If microfilaments function in dispersal of pigment granules, it is obligatory to reexamine the function of microtubules in translocation of pigment granules. Marsland's observation (*10*) that pigment granules were dispersed by high hydrostatic pressure suggested that a gel-to-sol transition occurred in darkening. Malawista (*11*) found that prior treatment of frog skin with colchicine enhanced the darkening effect of MSH and inhibited the lightening that follows removal of MSH; he proposed that colchicine affected cytoplasmic viscosity in frog melanocytes. Colchicine also disrupts microtubules (*12*) and decreases the rate of pigment aggregation in melanocytes in *Fundulus heteroclitus* scales in response to epinephrine (*13*). The darkening action of colchicine can be attributed either to interference with microtubular structure or with the sol-to-gel equilibrium.

Studies of *F. heteroclitus* melanocytes with the electron microscope (*14, 15*) indicate that pigment granules move through channels surrounded by microtubules. Because microtubules are arranged parallel to the long axis of the melanocytic process, it has been suggested that microtubules function as a cytoskeleton or perhaps provide tracks for the granules (*13, 14*). Wise (*16*) did not find such an orderly arrangement of microtubules in his electron microscopic examination of melanocytes of *Xenopus laevis* and *Hyla regilla.*

If colchicine inhibits pigment granule aggregation by disrupting microtubules and cytochalasin B inhibits pigment granule dispersion by disrupting microfilaments, then the following mechanism for pigment granule translocation can be tendered. Dispersion of pigment granules is effected by microfilaments; when microfilaments are destroyed, pigment granules move centripetally (*17*). Intact microtubules are required for pigment granule aggregation but not for dispersion. This model is supported by the inhibition by vinblastine of the lightening effect of cytochalasin on frog skin darkened with melanocyte-stimulating hormone (*18*).

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18. J. McGuire, unpublished observations.
19. Frog skins were fixed for electron microscopy at 30°C with 3 percent glutaraldehyde in 0.1*M* sodium cacodylate buffer, *p*H 7.2, containing 0.1 percent $CaCl_2$. Skins were refixed in osmium tetroxide and embedded in epoxy resin by the method of A. R. Spurr, *J. Ultrastruct. Res.* **26**, 31 (1969). Thin sections were counterstained with uranyl acetate and lead citrate.
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Tetraploid Origin of the Karyotype of Catostomid Fishes

Abstract. *Catostomid fishes appear to have* $2n(\rightarrow 4n?) \simeq 100$ *chromosomes. The Cyprinidae, from which catostomids probably diverged before the Eocene, usually have* $2n = 48$ *or 50 chromosomes. Preliminary cytophotometric measurements indicate an approximate doubling of DNA content of cells among catostomids.*

The fish family Catostomidae (suckers) comprises 12 genera and about 60 species of fishes confined to freshwaters of North America and eastern Asia. The jaw mechanism and other skeletal features indicate that the group evolved from an ancestor similar to the minnows, family Cyprinidae (*1*). The evolutionary divergence might have occurred in Asia (*2*) where cyprinids are especially diverse. Asian fossils of cyprinids from the Paleocene and catostomids from the Eocene (*3*) indicate separate evolutionary histories spanning at least 50 million years. All but two genera and species of catostomids are endemic to North America, where their fossil record also extends back to the Eocene. Cyprinids are unknown in North American fossil deposits earlier than the Oligocene, but the family is represented in North America by about 230 Recent species (*4*).

The chromosome number of cyprinids

Table 1. Diploid chromosome counts in catostomid fishes. Numerous cells with more than 85 chromosomes have been examined from each species. The tabulation of relatively countable spreads includes variability due to overlap and breakage of chromosomes. Columns indicate the number of fish in which dividing cells were examined and the frequency distribution of counts.

Taxon and locality	Fish (*N*)	Diploid cells (*N*)			
		<96	96–98	99–100	101–102
Ictiobus sp., Boone Co., Mo.	4	3	13	21	
Carpiodes carpio, Douglas Co., Kan.	3	1	12	19	
Cycleptus elongatus, Boone Co., Mo.	1		1	1	
Minytrema melanops, Monroe Co., Mich.	1	4			
Erimyzon sucetta, Van Buren Co., Mich.	3	2	2	4	
Hypentelium nigricans, Washtenaw Co., Mich.	6	2	8	9	3
Moxostoma duquesnei, Washtenaw Co., Mich.	2	3	2	1	
M. erythrurum, Washtenaw Co., Mich.	2	4	9	9	
M. macrolepidotum, Washtenaw Co., Mich.	2		2	2	
Catostomus commersoni, Washtenaw Co., Mich.	2	1	9		
C. latipinnis, Grand Co., Utah	3		2	4	
C. discobolus, Grand Co., Utah; Moffat Co., Colo.	3	1	4	4	
C. clarki, Coconino Co., Ariz.	3	2	4	1	
Hybrid *C. clarki* × *C. platyrhynchus*, Washington Co., Utah	2	1	4	3	

and most other fishes is usually $2n = 48$ or 50 chromosomes (*5*). A recent survey of 60 species representing 74 percent of North American cyprinid genera indicates uniformity in the occurrence of $2n = 50$ chromosomes (*6*). By contrast, a survey of 14 species of 8 genera of catostomids suggests near uniformity in the occurrence of $2n(\rightarrow 4n?) \simeq 100$ chromosomes (Table 1). Karyotypes (Fig. 1) are based on squashes of gill epithelium of juvenile specimens in which metaphase had been arrested by prior injection (2 to 4 hours) of 0.05 percent Velban (*7*). Meiotic figures (testicular) observed in one species (*Erimyzon sucetta*) displayed $n = 50$ and $2n = 100$ chromosomes, with no indication of quadrivalents.

Catostomid cells are somewhat larger than corresponding cells of cyprinids, and preliminary cytophotometric comparison by the two-wavelength method (*8*) indicates twice as much DNA in the catostomid cells. Ten absorption measurements of five cells from each of two species showed a range of 1.09 to 1.88 ($\bar{X}$=1.57) arbitrary units of DNA in the cyprinid *Clinostomus elongatus* and 2.48 to 3.17 ($\bar{X}$=3.08) units in *Catostomus commersoni*.

No cyprinids or catostomids show $2n$ chromosome numbers intermediate between about 50 and 100. However, three Asian cyprinids similar in size and habits to catostomids possess double the chromosome number and up to two times the DNA of other cyprinids. *Cyprinus carpio* (carp), *Carassius auratus* (goldfish), and *Barbus barbus* have $2n(\rightarrow 4n?) = 100$ to 104 chromosomes with about 50 percent of the DNA content of placental mammals. Comparative measurements of six other species of Asian cyprinids in five genera showed $2n = 48$ to 52 chromosomes with about 20 to 38 percent of the DNA content of placental mammals (*9*).

The apparent doubling of the chromosome number and DNA content of cells of catostomid fishes and the lack of intermediate numbers suggests that the catostomid karyotype evolved by tetraploidy from a cyprinid-like ancestor with $2n = 50$ chromosomes. (Unfortunately the primitive catostomid genus *Myxocyprinus*, restricted to China, is unavailable for karyotyping.) Carp, goldfish, and several other possibly tetraploid Asian cypriniform species (*10*) were similarly derived from ancestral cyprinids, and appear to be, along with salmonids (*11*), catostomids, and several kinds of frogs (*12*), the only

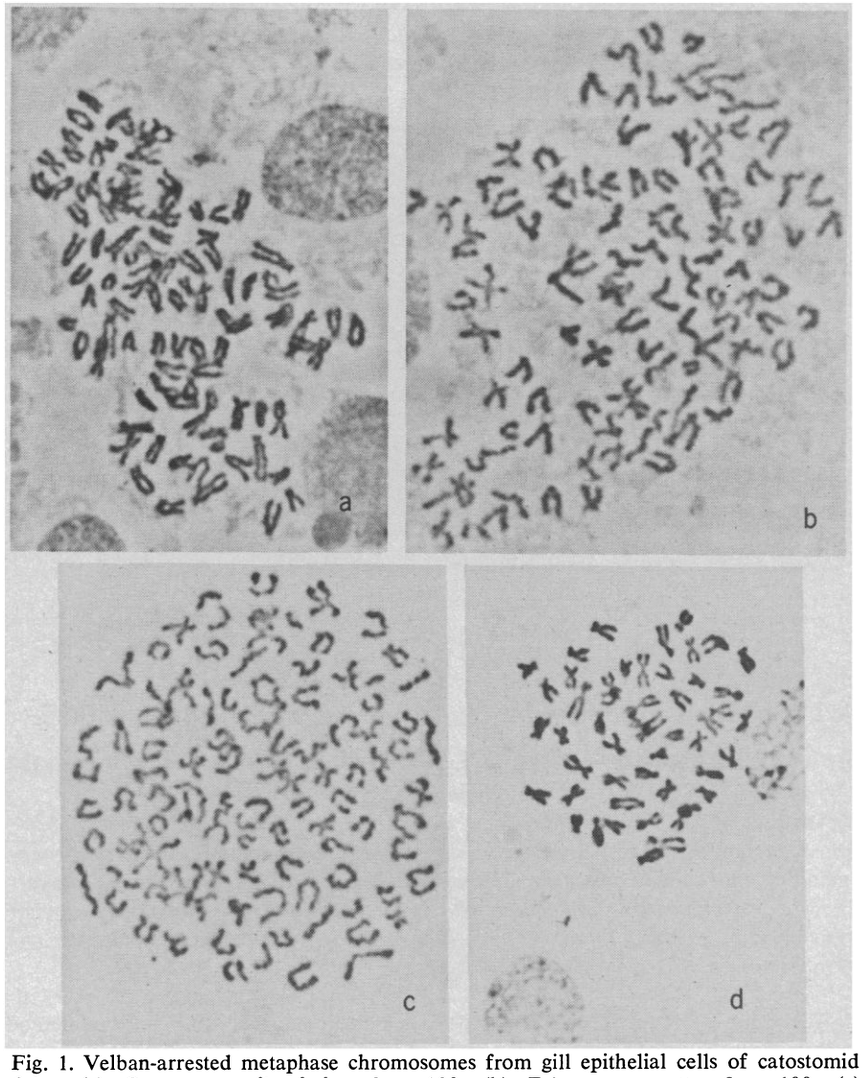

Fig. 1. Velban-arrested metaphase chromosomes from gill epithelial cells of catostomid fishes (a) *Catostomus discobolus*, $2n = 100$; (b) *Erimyzon sucetta*, $2n = 100$; (c) *Moxostoma duquesnei*, $2n = 100$; and (d) the cyprinid fish *Ptychocheilus lucius*, $2n = 50$. (× 1220)

known examples of naturally occurring, bisexual vertebrates with polyploid karyotypes. Other examples of polyploidy in vertebrates involve parthenogenetic or gynogenetic reproduction and probable hybrid origin (*13*). Examination of the gonads of samples of many species of catostomids, including those listed in Table 1, revealed the occurrence of both sexes in apparently normal ratios. This is also true for carp and goldfish, except that recently discovered examples of higher ploidy in goldfish near Tokyo included a population with $2n(\rightarrow 6n) = 154$ or 156 and $2n(8\rightarrow n?) = 208$ chromosomes that consists predominantly of females (*14*).

More active work will probably reveal other examples of polyploidy in vertebrates lacking well differentiated sex chromosomes. The mode of origin of cypriniform polyploids is unknown, but hybridization is unusually common in the group (*15*). The selective advantage attending tetraploidy is not clear. Increase in number of genes, recombination, and especially heterozygosity (*9*) might have contributed to more vigorous growth and adaptability. Catostomids and the tetraploid cyprinids are larger and have longer life, faster growth, and greater ecological adaptability than the majority of cyprinids. The carp is probably the world's most adaptable fish species (*4*), and the Catostomidae include some of the most ecologically labile North American fishes. In particular, *Catostomus* inhabits a broader geographical and ecological range in North America than any other freshwater fish genus.

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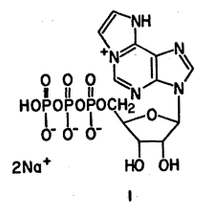
A Fluorescent Modification of Adenosine Triphosphate with Activity in Enzyme Systems: 1,N^6-Ethenoadenosine Triphosphate

Abstract. *A new, highly fluorescent adenosine triphophate (ATP) analog, 1,*N^6*-ethenoadenosine triphosphate, has been synthesized. Its fluorescence properties, including the long fluorescence lifetime and the possibility of detection at very low concentrations, in conjunction with its activity in the representative enzyme systems here reported, make it a valuable probe of enzymic mechanism and structure.*

Adenosine triphosphate (ATP) is the universal stoichiometric coupling agent between metabolic sequences, in addition to acting as a regulatory modifier of these sequences (*1*). Further information would be obtained on many biochemical problems of mechanism and conformation if the interactions of ATP with macromolecules could be examined more closely. The possible utility of fluorescent molecules in this connection has been mentioned by Stryer (*2*), and the power of fluorescence techniques in nucleic acid systems has been amply demonstrated (*3*). We have synthesized a highly fluorescent analog of ATP, 1,N^6-ethenoadenosine triphosphate (**1**, shown as the disodium salt), abbreviated "εATP" (*4, 5*), by reaction of ATP with chloroacetaldehyde according to the method described earlier for simpler adenine derivatives (*5*), and we are able to describe the first benefits to be derived from its fluorescence properties and behavior with several enzyme systems.

The fluorescence emission spectrum of εATP in neutral or acidic solution shows a maximum near 410 nm upon excitation at either 275 nm (the absorption maximum) or 300 nm. That the longer wavelength absorption band is responsible for the fluorescence is shown by the excitation spectrum, which exhibits a maximum near 300 nm. The presence of the long wavelength absorption is important because it permits excitation of the fluorophore without interference from most other ultraviolet-absorbing moieties in proteins and nucleic acids. Additionally, the fluorescence intensity is sufficiently great so that the εATP can be detected at concentrations in the range of $10^{-8}M$. The fluorescence lifetime of εATP is close to 23 nsec, which provides the possibility of utilization of more detailed fluorescence techniques, such as fluorescence polarization (*6*) and polarized decay (*7*).

The usefulness of εATP (**1**) depends, in fact, on its ability to substitute for ATP in enzyme systems. We chose to examine systems involving ATP in the roles of phosphoryl, pyrophosphoryl, and adenylyl donor, and as allosteric effector. For an initial study, the enzyme adenylate kinase (rabbit muscle) was selected, since equilibration can be followed easily by thin-layer chromatographic analysis. When the system εATP + AMP was used, reaction occurred in the presence of adenylate kinase as evidenced by the rapid appearance of εADP as well as ADP (*4*). Comparison with the ATP + AMP system indicated that the εATP + AMP system proceeded with a comparable rate, the reaction being completed within a matter of minutes. By contrast, when either the ATP + εAMP or the εATP + εAMP system was used, no reaction occurred; however, addition of AMP allowed both reactions to proceed. With the same enzyme εADP alone showed no activity, and εAMP did not function as an inhibitor of the reaction at concentrations up to 2.0 mM. These results indicate that specificity is greater at the AMP site than at the ATP site and that ADP utilization is dependent on the more stringent requirements of the AMP site.



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Substitution of εATP for ATP was next examined with an enzyme of greater specificity, hexokinase (yeast), which was assayed according to the standard procedure of coupling to glucose-6-phosphate dehydrogenase. The analog εATP replaced ATP in this system, with a K_m of 2.0 mM, while under identical conditions the K_m observed for ATP was 0.12 mM. In further comparison, V_{max} for ATP was equal to ~38 percent of the V_{max} of ATP (Table 1). Prior to this finding the only nucleoside triphosphate other than ATP that had been found to serve as a good substrate in this system was deoxyadenosine triphosphate (*8*).

Table 1. Binding and activity of the modified coenzyme.

Enzyme	Substrate	K_m* (mM)	V_{max}†
Hexokinase (yeast)	εATP	2.0(0.12)	0.38
Phosphofructokinase (rabbit muscle)	εATP	0.030(0.013)	0.95
Pyruvate kinase (rabbit muscle)	εADP	0.30(0.30)	0.80

* The K_m for normal substrate is shown in parenthesis. † Relative to normal substrate.