

cold. The extracts were centrifuged and the supernatants were dried. The residue was resuspended in scorpion ringer<sup>12</sup>. Each scorpion received by injection 50 µl of the extract (2 eyestalk equivalents of crab hormone or 1 eyestalk equivalent of prawn hormone); 1 batch of scorpions was injected with ringer solution to serve as a control. 2 h after injection the scorpions were sacrificed for the estimation of total sugar in the haemolymph, and free sugar and glycogen<sup>13</sup> in the hepatopancreas. The data were subjected to Student's t-test.

**Results and discussion.** Administration of eyestalk extracts from freshwater crab and marine prawn caused significant elevation of haemolymph sugar in the scorpion (table) indicating that the scorpion tissues respond to crustacean hyperglycemic principles. Crab eyestalk extract elicited a greater response than the prawn eyestalk extract. A parallel decrease in the free sugar and glycogen levels in the

hepatopancreas were observed, suggesting the possible mobilization of sugar from hepatopancreas to haemolymph. It has been established that the crustacean hyperglycemic hormone is glycogenolytic through activation of the phosphorylase system, leading to the elevation of tissue free glucose. This glucose leaks into the haemolymph, causing hyperglycemia<sup>2,4,14,15</sup>.

The hyperglycemic principles of the scorpion neuroendocrine system do not enhance the activity of the phosphorylase system<sup>6</sup>. However, the crustacean hormone molecule, when introduced into the scorpion, acts through the same mechanism as in crustaceans. Although the scorpion has a hyperglycemic hormone of a different nature, the tissues do respond to crustacean hormone. The findings of the present investigation unequivocally prove the interspecific action of crustacean hyperglycemic hormone and throw light on the parallel evolution of hormonal mechanisms in invertebrates.

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## Amino acid transport by small and large intestine of newborn pig

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**Summary.** Unidirectional fluxes of different amino acids have been determined across newborn pig small intestine and colon. The systems responsible for amino acid transport are present in the same proportion in both tissues. Colonic transport of amino acids appears to represent a transient overspill function of the small intestine.

The presence of at least 2 separate mechanisms for neutral amino acid entry into rabbit ileal mucosa has been established recently through the combined use of 3 different types of experimental approach. The first consists of mutual competition experiments, where it is found that serine is unable to inhibit fully the uptake of other neutral amino acids<sup>1</sup>. The range of apparent affinities of other amino acids for this serine-sensitive system varies from 0.3 to 3.7 mM. The characteristics of the amino uptake system resistant to serine inhibition cannot be determined from this type of experiment. Computer analysis of a 2nd series of uptake and inhibition experiments, involving the simultaneous fitting of 83 data points for 3 neutral amino acids to a 2-system model, leads to the generation of a similar series of high affinity constants for neutral amino acids (0.4–6.3 mM), together with a 2nd set of low affinity constants (22, 91 and 2931 mM for methionine, alanine and serine respectively)<sup>2</sup>. Subsequent work has shown this 2nd low affinity system to be present in the absence of sodium (23, 75 and 89 mM for methionine, alanine and serine respectively)<sup>3</sup>. The low affinity system for serine shows

most apparent variation due to its close resemblance to diffusion. Inhibition experiments with methionine show, however, that the diffusion of serine into this tissue is small or non-existent<sup>2</sup>. Similar mechanisms for amino acid entry have been described for the proximal colon of the newborn pig, but in this case the relative proportions of the 2 systems appear to differ from those reported for the rabbit<sup>4</sup>. These results are interesting, but it is not known whether the difference arises because of the developmental state of the animal, whether it represents a true difference between species or whether the amino acid transport characteristics of the large intestine are normally different from those found higher up the gastrointestinal tract. The present work was initiated to answer some of these questions by directly comparing the transport of different neutral amino acids across the small and large intestine of the newborn pig.

Short-term measurements of amino acid uptake generally provide the best means of analysing intestinal transport mechanisms. Endocytosis of immunoglobulins by newborn pig intestine makes this method of analysis unsuitable in the present case. For this reason, it was decided to measure

the steady state unidirectional fluxes of individual amino acids for subsequent comparison of transport properties. Pieces of proximal colon and small intestine, taken immediately from newborn pigs killed by decapitation, were mounted in Ussing-type chambers as described previously<sup>5</sup>. Steady state unidirectional fluxes of <sup>14</sup>C-labelled amino acids became established across the small intestine after 40–60 min incubation (60–90 min in the case of the colon). Incubation was in bicarbonate saline<sup>6</sup> containing 5.5 mM

glucose and 1 mM amino acid throughout. Mean mucosal to serosal fluxes for a whole range of amino acids are shown in figure 1. The basic amino acid lysine and the  $\alpha$ -amino acid proline are both transported poorly by the small and large intestine of the newborn pig. Small hydrophilic amino acids are transported less readily than the larger hydrophobic amino acids (compare the transport of Gly, Thr, Ser or Ala with that for Leu, Tyr, Val, Phe, Met or Ile). Colonic transport of all amino acids is less than that measured across the small intestine.

A critical way to test whether the proportional representation of different amino acid transport systems is similar in both small and large intestine, is to compare directly the transport by small intestine to that for colon. A linear relationship with zero intercept will be seen only when the proportional distribution of basic, amino and neutral amino acid transporting systems is identical. The results of plotting experimental data in this way are shown in figure 2. The transport of amino acids across the small intestine is related linearly to that measured across the colon. The straight line representing this relationship, calculated by regression analysis, has a slope of  $1.87 \pm 0.31$  and an intercept of  $-0.3 \pm 1.2$  nmoles  $\text{cm}^{-2} \text{min}^{-1}$  (mean  $\pm$  SE;  $r=0.88$ ). It is concluded from these results that both systems of amino acid uptake are present, in fixed proportion, in both the small and large intestine of the newborn pig.

Finally, it is interesting to compare the mucosal to serosal flux of amino acids in the proximal colon of the newborn pig with values reported previously for short-term influx across the brush border membrane<sup>4</sup>. These values are essentially equal, suggesting that the backflux of amino acid from serosa to mucosa is low and that the rate-limiting step to transport from a 1 mM solution of amino acid is the speed of entry across the brush border membrane. Independent measurements of amino acid backflux from a 1 mM solution show no major difference either between amino acids or between tissues (serosal to mucosal flux for Lys and Ile in the small intestine,  $0.40 \pm 0.05$  and  $0.64 \pm 0.06$  nmoles  $\text{cm}^{-2} \text{min}^{-1}$ ; for Met, Ala and Phe in colon,  $0.32 \pm 0.03$ ,  $0.40 \pm 0.05$  and  $0.23 \pm 0.01$  nmoles  $\text{cm}^{-2} \text{min}^{-1}$  respectively). Amino acid backflux is, on average, only 6% of the measured influx.

The finding of a similar pattern of amino acid transport by both small and large intestine in the present work lends credence to the idea that the proportions of different transport systems for neutral amino acids might vary from species to species<sup>4</sup>. These differences could provide important clues as to how amino acid transport is organized normally within the intestine. Much is known about the hormonal control of individual amino acid transport systems in a wide variety of tissues<sup>7</sup>. It now seems worthwhile investigating whether similar mechanisms of regulation might occur within the maturing enterocyte of the intestinal mucosa.

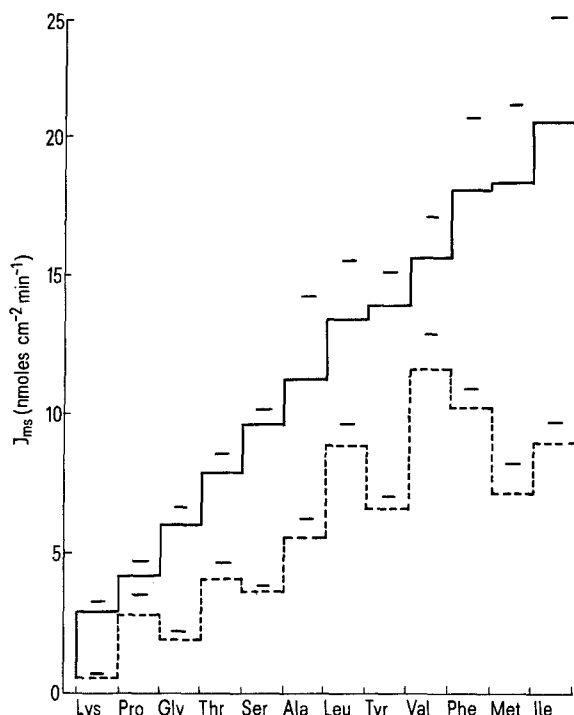


Fig. 1. Amino acid flux across newborn pig intestine. The unidirectional flux of each amino acid was measured separately from mucosa to serosa ( $J_{ms}$ ) using methods described in the text. The concentration of amino acid in the bathing medium was maintained constant at 1 mM throughout these experiments. Each value gives the mean  $\pm$  SE of from 5 to 15 estimations carried out on the small intestine (solid line) and proximal colon (broken line) of newborn unsuckled pigs.

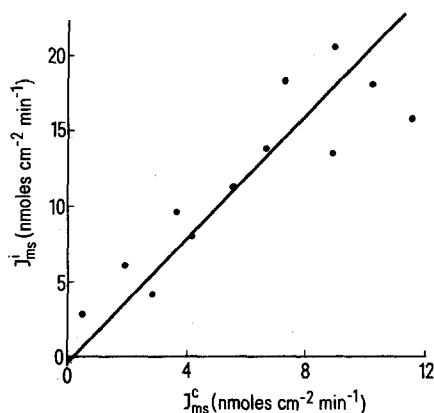


Fig. 2. Direct comparison of amino acid flux across the small intestine ( $J_{ms}$ ) and proximal colon ( $J_{ms}^c$ ) of newborn unsuckled pigs. The line describing the best fit to these experimental points had a slope of  $1.87 \pm 0.31$  and an intercept of  $-0.3 \pm 1.2$  nmoles  $\text{cm}^{-2} \text{min}^{-1}$ .

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