STATEMENT

The following signed statement has been received with a request that it be published in *FEBS Letters*:

In October 1977, Mr O'Halloran joined my laboratory as a graduate research assistant and was responsible for setting up an experimental system for investigating the transport of amino acids in pig kidney microvillar vesicles. The results he produced showed a statistically significant effect at low concentrations of a proteinase inhibitor, phosphoramidon, on L-leucine transport. Four compounds with chelating properties, EDTA, 1,10-phenanthroline, dithiothreitol and cysteine, were also shown to be strongly inhibitory and these results, too, were reproduced in several experiments. A paper based on these results, pointing out some similarities and differences between the properties of the leucine transport system and those of an endopeptidase in the same membrane, appeared in *FEBS Letters* [1].

In October 1979, another graduate joined my laboratory in order to extend these findings to the transport of other amino acids in the same vesicle preparation. Differences from the published results were soon apparent and a reinvestigation of the experiments on leucine transport was undertaken. Except for some positive results showing inhibition by 1,10-phenanthroline (which also inhibited the transport of L-proline, L-alanine and D-glucose), the other compounds did not inhibit at the published concentrations (I. S. Fulcher and A. J. Kenny, unpublished observations). Mr O'Halloran also performed four experiments in which the reagents were coded. In one, *o*-phenanthroline and dithiothreitol inhibited at 100 μM and phosphoramidon at 1 mM. The other three experiments were negative.

From the evidence now available, I no longer believe that the results published in [1] can be confidently attributed to the effectors studied (with the exception of 1,10-phenanthroline) but were instead derived from extraneous factors in the experiments, such that apparently significant data were produced. Attempts to identify the nature of these factors have failed. Phosphoramidon, EDTA, dithiothreitol and cysteine do not cause significant and reproducible inhibition of L-leucine transport, except at concentrations too high to be regarded as site-specific. Nor, too, has any effect of other chelating agents, e.g. 8-hydroxyquinoline and α,α'-bipyridyl, been demonstrated. There is therefore no evidence to suggest that the leucine transport system is dependent on a metalloprotein, nor that it is similar in any way to the microvillar membrane endopeptidase. I profoundly regret any confusion that the publication [1] may have caused.

Reference

[1] Kenny, A. J. and O'Halloran, D. M., Effect of chelating agents and phosphoramidon on the L-leucine transport system in microvillar vesicles from pig kidney, FEBS Letters (1979) 101, 407–410.

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Mr O'Halloran writes:

The recent experiments by me to confirm data used for the publication [1] were unsuccessful in consistently reproducing the results therein. I have been unable so far to elucidate further this discrepancy but have noted that the efficiency of the preparation to transport L-leucine has greatly increased in the interim period suggesting that the properties and/or composition of the vesicle preparation have changed. It may be that the inconsistencies recently obtained and the fluctuating effects of phosphoramidon and some chelating agents reported by me and my colleagues, are a reflection of this change.

I regret any inconvenience that may have been caused by the publication [1] but am hopeful that current experiments designed to characterize further the vesicle preparation and its production will answer this discrepancy and resolve the question of whether a metalloprotein is involved in amino acid transport.

20th December 1979

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