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# AMINO ACID TRANSPORT BY THE HELICOIDAL COLON OF THE NEW-BORN PIG

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## Summary

The proximal colon of the new-born pig maintains a stable short-circuit current which is partly dependent upon the presence of methionine. This interaction between methionine and short-circuit current shows Michaelis-Menten kinetics with a $K_m$ of 0.24 mM and a $V$ of 27 $\mu A \cdot cm^{-2}$. The net flux of methionine to the serosal surface of proximal colons also shows a hyperbolic relation to the external concentration of methionine ($K_m$ 0.38 mM, $V$ 10 4 $nmol \cdot cm^{-2} \cdot min^{-1}$). The proximal colon concentrates methionine within its epithelium giving a mucosal to medium ratio of 11.2 ± 1.9 (90 min incubation in 1 mM methionine)

The ability of the colon to transport methionine across and concentrate methionine within its mucosa is maintained for at least 24 h after birth Colonic transport of amino acids could be physiologically important in the pig, where the immediate post-natal transfer of immune globulins has been shown to cause a temporary inhibition of normal intestinal function

The pig at birth acquires a passive immunity to disease through the intestinal absorption of immune globulins Gross changes in mucosal morphology take place at this time [1] and the transport of sodium becomes much diminished [2,3]. This partial inhibition of normal intestinal function could lead to an incomplete absorption of sodium in the small intestine In this case the colon could become essential in controlling sodium balance in the whole pig Compensatory increases in the ability of the colon to absorb sodium during the first day of post-natal life have already been reported [4]. The aim of the present work was to see whether the colon could also actively transport amino acids at this stage in its development

Pieces of proximal colon, taken at birth or after a 24 h period of suckling, were mounted between two Lucite chambers so as to expose 2 4 $cm^2$ of

mucosal surface to the bathing medium Krebs-bicarbonate saline [5], gassed with 95% $O_2$ + 5% $CO_2$ and maintained at 37°C, was used to bathe both sides of the tissue Short-circuit current and open-circuit voltage was measured as described previously [3] Small volumes of bicarbonate saline containing high concentrations of methionine were added to the mucosal side of these preparations to test for changes in short-circuit current (maximal volume 100 $\mu$l added to 5 ml of bathing medium) Unidirectional methionine fluxes were also measured across short-circuited preparations of proximal colon In this case Krebs-bicarbonate saline contained different known concentrations of methionine and 5 5 mM glucose [$^{14}$C]Methionine was added to medium bathing either the mucosal or serosal side of the colon Fluid bathing both sides was replaced every 15 min and aliquots of the collected fluid counted for radioactivity Methionine fluxes were expressed as nmol transferred per $cm^2$ per min

The ability of the colonic mucosa to concentrate methionine was measured in a separate series of experiments Pieces of everted proximal colon were incubated in medium identical to that described above, containing [$^{14}$C]-methionine, and [$^3$H] inulin to enable subsequent correction for extracellular space Tissues removed after a 90 min period of incubation, were drained and blotted lightly The separated mucosa was weighed and a known volume of distilled water added The sample was frozen and thawed, the suspension centrifuged and samples of the supernatant then counted for $^3$H and $^{14}$C The wet to dry weight ratio was determined separately using colons incubated under identical conditions in non-radioactive medium Knowing this value, and using corrected values for [$^{14}$C] methionine uptake, it was then possible to calculate the true intramucosal methionine concentration

Table I shows the steady-state short-circuit currents, open-circuit voltages and conductances, measured across proximal colons in the presence and absence of methionine, after 15 and 90 min incubation Changes in electrical parameters over this period of time were not statistically significant The pig proximal colon is an extremely stable preparation used in vitro Including 0 1 or 1 0 mM methionine in the incubation medium caused the short-circuit

TABLE I

Values of short-circuit current, open-circuit voltage and tissue conductance obtained for the proximal colon of the new-born pig incubated in vitro in the presence of different concentrations of L-methionine Tissues were incubated at 37°C in Krebs-bicarbonate saline containing 5 5 mM glucose, gassed with a mixture of 95% $O_2$ + 5% $CO_2$ The final pH was 7 4 Values give means ± S E of from 12 to 13 observations

| Methionine conc (mM) | Time (min) | Potential difference (mV) | Short-circuit current ($\mu A \cdot cm^{-2}$) | Conductance (mmhos·$cm^2$) |
|---|---|---|---|---|
| 0 | 15 | 9 28 ± 0 76 | 53 5 ± 7 3 | 5 67 ± 0 52 |
| | 90 | 11 24 ± 3 13 | 57 1 ± 5 2 | 5 32 ± 0 55 |
| 0 1 | 15 | 12 44 ± 1 20 | 80 1 ± 11 1 | 7 03 ± 0 34 |
| | 90 | 12 06 + 1 10 | 67 2 ± 7 0 | 6 42 ± 0 44 |
| 1 0 | 15 | 11 29 ± 1 12 | 103 0 ± 10 7 | 8 27 ± 0 69 |
| | 90 | 9 47 ± 1 05 | 93 3 ± 10 1 | 9 19 ± 0 79 |

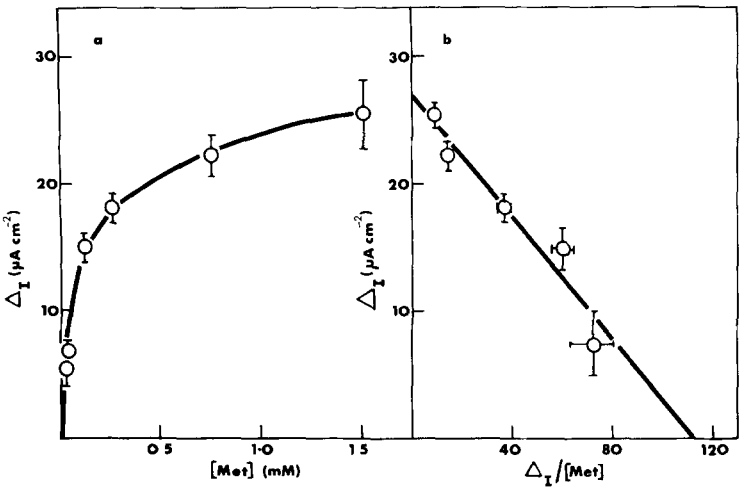


Fig 1 Methionine dependence of short-circuit current in the proximal colon of the neonatal pig (a) $\Delta_I$ gives the increase in short-circuit current when different concentrations of methionine (Met) are presented to the mucosal surface Increases in current were measured not later than 60 s following the addition of methionine (b) Hofstee plot of values shown in a. The maximal methionine effect is given by the intercept on the ordinate and the apparent $K_m$ for the methionine-membrane interaction by the negative slope Values give means ± S E of from 11 to 16 observations Glucose was not present in these experiments

current and conductance to increase ($P < 0\ 01$ for 1 mM versus no methionine in both cases). There was no significant effect on open-circuit voltage. These results were obtained under steady-state conditions using different populations of pigs

Fig. 1 shows the immediate effect of methionine on short-circuit current, a series of measurements being made on each colon immediately before and after the addition of methionine. The short-circuit current rose rapidly as the methionine concentration increased from 0 to 0.5 mM and then more slowly as the methionine concentration was further raised to 1.5 mM. A Hofstee plot of these results (Fig. 1b) showed this interaction to follow Michaelis-Menten kinetics with a $K_m$ for methionine of 0.24 ± 0.04 mM and a $V$ of 27 0 ± 1 7 $\mu A \cdot cm^{-2}$ Increases in short-circuit current were always accompanied by an immediate increase in open-circuit voltage. There was also a slight increase in tissue conductance, 5.24 ± 0 62 and 5 62 and 5 62 ± 0 59 mmho$\cdot cm^{-2}$ before and immediately after the addition of 1 mM methionine A paired $t$-test on these values showed the difference to be significant at the 5% level This result differed slightly from that shown in Table I where methionine was shown to have no effect on voltage. This discrepancy can be explained if one assumes that the main effect of methionine is to stimulate the electrogenic transfer of sodium into the intercellular space. This will cause an immediate rise in both short-circuit current and open-circuit voltage The continuation of this increased transport will, however, probably lead to greater swelling of the intercellular spaces which could then lower the open-circuit voltage by further increasing the shunt conductance

The unidirectional fluxes of methionine across pig proximal colon are shown in Fig. 2 The serosal to mucosal flux, which is shown to be linearly

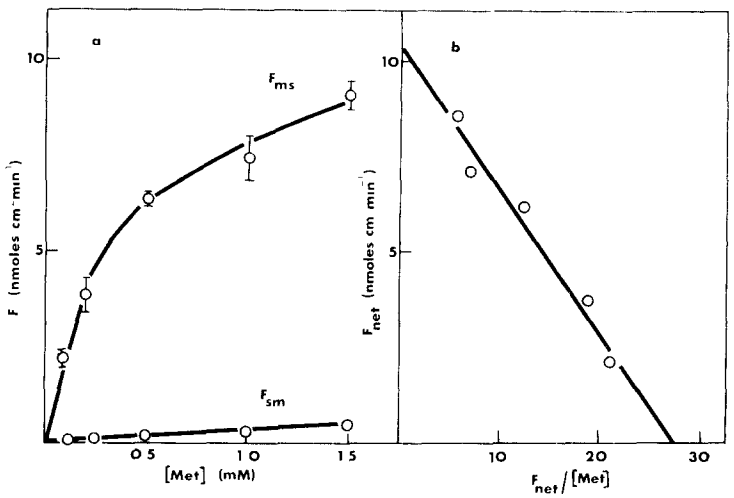


Fig 2 Methionine transport across the proximal colon of the neonatal pig Unidirectional methionine fluxes ($F$) were determined as described in the text Each preparation was short-circuited throughout the experiment Glucose was present at a concentration of 5 5 mM Each value gives the mean ± S E of from 8 to 18 determinations (a) unidirectional flux from mucosa to serosa ($F_{ms}$) and serosa to mucosa ($F_{sm}$) measured for different concentrations of methionine (Met ) (b) Hofstee plot of net methionine transfer ($F_{net}$)

dependent upon the concentration of methionine in the medium, remained some 20 to 50 times less than the corresponding mucosal to serosal flux Subtraction of the serosal to mucosal from the mucosal to serosal flux, for different concentrations of methionine, gives net fluxes showing a Michaelis-Menten dependence upon the external concentration of methionine. A Hofstee plot of these values gave an overall $K_m$ of methionine for its transport process of 0 38 ± 0.04 mM and a $V$ of 10.4 ± 0 62 nmol·cm$^{-2}$·min$^{-1}$

Everted pieces of colon were shown, in a separate series of experiments, to accumulate methionine over a 90 min incubation period The wet to dry weight ratio of colonic mucosa, measured after a 90 min incubation in non-radioactive medium was 7 67 ± 0 29 The corresponding accumulation ratio, with 1 mM methionite in the incubation medium was 7 18 ± 0 8

The above results show clearly that the colon of the new-born pig possesses an active transport system for methionine. The partly villous structure of the proximal colon at birth, the passing observation that the mucosa contains fat droplets [4] and the present demonstration of active amino acid transport in the colon, could lead one to suppose that this tissue is behaving, in the new-born pig, as a mere extension of the small intestine A closer comparison of transport parameters between these two tissues, however, suggests that this is too simple an interpretation of the experimental findings It is this comparative aspect of intestinal and colonic function which is being further investigated at the present time

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