Technical Comments

Glucose-6-Phosphatase Activity in Brain

Nelson *et al.* (*1*) have challenged our earlier finding (*2*) that the estimated rate of glucose metabolism in rat brain is greater when measured with [2-^{3}H]-glucose than when measured with [U-^{14}C]-glucose as a result of the apparent dephosphorylation of glucose-6-phosphate by brain in vivo. This challenge to our original report was based on (i) a comparison of the rates of glucose metabolism obtained between 2 and 7 minutes after intracarotid injection of variously labeled glucose (our original report measured the initial rates of glucose metabolism between 5 and 60 seconds after injection of the label) and (ii) a criticism of our original method of column fractionation of glucose.

As Table 1 and our previous report (*2*) show, the differences in the two measured rates of glucose metabolism are only significant within the first 30 seconds after intracarotid injection of label. Nelson *et al.* (*1*) report only the ^{3}H/^{14}C ratio of glucose from 2 to 7 minutes after injection. At 2 minutes, the specific activity of cerebral venous and systemic venous [2-^{3}H]glucose are already equilibrated, and the ^3H_2O formed in brain is already released (*2*). Without data from the first minute after injection, any conclusion about the magnitude of the rate of the dephosphorylation of glucose-6-phosphate, such as that drawn by Nelson *et al.* (*1*), is unwarranted.

Table 1. Observed average rates of the phosphorylation of glucose during the specified time interval after injection. The specific activity of brain glucose was determined at the end of the specified time period. Values are means ± SD; n = 4 or 5 (observations).

Time v from (sec)	v from [2-^{3}H]glucose (μmol min^{-1} g^{-1})	v from [U-^{14}C]glucose (μmol min^{-1} g^{-1})	Difference (μmol min^{-1} g^{-1})	P*
0–10	1.84 ± 0.1	1.48 ± 0.1	0.36	<0.01
0–20	1.38 ± 0.08	1.15 ± 0.06	0.24	<0.01
0–30	1.16 ± 0.24	1.02 ± 0.24	0.14	<0.08
0–60	1.12 ± 0.08	1.03 ± 0.09	0.09	<0.11

*Two-tailed t test.

The second methodological criticism raised by Nelson *et al.* (*1*) is entirely valid: in our original column fractionation of glucose we neglected to use a cation exchange column, with the consequence that we identified labeled glutamine and γ-aminobutyric acid in mass spectrometric studies of such extracts. Accordingly we have repeated our original study and included a cation exchange resin step similar to that used by Nelson *et al.* (*1*).

We herein report a repeat of our original experiments (*2*) in which we used this later method of fractionation of labeled glucose (*1*) from freeze-blown rat brains after intracarotid injection of radioactive label. All data were collected within 1 minute after injection of label. The rates of glucose phosphorylation (v) were calculated from $dP/dt = v \times SA(t)$, where dP/dt is the rate of formation of the labeled products of glucose phosphorylation, and $SA(t)$ is the specific activity of labeled glucose at time (t).

The initial rate of glucose utilization [$v = dP/dt/SA(t = 0)$] from data calculated at zero time for [2-^{3}H]glucose is 1.26 micromoles (μmol) per minute per gram of brain, while that from [U-^{14}C]glucose is 1.06 μmol per minute per gram, a difference of 0.20 μmol per minute per gram.

Our data show that the rate of the dephosphorylation of glucose-6-phosphate is about 19 to 24% of the rate of the phosphorylation of glucose estimated from [U-^{14}C]glucose.

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REFERENCES

1. T. Nelson *et al.*, *Science* **229**, 60 (1985).
2. M-T. Huang and R. L. Veech, *J. Biol. Chem.* **257**, 11358 (1982).

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Response: Huang and Veech concede that the "glucose" fraction they extracted from brain, on which they based their conclusion of high glucose-6-phosphatase (G6Pase) activity (*1*), was contaminated with metabolic products of [2-^{3}H, U-^{14}C]glucose. They no longer mention a fall in the ^{3}H/^{14}C ratio in the glucose fraction, but base their claim of high G6Pase activity in brain on a fleeting difference between apparent rates of glucose utilization calculated from [^{3}H]glucose and [^{14}C]glucose disappearance. These rates are calculated by dividing the radioactivity in the labeled metabolic products by the specific activity of the respective labeled glucose fractions recovered from brain by their purification procedure. They offer no proof of quantitative recovery of labeled products and purity of the glucose fractions. In fact, neither of these requirements is met. They have now added to their purification procedure a cation exchange step, presumably applied to the Dowex-1–borate eluate in their previous procedure (*1*), which removes two ^{14}C-labeled contaminants identified by mass spectrometry. They provide no evidence, however, for the purity of the remaining glucose fraction and incorrectly cite our report (*2*) as providing evidence that the cation exchange step purifies the "glucose" fraction. We (*2*) used a different, more extensive purification procedure in our studies and found no significant change in the ^{3}H/^{14}C ratio of the brain glucose pool; the cation exchange step applied to the Dowex-1-borate eluate only partially purified the glucose.

The purification procedure of Huang and Veech, including the cation exchange step, does not exclude completely ^{14}C-metabolites from the [^{14}C]glucose fraction. The procedure also produces detritiated ^{14}C-labeled acidic derivatives of glucose and its metabolites at the Dowex-1-borate eluate step that are not removed by cation exchange. The procedure clearly leads to low recovery of ^{14}C-metabolites and overestimates of [^{14}C]glucose specific activity. These deficiencies are sufficient to explain why the rates of glucose utilization calculated with [^{14}C]glucose are lower than those calculated with [^{3}H]glucose, for which the contaminants are largely unlabeled. This difference in calculated rates is transient. As the loss of [^{3}H]water derived from brain [^{3}H]glucose metabolism becomes progressively more significant, the rate calculated with [^{3}H]glucose diminishes rapidly toward the already artifactually lower rate calculated with [^{14}C]glucose. Moreover, both rates are, in fact, erroneously inflated, particularly at early times, because they were calculated with the specific activities of precursor pools at kill time rather than with the correct integrated specific activities over the entire experimental period.

Contrary to the statement of Huang and Veech, a fall in the ^{3}H/^{14}C ratio of the glucose pool due to G6Pase would not disappear with time. It can be readily calculated (with worst-case estimates of the kinetics of glucose transport between blood and brain) that the magnitude of G6P dephosphorylation claimed by Huang and Veech would lead to an easily measurable reduction in the ^{3}H/^{14}C ratio in the glucose pool in brain, even under steady-state conditions,

a change we did not observe (*2*) with adequately purified glucose fractions. Furthermore, in view of the extreme transiency of their observed effect (<30 seconds after the pulse), how do Huang and Veech exclude a more likely possibility? Their fleeting effect, if it were true, could be due to the known G6Pase activity in the vascular endothelial cells in which the injected glucose must equally transiently reside before transfer to the brain.

Huang and Veech provide no convincing evidence to support their notion of a futile cycle of glucose phosphorylation and G6P dephosphorylation in brain tissue. Their results are fully explicable by demonstrable artifacts and errors in their studies.

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REFERENCES

1. M-T. Huang and R. L. Veech, *J. Biol. Chem.* **257**, 11358 (1982).
2. T. Nelson *et al.*, *Science* **229**, 60 (1985).

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Metal-Rich Layers in Pelagic Sediments

T. R. S. Wilson *et al.* (*1*) present a model that predicts the depth and thickness of metal-rich layers in marine sediments. Reactions of the downward flux of oxidants (O_2, NO_3^-) in pore waters with reduced solid phases in the sediments (organic and inorganic) and with the upward diffusive flux of dissolved reductants (Fe^{2+}, Mn^{2+}) are modeled to predict (i) the rate at which redox fronts migrate downward into sediments, (ii) the depth in the sediments at which layers enriched in oxides of Fe and Mn are formed, and (iii) the length of time since deposition of turbidite layers in certain cases.

Essential components of the model include the reaction of dissolved O_2 with dissolved Fe^{2+} and Mn^{2+}. However, the chemical equations for these reactions given by Wilson *et al.* are not stoichiometrically balanced. In their table 1, equation 3 is written as

$$4\ Fe^{2+} + 3\ O_2 + 2\ H_2O = 4\ FeOOH$$

The correct equation is

$$4\ Fe^{2+} + O_2 + 6\ H_2O = 4\ FeOOH + 8\ H^+$$

Their equation 4 is written as

$$3\ Mn^{2+} + 2\ O_2 = Mn_3O_4$$

The correct equation for precipitation of Mn_3O_4 is

$$3\ Mn^{2+} + \tfrac{1}{2}\ O_2 + 3\ H_2O = Mn_3O_4 + 6\ H^+$$

Correctly balanced, the Fe^{2+}/O_2 and Mn^{2+}/O_2 molar ratios are 4 to 1 and 6 to 1, respectively, much larger than the ratios of 4 to 3 and 3 to 2 used by Wilson *et al.* in their model.

As a consequence of using these incorrect ratios, the model of Wilson *et al.* will (i) underestimate the rate of downward migration of the redox front, (ii) underestimate the depth of the redox front below the sediment-water interface when the downward migration of the front ceases, that is, the depth of the metal-rich layer, and (iii) overestimate the portion of total O_2 consumption attributable to oxidation of Fe^{2+} and Mn^{2+} (that is, underestimate O_2 consumption by oxidation of organic carbon and inorganic solid-phase reduced species).

Given the serious errors in reaction stoichiometries incorporated into the model, it is curious that model predictions can be made to match so well with the observed sedimentary profiles.

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REFERENCES

1. T. R. S. Wilson *et al.*, *Science* **232**, 972 (1986).

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Response: Anderson and Schiff (*1*) are correct in challenging two equations used in our report (*2*) since neither equation conserves charge. If hausmannite and goethite are the minerals formed (itself an assumption), their suggested stoichiometries are closer to reality than those represented by equations 3 and 4 in our original report.

Anderson and Schiff are incorrect, however, in their implication that this criticism undermines the report in any significant way, as they appear to misinterpret the relative importance of the various processes modeled. The main purpose of the model we developed (*2*) was to test the concept that a downward propagating oxidation front could form metal-rich layers in the available time, the inputs being "reasonable" parameters culled from the literature and from our own data. In fact, as is clear from the values quoted in the report, the gross behavior of the model is determined by the oxidation of solid-phase organic carbon at the front. In this context, the oxidation of Fe(II) and of Mn(II) is of minor quantitative importance.

To illustrate this point, we have rerun the model with the amendments suggested by Anderson and Schiff. For the Madeira Abyssal Plain station discussed, the estimated age of the turbidite changes from 217 years to 214 years. For the equatorial Atlantic base case the result is an increase of 5.3 cm in the depth of the iron maximum, from 55.6 cm to 60.9 cm. A consequent slight broadening of the peak results in a drop in the maximum Fe_2O_3 enrichment, from 5% to 3%. As the upward diffusional fluxes of the metals are unaltered, the integrated quantities of iron and of manganese oxidized during the 11,000-year run time remained unchanged.

Figure 2 of our original report (*2*) shows the range of the field data and the effects of various changes in the base case assumptions. Comparison shows that the results quoted above are well within the overall ranges of uncertainty presented in that figure. There is thus no need to revise the important conclusion of the modeling exercise, which is that the propagating oxidation front mechanism described is capable of producing iron enrichments of the type observed in sediments. These enrichments were previously unexplained.

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REFERENCES

1. R. F. Anderson and S. L. Schiff, *Science* **234**, 1129 (1986).
2. T. R. S. Wilson *et al.*, *ibid.* **232**, 972 (1986).

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