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LETTERS TO THE EDITOR

We are pleased to receive Letters to the Editor on appropriate subjects. These letters should be submitted in typewritten form, double-spaced, and are not to exceed 2½ pages. When appropriate, we will solicit comments from the original authors. All Letters to the Editor are subject to editing and possible abridgment.

RADIOASSAY OF ^{14}C LABELED MELANIN

To the Editor:

The article by White and Hu [1] has many weaknesses which open their conclusions to question:

1. Radioassay of C^{14} labeled melanin is ambiguous because of possible non-covalent binding of labeled substrate to melanin or metal. The authors assumed that use of detergent eliminated this potential error, but included no controls to monitor the effectiveness of this method.

2. The Pomerantz radioassay system [2] was not properly validated by him. He never standardized the method with known hydroxylating enzyme (e.g., mushroom tyrosinase) and incorrectly assumed that column chromatography could be used to quantitate reaction products (dopa and water)—and thereby obtain stoichiometric confirmation of its validity. Column chromatography, by its very nature, cannot result in total release or retention of substances [3]. Additional sources of error in his experiments were the use of ascorbate in assays of dopa, but not in assays of triated water (ascorbate can affect the rate of hydroxylation of tyrosine) and lack of evaporation controls to monitor the purity of his water fractions. Studies in our laboratory [4] have shown that measurement of tritiated water as an indicator of hydroxylation of tritiated tyrosine cannot be used with crude preparations (such as the preparation used by White and Hu), since hydrogen peroxide can generate tritiated water by non-enzymatic tritium exchange [4] and significant amounts of hydrogen peroxide can be formed by oxidases in crude preparations [5]; such oxidases could have been running concurrently in the gels of White and Hu. Formation of high concentrations of hydrogen peroxide in the gel band could, in turn result in non-enzymatic conversion of tyrosine to dopa. Under such circumstances, drop in tritiated water and tritiated dopa by boiling would be due to suppression of hydrogen peroxide-generating oxidases and would not reflect ability of melanoma "tyrosinase" to hydroxylate tyrosine.

Our experiments [4] have demonstrated that measurement of tritiated water as an indicator of hydroxylation of tritiated tyrosine is a valid *qualitative* method with *isolated* enzymes when purity of water fractions is controlled by evaporation.

3. The credibility of White and Hu is compromised by their use of inadequate time for heat-inactivation of enzymes and their assumption of total binding of amino acids to the column (see above). Both mammalian "tyrosinase" and peroxidase are relatively heat-resistant enzymes requiring longer periods of boiling than 5 min for proper heat-inactivation.

4. White and Hu suggest that their "peroxidase" band could represent hemoglobin (this could well be true, since melanoma peroxidase is membrane-bound in melanosomes), but conclude that peroxidase cannot hydroxylate tyrosine on the basis of activities of this band. Further, they ignored the requirement that adequate hydrogen peroxide concentration be present to demonstrate peroxidase activity. (At any rate, peroxidatic hydroxylation could not be determined with this system because of non-enzymatic tritium exchange produced by hydrogen peroxide.) Their inability to obtain soluble peroxidase from cultures of melanoma cells coincides with our experience; they neglected to mention, however, that the pellets have intense peroxidase activity.

The question of the function of mammalian "tyrosinase" need not be the subject of continuing controversy, since the enzyme can be easily isolated and its function can be determined with standard direct and indirect methods. We have carried out this isolation and assay [6] and have found that mammalian "tyrosinase" cannot hydroxylate tyrosine under any conditions, and is actually a dopa oxidase. This finding paralleled that of our histochemical studies with labeled [7] and unlabeled [8] substrates. It is unfortunate that White and Hu did not even mention our work with isolated enzyme in their paper. Older studies of Pomerantz [2] and others, claiming that isolated mammalian "tyrosinase" can hydroxylate tyrosine, can be dismissed since they did not carry out controls for peroxidase contamination. We have offered to exchange our enzyme preparation for that of Pomerantz and others, but to date this offer has been ignored. We would be pleased to exchange our isolated melanoma "tyrosinase" preparation for the "tyrosinase" preparation obtained by White and Hu from their gels, so that each of us can have an opportunity to examine their electrophoretic characteristics, purity and function.

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Milton R. Okun, M.D.
Department of Dermatology
Tufts University School of Medicine
Boston, Massachusetts 02111

REPLY TO DR. OKUN

1. We stated in our report our awareness of the possible ambiguity of the ^{14}C "radioassay" for dopa oxidase activity (fig 2b of ref. 1). This is one reason we also used the dopa staining technique (fig 1a of ref. 1), which verifies the coincidence of dopa oxidase activity with the peak of ^{14}C-dopa labeling.

2. Although Pomerantz did not use mushroom enzyme to standardize his method, he did report quantitative correlation of tritium release with tritiated dopa formation [2,3], and the tritiated dopa must have arisen by hydroxylation of tyrosine. Column chromatography is certainly a valid method for separation and quantification of tyrosine and dopa when the behavior of these compounds in such chromatographic systems is adequately characterized. Inspection of Pomerantz's papers [2,3] confirms that he carefully verified the identity of chromatographically purified dopa by evaporation, by paper electrophoresis, and by paper chromatography in 3 solvent systems. He also evaluated the recovery by measuring concurrently purified cold dopa.

Furthermore, Dr. Okun's statement that Pomerantz [3] used ascorbate in experiments for assay of one product but not the other does not seem accurate. Pomerantz measured the production of both ^{3}HOH and ^{3}H-dopa in the presence of ascorbate (ref: 3, fig 2), and he compared ^{3}HOH production in the presence and absence of ascorbate (ref. 3, fig 1). Also, Pomerantz himself pointed out the effects of ascorbate on the reaction. With respect to "purity" of water fractions, controls conducted by Pomerantz [2] and by us [1] showed that the Norit A columns quantitatively removed labeled tyrosine and dopa. Therefore, we feel that Pomerantz *did* properly validate his methods, and the criticisms of his method do not apply.

We can offer no opinion of the work of Shapiro et al [4], cited by Dr.