Some Additional Characteristics of Methionine Transport in the Chicken Intestine

In an earlier study the intestinal absorptions of D- and L-methionine in the chicken were shown to occur by attachment to a common L-preferring site, and that the following effects of L-ethionine on the transports of these isomers were observed[1]. (1) L-ethionine, in concentrations ranging between 1–4 mM, inhibits the transports of 0.1 mM D- or L-methionine giving K_i values of 2.7 and 3.5 mM, respectively (K_i = dissociation constant of the inhibitor-receptor site complex), when these constants are measured from the slopes of the lines in plots of reciprocal percent inhibition as a function of reciprocal inhibitor concentration. (2) The kinetics of these inhibitions obey this function for competitive inhibition at the steady state: $1/P = (K_i/100)(1 + S_1/K)(1/I) + 1/100$. [Equation 1], where K = apparent Michaelis constant; I = inhibitor concentration; P = % inhibition of substrate uptake[1]. In this study we have tested further the effects of L-ethionine on the transports of the methionine isomers under conditions of low ethionine concentration and have examined the supposition that equation 1 is obeyed at these concentrations.

The steady-state uptakes of D- or L-methionine into chicken intestinal sections were studied using the following techniques[1]. White Leghorn female chickens, 12–18 weeks old, were fasted 24 h and killed by decapitation. A portion of small intestine 15 cm on either side of the yolk stalk was removed to ice cold, oxygenated (5% CO_2–95% O_2) physiological saline enriched with 0.3% glucose. Without distinguishing between orad and caudad ends, the 30 cm portion was cut into 3 cm sections, each of which was further cut lengthwise and blotted dry. The even-numbered and odd-numbered sections of tissue were segregated and incubated at 37 °C with shaking in 25 ml portions of Krebs-Henseleit buffer, containing 0.1 mM D-^{14}C or L-^{14}C methionine, 0.3% glucose and an atmosphere of 5% CO_2–95% O_2. L-ethionine was added to flasks containing even-numbered sections in concentrations ranging from 0.1–0.9 mM. After incubation for 1 h the segments, as previously reported[1], were extracted with aqueous ethanol and the labeled methionine removed was assayed by liquid scintillation techniques.

K_i values for inhibitions by L-ethionine as determined from a weighted, least squares method (Figures 1 and 2) were found to be 2.2 and 3.5 mM for D- and L-methionine, respectively. The functions generated by equation 1 for these compounds fall within the 95% confidence limits of every point with one exception (Figures 1 and 2), thereby indicating that these points are drawn from the same population as the lines. Further confirmation that equation 1 is an adequate description of L-ethionine inhibition of either D- or L-methionine steady-state transports over the concentration range employed comes from an F test which showed the variances (of the D- and L-methionine data) due to internal and external consistencies[2] to be equal at the 95% level.

Some properties of L-methionine release were examined. The methods used to analyze this process differed from standard techniques in the following ways. Incubation of intestinal sections with radioactive L-methionine in Krebs-Kenseleit buffer (KHB) was terminated after 30 min. The sections were then blotted, weighed, and re-incubated at 37 °C in KHB either with or without addition of non-labeled L-methionine in various concentrations for a period of 30 min. The ratio of the amount of labeled L-methionine released from the tissue in the presence of non-labeled L-methionine in the medium to the amount released with no addition indicates the relative efficiency of the external amino acid in bringing about release. Aliquots of incubation solution were assayed by liquid scintillation techniques[1]. The results are shown in the Table. Radioactive L-methionine loss from preloaded sections is accelerated over loss in the controls when pre-

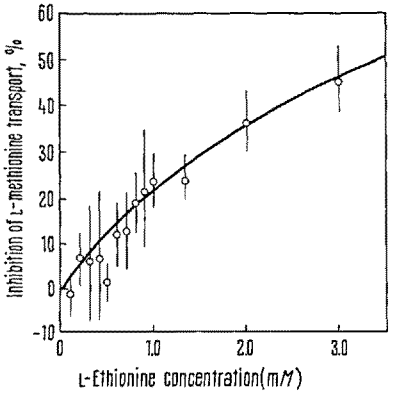

Fig. 1. Inhibition of L-methionine transport as a function of L-ethionine concentration. Each point represents an average % inhibition of uptake in 5 animals, where 5 pairs of 3-cm sections of tissue were analyzed per animal, with variability given by the 95% confidence limits for each average. The line was determined from equation 1, where S_1 = 0.1 mM and K = 4.1 mM[1], using a value for K_i from a weighted, least squares fit of the data (see text), including those points previously reported for L-ethionine concentrations 1–3 mM. In performing the least squares analysis, equation 1 was rewritten in the form $y = mx$, with $y = 1/P - 0.01$; $m = K_i/100\ (1 + S_1/K)$; $x = 1/I$. The weighted, least squares fit of the slope m was then found from:

$$m = \frac{\sum_{i=1}^{n} (y_i^4/\sigma_i^2)\, x_i\, y_i}{\sum_{i=1}^{n} (y_i^4/\sigma_i^2)\, x_i^2}\,,$$ where σ_i is the standard deviation of P.

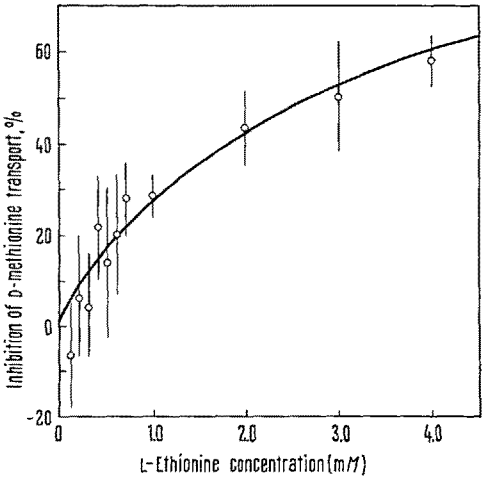

Fig. 2. Inhibition of D-methionine transport as a function of L-ethionine concentration. Conditions same as in Figure 1, except K = 10.0 mM. Points for L-ethionine concentrations 1–4 mM were previously reported (see text).

[1] J. Lerner and M. W. Taylor, Biochim. Biophys.Acta *135*, 991 (1967).

[2] W. E. Deming, in *Statistical Adjustment of Data* (John Wiley, New York 1943), p. 27.

loading is from a 1 m*M* L-methionine solution and when the non-labeled L-methionine is 1 m*M* ($1.21 \pm 0.03 > 1.00$ at the 0.01 level, Student $t = 5.97$). If labeled L-methionine is accumulated from a 0.1 m*M* solution, and if 0.1 m*M* L-methionine is present in the medium during re-incubation, no acceleration can be detected. However, increasing the non-labeled L-methionine concentration 10-fold results in significant enhancement ($1.23 \pm 0.06 > 1.00$ at the 0.02 level, Student $t = 4.625$). Concentrations of non-labeled L-methionine above 5 m*M* do not lead to greater enhancement of exit. Presumably, at high external concentrations, competition between the labeled and non-labeled species may take place at the inner surface of the mucosal membrane, thereby limiting the amount of labeled methionine leaving the cells. These observations on L-methionine release can be explained by the carrier concept which provides a model for experimental findings concerning counterflow or counter-transport[3].

Release of accumulated methionine into methionine-containing medium

Concentration labeled methionine initial incubation medium (m*M*)	No. of animals used	Concentration non-labeled methionine release medium (m*M*)	Ratio of amounts released ± S.E.
1.0	5	1.0	1.21 ± 0.03
0.1	5	0.1	1.00 ± 0.04
0.1	4	1.0	1.23 ± 0.06
0.1	5	5.0	1.32 ± 0.07
0.1	5	10.0	1.28 ± 0.07
0.1	4	20.0	1.28 ± 0.00

Tissues were incubated as described in text with L-^{14}C methionine and then transferred to KHB containing either L-methionine or no addition. Release conditions were 37 °C and 30 min. Release was represented by the ratio of amounts released, which is the µmoles of labeled methionine released by 5, odd-numbered sections of tissue in the presence of non-labeled L-methionine divided by the µmoles of labeled methionine released from 5, even-numbered sections into KHB.

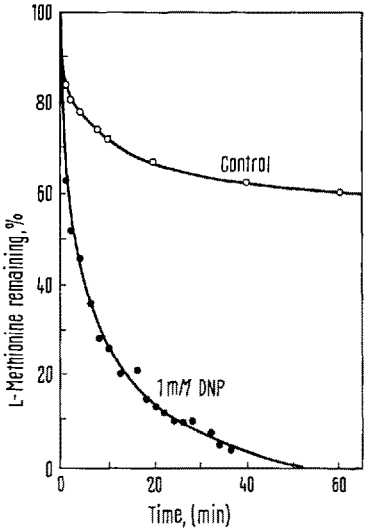

Fig. 3. Release of preloaded L-methionine. Control: 5, 30-cm portions of intestine from 5 animals were pooled and incubated in 250 ml of KHB containing 1 m*M* L-^{14}C methionine (37 °C) as described previously (see text). After 30 min, the tissues were removed to fresh KHB and incubated an additional 30 min (37 °C). Aliquots of the latter solution were assayed for labeled methionine. Total accumulation (100% remaining) = 133 µmoles. Release of preloaded L-methionine in a DNP-poisoned system: intestines were initially incubated in the same manner, except 1 m*M* DNP was added. Release was then followed into KHB containing 1 m*M* DNP. Total accumulation (100% remaining) = 21 µmoles.

Incubation of intestinal sections with 1 m*M* L-methionine plus 1 m*M* dinitrophenol (DNP) leads to reduction in the steady-state accumulation of methionine (1 h incubation) by about 70–80% compared to the uninhibited case[4]. Although this experiment demonstrates the requirement of metabolic energy, the degree of inhibition of the uptake and exit fluxes cannot be discerned. Other investigators have reported that DNP may affect both entry and exit by reducing the rate of the former while increasing the rate of the latter[3]. We elected to study the influence of DNP on the exit of L-methionine as a function of time by incubating tissues sections in 1 m*M* radioactive L-methionine for 30 min, either with or without addition of 1 m*M* DNP (Figure 3). The release of accumulated methionine into KHB containing either 1 m*M* DNP or no addition was then followed. The percent methionine remaining at time $t = 100\ (M_a - M_t)/M_a$, where $M_a =$ µmoles methionine accumulated in 30 min (determined by taking duplicate readings on aliquots of the initial and final incubation solutions); $M_t =$ µmoles methionine released into 250 ml KHB at time t. Figure 3 shows the release of preloaded methionine to be rapid during the first min; the process then slows asymptotically, reaching 60% remaining after 1 h. This loss is not described by a first order function of the log of the amount remaining with time. The loss of methionine in the DNP-poisoned system, on the other hand, is much more rapid during the first min, and it continues at a rate about 3-fold that found without added DNP. Almost total methionine loss occurs in the poisoned system within 50 min. In noting these results, it is of value to mention the findings of WINKLER and WILSON[5] on galactoside transport in *Escherichia coli*. These investigators reported that metabolic inhibitors reduce the affinity constant for exit without changing the affinity constant for entry of galactosides. Apparently, energy coupling in transport is needed to reduce the affinity of the substrate for its carrier on the inner side of the membrane. Our results seem to support this hypothesis[6].

Zusammenfassung. Aufnahme und Abgabe von Methionin wurden an Darmstücken des Huhnes gemessen und die Wirkung von Äthionin sowie von DNP studiert. Ferner wurde der Einfluss des kalten Methionins in der Aussenlösung auf die Abgabe von markiertem Methionin untersucht.

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[6] The authors express their appreciation to Dr. A. I. SCHEPARTZ for his interest in this work and to Mr. W. PENN for expert technical assistance.