4. L. W. Labaw, *J. Ultrastruct. Res.* **17**, 327 (1967).
5. J. B. Sumner and A. L. Dounce, *J. Biol. Chem.* **121**, 417 (1937).
6. A. V. Crewe, M. Isaacson, D. Johnson, *Nature* **231**, 262 (1971).
7. C. E. Hall, *J. Biol. Chem.* **185**, 749 (1950).
8. M. G. Rossmann and L. W. Labaw, *J. Mol. Biol.* **29**, 315 (1967).
9. W. Hoppe, R. Langer, G. Knesch, C. Poppe, *Naturwissenschaften* **55**, 333 (1968); A. E. C. Spargo, private communication.
10. J. M. Cowley, *Proc. Phys. Soc. London Sect. B* **66**, 1096 (1953); R. Nathan, *Advan. Electron Opt. Microsc.* **4**, 85 (1971); M. F. Moody, *Nature* **218**, 263 (1968).
11. D. J. DeRosier, *Contemp. Phys.* **12**, 437 (1971); H. P. Erickson and A. Klug, *Ber. Bunsenges. Phys. Chem.* **74**, 1129 (1970); N. A. Kiselev, D. J. DeRosier, A. Klug, *J. Mol. Biol.* **35**, 561 (1968).
12. R. W. Horne, *Lab. Invest.* **14**, 1054 (1965); R. C. Valentine, *Nature* **184**, 1838 (1964).
13. B. K. Vainshtein, V. V. Barynin, G. V. Gurskaya, in *Microscopie Électronique 1970*, P. Favard, Ed. (Société Française de Microscopie Électronique, Paris, 1970), vol. 1, p. 627.
14. Dr. B. W. Schumacher (Westinghouse Electric Corp.) provided valuable advice on the design of the water vapor flow system and the calculation of flow rates. We thank G. G. Hausner, Jr., for machining and design aid, and J. Subjeck and R. Slon for technical assistance. Supported by NIH grant GM16454.

29 March 1972; revised 27 April 1972

Coral Chronometers: Seasonal Growth Bands in Reef Corals

Abstract. *Autoradiographs and x-radiographs have been made of vertical sections through the centers of reef corals from Eniwetok. Radioactivity bands in the coral structure are caused by strontium-90 and are related to specific series of nuclear tests, thus making possible calculation of long-term growth rates. These data indicate that the cyclic variations in radial density revealed by x-radiography are annual.*

Growth rates and growth patterns of corals are of interest for several reasons. Wells (*1*) and Runcorn (*2*) have interpreted growth bands in fossil corals as indicators of past variations in the length of day, year, and lunar month. Chave *et al.* (*3*) have summarized the results of various investigations of the growth rates of contemporary reef corals. Other workers have commented on apparently annual bands in coral growth patterns (*4*), but their nature, origin, and periodicity have not been conclusively characterized or calibrated.

Bonham (*5*) has reported on the use of autoradiography in combination with optical thin-section photography to measure the growth rate of a giant clam, *Tridacna gigas*, which was sampled at Bikini Atoll and which contained fallout radioactivity from nuclear tests. He observed variations in shell translucence which he identified as annual and tentatively ascribed to seasonal variations in the water temperature.

We have used autoradiography to study the growth rates and x-radiography to identify structural density variations in a variety of reef corals listed in Table 1. Samples 1 to 5 from Eniwetok Atoll (also the site of nuclear tests, 1948 to 1958) were subjected to both types of radiography. Samples 6 and 7 (from Fanning Island and Fort Lauderdale, Florida, respectively) were x-radiographed only; these samples were included in the study in order to ensure that the observed growth bands were not peculiar to the Eniwetok environment.

We cut a vertical slice less than 2 cm thick through the center of each coral specimen. For autoradiography, no-screen x-ray film was placed in direct contact with the samples; lead backscatter plates or fluorescing intensifier screens, or both, were placed behind the films to increase exposure speeds. After 40 days, the autoradiograph of sample 1 showed four radioactivity bands, that of sample 2 showed two bands, and that of sample 3 showed one definite band and a possible second band. Radioactivity bands for samples 2 and 3 were near the base (origin) of the coral, an indication that the earlier test series occurred prior to the inception of growth. Samples 4 and 5 showed no discernible concentrations of radioactivity, thus indicating total ages younger than the elapsed time since the most recent nuclear test series.

Nuclear weapons test series at Eniwetok were conducted during April–May 1948, April–May 1951, October–November 1952, May 1954, May–July 1956, and May–July 1958 (*6*). In each radioactivity-containing specimen the

Table 1. Growth rates and dimensions of reef corals.

Sample No.	Species	Date collected	Location*	Average linear distance, origin to surface† (cm)	Average upward growth rate based on 1958 radioactivity band (cm/year)	Average upward growth rate based on density band count (cm/year)
1	*Favia speciosa*	Feb. 1971	Eniwetok (Chinimi)	18 ± 0.5	0.46 ± 0.02‡	0.46 ± 0.02
2	*Goniastrea parvistella*	June 1971	Eniwetok (Bogen)	16 ± 1	1.25 ± 0.05	1.25 ± 0.05
3	*Goniastrea retiforms*	June 1971	Eniwetok (Chinimi)	10 ± 0.5	0.78 ± 0.03	
4	*Porites lutea*	June 1971	Eniwetok (Chinimi)	15 ± 1	> 1.2	1.35 ± 0.05
5	*Psammocora togianensis*	June 1971	Eniwetok (Chinimi)	26 ± 0.5	> 2.0	2.9 ± 0.1
6	*Platygyra rustica*	Aug. 1971	Fanning Island (Suez Pond)	22 ± 1		2.2 ± 0.1
7	*Montastrea annularis*	Jan. 1970	Florida (Fort Lauderdale)	14 ± 2		1.7 ± 0.3

* All samples were taken from water depths ranging from 2 to 5 m; Eniwetok samples were all from the reef flat toward the lagoon, except for sample 2, which was taken from a pinnacle reef top. † Samples 1 through 4, 6, and 7 are approximately hemispherical; measurements are the average of those along several different radial transects; error limits represent the range of the extreme values determined. Sample 5 is a branching coral; the measurement is along the longitudinal axis of the stalks x-rayed, with error limits representing the difference between adjacent stalks. ‡ Growth rate between 1952 and 1958 radioactivity bands equals 0.47 ± 0.02 cm/year.

outermost band was by far the most intense (see Fig. 1); we correlate this band with the 1958 Hardtack test series, which not only was the last at Eniwetok but also had the highest announced total yield and the greatest number of surface and subsurface lagoon shots. The other radioactivity bands of sample 1 have been identified with the 1956, 1954, and 1952 tests. The 1951 and 1948 series were relatively low-yield tests and they apparently did not deposit detectable amounts of radioactivity in sample 1.

Chemical separation of strontium (*7*) from samples 1 and 2 followed by liquid scintillation counting of the recovered salt indicated that much if not all of the observed radioactivity is due to ^{90}Sr and its daughter ^{90}Y. This finding is in agreement with Bonham's observations (*5*).

All samples x-rayed (*8*) showed distinct and fairly regular alternating dark and light bands, reflecting a cyclic variation in the bulk density of the deposited skeletal material. The measurement does not make it possible to determine whether the density variations are the result of variations in the thickness of the corallite skeletal walls, variations in the spacing of the structural components of the skeleton, or some combination of the two. Pairs of consecutive dark and light bands were counted and measured, and the results are summarized in Table 1. In the case of sample 3 the 1958 radioactivity band was readily observable, but the specimen had been so badly perforated by boring organisms that accurate counts or measurements of density band pairs were not possible.

Figure 1 shows the x-radiographs and autoradiographs of samples 1 and 2, which contained both radioactivity bands and easily discernible density bands. We superposed the two radiographs for each sample and used the 1958 radioactivity band as a base line for counting density bands. For both samples there are 13 density band pairs between the 1958 radioactivity band and the surface, in agreement with the elapsed time (13 years) between the 1958 tests and the 1971 sampling of the corals.

These results, summarized in Fig. 1 and Table 1, indicate that the density banding is annual. Sample 1 provides further evidence of annual density banding, in that the radioactivity bands corresponding to the 1956, 1954, and 1952 tests are each separated by two density band pairs. This specimen thus contains a confirmed record of annual density bands for 19 years of growth, with radioactivity inclusions providing four separate chronology checks. The density band records from the presumably younger corals (samples 4 and 5) containing no radioactivity are in support of this conclusion; fewer than 13 band pairs are observed in these samples.

Growth rate data are presented in Table 1. The agreement between ages based on radioactivity inclusions and those determined by density band counting encouraged us to measure growth rates on the basis of density bands in those corals which do not contain radioactivity. The growth rates thus determined are in good agreement with determinations based on other techniques (*3*). Significantly, the growth rates are approximately constant in terms of linear yearly increments, but not in terms of the amount of mass deposited each year. Moreover, inclusion of the observed radioactivity had no apparent significant effect on growth rates and patterns; for example, the average annual growth rate of sample 1 for the period from 1952 to 1959 is virtually the same as its average growth rate between 1959 and 1971.

Seasonal growth patterns shown in the x-radiographs presumably reflect regular variations in growth rate in response to some cyclically fluctuating environmental parameter. The fact that seven different species from three different locations show similar characteris-

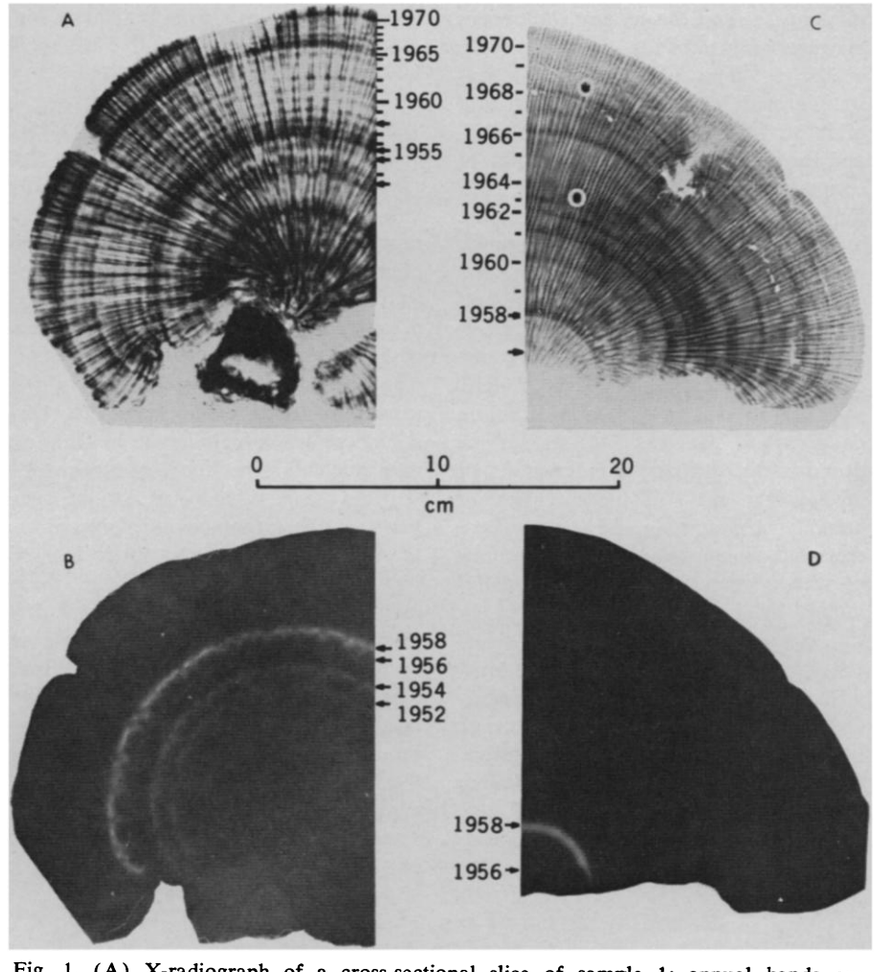


Fig. 1. (A) X-radiograph of a cross-sectional slice of sample 1; annual bands are indicated by index lines and dates; arrows show the location of (B) the dated radioactivity bands. (B) Autoradiograph of sample 1, with dates of test series associated with observed bands. (C) X-radiograph of sample 2; circles indicate the locations of intraseasonal secondary bands less intense or less continuous, or both, than the annual bands. (D) Autoradiograph of sample 2. The pictures are positives printed from x-ray negatives, so that dark areas correspond to the denser portions of the coral. In order to index bands near the center of the coral, half of each radiograph is shown.

tics suggests that the controlling seasonal fluctuations are not merely local phenomena. In addition, the controlling parameters should display intraseasonal variations capable of accounting for the occasional secondary bands observable in sample 2 (see Fig. 1).

Water temperature (*9*) and available sunlight (*10, 11*) have been the factors most frequently suggested as controlling coral growth rates. A recent study in Hawaii (*11*) has indicated that available light is a dominant factor in determining growth rates of reef corals there. Our results are consistent with this finding; seasonal variations in cloud cover in combination with variations in the length of day occur even in locations where water temperature is almost invariant, and can account for the annual banding mechanism. Year-to-year variations in the degree and dates of occurrence of cloudiness can account for both the secondary bands observed in sample 2 and the modest differences between annual bands.

The fact that there are internal, seasonal growth bands permits the retrieval of information on both the differential and integral long-term growth rates of corals without the necessity for real-time in situ experiments. The response of coral growth rates to imposed stresses such as storms, pollution, and radioactivity inclusions may also be preserved in the density pattern record.

Other possible applications of the coral internal calendar are the following: (i) retrieval of global distribution patterns of ^{90}Sr and ^{14}C in surface ocean waters during the early stages of the nuclear era; (ii) measurement of annual variations in cloudiness from the band dimensions and, concurrently, of water temperature variations determined by isotope thermometry (*12*); (iii) eventual construction of a "coral ring chronology" analogous to the long-term tree-ring chronologies successfully developed by dendrochronologists; and (iv) paleoclimatic studies and investigations of the natural historic variations in oceanic ^{14}C activity, provided that sufficiently old or preserved coral heads can be found and dated.

DAVID W. KNUTSON
ROBERT W. BUDDEMEIER
Department of Chemistry and
Hawaii Institute of Geophysics,
University of Hawaii,
Honolulu 96822

STEPHEN V. SMITH*
Department of Oceanography and
Hawaii Institute of Geophysics,
University of Hawaii

References and Notes

1. J. Wells, *Nature* **197**, 948 (1963).
2. S. Runcorn, *Sci. Amer.* **215**, 26 (October 1966).
3. K. Chave, S. Smith, K. Roy, *Mar. Geol.* **12**, 123 (1972).
4. T. Ma, *Proc. Acad. Jap.* **10**, 353 (1934); N. Abe, *Palao Trop. Biol. Sta. Stud.* **2**, 105 (1940).
5. K. Bonham, *Science* **149**, 300 (1965).
6. K. Telegadas, *U.S. At. Energy Comm. Res. Develop. Rep. HASL 111* (1961), pp. 176–182.
7. M. Wade and H. Seim, *Anal. Chem.* **33**, 793 (1961).
8. X-radiography was carried out with a variety of medical and industrial equipment and Kodak medical or industrial type M film. Exposures were taken with the head 2 to 3 m from the sample, with low voltage (60 to 75 kv), and with current and time adjusted to optimize contrast and resolution.
9. E. Shinn, *J. Paleontol.* **40**, 233 (1966); T. Ma, *Effect of Water Temperature on Growth Rate of Reef Corals* (Oceanographica Sinica, Taipei, Formosa, 1959), special volume 1, p. 1.
10. T. Goreau, *Endeavour* **20**, 32 (1961).
11. J. Maragos, in preparation.
12. J. Weber and P. Woodhead, *J. Geophys. Res.* **77**, 463 (1972).
13. We thank J. Wells, K. Chave, J. Maragos, and R. Kinzie for advice and aid and G. Liese, S. Shin, and M. Perez for assistance with the x-radiography. Some of the sampling efforts were supported by the U.S. Atomic Energy Commission through the Eniwetok Marine Biological Laboratory, and by the Symbios Expedition. Hawaii Institute of Geophysics Contribution No. 471.
* Present address: Office of Environmental Science, Smithsonian Institution, Washington, D.C. 20560.

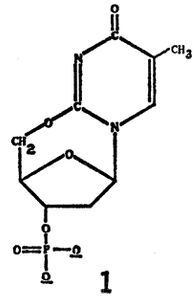
29 March 1972

Oligothymidylates: Formation by Thermal Condensation of O^2,5′-Cyclothymidine 3′-Phosphate

Abstract. *Thermal activation of* O^2,5′-*cyclothymidine 3′-phosphate in solution and in the solid state led to the formation of thymidine oligonucleotides containing up to approximately 12 nucleotide units. Only the 3′,5′ internucleotide diester bonds were formed. This polymerization occurs without the addition of any activating agent or catalyst.*

The chemical synthesis of oligonucleotides (*1*) has been undertaken by the stepwise condensation or polymerization of mononucleotides. Both of these processes involve the activation of the phosphate moiety of the suitably protected nucleotides. Even in the absence of activating agents, 2′-deoxynucleotides could be polymerized at elevated temperatures in anhydrous solution with a variety of mild acidic catalysts. Oligonucleotides containing up to eight or nine nucleotide residues have been obtained (*2*). Another route for the synthesis of the internucleotide linkage involves an intermediate formation of phosphotriesters (*3*). Besides these methods of phosphate activation, a different principle is based on the use of cyclonucleosides (*4*). Various pyrimidine and purine cyclonucleosides have been used for this purpose, but the products obtained were no larger than the dinucleoside monophosphate or the trinucleoside diphosphate.

We report our results on the self-condensation of O^2,5′-cyclothymidine 3′-phosphate (**1**), which led to the formation of a mixture of oligonucleotides. This molecule (**1**) contains both the nucleophile and the leaving group, and consequently is able to polymerize. The polymerization is not dependent on the addition of an activating agent or a catalyst, and it can be performed in solution as well as in the solid state.



1

O^2,5′-cyclothymidine 3′-phosphate was obtained after a series of reactions starting from 5′-*O*-tosylthymidine (*5*), which was phosphorylated with 2-cyanoethyl phosphate by the method of Tener (*6*). The cyanoethyl group was removed by alkali, and the 5′-*O*-tosylthymidine 3′-phosphate was purified by column chromatography on Dowex-1 formate with 0.05*M* ammonium formate in 50 percent alcohol (*p*H 4). This was converted into 5′-*O*-tosylthymidine 3′-phosphoromorpholidate by activating the phosphate moiety with diphenyl phosphorochloridate (*7*) in a mixture of dioxane and hexamethylphosphorotriamide, followed by the addition of morpholine. The product was purified on a silica gel column with *n*-butanol : water (86 : 14) as the eluant. Treatment of this with four equivalents of *N*,*N*′-dicyclohexyl-4-morpholinocarboxamidine (*8*) in a mixture of dimethylformamide and dioxane at 90°C for 18 hours led to the formation of O^2,5′-cyclothymidine 3′-phosphoromorpholidate in 55 to 60 percent yield. The phosphoromorpholidate was hydrolyzed in 0.5*N* HCl at 0°C for 5 minutes, and the *p*H of the solution was quickly adjusted to 4. The solution was placed on Dowex-1 chloride resin (10 ml of