J. Physiol. (1987), **393**, *pp.* 261–281
With 9 text-figures
Printed in Great Britain

GENETIC AND PHENOTYPIC ADAPTATION OF INTESTINAL NUTRIENT TRANSPORT TO DIET IN FISH

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(*Received 30 June 1986*)

SUMMARY

1. Herbivores have higher rates of intestinal sugar transport and lower rates of amino acid transport than carnivores, if each are studied while eating their respective natural diets. It was unclear whether these species differences involve a genetic contribution, since when omnivores are switched from a high-protein to a high-carbohydrate diet they reversibly increase sugar transport and suppress amino acid transport. Hence we studied eight fish species of differing natural diets while all were eating the same manufactured diet.

2. Na^+-dependent L-proline uptake and active D-glucose uptake, measured *in vitro* by the everted intestinal sleeve technique, followed Michaelis–Menten kinetics. Values of the apparent Michaelis–Menten constant increased with values of the maximal transport rate, probably as a result of unstirred layer effects.

3. The ratio of proline to glucose uptake decreased in the sequence: carnivores > omnivores > herbivores. The intestine's uptake capacity for the non-essential nutrient glucose was much higher in herbivores than in carnivores, correlated with species differences in carbohydrate content of the natural diet. Proline uptake varied much less among species, since species with different natural diets still have similar protein requirements.

4. Since all species were studied while eating the same diet, these species differences in uptake are not phenotypic but genetic adaptations to the different natural diets.

5. In two fish species which normally switch from carnivory towards herbivory or omnivory as they mature, we observe a 'hard-wired' developmental change in intestinal uptake. Larger animals had lower proline uptake relative to glucose uptake than did smaller animals, even though both were being maintained on the same diet in the laboratory.

6. Carnivorous fish tend to allocate absorptive tissue to pyloric caeca or a thick mucosa, while herbivorous fish tend towards a long thin intestine.

INTRODUCTION

The digestive systems of herbivorous and carnivorous species have long been known to differ in anatomy, enzyme concentrations (Barnard, 1973), and food transit rates (Fange & Grove, 1979). Recent studies have documented differences in

intestinal nutrient absorption as well. For example, within several vertebrate classes intestinal glucose absorption is highest in herbivores, intermediate in omnivores, and lowest in carnivores (Ferraris & Ahearn, 1983; Karasov, Buddington & Diamond, 1985*a*; Karasov, Phan, Diamond & Carpenter, 1986; Diamond & Buddington, 1987; Karasov & Diamond, 1987). Thus, intestinal glucose transport is matched to the carbohydrate content of the species' natural diet.

It is tempting to interpret these species differences in nutrient absorption as genetically fixed adaptations that evolved through natural selection. However, there is an alternative explanation: reversible phenotypic adaptations to diet. In the studies of nutrient absorption just cited, each species was studied while eating its natural diet or a manufactured one of similar nutrient composition: i.e. a high-protein ration for carnivores, a high-carbohydrate ration for herbivores. In mice (Diamond & Karasov, 1984; Karasov, Solberg, Chang, Hughes, Stein & Diamond, 1985*b*; Karasov, Solberg & Diamond, 1987), rats (Wolffram & Scharrer, 1984) and carp (Buddington, 1987) intestinal sugar or amino acid transport has been shown to increase reversibly on high-carbohydrate or high-protein rations, respectively. What contributions do such phenotypic responses make to the observed species differences in nutrient transport?

To separate the contributions of phenotypic responses and genetically fixed adaptations, one must know whether species differences observed while animals were consuming their various natural diets would persist when all species ate the same ration. This experiment presents practical difficulties, as it is difficult to devise a ration equally acceptable to animals with different natural diets, such as tigers and cows. However, some fish species with widely different natural diets will accept manufactured diets quite unlike their natural one in nutrient composition. Hence we compared intestinal absorption of D-glucose and the amino acid L-proline in eight fish species, of which three are herbivores (common carp = *Cyrinus carpio* (family Cyprinidae), grass carp = *Ctenopharyngodon idella* (Cyprinidae), tilapia = *Tilapia zillii* (Cichlidae)), two are omnivores (channel catfish = *Ictalurus punctatus* (Ictaluridae), white sturgeon = *Acipenser transmontanus* (Acipenseridae)), two are carnivores (striped bass = *Morone saxatilis* (Percichthyidae), rainbow trout = *Salmo gairdneri* (Salmonidae)), and one switches from carnivory to herbivory as it grows (monkeyface prickleback = *Cebidichthys violaceus* (Stichaeidae)). We were able to maintain all eight species in the laboratory on the same manufactured diet, thereby eliminating a possible contribution of phenotypic responses to observed species differences.

A further confounding factor in the published literature, the use of different methods by different authors to measure intestinal absorption in different species, was also eliminated in the present study by employing the same method for all eight species, namely the everted sleeve technique (Karasov & Diamond, 1983). This method has the virtues of minimizing unstirred layers, maintaining tissue viability, yielding uptake rates similar to those observed *in vivo*, and being readily applicable to intestines of many species that are difficult to study in Ussing chambers because of muscularity.

METHODS

Fish and their handling. Pricklebacks, our sole marine species, were netted from intertidal ponds along the southern California coast and held in a closed salt-water (32–35 parts per thousand) system at 19 ± 1 °C under continuous light. The other seven species, which inhabit fresh water, were held under a 12:12 h light:dark cycle in semi-closed recirculating water systems at 20–25 °C, except that trout, a cold-water form, was held at 12–15 °C. (How might these different holding temperatures affect our results? All our uptake measurements were performed *in vitro* at 20 °C. Smith (1966*a*,*b*) showed for goldfish intestine that glucose-dependent potential differences, measured *in vitro*, decrease with the acclimatization temperature at which the fish had been held before killing. If the same conclusions apply to our measurements of glucose and proline uptake in other fish species, Smith's results suggest that uptakes we measured at an incubation temperature of 20 °C for trout held at 12–15 °C would have been 37 % lower if the trout had instead been held at 20–25 °C. If this factor applied to both glucose and proline uptake, the uptake ratio depicted for the trout in Fig. 8 would be unaffected.)

Catfish, trout, tilapia and striped bass were donated by commercial fish growers. Our trout were those used by Buddington & Diamond (1987), while our striped bass were smaller than those used by Buddington & Diamond (1987). Common carp and grass carp were netted from experimental ponds operated by the University of California and the Coachella Valley Water District, respectively. Sturgeon were yearlings produced by the aquaculture programme at University of California, Davis. For at least 8 weeks before killing, all species were fed a commercial trout ration (Silvercup Trout Chow, Murray Elevators, UT, U.S.A.) with a minimum content of 45 % protein and 11 % lipid, maximum content of 15 % ash and 3 % fibre, and digestible carbohydrate content of *ca.* 25 % estimated by difference. Most of the study was conducted with one batch of the feed, to eliminate any possible effects of variation between batches.

Anatomical measurements. After a fish had been killed by a blow to the head, the body weight and fork length (distance from snout to fork in tail) were recorded (Table 1, rows 3 and 4), and the whole alimentary canal from oesophagus to vent was excised. In species with true acid-secreting stomachs (all species except common carp and grass carp) the gut was then severed at the pyloric sphincter, and only the post-gastric region was retained. The intestinal lumen was flushed with cold Ringer solution (2–4 °C) to remove digesta.

With the intestine in a relaxed, unstretched state, intestinal length (Table 1, row 7) was measured from vent to pyloric sphincter (species with stomachs) or to the constriction where the oesogaster and oesophagus meet (the two species without stomachs, which also lack pyloric sphincters). For species with pyloric caeca (trout, striped bass, sturgeon, prickleback) we measured or estimated the total length of the caeca (Table 1, row 6) as described elsewhere (Buddington & Diamond, 1987).

While in cold oxygenated Ringer solution, the intestine was cut into segments that were everted and transferred to fresh cold oxygenated Ringer solution. Definition of segments varied with species, as follows. For the common carp and grass carp we isolated the expanded proximal region (termed the oesogaster, 15–20 % of total intestinal length) and divided the remaining intestine into two equal segments, mid and distal, distinguished in the common but not grass carp by the greater pigmentation of the distal region. As intestines of tilapia and catfish lacked visually distinct regions, they were divided into three segments of equal lengths. Sturgeon intestine consisted of two very distinct regions of nearly equal lengths, the proximal intestine and spiral valve. Striped bass intestine had a distal region (30 % of intestinal length) isolated by a muscular constriction from the proximal region, which was cut into two segments of equal lengths. In trout the proximal intestine (30–35 % of intestinal length) was defined as the region along which the pyloric caeca were attached; the distal region (30–40 % of intestinal length) was distinguished from the mid region by the former's larger diameter, annular rings and darker colour. Prickleback intestine was divided into two regions of about equal lengths, the proximal region having a smaller diameter.

Solute uptake measurements. Solute uptakes across the brush-border membrane (*not* trans-epithelial fluxes) were measured *in vitro* at 20 °C by the everted sleeve method described previously (Karasov & Diamond, 1983). Briefly, an intestinal region was everted and cut into two to eight sleeves which were then mounted individually on grooved rods of a diameter chosen to yield a snug fit but not to stretch the tissue. Sleeves were secured to the grooves with surgical

thread, and trimmed to a length of 0·5–1·0 cm. Rods were of glass, or else stainless steel for intestines with outer diameters less than 4 mm. Control studies with adjacent tissues showed that glass and steel rods yielded the same uptake. Anatomical measurements and mounting took 30–45 min from the time of death, during which the tissues were kept in cold oxygenated Ringer solution. Electrophysiological measurements show that fish intestines maintain nutrient uptake rates for at least 4 h (Saul-Conn & Diamond, 1985).

Before an uptake determination we pre-incubated the mounted sleeve for 5 min in oxygenated Ringer solution at 20 °C. The sleeve on its rod was then transferred to a tube of 10 ml Ringer solution continuously oxygenated with 95% O_2–5% CO_2, and was positioned 3 mm over a stir bar rotating at 1200 r.p.m. to reduce unstirred layers and increase oxygenation. The solution contained L-proline or D-glucose at 25 mM and the uniformly labelled ^{14}C tracer, plus an ^{3}H-labelled solute in tracer quantities. From each intestinal region of each species half of the sleeves were used for L-proline studies, half for D-glucose studies. In L-proline studies the ^{3}H tracer was [1,2-^{3}H]polyethylene glycol (PEG, molecular weight 4000) to correct for L-[^{14}C]proline in adherent fluid. In D-glucose studies the ^{3}H tracer was L-[1-^{3}H]glucose to correct simultaneously for D-glucose in adherent fluid and D-glucose taken up passively, yielding active D-glucose uptake (Karasov & Diamond, 1983). The choice of incubation time (generally 4 min) and solute concentration (25 mM) will be discussed under Results.

Following the incubation we removed the rod from the incubation solution, lightly blotted off excess fluid without rinsing, cut out the sleeve between the ligatures, placed it in a tared vial, weighed it wet, solubilized it (TS-1, Research Products International), added scintillant (Econofluor, New England Nuclear), and determined tissue ^{14}C and ^{3}H by liquid scintillation counting (Karasov & Diamond, 1983).

Errors caused by metabolism of absorbed solutes to volatile products. If significant tissue metabolism of our labelled nutrients to volatile products occurred within our selected incubation time of 4 min, uptake values would tend to be underestimates. For intestines of several mammal and reptile species we previously described experiments showing that this error could be rendered negligible by using ^{14}C- instead of ^{3}H-labelled metabolites, and by not drying the tissues before counting (Karasov & Diamond, 1983, pp. 111–112; Karasov, Solberg & Diamond, 1985*c*, p. 280 and Table 7). Hence we repeated those experiments for four to eight individuals of each of three fish species (common carp, catfish and sturgeon) and obtained identical results. Briefly, we incubated one sleeve in Ringer solution with both D-[^{3}H]- and D-[^{14}C]glucose, and the adjacent sleeve in Ringer solution with both L-[^{3}H]- and L-[^{14}C]proline, split each sleeve in half, counted one half-sleeve wet and the other half-sleeve after drying overnight in an oven, and computed ratios among the four resulting tissue spaces (^{14}C and ^{3}H spaces in wet tissues, ^{14}C and ^{3}H spaces in dried tissue; see Fig. 1 legend for definition of tissue spaces). If there were no loss of volatile tracer, all four spaces for a given solute would be identical, and all ratios would be 1·00. This proved to be the case for the ^{14}C tracers of both solutes (dry space/wet space was $1{\cdot}00 \pm 0{\cdot}02$ ($n = 16$) for L-[^{14}C]proline, $1{\cdot}02 \pm 0{\cdot}04$ ($n = 18$) for D-[^{14}C]glucose), and nearly so for the ^{3}H tracer of L-proline ($0{\cdot}93 \pm 0{\cdot}03$ ($n = 16$)). The ratio of the ^{3}H to ^{14}C space for L-proline was also close to 1·00, both in wet ($0{\cdot}99 \pm 0{\cdot}01$ ($n = 16$)) and dry ($0{\cdot}95 \pm 0{\cdot}01$ ($n = 16$)) tissues. Thus, a 4 min incubation yields negligible amounts of volatile products of L-[^{14}C]proline, L-[^{3}H]proline, and D-[^{14}C]glucose. However, D-[^{3}H]glucose does yield significant amounts of volatile products (probably $^{3}H_2O$), as reflected in low tissue spaces, especially in dry tissues (D-[^{3}H]glucose space/D-[^{14}C]glucose space $0{\cdot}81 \pm 0{\cdot}03$ ($n = 18$) in wet tissues, $0{\cdot}27 \pm 0{\cdot}02$ ($n = 18$) in dry tissues; dry space/wet space $0{\cdot}36 \pm 0{\cdot}04$ ($n = 18$) for D-[^{3}H]glucose). Accordingly, we used D-[^{14}C]glucose and L-[^{14}C]proline and did not dry the tissues.

Expression of uptakes. Uptakes (Table 3, rows 2–4) were expressed relative to three alternative measures of the amount of tissue: wet weight (Table 3, row 4); sleeve length (row 2); and nominal surface area (row 3), calculated from the diameter of the rod on which the sleeve was mounted. Nominal surface area is the area of the equivalent smooth cylinder and neglects area amplification by villi and microvilli. For sturgeon distal intestine, however, we multiplied the nominal area by 3·3 to account for the contribution of the spiral valve (Andrews & Hickman, 1974). Nominal surface area/cm of each region was multiplied by the region's length and summed over all regions to yield the nominal surface area of the whole gut (Table 1, row 9). Uptake capacity of the entire gut (Table 3, rows 5*a* and *b*) was calculated by summing, for all gut regions, the product of uptake/cm times the region's length.

Solutions. Ringer solution composition (mM) was: NaCl, 117; $NaHCO_3$, 25; KCl, 5·8; KH_2PO_4,

1·2; $CaCl_2$, 2·5; $MgSO_4$, 1·2; 290 mosM; pH 7·4, when oxygenated with 95% O_2–5% CO_2. 25 mM-L-proline or D-glucose was incorporated by isosmotic replacement of NaCl. Na^+-free solutions were made by equimolar replacement of Na^+ with choline. To minimize contamination of Na^+-free solutions with Na^+ leached out of the tissue, we pre-incubated tissues in Na^+-free Ringer solution and changed both the pre-incubation and incubation solution after each use. Reagents were from Sigma Chemical Co. and were of the purest grade available. Radioisotopes were from New England Nuclear.

Statistics. Results are given as mean ± S.E.M. (n = sample size). The t test was used for tests of significance at the $P < 0.05$ level.

RESULTS

Anatomical results

Table 1, lines 5–11, gives measurements of the gut. Other features relevant to this paper are as follows.

Five species have pyloric caeca, which are narrow, blind-ended diverticula opening into the proximal intestine (Buddington & Diamond, 1987). The caeca are least numerous in prickleback (five) and striped bass (six), most numerous in trout (fifty-six), and are fused into a single structure in the sturgeon.

The intestine of tilapia is relatively much longer (5·8 times fork length) than in the other species and has numerous coils. In most species the proximal intestine has a thicker mucosa (higher value of mg/cm^2) than do the other regions. Apart from this mucosal thickening, there is little regional differentiation of the intestine in common carp, grass carp, tilapia, catfish and prickleback. The most differentiated intestine is that of the sturgeon (Buddington, 1983; Buddington & Doroshov, 1986), in which the distal intestine (termed the spiral valve) is expanded and includes an internal fold of spiralling tissue connected from the wall to a rod of tissue. The exposed epithelial surface of the post-gastric gut of sturgeon is dominated by mucus-secreting goblet cells, while deeper parts of the epithelium consist almost exclusively of columnar absorptive cells.

Choice of incubation time and concentration

We had adequate numbers of common carp, catfish, trout and sturgeon to study uptake as a function of incubation time and solute concentration. In these experiments we used mid intestine of the former three species, proximal intestine of sturgeon, since preliminary measurements with 4 min incubations and 50 mM-L-proline or D-glucose showed these regions to have relatively high, consistent uptakes.

Uptake vs. incubation time. The choice of incubation time involves a compromise among several considerations (Karasov & Diamond, 1983). On the one hand, the time must be long enough for the adherent fluid to equilibrate with tracers from the bathing solution, and for uptake to be measurable and high. On the other hand, the time must be short enough for the uptake/time relation to be still in the linear phase and for isotope appearance on the serosal surface to be still negligible. To identify the best compromise, we measured uptakes of tracer [^{3}H]PEG, tracer L-[^{3}H]glucose, L-[^{14}C]proline at 25 mM, and ^{14}C D-[^{14}C]glucose at 25 mM over incubation times of 1–16 min.

In all species the PEG space (Fig. 1) had not reached an asymptote at 1 min but had done so by 4 min, except in sturgeon, where the PEG space was still rising slowly at 16 min. Evidently the adherent fluid equilibrates within 4 min except in sturgeon, where mucus extrusion and deep crypts present a greater diffusion barrier (Buddington & Doroshov, 1986).

TABLE 1. Natural diet, body measurements, and intestinal measurements of the fish species studied

	Herbivores			Omnivores		Carnivores			
	Common carp	Grass carp	Tilapia	Catfish	Sturgeon	Striped bass	Trout	Herbivore prickleback > 12 cm	Carnivore prickleback < 12 cm
1 Protein in natural diet (% of dry wt)	10–20	10–20	10–20	20–50	30–60	> 70	> 70	10–20	> 70
2 No. of fish studied	13	14	10	6	27	9	10	18	12
3 Body wt. (g):									
mean	193±13	280±40	120±13	1260±150	1050±46	43±4	391±20	391±13·2	5·1±0·6
range	120–260	97–482	80–235	850–1590	775–3240	31–85	285–462	8–243	1·6–8·0
4 Fork length (cm)	21±1	27±1	19±1	40±5	55±1	16±1	31±1	17·5±1·5	10·3±0·4
5 No. of pyloric caeca	0	0	0	0	Many*	6±0	56±2	5±1	5±1
6 Total caecal length/ fork length	–	–	–	–	0·6*	0·20±0·01	3·4±0·2	0·1†	0·1†
7 Intestinal length/ fork length	2·1±0·1	1·9±0·1	5·8±0·2	1·6±0·1	0·87±0·08	0·46±0·01	0·49±0·01	0·65±0·03	0·60±0·03
8 Gut length/fork length	–	–	–	–	1·4±0·1	0·67±0·02	3·9±0·2	0·75	0·70
9 Gut area/body wt. (cm^2/g)	0·32±0·03	0·32±0·04	0·69±0·03	0·11±0·01	0·14±0·01	0·22±0·01	0·23±0·01	0·44±0·04	0·64±0·04
10 Gut wt./gut length (mg/cm)									
a Caeca	—	—	—	—	—	17‡	44±4	—	—
b Proximal intestine	316±41	309±31	73±4	557±25	212±13	118±10	101±9	45±3	16±1
c Mid-intestine	188±20	92±9	26±2	241±31	–	65±4	160±6	—	—
d Distal intestine	103±25	95±9	20±2	240±27	381±13	85±10	274±16	70±6	20±2
11 Mean gut 'thickness' (mg/cm^2)	89±8	111±7	46±2	130±12	101±8	226±14	111±7	67±3	37±2

Values in rows 3–11 are means±S.E.M. for the number of individual fish shown in row 2. Results for pricklebacks are broken down into two size classes based on fork length (> 12 cm or < 12 cm), since young pricklebacks are carnivores and old ones are herbivores. Values in row 1 are from Boyd & Goodyear (1971), McKechnie & Fenner (1971), and our observations. Row 4: length from tip of snout to fork in tail. Rows 6, 7 and 8: ratio of the total length of all pyloric caeca (row 6), length of the intestine without the caeca (row 7), and length of the whole gut = intestine + caeca (row 8), to fork length. Row 9: ratio of gut area = nominal surface area of intestine + caeca, considered as smooth-bore cylinders, to body wt. Row 10: wet wt. (mg) of a sleeve 1 cm long, from each section of the gut. Row 11: wet wt. (mg) per cm^2 of nominal surface area was averaged for all regions of the gut; this value approximates the gut thickness in $\mu m \times 10$ if the density of gut tissue is 1 g/ml.

* Sturgeon caeca are fused so that the number cannot be counted; the values for total length and area are estimated (Buddington & Diamond, 1987).

† Caecal length was estimated.

‡ Caecal wt./cm was estimated from Buddington & Diamond (1987).

The L-glucose space (Fig. 2) rose quickly in the first 2 min, then more slowly up to 16 min. Probably the initial rise represents equilibration of the adherent fluid, while the slow later rise involves passive permeation into the epithelium.

The L-proline space corrected for the PEG space (Fig. 3) rose quickly in the first 2 min and did not extrapolate through the origin, especially in trout and sturgeon. Since proline has a higher diffusion coefficient than PEG, the adherent fluid equilibrates more quickly with proline than with PEG, so that PEG underestimates the adherent proline and overestimates tissue-associated proline at early times, as previously found for mouse intestine (Karasov & Diamond, 1983). After 2 min the proline space continued to rise up to at least 8 or 16 min.

The D-glucose space corrected for the L-glucose space (Fig. 4) did not show proline's quick initial rise, because D-glucose and L-glucose have identical diffusion coefficients and L-glucose correctly estimates the D-glucose in the adherent fluid. D-glucose uptake continued to rise up to at least 8 or 16 min.

In intestines of mammals, birds, and reptiles, which have higher nutrient uptake rates than fish intestine (Karasov *et al.* 1985*a*), uptake rates level off as early as 2 or 4 min because the high uptake rates soon result in either appreciable back-flux of isotope to the luminal surface or else isotope exit to the serosal surface (Karasov & Diamond, 1983; Karasov *et al.* 1985*c*). Preliminary measurements with everted fish intestine sleeves mounted on perforated hollow rods confirmed that isotope transfer to the serosal compartment was still negligible at 10 min (less than 2% of tracer levels in the luminal compartment). This observation also indicates that negligible isotope reaches the serosal surface through damaged tissue.

Based on these measurements, we chose 4 min incubations as the best compromise, except for sturgeon in which we utilized 8 min incubations. At 4 min PEG has equilibrated with adherent fluid in all species except sturgeon, and L-glucose has passed its quick initial rise, also indicating equilibration of adherent fluid. On the other hand, a 4 min incubation reduces the risk of the absorbed tracer being transformed metabolically, or of the uptake/time relation becoming non-linear.

Uptake vs. concentration. We measured uptakes of L-proline and D-glucose as a function of concentration from 0·1 to 50 mM. When L-glucose was used to correct D-glucose uptake for passive uptake as well as for glucose in adherent fluid, the resulting active D-glucose uptake exhibited saturable kinetics, and its concentration dependence conformed to the Michaelis–Menten equation (Fig. 5). The Na^+-independent component of L-proline uptake, determined as uptake in Na^+-free solutions, varied approximately linearly with concentration (Fig. 6), suggesting that this component results mainly from passive diffusion. The Na^+-dependent component of L-proline uptake, calculated as total uptake minus Na^+-independent uptake, exhibited saturable kinetics (Fig. 7).

Table 2 summarizes values of the maximal transport rate (V_{max}) and the apparent Michaelis–Menten constant K_m* (apparent, because uncorrected for effects of unstirred layers). These values were obtained by fitting uptakes as a function of concentration to the Michaelis–Menten equation. Values of V_{max} for D-glucose decrease in the sequence herbivore (common carp) > omnivore (catfish, sturgeon) > carnivore (trout), while values of V_{max} for L-proline are in the reverse sequence except for the low value in sturgeon. (Both differences between the carnivore and the

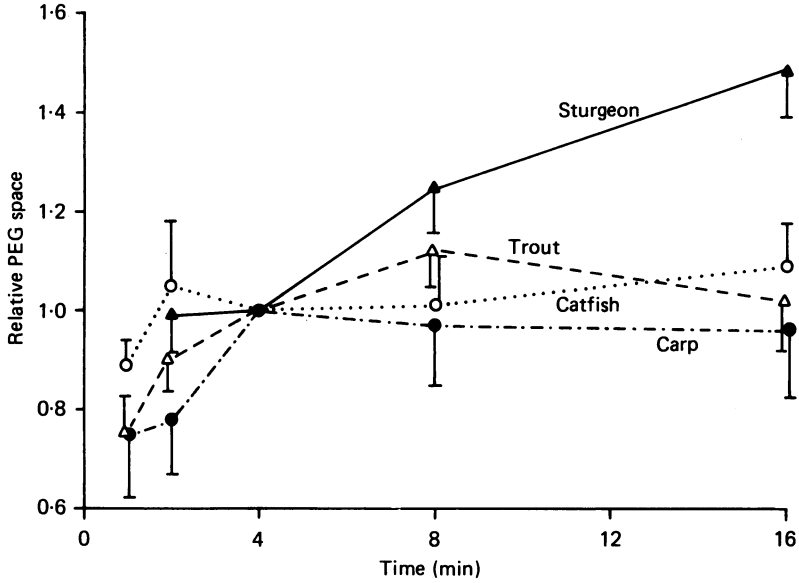

Fig. 1. Tissue uptake of tracer [^{3}H]PEG as a function of incubation time. PEG uptake is expressed as the tissue PEG space in μl/mg; i.e. d.p.m. of PEG per mg tissue, divided by d.p.m. of PEG/μl incubation solution. The ordinate gives the PEG space at the indicated time, divided by the PEG space at 4 min. The absolute space at 4 min, in μl/mg, was $0{\cdot}11 \pm 0{\cdot}01$ ($n = 32$) for sturgeon, $0{\cdot}26 \pm 0{\cdot}02$ ($n = 29$) for trout, $0{\cdot}14 \pm 0{\cdot}01$ ($n = 20$) for catfish, and $0{\cdot}15 \pm 0{\cdot}02$ ($n = 35$) for carp.

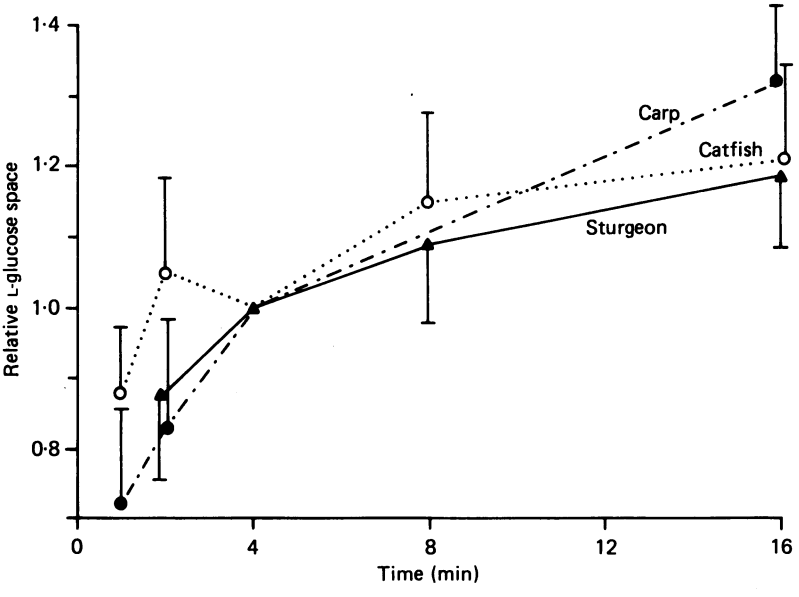

Fig. 2. Tissue uptake of tracer L-[^{3}H]glucose as a function of incubation time. Uptake is expressed as the tissue L-glucose space at the indicated time relative to the space at 4 min (ordinate), as explained for PEG in the legend to Fig. 1. The absolute space at 4 min, in μl/mg, was $0{\cdot}29 \pm 0{\cdot}05$ ($n = 22$) for carp, $0{\cdot}20 \pm 0{\cdot}03$ ($n = 20$) for catfish, and $0{\cdot}17 \pm 0{\cdot}02$ ($n = 34$) for sturgeon.

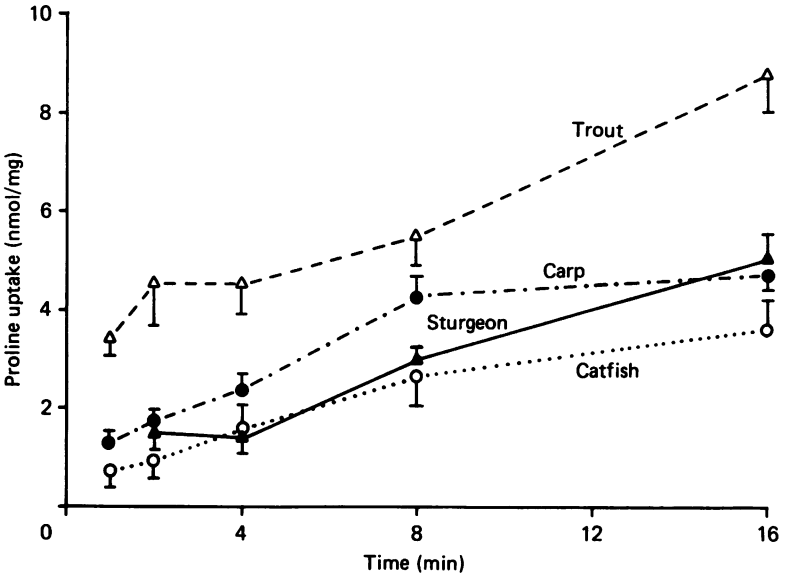

Fig. 3. L-proline uptake at 25 mM (nmol/mg wet intestinal tissue), as a function of incubation time. [^{3}H]PEG was used to correct for proline in adherent fluid.

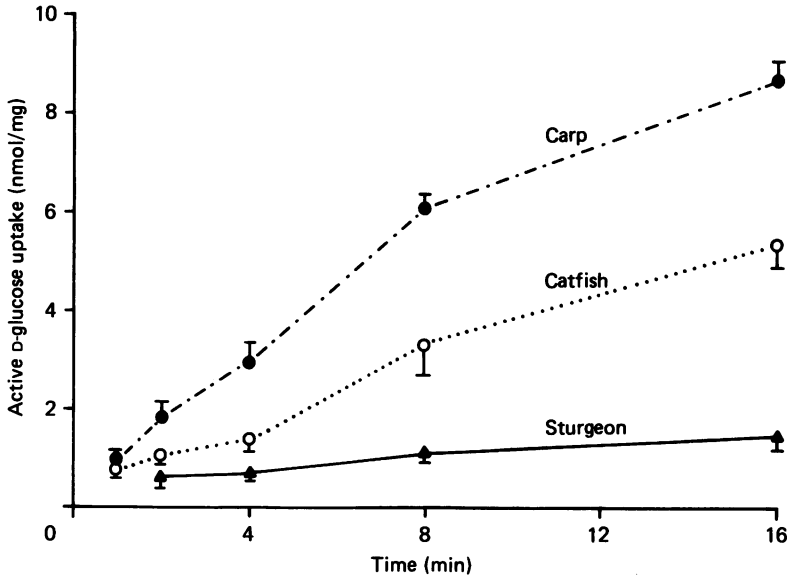

Fig. 4. Active uptake of D-glucose at 25 mM (nmol/mg wet intestinal tissue), as a function of incubation time. L-[^{3}H]glucose was used to correct for D-glucose in adherent fluid and for D-glucose taken up passively.

herbivore, and five of the eight differences between the two omnivores and the carnivore or herbivore, are significant at levels ranging from $P < 0{\cdot}05$ to $P < 0{\cdot}002$.) Values of K_m* for each solute fall in approximately the same sequence as the V_{max} values, except that in sturgeon the K_m* values for both D-glucose and L-proline are anomalously high.

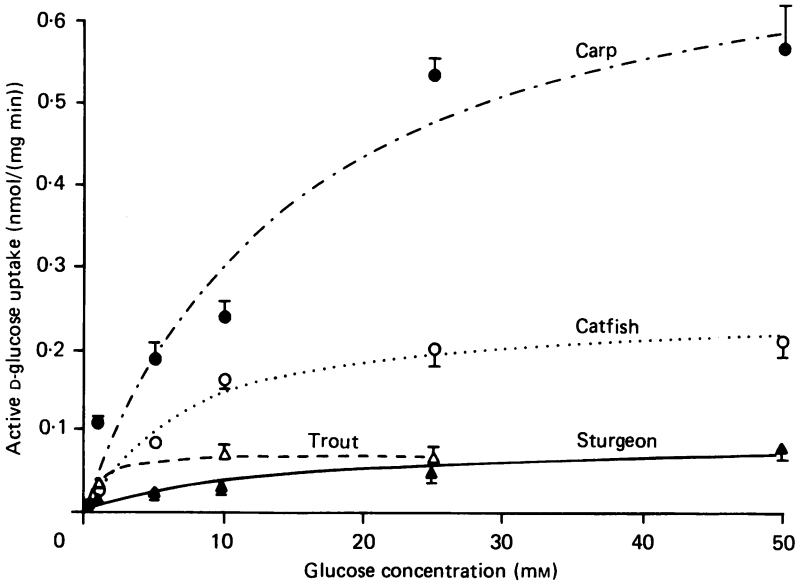

Fig. 5. Active uptake of D-glucose in nmol/(mg wet tissue min) as a function of glucose concentration, corrected for D-glucose in adherent fluid and for passive uptake by means of L-[^{3}H]glucose. Incubation time was 4 min. Curves shown are best fits of the Michaelis–Menten equation by the Gauss–Newton method of non-linear least-square regression (Duggleby, 1981).

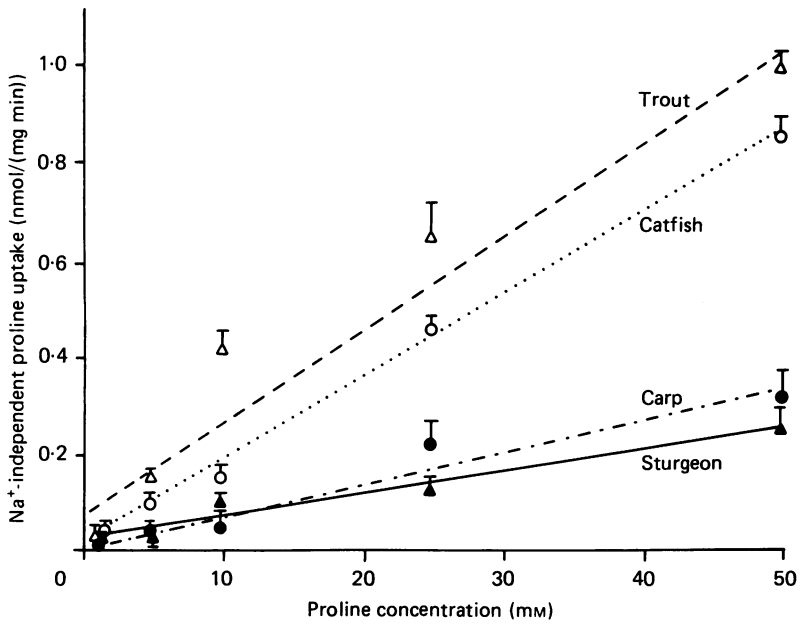

Fig. 6. Na^+-independent proline uptake (i.e. proline uptake measured in solutions in which the Na^+ had been replaced by choline), as a function of proline concentration. Uptake is expressed as nmol proline/(mg wet tissue min). Incubation time was 4 min, and [^{3}H]PEG was used to correct for proline in adherent fluid. Lines are the straight lines of best fit.

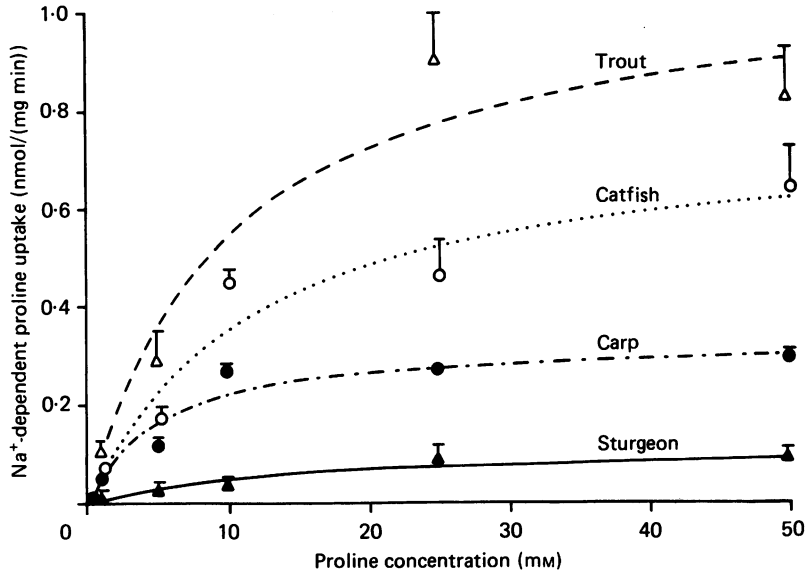

Fig. 7. Na^+-dependent proline uptake as a function of proline concentration, calculated as proline uptake measured in one sleeve in a Na^+-containing solution minus proline uptake measured in an adjacent sleeve in a Na^+-free solution. Curves shown are best fits of the Michaelis–Menten equation (see legend to Fig. 5). Other details as in Fig. 6 legend.

TABLE 2. Kinetic constants for Na^+-dependent uptake of L-proline and active uptake of D-glucose by intestines of four fish species

	Proline			Glucose		
	V_{max}	K_m*	n	V_{max}	K_m*	n
Common carp	0·33±0·02	4·9±1·2	22	0·77±0·05	15·4±2·6	39
Catfish	0·75±0·06	11·0±2·6	42	0·25±0·01	7·7±1·1	22
Sturgeon	0·13±0·01	16·4±2·9	47	0·09±0·01	14·6±4·4	33
Trout	1·08±0·10	10·0±3·2	32	0·068±0·003	1·1±2·9	45

The maximal transport velocity, V_{max} (nmol/(mg tissue wet wt. min)), and apparent Michaelis–Menten constant K_m* (nM) were calculated from best fits of the Michaelis–Menten equation to uptakes as a function of concentration (Figs 5 and 7). Sample size (n) refers to the number of uptake determinations on which each fit was based.

Although unstirred layers do not affect V_{max} determinations, they do cause measured apparent K_m values (i.e. K_m*) to exceed true K_m values (Thomson & Dietschy, 1977; Barry & Diamond, 1984). The magnitude of the error in K_m* increases with V_{max}. A previously noted result of these facts is that, if one compares K_m* and V_{max} values of intestinal D-glucose transport for many vertebrate species (or for the same species in different physiological states), K_m* is found to increase regularly with V_{max} (see Fig. 4 of Karasov *et al.* 1985*a*), suggesting that measured K_m* values are largely determined by unstirred layers and do not reflect true K_m values. Table 2 conforms to this pattern, both for proline transport and for glucose

transport. The anomalously high K_m* values for sturgeon are exceptions that prove the rule: sturgeon intestine is likely to have the thickest unstirred layers because of mucus production and because the mucus-secreting cells lie between the absorptive cells and the bathing solution.

In short, the V_{max} values of Table 2 are matched to natural diet: species whose diets normally contain much carbohydrate or protein tend to have many sugar or amino acid transporters, respectively. The K_m* values, on the other hand, appear to be unstirred-layer artifacts, as are most other K_m* values for nutrient transport measured in the intact intestine (Barry & Diamond, 1984; Karasov *et al.* 1985*a*).

For practical purposes, the results of Figs 5–7 convinced us to compromise on a concentration of 25 mM for studying interspecies differences in L-proline and D-glucose transport. This concentration yields uptakes close to the V_{max} values for proline and glucose transport in the four species used for uptake and concentration studies. If we had raised the concentration to 50 mM in order to obtain uptakes slightly closer to the V_{max}, self-inhibition of tracer uptake by unlabelled solute would sometimes have yielded tracer D-glucose uptakes indistinguishable from zero, especially in carnivorous fish with low glucose uptakes.

Species differences in uptakes

Table 3 gives for each species the proline and glucose uptake in each intestinal region, related to mg, cm or cm^2 of the intestinal sleeve. The Table also presents summed uptake capacities of the whole gut for each solute in each species.

Consider first the values of uptake/cm tissue (Table 3, row 2). These values, averaged over all gut regions of a given species, vary widely among species and bear no clear relation to natural diet, except that values of glucose uptake in carnivores are low. The reason is that our fish species vary greatly in body size (Table 1, row 3), hence also in intestinal thickness and diameter, and thus in quantity of absorptive tissue/cm (Table 1, row 10). Catfish have uptakes/cm for proline or glucose 10 times those of tilapia, simply because our catfish are big and have thick wide-bore intestines while our tilapia are small and have thin small-bore intestines. For this reason the uptake/cm^2 or uptake/mg are more informative.

Calculating uptake/cm^2 of nominal mucosal area removes the effect of species differences in gut diameter, though it does not remove the effect of species differences in gut thickness. For each species we averaged proline or glucose uptake values over all gut regions, then averaged the resulting values for all species of the same natural diet (e.g. all herbivores). The proline uptake/cm^2 averages 45 ± 11 nmol/(cm^2 min) for our four herbivores, 73 ± 15 for our two omnivores, and 65 ± 20 for our three carnivores, while corresponding values for glucose are 36 ± 14, 37 ± 23 and 11 ± 2 nmol/(cm^2 min). Thus, carnivores exceed herbivores in proline uptake/cm^2 while the reverse is true for glucose uptake/cm^2, but these differences do not reach the $P < 0.05$ level of significance.

Calculating uptake/mg intestinal weight removes effects of species differences in gut thickness as well as in diameter. Averaged over gut regions and then over species as in the case of uptake/cm^2, proline uptake/mg averages 0.59 ± 0.07 nmol/(mg min) for our four herbivores, 0.31 ± 0.06 for our two omnivores, and 0.96 ± 0.11 for our three carnivores (difference between each pair of classes significant at the $P < 0.05$

TABLE 3. Proline and glucose uptakes

	Herbivores				Omnivores					
	Common carp		Grass carp		Tilapia		Catfish		Sturgeon	
	Proline	Glucose	Proline	Glucose	Proline	Glucose	Proline	Glucose	Proline	Glucose
(1) No. of fish studied	5	4	14	14	10	10	3	3	9	11
(2) nmol/(cm min):										
Caeca	—		—		—		—		—	
Prox. int.	97±18	164±31	170±37	131±30	39±5	32±2	467±26	249±16	61±7	14·1±2·5
Mid int.	115±23	78±2	54±9	38±4	8·9±2·6	5·9±1·1	184±37	125±17	—	
Dist. int.	80±9	82±6	28±5	29±4	6·1±1·6	3·6±0·7	29±9	53±7	91±8	10·6±2·3
(3) nmol/(cm² min):										
Caeca	—		—		—		—		—	
Prox. int.	51±10	87±16	95±24	77±22	28±4	25±2	166±8	99±7	50±4	20±4
Mid int.	92±18	65±4	53±13	29±2	16·7±2·3	12·2±2·1	84±6	57±8	—	
Dist. int.	64±7	66±5	23±4	23±3	10·9±3·0	6·8±1·3	13±2	24±3	66±9	7·9±2·1
(4) nmol/(mg min):										
Caeca	—		—		—		—		—	
Prox. int.	0·52±0·08	1·02±0·15	0·63±0·09	0·43±0·06	0·56±0·07	0·45±0·02	0·47±0·07	0·20±0·03	0·35±0·02	0·10±0·01
Mid int.	1·03±0·26	0·83±0·02	0·61±0·07	0·42±0·03	0·67±0·10	0·22±0·03	0·24±0·02	0·15±0·02	—	
Dist. int.	0·72±0·07	0·81±0·05	0·36±0·06	0·31±0·03	0·10±0·03	0·18±0·03	0·05±0·01	0·06±0·01	0·39±0·03	0·03±0·01
(5) Uptake capacity (nmol/(g body wt. min)):										
a Proline	21±3		13·9±1·2		24±2		9·3±1·0		7·3±0·5	
b Glucose	29±4		10·0±1·1		17·6±2·0		5·8±0·3		2·0±0·2	
c Proline/glucose	0·8±0·2		1·5±0·2		1·3±0·2		1·6±0·3		4·0±0·5	

TABLE 3. (*cont.*)

	Carnivores				Herbivore prickleback > 12 cm		Carnivore prickleback < 12 cm	
	Striped bass		Trout					
	Proline	Glucose	Proline	Glucose	Proline	Glucose	Proline	Glucose
(1) No. of fish studied	3	3	10	10	18	18	12	12
(2) nmol/(cm min):								
Caeca	17	2	55±8	4·8±0·9	—		—	
Prox. int.	115±5	19·8±0·4	197±17	12·5±2·8	—		—	
Mid int.	20±5	7·4±1·0	155±14	14·9±2·6	29±4	11·0±1·3	23±4	6·4±1·5
Dist. int.	46±9	0·2±0·2	162±12	11·1±2·3	—		—	
(3) nmol/(cm² min):								
Caeca	69	22	115±13	7·7±2·3	—		—	
Prox. int.	91±4	15·8±0·3	125±11	7·9±1·8	42±6	14·9±1·3	54±9	16·0±3·8
Mid int.	21±5	7·7±0·8	96±9	9·5±1·7	—		—	
Dist. int.	20±3	1·0±1·0	86±7	5·9±1·2	25±5	14·8±2·7	28±4	10·6±2·0
(4) nmol/(mg min):								
Caeca	1	0·1	1·3±0·1	0·1±0·01	—		—	
Prox. int.	1·07±0·7	0·13±0·02	1·1±0·1	0·09±0·02	0·76±0·11	0·29±0·02	1·44±0·23	0·46±0·11
Mid int.	0·38±0·05	0·11±0·01	0·9±0·1	0·09±0·02	—		—	
Dist. int.	0·60±0·22	0·01±0·01	0·6±0·1	0·04±0·01	0·51±0·09	0·25±0·04	0·84±0·16	0·34±0·05
(5) Uptake capacity (nmol/(g body wt. min)):								
a Proline	10·0±0·8		20·9±3·2		15·8±3·9		23·1±3·3	
b Glucose	1·3±0·4		1·8±0·4		6·3±1·2		6·9±1·7	
c Proline/glucose	7·7±1·8		13·5±2·7		2·7±0·4		4·7±0·7	

Uptake rates normalized to length (row 2), nominal surface area (row 3), or wet wt. (row 4) of the intestinal sleeve. Rows 5*a* and *b* give the summed uptake capacity for the whole gut, calculated by multiplying the uptake/cm for each gut region by the region's length and summing over all regions. Values are means ± S.E.M. for the number of individual fish shown in row 1. The ratio of the proline uptake capacity to the glucose uptake capacity was also calculated for each individual fish, and the mean ± S.E.M. of this ratio is given in row 5*c*, which therefore differs slightly from the ratio of row 5*a* to row 5*b*. Values for uptake by striped bass caeca were not measured in this study but were instead calculated from the data of Buddington & Diamond (1987), on the assumption that ratios of caecal uptake to intestinal uptake measured in that study also apply to our fish. To calculate uptake capacity of sturgeon gut, we assumed that the caecum and proximal intestine have equal areas and uptake capacities (Buddington & Diamond, 1987). Caeca of pricklebacks were so few in number and small that their possible contribution to uptake capacity was assumed negligible.

level), while corresponding values for glucose are $0{\cdot}46 \pm 0{\cdot}15$ nmol/(mg min) (herbivores), $0{\cdot}10 \pm 0{\cdot}04$ (omnivores) and $0{\cdot}19 \pm 0{\cdot}11$ (carnivores) (differences not significant). Thus, proline uptake again increases, and glucose uptake decreases, in herbivores compared to carnivores. The low values for omnivores are probably due to the fact that our two omnivorous species were considerably larger than any of our herbivores or carnivores; uptake/mg of intestine tends to decrease with body weight within the species for which we had a sufficient range of weights to examine the relationship.

The whole gut's summed uptake capacity for proline (Table 3, row 5*a*) differs little between herbivores ($18{\cdot}7 \pm 2{\cdot}3$ nmol/(g body wt. min)($n = 4$ species)), omnivores ($8{\cdot}3 \pm 1{\cdot}0$ ($n = 2$ species)), and carnivores ($18{\cdot}0 \pm 4{\cdot}1$ ($n = 3$ species)). (The somewhat lower values for omnivores is probably again due to the large size of those fish, as explained in the preceding paragraph for values of uptake/mg.) For glucose, however, the summed uptake capacity (Table 3, row 5*b*) decreases steeply from herbivores ($15{\cdot}8 \pm 5{\cdot}1$ nmol/(g body wt. min) ($n = 4$)) to omnivores ($3{\cdot}9 \pm 1{\cdot}9$ ($n = 2$)) to carnivores ($3{\cdot}3 \pm 1{\cdot}8$ ($n = 3$)); the difference between herbivores and carnivores is significant ($P < 0{\cdot}05$).

Interspecies comparisons of the four uptake measures discussed in the preceding four paragraphs suffer from the disadvantage that those measures may be influenced by body size, which varies among our species. The ratio of the summed uptake capacity for proline to that for glucose has the virtue that there is no necessary relation between it and body size (see also the next section on developmental changes). Figure 8 depicts this ratio for each species (Table 3, row 5*c*) plotted against the proportion of protein in that species' natural diet (Table 1, row 1). The ratio increases from herbivores ($1{\cdot}6 \pm 0{\cdot}4$) to omnivores ($2{\cdot}8 \pm 1{\cdot}2$) to carnivores ($8{\cdot}6 \pm 2{\cdot}6$) (slope of Fig. 8 significantly different from 0 ($P < 0{\cdot}005$); Spearman rank correlation of the data significant ($P < 0{\cdot}02$); ratio for herbivores significantly lower than that for carnivores ($P < 0{\cdot}05$)). There is no overlap among our herbivore, omnivore and carnivore species in their mean values of the ratio for each species, except for the prickleback values, which change developmentally (points ▲ and ◑ in Fig. 8; see next paragraph). The shift of the ratio from herbivores to omnivores to carnivores is due mainly to species differences in glucose uptake rather than in proline uptake.

Developmental changes in uptake

We initially noticed that uptakes were more variable for pricklebacks than for our other fish species. Eventually we realized that this variation was correlated with body size; in Tables 1 and 3 the prickleback results are grouped according to whether the fish was longer or shorter than 12 cm. Hence we obtained more pricklebacks covering a wider size range and measured their uptakes, with the results shown in Fig. 9. The ratio of proline uptake capacity/glucose uptake capacity decreases greatly with increasing body size, from $4{\cdot}7 \pm 0{\cdot}7$ ($n = 12$) for fish shorter than 12 cm to $2{\cdot}7 \pm 0{\cdot}4$ ($n = 18$) for fish longer than 12 cm ($P < 0{\cdot}005$; slope of Fig. 9 significantly different from 0 ($P < 0{\cdot}001$)). The function of this shift is presumably related to a shift in pricklebacks' natural diet with size: small pricklebacks are carnivores but become increasingly herbivorous as they grow larger (Montgomery, 1977).

The results of Fig. 9 for prickleback prompted us to examine whether there could

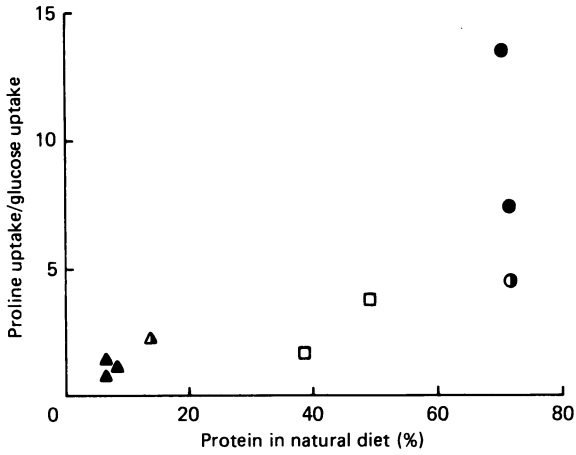

Fig. 8. The intestine's summed uptake capacity for proline divided by that for glucose (ordinate: from Table 3, row 5*c*), as a function of the percentage of protein in the natural diet (abscissa: from Table 1, row 1), for eight fish species. ●, carnivores; □, omnivores; ▲, herbivores; ◭ and ◑, pricklebacks longer and shorter than 12 cm, respectively (predominantly herbivores and carnivores, respectively). Note that the ratio of proline uptake/glucose uptake increases with the proportion of protein in the natural diet, even though all eight species were studied while eating the same artificial diet.

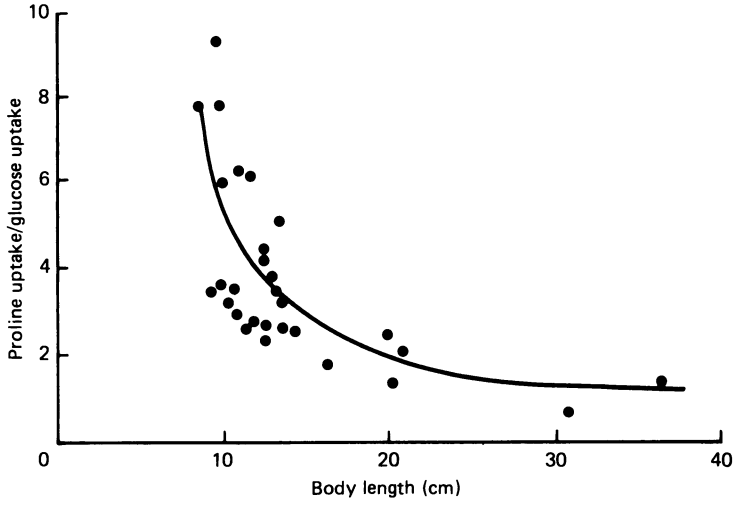

Fig. 9. The intestine's summed uptake capacity for proline divided by that for glucose (ordinate), divided by body length (fork length: abscissa), for pricklebacks. Large pricklebacks (herbivorous in the wild) have lower rates of proline uptake relative to glucose uptake than do small pricklebacks (carnivorous in the wild), even though both were studied while eating the same artificial diet in the laboratory.

also be a body size dependence of the proline/glucose uptake capacity ratio in our other seven fish species, none of which shifts in diet over the size range that we studied. We found no such dependence: in all seven species the uptake ratio for larger and smaller individuals was the same ($P > 0{\cdot}25$ or $P > 0{\cdot}50$). Nor did the ratio differ ($> 0{\cdot}50$) between the small striped bass (31–85 g) used in the present study (uptake

ratio 7·7 ± 1·8 ($n = 3$)) and the much larger striped bass (1164–3153 g) that we had studied previously (uptake ratio 9·1 ± 4·0 ($n = 11$): Buddington & Diamond, 1987). We therefore measured uptakes in an additional series of fifteen catfish including ones much smaller (3·7–949 g) than those used in our main study reported in Tables 1–3 (850–1590 g), since catfish weighing less than 30 g have relatively shorter intestines and are evidently more adapted to carnivory than are larger, more omnivorous catfish (Kline, 1978). The proline/glucose uptake capacity ratio proved to be independent of size from 32 to 949 g (1·34 ± 0·40 ($n = 7$) for 32–169 g fish, 1·35 ± 0·11 ($n = 7$) for 343–949 g fish; $P > 0·5$), but was much larger (6·3 ± 2·0 ($n = 6$): $P < 0·01$) for the smallest fish weighing 3·7–31 g. The ratio for the 32–949 g fish (1·4 ± 0·2 ($n = 14$)) does not differ ($P > 0·25$) from that (1·6 ± 0·3 ($n = 6$)) for the 850–1590 g catfish of Table 1.

Thus, the proline/glucose uptake capacity ratio changes developmentally in both prickleback and catfish, but only over the body size range over which natural diet also changes developmentally. With those two exceptions, the uptake ratio does not vary *intra*specifically with body size in our eight fish species, over the size ranges that we studied, even over a 100-fold weight range in the case of striped bass. One might also wonder whether the differences between herbivores, omnivores, and carnivores in uptake ratio (Fig. 8) could actually reflect *inter*specific differences in body size rather than in natural diet. However, the weight ranges of the herbivores (8–482 g), omnivores (30–3240 g), and carnivores (2–3153 g) that we studied overlap broadly. Nor could the differences between herbivores, omnivores and carnivores be a coincidence due to taxonomy, as our eight fish species belong to four orders, seven families, and eight genera.

DISCUSSION

This section examines why fish species with differing natural diets have differing glucose and proline uptakes, and why they also differ in intestinal morphology.

Species differences in nutrient transport

Herbivorous fish species proved to have higher rates of intestinal glucose uptake, and lower rates of proline uptake, than did carnivorous species. These species differences are expressed to varying degrees in the values of V_{max} (Table 2), uptake/mg or uptake/cm^2 (Table 3, rows 3 and 4), and summed uptake capacities (Table 3, rows 5*a* and 5*b*). Since species differences in uptake of a given solute may be influenced by species differences in body size as well as in diet, the clearest effect of diet emerges from the ratio of proline uptake capacity to glucose uptake capacity (Figs 8 and 9). This ratio is highest in carnivores, lower in omnivores, and lowest in herbivores.

As a result, uptakes are matched to natural diets. Herbivores, whose diets are low (10–20%) in protein but high in carbohydrates, have relatively lower amino acid uptakes and higher sugar uptakes. Carnivores, whose diets are high (> 70%) in protein but contain negligible carbohydrate, have relatively higher amino acid uptakes and lower sugar uptakes. Among measurements of intestinal glucose or amino acid transport in other fish species by other authors, the only instances in which no transport could be detected at all were for glucose transport in carnivores

(e.g. northern puffer, *Spheroides maculatus*: Wilson, 1957; winter flounder, *Pseudopleuronectes americanus*: Rout, Lin & Huang, 1965).

The uptake measure of physiological significance to the whole animal is the gut's summed uptake capacity. Why is it that carnivore *vs.* herbivore differences in summed uptake capacity are pronounced for glucose but not for proline? Sugars are not essential nutrients; they are merely used for calories if available. Carnivores require no dietary carbohydrate, consume little of it, and devote little biochemical machinery to processing it. Protein, however, is an indispensable nutrient that supplies essential nitrogen and essential amino acids, in addition to being available for use as calories. Herbivorous and carnivorous fish alike need protein, and their daily protein requirements are fairly similar (National Research Council, 1977). Herbivores exploit a diet with much lower protein levels than do carnivores, but compensate by ingesting much larger quantities of food. Thus, daily protein intake and intestinal amino acid uptake are only slightly, if at all, lower in herbivores than in carnivores.

Similar differences between herbivores and carnivores in sugar and amino acid uptake have been noted for other classes of vertebrates (Karasov *et al.* 1985*a*; Diamond & Buddington, 1987; Karasov & Diamond, 1987). However, as discussed in the Introduction, those intestinal differences might previously have been attributed to reversible phenotypic adaptations rather than to genetic differences, since earlier studies employed animals eating their natural diets (high in carbohydrate for herbivores, high in protein for carnivores). The present study excludes this possible interpretation based on phenotypic adaptations, because herbivores and carnivores proved to differ in uptake even while both were consuming the same diet. Evidently (subject to one caveat mentioned below), the intestinal uptake differences that we observed among fish species of different natural diets are fixed genetically. While at least some omnivores have retained some capacity for phenotypic adaptation to diet, natural selection has moulded the activities (or range of activities) of each species' intestinal transport systems to that species' usual natural diet.

The one caveat is that there is in principle an alternative explanation: early critical-period programming. That is, transporter activity in adulthood might be fixed irreversibly by an animal's diet early in life, so that a young herbivore programmed to eat plant food would as a result attain fixed high sugar transport activity and low amino acid transport activity. This developmental fixation seems unlikely for two reasons. First, an explicit search for critical-period programming of intestinal transport in mice failed to detect any such effect: mice reared throughout infancy on high-protein or high-carbohydrate diets ended up with similar intestinal transport rates as adults (Karasov *et al.* 1985*b*). Secondly, one fish species that we studied, the prickleback, is a carnivore when young and only later becomes a herbivore, yet adult pricklebacks are still typical herbivores in their rates of intestinal sugar and amino acid absorption.

The prickleback results are interesting from another point of view. Many animals undergo developmental changes in diet, the most familiar example being the shift from milk to the adult diet in mammals. In response to this change in diet, the mammalian digestive tract undergoes changes in anatomy and enzyme levels

(Henning, 1981). For each such response a question arises: is the change in diet required as the proximate signal for the response, or is the response 'hard-wired' to occur at the usually appropriate age even if the change in diet is prevented (Diamond, 1986)? Little information is available about these questions with respect to intestinal nutrient transport. The small and large pricklebacks that we studied had been maintained in our laboratory on identical diets for at least 8 weeks prior to killing. Thus, the ontogenetic change in uptake that we observed for intestines of pricklebacks (and catfish) must have been hard-wired with some unidentified internal trigger and did not require the natural dietary change itself as an external trigger.

Species differences in intestinal morphology

It is commonly emphasized that herbivorous fish species tend to have longer, thinner and narrower intestines than do carnivores (Fange & Grove, 1979). Our data bear this out: for instance, relative intestinal length increases from carnivores to omnivores to herbivores among our species (Table 1, row 7). However, Table 1 also shows that this is not the whole story. When one adds length of pyloric caeca and intestinal length to obtain the length of the whole gut, one of our three carnivores (the trout) actually has a relatively longer gut than all but one of our four herbivores (Table 1, row 8). Furthermore, within any given trophic class of fish the gut thickness tends to vary inversely with gut length (Fig. 2 of Buddington & Diamond, 1987): for example, tilapia has relatively the longest and thinnest intestine among the herbivores (rows 7 and 11 of Table 1). If one considers total gut weight as a measure of a fish's total quantity of absorptive tissue, fish of a given body weight have similar gut weights, regardless of whether they are carnivores, omnivores or herbivores (Fig. 3 of Buddington & Diamond, 1987). In effect, herbivores distribute that absorptive tissue along a long thin intestine, while carnivores concentrate it in a short thick intestine or allocate much of it to the pyloric caeca.

We suggest that these differences in allocation of absorptive tissue between carnivores and herbivores arise from the herbivores' very high food ingestion rates and fast intestinal transit times. Allocation of absorptive tissue to pyloric caeca is incompatible with fast transit times, because digesta would have to transit the blind caeca twice (once in each direction) whereas digesta transit the intestine only once. Distributing absorptive tissue in a thick mucosa would also be inefficient for species with fast transit times: nutrients would have little time to diffuse down to the deeper-lying absorptive cells, which would thus be under-utilized.

It is a pleasure to record our debts to Serge Doroshov, Michael Horn, Coachella Valley Water District, Fish Breeders, The Fishery, Pacific Aquafarms, University of California at Davis, Whitewater Trout Co., and Widman Fish Farm, for supplying fish. This work is a result of research sponsored in part by the National Oceanic and Atmospheric Administration, National Sea Grant College Program, Department of Commerce, under grant number NA85AA-D-SG140, Project number R/A 63, through the California Sea Grant College Program, and in part by the California State Resources Agency. The study was also supported by National Institutes of Health grants GM-14772 and AM-17328 (Center for Ulcer Research and Education).

REFERENCES

ANDREWS, W. & HICKMAN, C. P. (1974). *Histology of the Vertebrates, a Comparative Text.* St Louis: A. V. Mosby.

BARNARD, E. A. (1973). Comparative biochemistry and physiology of digestion. In *Comparative Animal Physiology*, ed. PROSSER, C. L., pp. 133–164. Philadelphia: W. B. Saunders.

BARRY, P. H. & DIAMOND, J. M. (1984). Effects of unstirred layers on membrane phenomena. *Physiological Reviews* **64**, 763–872.

BOYD, C. E. & GOODYEAR, C. P. (1971). Nutritive quality of food in ecological systems. *Archiv für Hydrobiologie* **69**, 256–270.

BUDDINGTON, R. K. (1983). Digestion and feeding of the white sturgeon, *Acipenser transmontanus*. Ph.D. Dissertation, Davis, University of California.

BUDDINGTON, R. K. (1987). Does the natural diet influence the intestine's ability to regulate glucose absorption? *Journal of Comparative Physiology* (in the Press).

BUDDINGTON, R. K. & DIAMOND, J. M. (1987). Pyloric caeca of fish, a 'new' absorptive organ. *American Journal of Physiology* **252**, G65-76.

BUDDINGTON, R. K. & DOROSHOV, S. I. (1986). Structural and functional relations of the white sturgeon alimentary canal (*Acipenser transmontanus*). *Journal of Morphology* **190**, 201–213.

DIAMOND, J. M. (1986). Hard-wired local triggering of enzyme expression. *Nature* **324**, 408.

DIAMOND, J. M. & BUDDINGTON, R. K. (1987). Intestinal nutrient absorption in herbivores and carnivores. In *Terrestrial vs. Aquatic Life*, ed. DEJOURS, P., BOLIS, L., TAYLOR, C. R. & WEIBEL, E. R. New York: Springer (in the Press).

DIAMOND, J. M. & KARASOV, W. H. (1984). Effect of dietary carbohydrate on monosaccharide uptake by mouse small intestine *in vitro*. *Journal of Physiology* **349**, 419–440.

DUGGLEBY, R. G. (1981). A nonlinear regression program for small computers. *Analytical Biochemistry* **110**, 9–18.

FANGE, R. & GROVE, D. (1979). Digestion. In *Fish Physiology*, vol. 8., ed. HOAR, W. S., RANDALL, O. J. & BRETT, J. R., pp. 162–260. New York: Academic Press.

FERRARIS, R. P. & AHEARN, G. A. (1983). Intestinal glucose transport in carnivorous and herbivorous marine fishes. *Journal of Comparative Physiology* **152**, 79–90.

HENNING, S. J. (1981). Postnatal development: coordination of feeding, digestion, and metabolism. *American Journal of Physiology* **241**, G199–214.

KARASOV, W. H., BUDDINGTON, R. K. & DIAMOND, J. M. (1985*a*). Adaptation of intestinal sugar and amino acid transport in vertebrate evolution. In *Transport Processes, Iono-, and Osmoregulation*, ed. GILLES, R. & GILLES-BAILLIEN, M. G., pp. 227–239. Berlin: Springer-Verlag.

KARASOV, W. H. & DIAMOND, J. M. (1983). A simple method for measuring solute uptake by intestine *in vitro*. *Journal of Comparative Physiology* **152**, 105–116.

KARASOV, W. H. & DIAMOND, J. M. (1987). Adaptation and evolution of intestinal transport in vertebrates. *American Journal of Physiology* (in the Press).

KARASOV, W. H., PHAN, D., DIAMOND, J. M. & CARPENTER, F. L. (1986). Food passage and intestinal nutrient absorption in hummingbirds. *Auk* **103**, 453–464.

KARASOV, W. H., SOLBERG, D. H., CHANG, S., HUGHES, M., STEIN, E. D. & DIAMOND, J. M. (1985*b*). Is intestinal transport of sugars and amino acids subject to critical-period programming? *American Journal of Physiology* **249**, G770–785.

KARASOV, W. H., SOLBERG, D. H. & DIAMOND, J. M. (1985*c*). What transport adaptations enable mammals to absorb sugars and amino acids faster than reptiles? *American Journal of Physiology* **249**, G271–283.

KARASOV, W. H., SOLBERG, D. H. & DIAMOND, J. M. (1987). Dependence of amino acid uptake by mouse intestine on dietary protein levels. *American Journal of Physiology* (in the Press).

KLINE, K. F. (1978). Aspects of digestion in stomachless fishes. Ph.D. Dissertation, Davis, University of California.

MCKECHNIE, R. J. & FENNER, R. B. (1971). Food habits of white sturgeon, *Acipenser transmontanus*, in San Pablo and Suisun Bays, California. *California Fish and Game* **57**, 209–212.

MONTGOMERY, W. L. (1977). Diet and gut morphology in fishes, with special reference to the monkeyface prickleback, *Cebidichthys violaceus* (Stichaeidae: Blennoidei). *Copeia* **1977**, 178–182.

NATIONAL RESEARCH COUNCIL. (1977). *Nutrient Requirements of Warmwater Fishes*. Washington, DC: National Academy of Sciences.

ROUT, W. R., LIN, D. S. & HUANG, K. C. (1965). Intestinal transport of amino acids and glucose in flounder fish. *Proceedings of the Society for Experimental Biology and Medicine* **118**, 933–938.

SAUL-CONN, C. & DIAMOND, J. M. (1985). Na^+-coupled glucose and amino acid transport in carp intestine. *Federation Proceedings* **44**, 812.

SMITH, M. W. (1966*a*). Influence of temperature acclimatization on sodium–glucose interactions in the goldfish intestine. *Journal of Physiology* **182**, 574–590.

SMITH, M. W. (1966*b*). Time course and nature of temperature-induced changes in sodium–glucose interactions of the goldfish intestine. *Journal of Physiology* **183**, 649–657.

THOMSON, A. B. R. & DIETSCHY, J. M. (1977). Derivation of the equations that describe the effects of unstirred water layers on the kinetic parameters of active transport processes in the intestine. *Journal of Theoretical Biology* **64**, 277–294.

WILSON, T. H. (1957). *In vitro* studies on intestinal absorption of fish. *Biological Bulletin, Marine Biological Laboratory, Woods Hole, Mass.* **113**, 362.

WOLFFRAM, S. & SCHARRER, E. (1984). Effect of feeding a high protein diet on amino acid uptake into rat intestinal brush border membrane vesicles. *Pflügers Archiv* **400**, 34–39.