all cases, the ATP-contents were normal. It has been suggested that such a CP storage is due to imbalance between production and utilization. Here the situation may appear different, since the hearts were neither fully empty nor completly arrested and the ATP levels were low. A blockage in the transfer between the 2 CP compartments is more likely[18]. If we refer to Lohmanns reaction:

$$ATP + C \rightleftharpoons CP + ADP.$$

The optimum pH in the direction of CP formation is 8.00 and 6.4 for the reverse reaction. After ischemia, the intracellular pH is lowered[19] mainly by accumulation of lactic acid. The washout curves presented here show that CP resynthesis is achieved at a time when high levels of this metabolite are still present. It then appears paradoxical that the results should favour the forward reaction. Concerning the mitochondria one may postulate either a local rise in creatine[20], or more probably the fact that ADP store is progressively reduced, implying its rapid utilisation as soon as it is generated for oxidative phosphorylation.

Nevertheless, this resynthesis of CP is indicative of an active oxidative phosphorylation as both this latter reaction and creatine kinase activity are linked. The inability to recover normal ATP contents may then be due to rapid degradation at the end-product stage of ATP rather than to mitochondrial damage as observed after longer periods of ischemia. So intermittent coronary perfusion seems to protect the mitochondria from the effect of anoxia. Whether this is beneficial to the heart is debatable. Kammermier[21] has observed a normal function on hearts with high CP and low ATP levels. In our experiments, hearts with such a high energy phosphate pattern failed.

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The function of the intestine in the pulmonate mollusc Helix pomatia L.

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Summary. Everted sacs of intestine from Helix pomatia do not actively transfer glucose, galatose, methionine, alanine, asparagine, aspartic acid or proline from the mucosal to serosal surfaces. The principal function of the intestine appears to be the reabsorption of water.

The molluscan intestine is usually a long and conspicuous organ[3] which a consensus of opinion, summarized by Yonge[4,5], considers to be a passive conduit concerned almost exclusively with the formation and elaboration of faecal pellets and their transport to the anus. This 'classic theory'[6] of the function of the molluscan alimentary canal, in which the intestine is assigned a prosaic role, has been reiterated – or not seriously questioned – in a number of recent reviews[7–10].

The principal evidence against this view has come from studies by Lawrence and his colleagues[11–18] on the chiton Cryptochiton stelleri where the intestine was found to actively absorb amino acids, sugars, organic bases and probably inorganic ions, by mechanisms coupled to cellular metabolism and showing similarities to those of the vertebrate gut.

In refuting conclusions from earlier work[19] on the chiton it was shown that, without supporting quantitative data, histological and histochemical studies are not adequate for a rigorous demonstration of nutrient absorption. Consequently, the findings of other studies, similarly based upon qualitative or semi-quantitative methods were also questioned. It has been proposed that absorptive mechanisms, similar to those in chitons, may occur in the gut of other molluscs[12], including pulmonates[20].

This paper considers the competence of the intestine of the pulmonate mollusc (Helix pomatia) to absorb amino acids and hexoses in vitro. There is a distinction to be made between absorption into the tissue, a general feature of living cells, and absorption into and across the gut wall, a function of tissue specialized for the assimilation of nutrients into the animal. Failure to recognize

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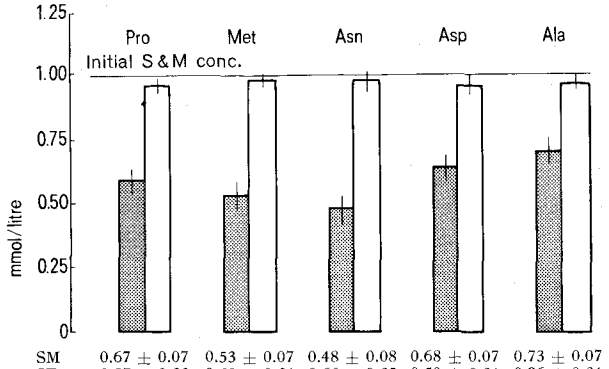
this difference has led to confusion over the meaning of the term absorption in earlier studies on the invertebrate gut (see discussion in Forester[3]). Absorption is defined here as the net movement of solute or solvent through the gut wall from the mucosal to serosal surface.

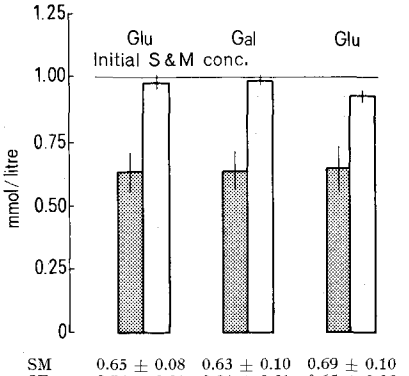
Methods. Experiments were made with active, feeding animals. The longest, undamaged portion of the intestine (Dünndarm)[21] between the stomach (Blindsack) and rectum (Enddarm) was used and no regional distinctions within the intestine were made.

Absorption was studied using an everted sac preparation[3, 22–24] in which the media comprising the serosal and mucosal fluids (saline containing a ^{14}C labelled amino acid or hexose) were the same so that, initially, no concentration gradients existed across the wall of the intestine. Sacs were incubated for 30 min at 25 °C after which time fluid movements were estimated by weight and the solute content of the tissue and bathing fluids were measured by liquid scintillation analysis.



	Pro	Met	Asn	Asp	Ala
SM	0.67 ± 0.07	0.53 ± 0.07	0.48 ± 0.08	0.68 ± 0.07	0.73 ± 0.07
SE	0.57 ± 0.22	0.69 ± 0.24	0.66 ± 0.25	0.50 ± 0.24	0.96 ± 0.24
TS	0.34 ± 0.10	0.53 ± 0.10	0.29 ± 0.11	0.33 ± 0.10	0.61 ± 0.10
N	12	10	9	10	10
M	a	a	a	a	a

Fig. 1. The amino acids: proline; methioine; asparagine; aspartic acid and alanine. Histograms show final serosal (hatched) and mucosal (open) concentrations. SM, final serosal/mucosal concentration ratio. SE, efflux of solute from serosal compartment (nmoles mg wet wt tissue^{-1} h^{-1}). TS, accumulation of solute by tissue^{-1} h^{-1}). Data expressed as mean ± (p = 0.05) and numbers of replicates by N. The incubating medium M was *a* Baldwin's saline.



	Glu	Gal	Glu
SM	0.65 ± 0.08	0.63 ± 0.10	0.69 ± 0.10
SE	0.75 ± 0.31	0.84 ± 0.31	0.65 ± 0.36
TS	0.50 ± 0.10	0.66 ± 0.10	0.62 ± 0.12
N	6	6	5
M	a	a	b

Fig. 2. The hexoses: glucose and galactose. Histograms show final serosal (hatched) and mucosal (open) concentrations. SM, final serosal/mucosal concentration ratio. SE, efflux of solute from serosal compartment (nmoles mg wet wt tissue^{-1} h^{-1}). TS, accumulation of solute by tissue (μmoles mg wet wt tissue^{-1} h^{-1}). Data expressed as mean ± (p = 0.05) and numbers of replicates by N. The incubating media M were *a* Baldwins' saline, *b* Tris saline containing succinate and ATP.

A phosphate buffered saline, containing no calcium or magnesium, was used for most experiments[25]. An alternative, containing divalent cations and buffered to pH 7.60 with TRIS (concentrations in mmoles l^{-1}: Na^+ 80.0; K^+ 4.0; Ca^{2+} 0.70; Mg^{2+} 0.5; anion component as Cl^-) was used for comparison. When used, other compounds were added to both serosal and mucosal fluids at the following concentrations (in mmoles l^{-1}): glucose 1×10^{-2}; succinate and pyruvate 1×10^{-3}; ATP 1×10^{-5}. Amino acids and hexoses under study were at an initial concentration of 1×10^{-3} mmoles l^{-1} except for some experiments using 5×10^{-4} mmoles l^{-1} alanine. These solutes were labelled by the addition of their ^{14}C analogues, supplied by the Radiochemical Centre, Amersham (England): L-methionine (methyl-C14); L-asparagine-C14 (U); L-aspartic acid-C14 (U); L-alanine-C14 (U); L-proline-C14 (U); D-glucose-C14 (U); L-galactose-1-C14.

The respiration of intestinal segments was determined in a Warburg apparatus by the direct method and the results expressed as Q_{02}^{28} °C = μl 0_2 mg dry wt^{-1} h^{-1}.

Results. Data from all experiments are similar (figures 1–4) conforming to a common pattern, irrespective of the experimental conditions or solutes chosen. No general differences were evident between results obtained with amino acids and hexoses.

In all cases there was a net efflux of solute (SE) from the serosal compartment (SE 0.50–0.96 nmoles mg wet tissue^{-1} h^{-1}) into the tissue. This is too small to account for the tissue solute (TS) concentrations (TS 0.29–1.14 mmoles mg wet wt^{-1} h^{-1}), the greater portion of which came from the mucosal fluid. The small contribution of solutes from the serosal compartment probably reflects the impermeability of the outer surface of the tissue compared with that of the mucosal epithelium.

Solute concentrations fell on both sides of the gut partly as a result of this movement of solutes from the bathing media into the tissue. The change in mucosal fluid was small, reflecting the large volume of the compartment (5 ml) whilst the serosal concentration (compartment volume 30–100 μl) fell to between 70 and 50% of their initial values, giving serosal/mucosal concentration ratios (SM) in the SM 0.48–0.96 range.

The fall in serosal concentration also results from the simultaneous transfer of water from the mucosal fluid, through the tissue, into the sac. Water was absorbed at the rate of 2–3 μl mg dry wt tissue^{-1} h^{-1} (aproximately equivalent to 25–30% tissue volume h^{-1}). This represents a physiological absorption of water from the gut lumen (*in vivo*) into the animal. The process is not considered in detail here, being the subject of a separate publication (in preparation).

The absence of a net accumulation of amino acid or hexose in the serosal compartment suggests that the intestine is not concerned with the absorption of nutrients. In this respect it differs from the chiton intestine where, under similar experimental circumstances, there is a net serosal transport of solute and the generation of SM ratios greater than unity.

It has not been possible to attribute this inability of the intestine to absorb nutrients to experimental artefacts.

21 Synonyms in parentheses refer to the definitive accounts by J. Meisenheimer, Die Weinbergschnecke (Helix pomatia L.), Leipzig 1912, and K. von Haffner, Z. wiss. Zool. *121*, 126 (1924).

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Baldwin's saline was previously shown[26] to be suitable for use with all tissues of H. pomatia, supporting respiration at a rate commensurate with that of the intact animal. Similar values for Q_{02} were found and the respiration, measured directly on removal of the intestine from the animal ($Q_{02} = 3.47 \pm 0.21$) did not decline after 4 h in saline ($Q_{02} = 3.29 \pm 0.25$). The integrity and viability of the preparation, after reversal of the gut wall, is further suggested by the unchanged respiration ($Q_{02} = 3.39 \pm 0.25$), persistence of a metabolically coupled fluid transport and a transmural PD whose size and polarity is characteristic of non-everted tissue (work in preparation). All the compounds tested have been reported in molluscan tissues and, with the exception of aspartic acid, were chosen for their ability to participate in the transport processes of other tissues and organisms. The distribution of solutes between the compartments of the everted sac preparation did not appear to be altered by the use of a different saline, the presence of ATP and metabolic substrates or by prolonging the experimental period to 150 min.

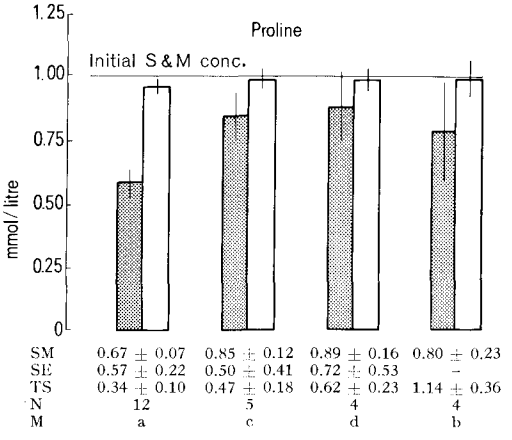

Fig. 3. The amino acid proline. Histograms show final serosal (hatched) and mucosal (open) concentrations. SM, final serosal/mucosal concentration ratio. SE, efflux of solute from serosal compartment (nmol mg wet wt tissue^{-1} h^{-1}). TS, accumulation of solute by tissue (μmoles mg wet wt tissue^{-1} h^{-1}). Data expressed as mean ± (p = 0.05) and numbers of replicates by N. The incubating media M were *a* Baldwin's saline, *b* Tris saline containing succinate and ATP, *c* Baldwin's saline containing pyruvate and *d* Baldwin's saline containing pyruvate and ATP.

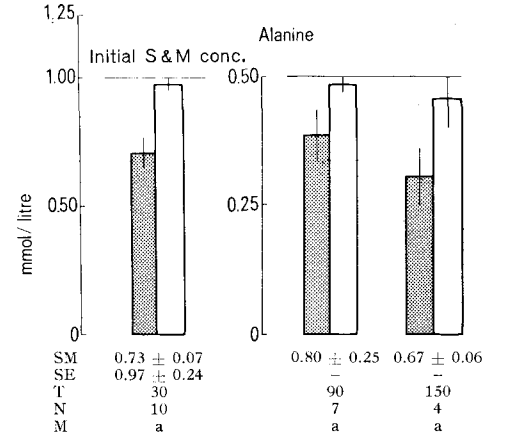

Fig. 4. The amino acid alanine. Histograms show final serosal (hatched) and mucosal (open) concentrations. SM, final serosal/mucosal concentration ratio. SE, efflux of solute from serosal compartment (nmoles mg wet wt tissue^{-1} h^{-1}). TS, accumulation of solute by tissue (μmoles mg wet wt tissue^{-1} h^{-1}). Data expressed as mean ± (p = 0.05) and numbers of replicates by N. The incubating medium M was *a* Baldwin's saline and the duration of the experiment in min is given by T.

Discussion. The intestine of H. pomatia appears unable to absorb nutrients by any mechanism resembling those of the chiton intestine[11–15,17], holothurian gut[27], locust rectum[28] or vertebrate small intestine.

It is possible that assimilation might occur through some mechanism which is not amenable to study using an everted sac technique. For example, the proposed permeability of the gut wall to small organic molecules[29–31], the presence of trehalose in the blood of some species[32] and the ability of the intestine to absorb water[3] are properties shared with the locust mid-gut in which amino acids and glucose are absorbed by facilitated diffusion[33]. However, the existence of similar mechanisms in molluscs is speculative.

I conclude that the intestine of H. pomatia is principally, and perhaps exclusively, concerned with the reabsorption of water from the lumen and that this is an adaptive feature of a soft bodied, terrestrial animal whose major physiological stress is water loss by evaporation through the integument. The earlier concept of the function of the molluscan alimentary system[4,5] appears adequate to account for terrestrial pulmonates in which the intestine has little function, at least with respect to the digestion and assimilation of nutrients.

The differences between pulmonates and chitons raise questions concerning the function of the intestine throughout the phylum and a number of alternatives to the accepted theory can be proposed. Active absorption of nutrients by mechanisms similar to those in the chiton may occur sporadically (but not in pulmonates), having arisen through parallel evolution as an adaptive feature with little explanation in phylogeny.

Alternatively, these mechanisms may be ubiquitous throughout the phylum with the exception of the terrestrial pulmonates. This might have occurred if the physiological requirements for nutrient transport were in conflict with those for water absorption and the selection pressures favouring water conservation were sufficiently great. Inference from comparative studies suggest, prima facie, that this is unlikely because solute and solvent transfer frequently occur together and do not appear to be mutually exclusive.

Finally, it is known that the Polyplacophora diverged early in molluscan evolution[8,9] and it is conceivable that the intestinal physiology of the group is unique amongst the phylum, the classic theory remaining adequate to account for other groups. Whilst recognizing that the accepted theory rests almost entirely upon evidence obtained in the first half of the century, before current concepts of epithelial transport (and the technical means for their study) were developed, I consider the last to be the most plausible hypothesis and, at least on the basis of available evidence, the most acceptable.

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