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 postsynaptic 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 emphasize 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 phenomena 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.

ROBERT M. POST
Section on Psychobiology, Adult Psychiatry Branch, National Institute of Mental Health, Bethesda, Maryland 20014

FREDERICK K. GOODWIN
Section on Psychiatry, Laboratory of Clinical Science, National Institute of Mental Health

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22. September 1975; revised 2 February 1975

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 formaldelay-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-

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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.

Marc A. Dichter
Beth Israel Hospital,
Boston, Massachusetts 02215

References

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 E2 or F3α, 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 pathophysiologic 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 deterrent 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 deterrent 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 diphasphate–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 (15) 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 diphasphate–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 0.8 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 deterrent action because, as compared with other long-chain fatty acids, 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 deterrent 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 questions because of the manner in which arachidonate was administered to the animals.

Arthur A. Spector
John C. Hoak
Departments of Biochemistry and Medicine, University of Iowa,
Iowa City 52242

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Science, vol. 190
Letter: Identification of neurons in cultures

MA Dichter

Science 190 (4213), 489-490.
DOI: 10.1126/science.1166322