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Differences in the uptake and efflux of two non-utilizable amino acids, α-aminoisobutyric acid and 1-aminocyclopentane carboxylic acid, by the "cut" rat diaphragm

α-Aminoisobutyric acid and 1-aminocyclopentane carboxylic acid (cycloleucine) have both been extensively used in the study of amino acid transport *in vitro*[1,2]; however, experiments on α-aminoisobutyric acid uptake where the "cut" diaphragm has been employed show that the accumulation of this amino acid continues at a maximal rate even 4 h after the beginning of an incubation at 0.05 mM (ref. 3). Moreover, the rate of uptake was found to be much greater than when the "intact" diaphragm was used, although, in the latter instance, there was also no evidence that after 4 h there was any reduction in the rate of amino acid accumulation[3]. By analogy with the observation of OXENDER AND CHRISTENSEN[4] that a failure to achieve equilibrium of distribution of amino acid between the extra- and intracellular phase denotes a diminished efflux of amino acid from the cell, it seemed likely that α-aminoisobutyric acid might have a relatively slow rate of exit from the diaphragm.

In order to investigate this possibility, "cut" hemidiaphragms from male Sprague–Dawley rats weighing 120–150 g were prepared by the method of KIPNIS AND CORI[5] and were incubated for varying times with either α-amino[^{14}C]isobutyric acid or [^{14}C]cycloleucine in oxygenated Krebs–Ringer bicarbonate buffer (pH 7.4) at 37° with a concentration of amino acid in the medium of 0.065 mM. Amino acid accumulation and efflux were then studied by using methods previously described[6,7].

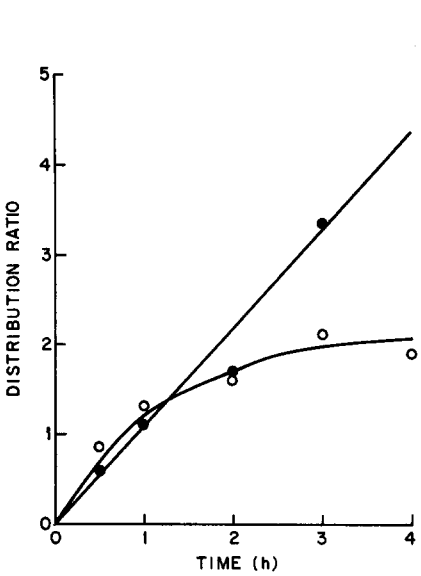

Fig. 1. Accumulation of α-aminoisobutyric acid (●—●) and cycloleucine (○—○) in "cut" rat diaphragm.

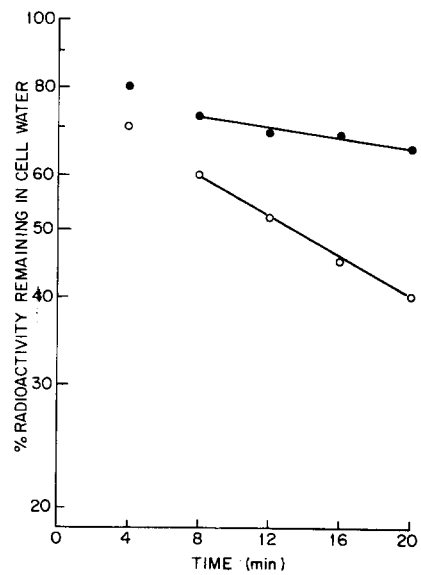

Fig. 2. Efflux of α-aminoisobutyric acid (●—●) and cycloleucine (○—○) from "cut" rat diaphragm.

Total cell water and inulin spaces were determined and were similar to those found by others[5]. Paper chromatography of medium and watery extract of tissue showed that the position of the radioactivity corresponded with that of the authentic compound.

Fig. 1 shows the accumulation of α-aminoisobutyric acid and cycloleucine in paired halves of "cut" diaphragms. The ordinates represent the distribution ratio of radioactivity between intracellular water and the medium and the abscissae show time. It is apparent that, while the accumulation of cycloleucine approaches equilibrium, the net uptake of α-aminoisobutyric acid continues linearly for the duration of the experiment.

In Fig. 2 are shown the efflux curves for each amino acid plotted semilogarithmically. There is a rapid initial loss of radioactivity from the tissue followed by a slower linear loss. Comparison of the latter component of each curve shows that the rate of loss of radioactivity from the tissue was much greater with cycloleucine than with α-aminoisobutyric acid; the $t_{1/2}$ of cycloleucine efflux was 21 min compared with 74 min for α-aminoisobutyric acid. This slow rate of efflux of α-aminoisobutyric acid is similar to that which has been observed in the intact diaphragm[8].

Thus, although the initial rate of uptake of α-aminoisobutyric acid is slower than that of cycloleucine (Fig. 1), the difference in efflux rate could account for the continued intracellular accumulation of α-aminoisobutyric acid in contrast to the pattern of the accumulation curve of cycloleucine which approaches equilibrium. Furthermore, it may be concluded that in the study of situations where intracellular accumulation may be affected by changes in efflux, cycloleucine is a better choice of non-utilizable amino acid than α-aminoisobutyric acid.

National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md. (U.S.A.)

D. R. LONDON*
S. SEGAL**

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* Performed while in receipt of an M.R.C. Travelling Fellowship. Present address: St. Thomas's Hospital, London, S.E.1., England.
** Present address: Department of Pediatrics, University of Pennsylvania, Philadelphia, Pa., U.S.A.