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Utilization of ascorbate-2-sulfate in fish

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Abstract

Although most vertebrate animals synthesize L-ascorbic acid (C_1), some animal species lack the ability to produce L-gulonolactone oxidase and are thus dependent upon a dietary source of vitamin C. Fish are unique among this latter group in that they store a chemically stable form of vitamin C and appear to metabolize this compound differently from other vitamin C-requiring organisms. Ascorbate-2-sulfate (C_2) contributes to total body stores of ascorbate, but the commonly used assays for ascorbate concentrations in tissues and body fluids do not generally measure C_2. An HPLC assay distinguishes between and measures both C_1 and C_2. Modification of the less exact but commonly used DNPH method can provide adequate data to estimate total vitamers C, C_1, and (by difference) C_2. Since vitamin C is a required component of feed for salmonids, catfish, eels, shrimp and carp, use of C_2 in feed formulation would provide a bioavailable form of ascorbate which is heat and water stable at pH 4-13.

Vitamin C seems to be ubiquitous in eucariotic cells of both plants and animals (Hodges and Baker 1973). Although ascorbic acid (C_1) has been identified chemically as having vitamin C activity only during the past 50 years, the effects of ascorbate deficiency have plagued man since ancient times. Scurvy (scorbutus) results from inadequate collagen formation – a process requiring C_1 as a cofactor. Non-human primates, guinea pigs, and numerous species of bats, birds, insects, and fish also are known to be scurvy susceptible. The common enzyme required for C_1 synthesis and lacking in these animals is L-gulonolactone oxidase. Because symptoms and signs of scurvy are not only dramatically obvious but also potentially lethal, possible roles for vitamin C other than those apparent in scurvy were largely ignored until recently.

The nearly universal appearance of this compound suggests a functional role, or roles, of major value to living organisms. Subclinical manifestations of ascorbate deficiency suggest numerous uses for C_1 by various cells. Although an underlying mechanism has yet to be elucidated, some of the identified functions link C_1 not only with collagen synthesis (Barnes and Kodicek 1972), but also with lipid and amino acid catabolism (Cooper 1961; Stone and Meister 1962; Weis 1975; Ginter 1979; Holloway and Rivers 1981), steroidogenesis (Kitabchi 1967; Datta and Sanyal 1978; Pintauro and Bergen 1982), catecholamine synthesis (Freidman and Kaufman 1966; Goldstein *et al.* 1968; Deana *et al.* 1975; Saner *et al.* 1975; Daniels *et al.* 1982), the cytochrome P_{450} system and detoxification (Liposchitz *et al.* 1971; Zannoni and Sato 1975;

Degkwitz *et al.* 1975; Zannoni *et al.* 1982; Tolbert 1985), and the immune system (Seigel and Morton 1977; Prinz *et al.* 1979; Anderson *et al.* 1980; Li and Lovell 1985).

L-ascorbic acid is a C_6 dibasic acid with a bifunctional enediol group built into a heterocyclic lactone ring with a molecular weight of 176.13. When dry, it is a stable, white crystalline substance, but is easily oxidized in neutral or alkaline solution and this oxidation is accelerated on exposure to heat, light, and traces of metals especially copper or iron. Its first oxidation to dehydroascorbic acid is reversible, but further hydrolysis to 2,3-diketogulonic acid is not.

In aqueous solution the bicyclic form of dehydroascorbate is always formed. It is rapidly hydrolyzed even if pure. In plant and animal tissues dehydroascorbate is either reduced to C_1 by dehydroascorbate reductase using glutathione as the reactant or is hydrolyzed to diketogulonate which appears to be rapidly metabolized to a variety of compounds including threonate and CO_2 (Bannerjee 1977, Tolbert and Ward 1982). Although tissue distribution of C_1 appears similar in guinea pigs and man, metabolic and catabolic pathways differ among animals. Rats and guinea pigs have an active C-1-decarboxylase which causes 20–70% of ascorbic acid to be excreted as CO_2 and intermediates. This occurs after the irreversible hydrolysis of dehydroascorbate to 2,3-diketogulonate probably regulated by dehydroascorbic acid lactonase. The activity of this enzyme is not functional or is present at very low levels in primates and, apparently, fish. Tolbert (1979) suggested this to be compensation to conserve ascorbate. Ginter (1979) reported high levels of this lactonase in the liver of ascorbate-synthesizing animals and low levels in primates which would explain why the half-life of vitamin C is several times longer in man and fish than in animals readily hydrolyzing dehydroascorbate. Redox properties of the ascorbate/dehydroascorbate system provide unusual biological protective properties against free radical damage. Halliwell (1982) suggests the role of this system in chloroplasts may be to protect against H_2O_2, HO_2, lipid peroxides, superoxide, and the hydroxyl radical. Similar functions have been suggested in insects, mammals, and foods (Kramer and Seib 1982). The dehydroascorbate/ascorbate ratio present at any given time appears to be associated with the metabolic health of the cells and may be related to cell division as a growth regulator (Tolbert and Ward 1982). In healthy, normal tissue of primates and fish the ratio remains low due to the reduction to C_1. This serves to conserve ascorbate and has been suggested as the existing mechanism which maintains C_1 levels. Yamamoto *et al.* (1977) found active dehydroascorbate reductase in trout liver and kidney tissues. Because of its non-ionic character and lipid solubility dehydroascorbate is suggested to be a form in which ascorbate is transported across membranes (Bannerjee 1977).

Fish require dietary ascorbate, but dietary deficiencies were not often reported until the advent of aquaculture which required manufactured or artificial feed (Halver *et al.* 1969). McCay and Tunison (1934) observed scoliosis and lordosis (broken back syndrome) in brook trout fed formalin-preserved meat. Since these signs took a year to develop they were not then recognized as being related to vitamin C deficiency. Poston (1967) later correlated this syndrome with spinal deformation induced in brook trout by a diet low in vitamin C. Trout and salmon were added to the list of scurvy susceptible animals (Kitamura *et al.* 1965; Halver *et al.* 1969). More recently other species of fish requiring dietary vitamin C have been identified.

Deficiency signs in fish generally relate to impaired collagen formation which become obvious after 20–30 weeks without dietary ascorbate. Sato *et al.* (1978) demonstrated that if hydroxylation of proline is decreased more than 10%, impaired collagen formation in rainbow trout occurred before signs of scurvy appeared. They suggested that the hydroxyproline/proline ratio is a useful indicator of vitamin C deficiency at an early stage. In acute scurvy, spinal anomalies (scoliosis, lordosis) are accompanied by internal hemorrhage, resorbed opercles, abnormal support cartilage in gills, spine, and fins with hyperplasia of jaw and snout (Halver *et al.* 1969). Wounds fail to heal (Halver 1972). Other signs are anemia, anorexia and lethargy (Hilton *et al.* 1978; Agrawal and Mahajan 1980),

ascites and hemorrhagic exophthalmia (Poston 1967), low serum triiodo-thryonine levels (Leatherland *et al.* 1980; Agrawal and Mahajan 1981), and high levels of plasma triglycerides and cholesterol (John *et al.* 1979).

Recommended supplemental levels to provide vitamin C requirements for fish were determined by noting the levels in purified diets necessary to prevent or cure signs of scurvy, Table 1 indicates the recommended dietary supplementation levels for several species. However, Halver (1972) demonstrated that six to eight times the maintenance levels were needed for most rapid wound repair. Fish also utilize ascorbate at higher rates when subjected to other stressors: rapid growth, handling, crowding, temperature change, infection, and pollutants in feed or the environment (Halver 1969, 1972).

Actual requirements are related to stress, growth rate, age and size of animal, and other nutrients in the diet. Adequate wound repair increases requirements several times. A protective role for ascorbate in fish exposed to environmental pollutants or infectious agents has been demonstrated (Eichbaum *et al.* 1977); Agrawal *et al.* 1978). Durve and Lovell (1982) found increased resistance to bacterial infection of channel catfish at 23°C with 150 mm/kg feed. Less supplementation was needed for resistance at 33°C when the natural resistance mechanisms of the fish were functional. Several studies suggest that there is an increased tolerance by fish to environmental pollutants when ascorbate stores are high. Effects of organochlorine pesticides (Agrawal *et al.* 1978), strychnine poisoning (Eichbaum *et al.* 1977), and toxaphene contamination of feed (Mayer *et al.* 1978) all have a diminished effect when ascorbate nutriture is increased. Among the pollution-associated diseases and abnormalities of fish are stress-invoked latent infections and spinal distortions (Sinderman 1979). We believe these effects directly relate to depleted ascorbate stores.

In aquaculture, vitamin C is a required component of feed for salmonids, catfish, eels, shrimp, and carp. Artificial fish feeds present a problem in ascorbate nutriture as unstable C_1 is readily lost during processing and storage due to oxidation and hydrolysis upon exposure to heat, oxygen, neutral or high pH, and/or traces of certain metals. Pelleting and extrusion of feeds adds moisture and heat resulting in destruction of vitamin C. Four to five times a reasonable dietary requirement of C_1 is added in feed production to allow for this destruction. To increase stability, alternate methods of adding ascorbic acid to fish feeds have been investigated. Hoffmann-LaRoche produces an ethyl cellulose coated ascorbic acid. Lovell and Lim (1978) reported significantly increased vitamin C retention with both pelleted and extruded feeds containing a coated ascorbic acid as compared to crystalline C_1. Although storage tests indicated better retention of the coated ascorbate at both room temperature and at 37°C, losses were still significant. An ascorbic acid-fat suspension sprayed on the outside of extruded pellets showed greater stability than coated ascorbic acid added before pelleting, but a nearly 50% decrease occurred after 60 days at room temperature (Krautmann and Frye 1981). Hilton and co-workers (1977) found coated ascorbic acid in fish feed mash to be depleted by 26% vs. 47% loss from the powdered form. Further cold pelleting and drying resulted in cumulative losses of nearly 90% with both forms of vitamin C. After 6 weeks of storage at room temperature, essentially all vitamin C had been destroyed, but was retained when stored at −20°C for 16 weeks. They suggested that moisture and alkalinity increase ascorbic acid instability. Although exponential losses of vitamin C occurred with increasing supplemental amounts, the coated product permitted significant retention longer than the powdered. Leaching losses during feeding measured 10% from crumbled pellets in 10 seconds.

L-ascorbyl-6-palmitate, L-ascorbate-2-sulfate, and L-ascorbate-2-phosphate were compared to L-ascorbic acid by Brandt *et al.* (1985) who sprayed these compounds (in a 1% gelatinized starch solution) onto feeds for channel catfish. They concluded that equimolar amounts of all of these derivatives provided adequate vitamin C activity. Murai *et al.* (1978) compared the effects of purified diets with supplemental C_1 or equimolar levels of ethocel coated C_1 or ascorbate-2-sulfate fed to channel catfish. A dietary level of 25 mg/kg of all three forms prevented signs of scurvy.

The desirability of finding a stable antiscorbutic form of vitamin C is obvious. Isoascorbic acid will prevent scurvy in salmonids only as long as a dietary source is present. It is not converted into a storage form as is C_1 and scurvy occurs rapidly with dietary deletion of this form of vitamin C (Halver *et al.* 1975). Sulfated ascorbate was isolated and purified from the undeveloped cysts of brine shrimp by Mead and Finamore (1969) who speculated that this may be a storage form of vitamin C. The compound was stable in aqueous solutions under a variety of conditions but was hydrolyzed to C_1 at $pH < 3$.

Ascorbate-2-sulfate (C_2) has been produced as the dipotassium salt by Paul A. Seib at Kansas State University (Quadri *et al.* 1973). It is stable in both air and aqueous environments at pH 3–13. It is stable to most heat treatments at these pH's and does not serve as a substrate for L-ascorbic acid oxidase (Quadri *et al.* 1975). Our assay for C_2 involves prolonged boiling in strong acid to hydrolyze C_2 to C_1 for analysis (Tucker 1983). Seib *et al.* (1974) demonstrated heat and water stability of C_2. Stuber and Tolbert (1978) characterized dipotassium ascorbate-2-sulfate as stable in aqueous solution for weeks, stable to mild oxidation and basic hydrolysis, stable to atmospheric oxidation and hydrolysis at pH 4–13.

Ascorbate-2-sulfate is antiscorbutic for fish and radioautographs of ingested labelled C_2 in salmonids show its tissue deposition to be the same as labelled C_1 (Halver *et al.* 1975; Tsujimura *et al.* 1978). While absorption of C_2 is minimal (3–5%) in primates, it appears to be more efficient in fish since orally ingested C_2 prevents scurvy in trout and channel catfish (Halver *et al.* 1975, Murai *et al.* 1978). In most mammals, parental administration of C_2 is rapidly excreted indicating little or no transport into tissues (Omaye *et al.* 1982).

Brine shrimp, a traditional feed for aquarium fish as well as prawn culture, provide vitamin C at least partially as C_2. The C_2 present in cysts is apparently converted to C_1 for use during embryonic development and larval emergence (Golub and Finamore 1972). Several species of fish larvae have been reared on dry flaked diets using C_2 as the sole vitamin C source (Tucker *et al.* 1980). Equimolar amounts of C_1, purified, or technical grade C_2 in diets fed to fingerling rainbow trout (RBT), under the same conditions used for C_1 quantitative studies (Halver *et al.* 1975) resulted in no differences in growth, diet conversion or mortality between treatments after 24 weeks. No clinical or histological deficiency signs were found. HPLC analysis showed both C_1 and C_2 in whole body homogenates (Halver *et al.* 1983).

Ascorbate-2-sulfate (C_2) has been identified in animal tissues, both aquatic and terrestrial (Mead and Finamore 1969; Mumma and Verlangieri 1972, Halver *et al.* 1983). It is antiscorbutic for all fish tested and is utilized on an equimolar basis with C_1 by salmonids (Halver *et al.* 1975). Murai *et al.* (1978) found a four-fold increase in supplemental C_2, as compared with C_1, was required for maximal growth and feed efficiency by channel catfish and suggested an enzyme (sulfatase) limiting theory. It may well be that warm water omnivores do not induce this enzyme activity when dietary C_1 is plentiful. Additional studies of C_2 metabolism in other fish species are needed as there is probably species variation. Ascorbic acid-sulfotransferase catalyzes the synthesis of C_2 from C_1 (Farooqui 1980, Benitez and Halver 1982. Baker *et al.* (1975) reported conversion of C_1 to C_2 in primates. Monkeys given labelled C_1 and C_2 excreted interchangeably labelled metabolites. Tissues of rats also contain C_2, the concentration apparently depending on the size of the ascorbate pool. Mumma and Verlangieri (1972) demonstrated *in vivo* sulfation of C_1. Hornig and co-workers (1973) observed orally ingested C_1 to be partially converted to C_2 in an intestinal site in rats with enterohepatic and lymph circulation. That ascorbate is present in tissues in some type of bound form has been reported for many years (Holtz and Walter 1948; Sumerwell and Sealock 1952). The ^{35}S of labelled C_2 injected into rats was subsequently found in a non-soluble form in the cell membrane fraction of brain, liver, and kidney (Campeau and March 1972).

Although the presence of C_2 as a urinary metabolite has been acknowledged for some time, postulations of a C_2 body pool, via sulfation of C_1 for tissue storage, have only recently become

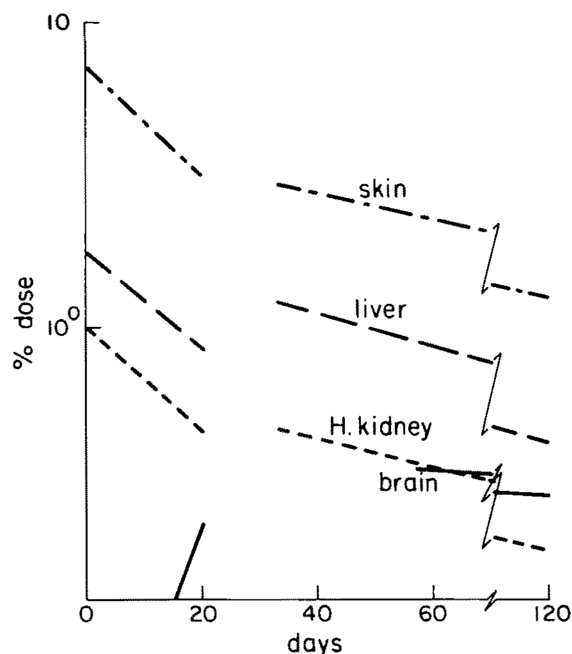


Fig. 1. ^{14}C in whole organs. Best fitting linear regression lines showing change in slope with decrease in vitamin C intake after 20 days. (H. kidney = head kidney). (By permission of J. Nutr.)

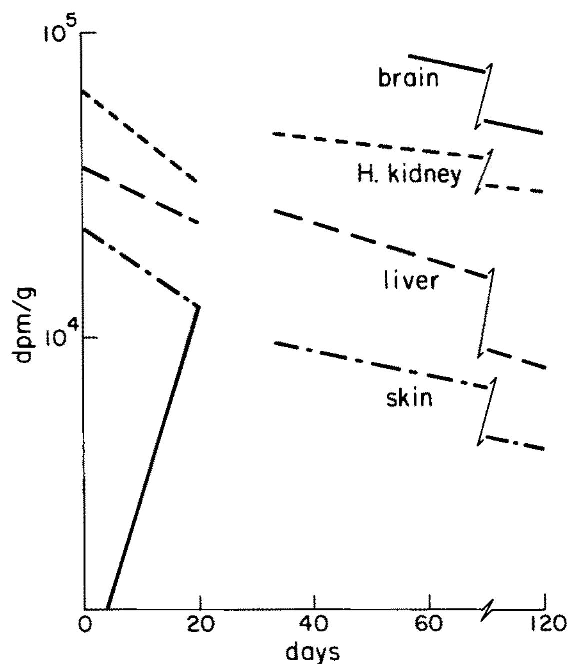


Fig. 2. ^{14}C in tissues. Best-fitting linear regression lines showing change in slope with decrease in vitamin C intake after day 20. (By permission J. Nutr.)

generally accepted and then only in fish (Baker *et al.* 1971, Tolbert *et al.* 1975) Omaye *et al.* (1982) questioned the significance of C_2 as a metabolite and correlated the high levels found in aquatic organisms with its stability in aqueous environments lending itself to a role as a storage form.

We recently reported on studies following a single ingested dose of 1-^{14}C-ascorbic acid in RBT (250 g) for 4 months (Tucker and Halver 1984a, b). The fish had been prefed for 3 months a C_1-depleted diet (25 mg/kg dry diet). Following the ^{14}C intake the fish were fed the normal diet (100 mg C_1/kg) twelve times/week for 1 month then three times/week for an additional 3 months. Excretion data as well as periodic sampling of selected tissues provided information for estimation of half-life, tissue turnover times, and body pool size. At this dietary C_1 level, the calculated $t_{1/2}$ for the ^{14}C was 42 days with a small, but significant, amount present in the urine after 4 months. We believe the 1-^{14}C-ascorbic acid was fixed in tissues with slow turnover rates as C_2. Semi-log plots of ^{14}C activity of each selected organ vs. days following the ^{14}C ingestion indicated a change in the slope of the regression line with the change in feed intake (Figs. 1 and 2). These changes are significant ($p < .001$) for skin and brain. The rate of ^{14}C release from the tissues was inversely proportional to dietary intake of C_1 (Table 2).

Table 1. Recommended ascorbic acid supplementation levels

Species	mg C_1/kg dry diet
Rainbow Trout	100
Coho Salmon	200
Catfish	50
Carp	50
Shrimp	100

Table 2. ^{14}C Half-life in organs[a] (Days)

Feeding[b]	Liver	Anterior Kidney	Skin	Brain
12 ×/wk	28	17	18	–
3 ×/wk	66	79	102	142

[a] by linear regression from dpm/g data
[b] 100 mg C_1/kg dry diet

When excess dietary C_1 is available to the fish it is readily converted to C_2 for storage. Total body C_2 was highest in the control fish maintained only on the standard diet. The skin, with its heavy collagen area, appears to be a storage pool which contains about 5% of the total body ascorbate. Concentrations of both forms of vitamin C in the tissues reflected dietary intake of C_1. Hornig and Hartmann (1982) reported a high liver turnover of ascorbate in excess of total body turnover and suggested a multiple circulation through the liver. In our study the rate of release of ^{14}C from the liver was not altered significantly (Fig. 1) with the decreased intake of dietary C_1 suggesting that a source of stored ascorbate is available for maintenance of liver levels. It appears that the skin provides an ascorbate reservoir for metabolizing organs.

Body pool estimates reflect dietary C_1 intake. Based upon isotope dilution analysis, the C_1 concentration is not significantly different at either feeding level (Table 3). The total ascorbate (C_T) is, however, directly related to C_1 intake. We found a 34% decrease after changing to the 3×/week feeding indicating a conversion of stored C_2 to maintain the C_1 levels.

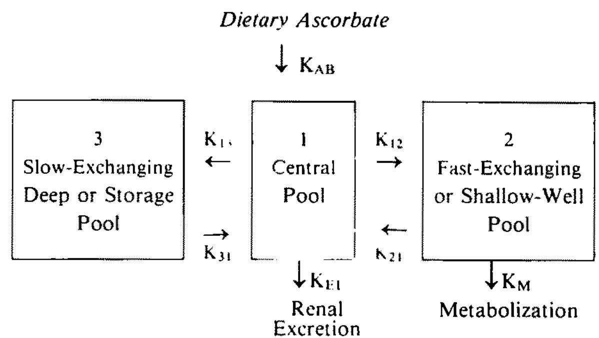
Ascorbate-2-sulfate sulfohydrolase (ascorbate sulfatase) and arylsulfatase were copurified and characterized from liver of a arylsulfatase were copurified and characterized from liver of a marine gastropod and from bovine liver (Hatanaka *et al.* 1975, Carlson *et al.* (1977). Benitez and Halver (1982) proposed ascorbate-2-sulfate sulfohydrolase (C_2 sulfatase), which they purified from rainbow trout liver, to be the modulator of cellular levels of C_1 in fish. The activity of this enzyme is controlled by feedback inhibition by C_1. The C_2 stores are

Table 3.

	Body pool size[a]		
Feeding[b]	C_1	C_2	C_T
12 ×/wk	28.9	63.9	92.8
3 ×/wk	27.7	33.8	61.5

[a] by I.D.A.
[b] 100 mg C/kg dry feed

Kallner's 3 Compartment Model



hydrolyzed to C_1 to maintain adequate levels for metabolic functions. Liver levels of C_1 in fish seem to stabilize for some time even under continuing vitamin C deprivation (Hilton *et al.* 1979). Since ascorbate sulfatase is a glycoprotein it could be membrane associated where C_2 hydrolysis could occur (Carlson *et al.* 1977).

The calculated half-life of ascorbate varies with nutritional status and indicates that the kinetic behavior of vitamin C is very complicated. Kallner *et al.* (1979) proposed a three compartment model to describe the ascorbate body pool in man (Fig. 3). The overall half-life of C_1 was found to decrease with increasing total turnover. Tillotson and O'Connor (1981) concluded that several ascorbate compartments with different exchange rates are present in monkeys and probably in man as previously suggested. Not all the compartments would be measured in a single study with a single-dose technique. The very slow-exchanging compartment would be measured only with minimal ascorbate supplementation with adequate time for equilibration in the tissues.

We propose a similar situation exists for RBT: multi-compartments with fast and slow exchanging pools and a C_2 storage pool. The C_2 storage seems to be associated with both the slow and fast-exchanging C_1 compartments. This pool is large compared to the size of the C_1 pool. The exchange rate between C_1 and C_2 is rapid compared with the half-life of the labelled C_1. Dietary excesses of C_1 stimulate rapid conversion in the cells to C_2 which the cell can call upon for a quick source of C_1. This exchange is modulated by an enzyme system re-

sponsive to dietary levels of C_1. This hypothesis can be applied to explain data reported by Tsujimua *et al.* (1978). They fed newborn RBT C_1, C_2, or ascorbate-free diets and measured C_1 in liver and muscle and hydroxyproline in bone after 119 days. Hydroxyproline levels were essentially the same in both the C_1 and C_2 fed groups while the tissue C_1 levels of the C_2 fed group were only 65–70% of those of the C_1 fed group.

In our study, the long turnover times in the selected tissues after the feeding change would be representative of the slow-exchanging compartment with the fast-exchanging pool essentially empty. The control fish illustrated measurement of the fast-exchanging compartment. The various selected tissues appear to store ascorbate differently. Liver seems to be the site where circulating C_1 levels are maintained even when dietary C_1 is decreased. Skin had the highest ^{14}C uptake with subsequent rapid release, but C_1 and C_2 levels, as measured by DNPH, indicate a stable pool with C_1 increasing slightly after the feeding change with less stockpiling of C_2. In contrast, the anterior kidney had no significant C_2 when the fish consumed high levels of C_1, but showed similar C_1 and C_2 patterns before and after the feeding change from moderate to low levels of C_1 (Table 4).

The identity of the molecules into which ^{14}C was incorporated appeared to be mainly C_1 and C_2 as indicated by HPLC separation and subsequent counting of the collected fractions. The decrease in labelled C_1 in the metabolizing tissues and the decrease in labelled C_2 in the skin and carcass with the dietary intake change is further evidence to support the hypothesis of a C_2 storage pool which is used to maintain circulating levels of C_1. That the labelled molecules were still ascorbate rather than metabolites is further supported by previous work (Halver *et al.* 1975; Tolbert *et al.* 1975).

Ascorbate-2-sulfate may contribute to total body stores of ascorbate, but the commonly used assays for ascorbate concentations in tissues and body fluids do not generally measure C_2. Therefore, estimates of the ascorbate body pool size in fish should include both C_1 and C_2 (C_{Total}). Unmeasured C_2 in practical diets may well explain the "appearance" of piscine ascorbate synthesis discussed by several authors. We have published a modified DNPH method for determination of total C vitamers, C_1, and C_2 (by difference) (Tucker 1983). The increasing availability of HPLC analysis provides far greater accuracy than has been possible with "wet chemistry" methods. Bigler and Kelly's (1975) method using a strong anion exchanger with an ultraviolet detector gives a good separation of C_1 and C_2.

We concluded that C_2 is a major storage form of vitamin C in RBT; the kinetics of ascorbate metabolism and body pool size are influenced by dietary intake levels and the stressors present; RBT require and utilize ascorbic acid similarly to man and other animals; RBT are a better test animal for vitamin C studies than guinea pigs because of similarities to humans in body pool size, turnover time and half-life (Table 5); the body pool has two or more compartments which include a store of C_2. These

Table 4.

Feeding[b]		Ascorbate in tissues[a] (μg/kg)		
		Liver	Skin	Anterior Kidney
12 ×/wk	C_1	67.5 ± 9.9	19.8 ± 3.7	51.0 ± 9.0
	C_T	311.7 ± 48.0	71.3 ± 12.0	125.8 ± 29.4
3 ×/wk	C_1	46.7 ± 8.9	35.0 ± 12.1	49.7 ± 18.6
	C_T	163.0 ± 7.8	56.0 ± 2.6	104.7 ± 35.2

$C_T = C_1 + C_2$
[a] = by DNPH analysis
[b] = prefed @25 mg C/kg dry diet

Table 5. Turnover time and body pool comparisons

	Man	Rainbow Trout	Guinea Pig	Rat
Diet. req.	60–120 mg/da	3–5 mg/kg b.w. 100 mg/kg diet	5–30 mg/kg b.w. 300 mg/kg diet	0
Turnover (days)	20	30	4	5
B. pool (mg/kg b.w.)	22	20–30	54	107
% B. pool catabolized in 24hrs	3	4–5	17–20	24–29
Days to signs of deficiency	90–160	140–160	20	–

two forms of vitamin C are readily interconverted in metabolizing tissues to and from reduced L-ascorbic acid.

Perhaps the finding of most practical importance is the establishment of C_2 as a major and usable storage form of ascorbate in RBT (Tucker and Halver 1984a, b). This stable and reliable vitamin C source, incorporated into fish feed, should improve fish growth and fish health in aquaculture. It is functional in special feeds, lending itself to making powders, flakes, micropellets, and semi-moist feeds. Stability during heat processing, as well as deletion of the need to add great excesses, as is necessary when formulating with C_1, make ascorbate-2-sulfate an especially attractive component of aquaculture rations. The disodium salt of ascorbate-2-sulfate is now being produced by Nikko Chemicals Co. Ltd., Tokyo, and is being incorporated into some fish feeds.

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