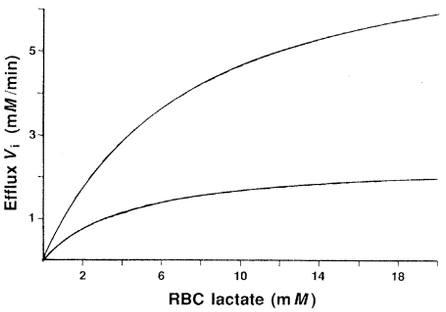

Fig. 5. Best-fit rectangular hyperbolas (Michaelis-Menten plots) for the mean normal values (upper curve) and for the patient's data (lower curve) in the red blood cell (RBC) efflux assay, extrapolated to 37°C and to high lactate levels. The K_m values are the same as in Fig. 4; the V_m values are 8.0 mM/min (SEM 11%) for the control subjects and 2.4 mM/min (SEM 16%) for the patient. At lactate levels above 8 mM (which normally occur only in muscle), the patient's cells cannot significantly increase lactate efflux to limit further elevations.

blood cell lactate transporter and a failure of muscle lactate to decline normally after exercise. The only parsimonious explanation is that human skeletal muscle and erythrocytes share the same genetic lactate transporter, or at least a common genetic subunit, which is defective in this patient.

Why call this a disease of muscle, when the defect is most convincingly demonstrated in the erythrocyte? The reason becomes clear from a comparison of the mean normal and the patient's hyperbolic plots at higher lactate levels (and at body temperature). This is easily done, since the binding constant changes little with temperature, and the activation energy is known (*11*). The discrepancy from normal only becomes marked above 8 mM lactate, where the patient's efflux can no longer increase significantly upon further lactate increase (Fig. 5). The only tissue in the body normally harboring such high levels is skeletal muscle, wherein the lactate may reach 25 to 30 mM upon extreme exercise. Indeed, an important role of the erythrocyte carrier may be to assist the muscle's lactate excretion. Since the red blood cells contain a third of the total water volume of blood, their rapid uptake of lactate egressing from muscle to plasma would retard the rise of plasma lactate concentration and facilitate continued efflux from muscle.

After extreme exercise, then, this patient may be unable to decrease his intramuscular acidosis with sufficient rapidity and might therefore be at risk of acute rhabdomyolysis and myoglobinuria. In the absence of extreme stress, however, he might encounter no difficulties; and this defect, like myoadenylate deaminase deficiency (*8*), may well be encountered in asymptomatic subjects, once the methods for detection are widely applied. Lactate transporter defect may ultimately be found to be a common cause of chronically elevated serum CK levels and of unexplained attacks of rhabdomyolysis and myoglobinuria in patients undertaking extreme muscular activity.

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14. Venous trapping at half-diastolic pressure is used for 10 seconds before and during each specimen collection, for a total stasis time of 20 to 25 seconds. This is a reasonable time for equilibration with muscle by diffusion, as judged by our erythrocyte transport data (see text). Since lactate transport is not energy-coupled, the level of lactate in plasma cannot exceed that in the muscle and may well underestimate it. However, the main objective is to see how much the level falls during free blood flow between samplings.
15. Aliquots of the stirred sample are removed at 30- or 60-second intervals and diluted in ice-cold buffer (*p*H 5) to stop efflux. The cells are harvested and washed by centrifugation at 0°C, then lysis and assay follow (*11*). A detailed procedure will be published elsewhere.
16. I am indebted to S. M. Muldoon for referring this patient. Informed consent was obtained from all participants in the study, in accordance with institutional Human Use Committee guidelines. The control subjects were all healthy normal adult volunteers. The opinions or assertions contained herein are the private views of the author, and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

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Energy Sources for Detritivorous Fishes in the Amazon

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Detritivorous fishes form an important part of the ichthyomass in the Amazon basin. Most of these fishes are contained in the orders Characiformes and Siluriformes (catfishes). The Characiformes constitute more than 30% of the total fish yield in the Amazon basin, whereas the catfishes are of minor importance. Stable isotope data indicate that Characiformes species receive most of their carbon through food chains originating with phytoplankton, while the Siluriformes receive a significant part of their energy from other plant sources.

THE PRIMARY PROTEIN SOURCE FOR the human population in the Amazon basin is fish (*1*). More than 30% of the fish consumed in the Amazon basin are detritivores (*2–4*), and evidence shows that this percentage has risen in recent years (*4, 5*). Most of these detritivorous fishes are contained in the order Characiformes (families Prochilodontidae and Curimatidae), whereas the catfishes (Siluriformes) form a second minor group.

Effective management of these populations will require an understanding of factors controlling their production. A critical first step will be to identify the plant carbon sources that fuel the detritivore food chain. There are four general groups of autotrophs in the Amazon region that could support detritivorous fishes, either directly or indirectly: trees, phytoplankton, periphyton, and aquatic macrophytes. Both aquatic macrophytes (*5, 6*) and trees (*7*) have been suggested as the principal carbon source for detritivorous fishes in the Amazon. There is also some evidence that algae can be important in the detritivore diet (*5, 7*). These hypotheses, however, are largely based on visual analyses of stomach contents, which can be misleading. These fishes are bottom feeders, and the stomach analyses generally reveal a complex mosaic of food items in their diet, including microinvertebrates, al-

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gae, bacteria, and detritus, usually dominated by detritus (*5, 7, 8*). It is not clear, though, which of these food items is digested and assimilated by the fish (*8*) and, with the exception of live algae, it is not possible to identify the autotrophic carbon source from which the material originated.

Stable isotope tracers offer an alternative and more direct approach to this question. Isotope analysis based on the use of $\delta^{13}C$, an effective tracer of carbon flow through food chains (*9*), has been especially useful in identifying the plant carbon sources that support production at higher trophic levels (*9*). We present here the results of a $\delta^{13}C$ tracer study of carbon flow through fish detritivore food chains in the central Amazon basin. Our objective was to identify the principal autotrophic carbon sources supporting detritivorous fish production in the Amazon.

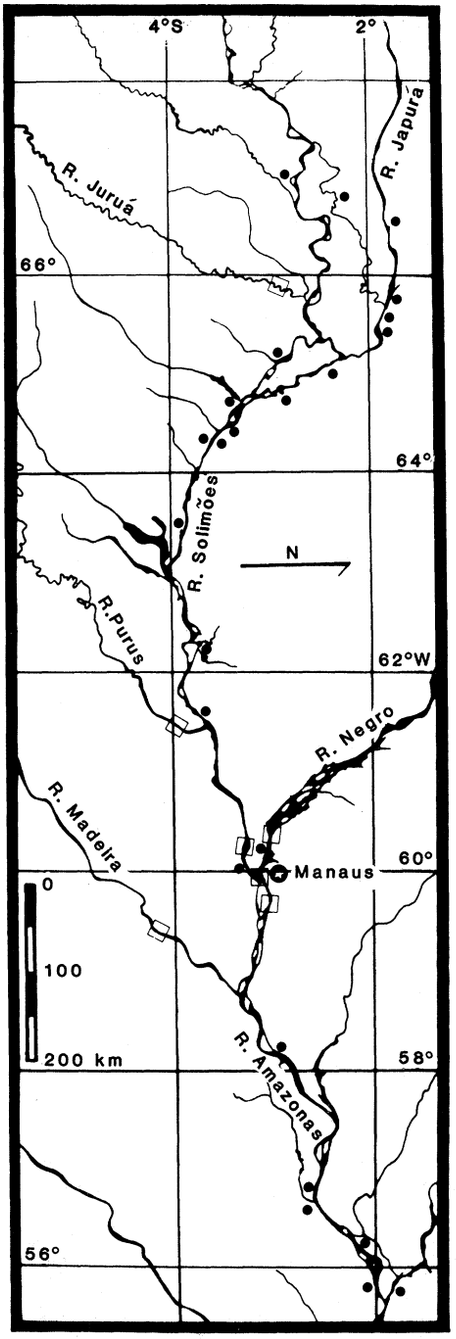

Fig. 1. Plant and POC (closed circles) and fish (open squares) collection sites in the central Amazon basin.

Plant (n = 147) and fish (n = 56) samples were collected during high- and low-water seasons in 1984 and 1985 (*10*). Suspended particulate organic carbon (POC), phytoplankton, periphyton, tree parts, and aquatic macrophytes were sampled at a variety of sites in the central Amazon basin (*11*) (Fig. 1). Fish samples were collected near Manaus and over most of the Amazon River system by the Manaus fishing fleet (Fig. 1). Preliminary processing of the samples was carried out at Instituto Nacional de Pesquisas da Amazônia (INPA) in Manaus (*12*), and isotope analyses were performed at Centro de Energia Nuclear na Agricultura (CENA) in Piracicaba (*13*).

The results of the isotope analyses are shown in Fig. 2. Some published values for Amazonian plants were also used in determining the means, ranges, and 95% confidence intervals for the plant end members (*14*).

The C_4 macrophytes (principally *Echinochloa polystachya* and *Paspalum repens*) were the heaviest (that is, most positive) plant end members with a mean $\delta^{13}C$ value of −12.9 per mil. The next heaviest plant groups were periphyton, C_3 macrophytes, tree wood, and tree seeds with mean $\delta^{13}C$ values of −26.8, −27.6, −27.6, and −28.3 per mil, respectively. A Student-Newman-Keuls (SNK) test indicated no significant difference after paired comparison of these four means ($P \geq 0.05$). Tree leaves were significantly lighter than tree wood and seeds (SNK test; $P < 0.05$) with a mean value of −30.0 per mil. The mean $\delta^{13}C$ value for POC samples containing more than 60% live phytoplankton was −33.3 per mil, and the mean for all POC samples was identical. The similarity of these two means suggests that POC in the lakes is derived primarily from phytoplankton. Since phytoplankton is the most negative plant end member, this result is unambiguous: the contribution of carbon from other plant end members must be relatively small.

The mean $\delta^{13}C$ values for the characiform detritivore species ranged between −32.5 to −34.2 per mil and were not significantly different (SNK test; $P \geq 0.05$). The siluriform catfishes were significantly heavier (SNK test; $P \leq 0.05$) with a mean $\delta^{13}C$ value of −26.0 per mil.

The $\delta^{13}C$ values for the catfishes fell in the middle of the plant end-member values. Since there are more than two plant end members, the carbon source for this fish group is uncertain. Catfishes could be receiving carbon primarily from periphyton or from an unknown mixture of plant end members. We can only conclude that neither phytoplankton nor C_4 macrophytes provide all the energy for this group. The results for the characiform species are easier to interpret since their $\delta^{13}C$ values fell close to the most negative end member, phytoplankton. In this case the result is unambiguous. The Characiformes must receive a large fraction of their carbon from phytoplankton and very little from the other plant groups (*15, 16*). The maximum contribution of tree leaves (the lightest alternative carbon source) to the carbon balance of *Semaprochilodus taeniurus*, and the other characiform species is 30 and 0%, respectively, whereas the contribution of C_4 macrophytes (the heaviest alternative carbon source) does not exceed 5% for any of the five species (*17*).

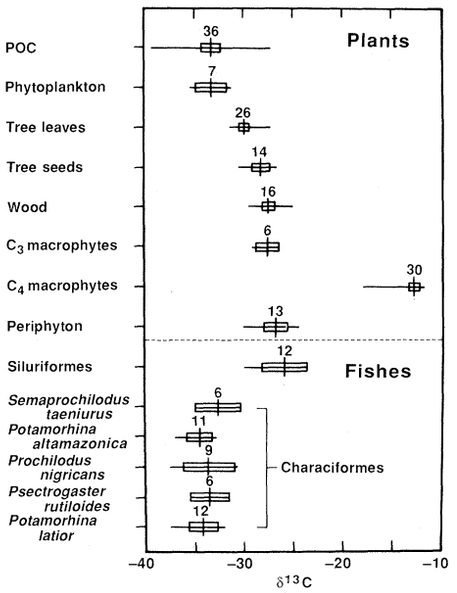

Fig. 2. Ranges (horizontal lines), means (vertical lines), and confidence intervals (rectangles) of $\delta^{13}C$ values (per mil) for POC, the seven autotrophic end members, Siluriformes, and five Characiformes fish species. Four Siluriformes fish species were pooled.

Although it was possible to identify the carbon source only for the characiform species, this result is important. The characiform detritivores are the most important group of food fish in the Amazon, accounting for over 99% (*2*) of the detritivorous fish yield and over 30% of the total fish harvest (*2, 3*). Thus, our results suggest that a large portion of the fish consumed in the Amazon is derived through food chains beginning with phytoplankton. Macrophytes, which have been proposed as a major energy source for detritus-based food chains, appear to be relatively unimportant.

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10. Nine fish and 37 plant species were sampled. The Characiformes fish species were *Semaprochilodus taeniurus, Prochilodus nigricans, Potamorhina latior, Potamorhina altamazonica*, and *Psectrogaster rutiloides*. The catfish species were *Pterygoplichthys multiradiatus, Loricariichthys platimetopon, Hoplosternum thoracatum*, and *Hemiancistrus scaphirhynchus*. Only adult fishes were sampled.
11. POC samples were collected exclusively in lakes. We collected POC samples to estimate phytoplankton $\delta^{13}C$ values. POC samples with more than 60% phytoplankton were classified as pure phytoplankton samples. POC samples were not collected in the river because phytoplankton biomass is typically very low [T. R. Fisher, *Comp. Biochem. Physiol.* **62A**, 1 (1979); R. C. Wissmar, J. E. Richey, R. F. Stallard, J. M. Edmond. *Ecology* **62**, 1622 (1981)]. The samples were concentrated by continuous centrifugation or by filtering whole-water samples through precombusted glass fiber filters (Gelman, GFF). Periphyton and macrophytes were collected in lakes and rivers. Tree parts were collected in lake drainage basins.
12. Plant samples were washed with tap water and a 1*N* HCl solution and then rinsed several times with deionized water. All samples were dried at 60°C. After drying, vascular plants were ground to a fine powder with a mortar and pestle. A skinless, boneless sample of muscle was taken from the upper body of each fish, dried, and ground as above.
13. Samples for isotopic analysis were prepared by combustion of ~10 mg of dry matter with CuO, in sealed, evacuated Pyrex tubes. The samples were burned overnight at 550°C and purified by passage through alcohol–dry ice traps. The purified sample was collected in a tube under liquid nitrogen, in a special vacuum line. A set of CENA's substandards [pure charcoal from C_3 and C_4 plants, calibrated to Pee Dee belemnite standard (PDB) limestone] was prepared with each sample batch; thus, no correction for oxygen was necessary, as its source was the same for the samples and the substandards. All carbon-13 values represent the mean of two separate analyses from the same sample (SEM = 0.25 $\delta^{13}C$ unit) and are reported relative to PDB limestone:

$$\delta^{13}C = \frac{(^{13}C/^{12}C_{sample} - {}^{13}C/^{12}C_{PDB})}{(^{13}C/^{12}C_{PDB})} \times 1000$$

Analyses were performed in a Micromass model 602-E mass spectrometer fitted with double inlet and double collector systems.
14. J. I. Hedges *et al.*, *Limnol. Oceanogr.*, in press. (Sixteen wood, 15 leaf, and 4 macrophyte values from their table 1 were included in the analysis.)
15. Since the organic matter encountered in stomach analyses is largely unrecognizable, it is not clear whether these fishes are obtaining their energy directly from phytoplankton by consuming their sedimented remains or indirectly by consuming the remains of organisms at higher trophic levels. The $\delta^{13}C$ results merely indicate that the carbon in these fishes is derived from a food chain of which the initial stage is phytoplankton.
16. One caveat should be mentioned here. The existence of a major unrecognized autotrophic energy source with a $\delta^{13}C$ value lower than phytoplankton would tend to invalidate this conclusion, making it impossible to determine the relative energy contribution of phytoplankton. The only autotrophs known to have values significantly lower than those that we report for phytoplankton are the chemolithotrophic bacteria [B. Fry and E. Sherr, *Contrib. Mar. Sci.* **27**, 15 (1984)]. The importance of autotrophic bacteria in Amazon waters is unknown. However, given their strict growth requirements, it is unlikely that they contribute significant quantities of organic carbon to the system.
17. The maximum carbon contribution from alternative plant sources was calculated as:

$$x = (\delta_3 - 1 - \delta_2)/(\delta_1 - \delta_2)$$

where x is the proportional contribution of the alternative carbon source, δ_2 and δ_3 are the upper limits of the 95% confidence intervals for the mean $\delta^{13}C$ values of phytoplankton and fish species, respectively, and δ_1 was the lower limit of the 95% confidence interval for the mean $\delta^{13}C$ value of the alternative carbon source. An increase of 1 delta unit per trophic level was assumed to be due to fractionation [M. De Niro and S. Epstein, *Geochim. Cosmochim. Acta* **42**, 495 (1978); G. H. Rau *et al.*, *Ecology* **64**, 1314 (1983)].

18. We thank L. Py-Daniel for identifying the siluriform species, C. C. Fernandes and T. Pimentel for help in collecting and preparing the samples, and N. A. Menezes for critically reviewing the manuscript. We also thank M. N. Goes, P. Vose, J. Richey, J. Hedges, P. Bayley, and P. Quay for valuable advice and support during the course of this study. This research was supported by NSF grant DEB 81-07522, Sudam-Polamazonia and International Atomic Energy Agency grant Amazonia I/BRA/0/010. Contribution 16 of the Carbon in the Amazon River Experiment Project and contribution 1 of the Amazonia I Project.

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Expression of Bovine 17α-Hydroxylase Cytochrome P-450 cDNA in Nonsteroidogenic (COS 1) Cells

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Cortisol production requires the activity of only 17α-hydroxylase, whereas the formation of sex steroids requires both 17α-hydroxylase and 17,20-lyase activities. Studies in reconstituted enzyme systems have suggested that a single steroid hydroxylase, 17α-hydroxylase cytochrome P-450 (P-450$_{17\alpha}$), catalyzes both activities. By expression of bovine adrenocortical P-450$_{17\alpha}$ in COS 1 (transformed monkey kidney) cells, which normally contain no detectable P-450$_{17\alpha}$, it has now been established in situ that a single polypeptide chain does catalyze both the 17α-hydroxylase and the 17,20-lyase reactions. This heterologous system supports 17α-hydroxylation of pregnenolone and progesterone with equal efficiency, but catalyzes about five times as much 17,20-lyase activity when 17α-hydroxypregnenolone is the substrate than when 17α-hydroxyprogesterone is the substrate. For these activities to be observed in COS 1 cells, newly synthesized apocytochrome P-450$_{17\alpha}$ must bind heme and insert into the endoplasmic reticulum such that endogenous cytochrome P-450 reductase can support hydroxylation. Thus, COS 1 cells are a useful system for expression and study of various forms of cytochrome P-450.

STUDIES OF MICROSOMAL 17α-HYdroxylase cytochrome P-450 (P-450$_{17\alpha}$), as with other eukaryotic cytochromes P-450, have been complicated by problems associated with purification from their membrane (microsomal or inner mitochondrial) environments and subsequent reconstitution of their activities in vitro. The similarities in physical and biochemical characteristics of different cytochromes P-450 (*1*) together with potential artifacts generated during solubilization, purification, and reconstitution procedures (*2*) have made it difficult to unambiguously assign one or more activities to an individual form of cytochrome P-450. Preparations of purified adrenocortical and testicular P-450$_{17\alpha}$ possess 17α-hydroxylase activity necessary for the production of cortisol, as well as 17,20-lyase activity required for sex steroid formation (*3*). However, the ratio of these activities changes during purification procedures (*3*) and in vivo under differing physiological conditions (*4*). Also, in humans, deficiencies associated with these activities have been reported for either 17α-hydroxylase activity (*5*) or 17,20-lyase activity (*6*). Thus, after identifying and characterizing a complementary DNA (cDNA) clone specific for bovine P-450$_{17\alpha}$ (*7*), we sought to clarify the uncertainty surrounding the reported dual activities associated with the P-450$_{17\alpha}$ polypeptide chain.

Our strategy was to analyze the activities

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