Letters

Cholinesterase Activity

The recent article by L. S. Rubin (*1*) suggesting a relationship between "acetylcholine-cholinesterase imbalance" and "functional psychoses" raises interesting possibilities. We are particularly interested in this suggestion since we have reported studies (*2, 3*) which indicate a relationship between cholinesterase activity in the cerebral cortex and adaptive behavior among normal animals. However, there seem to be serious difficulties with Rubin's interpretation of his data, and with his proposed "experimental therapy" with schizophrenic patients.

Although Rubin measured only cholinesterase activity, he states that there are differences in acetylcholine-cholinesterase *balance* among his groups of subjects. Unless the amount of acetylcholine is identical for all subjects, or unless there is a negative relationship between the amounts of acetylcholine and cholinesterase (both rather doubtful assumptions), it would seem necessary to measure acetylcholine as well as cholinesterase in order to determine the balance between them. From Rubin's data, all that can be said is that groups of subjects appear to differ in cholinesterase activity. Two subjects who differ in cholinesterase activity might, in fact, have identical acetylcholine-cholinesterase balances if the relationship were linear. At the present time we are attempting to determine the acetylcholine-cholinesterase balance by measuring both acetylcholine and cholinesterase activity in the same animal subjects.

An even more puzzling question is Rubin's use of *erythrocyte* cholinesterase activity as a measure of the enzyme activity in the central nervous system. In our own work (*3*) we have consistently found that even within the central nervous system the correlations of cholinesterase activity among different loci (for example, cerebral cortex and subcortical brain), although generally positive, vary from −0.08 to 0.52, depending upon the strain of animals used. It would therefore be surprising if cholinesterase activity obtained from a blood sample provided a highly valid index for cholinesterase activity of neural tissue—yet this is the assumption which Rubin appears to make without giving any justification for it.

On the basis of both of these assumptions—that erythrocyte cholinesterase activity is a measure of central nervous system cholinesterase activity and that the level of cholinesterase activity is a valid index of acetylcholine-cholinesterase balance—Rubin proposes diisopropylfluorophosphonate therapy for schizophrenic patients showing a high level of erythrocyte cholinesterase activity. It would seem questionable whether such therapy should be undertaken as "the next step," prior to a more careful experimental determination of the validity of the assumptions. Diisopropylfluorophosphonate is, in any effective dose, a poison which inflicts a biochemical lesion on the central nervous system. The field of chemotherapy of mental disease may well benefit from a more cautious and systematic experimental approach.

DAVID KRECH
MARK R. ROSENZWEIG
EDWARD L. BENNETT
University of California, Berkeley

References

1. L. S. Rubin, *Science* **128**, 254 (1958).
2. D. Krech, M. R. Rosenzweig, E. L. Bennett, B. Krueckel, *Science* **120**, 994 (1954); *J. Comp. Physiol. Psychol.* **49**, 261 (1956).
3. M. R. Rosenzweig, D. Krech, E. L. Bennett, in *Neurological Basis of Behaviour* (Churchill, London, 1958).

Krech and his associates have raised several pertinent criticisms of the interpretation of the data which I presented in a recent article (*1*) in which significant differences in the hydrolysis rate of acetylcholine by erythrocyte cholinesterase were found between the blood samples obtained from psychiatric patients and those obtained from normal human beings.

The first objection pertains to use of the concept of an *imbalance* between acetylcholine and cholinesterase. The in vitro study was in fact restricted to the action of cholinesterase on a measured, constant quantity of acetylcholine chloride. It must be admitted that in interpreting the data, I was reminded of the finding of Nachmansohn and Rothberg (*2*) that the specific cholinesterase of nervous tissue has an optimal substrate concentration (about 10^{-2} *M* for acetylcholine) and that at higher or lower substrate concentrations the cholinesterase activity drops off markedly. The in vivo interaction between cholinesterase and acetylcholine has also been demonstrated by Early and his associates (*3*). They found that after intravenous injection of from 4 to 8 mg of acetylcholine into rabbits, the true cholinesterase content of serum, 4 minutes after injection, decreased in four experiments, was unchanged in one, and increased in a sixth. In spite of the above suggested interaction between cholinesterase and acetylcholine in vivo, it is admitted that an experimental demonstration of this presumed balance in human beings was not demonstrated in the reported study.

The second objection of Krech *et al.* to my interpretation is one with which researchers in the area of psychophar-

macology are familiar. Nevertheless, familiarity does not preclude embarrassment when one is asked to establish the identity between peripheral and central enzymatic activity. There is evidence, however, which does suggest a relationship between the specific cholinesterase of erythrocytes and the specific cholinesterase of the brain. Mazur and Bodansky (*4*) in their work on the rabbit and monkey and in work with human beings found that the cholinesterases of the red cells and brain are nearly equally sensitive to diisopropylfluorophosphate. This finding is in agreement with the report of Nachmansohn and Rothberg (*2*) in which it was demonstrated that nervous tissue and erythrocytes contained specific cholinesterase. Oberst and Christensen (*5*) studied the rate of regeneration of erythrocyte and brain cholinesterase in the rat following exposure to isopropylmethylphosphonofluoridate and found that although the rate of regeneration of erythrocyte cholinesterase is somewhat faster than that of brain cholinesterase activity, still the pattern of recovery was similar for both. Cohen *et al.* (*6*) concluded from their animal study that significant depression of erythrocyte cholinesterase activity by isopropylmethylphosphonofluoridate preceded significant depression of brain cholinesterase.

The third objection of Krech *et al.* requires but a brief comment. The objection is directed against the suggestion that diisopropylfluorophosphate be employed experimentally in the treatment of schizophrenic patients showing a high level of erythrocyte cholinesterase activity. Actually, reference was made to the use of members of the class of anticholinesterases. In selecting diisopropylfluorophosphate as the focal point for their objection, the authors caution against its use because "in any effective dose [it is] a poison which inflicts a biochemical lesion on the central nervous system." It is well known that diisopropylfluorophosphate has been used in the treatment of glaucoma and myasthenia gravis. Furthermore, it has been employed by Rowntree *et al.* (*7*), who administered from 1 to 2 mg/day to psychotic patients for as long as 37 days without untoward, measurable, permanent effects.

LEONARD S. RUBIN

Eastern Pennsylvania Psychiatric Institute, Philadelphia

References

1. L. S. Rubin, *Science* 128, 254 (1958).
2. D. Nachmansohn and M. A. Rothberg, *J. Biol. Chem.* 158, 653 (1945).
3. D. F. Early, R. E. Hemphill, M. Reiss, E. Brummel, *Biochem. J.* 45, 552 (1949).
4. A. Mazur and O. Bodansky, *J. Biol. Chem.* 163, 261 (1946).
5. F. W. Oberst and M. K. Christensen, *J. Pharmacol. Exptl. Therap.* 116, 216 (1956).
6. B. S. Cohen, F. W. Oberst, J. W. Crook, C. Harris, *ibid.*, p. 209.
7. D. W. Rowntree, S. Nevin, A. Wilson, *J. Neurol. Neurosurg. Psychiat.* 13, 47 (1950).