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SHORT COMMUNICATIONS

Nontransport of certain substituted amino acids*

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THE suggestion has been made in this journal that actively transported amino acids be utilized as 'carriers' to bring various cytoactive groupings into cells.[1] For such substituted materials to perform this function, it is apparent that the added grouping must not interfere with the transport event. It is conceivable that the addition could alter the pK values of the parent compound. Recent studies by

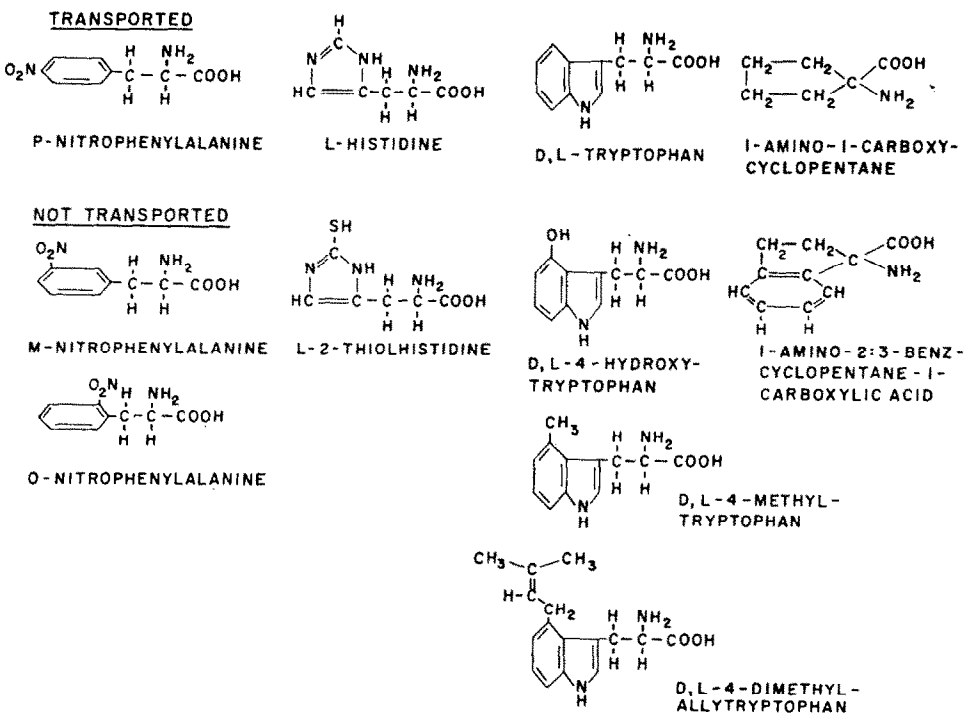

FIG. 1. Amino acids studied for transport against a concentration gradient.

Christensen and Oxtender[2] have indicated, however, that modest alteration of pK values have but slight effect on transport. A second possibility is that, by steric bulk or local interactions, the addend might cause diminution or cessation of transport. We wish to point out possible examples of this phenomenon, namely interference with amino acid transport by substitutions on their side chains.

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This emphasizes that additions to amino acids must be carefully chosen. The results appear all the more interesting since the statement has been made that the size of the side chain appeared of little importance in amino acid transport.[3]

Everted sacs were prepared from the small intestines of adult golden hamsters fed *ad libitum* until time of sacrifice.[4] Three sacs were made from each hamster and at least two animals utilized per compound. Sacs were filled (serosal fluid) with 1 ml of the compound dissolved in Krebs-Henseleit buffer (pH 7·4). Sacs were incubated in 5 ml of identical composition (mucosal fluid) contained in stoppered 25-ml Erlenmeyer flasks that had been gassed with 95% O_2 + 5% CO_2. After 1-hr incubation at 37° in an oscillating water bath, sacs were drained and mucosal and serosal solutions centrifuged to remove sloughed tissue. Solutions were then analyzed for the compounds. Sacs were blotted and weighed. Controls consisted of sacs incubated with buffer alone (to correct for materials sloughed by the sacs). The amino acid concentrations employed were of the order of 1×10^{-3} M. Listed below† are the sources of the compounds, analytic methods, actual concentrations, and amount transported in those cases in which net movement did occur (transport was expressed in terms of micromoles of compound gained in the serosal fluid per 400 mg sac weight).

Compounds utilized are shown in Fig. 1, where they are divided into two groups: those transported against a concentration gradient, and those not transported. It can be seen that alterations on the rings to produce an increase in bulk (principally in the *ortho-* and *meta-* positions) caused cessation of amino acid transport. Aside from the nitrophenylalaines, the additions had little effect on pK values, and other explanations for the lack of transport must be sought. Such factors as steric bulk and the effects of polar and nonpolar groups on transport affinity have to be considered. One point, however, is clear—some additions to amino acids result in nontransport of the molecule. The 'selection rules' determining which of these noncharged groupings result in the halting of transport remain to be demonstrated. It can likely be predicted that addition of certain pharmacologically interesting groupings to amino acids will be accompanied by cessation of active transport.

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† L-Histidine (CalBiochem) was assayed by means of L-histidine-^{14}C, generally labeled (New England Nuclear Co.), and liquid scintillation counting; at 1×10^{-3} M, transport was 0·38 μmole/sac. L-2-Thiolhistidine (CalBiochem) was used at 2×10^{-3} M and assayed at 258 mμ. 1-Amino-1-carboxycyclopentane (CalBiochem) was quantitated by means of the carboxyl-^{14}C compound (CalBiochem); at $0{\cdot}5 \times 10^{-3}$ M, 0·37 μmole was transported/sac. The 1-amino-2:3-benzcyclopentane-1-carboxylic acid was kindly supplied by Dr. T. A. Connors. At 5×10^{-3} M it was quantitated by its absorbance at 220 mμ. In separate experiments, using the compound at 4×10^{-3} M, aliquots of the fluid were dried at 105°, and 0·1% ninhydrin in glacial acetic acid added; after 1 hr the green color produced was read at 622 mμ. DL-Tryptophan (CalBiochem) was quantitated at 280 mμ; at 2×10^{-3} M, transport was 0·50 μmole/sac. DL-4-Dimethylallyltryptophan was provided by Dr. H. Plieninger and at maximal solubility (about $0{\cdot}5 \times 10^{-3}$ M) it was assayed at 280 mμ. DL-4-Methyltryptophan (Mann), studied at 5×10^{-3} M, was assayed at 275 mμ. DL-4-Hydroxytryptophan (CalBiochem) was utilized at 4×10^{-3} M and assayed at 280 mμ (the outer fluid was slightly discolored at the conclusion of the experiment, indicating some instability). The nitrophenylalanines were kindly provided by Dr. Alvie L. Davis. The DL-*o*-Nitrophenylalanine was run at $2{\cdot}5 \times 10^{-3}$ M and assayed at 270 mμ. DL-*p*-Nitrophenylalanine (4×10^{-3} M) was also assayed at 270 mμ. DL-*p*-Nitrophenylalanine (4×10^{-3} M) was estimated at 276 mμ; transport was 3·2 μmoles/sac.