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Lack of Blindness Procedures in Alzheimer's Study

TO THE EDITOR: The recent article by Fukuyama et al. (*1*) raises the intriguing possibility that relative preponderance of synaptic versus cellular damage in Alzheimer's disease (AD) could be detected by the metabolic ratio between oxygen and glucose consumption. It is unclear, however, that the sample consisted of typical AD patients, considering their young age (58 ± 8 yr). Furthermore, the authors' use of the term "age-matched" is highly questionable. The controls were over 10 yr younger than the patients (47 ± 16 yr) and only the small sample size and high variance prevented this difference from being highly significant ($p < 0.07$). Finally, it is unfortunate that Dr. Hachinski's name was consistently misspelled and a citation to his scale was not provided. Readers who want to follow-up on this vague and misleading reference would be unable to find it as spelled.

My greatest concern, however, is with the apparent lack of any blindness procedures or other efforts to reduce bias. In this instance, the most interesting finding of this paper consists of a mismatch between oxygen and glucose utilization in specific cortical regions derived by placing small, circular ROIs in relevant areas by visual examination. If the investigators knew which scans belonged to patients and which were controls (and, in the case of AD, this is not a difficult distinction), it is hard to ensure the absence of bias in the manual placement of these ROIs. Furthermore, 4/9 patients and all controls had the two PET procedures on separate days, so even the choice of ROIs for CMRgl and CMRo2 determinations within a subject cannot be considered bias-free. Finally, were we to try and reproduce this finding, in the absence of rigorous definitions of these ROIs any lack of reproducibility would be hard to interpret. In other words, other investigators trying to replicate this study would be hard-pressed to place the ROIs in the same regions as used here, due to the lack of rigorous criteria for ROI placement.

The findings are intriguing, and sufficiently important to require replication and follow-up. However their interpretation, and eventual confirmation, would benefit from greater clarity.

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Ascorbate Plays a Major Role in Labeling Proteins with Technetium-99m

TO THE EDITOR: I read with interest the recent article by Hnatowich et al. (*1*) in which the authors studied the role of ascorbate in the reduction of antibody disulfide groups to sulfhydryls which provide binding sites for reduced ^{99m}Tc. The method, developed in our laboratory several years ago (*2,3*), showed that certain concentrations of sodium ascorbate (AA) was preferred for controlled generation of antibody sulfhydryls. Dithionite-reduced ^{99m}Tc was then incubated with AA-treated protein at pH 7.8–9 for subsequent radiolabeling in high yields. We also observed that under these experimental conditions, AA stabilized reduced ^{99m}Tc which bound to protein and prevented colloid formation.

In 1990, we determined the number of cysteine residues generated by the variety of reducing agents used in direct methods of labeling monoclonal antibodies with ^{99m}Tc (*4*). Although Ellman's reagent was readily available and commonly used to determine cysteine residues in protein, we chose not to use it in this assay because both AA and dithionite seriously interfered with the assay (*4,5*) and it was insensitive ($E_{412} = 1.36 \times 10^4$ cm^{-1} M^{-1}) to accurately determine the low levels of cysteine residues generated in reasonable quantities of expensive antibodies. We considered several other spectrophotometric methods and chose the Ninhydrin method (*5*), for it had less interference from the reagents in question and was more sensitive ($E_{520} = 3.025 \times 10^4$ cm^{-1} M^{-1}).

Using different chemical conditions, higher concentrations of protein, AA and dithionite, Hnatowich et al. (*1*) have determined the quantity of cysteine residues generated by Ellman's assay and ^{99m}Tc labeling efficiency by gel and paper chromatography. The basic findings of their investigations were that only 7% of the available cysteine residues were generated which were much less than we had observed with Ellman's assay of protein, incubated with 10 times higher concentrations of AA, (*4*) and that using protein incubated with 5 times the recommended concentration of dithionite, 5% of the cysteine residues were generated. In both cases, following incubation with protein, the reducing agents were removed since they interfere with Ellman's assays. Furthermore, Hnatowich et al. observed that when protein was treated with AA and labeled with ^{99m}Tc, after removal of AA, the labeling efficiency was much lower than we had reported; and that when protein was not treated with AA and incubated with ^{99m}Tc reduced with 5 times the dithionite concentrations than we had recommended, the labeling efficiency was 90%–95%. Based on these results, they have basically concluded that in reduction of protein disulfides, AA plays a minor role and dithionite a major one.

With the use of a relatively less sensitive Ellman's assay, with strong interference from the reagents involved, the different chemical conditions, the higher concentrations of dithionite (that may not have been fully eliminated by dialysis) and the low optical density readings, such results are plausible but not convincing. Furthermore, Mather et al. (*6*) and Pimm et al. (*7*) have independently observed that when protein was incubated with 2-mercaptoethanol, the other prominent agent used for reduction of protein disulfides, and removed, Ellman's assay showed no increase in cysteine residues and that the labeling efficiency with ^{99m}Tc was poor. The authors have attributed these results to reoxidation of reduced sulfhydryl groups and postulated that, because of the close proximity of adjacent thiol groups, reoxidation occurred too rapidly and prevented detection of sulfhydryls and ^{99m}Tc binding