primary symptoms of schizophrenia (21). The initial, incomplete clinical response to phenothiazines might be explained by the immediate onset of dopamine receptor blockade. However, the decrease in dopamine receptor function may be partially offset by the compensatory increase in presynaptic dopamine synthesis and release, which perhaps stimulates receptors not adequately blocked. When this compensatory increase in dopamine synthesis is terminated by as yet unknown mechanisms (in about 3 weeks), the combined pre- and postsynaptic dopamine function would be optimally reduced, at a time correlating with maximum therapeutic effectiveness of the phenothiazines. This view is consistent with the report that α-methyl-paratyrosine, an inhibitor of tyrosine hydroxylase, may potentiate neuroleptic efficacy in schizophrenia (22). It may do so by preventing the compensatory increase in catecholamine synthesis occurring secondary to receptor blockade with phenothiazines.

An alternate hypothesis suggests that the phase of maximal clinical efficacy may be related to adaptation of the dopamine receptor initially blocked by the phenothiazines, the adaptation being reflected by the return to normal of the presynaptic synthesis and release. The possibility also exists that direct involvement of presynaptic dopaminergic receptors (23), coupling mechanisms, or dopamine release (24) may mediate the phenothiazine-induced changes in HVA accumulations. Finally, the evidence that tolerance develops to the phenothiazine effect on HVA accumulation could suggest that the long-term antipsychotic effects of the phenothiazines, to which there is no tolerance in the clinical sense, are not mediated by dopaminergic mechanisms. Bowers (20), however, reports that tolerance may occur to the neuroleptic effect on HVA only in the striatum (from which most HVA in the CSF is derived) and not in the limbic system, which may be more directly involved in the antipsychotic effects of the neuroleptics.

Our data are as yet inadequate to conclude whether such time-related effects may also occur at noradrenergic synapses (19). Moreover, a purposefully simplified "one transmitter" model is discussed here for the sake of clarity, but not with the illusion that dopamine function alone is related to schizophrenic and manic psychoses and their treatment (25).

Our findings reemphasize the potential significance of time-related compensatory and regulatory changes in neurotransmitter functions in relation to behavioral change after psychotropic drug administration, as emphasized by Mandell (26). It is also possible that such regulatory phe-

nomena and long-term adjustments occur after endogenous biochemical alterations that are not drug-mediated; this would make the time elapsed from the initial biological insult a critical variable in biological psychiatric studies.

Note added in proof. Since submission of this manuscript, there have been two other reports (27) of decreased HVA in CSF after long-term compared to short-term neuroleptic administration in psychiatric patients.

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References and Notes

1. T. Persson and B. E. Roos, *Nature (Lond.)* 217, 854 (1968); *Br. J. Psychiatry* 115, 95 (1969); T. N. Chase, J. A. Schur, E. K. Gordon, *Neuropharmacology* 9, 265 (1970); M. B. Bowers, *Psychopharmacologia* 28, 309 (1973); *Arch. Gen. Psychiatry* 31, 50 (1974); B. Fyro, B. Wode-Helgodt, S. Borg, G. Sedvall, *Psychopharmacologia* 35, 287 (1974).
2. M. B. Bowers, *Clin. Pharmacol. Ther.* 17, 73 (1975).
3. A. Carlsson and M. Lindqvist, *Acta Pharmacol. Toxicol.* 20, 140 (1963); M. Da Prada and A. Pletscher, *Experientia* 22, 465 (1966); H. Nyback and G. Sedvall, *J. Pharmacol. Exp. Ther.* 162, 294 (1968); N. E. Anden, S. G. Butcher, H. Corrodi, K. Fuxe, U. Ungerstedt, *Eur. J. Pharmacol.* 11, 303 (1970).
4. F. Gerbode and M. B. Bowers, *J. Neurochem.* 15, 1053 (1968); N. E. Anden, B. E. Roos, B. Werdinius, *Life Sci.* 2, 448 (1963).
5. Duplicate HVA assays by mass fragmentography correlated well with the spectrofluorometric values [E. K. Gordon, J. Oliver, K. E. Black, I. J. Kopin, *Biochem. Med.* 11, 32 (1974)].
6. F. K. Goodwin, R. M. Post, D. L. Dunner, E. K. Gordon, *Am. J. Psychiatry* 130, 73 (1973).
7. N. H. Neff, T. N. Tozer, B. B. Brodie, *J. Pharmacol. Exp. Ther.* 158, 214 (1967); H. C. Guldberg, G. W. Ashcroft, T. B. B. Crawford, *Life Sci.* 5, 1571 (1966).
8. R. Papeschi, T. L. Sourkes, L. J. Poirier, R. Boucher, *Brain Res.* 28, 527 (1971).
9. H. C. Guldberg and C. M. Yates, *Br. J. Pharmacol.* 33, 457 (1968).
10. R. Olsson and B. E. Roos, *Nature (Lond.)* 219, 502 (1968); T. N. Chase, *Neurology* 20, 36 (1970).
11. A. Pletscher, G. Bartholini, R. Tissot, *Brain Res.* 4, 106 (1967).
12. G. Curzon, E. J. W. Gumpert, D. M. Sharpe, *Nat. New Biol.* 231, 189 (1971); R. M. Post, F. K.

13. Goodwin, E. Gordon, D. M. Watkins, *Science* 179, 897 (1973); T. L. Sourkes, *J. Neurol. Transm.* 34, 153 (1973); E. Garelis and T. L. Sourkes, *J. Neurol. Neurosurg. Psychiatry* 34, 625 (1973).
13. The lower HVA accumulations after prolonged treatment with phenothiazines do not appear related to differences in probenecid blockade, since a similar trend occurred in our baseline HVA levels without probenecid administration. Values for HVA early in treatment were 46 ± 22 ng/ml ($N = 3$) which decreased to 22 ± 8 ng/ml ($N = 4$) later in treatment. Also, J. M. Perel, M. Levitt, and D. L. Dunner [*Psychopharmacologia* 35, 83 (1974)] showed no significant correlation between HVA accumulation and CSF probenecid levels after the high-dose probenecid technique as employed here. Bowers (2) did report lower CSF probenecid levels during thioridazine treatment, but this would not explain differences between short- and long-term phenothiazine treatment; he found HVA increases over baseline values in spite of lower probenecid levels.
14. Patients recovered from acute schizophrenic episodes have lower HVA accumulations than recovered manic patients, however (R. M. Post, E. Fink, W. T. Carpenter, F. K. Goodwin, *Arch. Gen. Psychiatry* 30, 1063 (1975).
15. B. S. Bunney, J. R. Walters, R. H. Roth, G. K. Aghajanian, *J. Pharmacol. Exp. Ther.* 185, 650 (1973); G. K. Aghajanian and B. S. Bunney, in *Frontiers in Catecholamine Research*, E. Usdin and S. H. Snyder, Eds. (Pergamon, New York, 1974), pp. 643–648.
16. Y. C. Clement-Cormier, J. W. Kebabian, G. L. Petzold, P. Greengard, *Proc. Natl. Acad. Sci. U.S.A.* 71, 1113 (1974); A. S. Horn, A. C. Cuello, R. J. Miller, *J. Neurochem.* 22, 265 (1974).
17. A. S. Horn and S. H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.* 68, 2323 (1971).
18. F. S. Messiha, *J. Neurol. Sci.* 21, 39 (1974).
19. R. O'Keefe, D. F. Sharman, M. Vogt, *Br. J. Pharmacol.* 38, 287 (1970); H. Asper, M. Baggiolini, H. R. Burki, H. Lanener, W. Ruch, G. Stille, *Eur. J. Pharmacol.* 22, 287 (1973); H. R. Burki, W. Ruch, H. Asper, M. Baggiolini, G. Stille, *ibid.* 27, 180 (1974); J. Glowinski, in *Association for Research in Nervous and Mental Diseases*, Research Publication No. 64, in press.
20. M. B. Bowers, *J. Pharm. Pharmacol.* 26, 743 (1974).
21. National Institute of Mental Health–Psychopharmacology Service Center Collaborative Study Group, *Arch. Gen. Psychiatry* 10, 246 (1964); *Publication Report No. 6* (Public Health Service, Bethesda, Md., April 1966).
22. A. Carlsson, T. Persson, B. E. Roos, J. Walinder, *J. Neurol. Transm.* 33, 83 (1972).
23. R. H. Roth, J. R. Walters, V. H. Morgenroth, in *Neuropsychopharmacology of Monoamines and Their Regulatory Enzymes*, E. Usdin, Ed. (Raven, New York, 1974), pp. 369–384.
24. P. Seeman and T. Lee, *J. Pharmacol. Exp. Ther.* 190, 131 (1974).
25. R. M. Post, *Am. J. Psychiatry* 132, 225 (1975).
26. A. J. Mandell, *Am. Sci.* 61, 565 (1973).
27. J. Gerlack, K. Thorsen, R. Fog, *Psychopharmacologia* 40, 341 (1975); G. Sedval, L. Bjerkenstedt, B. Fryo, C. Harnryd, F. Wiesel, B. Wode-Helgodt, in *Abstracts, Sixth International Congress of Pharmacology, Helsinki, 1975*, p. 597.
28. We thank K. Black, C. Jones, D. Runkle, and A. Fairfax for their expert technical assistance.

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Identification of Neurons in Cultures

Wahn *et al.* (1) claim to have induced "neural differentiation" in cultures of undetermined presumptive epidermis by treatment with adenosine 3',5'-monophosphate (cyclic AMP) derivatives. The basis of this claim rests on the identification of "neurons" in their explant cultures. This was apparently done by calling any cell which extended a process a "neuron" and then "confirming" the neuronal nature of the cell by formaldehyde-induced fluorescence of biogenic amines. (This latter point was mentioned but no data were illustrated.)

The actual definition of a neuron is hard to specify with precision, especially when cells are no longer seen in their normal surroundings. However, there are a number of generally recognized criteria for neuronal identification which include cell morphology, ability to react with silver stains, ability to generate action potentials, ability to form synaptic connections with other cells, and the ability to synthesize and store specific neurotransmitters. The least satisfactory criterion, especially in tissue culture where cells are growing in a two-dimensional substrate and often assume unusual shapes, is cellular morphology. Similarly, the classification of cells in cul-

ture as "glial" by morphological criteria must be considered as quite tentative.

I submit that Wahn et al. have possibly demonstrated that certain cyclic AMP derivatives influence some of the cells in their explant cultures to extend long thin processes, but the "neuronal" nature of these cells has yet to be established.

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References

1. H. L. Wahn, L. E. Lightbody, T. T. Tchen, J. D. Taylor, *Science* **188**, 366 (1975).

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The identifications of simple motor neurons, melanophores, and nerve bundles [(*1*), figure 1, c, e, and g] are unambiguous. The identifications of glial cells and complex neurons [(*1*), figure 1, d and f] are more tentative. We have additional evidence which could not be included in a short communication owing to space limitations. These include a comparison of cultured cyclic AMP–induced cells with cultured neural fold or neural tube cells. The morphologies seen in the former are identical to those seen in the latter. In addition we have made ultrastructural identification. Setting aside the tentative identification of glial cells and complex neurons, the formation of simple neurons, melanophores, and nerve bundles clearly establishes that neural differentiation took place.

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References

1. H. L. Wahn, L. E. Lightbody, T. T. Tchen, J. D. Taylor, *Science* **188**, 366 (1975).

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Fatty Acids, Platelets, and Microcirculatory Obstruction

Furlow and Bass (*1*) observed that sodium arachidonate injection into the carotid artery of rats produced unilateral cerebrovascular occlusion due to obstruction of the hemispheric microcirculation by platelet aggregates. In a similar study, Silver et al. (*2*) found that arachidonate infusion into the ear vein of rabbits produced sudden death due to occlusion of the lung microcirculation by platelet aggregates. We are concerned that these observations may be misinterpreted by those unfamiliar with the biological effects of fatty acid solutions. For example, a reasonable inference is that arachidonate or the prostaglandins E_2 or $F_{2\alpha}$, which are synthesized from arachidonate and known to cause platelet aggregation (*3*), may be involved in the pathogenesis of some types of microvascular occlusive disease. Because of the way in which the fatty acid was administered in these studies (*1, 2*), however, we question whether the responses observed have any pathophysiological significance.

In the stroke study, 50 μl of sodium arachidonate was injected rapidly in concentrations ranging from 0.33 to 33 mM (*1*). When a fatty acid salt is infused in this manner, it enters the plasma unesterified fatty acid pool, known commonly as plasma free fatty acid. Normally, 99 percent or more of these free fatty acids exists as a physical complex with plasma albumin (*4*). In the concentrations usually present in the plasma, 0.2 mM to about 1.7 mM (*5*), free fatty acids are bound very tightly by albumin, so that the concentration of unbound fatty acid anions is in the range of 0.01 to 10 μM (*6*). Fatty acids are not toxic when complexed with albumin; in fact, they are excellent substrates for a variety of cells and tissues (*7*). By contrast, fatty acids that are not firmly bound to albumin exhibit detergent actions, denaturing proteins and damaging cells and organelles (*8*). Therefore, when fatty acids are said to produce toxic effects in biological systems, special care must be taken to be certain that these effects are not due to the nonspecific detergent actions of a soap solution. The key point is that the fatty acid must be combined with albumin or another carrier protein *prior* to contact with cells or tissues. Suitable methods have been developed for the injection of fatty acid solutions into animals (*9*). We have reservations about the interpretation of the studies of Furlow and Bass (*1*) and Silver et al. (*2*) because these precautions were not taken when arachidonate was injected.

That fatty acid soap solutions cause platelet aggregation actually is not a new finding. The initial observation was that fatty acid infusion or massive mobilization produced thrombosis (*10*). Subsequent in vitro studies established that fatty acids can activate the plasma clotting system (*11*) and cause platelet aggregation (*12*). When human platelets are incubated with fatty acids that are properly complexed with plasma albumin, the platelets take up, oxidize, and esterify large quantities of fatty acid (*13*). Incubation with albumin-bound saturated fatty acids such as palmitate and stearate made the platelets more sensitive to adenosine diphosphate–induced aggregation, but only when the molar ratio of fatty acid to albumin was greater than 2 (*14*). This is due presumably to fatty acid binding to the platelet membrane (*13*) and perhaps destabilizing the lipid bilayer structure (*15*). In no case, however, did any of the fatty acids themselves cause the platelets to aggregate (*14*). Even at molar ratios of 6, the unsaturated fatty acids oleate and linoleate did not enhance adenosine diphosphate–induced platelet aggregation when they were added as a complex with albumin.

Arachidonate was not tested in our experiments because it comprises only a very small fraction of the plasma free fatty acids (*7, 16*). Even if the plasma free fatty acid concentration is elevated greatly, such as after exercise or injection of heparin (*17*), the total arachidonate concentration will be less than 0.1 mM, and most of it will be bound to albumin. The dose of arachidonate employed to produce microvascular occlusion in the rabbit was 1.4 mg/kg (*2*). Of the six fatty acids tested, only arachidonate caused this effect. In interpreting these observations, one must remember that arachidonate, because of its four double bonds, is much more water-soluble than the sodium salts of other long-chain fatty acids. Therefore, sodium arachidonate in high concentrations may have a greater detergent action because, as compared with other long-chain fatty anions, much more of it remains in solution after entering the plasma.

In conclusion, the recent studies with sodium arachidonate provide a potentially useful method for producing microcirculatory obstruction in experimental animals (*1, 2*). We believe, however, that neither of these studies provides any conclusive evidence that arachidonate or prostaglandins are involved in the pathogenesis of platelet aggregation or microcirculatory obstruction. A more likely interpretation is that arachidonate produced a nonspecific detergent effect because it was injected into the blood as a soap solution. This is not to say that elevations in arachidonate or other fatty acids are completely innocuous. Our point is that these studies are not valid tests of the pathophysiological question because of the manner in which arachidonate was administered to the animals.

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References and Notes

1. T. W. Furlow, Jr., and N. H. Bass, *Science* **187**, 658 (1975).
2. M. J. Silver, W. Hoch, J. J. Kocsis, C. M. Ingerman, J. B. Smith, *ibid.* **183**, 1085 (1974).
3. B. B. Vargaftig and P. Zirinis, *Nat. New Biol.* **244**, 114 (1973); M. J. Silver, J. B. Smith, C. In-