, α-methyl-*p*-tyrosine, thought to be a specific inhibitor of tyrosine hydroxylase [it also inhibits phenylalanine hydroxylase resulting in phenylketonuria (*35*)], in doses which significantly impair catecholamine synthesis in man (*36*), failed to produce depression of mood in a group of schizophrenics. Because the nonspecificity of metabolic systems effected by a specific drug is exemplified by the broad range of chemical systems affected by reserpine, perhaps it is worthwhile to demonstrate the kinds of problems encountered when we work with a behaviorally active drug within a particular system. This can be done by examining some studies, including our own, of the effects of tricyclic antidepressant drugs on behavior and dynamics of norepinephrine.

The "tricyclic" psychotropic drugs, such as imipramine, appear to be the most clinically efficacious antidepressants available in the treatment of depression (*37*). Rather compelling research, with intraventricular C^{14}-labeled norepinephrine in rats, shows that this drug leads to a relative increase in the *O*-methylated metabolite, normetanephrine as well as to a decrease in brain uptake of intraventricularly administered ^{14}C-norepinephrine (*33*). This has been interpreted as suggesting that this drug family potentiated the central effect of norepinephrine by preventing its reuptake by presynaptic terminals with increase of concentration at the postsynaptic site. Findings from two major areas of research serve as impediments to the complete acceptance of this interpretation. The first group of studies revealed that imipramine in the dose selected and the time chosen to use animals for biochemical studies produced lethargy, psychomotor retardation, and reduced alertness in mice, rats, cats, and man (*37*). The second group of apparently discontinuous studies is summarized in Fig. 1.

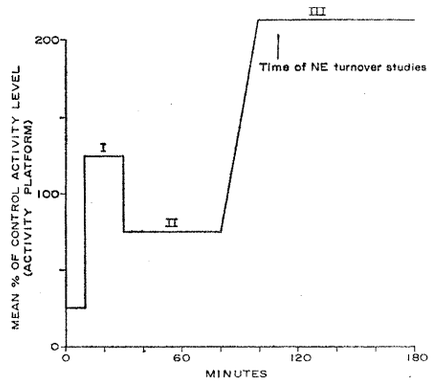

Fig. 2. Triphasic effect of imipramine on activity. The elapsed period after a psychopharmacological drug is administered appears to be critical for the timing of neurochemical studies. Whereas most chemical studies of imipramine (an antidepressant) appear to be done in behavioral state II of the animal, the metabolic results seen in Fig. 3 were obtained during phase III, when the animal was hyperactive.

There has been a body of research since 1914 in which epinephrine or norepinephrine, when given so that it passes the blood-brain barrier (that is, intracisternally, intraventricularly, or intravenously into animals with immature blood-brain barriers), produces a monotonic dose-response pattern of behavioral depression, lethargy, sleep, sedation, and stupor even in man (*22–26*). Most iontophoretic applications of norepinephrine directly into brain have shown predominantly a suppression of units (*28*). With these and other considerations in mind our group began a series of behavioral and biochemical studies to reevaluate both the role of norepinephrine in behavior and the effects of the tricyclic antidepressant imipramine on behavioral and biochemical parameters.

A problem in doing animal research with psychotropic agents like imipramine, which require long-term administration in order to achieve a clinical effect, is the timing of the behavioral and biochemical measurement after the administration of the drug or both. It has frequently been reported that in man imipramine has a biphasic action characterized by a period of mild sleepiness and lethargy before the antidepressant action begins. Wallach and coworkers (*38*) reported a triphasic action of a single dose of imipramine on reticular units in the cat. After administration of imipramine, in phase I there was a brief increase in reticular unit activity lasting 20 to 30 minutes, then a prolonged decrease in reticular units lasting up to 18 hours in phase II, and then a marked and general increase in unit activity in phase III, lasting a few days. Behavioral changes in our experimental animal, the newborn chick, mirror this triphasic sequence (Fig. 2). Previous work on the rat showed that when labeled norepinephrine was administered intraventricularly and preceded (or followed) by imipramine, there was a relative increase in the *O*-methylated product, normetanephrine (*33*). The time course of these studies suggests that the rat was, if anything, behaviorally depressed by the imipramine at the time of the norepinephrine turnover. When norepinephrine is administered intravenously to the chick [with an immature blood-brain barrier, an "adult" electroencephalogram, and typical responses to a wide range of psychotropic agents (*39*)], it produces behavioral depression and antagonizes the behavioral activating effect of imipramine (*24, 25*).

The pattern of radioactivity of the metabolites (Fig. 3) is consistent with a decrease in synaptically active norepinephrine (a decrease in normetanephrine) (*40*). Thus, the story about imipramine, antidepressant action, and brain norepinephrine becomes quite complicated. The elision of behavioral depression in animals with clinical depression in man exemplifies some problems in the use of animal models for human behavioral states as well as the complexities of species differences. Even the clinical syndrome used as the target behavior to modify with drugs may be quite heterogeneous. Kielholz and Poeldinger

	CONTROL (N=5) DPM/gm Brain ± S.E.M.	IMIPRAMINE (N=5) DPM/gm Brain ± S.E.M.	P
NE-C^{14}	3595.0 ± 579.6	2800.2 ± 510.8	Not sig.
NME-C^{14}	846.6 ± 167.7	230.6 ± 58.9	< .01
VMA-C^{14}	177.5 ± 33.4	154.0 ± 39.3	Not sig.

Fig. 3. Patterns of radioactivity of brain norepinephrine and metabolites as effected by imipramine when studied during the hyperactive phase of the drug's action. These results suggest that imipramine may *block* norepinephrine access to the postsynaptic membrane [a decrease in brain normetanephrine (NME)] at this time. This finding appears to be at variance with current concepts of the central role of imipramine and norepinephrine (NE). VMA, 3-methoxy-4-hydroxyvanylmandelic acid.

(*41*) and others demonstrated a spectrum of clinical depressive syndromes differentially responsive to categories of antidepressant drugs by the use of indices of agitation and anxiety as predictors. Imipramine, for example, appears to be more useful for the retarded depressives than for the patients with agitated depressions. Yet both types of patients belong clinically to the same diagnostic categories and have the same range of genetic histories (*42*). At the present time, the neuropharmacological-psychochemical research approach rests on insecure bases both in terms of the establishment of what are necessary and sufficient conditions in brain chemistry to explain the metabolic effects of the drugs and in terms of the connection of these changes with predictable behavioral phenomena.

2) *The administration to man of loads of precursors of metabolic substances hypothesized to have central nervous system action with behavioral ramifications.* Very generally, the logic of this approach is that, by selectively increasing the amount of substrate available for a metabolic pathway, there will be an increase in the end product (or end products) of interest in the brain; that an increase in this product will result in an exaggerated expression of its normal central effect which will reflect itself in observable changes in behavior. Because many centrally active substances will not pass the blood-brain barrier and their precursors will, this approach seems especially suited to man.

Perhaps the classic group of studies with this strategy in animals and man made use of 3,4-dihydroxyphenylalanine loads (the precursor of both dopamine and norepinephrine) to reverse reserpine-induced behavioral depression. Carlsson, Lindqvist, and Magnusson demonstrated this in animals in 1957 (*43*). This effect has been attributed by many workers to the replenishment of brain norepinephrine levels (*4*). Degkwitz and co-workers (*44*) reported that the reversal of reserpine induced depression in man with 3,4-dihydroxylalanine loads. However, another group of investigators, using a monoamine oxidase inhibitor in conjunction with 3,4-dihydroxyphenylalanine to retard the degradation of the hypothetical mood elevator, norepinephrine, failed to demonstrate any elevation of mood in a population of depressed patients (*45*). In addition, some recent work casts some serious doubt on the applicability of the 3,4-dihydroxyphenylalanine-reserpine reversal phenomenon to a norepinephrine theory of mood. Havlicék and associates (*22*) demonstrated behavioral depression after they administered dihydroxyphenylserine (which is decarboxylated to norepinephrine without dopamine as an intermediate). Creveling (*26*) confirmed and extended these observations with the finding that in animals behavioral depression induced by reserpine was not reversed when the brain norepinephrine levels were restored to their previous levels by dihydroxyphenylserine.

Whereas the 3,4-dihydroxyphenylalanine load approach to a norepinephrine theory of mood includes the possibility that an intermediate, dopamine, may be the substance responsible for the observed induced changes in behavior, the strategy of the indole-amino acid load (with and without monoamine oxidase inhibitors) brings with it another problem of product specificity. During the early days of the "monoamine game," there was an initial focus on serotonin, the decarboxylated product of 5-hydroxytryptophan, rather than on norepinephrine. Reserpine had been shown to deplete this brain amine associated with the production of behavioral depression in animals and man; the hydrazine monoamine oxidase inhibitors, the first effective antidepressants, increased brain serotonin (*46*). Since that time, the story has become very much more complex. Carlsson and co-workers (*43*), in the same series of experiments that demonstrated reversal of reserpine depression by 3,4-dihydroxyphenylalanine, showed that 5-hydroxytryptophan loads failed to reverse this effect. In spite of the dearth of studies uniquely implicating serotonin in studies of antidepressant drug action and the apparent sedative effects of serotonin when given so that it gets into the brain (*47*), the role of serotonin or other indoleamines in the elevation of mood is still of great interest. Rather than the findings of basic neuropharmacology keeping alive a theory in the face of negative precursor-load findings (as in the case of the norepinephrine theory of mood), the serotonin theory of mood elevation is being sustained by precursor-load studies even though basic neuropharmacological findings are absent or negative. Since the work of Zeller and collaborators (*48*) in 1957, a large number of investigators have reported various kinds of behavioral activation and antidepressant action of either tryptophan or 5-hydroxytryptophan loads when preceded by a monoamine oxidase inhibitor (which presumably retards the degradation of serotonin) (*49*). In perhaps the most careful series of studies with amino acid loads and monoamine oxidase inhibitors, Kety and his group (*50, 51*) found that, whereas glycine, histidine, phenylalanine, and tyrosine did not produce many significant behavioral effects when preceded by a monoamine oxidase inhibitor, tryptophan and methionine led to behavioral activation in a group of schizophrenic patients. Coppen, Pare, Kline, and others (*49*) have shown that the antidepressant action of the monoamine oxidase inhibitors is potentiated by indoleamino acid loads in depressed patients. This kind of evidence has been used (*49*) to suggest that the amount of serotonin in the brain may be related to mood. Other studies, in which methionine, or methionine in conjunction with tryptophan, was used in patients that had been treated with monoamine oxidase inhibitor (*51, 52*) also demonstrated behavioral activation in schizo-

phrenic patients. These findings suggest that a centrally active methylated indoleamine, such as bufotenine or dimethyltryptamine, rather than serotonin might be responsible for the indoleamino acid potentiation of the effect of monoamine oxidase inhibitors. This kind of suggestion would serve as an alternative hypothesis in explaining the poor relationship reported between the amount of behavioral activation and the brain levels of serotonin in some studies on animals (*53*), which had previously been explained by the better relationship observed between activation and amounts of norepinephrine (*54*). In a more general way, according to this kind of hypothesis, when the normal metabolic pathway of a substance is chemically blocked, there are many possibilities for the production of usually absent or insignificant by-products as well as the increase in more normal products; any or all of these by-products may well be responsible for the observed behavioral change. In the case of the *N*-methylated indoleamines, Fig. 4 represents the conjectured metabolic results of blocking the enzyme responsible for the degradation of the indoleamines in combination with appropriate precursor loads. Axelrod (*55*) has reviewed the enzymatic capacity of various organisms (including man) for such phenomena. Behavioral activation when the indoleamino acid loads are combined with a monoamine oxidase inhibitor is not just seen in depressed or schizophrenic man, but has been recently reported for normal human sleep in a series of studies by Williams and co-workers (*56*). They found that, whereas the soporific effects of phenylalanine loads were not changed by prior treatment with a monoamine oxidase inhibitor, those produced by tryptophan were reversed into signs of activation with the night's sleep marked by a large increase in spontaneous arousals. This same kind of reversal of indoleamino acid behavioral depression by prior treatment with a monoamine oxidase inhibitor has been reported (*57*) in the chick. This activation was correlated with an increase in labeled bufotenine (when the chick was administered ^{14}C-labeled 5-hydroxytryptophan). This increase in the *N*-dimethylated indoleamine was most marked in the lung, which Axelrod (*55*) reported to be the site of the nonspecific *N*-methyltransferases. Although the central action of the postulated indole hallucinogen, bufotenine, in man is far from established (*58*), it appears to be an interesting model with which to view the problem of nonspecificity of centrally active products when using the "precursor load strategy."

The previous discussion elucidates in some detail two current areas in which loads of precursors have been used. The possibility has been suggested that centrally active intermediate (for example dopamine with 3,4-dihydroxyphenylalanine loads) or by-products (like dimethyltryptamine and bufotenine with indoleamino acid loads) may be responsible for the observed changes in behavior rather than the substance to which these changes were attributed.

In addition to these relatively circumscribed sources of nonspecificity in such experiments, there are perhaps more important general ones. Studies of experimental phenylketonuria and of branch-chain amino acid loads in animals, for example, have shown that loads of one kind of precursor may inhibit the transport into the brain of others (*59*). These unbalanced amino acid loads may interfere with protein synthesis with resulting changes in the central nervous system. Loads of one substance may bind cofactors associated with the metabolism of other substances (*60*). Even in those cases in which there has been evidence of the desired effect of the load [for example, the measured increase in the *S*-adenosylmethionine pool with methionine loads (*61*)], there is always the possibility that many other concomitant effects are responsible for observed behavioral changes that follow loads of precursors.

3) *Sleep stage—an example of a unifying cross-species dependent variable.* It is quite difficult to find behavioral events that both man and animals share in a specific enough way to allow data and hypotheses on animals to be used in human research. Conditioning situations, which have been frequently used in this regard in the past, appear to be different events in animals and man, as recent trends in human behavioral analyses seem to indicate (*62*). Various emotional responses, such as fright, avoidance, and depression, have been used with equal problems of generalizations from animals to man. The advent of sleep stage analysis by Dement and Kleitman (*63*) and others, and especially the emphasis on a division between slow-wave and fast-wave electroencephalogram in sleep, presented an electrophysiological organization that

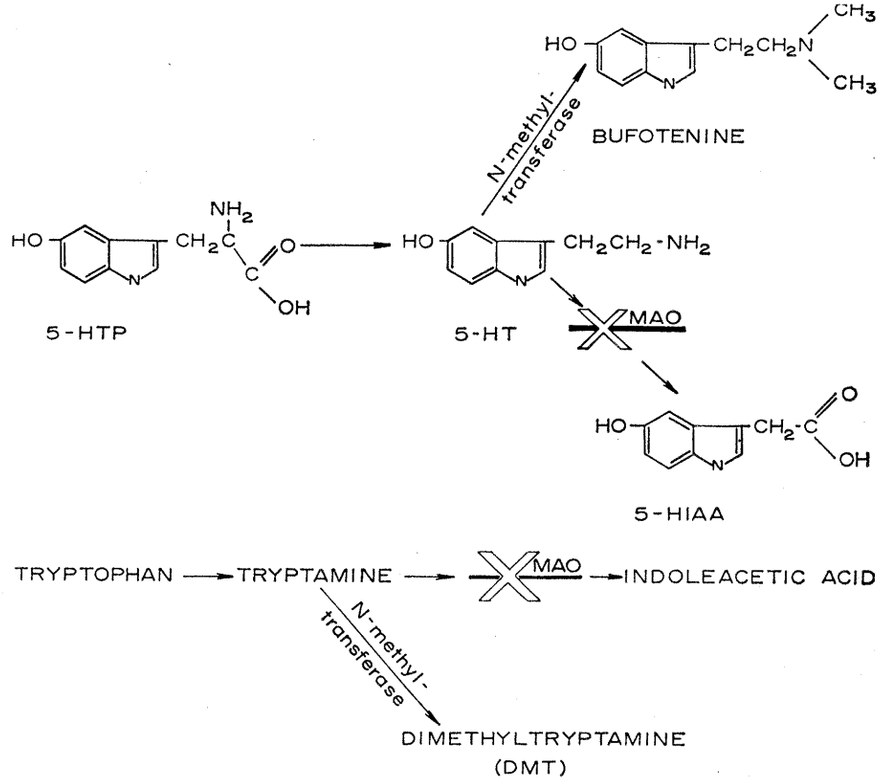

Fig. 4. A speculative metabolic scheme suggesting the possibility that by blocking a normal degradative pathway with a drug (a monoamine oxidase inhibitor), previously insignificant alternative pathways may become important (in addition to the production of an increment in the normal substrate).

is relatively constant across species. The episodic occurrence of "rapid eye movement sleep" is associated with a shift to low-voltage, fast-electroencephalogram, spikes along the pontogeniculate to occipital cortex pathways, loss of branchial muscular tone, respiratory and cardiovascular irregularities, erections, increases in urinary steroids, evidence for antidiuretic hormone release, and many other objective events in a wide variety of species (*5*). From man to chick, there appears to be oscillations from slow- to fast-wave sleep throughout the periods of sleep. Rebound in rapid eye movement sleep (REMS) time, pontogeniculo-occipital spikes, and other such measures after REMS deprivation as well as a refractory period for repeated electrical induction of REMS had suggested a neurohumoral substrate (*5*). A report of the increased ease with which this REMS rebound phenomenon can induce deprivation in schizophrenics in remission makes this postulated neurohumoral substrate of acute clinical interest (*64*). Other suggestions of changes in brain chemistry derive from studies of the deprivation of REMS in animals that lead to evidence of long-lasting increases in excitability of the central nervous system including lower seizure threshold, changes in the auditory system, and long-lasting increases in heart rate (*65*). Since the biogenic amines that have been a principal focus of general neurochemical attention during the past several years have been serotonin and norepinephrine, it was only too predictable that these substances be used in research directed toward deriving concepts of the chemical substrates of sleep stage. Two groups of representative studies centered around these two monoamines will be reviewed.

Oswald and co-workers (*66*) found that L-tryptophan, when administered to humans, led to a decrease in latency to the first epoch of REM sleep. Mandell and colleagues (*67*) reported that total REM time (percentage) increased during the all-night infusion of 5-hydroxytryptophan. Hartman (*5*), using L-tryptophan, also reported a small increase in the relative percentage of the night spent by human subjects in rapid eye movement sleep. In addition, he found that a small dose of reserpine (which releases biogenic amine) also resulted in a relative increase in REMS. Williams and others (*56*) found that L-tryptophan increased the amount of slow-wave sleep in all subjects and REMS tendency (latency to the first REMS period) in a few. In most of these studies it has been assumed that, by increasing the amount of available indoleamino acid substrate, the amine product serotonin would be increased in the brain. Spooner and Winters (*68*) in a series of studies in the young chick (with a reduced blood-brain barrier to the biogenic amines), were able to demonstrate more directly the induction of behavioral and electrophysiological sleep by injection of serotonin in a monotonic, dose-response manner. Weitzman (*69*), administered an inhibitor of tryptophan hydroxylase, *p*-chlorphenylalanine, to an animal which then, as a result, showed reduced slow-wave sleep and markedly lowered brain serotonin levels. Jouvet (*5*), in a series of studies with cats, has shown that reserpine, given at a "unique" dose of 0.5 milligram per kilogram of body weight, suppressed both slow-wave sleep and rapid eye movement sleep for several hours. Slow-wave sleep was returned with the administration of the serotonin precursor, 5-hydroxytryptophan. These and similar studies had led to a "serotonin school" of sleep with an apparent disagreement about which sleep stage is most influenced by this biogenic amine.

As noted, work going back as far as that of Bass in 1914 implicated epinephrine and norepinephrine in the production of soporific states in various animals including man, when these substances were given so that they get into the brain (*22*). Jouvet emphasized the role of norepinephrine in REM sleep as the result of his experiments showing that dihydroxyphenylalanine (a dopamine and norepinephrine precursor) returns that stage of sleep in cats given reserpine. Lester and co-workers (*70*) reported that patients on a diet deficient in phenylalanine and tyrosine have reduced REM sleep with no reduction in slow-wave sleep; this state was reversed with the reintroduction of the missing amino acids into their subjects' diet. They speculated that these changes were due to a decrease in norepinephrine induced by substrate deficiency. These kinds of studies have buttressed a "norepinephrine school" of sleep with some continuing conflict about the particular sleep stage involved.

In some of our work we have used the newborn chick with an immature blood-brain barrier and a mature electroencephalogram. Using an activity platform, behavioral rating scales, electroencephalograms, and evoked potentials (*71*), we showed that a number of amino acids produce many elements of the sleep syndrome. All these amino acids have been shown to be present in free form in the central nervous system (*72*) (Fig. 5). Mandell and Mandell reviewed studies implicating a number of other chemicals in the role of "sleep substances" including acetylcholine, aliphatic or hydroxy organic acids, steroid and polypeptide hormones, and aromatic amines (*5*). Thus, it appears that what initially seemed to be a relatively standard, cross-species system of dependent variables (sleep and sleep stage) for use in the study of brain chemistry that would be applicable to man seems to be a very complex situation. These electrophysiological states appear to be the end product of the action of a large number of potential brain-chemical systems. Whether further study will reveal a single common chemical pathway to explain the action of all these chemical compounds or simply reflect the limits of the number of response patterns the central nervous system can make to a variety of chemical manipulations remains to be seen.

4) *The search for metabolic errors that are reflected in changes in both body chemistry and behavior.* Looking for evidence of metabolic disease, workers in this area study the body fluids of various behaviorally defined groups of humans (that is, the mentally retarded, epileptics, psychotics, depressives, autistic children, and other groups with relatively stereotyped behavioral deformations). Attempts are made to relate changes in amounts of normal or abnormal metabolites or deviations from normal in the metabolic dynamics (such as turnover of administered load) with behavioral patterns. The goal of this approach is to establish the underlying metabolic defect and to relate the observed behavior to some direct (in the brain itself) or indirect (the brain's response to the alteration in its milieu) biochemical mechanism.

The most influential problem, with attempts at solution based on this approach that has served as a model for research workers using this strategy, was broken open by Fölling in 1934 (*73*). His discovery of marked increases in acid products of phenylalanine in the urine in a familial syndrome characterized by mental deficiency, defects in pigmentation, growth retardation, and a characteristic body aroma has led to a most extensive program of re-

search. Phenylketonuria was demonstrated to be an autosomal recessive genetic disease manifested by a marked decrease in the enzyme phenylalanine hydroxylase (*74*). This disease has served as a goad and inspiration to those who have been looking for metabolic errors in the more nebulous syndromes, such as depression or schizophrenia (which also have, though less well-defined, evidence of significant genetic factors). In attempting to understand some of the problems that exist in applying the "metabolic error" strategy to an understanding of the chemistry of the brain and behavior, it might be worthwhile to examine some behavioral and chemical studies derived from this thoroughly researched area. With increasing ease and application of case-finding techniques, a far broader range of behavioral manifestations is being reported as part of this syndrome. These include not only the range of mental deficiency that was part of the original descriptions, but also such features (varying from case to case) as seizures, agitation, hyperkinesis, apathy, passivity, emotional lability, destructiveness, even-temperedness, manifestations of a schizophrenic thought disorder, neurosis, psychosis, and normal intelligence (*75*). In heterozygotes for this syndrome an increased incidence of a wide diversity of psychopathological syndromes (*76*) has been reported. Even this completely identifiable metabolic disease altering brain function is manifested by a remarkable array of human behavior. An understanding of the neurochemical side of this disease is at least equally difficult at the present time. After the demonstration of the absence of a labile protein component of the liver-hydroxylating system in this syndrome and the elucidation of some of the changes in body fluids that resulted, the question remains—How are these abnormalities translated into the variegated behavioral syndromes that have been described? The specific brain biochemical system that is necessarily affected for the expression of the clinical syndrome has not yet been established. Studies of peripheral biochemical systems in man, and neurochemical studies in animals made "phenylketonuric" by diets with increases in phenylalanine, have suggested that the behavioral manifestations may be due to toxic effects of abnormal amines, as *O*-tyramine and phenylethylamine, of amino acid decarboxylase inhibition resulting in abnormalities in tryptophan, tyrosine, and glutamic acid metabolism in brain, of deficiencies in catecholamines, of increases in metabolism of tryptophan (along the pathway to indican) leading to tryptophan wastage, of serotonin deficiency, of abnormalities in protein and lipid synthesis, and of abnormalities in transport of a number of amino acids into the brain (*74*). A review of experimental work focused on just one of these systems leads to another splay of potential mechanisms. Using Auerbach's model for experimentally simulating this syndrome in rats by a diet high in phenylalanine, Yuweiler and Louttit demonstrated that the animal was impaired in some problem-solving tasks and associated with a significant decrease in their brain serotonin (*77*). This finding is consistent with the clinical chemical findings of a decrease in the urinary end product of serotonin, 5-hydroxyindoleacetic acid and a decrease in blood serotonin in patients with this disease (*78*). In a review and experimental test of the mechanisms that might produce this decrease in brain serotonin, Yuweiler and co-authors (*79*) considered the following specific and nonspecific inhibition of amino acid decarboxylases and tryptophan hydroxylase, inhibition of precursor transport through the blood-brain barrier, increases in monoamine oxidase activity, decrease in serotonin binding or storage capacity, and steroid-induced changes in tryptophan pyrrolase and amino transferase. Only some of these possibilities were ruled out. Thus it appears that little about the specific way a chemical abnormality can influence brain function and behavior can be adduced from this well-identified focus of research when the "metabolic error" strategy is used.

NO EFFECT	BEHAVIORALLY DEPRESSING AND/OR SOPORIFIC	BEHAVIORALLY ACTIVATING
Alanine	*5-hydroxytryptophan	*5-hydroxytryptophan
Arginine	*Tryptophan	*Tryptophan
Proline	Phenylalanine	β-alanine
Lycine	Tyrosine	Methionine
Serine	Glutamic acid	Dihydroxyphenylalanine
Valine	Aspartic acid	
Threonine	Histidine	
*Glycine	Leucine	
	Isoleucine	
	*Glycine	
	GABA	

Fig. 5. A summary of our studies on the behavioral effects of amino acid loads in the chick. Asterisk indicates dose- and time-dependent mixed responses.

The history of research in another metabolic disease characterized by intermittent psychosis and a number of indole compounds in the urine is an example of an even more seductive model for those working with biochemical methods in the so-called "psychogenic" psychoses. With the synthesis and study of a number of indole psychotogens (such as dimethyltryptamine, psilocybin, and lysergic acid diethylamide), it is no wonder that the first reports of Hartnup's syndrome (*80*), manifested by schizophrenic-like symptoms and various indole products in the urine, was looked upon as a potential model for the psychochemist (*81*). As research progressed, however, it became clear that this syndrome was probably the result of a congenital error in the renal and intestinal membrane transport of amino acids. The indoluria is probably the result of bacterial action on the unabsorbed tryptophan in the gut, and the central nervous system changes are probably due to a pellagra-like deficiency of nicotinic acid resulting from a reduction in one of its exogenous precursors, tryptophan (*82*). During the past decade, various macro- and micromolecular chemical moieties in the body fluids, which have been used to discriminate among groups of people with "psychiatric" disease, have come and gone. These studies have suffered from the same kinds of initially oversimplified theoretical assumptions, uncontrolled studies, unestablished mechanisms, and inept behavioral and chemical analyses, which Kety over a decade ago outlined (*83*). A possible exception to this trend is the relatively frequently repeated finding, first reported by Friedhoff and Van Winkle, of an abnormal methylated catecholamine (dimethoxyphenylethylamine) in the urine of schizophrenic patients (*84*). This observation converged with a neuropharmacologically derived hypothesis first put forward by

Harley-Mason (*85*), namely, that phenolic amines which are normally active only peripherally because of their inability to get through the blood-brain barrier could transcend this barrier and be centrally active if "hypermethylated" (*85, 86*). The demonstration of the enzymatic capacity of the liver from schizophrenics to synthesize dimethoxyphenylethylamine and its positive identification by mass spectrometry (*87*) are promising. The large amount of this compound required to demonstrate central action when compared with the amount of this amine and its acid end product in urine suggests that the meaning of this finding is probably quite complex (*88*). Does a single dose, for example, really mimic what might be an exposure to an abnormal metabolite over a long period of time? Perhaps the use of such a disease as familial high-density lipoprotein deficiency (*89*) which gradually becomes more symptomatic over the years, rather than the use of an acute toxicity syndrome, such as gout (*90*) as a model would make this single test of the potency of dimethoxyphenylethylamine in the central nervous system inappropriate. Perhaps the discovery of this substance in urine is just one of the manifestations (but not a sufficient one) of a more general defect in transmethylation (*86*). Thus, there is a possibility that a number of abnormally methylated amines may be responsible for the observed behavioral state. The report of the appearance of bufotenine in the urine of schizophrenics 2 weeks before the appearance of acute symptomatology when given monoamine oxidase inhibitors might be another example of the same general phenomenon (*91*). Another theoretical possibility that would account for the irregular finding or effect of this kind of abnormal metabolite derives from a source too often neglected in psychochemical research—changes in regulatory dynamics of the kinds being elucidated in molecular biology. That the group involved with relating chemistry to behavior should be thus far rather insensitive to the suggestions growing out of studies in metabolic regulation is especially difficult to understand in light of the apparent theoretical coherence between concepts of the phasic manifestations of human behavioral states and their sensitivity to environmental conditions and observations explaining the graded shifts from latency to expression of metabolic mechanisms in such areas as enzyme regulation.

Metabolic Regulation: Psychochemical Research Strategies

In psychochemical studies the researcher who looks for metabolic factors concomitant with behavioral states, if he uses the "metabolic error" strategy, appears to be using the "one gene, one enzyme" concept (*92*). This formulation suggests that a single gene is responsible for the presence or absence of a cell's capacity to carry out a single metabolic process. This inheritable characteristic was thought to be either present or absent, and the chemical manifestations of either of these states could be demonstrated in an all-or-none way. The implications of such a position for the research scientist looking for peripheral evidence of metabolic abnormalities in behavioral states with significant genetic histories (such as some forms of mood disorders or schizophrenia) are clear. Evidence of a metabolic error should always be demonstrable, independent of the time, environmental situation, or psychological status. With the advent of the new era in thinking about metabolism and the shift in emphasis from mere concern for the delineation of the sequential mechanisms for the synthesis, utilization, and degradation of metabolites to a focus on how these processes are regulated and integrated, a myriad of regulatory mechanisms began to be elucidated. Velocities of enzymatic reactions are seen as a joint function of a number of important factors including transport of substrate into cells or organs, amounts of available enzymes, characteristics of these enzymes, responsitivity of enzymatic mechanisms to activators or inhibitors and intracellular location or attachment of enzymes (*93*). In addition, numerous scientists focus on the operation of a hypothetical system of mechanisms involving nucleic acid polymers regulating in a graded way the expression of enzymatic potential as portrayed by Jacob and Monod (*94*). Information influencing these processes could apparently be derived from within the cell (such as the effective concentration of metabolic intermediates) as well as influences from one organ system to another (such as neural control of the pituitary-adrenal axis) with resulting changes in remote organs such as the liver (*95*). One important implication of these newer concepts of genetically derived modulatory complexity (one gene–one polypeptide, one protein, one enzyme, one regulatory mechanism, or some combination) is that both behavioral state and metabolic evidence can traverse a wide range of manifestations depending upon a number of circumstances impinging on the organism at the time of any particular study. Both abnormalities in structural genes (for example, glucose-6-dehydrogenase deficiency) as well as a mutation in a regulatory gene (such as in acute intermittent porphyria) often required a definite environmental trigger (the ingestion of particular drugs) for the manifestation of both the clinical syndrome and its concomitant metabolic changes (*96*). The various nutritional, hormonal, drug, precursor load, and other influences on mammalian enzyme regulation have been well studied (*95*). These graded metabolic responses to various kinds of alterations in base line conditions seem to fit human behavioral patterns as we know them: phasic shifts in mood, temporary changes in life style, intermittent attacks of psychosis, and psychosomatic illness apparently precipitated by alterations in life's stresses (*97*). These changes in human behavior last varying lengths of time and some peripheral biochemical correlates have already been tied to them: a transient emotional change leads to an increase in plasma-free fatty acids (*98*); the breakdown of psychological defenses in psychotherapy or in real life stresses, such as the experience by a parent of the irregular clinical course of a leukemic child, is reflected by indices of adrenocortical function (*99*); acute, intermittent catatonic episodes lasting several days are associated with changes in the metabolism of nitrogenous substances (*100*); mood shifts lasting many months, such as seen in the manic-depressive psychoses, are characterized by changes in body electrolyte metabolism and plasma and urine corticosteroids (*49*). The strategy dictated by this kind of thinking leads to the use of repeated measures on the same subject made over a length of time in which he passes in and out of the behavioral state of interest. A presently irrefutable objection to such work with respect to the elucidation of links in causal chains is that these chemical findings may be in response to rather than the determiner of behavioral states. The investigator is left with correlates rather than causes, but, as adduced from the foregoing discussion of more basic neurochemical data, this is the same for the present state of the art in brain chemistry as well.

We now present some data from

studies exemplifying the use of both repeated measures before and after the onset of a behavioral state and an attempt to capture some of the complexity possible in psychochemical research when metabolic regulatory factors are considered. Previous work suggested that release of adrenal glucocorticoid increased during depression (*99*). In addition, a series of studies beginning with that of Knox in 1951 (*101*) suggested that adrenal corticosteroids altered some hepatic amino acid enzymes. We endeavored to combine these two research themes. At first, an attempt was made to adapt turnover techniques to obtain a peripheral reflection of stress-induced metabolic adaptations. The goal was to detect parallel peripheral biochemical changes reflecting those that have been measurable with the use of direct kinetic studies of hepatic enzymes or precipitable protein, without serial tissue biopsies in human subjects. We studied the radioactivity of products of several alternative pathways of tryptophan metabolism simultaneously, under control and artificial stress conditions (adrenocorticotropic hormone injection) after infusion of metabolically insignificant amounts of ^{14}C ring-labeled tryptophan (*102*) in man.

After the administration of adrenocorticotropic hormone there was a significant rise in the radioactivity of the products of the two inducible pathways (kynurenine and indole-3-acetic acid), but not in the products of the noninducible pathways, 5-hydroxyindoleacetic acid, indolylacroylglycine, and *N*-acetyltryptophan. Having demonstrated that a rise induced by adrenocorticotropic hormone in circulating corticoids produced a demonstrable metabolic shift in tryptophan metabolism, our next step was to see if this relation could be demonstrated in patients with psychiatrically related corticoid changes. For this study, rapidly cycling manic-depressive patients were used. Multiple biochemical and behavioral measures were made daily for several months in which intermittent studies on ^{14}C-tryptophan turnover were done. Figure 6 is a representation of the daily values of a number of determinations on one patient. The turnover of ^{14}C-tryptophan to kynurenine on 16 December and 6 January, during a depressive phase, was significantly increased and associated with a rise in 17-hydroxycorticosteroids. A report of a hydrocortisone-induced decrease in brain serotonin may be consistent with our demonstration of a steroid-related increase in the breakdown of the indole nucleus through the kynurenine pathway (*103*).

Studies of peripheral biochemical correlates of behavioral states under various conditions, may, in the future, lead to the demonstration of differences in metabolic adaptive capacity rather than stable defects. The long history of psychiatric clinical research in which attempts were made to tie premorbid psychological strength and amount of environmental stress to subsequent deformations in behavior (called mental illness) might reveal some biochemical factors associated with, in part, abortive behavioral adaptation. It is of interest that in spite of the demonstration that glucocorticoids influence the intermediary metabolism of a number of amino acids (*95*), such metabolic hypotheses of mental disease as Harley-Mason's "transmethylation hypothesis" (*85*) have not been studied relative to the possible corticoid influence on the enzymatic system involved. Studies demonstrating the corticosteroid sensitivity of a specific methyltransferase, phenylethanolamine-*N*-methyltransferase in the adrenal medulla which converts norepinephrine to epinephrine (*104*) may also apply to the nonspecific methyltransferases that can carry out such reactions as the conversion of serotonin to bufotenine. Might temporary steroid or other hormonal influences on such metabolic mechanisms explain the intermittency of "attacks" of schizophrenia precipitated by life stresses? It would seem that the era of research in biochemical regulation is soon to influence styles in psychochemical research strategies.

Summary

The current state of the art in theory and methodology in some areas of research attempting to relate biochemical phenomena to brain function in man with particular relevance to behavioral states is presented. The reservations that a critical witness must have about the establishment of any substance as a physiological modulator or transmitter in the central nervous system are discussed. Four of the many research strategies currently in use were described, with examples.

As compared with other areas of re-

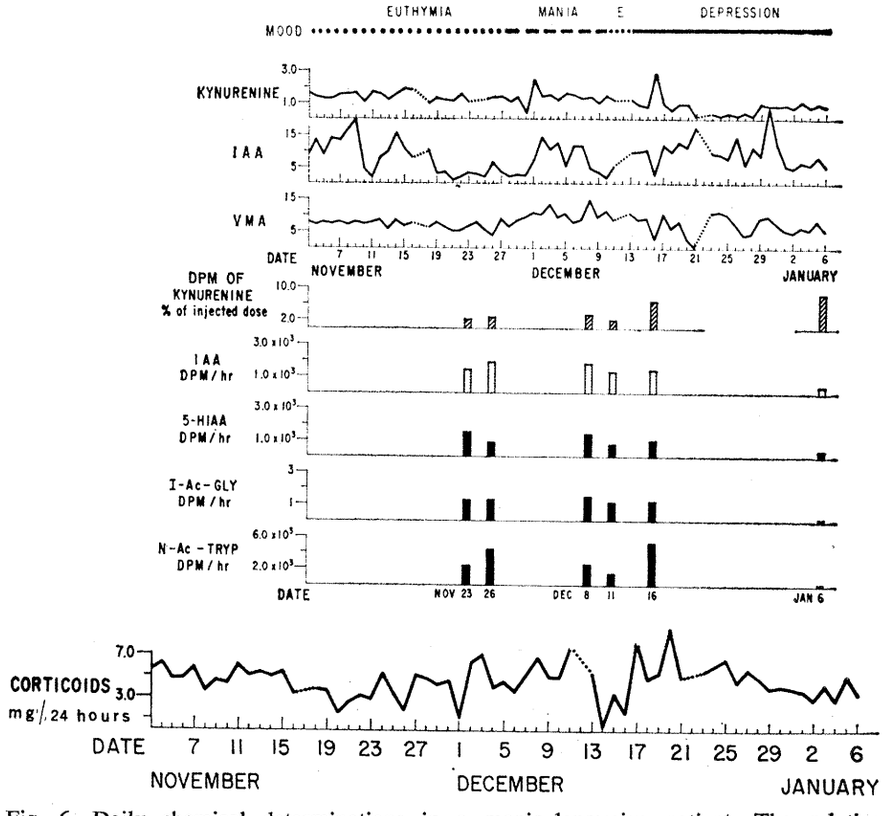

Fig. 6. Daily chemical determinations in a manic-depressive patient. The relative increase in the turnover of C^{14}-labeled tryptophan to kynurenine when compared with the products of alternative pathways of tryptophan is seen on 16 December and 6 January during the depressive phase. The cortecoid excretion is shown in milligrams per 24 hours.

search in biology the kinds of assumptions, operations, and conclusions extant in this field are crude. However, when one compares work from earlier decades in this area with current activity, it is clear that the psychochemist is benefiting from the basic advances in chemistry and biology.

References and Notes

1. T. S. Kuhn, *The Structure of Scientific Revolutions* (Univ. of Chicago Press, Chicago, 1962).
2. J. F. Fulton, *A Textbook of Physiology* (Saunders, Philadelphia, 1955).
3. P. A. Shore, *Pharmacol. Rev.* **14**, 531 (1962).
4. J. J. Schildkraut and S. S. Kety, *Science* **156**, 21 (1967).
5. M. Jouvet, *Physiol. Rev.* **47**, 117 (1967); H. Roffwarg, J. Muzio, W. Dement, *Science* **152**, 604 (1966); E. Hartman, *The Biology of Dreaming* (Thomas, Springfield, Ill., 1967); F. Snyder, *Amer. J. Psychiat.* **122**, 377 (1965); A. J. Mandell and M. P. Mandell, *ibid.*, p. 391.
6. A. J. Mandell and M. P. Mandell, Eds., *Methods and Theory in Psychochemical Research in Man* (Academic Press, New York, in press).
7. H. Dale, *J. Mt. Sinai Hosp.* **4**, 401 (1938).
8. B. Katz, *Proc. Roy. Soc. (London)* **155**, 455 (1962).
9. P. Fatt and B. Katz, *J. Physiol.* **117**, 109 (1952).
10. C. O. Hebb, *Physiol. Rev.* **37**, 196 (1957).
11. J. Del Castillo and B. Katz, *J. Physiol* **124**, 560 (1954).
12. V. P. Whittaker, *Progr. Biophys. Mol. Biol.* **15**, 39 (1965).
13. R. M. Krupka, *Biochemistry* **2**, 76 (1963).
14. I. B. Wilson and S. Ginsburg, *Biochim. Biophys. Acta* **18**, 168 (1955).
15. R. Birks and F. C. MacIntosh, *Can. J. Biochem. Physiol.* **39**, 787 (1961).
16. V. S. von Euler, S. Rosell, B. Uvnäs, Eds., *Mechanisms of Release of Biogenic Amines* (Pergamon, New York, 1966).
17. D. R. Curtis, in *Nervous Inhibition*, E. Florey Ed. (Pergamon, New York, 1961); V. J. Wilson, W. H. Talbot, M. Kato, *J. Neurophysiol.* **27**, 1063 (1964).
18. R. Galambos *Proc. Nat. Acad. Sci. U.S.* **47**, 129 (1961); G. Svaetichin, R. Fatehchand, B. D. Drujan, M. Laufer, P. Witkovsky, K. Negishi, A. Selvin de Testa, *Acta Cient. Venezolana, Suppl.* **1**, 135 (1963).
19. M. Vogt, *J. Physiol.* **123**, 451 (1964); A. Carlsson, *Pharmacol. Rev.* **11**, 490 (1959); M. McLennan, *Synaptic Transmission*, (Saunders, Philadelphia, 1963).
20. J. A. Michaelson, V. P. Whittaker, R. Laverty, D. F. Sharman, *Biochem. Pharmacol.* **12**, 1450 (1963); E. De Robertis, *Pharm. Rev.* **18**, 413 (1966); V. P. Whittaker, *ibid.*, p. 401.
21. A. Dahlstrom and K. Fuxe, *Acta Physiol. Scand.* **60**, 293 (1964); K. Fuxe, *Acta Physiol. Scand.* (*Suppl.*) **64**, 247 (1965).
22. A. Bass, *Z. Ges. Neurol. Psychiat.* **26**, 600 (1914).
23. G. Marinesco, O. Sager, A. Kreindler, *ibid.* **119**, 277 (1929); A. Leimdorfer and W. R. T. Metzner, *Amer. J. Physiol.* **157**, 116 (1949); N. V. Clymer and J. Seifter, *J. Pharmacol.* **89**, 149 (1947); W. Feldberg, *A Pharmacological Approach to the Brain from its Inner and Outer Surface*, (Arnold, London, 1963); S. L. Sherwood, *Proc. Roy. Soc. Med.* **48**, 855 (1955); H. Reitter, *Anaesthesist* **6**, 131 (1957); T. J. Haley and W. G. McCormick, *Brit. J. Pharmacol.* **12**, 12 (1957); A. B. Rothballer, *Pharmacol. Rev.* **11**, 494 (1959); A. C. Palmer, *J. Physiol.* **149**, 209 (1959); E. Marley and B. J. Key, *Electroencephalogr. Clin. Neurophysiol.* **15**, 620 (1963); G. Milhand and J. Glowinski, *Compte Rendu Acad. Sci. Paris* **236**, 1033 (1963) R. D. Myers, *Can. J. Psychol.* **18**, 6 (1964); C. E. Spooner and W. D. Winters, *Experientia* **21**, 256 (1965); *Int. J. Neuropharmacol.* **5**, 217 (1966); J. W. Maas and D. H. Landis, *Psychosom. Med.* **27**, 399 (1965); F. S. Abbuzzahab, *Fed. Proc.* **25**, 451 (1966); V. Havlicék and A. Sklenovsky, *Brain Res.* **4**, 345 (1967); J. D. Findlay and G. E. Thompson, *J. Physiol.* **194**, 809 (1968); E. Morley, *Pharm. Rev.* **18**, 753 (1966).
24. R. T. Britain, *J. Pharm. Pharmacol.* **18**, 621 (1966).
25. A. J. Mandell, C. E. Spooner, W. D. Winters, M. Cruikshank, I. M. Sabbot, *Proc. West. Pharmacol. Soc.* **11**, 81 (1968).
26. C. R. Creveling, J. Daly, T. Tokuyama, B. Witkop, *Biochem. Pharmacol.* **17**, 65 (1968).
27. D. J. Reis and L. M. Gunne, *Science* **149**, 450 (1965); F. Javoy, A. M. Thierry, S. S. Kety, J. Glowinski, *Com. Beh. Biol.* **43** (1968); R. J. Baldessarini and I. J. Kopin, *Science* **152**, 1630 (1966).
28. G. C. Salmoiraghi, *Pharm. Rev.* **18**, 717 (1966); J. W. Phillis, A. K. Tebecis, D. H. York, *J. Physiol.* **190**, 563 (1967); D. W. Stranghan, in *Amines and Schizophrenia*, H. E. Himwich, S. S. Kety, J. R. Smythies, Eds. (Pergamon, New York, 1967).
29. R. Werman, *Comp. Biochem. Physiol.* **18**, 745 (1966); K. Kuriyama, B. Haber, B. Sisken, E. Roberts, *Proc. Nat. Acad. Sci. U.S.* **55**, 846 (1966); L. T. Graham, R. P. Shank, R. Werman, M. H. Aprison, *J. Neurochem.* **14**, 465 (1967); L. Salgaincoff and E. De Robertis, *J. Neurochem.* **12**, 287 (1965); N. A. Hillorp, K. Fuxe, A. Dahlstrom, in *Mechanisms of Release of Biogenic Amines*, U. S. von Euler, S. Rosell, B. Uvnäs, Eds. (Pergamon, New York, 1966).
30. T. L. Perry, S. Hansen, L. MacDougall, *J. Neurochem.* **14**, 775 (1967).
31. M. D. Day and M. J. Rand, *J. Pharm. Pharmacol.* **15**, 221 (1963); I. J. Kopin, *Pharm. Rev.* **18**, 513 (1966); I. J. Kopin, in *The Neurosciences*, G. C. Quarton, T. Melnechuk, F. O. Schmitt, Eds. (Rockefeller Univ. Press, New York, 1967).
32. J. Glowinski and R. J. Baldessarini, *Pharm. Rev.* **18**, 1201 (1966).
33. I. J. Kopin and E. K. Gordon, *J. Pharmacol. Exp. Therap.* **138**, 351 (1962); J. J. Schildkraut, *Amer. J. Psychiat.* **122**, 509 (1965); J. J. Schildkraut, S. M. Schanberg, I. J. Kopin, *Life Sci.* **5**, 1479 (1966); S. M. Shanberg, J. J. Schildkraut, I. J. Kopin, *Biochem. Pharmacol.* **16**, 393 (1967); S. S. Kety, in *The Neurosciences*, G. C. Quarton, T. Melnechuk, F. O. Schmitt, Eds. (Rockefeller Univ. Press, New York, 1967); J. Glowinski and J. Axelrod, *Pharm. Rev.* **18**, 775 (1966).
34. P. C. Baastrup and M. Schou, *Arch. Gen. Psychiat.* **16**, 162 (1967).
35. M. A. Lipton, R. Gordon, G. Guroff, S. Udenfriend, *Science* **156**, 248 (1967).
36. S. Spector, A. Sjoerdsma, S. Udenfriend, *J. Pharmacol. Exp. Therap.* **147**, 86 (1965); S. Gershon, L. J. Hekimian, A. Floyd, L. E. Hollister, *Psychopharmacologia* **11**, 189 (1967).
37. G. L. Klerman and J. O. Cole, *Pharm. Rev.* **17**, 101 (1965); W. Banst, H. Niemczyk, J. Vieth, *Electroencephalogr. Clin. Neurophysiol.* **15**, 63 (1963); C. E. Spooner and W. D. Winters, *Int. J. Neuropharmacol.* **6**, 109 (1976); ———, A. J. Mandell, *Fed. Proc.* **25**, 451 1966).
38. M. B. Wallach, W. D. Winters, A. J. Mandell, C. E. Spooner, *Proc. Western Pharm. Soc.* **11**, 61 (1968).
39. C. E. Spooner and W. D. Winters, *Int. J. Neuropharmacol.* **5**, 217 (1966).
40. A. J. Mandell, C. E. Spooner, W. D. Winters, M. Cruikshank, I. M. Sabbot, *Life Sci.*, in press.
41. P. Kielholz and W. Poeldinger, *Compr. Psychiat.* **9**, 179 (1968).
42. G. Winokur and P. Clayton, in *Recent Advances in Biological Psychiatry*, J. Wortis, Ed. (Plenum, New York, 1967).
43. A. Carlsson, M. Lindqvist, T. Magnusson, *Nature* **180**, 1200 (1957).
44. R. Degkwitz, R. Fromein, C. Kulenkampff, V. Mohs, *Klin. Wochenschr.* **38**, 120 (1960).
45. G. L. Klerman, J. J. Schildkraut, L. L. Hasenbush, M. Greenblatt, D. G. Friend, *J. Psychiat. Res.* **1**, 289 (1963).
46. See historical material in B. B. Brodie and E. Costa, *Psychopharm. Serv. Ctr. Bull.* **1**, 1 (1962).
47. C. E. Spooner and W. D. Winters, *Experientia* **21**, 256 (1965).
48. E. A. Zeller, J. Bernsohn, W. M. Inskip, J. W. Lauer, *Naturwissenenschaft* **15**, 427 (1957).
49. Reviewed by A. Coppen, in *Brit. J. Psychiat.* **113**, 1237 (1967); and N. S. Kline, G. Simpson, W. Sacks, in *Neuropsychopharmacology*, H. Brill, J. O. Cole, P. Deniker, H. Hippius, P. B. Bradley, Eds. (Excerpta Medica, New York, 1967), p. 343–346.
50. S. S. Kety, *Fed. Proc.* **20**, 4 (1961).
51. W. Pollin, P. V. Cardon, S. S. Kety, *Science* **133**, 104 (1961).
52. H. Sprince, C. M. Parker, D. Jameson, F. Alexander, *J. Nerv. Ment. Dis.* **137**, 246 (1963); F. Alexander, G. C. Curtis, H. Sprince, A. P. Crosley, *ibid.*, p. 135; H. H. Berlet, C. Bull, H. E. Himwich, H. Kohl, K. Matsumoto, G. R. Pscheidt, J. Spaide, T. T. Tourlentes, J. M. Valverde, *Science* **144**, 311 (1964); J. Spaide, H. Tanimukai, J. R. Bueno, H. E. Himwich, *Arch. Gen. Psychiat.* **18**, 658 (1968).
53. A. Pletscher, K. F. Gey, W. P. Burkard, in *5-Hydroxytryptamine and Related Indolealkylamines*, O. Eichler and A. Farah, Eds. (Springer, New York, 1966).
54. B. B. Brodie, S. Spector, P. A. Shore, *Ann. N.Y. Acad. Sci.* **80**, 609 (1959).
55. J. Axelrod, in *Transmethylation and Methionine Biosynthesis*, S. K. Shapiro and F. Schlenk, Eds. (Univ. of Chicago Press, Chicago, 1965).
56. H. L. Williams, B. K. Lester, J. D. Coulter, paper presented at the Annual Meeting of the Association for Psychophysiological Study of Sleep, Denver, March 1968.
57. C. E. Spooner, A. J. Mandell, D. Brunet, M. Cruikshank, I. M. Sabbot, *Fed. Proc.* **27**, 540 (1968).
58. H. D. Fabing and J. R. Hawkins, *Science* **123**, 886 (1956); W. J. Turner and S. Merlis, *Arch. Neurol. Psychiat.* **81**, 121 (1959).
59. S. M. Schanberg and N. J. Giarman, *Biochim. Biophys. Acta* **41**, 556 (1960); S. W. Smith, *Brit. J. Pharmacol.* **20**, 173 (1963); K. D. Neame, *Nature* **192**, 173 (1961).
60. R. E. Tashien, *Metabolism* **10**, 393 (1961).
61. R. J. Baldessarini, *Biochem. Pharmacol.* **15**, 741 (1966).
62. I. Maltzman, paper presented at the Biometrics Research Workshop on Objective Indicators of Psychopathology, Tuxedo, New York, February 1968; G. Mandler, *New Directions in Psychology* (Holt, New York, 1967), vol. 3.
63. W. Dement and N. Kleitman, *Electroencephalogr. Clin. Neurophysiol.* **9**, 673 (1957).
64. V. Zarcone, G. Gulevich, T. Pivok, W. Dement, *Arch. Gen. Psychiat.* **18**, 194 (1968).
65. H. Cohen and W. Dement, *Science* **150**, 1318 (1965); W. Dement, P. Henry, H. Cohen, J. Ferguson, in *Sleep and Altered States of Consciousness*, S. S. Kety, E. Evarts, H. Williams, Eds. (William and Wilkins, Baltimore, 1967); J. Dawson, W. Dement, T. Wagener, K. Nobel, *Science* **156**, 403 (1967); V. Karadzic and W. C. Dement, *Brain Res.*, in press.
66. I. Oswald, R. Berger, J. Evans, V. Thacore *Electroencephalogr. Clin. Neurophysiol.* **17**, 603 (1964).
67. M. Mandell and A. Mandell, *Rec. Advan. Biol. Psychiat.* **7**, 115 (1964).
68. C. E. Spooner and W. D. Winters, *Brain Res.* **4**, 189 (1967).
69. E. D. Weitzman, M. M. Rapport, P. McGregor, J. Jacoby, *Science* **160**, 1361 (1968).
70. B. K. Lester, H. L. Williams, R. E. Chanes, P. T. Condit, *Amer. J. Psychiat.*, in press.
71. A. J. Mandell, C. F. Spooner, D. Brunet, in *Adv. Biol. Psychiat.*, in press.
72. H. H. Tallan, in *Amino Acid Pools*, J. T. Holden, Ed. (Elsevier, New York, 1962).
73. A. Folling, *Zeitsber. Physiol. Chem.* **227**, 169 (1934).
74. Relatively recent reviews of the clinical, genetic, and chemical features of this syndrome are in: F. L. Lyman, Ed., *Phenylketonuria*, (Thomas, Springfield, Ill., 1963); W. E. Knox, in *The Metabolic Basis of Inherited Disease*, J. B. Stanbury, J. B. Wyngaaden, D. S. Frederickson, Eds. (McGraw-Hill, New York, ed. 2, 1966); K. Closs, in *Molecular Basis of Some Aspects of Mental Activity*, O. Walaas, Ed. (Academic Press, New York, 1967), vol. 1, p. 231.
75. G. A. Jervis, *Res. Publ. Ass. Nerv. Ment. Dis.* **33**, 259 (1954); S. W. Wright and G. Tarjan, *Amer. J. Dis. Child.* **93**, 405 (1957); B. S. Sutherland, H. K. Berry, H. C. Shirkey, *J. Pediat.* **57**, 521 (1960); V. A. Cowie, *Lancet* **1957-II**, 272 (1957); R. J. Allen, R. M. Gibson, H. E. Sutton, *Amer. J. Dis.*

Child. **100**, 563 (1960); L. I. Woolf, C. Ounsted, D. Lee, M. Humphry, N. M. Cheshire, G. R. Steed, *Lancet* **1961-II**, 464 (1961); J. Bjornson, *Arch. Gen. Psychiat.* **10**, 65 (1964).
76. T. A. Munro, *Ann. Eugen.* **14**, 60 (1947).
77. A. Yuwiler and R. Louttit, *Science* **134**, 831 (1961).
78. M. D. Armstrong and K. S. Robinson, *Arch. Biochem.* **52**, 287 (1954); C. M. Pare, M. Sandler, R. S. Stacey, *Lancet* **1957-I**, 551 (1957).
79. A. Yuwiler, E. Geller, G. G. Slater, *J. Biol. Chem.* **240**, 1170 (1965).
80. D. N. Baron, C. E. Dent, H. Harris, E. W. Hart, J. B. Jepson, *Lancet* **1956-II**, 421 (1956).
81. A. J. Mandell, in *Recent Advances in Biological Psychiatry*, J. Wortis, Ed. (Plenum Press, New York, 1962).
82. J. P. Jepson, in *The Metabolic Basis of Inherited Disease*, J. B. Stanbury, J. B. Wyngaarden D. S. Frederickson, Eds. (McGraw-Hill, New York, ed. 2, 1966), chap. 57.
83. S. S. Kety, *Science* **129**, 1528 (1959); *ibid.* **129**, 1590 (1959).
84. A. J. Friedhoff and E. Van Winkle, *J. Nerv. Ment. Dis.* **135**, 550 (1962); *Nature* **194**, 897 (1962).
85. As discussed by H. Osmond and J. R. Smythies, *J. Ment. Sci.* **98**, 309 (1952).
86. Many of these issues are discussed by various authors in *Amines and Schizophrenia*, H. E. Himwich, S. S. Kety, J. R. Smythies, Eds. (Pergamon, New York, 1967).
87. A. J. Friedhoff and E. Van Winkle, *Nature* **199**, 1271 (1963); C. R. Creveling and J. W. Daly, *ibid.* **216**, 190 (1967).
88. J. P. Appel and D. X. Freedman, *Life Sci.* **4**, 2181 (1965); L. E. Hollister and A. J. Friedhoff, *Nature* **210**, 1377 (1966).
89. D. S. Frederickson, in *The Metabolic Basis of Inherited Disease*, J. B. Stanbury, J. B. Wyngaarden, D. S. Frederickson, Eds. (McGraw-Hill, New York, ed. 2, 1966), chap. 23.
90. S. È. Malawista and J. E. Seegmiller, *Ann. Int. Med.* **62**, 648 (1965).
91. H. Tanimukai, R. Ginther, J. Spaide, J. R. Bueno, H. E. Himwich, *Life Sci.* **6**, 1697 (1967).
92. E. L. Tatum, *Science* **129**, 1711 (1959); A. E. Garrod, *Lancet* **2**, 1 (1908); M. Wheldale, *J. Genet.* **4**, 109 (1914).
93. Elegant discussions of the historical evolvement of such concerns are reviewed by J. B. Stanbury, J. B. Wyngaarden, D. S. Frederickson, Eds., in *The Metabolic Basis of Inherited Disease* (McGraw-Hill, New York, ed. 2, 1966), chap. 1; D. Atkinson, in *The Neurosciences*, G. C. Quarton, T. Melnuchuk, F. O. Schmitt, Eds. (Rockefeller Univ. Press, New York, 1967).
94. F. Jacob and J. Monod, *J. Mol. Biol.* **3**, 318 (1961).
95. For numerous studies of hormonal effects on hepatic amino acid and carbohydrate enzymes, see F. Weber, Ed., *Advances in Enzyme Regulation* (Pergamon, New York, 1963–68), vols. 1–5.
96. W. C. Parker and A. G. Bearn, *Amer. J. Med.* **34**, 680 (1963).
97. A. J. Mandell and R. T. Rubin, in *Stress and Adaptation* (Forrest Hospital Publications, Des Plaines, Ill., 1965).
98. L. A. Gottschalk, J. M. Gleghorn, G. C. Gleser, J. M Iacono, *Psychosom. Med.* **27**, 102 (1965).
99. A. J. Mandell and R T. Rubin, *Amer. J. Psychiat.* **123**, 387 (1966); E. Sacher, *Arch. Gen. Psychiat.* **16**, 461 (1967); *ibid.* **17**, 544 (1967).
100. R. Gjessing, *J. Ment. Sci.* **84**, 608 (1938).
101. W. E. Knox, *Brit. J. Exp. Pathol.* **32**, 462 (1951).
102. A. J. Mandell and R. T. Rubin, *Life Sci.* **5**, 1153 (1966).
103. G. Curzon and A. R. Green, *ibid.* **7**, 657 (1968).
104. R. J. Wurtman and J. Axelrod, *Science* **150**, 1464 (1965).
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Genotype, Environment, and Population Numbers

Animal numbers are regulated by the genetic composition of the population and by environmental factors.

Francisco J. Ayala

In his work *On the Origin of Species*, Darwin wrote that "the causes which check the natural tendency of each species to increase in numbers are most obscure. . . . We know not exactly what the checks are even in a single instance." The regulation of population numbers is of major importance for the understanding of natural selection and biological evolution. It has implications of economic interest, particularly for the control of animal pests. Finally, it is a major problem for modern man who has become aware that the quality of human life is seriously threatened by the so-called "population explosion."

Population biology is concerned with the distribution and abundance of organisms. The factors considered when studying a particular population are the relationship of the animals to their food, to the places where they live, to the weather, and to other animals that share the same food or place to live, that prey on them, or that are related to them in any way. Unfortunately the genetic constitution of the population is usually not given sufficient attention. Populations of a species are treated as if they were genetically homogeneous in space and in time. Yet, to understand the causes which regulate animal numbers, both genetic and environmental factors must be considered.

Students of natural populations of animals encounter many difficulties, particularly in the estimation of adult numbers and the causes of mortality (*1*). Some problems can more easily be approached in laboratory studies, which permit control of the more important factors, while one or a few variables are manipulated at a time. Models can be produced; the validity of which must, of course, be ultimately tested in the field.

Drosophila flies are particularly favorable organisms for laboratory studies of some population problems. They multiply rapidly in cultures which are easy to maintain at moderate expense. Moreover, much is known about their biology, since they have been intensively studied for the last 60 years. I now describe some experimental approaches using drosophila that have provided information on the factors which regulate population numbers.

Innate Capacity for Increase

All components of the life cycle of drosophila are influenced by the genetic constitution of the flies. Genetic variation has been found to affect fertility of females and hatchability of eggs (*2*), fertility and mating activity of males (*3*), rate of development (*4*, *5*), longevity (*6*), and others. The ability of a population to increase in numbers or to maintain a certain size is related to these properties of the flies. However, it is not clear how they interact with each other to determine reproductive capacity. A statistic variously named the Malthusian parameter, intrinsic rate of natural increase, or innate capacity for increase has been proposed which

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