Abstract—1. The intestinal absorption of methionine has been studied by an in vitro tissue-accumulation technique in the chicken.
2. Carrier-mediated transport for this amino acid was found in the duodenum, jejunum, ileum and colon in week-old chicks; the affinity and capacity of the small intestine for methionine absorption are relatively constant from proximal duodenum through the ileum.
3. The colon in hens can absorb methionine by a saturable process, suggesting that the large intestine functions in the adult.
4. The caecum in day-old chicks transports methionine by way of two kinetically distinct processes, one with high affinity and low capacity, and another with low affinity and high capacity.
5. By the end of the first week of life, the caecum no longer possesses the ability to transport methionine or alanine by a carrier mechanism.
6. A comparison of the specificity limits of methionine uptake at low substrate concentration in the colon and caecum revealed a contrasting order of maximal inhibitions brought about by a series of amino acids. This information, along with the kinetic findings, suggests the presence of different mechanisms for methionine absorption in the caecum and colon.

INTRODUCTION
We have reported in depth on the various aspects of the in vitro intestinal absorption of amino acids in the chicken (Miller et al., 1973). As a matter of convenience all former studies employed sections of intestine from the region about the yolk stalk, which is to be found midway in the bowel. In contrast, in the present investigation we were concerned with elucidating kinetic characteristics of methionine influx at a number of different levels in the gut. In particular, we have attempted to answer questions relating to whether (a) methionine is absorbed by a carrier mechanism at every level of the intestine; (b) there is a region of focus of absorption sites; (c) the affinity of the carrier varies with location; (d) the kinetic characteristics of methionine influx are the same in select regions of chick gut and the bowel of the hen; and (e) the caecum and large intestine possess carrier sites for methionine.

MATERIALS AND METHODS
Male chicks (Gallus domesticus) either day-old or "week-old" (7-15 days) and female chickens 90-92 weeks old were used as a source of intestinal tissue. The tissue handling and manipulations involved in the preparation and incubation of the intestinal segments have been previously described (Miller et al., 1973). A portion of the appropriate region of the intestine 5-15 mm in length was excised and immersed in previously gassed (O₂-CO₂, 95 : 5 by volume) physiological saline enriched with 0.3% glucose. This solution was maintained at 41°C. The intestinal section was stripped of mesentery and fatty tissue, cut lengthwise and allowed to contact towel paper to remove excess fluid. In the inhibition studies an individual segment of tissue was incubated at 37°C with shaking in a 25-ml Erlenmeyer flask containing a gassed (O₂-CO₂, 95 : 5 by volume) 5-ml portion of Krebs-Henseleit buffer, 0.3% glucose, L-([³⁵]C) amino acid (New England Nuclear Corp., Boston) and unlabeled L-amino acid(s) (Sigma Chemical Co., St. Louis; Nutritional Biochemical Corp., Cleveland). The incubation period was 1 min. The reaction was terminated by pouring the flask contents onto a Hirsch funnel (maintained under suction). The segment was then rapidly washed with physiological saline, removed to towel paper and then weighed. Tracer amino acid was extracted with 2-5% trichloroacetic acid. The tissue extract was clarified by centrifugation and the tracer assayed by standard liquid-scintillation counting techniques. The results are presented as percentage inhibition of amino acid transport and have been calculated from the ratio of uptake in the presence of inhibitor to that in the absence of inhibitor using paired segments of tissue from one animal (Miller et al., 1973). An incubation period of 5 sec was used to determine kinetic constants (apparent Michaelis constant, \( K_m \); maximum velocity, \( V_{max} \); and apparent diffusion coefficient, \( K_d \)). Tissue was incubated for 5 sec on a water-jacketed Hirsch funnel (maintained at approximately 37°C) which was mounted on a vacuum flask (LaBelle et al., 1971). The reaction was monitored with a timer-timeswitch (Fisher Scientific Co., Boston) that was coupled electronically to a relay, which controlled a vacuum line communicating with the flask. The incubation period was started by pouring the preheated (37°C)
and pregassed (O$_2$-CO$_2$) 5-ml portion of glucose-enriched Krebs-Henseleit buffer, which contained substrate, onto the filter and was terminated when the relay allowed a vacuum to evacuate substrate solution from the funnel. The tissue was simultaneously irrigated with physiological saline.

RESULTS

Figure 1 shows the rate of methionine uptake in 5 sec into jejunum of week-old chicks as a function of methionine concentration in the bathing medium. The curvilinear function is a composite of mediated transport and uptake by diffusion. The linear function was generated in the presence of 40 mM leucine, which was used to block the mediated portion of methionine entry, and is interpreted to represent the rate of uptake by diffusion. The kinetic constants listed in Table 1 were derived from Lineweaver & Burk plots which were generated from rates obtained when the nonmediated uptakes were subtracted from the rates found in the absence of leucine. Methionine transport is mediated at all levels of the intestinal tract in week-old chicks including the colon (Table 1; Fig. 2). The mechanism in the colon apparently remains through maturation, because it is still present in hens. A carrier mechanism, however, is absent in the caecum as indicated by data in Fig. 3 which show the rate of uptake to be the same when tested in the presence or absence of leucine. In contrast to week-old chicks, the day-old animal transports methionine in the caecum by way of two kinetically distinct processes, one with high affinity,

![Fig 2. Lineweaver & Burk plot of methionine transport in the colon. Incubation period was 5 sec. Rates were corrected for entry by diffusion (see text). O, 7-15-day-old chick; A, 90-92-week-old hen. Each value is the mean of approximately ten experiments using tissue from ten animals.](image)

### Table 1. Kinetic constants for methionine uptake

<table>
<thead>
<tr>
<th>Region</th>
<th>Age of bird</th>
<th>$K_m$ (mM)</th>
<th>$V_{max}$ (nmoles/g)*</th>
<th>$K_d$ (nmoles/g x mM)*</th>
<th>$V_{max}/K_d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal duodenum</td>
<td>7-15 days</td>
<td>1.5</td>
<td>142</td>
<td>12.5</td>
<td>11</td>
</tr>
<tr>
<td>Distal duodenum</td>
<td>7-15 days</td>
<td>1.8</td>
<td>200</td>
<td>16.5</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>90-92 weeks</td>
<td>1.4</td>
<td>160</td>
<td>11.4</td>
<td>14</td>
</tr>
<tr>
<td>Jejunum</td>
<td>7-15 days</td>
<td>1.2</td>
<td>100</td>
<td>12</td>
<td>8.3</td>
</tr>
<tr>
<td>ileum</td>
<td>7-15 days</td>
<td>2.2</td>
<td>111</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Colon</td>
<td>7-15 days</td>
<td>3.6</td>
<td>166</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>90-92 weeks</td>
<td>0.8</td>
<td>50</td>
<td>9.8</td>
<td>5.1</td>
</tr>
<tr>
<td>Caecum</td>
<td>7-15 days</td>
<td>0.3</td>
<td>31</td>
<td>20</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>1 day</td>
<td>5.6</td>
<td>166</td>
<td>20</td>
<td>8.3</td>
</tr>
</tbody>
</table>

* Incubation period 5 sec.

Experimental conditions are described in the text. $K_d$ values were determined from the slope of the line in the velocity vs. substrate concentration plot for methionine uptake in the presence of 40 mM leucine. $K_m$ and $V_{max}$ values were determined from Lineweaver & Burk plots of rates of methionine uptake corrected for diffusion by subtraction of the leucine-uninhibitable rate from the total uptake.
Methionine influx into chicken intestine

Fig. 3. Rate of methionine uptake vs. methionine concentration in the bathing medium for transport into segments of 7–15-day-old chick caecum. Incubation period was 5 sec. Rates were not corrected for diffusion. O, Methionine plus 40 mM leucine; △, methionine with no addition. Each value is the mean of approximately ten experiments using tissue from ten animals. Variability is given by S.E.

The affinity of methionine for transport as judged from K_m values (Table 1) is relatively constant along the length of intestine. It is, however, comparatively low in the chick colon and high in the colon of the hen. On the other hand, the affinity in the duodenum of the hen is equal to that in the chick duodenum.

Fig. 4. Lineweaver & Burk plot of methionine transport in the day-old chick caecum. Incubation period was 5 sec. Rates were corrected for diffusion (see text). Each value is the mean of approximately ten to twenty experiments using tissue from ten to twenty animals.

Fig. 5. Inhibition of alanine transport from 0.1 mM solution in chick caecum. Incubation period was 1 min. Rates of uptake used to calculate inhibition were not corrected for diffusion. Inhibition was calculated from the fraction uptake (ratio of inhibited to uninhibited velocity) using paired tissue from both caeca in one animal. Each value is the mean of approximately ten experiments. O, 40 mM methionine; △, 40 mM leucine; ●, 100 mM glycine; ○, 100 mM proline.

The relative "capacity" of intestine to absorb methionine is given by the ratio of V_max to the apparent diffusion coefficient (K_d) and represents a normalized estimate of maximum uptake when comparisons are made between levels of intestine in which variation occurs in the absorptive cross-sectional area per g of tissue. In this respect, a region of intestine with a large cross-sectional area per g of tissue would be expected to have a large number of sites for mediated transport as well as a large number of pores serving entry by diffusion. Thus, in this case K_d and V_max would both be large. The intestinal tract in the week-old chick has a relatively uniform capacity to absorb methionine as demonstrated by V_max/K_d values. The capacity of the hen colon to transport this compound is much reduced when compared with the hen duodenum or with various levels in the week-old chick intestine. In theory, the ability of the hen colon to absorb methionine would be partially compensated by the high relative affinity measured for this region of the bowel. Similarly, the high-affinity mediator proposed for methionine absorption in the caecum of the day-old chick was found to have a very small capacity.

The inhibition of 0.1 mM methionine uptake (1-min incubations) in week-old chick colon as a function of inhibitory amino acid concentration is illustrated in Fig. 6. In these experiments, a substrate concentration far below the K_m of methionine was selected to facilitate blockage of transport. Mannitol was used as an osmotic control in concentrations to 100 mM. The data show that methionine can be specifically inhibited by various amino acids. Leucine abolishes approximately 90 per cent of the
Fig. 6. Inhibition of 0.1 mM methionine in week-old chick colon. Incubation period was 1 min. Paired sections of tissue from one animal were used. Each point is the mean of approximately ten experiments from ten animals. ○, Leucine; ●, alanine; ●, proline; △, glycine; A, AIB; □, β-alanine; ×, mannitol.

Fig. 7. Inhibition of 0.1 mM methionine in week-old chick colon. Tissues were preincubated for 30 min in Krebs-Henseleit buffer enriched with 0.3% glucose, washed with physiological saline and then reincubated 1 min with substrate solution alone, or with the inhibitors shown in Fig. 6.

flux, and the remaining uptake can be attributed to diffusion. Alanine appears to saturate the sites at a level of inhibition somewhat below that of leucine, while the effects of proline, glycine and α-aminoisobutyric acid (AIB) are circumscribed. In addition, the action of β-alanine does not differ from the mannitol control. The same experiments were performed after a pre-incubation of tissue for 30 min in Krebs-Henseleit buffer. The patterns of inhibition generated in this manner are identical to those found without pre-incubation, perhaps with the exception of a slight change in the alanine curve (Fig. 7). The data show that the patterns are essentially independent of the metabolic status of the cells as the system approaches steady state in vitro. The inhibition patterns at a substrate concentration which favors operation of the low $K_m$ process of methionine transport in the day-old caecum are presented in Fig. 8. The saturation curves produced by leucine, alanine and proline are identical in magnitude to those found in the week-old colon. On the other hand, glycine and AIB are significantly more effective in the caecum. In Table 2 are shown data for the inhibition of methionine transport in the day-old caecum at a substrate concentration favoring operation of the high $K_m$ process. Of the substances tested, only leucine, alanine and perhaps proline inhibit. The inhibition experiments for the colon and caecum indicate therefore that the specificity of the sites centers primarily about compounds such as leucine and alanine, although other amino acids (proline, glycine and AIB) do react at high inhibitor to substrate concentration ratios. Moreover, the contrasting patterns of maximal inhibition by glycine and AIB in particular suggest that different sites transport methionine in the caecum and colon.

**DISCUSSION**

Various regions of the intestinal tract in higher animals appear to have discrete absorptive functions.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Inhibition of 1-min uptake (% ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate</td>
<td>Substrate (5 mM)</td>
</tr>
<tr>
<td>40 mM Leucine</td>
<td>48.8 ± 4.3 (10)</td>
</tr>
<tr>
<td>80 mM Alanine</td>
<td>23.7 ± 4.0 (10)</td>
</tr>
<tr>
<td>100 mM Proline</td>
<td>18.9 ± 6.2 (10)</td>
</tr>
<tr>
<td>100 mM Glycine</td>
<td>-6.6 ± 6.7 (10)</td>
</tr>
<tr>
<td>100 mM AIB</td>
<td>0.8 ± 3.9 (9)</td>
</tr>
<tr>
<td>100 mM β-Alanine</td>
<td>-12.0 ± 6.2 (10)</td>
</tr>
<tr>
<td>100 mM Mannitol</td>
<td>-10.0 ± 5.8 (10)</td>
</tr>
</tbody>
</table>

Experimental conditions are given in the text. The number of animals used to obtain the mean value is given in parentheses. Animals were used on day 1. Values for percentage inhibition of uptake were found from the fraction uptake $u'/u$, where $u'$ and $u$ were determined using paired caeca from one animal. Uptake values were not corrected for diffusion.
The patterns of uptake along the rat intestine for methionine, leucine, betaine, proline, sections of tissue from one animal were used as described in Fig. 5. Each value is the mean of approximately ten experiments from ten animals. Symbols used are given in Fig. 6.

The mammalian ileum has been reported to maximally absorb bile salts and vitamin B12 (Booth, 1967) and ascorbic acid was shown to be best absorbed in the duodenum and proximal small bowel of the guinea pig and in the ileum of the rat (Hornig et al., 1973). Matthews (1972) has observed the greatest uptake of methionine near the ileocecal valve in tied loops of rat intestine and has compared this with methionyl-methionine which is taken up best in the jejunum. The patterns of uptake along the rat intestine for methionine, leucine, betaine, proline, alanine, glycine and AIB have been noted by Baker & George (1971) to be similar in one sense in that the distal jejunum and (or) proximal ileum transport more of each amino acid than do either the duodenum or distal ileum. These results are in agreement with those of Schedl et al. (1969) for AIB and of Larsen et al. (1967) for leucine, but differ in principle from those of Reiser & Christiansen (1965), who found that the leucine analog valine is absorbed most rapidly by the proximal gut. Maximal absorption of cysteine and cystine was observed to occur in the rat duodenum (Neill, 1959). In the hamster, glycine, alanine, valine, leucine and AIB were noted to be maximally absorbed in midintestinal segments (Matthews & Laster, 1965). Additionally, the transport $K_i$'s for glutamate influx in rabbit ileum and jejunum are very similar as are the decreases in $K_i$ as a function of medium Na concentration in both regions (Schultz et al., 1970). With reference to the chicken, Fearon & Bird (1967) have discovered that active lysine transport is confined to the jejunum and ileum, and Scharrer (1972) found that a decrease in pH of the incubation medium from 7.4 to 6.4 was sufficient to cause an increase in galactose transport in chicken jejunum but not in the ileum.

There does not appear to be a localized region of maximal methionine transport in the week-old chick as judged from the ratio of $V_{\text{max}}/K_d$. We have interpreted these results to mean that the population of sites for methionine absorption is approximately uniformly distributed along the intestine from proximal duodenum through the colon. Moreover, if we calculate normalized rates with the constants in Table 1 and with a substrate level of 0·1 mM methionine by the equation

$$v = \frac{0·1V_{\text{max}}/K_d}{0·1 + K_m}$$

the following order is found: 0·69 mM/sec (proximal duodenum); 0·63 (distal duodenum); 0·64 (jejunum); 0·43 (ileum); and 0·30 (colon). Thus, for rates of transport where the substrate concentration is much smaller than the $K_m$, absorption is uniform along the small bowel but falls off in the most distal regions.

The widespread distribution of methionine uptake seen in our studies can be compared with the results of Baker & George (1971) which indicate that although maximal uptake occurs in the rat ileum, absorption at no segment (duodenum through distal ileum) differs from the mean of all segments by more than 15 per cent. On the contrary, betaine uptake was found to be maximal in the distal jejunum and was 153 per cent of the mean, while uptakes at the proximal and distal extremes were shown to be very small. These investigators have observed, in fact, that both methionine and betaine generate transport patterns along the gut which can be considered to be extremes; most neutral amino acids have intermediate patterns. Another comparison can be made with methionine transport in human small intestine. The intubation technique has been used to reveal a $K_m$ of 12·28 mM for the proximal bowel and 2·0–5·7 mM for the distal bowel; corresponding $V_{\text{max}}$ values were 16–33 m-moles/hr per segment and 5–5 m-moles/hr per segment, respectively (Schedl et al., 1968). On the basis of these findings, the kinetic characteristics for chick gut would appear to differ considerably. However, the most distal section of the hen bowel transports methionine by a process with significantly lower capacity than observed for the hen duodenum. Schedl et al. (1968) have suggested that the different kinetic constants of the two regions in the human may reflect the presence of different transport mechanisms (carriers). Likewise, Baker & George (1971) have theorized that the two patterns of uptake generated by methionine and betaine are indicators of specific longitudinal distributions of two distinct membrane carriers. In this regard further work with other substrates will be required before we are in a position to postulate ideas on the presence and longitudinal distribution of carriers in the chicken.

The apparent Michaelis constants for the absorption of methionine into chick and hen duodenum are the same within experimental error. These data suggest that the transport mechanism has not
changed during the course of development. Moreover, identical values for $K_m$ were found for cysteine and valine accumulation in neonatal and adult rat small intestine (States & Segal, 1968; Reiser et al., 1970).

In addition to our findings on methionine transport in the chick colon, a number of reports have been published on the ability of the neonatal colon to absorb substances. Thus, the large intestine of the chick can accumulate $\alpha$-methylglucoside to a concentration gradient greater than one (Holdsworth & Wilson, 1967). This process was found to be phlorizin sensitive and to require Na. In the newborn rat 3-O-methylglucose and proline were observed to be accumulated throughout the small intestine and colon, but that after 30 days sugar transport was no longer seen in the colon or distal ileum and net proline transport was lost in the colon (Batt & Schachter, 1969). In another study, Batt (1969) reported that sulfate accumulation occurs along the entire length of small intestine and colon at birth in the mouse, but at the time of weaning (third week) it becomes restricted to the distal ileum.

The adult colon of a number of animals does, however, have the ability to transport certain inorganic salts and water (Wilson, 1962). As an example, Harrison & Harrison (1969) have shown that an in vitro preparation of rat large intestine absorbs Ca against a concentration gradient from the mucosal to the serosal compartment, and that this process is similar to the one in small intestine in requiring vitamin D. Several studies have demonstrated, in corroboration of the developmental work reported above, that the mature colon lacks mechanisms for sugar and amino acid absorption. Parsons & Paterson (1965) found no net transport of glucose, galactose or 3-O-methylglucose against a gradient in muscle-free sacs prepared from the rat, and a concentrative mechanism was noted to be absent for monolodotyrosine (Nathans et al., 1969), valine, citrulline and histidine (Evered & Nunn, 1968) in sacs. Similar results were obtained in vivo with AIB and cycloleucine (Christensen et al., 1963). A detailed study by Binder (1970) on alanine accumulation in the rat failed to reveal evidence of either active transport or facilitated diffusion. Saturation kinetics could not be demonstrated for alanine; its entry was not inhibitable by metabolic inhibitors or by other neutral amino acids; and accumulation was similar at both 25 and 37°C. In the same study, glycine influx into the large intestine of the rabbit was observed to be nonstaturable up to concentrations of 300 mM. There are two exceptions to these findings in addition to our own. One is the observation by Evered & Nunn (1968) on the accumulation of valine against a slight concentration gradient into muscle-free rings of rat intestine; by this preparation valine was also shown to be inhibited by methionine and glycine. Because of the discrepancy between these results and those obtained with the sac preparation (reported above), these authors have concluded that the process of accumulation in rings is probably more closely concerned with amino acid metabolism and requirements of the colon itself than with a specific transport mechanism which operates from the lumen to the blood. Recent findings indicate, on the contrary, that the dog colon has the capacity to absorb both sugars and amino acids by systems which are dependent upon cellular metabolism and Na (Luisier & Robinson, 1973).

The presence of transport carriers for methionine in the mucosa of the chicken large intestine raises the question of their possible contribution to the absorptive capacity of the intestinal tract as a whole. Perhaps it is reasonable to assume that during the first week of life the chick would utilize the most distal regions for absorption, since the colon is anatomically close to the point where the yolk sac communicates with the ileum. On the other hand, the relatively short colon of the adult is remote from the proximal regions which transfer the bulk of endogenous and exogenous nutrients. Nevertheless, the unexpectedly high affinity for methionine transport seen in the hen would suggest that this portion of intestine serves to scavenge the remaining quantities of amino acid which escape uptake in more proximal regions.

Holdsworth & Wilson (1967) have demonstrated that $\alpha$-methylglucoside can be accumulated against a concentration gradient in day 1 to day 3 chick caecum; this activity is subsequently lost. They showed further that before hatch the capacity of the caecum for active glycine transport is very similar to that of the small intestine, but that by day 8 posthatch, the concentrative mechanism is lost. Our work on the loss of alanine and methionine transport sites during the first week corroborates these findings. As the chick matures, the caecum loses columnar absorptive cells; villi become more stunted and the proportion of goblet cells increases (Holdsworth & Wilson, 1967). The discovery of the high affinity, low capacity and the low affinity, high capacity processes for methionine transport in the day-old caecum suggests that this portion of the alimentary canal plays a complex role in absorption of yolk material in the prehatch and neonatal organism. The occurrence of multiple saturable processes that operate in parallel has been reported for both lysine and leucine influx into rabbit ileum, where the kinetic constants for leucine ($K_i$'s equal 6.0 and 0.3 mM) are perhaps fortuitously similar to those in the chick caecum (Munck & Schultz, 1969). Two-limbed curves have also been found for glycine and proline transport in rabbit kidney tubules (Hillman & Rosenberg, 1969), for glutamate and aspartate in cerebral cortex and spinal cord of the rat (Logan & Snyder, 1972), and for $\gamma$-aminobutyric acid in brain of the 8-19-day-old chick embryo (Levi, 1970).

The results of Figs. 6-8 demonstrate that the inhibitions of methionine transport by various amino
acids in both the colon and cecum probably do not occur at a single carrier site, because discrete levels of saturating inhibition are seen. We have observed similarly circumscribed inhibition patterns for a number of neutral amino acids in the distal jejunum of the chick, and a rigorous analysis of the data has led us to suggest the existence of seven possible modes of substrate entry (Miller et al., 1973). The data in Figs. 6–8 show, moreover, that methionine absorption is apparently partitioned between processes shared with proline, glycine and (or) AIB, on the one hand, and those which exclude these substances, on the other. In view of these findings the double reciprocal plot of methionine entry in colon may, in fact, be a composite describing rates in several systems. The same argument applies to the double-limbed plot found for caecal transport. In this regard, Christensen (1966) has cautioned that a Lineweaver & Burk plot which represents the parallel action of two catalytic sites, one with a $K_m$, for example, four to ten times the other and with a similar $V_{max}$ values, can yield a linear graph. As a final point, the characteristic inhibition patterns generated on methionine transport in the caecum, especially with regard to the levels seen with glycine and AIB, serve to emphasize that the absorption mechanism(s) of methionine in this portion of the bowel differs fundamentally from the process(es) in the colon.

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REFERENCES


Key Word Index—Intestinal absorption of amino acids; amino acids; absorption; chicken; Gallus domesticus; large intestine; caecum; transport.