Correspondence

Correlation of Blood Levels of 4-Hydroxybutyrate and Consciousness

To the Editor:—We were prompted to write this letter in the interest of scholarly scientific communication, after having read an article by M. Helrich, T. C. McAslan, S. Skolnik and S. P. Bessman, entitled "Correlation of Blood Levels of 4-Hydroxybutyrate with State of Consciousness," which appeared in ANESTHESIOLOGY **25**: 771, November–December, 1964. We found this paper deficient in two respects: in addition to the fact that there is strong evidence directly opposed to some of the statements made in the paper, which the authors were either unaware of or ignored, there are also statements of alleged fact which are in need of documentation.

Roth and Giarman (Fed. Proc. **23**: 148, 1964; Science **145**: 583, 1964) have shown that, in the rat, the onset and duration of anesthesia produced by the intravenous administration of either γ-butyrolactone or γ-hydroxybutyrate correlate strictly with the level of γ-hydroxybutyrate in the brain, and not, as indicated in this and in another paper by Bessman and Skolnik (Science **143**: 1045, 1964), with the level of lactone in the brain. Helrich *et al.*, in this paper, explain their finding that the greatest clinical effect after γ-hydroxybutyrate lagged behind peak blood levels by about 15 minutes on the basis that a metabolite of 4-hydroxybutyrate (presumable the lactone, they state later) is the active compound. Another explanation, in view of the finding of Roth and Giarman, is that γ-hydroxybutyrate is the active form, but that this molecule traverses the blood-brain barrier slowly, and requires some time after peak levels in blood are reached to achieve the concentration in brain critical for the induction of anesthesia.

Helrich and his associates state on page 774: "The evidence presented (in reference 8) suggested that the lactone is formed in the liver. . . ." Apart from the fact that no such evidence was presented, the suggestion is hardly likely since Roth and Giarman have found that blood and liver (but not brain) contain a highly active lactonase which rapidly hydrolyzes the lactone to the acid form (Roth, R. H., and Giarman, N. J.: Biochem. Pharmacol. **14**: 177, 1965). In their discussion Helrich *et al.* again return to this theme with the statement: "From reports with rats, it seems most likely that the active metabolite is the lactone." This statement is not documented, but from our close contact with the literature on this subject there is only *one* report in which such a suggestion is made, i.e., the above-mentioned paper by Bessman and Skolnik. In point of fact the situation is made more confused by Bessman who states in an abstract presented in the proceedings of the Sixth International Congress of Biochemistry (Abstracts V, p. 412, July 26 to Aug. 1, 1964): "The brain levels of γ-hydroxybutyric acid are related to the sleep of the animal."

A statement in the paper by Helrich *et al.*, which is in need of documentation, is the following: "It is of interest that this material (γ-hydroxybutyrate) is formed and degraded through a pathway leading to gamma-aminobutyric acid." While we are acquainted with Bessman and Fishbein's observation of the conversion of succinic semialdehyde to γ-hydroxybutyrate, we are not aware of any evidence supporting the part of the above statement which suggests that γ-hydroxybutyrate *leads to* the formation of GABA. In point of fact Giarman and Schmidt (Brit. J. Pharmacol. **20**: 563, 1963) could not find any increase in gamma-aminobutyric acid in brains of animals deeply anesthetized with γ-butyrolactone.

It is clear that the differences cited above in results of Bessman and his co-workers in

comparison with those of Giarman and Roth arise from differences in techniques used for the estimation of γ-hydroxybutyrate and γ-butyrolactone in body tissues. Giarman and Roth have described in detail their highly specific and sensitive gas chromatographic method (Science **145**: 583, 1964). Bessman and his colleagues, on the other hand, have reported using several modifications of a colorimetric procedure based on the Hestrin reaction, which is poorly specific and can be demonstrated with a variety of naturally occurring esters.

The important issue in publications involving an area with such methodologic difficulties, it seems to us, is the necessity of paying scrupulous attention to discrepant results and of documenting appropriately all statements of fact so that other investigators will be in a position to evaluate the work properly, and, in certain cases, so that duplication and extension of the work can be made in an efficient manner.

NICHOLAS J. GIARMAN, PH.D., and
ROBERT H. ROTH, PH.D.
Department of Pharmacology
Yale University School of Medicine
New Haven, Connecticut

To the Editor:—It seems advisable to point out at the beginning that our report relates the levels of 4-hydroxybutyrate in the blood to the state of consciousness in human beings. We are not dealing with *rats* nor with the level of *lactone* in tissues or blood.

The complaint appears to be that we did not discuss adequately the findings by Roth and Giarman which appeared in print almost two months after our article was accepted for publication. One of us had discussed with Roth and Giarman their communication at the Federation of Biological Sciences meeting and had raised several questions concerning their method of gas chromatography for volatile material resembling gamma-hydroxybutyrate which we felt, according to their methodology, did not reflect the actual state of affairs in the tissue. When the paper in Science **145**: 583, 1964, was published we found, on attempting to employ their method, that there were considerable losses.

The discussion then becomes a matter of methodology. By one method, one set of results is obtained; by another, another is seen. We are dealing here, however, with a rather direct difference of opinion and we are able to verify our findings in this type of experiment by measurement of blood level compared to standard materials. The physical state of the material which is in the blood and in the tissues may very well lead to the differences in results obtained. We do not believe, however, that a putative diffusion rate which leads to a lag of 15 minutes in the development of a brain level from a very rapidly falling blood level (according to Giarman and Roth, Science, *loc. cit.*) is any better explanation of our data than the postulation of a possible intermediate compound.

We further cannot understand how Giarman and Roth have proved that there is no lactone formation in the liver and refuse to accept their explanation of why there should not be lactone formed in the liver on the basis of their as yet unpublished material. They seem again to call us to task for not knowing what they are about to publish.

The statement that gamma-hydroxybutyrate is formed and degraded through a pathway leading to gamma-hydroxybutyric acid is inverted by Giarman and Roth. We did not say, as their own quotation from our paper makes clear, that gamma-hydroxybutyrate leads to gamma-aminobutyrate, but that it is formed and degraded through a pathway leading to gamma-aminobutyrate. The data on net synthesis of GABA which they present is in no way evidence that this does not take place.

We agree with the last two paragraphs of the letter under consideration and believe that it would be worthwhile for the writers to inspect the possibility that their own method may be observing only one aspect of the problem. A key to the mode of action of this strange compound may be the very discrepancies in method which are plaguing the field at this time.

M. HELRICH, M.D., T. C. MCASLAN, M.D.,
S. SKOLNIK, B.S., S. P. BESSMAN, M.D.
Departments of Anesthesiology
and Pediatrics,
University of Maryland School of Medicine
Baltimore