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METHIONINE TRANSPORT BY PIG COLONIC MUCOSA MEASURED DURING EARLY POST-NATAL DEVELOPMENT

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SUMMARY

1. New-born pig proximal colon, incubated *in vitro*, transports methionine with a K_m of 0·33 mM and a V_{max} of 0·62 μmole cm^{-2} h^{-1}. There is still a net transport of methionine on day 4, but the K_m now increases to 10mM and the V_{max} falls to 0·15 μmole cm^{-2} h^{-1}. There is no net transport of methionine across proximal colons taken from 10-day-old pigs.

2. The mean intramucosal concentration of methionine, following incubation in medium containing 1 mM methionine, is 7·18 ± 0·8 mM for the new-born, 0·55 ± 0·05 mM for the 4-day old and 0·31 ± 0·06 mM for the 10-day-old pig.

3. Both methionine and glucose cause an immediate increase in the short-circuit current of new-born and 1-day-old pig colons. The kinetics for this interaction with methionine gives a K_m for methionine of 0·24 mM and a maximum effect of 27 μA cm^{-2}. This effect is not seen in 4- or 10-day-old pigs.

4. Net Na^+ transport across the new-born pig proximal colon, measured in the absence of methionine, is about three times that calculated from the measured short-circuit current. Methionine increases the mucosal to serosal flux of Na^+ by an amount roughly equal to that predicted from the increase in short-circuit current. The ability of glucose and methionine to affect short-circuit current is lost by day 4.

5. Short-circuit current, measured in the absence of methionine or glucose, increases between day 1 and 2 of post-natal life. This increased electrogenicity is maintained for up to at least 10 days after birth.

6. The pig proximal colon has many of the properties of a small intestine at birth. It actively transports methionine and the presence of methionine stimulates the absorption of Na^+. These effects could be physiologically important in the pig, where the normal absorptive function of the intestine is temporarily inhibited at birth by the intestinal transmission of immune globulins.

INTRODUCTION

It is universally accepted, by present day writers of textbooks of physiology, that the large intestine is unable to actively transport different amino acids. This statement is based on exhaustive experimental work carried out on species as different as the rat (Nathans, Tapley & Ross, 1960; Christensen, Feldman & Hastings, 1963; Binder, 1970), the hamster (Nathans *et al.* 1960; Cordero & Wilson, 1961); the rabbit (Binder, 1970) and humans and guinea-pigs (Robinson, Luisier & Mirkovitch, 1973). It also applies to the large intestine of the greek tortoise (Baillien & Schoffeniels, 1961). Until recently the only work contradicting this general conclusion came from tissue accumulation experiments. Adult rat colon accumulated valine against a concentration gradient (Evered & Nunn, 1968) and new-born, though not 31-day-old, rat colon accumulated proline (Batt & Schacter, 1969). More recent work shows methionine entry into chick and hen colonic mucosa to be by a saturable process which can be inhibited by the presence of other amino acids (Lerner, Sattelmeyer & Rush, 1975). Methionine can also be accumulated by the colonic mucosa of the new-born pig and in this case accumulation can be shown to be associated with the net absorption of amino acid (Smith & James, 1976). The first part of the present work examines the kinetics of this transport process in detail and tests for any interaction that might exist between the transport of methionine and sodium.

Studies of absorptive function in some colons of neo-natal animals show them to be capable of accumulating sugars as well as amino acids, this facility being lost as the animals grow older (Holdsworth & Wilson, 1967; Batt & Schacter, 1969). The dog may be an exception here in that the adult colon can absorb galactose by an energy and Na^+-dependent process (Robinson *et al.* 1973). The second part of the present work was undertaken to see whether methionine transport would continue during the early post-natal period of development. Changes in the nature of methionine transport did occur and these have been further investigated. A preliminary communication of part of these later findings has already been published (James, Smith & Wooding, 1976).

METHODS

Animals. Pigs were taken from a herd of Large Whites bred at Babraham. Parturition was induced routinely by the intramuscular injection of prostaglandin analogues on day 109–111 of gestation. Birth occurred some 24 h later. Piglets were used either at birth, before they could suck the sow, or on days 1, 2, 4 or 10. The method of induction has been described in detail elsewhere (Ash & Heap, 1973).

Preparation of tissue and measurement of short-circuit current. Pieces of colon, taken from the proximal region only, were rinsed and mounted in Krebs-bicarbonate

saline (Krebs & Henseleit, 1932) in Ussing-type chambers, so as to expose 2·4 cm^2 surface to the bathing media. Incubation was at 37° C in medium gassed with 95% O_2 + 5% CO_2. One piece of tissue was taken from the colons of 0-, 1- and 2-day-old pigs. Two pieces of tissue were obtained from the colons of 4- and 10-day-old pigs. This allowed one to make a paired comparison of mucosal to serosal and serosal to mucosal fluxes in the same animal. Short-circuit current was measured using a conventional system of voltage clamp. Correction was made for the resistance of bathing fluid and the final short-circuit current recorded on an Oxford pen recorder. Open-circuit voltages were measured between calomel half-cells, connected to the bath through agar bridges, at approximately 15 min intervals throughout an experiment. The open-circuit potential between these half-cells, measured in the absence of tissue, never exceeded 0·2 mV.

Changes in short-circuit current, produced by the addition of up to 100 μl of glucose or methionine solution (300 mM in each case) to the mucosal solution only, were recorded after a period of 1 min.

Flux measurements. Methionine at a known concentration was present in solutions bathing both mucosal and serosal surfaces of pig colon throughout the flux measurements. Incubation was originally in non-radioactive medium, [*methyl*-^{14}C] methionine (0·2–0·6 μCi/ml) being added after a preliminary incubation of 10–15 min. Both mucosal and serosal solutions were replaced every 15 min, aliquots of these solutions then being taken for conventional counting on a Packard scintillation spectrometer.

Sodium fluxes were also measured, using ^{22}NaCl (0·4 μCi/ml) in mucosal or serosal solution, as described for methionine, but using 10 instead of 15 min periods for collection. All flux measurements were carried out across tissues maintained under short-circuit conditions.

Measurement of intramucosal methionine concentration. Pieces of proximal colon, taken from 0-, 1-, 4- and 10-day-old pigs were everted and divided into two. One piece was incubated at 37° C for 90 min in Krebs-bicarbonate saline, containing 5·5 mM glucose, gassed with 95% O_2 + 5% CO_2. The second piece was incubated under identical conditions, in the presence of 1 mM methionine labelled with [*methyl*-^{14}C]methionine (0·01–0·05 μCi/ml), with [^{3}H]inulin (0·5–2 μCi/ml) added as a marker of extracellular space. The mucosa was scraped from both preparations at the end of incubation, that from the non-radioactive medium being weighed, dried at 95° C overnight, and re-weighed to give a wet to dry weight ratio for the tissue. Scrapings from the tissue incubated in radioactive medium were also weighed, 1 ml 0·1 N-HNO_3 then being added and the suspension frozen. These preparations were frozen and thawed twice before being centrifuged. Aliquots of the supernatant were then taken for dual isotope counting using a Packard scintillation spectrometer.

Wet to dry weight ratios were found to be independent of the age of the animal and so a grand average for all these measurements was used to calculate the water content of each tissue incubated in radioactive methionine. This was divided into the total amount of methionine present minus that associated with extracellular space, to give the calculated methionine concentration in mucosal cell water.

Chemicals. All radiochemicals used in this work were purchased from the Radiochemical Centre, Amersham, Bucks. They included ^{22}Na as an aqueous solution of NaCl, carrier free (> 100 mCi/mg Na), and L-[*methyl*-^{14}C]methionine as a freeze-dried powder (> 50 mCi/mmole). All other reagents were of Analytical Grade.

RESULTS

Effect of L-methionine and D-glucose on the electrical properties of new-born pig proximal colon

Pieces of new-born pig proximal colon maintain stable short-circuit currents when incubated *in vitro* for periods of up to 100 min (Bentley & Smith, 1975). These experiments were carried out in the presence of glucose. Table 1 summarizes results showing that glucose can itself change both the short-circuit current and the transmural potential difference of new-born pig colon.

TABLE 1. Effect of L-methionine and D-glucose on short-circuit current and open-circuit voltage of pig proximal colon. Pieces of colon taken from new-born pigs were incubated *in vitro*, in Krebs-bicarbonate medium, in the absence and presence of these two non-electrolytes. Values give means ± S.E. of from ten to twenty determinations. Glucose and methionine were used at concentrations of 5·5 and 1·0 mM respectively

Experimental conditions	Potential difference (mV)	Short-circuit current (μA cm^{-2})
No glucose, no methionine	6·1 ± 0·6	32·9 ± 2·0
Glucose	9·3 ± 0·8	53·5 ± 7·3
Methionine	10·3 ± 1·6	51·4 ± 3·7
Glucose plus methionine	11·3 ± 1·1	103·0 ± 10·7

Pieces of proximal colon taken from new-born, unsuckled, pigs were incubated for a period of 15 min in Krebs-bicarbonate medium containing: no glucose or methionine; glucose; methionine; glucose plus methionine. The potential differences and short-circuit currents recorded immediately after this initial period of equilibration are summarized in Table 1. Both potential difference and short-circuit current increased by about 50% in the presence of 5·5 mM glucose or 1 mM methionine. The effect of these two non-electrolytes was additive, current measured in the presence of both being twice that measured in the presence of either. The potential difference across colons incubated in glucose plus methionine-containing medium was also greater than that measured in the presence of either but the percentage change was less than that seen with short-circuit current. The combined presence of methionine and glucose causes a significant increase in tissue conductance.

Further experiments were carried out to determine the concentration dependence of non-electrolyte effects on colonic short-circuit current.

Methionine was used in preference to glucose in these experiments. The effect of glucose is generally similar to that of methionine but its possible metabolism within the mucosa could produce secondary effects on short-circuit current which would be difficult to interpret. Methionine was added to the mucosal side only, the effect on short-circuit current then being measured over a period of 5 min. The results obtained are shown in Fig. 1. The short-circuit current increased rapidly as the concentration of

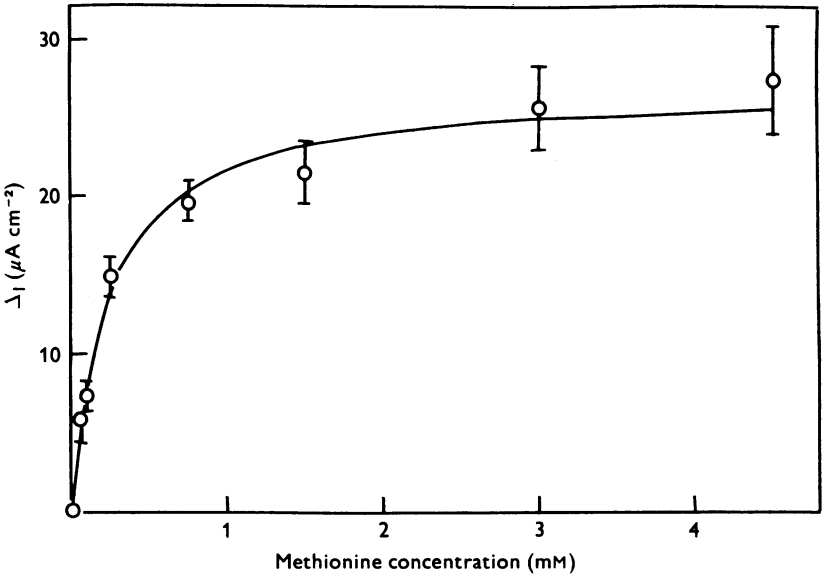

Fig. 1. Effect of methionine on the short-circuit current of pig proximal colon. Conditions of the experiment were as described in the text. Each value gives the mean change in short-circuit current (Δ_I) $\pm$ S.E. of from five to sixteen determinations. Experiments were carried out on colons taken from new-born and 1-day-old pigs. No difference in the magnitude of methionine effect could be detected between these two groups of animals. The line is calculated assuming Michaelis–Menten kinetics with a V_{max} of 27μA cm^{-2} and an apparent K_m of 0·24 mM.

methionine was raised to 1 mM and then more slowly as the concentration was further raised to 4 mM. The interaction between methionine and short-circuit current shows Michaelis–Menten kinetics. The curve drawn through the points is a theoretical one, calculated assuming an apparent K_m and V_{max} for methionine of 0·24 mM and 27 μA cm^{-2} respectively.

These results are similar to those reported previously for the small intestine, where the non-electrolyte dependent increase in short-circuit current was shown to be associated with a net increase in Na^+ transport (Schultz & Zalusky, 1964).

Effect of methionine on Na^+ transport across new-born pig proximal colon

Pieces of pig proximal colon were incubated initially in Krebs-bicarbonate medium containing no glucose or methionine. Trace quantities of $^{22}NaCl$ were added to the mucosal or serosal solution after a 15 min

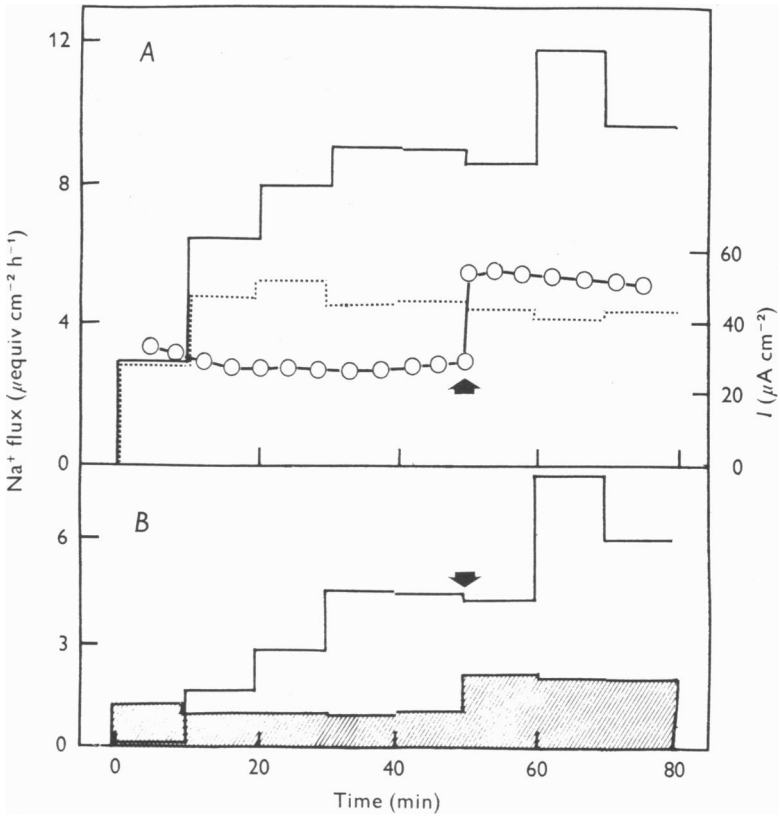

Fig. 2. Effect of methionine on Na^+ flux across the new-born pig colon. *A*, unidirectional fluxes of Na^+ were determined from mucosa to serosa (—) and from serosa to mucosa (- - -). Conditions of incubation are as described in the text. (—○—), short-circuit current (*I*). Values in all cases give the means of three experiments. *B*, net flux of Na^+ from mucosa to serosa (—) compared with equivalent theoretical flux of Na^+ calculated from short-circuit measurements (hatched areas). Arrows in *A* and *B* show where methionine was added to both serosal and mucosal solutions to give a final concentration of 3 mM.

equilibration period. This is given as time 0 in Fig. 2. The unidirectional fluxes of Na^+ were then measured at 10 min intervals, 3 mM methionine being added to the mucosal solution half-way through the experiment. Steady-state fluxes of Na^+ become established 20–30 min after addition

of isotope (Fig. 2*A*). Adding methionine caused an immediate increase in short-circuit current followed, after a 10 min lag period, by a significant increase in the mucosal to serosal flux of Na^+. The serosal to mucosal flux of Na^+ was not affected by the presence of methionine in the mucosal solution.

The net flux of Na^+ is compared to the calculated flux, assuming short-circuit current to arise solely from the electrogenic transport of Na^+, in Fig. 2*B*. The net flux of Na^+, measured under steady-state conditions in the absence and presence of methionine, is considerably greater than that calculated from the measured short-circuit current. Similar results have been reported for the pig colon incubated in glucose-containing medium (Bentley & Smith, 1975). The methionine-dependent increase in Na^+ transport, measured 20 min after the addition of methionine, is of the same order as that calculated from the immediate increase in current. Experimental variability makes it impossible at present to gauge accurately whether these fluxes are exactly equivalent.

Taking the example of the small intestine one might suppose that the ability of methionine to stimulate Na^+ transport is associated with its own active transport across the tissue. This was tested for in the following series of experiments.

Methionine transport across new-born pig proximal colon

Fig. 3 shows the time course of methionine flux across the new-born pig proximal colon. Methionine was present in both mucosal and serosal solutions at a concentration of 1 mM. Samples were taken for counting at 15 min intervals. The unidirectional mucosal to serosal flux of methionine increased steadily with time, approaching a constant value after about 60 min incubation. The serosal to mucosal flux of methionine, which was considerably less than the mucosal to serosal flux, had reached a steady state after only 30 min incubation. The short-circuit current remained essentially constant throughout, verifying the stability of these preparations used *in vitro*.

These experiments establish the ability of the new-born pig colon to actively transport methionine from mucosa to serosa. It was decided to use the last two periods of measurement to calculate methionine flux in future and to extend these measurements to further experiments designed to determine the concentration dependence of methionine transport. The results of these experiments are shown in Fig. 4. The mucosal to serosal flux of methionine rises rapidly as the external concentration increases from 0·1 to 0·5 mM. There is a continuing, though less pronounced, increase in transport as the external concentration further increases from 0·5 to 1·5 mM.

The serosal to mucosal flux of methionine is linearly dependent upon the external concentration of methionine over the whole range tested (0·1 to 1·5 mM). Regression analysis of these results gives a straight line of slope $25{\cdot}7 \pm 1{\cdot}3$ nmole $cm^{-2}\ h^{-1}\ mM^{-1}$ with a correlation coefficient of 0·93. The flux of methionine from blood to lumen, which varies from 2 to 5% of the influx over the concentration range tested, probably occurs by diffusion

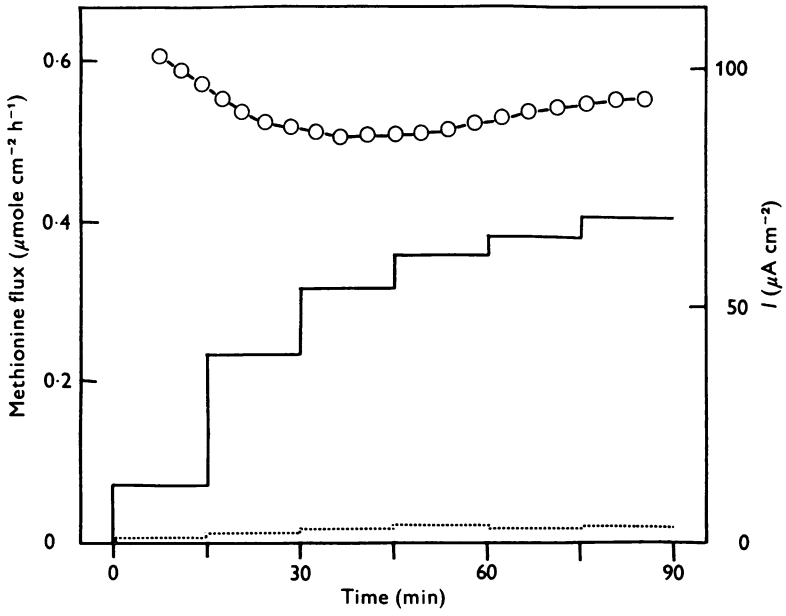

Fig. 3. Time-dependence of methionine flux across new-born pig colon. Unidirectional fluxes of methionine were determined from mucosa to serosa (—) and from serosa to mucosa (---). 1 mM methionine was present in all incubation solutions. Values give the means of five experiments. (—○—), short-circuit current (I). Glucose was present throughout incubation at a final concentration of 5·5 mM.

between or through the epithelial cells. The net flux of methionine shows a Michaelis–Menten dependence on external methionine concentration (Fig. 4B). The line drawn through these points has been constructed assuming an apparent K_m and V_{max} of methionine for its transport process of 0·38 mM and 0·62 μmole $cm^{-2}\ h^{-1}$ respectively. The concentration of methionine to half saturate its transport process is very similar to that needed to produce half maximal response of short-circuit current (0·38 and 0·24 mM respectively).

Pieces of new-born pig colon were finally incubated in Krebs-bicarbonate medium containing ^{14}C-labelled methionine, using [^3H]inulin as a space

marker, to test whether the colon at birth could concentrate this amino acid within its mucosa. Incubation was for 90 min to allow time for equilibrium to become established (see Fig. 3). The concentration of methionine used in the medium was fixed at 1 mM to ensure near maximal rates of transport (Fig. 4). The wet to dry weight ratio of mucosal scrapings taken at the end of incubation was $7{\cdot}67 \pm 0{\cdot}29$. The corresponding accumulation ratio was $7{\cdot}18 \pm 0{\cdot}8$. This sevenfold increase in methionine concentration should be regarded as a minimum, since not all cells in the colonic epithelium can be expected to accumulate methionine with equal facility.

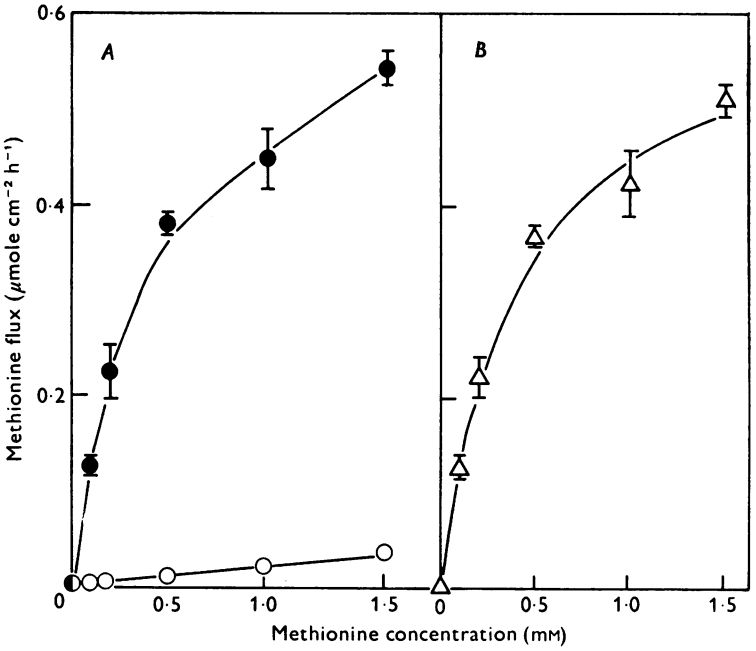

Fig. 4. Transport of methionine by new-born pig colon. *A*, unidirectional flux for methionine measured from mucosa to serosa (—●—) and serosa to mucosa (—○—) using conditions described in the text. Glucose was present throughout these experiments at a concentration of 5·5 mM. *B*, net methionine flux (—△—) calculated from Fig. 4*A*. The line has been constructed assuming that Michaelis–Menten kinetics operate with a K_m for methionine of 0·38 mM and a V_{max} of 0·62 μmole cm^{-2} h^{-1}.

Colonic transport of methionine measured during the first 10 days of post-natal life

The ability of the pig proximal colon to transport Na^+ changes during the first day of post-natal life (Bentley & Smith, 1975) and there are later changes in the ability of methionine to depolarize the microvillar

membrane potential (Hénin & Smith, 1976). The following experiments were carried out to see whether these developmental changes in transport were related in any way to changes in the ability of the colon to actively transport methionine.

Pieces of proximal colon from 0-, 1-, 2-, 4- and 10-day-old pigs were incubated in glucose-containing Krebs-bicarbonate medium. Mucosal to serosal and serosal to mucosal fluxes of methionine were measured across adjacent pieces of tissue for the older animals (2-, 4- and 10-day-old pigs). Only one piece of tissue could be taken for flux measurement from 0- and

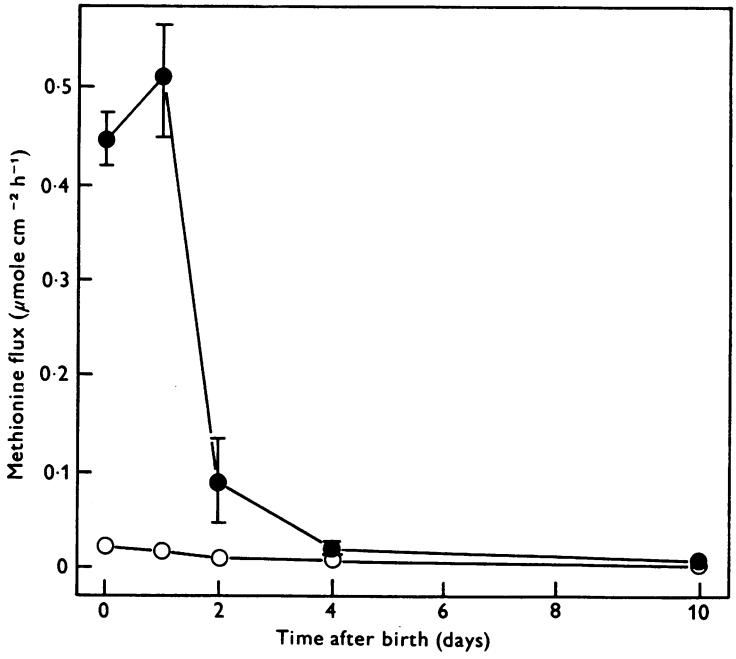

Fig. 5. Transport of methionine across pig proximal colon measured during the first 10 days of post-natal life. Tissues were incubated in the presence of 1 mM methionine under conditions as described in the text. Glucose was present throughout at a concentration of 5·5 mM. (—●—) and (—○—) mucosal to serosal and serosal to mucosal flux of methionine. Each value gives the mean ± S.E. of from six to twelve determinations.

1-day-old animals. The concentration of methionine used in these experiments was 1 mM. The results obtained are shown in Fig. 5. The mucosal to serosal flux of methionine, which is high on day 0, falls to what appears to be a new steady level by day 4. This fall, which is very abrupt, appears to be nearly complete by day 2. The serosal to mucosal flux of methionine also falls slightly, the values on day 4 and 10 being only half that measured

on day 0. Both muscle and connective tissues have grown quickly during this period of post-natal development. The tissue is considerably thicker by day 4. This could constitute a greater barrier to diffusion of methionine from blood to lumen.

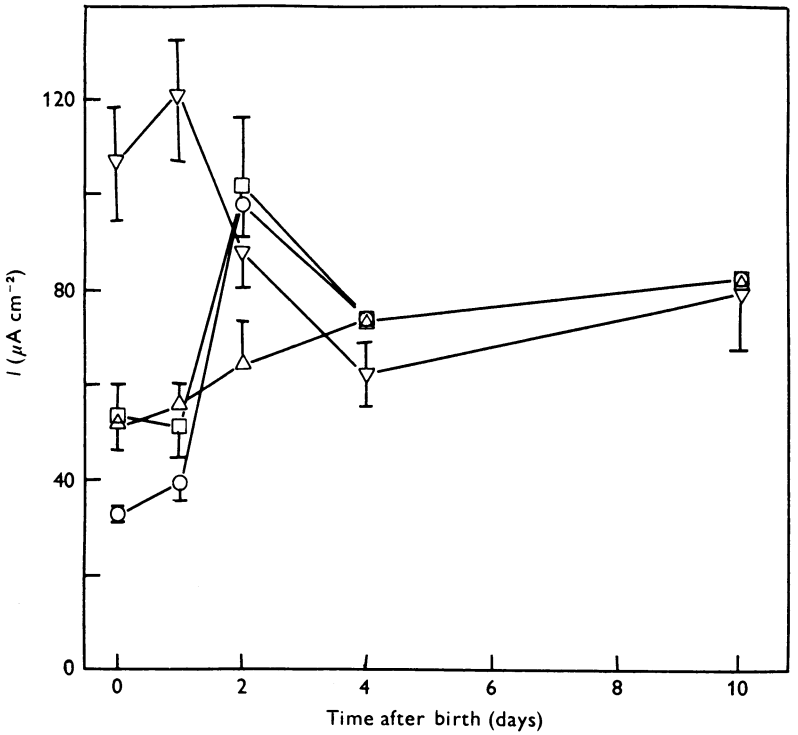

Fig. 6. Glucose and methionine dependence of short-circuit current measured across pig proximal colon during the first 10 days of post-natal life. The conditions of the experiment were as stated in the text. Incubation was in medium containing no glucose or methionine (—○—); in medium containing either 1 mM methionine or 5·5 mM glucose (—△— and—□— respectively) or in medium containing 1 mM methionine plus 5·5 mM glucose (—▽—). Values give means ± S.E. of from six to eighteen observations. I, short-circuit current.

The fall in mucosal to serosal transport, seen to occur on day 2, is too large to be accounted for by any gross change in tissue structure. It could, however, be connected with a changed ability of methionine to increase microvillar membrane permeability to Na^+ at this time (Hénin & Smith, 1976). The short-circuit current of proximal colon was therefore measured to see if changes in this parameter would correlate with changes in methionine transport during this period. The results of these experiments are

summarized in Fig. 6. Pieces of proximal colon were incubated for 15 min in Krebs-bicarbonate medium containing no glucose or methionine; 5·5 mM glucose; 1 mM methionine; or 5·5 mM glucose plus 1 mM methionine. The short-circuit current was then recorded. The values for the new-born pig colon correspond closely to those recorded from colons taken from 1-day-old pigs. Colons taken from 2-day-old pigs incubated in the presence of glucose plus methionine show a significant drop in short-circuit current. Control colons incubated in the absence of non-electrolyte show a compensatory rise in current. The control tissue is becoming more electrogenic at a time when the ability of glucose and methionine to stimulate short-circuit current is decreasing. The net effect of these opposing changes, seen in the presence of methionine or glucose, is to produce a modest increase in short-circuit current between days 1 and 2 of post-natal life. The short-circuit current of control colons taken from 4- and 10-day-old animals remains high relative to days 0 and 1. The presence of glucose; methionine; or glucose plus methionine, is without effect on short-circuit currents measured across colons taken from these older animals.

Methionine transport across colons taken from 4- and 10-day-old pigs

A cursory glance at Fig. 5 suggests negligible net transport of methionine by colons taken from 4- and 10-day-old pigs. The ability to use adjacent pieces of proximal colon from these larger animals shows, however, in the 4-day-old animal at least, that the mucosal to serosal flux of methionine is consistently greater than the serosal to mucosal flux measured in the same animal. It was decided to investigate this further, using a wider range of methionine concentrations, to determine the kinetics of this residual transport system. The results for proximal colons taken from 4-day-old pigs is shown in Fig. 7.

The mucosal to serosal flux, measured over a concentration range for methionine of 0·75 to 24 mM, is higher than the serosal to mucosal flux. There is a tendency for the mucosal to serosal flux of methionine to approach that of the serosal to mucosal flux at high methionine concentrations. The whole population of results is used to calculate the errors in Fig. 7*A* and values appear to be not significantly different at methionine concentrations of 16 and 24 mM. Even at these high concentrations, however, the paired comparison in individual pigs consistently shows a small positive net flux to the serosa. The serosal to mucosal flux is linearly dependent on the concentration of methionine. The calculated slope for this line is $11{\cdot}7 \pm 0{\cdot}7$ nmole cm^{-2} h^{-1} mM^{-1} and the correlation coefficient is 0·89. The net transport of methionine by colons taken from 4-day-old pigs is plotted in Fig. 7*B*. There is an apparent saturation of net transport when the concentration of methionine in the medium reaches about

16 mM. A line has been fitted to these points giving methionine an apparent K_m for its carrier of 10 mM and a V_{max} value of 0·15 μmole cm^{-2} h^{-1}. The apparent affinity of methionine for its transport system in the 4-day-old pig colon is some thirty times less than that determined in the new-born animal.

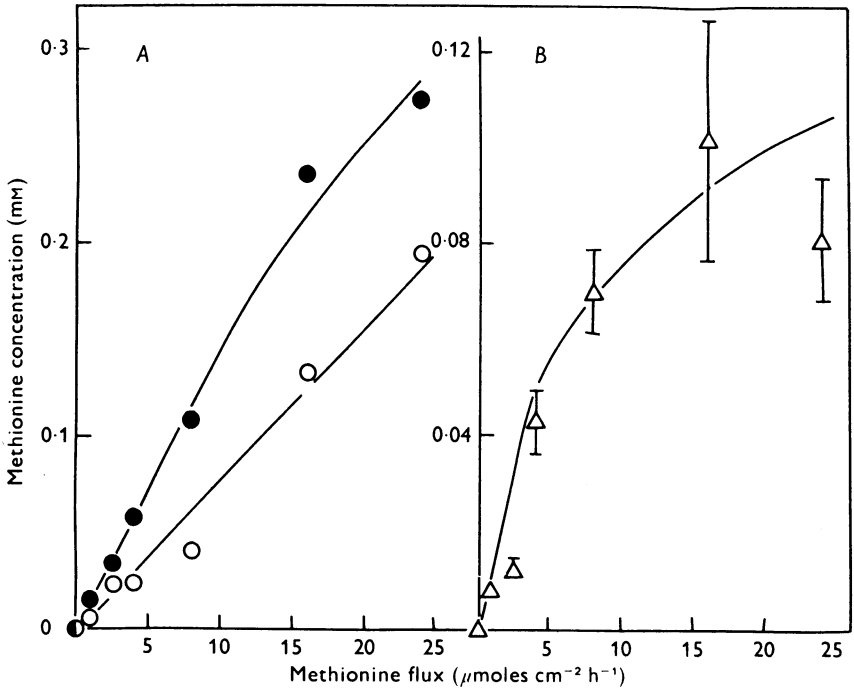

Fig. 7. Transport of methionine across proximal colons taken from 4-day-old pigs. Tissues were incubated in different concentrations of methionine as described in the text. Glucose was present throughout at a concentration of 5·5 mM. *A*, mucosal to serosal (—●—) and serosal to mucosal (—○—) flux of methionine. *B*, net flux of methionine (—△—). Each value gives the mean ± S.E. of from eight to seventeen determinations. The curve is drawn assuming a K_m for methionine of 10 mM and a V_{max} of 0·15 μmole cm^{-2} h^{-1}.

Pieces of proximal colon taken from 4-day-old animals were shaken in medium containing 1 mM methionine for 90 min and the mucosal scrapings then processed and counted for ^{14}C-labelled methionine. These experiments were identical to those described previously for colons taken from new-born pigs. The calculated intramucosal concentration of methionine at the end of incubation was 0·55 ± 0·05 mM. Thus the colon of the 4-day-old pig cannot concentrate methionine. This over-all average intramucosal concentration could, of course, mask concentration by a relatively small number of epithelial cells. It is interesting that the final intramucosal

concentration in 4-day-old pig colon is still higher than that found in colons taken from 10-day-old pigs.

There was no significant net flux of methionine across the proximal colon of 10-day-old pigs, using 1 mM methionine in the incubation medium (see Fig. 5). Nevertheless, in view of the results obtained with 4-day-old pigs, it was thought important to measure unidirectional fluxes of

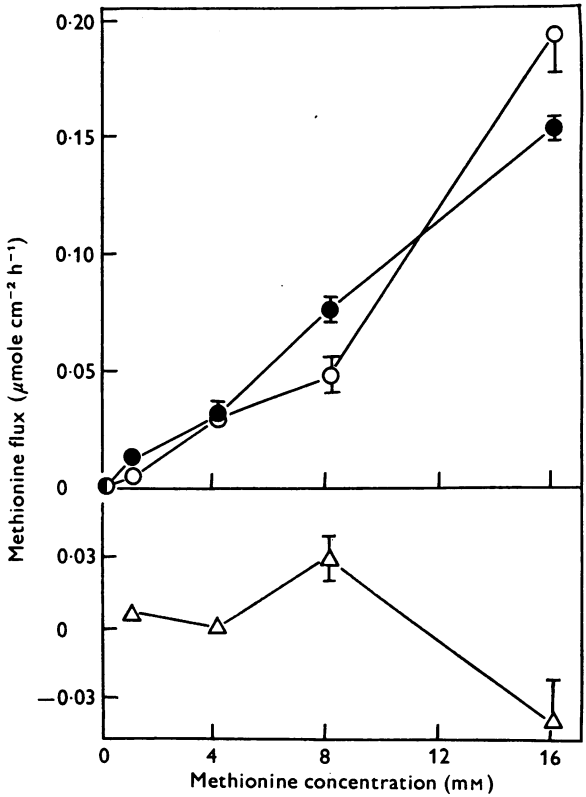

Fig. 8. Transport of methionine across proximal colons taken from 10-day-old pigs. Tissues were incubated in different concentrations of methionine as described in the text. Glucose was present throughout at a concentration of 5·5 mM (—●—), (—○—) and (—△—), mucosal to serosal, serosal to mucosal and net flux of methionine respectively. Each value gives the mean ± S.E. of from six to ten determinations.

methionine over a whole range of concentrations before coming to any firm conclusion concerning the transporting ability of the colon. The results obtained are shown in Fig. 8.

The flux of methionine from mucosa to serosa was very similar to the serosal to mucosal flux at all concentrations of methionine tested. Both fluxes were linearly dependent on the external concentration of methionine.

The regression line describing the concentration dependence of the serosal to mucosal flux of methionine had a slope not significantly different from that determined for the 4-day-old animal ($13{\cdot}9 \pm 1{\cdot}0$ and $11{\cdot}7 \pm 0{\cdot}7$ nmole $cm^{-2}\,h^{-1}\,mM^{-1}$ respectively). The correlation coefficient was 0·92. Including the mucosal to serosal with the serosal to mucosal fluxes for determination of a single line gave a slope of $12{\cdot}4 \pm 0{\cdot}6$ nmole $cm^{-2}\,h^{-1}\,mM^{-1}$ with a correlation coefficient of 0·93, values not significantly different from those produced by simple analysis of the serosal to mucosal fluxes. There is then no evidence for saturation of methionine influx as seen with colons taken from 0- and 4-day-old pigs. Paired comparison of fluxes gave net fluxes not significantly different from nought (Fig. 8). Accumulation experiments involving incubation of proximal colons from 10-day-old pigs in ^{14}C-labelled methionine, medium concentration 1 mM, gave a final calculated intramucosal concentration for methionine of $0{\cdot}31 \pm 0{\cdot}06$ mM. The epithelial cell membranes become highly impermeable to this amino acid during the first 10 days of post-natal life.

DISCUSSION

The general ability of the gastro-intestinal tract to modify function in response to changes in diet or environment is now well established. Adaptation initiated within the same cell population takes place within a few hours. Longer term adaptation occurs as the epithelium replaces itself over a period of a few days. One form of adaptation involves qualitative changes in epithelial cell function, while another involves hypertrophy of the same type of cell population. Virtually all work concerned with this aspect of intestinal function has, up till now, been carried out in the small, rather than the large, intestine. There are good reasons why this should be so. The number of specialized transport systems susceptible to adaptation are far more numerous in the small intestine, the metabolic activity of the small intestine is greater and the mucosal epithelium takes a shorter time to renew itself. Present work with the pig colon, however, shows unexpected and interesting exceptions to these general findings. The proximal colon actively transports methionine at birth in a way similar, if not identical, to that normally seen to take place in the small intestine. Glucose is equally effective with methionine in increasing the trans-tissue short-circuit current of new-born and 1-day-old pig colons. Assuming for the moment that this increase in current arises from an electrogenic influx of Na^+ in association with glucose, one is forced to conclude that an active transport system for hexoses also exists in this tissue. Lipid droplets appear in the cytoplasm of the apical cells of the colonic epithelium in the 1-day-old pig (Bentley & Smith, 1975). It may be that this tissue

also transports fatty acids. There is no reason to suppose that this list of transport systems is in any way complete. The small intestine absorbs immune globulins at birth and the epithelial cells become distorted with swollen, protein-filled, vesicles. These vesicles do *not* appear in the colonic mucosa but whether this is because the cells lack the ability to transport proteins, or because protein never reaches the colon *in vivo*, is not yet known.

The ability of the new-born pig colon to actively transport non-electrolytes changes during post-natal development. This provides a rare example of adaptational or developmental processes at work in the mammalian large intestine. The changes seen to take place occur in two stages. The first stage, between days 1 and 4, involves a loss in the ability of methionine to increase the microvillar membrane permeability to Na^+ (see also Hénin & Smith, 1976). A transport system for methionine can still be detected at this time, but its affinity is some thirty times less than on day 0. The maximal rate of transport also falls. It is tempting to suggest that changes in microvillar membrane properties occur at this time and that these inhibit a methionine-induced binding of Na^+ to the carrier. An equally acceptable alternative explanation of these findings is to suggest that the original transport system disappears entirely, revealing a secondary transport system present in the new-born as an insignificant component of the total methionine transport. The determination of affinity constants for methionine transport in the absence of Na^+ in the new-born pig colon might help to distinguish between these two possibilities. Net transport of methionine ceases completely by day 10 of post-natal life. This final change to an adult colon-like behaviour could arise from cellular renewal. Thymidine labelling experiments are being carried out currently to test this hypothesis. The ability to split colonic function into three distinct phases, one where amino acids are not transported, one where there is a non-coupled transport and one where transport is coupled to Na^+, could prove a powerful tool in understanding how these functions relate to membrane structure. Chemical analysis of brush border fractions might eventually allow one to link these differences in function to the presence or absence of specific chemical components within the transporting membrane.

The transport of Na^+ is shown, in the present work, to be closely connected with the transport of methionine (and presumably with other amino acids and hexoses), so that the absorption of Na^+ in the colon will be high at birth if these non-electrolytes succeed in reaching this area of the gastro-intestinal tract. An increase in net Na^+ transfer also occurs across the proximal colon between day 0 and day 1 of post-natal life (Bentley & Smith, 1975). This is in no way related to the non-

electrolyte-Na^+ interaction. This increase in Na^+ transport appears to be electrically neutral. The short-circuit current also increases between days 1 and 2, at a time when the ability of methionine and glucose to change current is fast disappearing. Na^+ transport in pigs older than 1 day has not been determined, but it could be that Na^+ transport in the absence of non-electrolyte is becoming more electrogenic in these older animals. This might explain why the discrepancy between short-circuit current and net Na^+ transport is so great in the new-born pig colon compared with colons from other mammalian species. More information is needed before coming to any more definite conclusion on how Na^+ transport is regulated across this tissue.

There is some indirect evidence to suggest that the functions of the colon and small intestine might interchange, to some extent, following different surgical procedures. Removal of the colon results in intestinal hypertrophy which cannot be entirely explained on the basis of an increased food intake (Wright, Poskitt, Cleveland & Herskovic, 1969; Masesa, 1976). Intestinal re-section may also cause the colon to take over some of the transport functions of a small intestine (Grenier, Sava & Gillet, 1970; Hollender & Sava, 1970) as well as changing the function of the remaining part of the small intestine (Wright, Cleveland & Tilson, 1969). The absorptive function of the small intestine in the new-born pig is partly inhibited, following ingestion of colostrum, by the pinocytotic transport of immune globulins. The ability of the colon to transport a wide range of nutrients normally handled by the small intestine could be important under these circumstances. There is, however, one important difference between this type of compensation and that seen to follow surgical re-section. Adaptation following surgery is assumed to be *induced* by changes in the composition and quantity of the intestinal contents, whereas in the pig the mechanisms for colonic transport of nutrients *pre-date* the possible appearance of these substances in the tract. One could speculate that it might be the steroid levels in the foetal blood which orchestrate intestinal and colonic function, so that they complement each other at birth, but this idea has yet to be tested.

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