

interval used, Q_{10} must vary within the limits of 0.83 to 1.17.

In sum, the experiments demonstrate the existence of a rhythm of gas uptake in dry seeds which persists under constant conditions, and whose period appears to be independent of temperature. The rhythm therefore satisfies two prime requisites of circadian character and is assumed to be circadian. Allowing for the 4-hour interval, the curves resemble those already established for potato tubers and germinating beans (5).

Previous studies with this system have shown that DNA, RNA, and protein synthesis are initiated at discrete points following exposure to germinative conditions. The DNA replication is initiated at approximately 36 hours (6), RNA synthesis appears at 12 to 18 hours (7), and protein synthesis is detected at 12 to 18 hours of germinative conditions (8). Replication, transcription, and translation are not detectable in the quiescent seed. These results are in accord with those of others (9), which demonstrate a lack of genomic transcription in dormant seeds (10). Further evidence may be marshaled which shows the same sort of sequential initiation of synthesis in comparable dormant phases of other organisms (11). Additionally, visual evidence from electron microscopy supports the conclusions by the exclusive or near-exclusive occurrence of ribosomes in the monosomic rather than the polysomic form (12).

Finally, it should be emphasized that this is a demonstration of a circadian rhythm in a dormant system. The only process we know to be occurring in the dormant seed is the minimal level of respiration shown here. Since no other sequences occur to interfere with this single rhythm, one of the disadvantages of active systems is obviated. In fact, since only this phenomenon is known to occur in the dormant seed, it presents a logical argument for consideration of energy cycling as the basic oscillator. It occurs in all living cells and is coupled directly or indirectly to all metabolic pathways of the organism.

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3. This is not to say no CO_2 is released. It may simply be immediately recycled without leaving the tissue. It is conceivable on the other hand, that a mechanism (polyphenol oxidase or something akin to luminescence, for example) which does not yield CO_2 is involved.
4. Preliminary measurements indicate that seed temperature may increase to 40° or 45°C when held at constant temperature (25°C) and illuminated. Seeds heated to 120°C for 48 hours, allowed to recover for 24 hours, and then measured for gas uptake show greatly increased uptake rates which gradually (over a period of several days) decrease to normal levels. The rhythmic fluctuation is still present, which in itself would contraindicate an oscillation based on transcription-translation processes.
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11. See, for example, *Dormancy and Survival* (proceedings of the 23rd symposium of the Society for Experimental Biology, Norwich, England), H. W. Woolhouse, Ed. (Academic Press, New York, 1969).
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Mercury Concentrations in Recent and Ninety-Year-Old Benthopelagic Fish

Abstract. Several species of bottom-dwelling fish from 2500 meters with similar feeding habits had mercury concentrations that differed by an order of magnitude. Within one species there was a correlation between size and concentration, with the larger individuals having mercury concentrations as high as 0.8 part per million (wet weight). The mercury content of the water in the deep-ocean habitat of these fish appears not to determine the mercury content of a particular fish; species-specific factors and size do appear to determine this concentration. The species-specific variation between the recent fish also existed between the same two species in specimens collected 90 years ago from a depth of 2000 meters, and a 90-year-old specimen fit closely the size-concentration regression curve for nine recent individuals of the same species.

A knowledge of the concentration of mercury in the biological reservoirs of the ocean is necessary to understand the natural biogeochemical flux of this element through the marine ecosystem and to predict the consequences of cultural changes in the global flux. We measured the Hg concentration in one biological reservoir, the bottom-dwelling deep-sea fish, to provide one portion of the information needed for a predictive model.

Interest in the Hg concentrations in deep-sea fish is heightened because of the popular assumption that the deep ocean is the portion of the biosphere that has been least affected by chemical pollutants. The assumption that the deep sea is still relatively uncontaminated is almost certainly not valid for certain chemicals; for example, DDT and DDE (dichlorodiphenyltrichloroethane and dichlorodiphenyldichloroethylene) were detected in the axial muscle in most fish we collected at 2500 m (1). Yet, the idea that indus-

trial and agricultural activities have not significantly increased the concentration of Hg in deep-ocean water can be supported by calculating the change in Hg concentrations which would result if the total increase in Hg flux due to human activities were to be evenly mixed into the oceans. Using this line of argument, Weiss *et al.* (2) have predicted that, at most, a doubling in the upper 100 m of the oceans could result from culturally enhanced degassing of Hg from disturbed land to the atmosphere and then to the oceans. If the prediction of these authors is correct, we would assume that man has not significantly increased the Hg content of deep-ocean water and, therefore, that fish living permanently at depth will have "natural" or preindustrial levels of mercury. However, an untested assumption in this reasoning is that there are no special food chain phenomena or physical mechanisms that concentrate and transport Hg rapidly from cultural sources to the deep-

ocean floor in a form available to fish that feed by rooting in the sediments. Certain metals do move rapidly from the atmosphere, through the water column and into benthic animals; the fallout radionuclides ^{141}Ce , ^{144}Ce , ^{95}Zr , and ^{95}Nb rapidly entered sea cucumbers living at 2800 m, but ^{65}Zn did not (3). We report here the variations in Hg concentrations in five species of large benthopelagic fish collected at 2500 m.

Compared to collecting water and sediment from the deep ocean, it is difficult to collect the large amount of biological material required for chemical analyses. We used an otter trawl (4) to collect a variety of large benthopelagic fish from 2500 m. Two trawls were made southeast of Cape Hatteras on the upper continental rise (5); the net was towed at depth for 2 hours over a distance of 5.5 km according to high-precision loran navigation fixes. Both tows straddled the 2500-m contour. After the trawl was retrieved the contents of the cod end were sorted into plastic bags and immediately frozen at -80°C . The fish catch consisted of five species of benthopelagic fish (6, 7). In addition to the fish, the 1971 trawl collected an empty catsup bottle, a rusted out Spam tin can, and half a banana. The banana was green and hard when collected, but darkened quickly after being stored at 5°C . The fresh condition of the banana followed by fast decay agrees with the observations of Jannasch *et al.* (8) on food preservation in the deep sea. The 1972 catch included a pair of men's shorts, a coal lump, and a large mass of oily cheesecloth.

To reduce the probability that systematic errors would bias our Hg measurements, separate teams used two different analytical procedures. One team was at the Atlantic Estuarine Fisheries Center (AEFC) of the National Marine Fisheries Service, and the other was at Duke University Marine Laboratory (DUML) (9). In the DUML method, digestion by a mixture of nitric and sulfuric acids was followed by oxidation by a mixture of permanganate and persulfate, reduction by stannous chloride, and subsequent measurement of the Hg vapor with a Coleman flameless atomic absorption analyzer (model MAS-50). In the AEFC method, sulfuric acid digestion was followed by permanganate and peroxide oxidation, stannous sulfate reduction, and subsequent measurement with a Perkin-Elmer atomic absorption spectrophotometer (model

303). Axial muscle was removed in a block from the mid-dorsal region. All values were determined as parts per million (wet weight). The recovery of inorganic Hg spikes was checked repeatedly in the DUML method, especially with the *Aldrovandia* samples which gave very low values; the percentage of recovery ranged from 85 to 95. Intercalibration of the DUML and AEFC methods with those used in other laboratories was performed by analyzing a homogenized-fish check sample (AA-4), which was prepared and distributed by the Fishery Products Technology Laboratory of the National Marine Fisheries Service (10). The Hg concentrations determined by the various laboratories are: DUML, 0.49 ± 0.06 ; AEFC, 0.52 ± 0.03 ; Phoenix Laboratory, 0.49 ± 0.02 ; Fishery Products Technology Laboratory, 0.59 ± 0.03 ppm (wet weight). (Neutron activation was used by the Phoenix Laboratory, and flameless atomic absorption by the other three.) The values reported are means and one standard deviation of five replicates. Further intercalibration was performed by analyzing the check sample distributed by Uthe, Armstrong, and Tam (11) to 29 North American laboratories.

The first observations deal with the differences in Hg concentration between the halosaur, *Aldrovandia macrochir*, and the *Chalinura* (macrourids) and *Antimora* (morids). The Hg con-

centration in the recent *A. macrochir* varied from 0.03 ± 0.02 to 0.08 ± 0.007 ppm, about an order of magnitude lower than the range for the macrourids and morids (Table 1 and Fig. 1). The 1886 museum specimen of *A. macrochir* from approximately the same depth as the recent fish had a Hg concentration of 0.11 ± 0.02 , which was less than half of the lowest value for other species of benthopelagic fish. According to Marshall (7), these three benthopelagic groups—morids, macrourids, and halosaurs—show morphological convergences which are related to existence in the deep ocean. The long tails, projecting snouts, and under-slung mouths are adaptations for hovering over the bottom and rooting in the ooze for food. Marshall and Bourne (12) have reported, on the basis of deepwater photographs, that the feeding behavior of these groups of fish appears to be the same; probably they eat much the same food. In view of the similar ecology of the three groups, it is puzzling to find the differences in Hg concentration among them. We must assume that the *A. macrochir* are exposed to the same environmental concentrations of Hg as the other benthopelagic fish collected in the same net haul. The differences in Hg concentration must result from species-specific metabolic properties, because these differences were maintained even though the fish occurred in the same chemical

Table 1. Mercury concentrations in the axial muscle of benthopelagic fish. The values given are the means of three to six replicates, ± 1 standard deviation. The recent fish were caught on 28 July 1971 and 5 July 1972 (5). For a description of the DUML and AEFC methods, see text.

Species	Length (cm)	Hg (ppm, wet weight)	
		DUML	AEFC
	<i>Recent fish</i>		
<i>Aldrovandia macrochir</i>	43.5	0.07 ± 0.01	0.09 ± 0.04
	55.0	.03 ± .02	
	58.0	.04 ± .016	.04 ± .009
	66.0	.04 ± .009	.02 ± .004
	67.5	.08 ± .007	.09 ± .018
<i>Antimora rostrata</i>	32.3	.32 ± .01	.30 ± .02
	33.4	.43 ± .02	.45 ± .02
	33.5	.24 ± .02	.26 ± .05
	35.5	.31 ± .04	.40 ± .04
	39.0	.53 ± .04	
	42.8	.58 ± .02	.59 ± .04
	45.3	.65 ± .06	
	45.8	.71 ± .004	.71 ± .09
	52.2	.76 ± .02	.74 ± .02
<i>Bathysaurus agassizi</i>	42.4	.36 ± .07	
<i>Chalinura brevibarbis</i>	28.8	.38 ± .03	
	39.1	.42 ± .02	.45 ± .01
<i>Chalinura carapina</i>	27.5	.36 ± .02	
	<i>Old fish</i>		
<i>Antimora rostrata</i> *	45.7	0.50 ± 0.03	
<i>Aldrovandia macrochir</i> †	43.5	.11 ± .02	

* Collected 1 August 1883. † Collected 24 October 1886.

environment. The Hg content of the water appears not to be the most important factor determining the Hg concentration in a particular fish. Halosaurs are considered to be phylogenetically distinct from morids and macrourids (7); therefore, the existence of fundamental metabolic differences is not unreasonable. Large variations in Hg concentration between groups of fish living in the same environment have not, to our knowledge, been reported previously.

The next observation deals with the relationship between size and Hg concentration within nine individuals of *Antimora rostrata*. Work on lake trout (13) and pike (14) established that the total Hg concentration increases with age, although the accumulation rate decreases with size (15). Specialists on scale aging were unable to determine the age of our benthopelagic fish, but the wide size range of the nine *Antimora rostrata* enabled us to examine Hg concentration as a function of size (Fig. 1); presumably, size is related to age, especially for a group of fish collected in a single net haul. The correlation between length and Hg concentration was high ($r^2 = .855$) for all the samples from the nine recent fish. If there is a level at which the Hg concentration begins to impair the fitness of the fish, attainment of this level would limit the upper size (or age) for these fish. The increase in Hg concentration in the axial muscle with size for benthopelagic fish contrasts with the results of Cross and Brooks (16) for Fe, Zn, and Mn concentrations in whole specimens in three species of juvenile estuarine fish; for these essential metals there was a decrease in concentration as weight increased. Analyses of muscle from various sized bluefish for Hg, Fe, Cu, Zn, and Mn have shown that the Hg concentration increases with size, whereas the concentrations of the other metals remain the same (17). Apparently the physiological processes that effectively regulate the levels of Fe, Cu, Mn, and Zn do not regulate that of Hg effectively.

To determine if the Hg concentrations in these fish have changed during

the last century we analyzed small portions of 90-year-old museum specimens, which were collected from the same region and approximately the same depths (18). Only enough material was available for one set of analyses; these were done with the DUML method. As noted above, both the recent and old fish of *Aldrovandia macrochir* had much lower concentrations than the other four benthopelagic species (Table 1).

The 1833 *Antimora rostrata* had a Hg concentration very close to that of recent *Antimora* of the same size (Fig. 1). The relatively good fit of the 1883 *Antimora* to the size-concentration regression line for nine 1971 fish is evidence that there has not been a change in Hg concentration in these benthopelagic fish during the last century, when the global flux of Hg has been enhanced by man (2). The maintenance of the species-specific differences between the morids and the halosaurs is evidence that the preservation method did not alter the Hg concentration of the museum specimens. It is difficult to propose a preservation artifact that would maintain the differences between the taxonomic groups.

In a report of the International Decade of Ocean Exploration (IDOE)

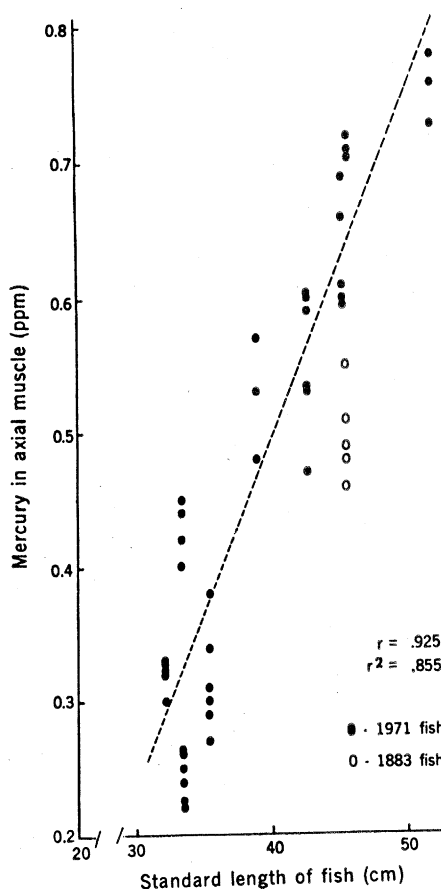


Fig. 1. The relationship between Hg concentration and length in *Antimora rostrata* collected in a single trawl on 28 July 1971. Each point represents one analysis; $r^2 = .855$ was calculated for all analyses on the nine fish. The data graphed here are given in Table 1 under the heading DUML.

(19), Hg levels were found not to exceed the limit of 0.5 ppm (wet weight) set by the U.S. Food and Drug Administration for common marine food fish, except for tuna and swordfish and a few individual fish from "localized hot spots" (20). Our observations indicate that values exceeding 0.5 ppm (wet weight) are to be expected in the larger fish of certain species, and these levels are not associated with any known localized inputs. Benthopelagic fish are not, of course, harvested for human consumption; our purpose is to emphasize that concentrations exceeding 0.5 ppm (wet weight) may occur "naturally" in some species and may not be related to anthropogenic inputs of Hg to the oceans.

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4. The authors will provide net and door specifications and a description of our deepwater trawling tactics to those interested.
5. The trawls were made from the R.V. *Eastward* at station 17544, $34^{\circ}45.3'N$, $75^{\circ}11.8'W$, and at station 20536, $34^{\circ}18.2'N$, $75^{\circ}32.6'W$.
6. The species collected were: *Antimora rostrata* (Günther) 1878, *Bathysaurus agassizi* (Goode and Bean) 1885, *Chalinura brevibarbis* (Goode and Bean) 1895, *Chalinura carapina* (Goode and Bean) 1883, *Aldrovandia macrochir* (Günther) 1878. These fish are permanent bottom dwellers of the deep sea (benthopelagic), according to N. B. Marshall (7).
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in the Hg analysis. We acknowledge the technical assistance of L. H. Hardy and N. Y. Jones of the Atlantic Estuarine Fisheries Center, and the Oceanographic Program of Duke University Marine Laboratory for the use of R.V. *Eastward* on cruises E-19-71 and E-12-72. The Oceanographic Program at DUML is supported by NSF grants GA-27725 and GD-28333; other DUML support was from NSF grant GA-28742. The research conducted at AEFC was supported jointly by the National Marine Fisheries Service and the AEC, agreement No. AT(49-7)-5.

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coli (5) or from calf thymus nuclei (6). The capacity of the enzyme preparation to incorporate labeled cytosine triphosphate (CTP), guanosine triphosphate (GTP), or uridine triphosphate (UTP) was relatively insignificant. The enzyme activity was 380 units for [³H]UTP, 300 units for [³H]CTP, and 40 units for [³H]GTP. The enzyme preparations obtained from the same amount of tumor tissue had no significant poly(A)-forming activity. The material that was not solubilized by our procedure did not contain significant poly(A) polymerase activity (< 1000 units for liver and < 50 units for hepatomas).

The product was characterized as poly(A) on the basis of the following properties (Table 1). (i) Pancreatic ribonuclease had no inhibitory effect if it was incubated with the enzyme preparation before the polymerization reaction or if it was incubated with the reaction product. (ii) The product was retained on a Millipore filter in the presence of 0.5M KCl, which dissolves the bulk of cellular RNA (7). (iii) Deletion of the remaining three nucleotides did not inhibit enzyme activity. The internucleotide or nonterminal incorporation of adenylate was tested by estimating the radioactivity in the nucleoside mono-

Mitochondrial Polyriboadenylate Polymerase: Relative Lack of Activity in Hepatomas

Abstract. An enzyme that polymerizes adenylate residues from adenosine triphosphate was prepared from rat liver mitochondria and compared to similar preparations from the mitochondria of three hepatomas. Enzyme activity in the hepatomas was only 1 to 2 percent of that in normal liver.

An enzyme in eucaryotes that can incorporate adenylate residues into non-terminal internucleotide linkages has been characterized in the nuclei (1) and in the supernatant from microsomal centrifugation (2). During our studies on DNA-dependent RNA polymerase of rat liver mitochondria, we solubilized and partially purified an enzyme that can synthesize polyadenylate[poly(A)] chains from adenosine triphosphate (ATP). The activity of this enzyme was considerably reduced in preparations from Morris hepatomas 7777 (well differentiated), 3924A (poorly differentiated), and 3683F (undifferentiated) (3).

Mitochondria were isolated by a procedure similar to that of Sordhal and his colleagues (4). The mitochondrial pellet was suspended in 50 mM tris(hydroxymethyl)aminomethane (tris) buffer (pH 9.0) containing 5 mM MgCl₂, 0.1 mM ethylenediaminetetraacetic acid (EDTA), and 1 mM dithiothreitol (TMED buffer), and sonicated for 1 minute (four 15-second periods) in a Branson Sonifier (model W 140) at full output. The suspension was mixed with glycerol (final concentration, 20 percent, by volume), incubated at 37°C for 30 minutes, and centrifuged at 105,000g for 1 hour. The supernatant, which contained enzyme activity, was dialyzed against several volumes of TMED buffer (pH 7.5) containing glycerol (20 percent, by volume) to deplete the nucleotides. In some experiments, the enzyme was further purified by differential precipitation with ammonium sulfate. The fraction precipitated by increasing the ammonium sulfate saturation from 40 percent to 50 percent had most of the activity, although this pro-

cedure resulted in a considerable loss of activity.

The enzyme from liver had a specific activity of 13,000 units (1 unit = 1 pmole of nucleotide incorporated per milligram of protein per 30 minutes) (Table 1). The reaction was linear for 30 minutes. The specific activity of the liver enzyme was comparable to the highest values reported for the corresponding enzymes from *Escherichia*

Table 1. Comparison of poly(A) polymerase activities in the mitochondrial preparations of normal rat liver, host liver, and hepatomas. The enzyme was prepared from 30 g each of liver and hepatoma. The standard reaction mixture (0.35 ml) contained 10 μ mole of tris-HCl (pH 8.0), 2 μ mole of MgCl₂, 1 μ mole of MnCl₂, 13 μ mole of KCl, 3 μ mole of phosphoenolpyruvate, 20 μ g of pyruvate kinase, 0.1 μ mole of unlabeled ATP, 0.00075 μ mole of [³H]ATP [generally labeled, 9.53 to 15 c/mmole (New England Nuclear)] and 0.8 to 1.0 mg of enzyme protein. After incubation at 30°C for 30 minutes, the reaction was stopped by the addition of 0.1 μ mole of unlabeled ATP and a cold solution of 10 percent trichloroacetic acid and 0.04M sodium pyrophosphate. The precipitate was collected either on Whatman GF/C filters or on Millipore filters (1.2- μ m pore size), washed three times with a cold solution of 5 percent trichloroacetic acid and 0.02M sodium pyrophosphate, and finally with cold glass-distilled water. In some experiments, the product was filtered in the presence of 0.5M KCl to test for poly(A) (7). The radioactivity in the filters was determined by counting in 10 ml of a toluene-based scintillation solution containing 4 g of Omnifluor (New England Nuclear) and 0.1 g of 1,4-bis-[2-(4-methyl-5-phenyloxazolyl)]-benzene (POPOP) per liter of solution. The enzymes from liver and tumor were used in saturating amounts and were assayed in triplicate (1 unit is the amount of enzyme that incorporates 1 pmole of nucleotide into trichloroacetic acid-insoluble product per milligram of protein per 30 minutes; N is the number of experiments).

Enzyme source	Additional treatment	Enzyme activity (10 ³ units)	N
Host liver, rats with 7777		13.80 \pm 0.98	4
Host liver, rats with 3924A		13.10 \pm 0.51	3
Host liver, rats with 3683F		10.00 \pm 0.64	2
Hepatoma 7777		0.25 \pm 0.05	4
Hepatoma 3924A		0.15 \pm 0.09	3
Hepatoma 3683F		0.10 \pm 0.03	2
Hepatoma 3683F	Unlabeled ATP + (0.1 μ mole)	0.11 \pm 0.03	2
Normal liver		13.50 \pm 1.70	10
Normal liver	Prior 30-minute incubation with ribonuclease (100 μ g/ml)	12.90 \pm 0.94	2
Normal liver	Product incubated 30 minutes with ribonuclease (100 μ g/ml)	13.40 \pm 0.90	3
Normal liver	+ GTP, CTP, UTP	13.10 \pm 0.61	2
Normal liver	+ 0.5M KCl during filtration	12.90 \pm 1.08	3
Normal liver	+ Deoxyribonuclease (100 μ g/ml)	13.10 \pm 0.71	2
Normal liver	+ Mitochondrial RNA (50 μ g/ml)	13.70 \pm 0.75	2