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Kinetics of the accelerated intestinal valine transport in 2-day-old rats

Previous studies in our laboratory have suggested that the active intestinal transport of valine decreases in rats as a function of age[1]. For both amino acids[2] and sugars[3], transport peaks have been observed immediately after birth. These studies are not conclusive since initial velocity conditions were not employed and increases, decreases, or maximal rates of transport should be based on maximal rate data. The purpose of the present study was to compare the kinetic characteristics of valine transport in 2-day-old rats with those of adult rats in order to confirm the presence of an accelerated rate of transport in young animals and to determine whether the increased transport represents an increase in the number of transport carriers (*i.e.* an increase in v_{max}) or the transient presence of transport carriers having a greater efficiency to bind the amino acid (*i.e.* a decrease in the apparent K_m).

2-day-old Wistar rats were used as the experimental animals. The rats were killed by decapitation and the small intestine quickly removed. The intestine was then split longitudinally and the luminal contents removed by washing with oxygenated saline. The intestinal segment from each rat was then placed in 5 ml of an oxygenated Krebs–Tris buffer (pH 7.4)[4] containing 8000–15000 counts/min per ml of uniformly labeled L-[^{14}C]valine (Amersham Searle Corp., specific activity 14.9 mC per mmole) and nonradioactive valine to the desired final concentration. In addition, 5000–12000 counts/min per ml of methoxy-[^{3}H]inulin (New England Nuclear Corp., specific activity 672 mC/mmole) was added to the medium to measure the extracellular space of the tissue[5]. The segments were incubated at 37° for the desired time periods after which they were washed quickly in saline, blotted and weighed. The segment then was homogenized in 4 times its weight of 5% trichloroacetic acid and the homogenate centrifuged. Aliquots of the supernatant were counted in a TriCarb liquid scintillation spectrometer (Packard) in a xylene, dioxane, ethanol, naphthalene, 2,5-diphenyloxazole, and 1,4-bis-2-(5-phenyloxazolyl)benzene system. The spectrometer was adjusted to permit 60% ^{14}C efficiency, 22% ^{3}H efficiency, less than 0.01% ^{3}H efficiency on the ^{14}C channel, and 10% efficiency of the ^{14}C on the ^{3}H channel. Valine transport is expressed as intracellular accumulation which is defined as the millimolar concentration of valine in the cellular water after a given incubation period. The formula used to calculate intracellular accumulation has been previously reported[4,6]. Each accumulation value represents the average of the indicated number of determinations each of which is the mean of at least three individual experiments. All regression lines were calculated by the method of least squares and the confidence limits of the x and y intercepts and slopes of these lines were calculated by the method of JERVIS AND SMYTH[7].

Valine accumulation was essentially at initial velocity through the first 5 min and reached a steady state after 10 min. Table I shows the effect of valine concentration over the range 0.5–5 mM on the intracellular accumulation of valine after 5 min. Valine accumulation in the intestine from 2-day-old rats was increased 50–70% over that found for the same valine concentration in the intestine from adult rats[4,6].

Fig. 1 presents the data of Table I plotted as the velocity or intracellular accumulation as the ordinate against the velocity divided by the initial substrate concentration as the abscissa. For comparison, a similar plot of valine accumulation from

adult rats is included[4]. This type of curve has been reported to give a more uniform distribution of points than does a Lineweaver–Burk plot[8]. The equation of the line representing valine accumulation in the 2-day-old rats was $y = -5.830\,x + 32.848$.

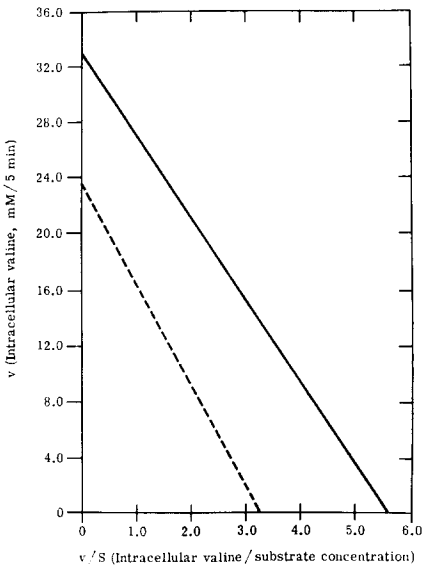

Fig. 1. Velocity *vs.* velocity/initial substrate concentration plot of data shown in Table I. The equations of the regression lines with the 95% confidence limits of their y intercepts and slopes are as follows: 2-day-old rats (———), equation to the line: $y = -5.830\,x + 32.848$; intercept on y axis = 32.848 ± 2.346; slope = −5.830 ± 0.632. Adult rats (– – – –), equation to the line: $y = -7.296\,x + 23.752$; intercept on y axis = 23.752 ± 4.164; slope = −7.296 ± 1.711.

TABLE I

THE EFFECT OF VALINE CONCENTRATION ON THE INTRACELLULAR ACCUMULATION OF VALINE AFTER 5 min

Intestinal segments were incubated for 5 min at 37° in a Krebs–Tris medium containing valine at the indicated initial concentrations. Each accumulation value represents the average of six determinations, each of which is the mean of four individual experiments ± S.E.

Valine concn. (*mM*)	*Intracellular accumulation* (*mM valine per 5 min*)
0.5	2.580 ± 0.173
1.0	4.840 ± 0.318
2.0	8.424 ± 0.556
5.0	15.146 ± 1.265

The intercept on the y axis, v_{max}, was 32.848 with 95% confidence limits of ± 2.346. The slope of the line, $-K_m$, was −5.830 with 95% confidence limits of ± 0.632. The equation of the line representing valine accumulation in the adults was $y = -7.296\,x + 23.752$. The y intercept and slope with the 95% confidence limits were 23.752 ± 4.164 and −7.296 ± 1.711, respectively[4]. A Lineweaver–Burk plot of the data shown in Table I and corresponding data from adult rats[4] showed that on the

basis of 90% confidence limits, the y intercepts ($1/v_{max}$) were different while the x intercepts ($-1/K_m$) were the same. These kinetic plots indicate that the apparent K_m for valine accumulation by 2-day-old rats (average value from Fig. 1 and the Lineweaver–Burk plot of 5.9 mM) is not statistically different from that of adult rats (average value, 6.8 mM). However, the v_{max} of valine accumulation in 2-day-old rats (average value, 32.5 mM/5 min) is statistically greater than the v_{max} in adult rats (average value, 22.5 mM/5 min).

The increase in valine intracellular accumulation in the 2-day-old rats as compared to adult values can best be explained by the presence of more transport sites rather than by a more efficient binding of valine by the carrier. The progressive decrease in transport activity from newborn to adult levels may be a consequence of the disappearance of the widespread and locationally nonspecific transport sites found in the intestine and colon of newborn rats[9] eventually resulting in the specific distribution patterns for sugar and amino acid transport found in adult intestine.

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