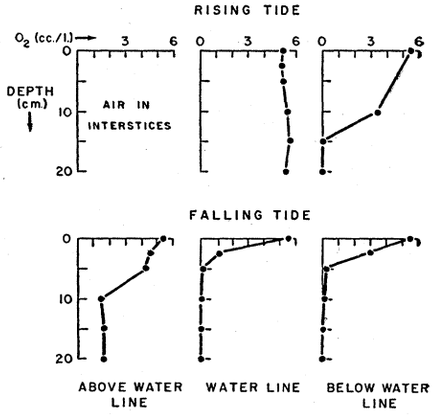

Fig. 1. Interstitial oxygen concentrations in the beach at Corona del Mar during both rising and falling tides. Sampling sites 3 to 5 m apart horizontally normal to the surf line. "Above water line," highest area on the beach reached by wash of breaking waves at time of sampling; "water line," area on the beach covered by wash of breaking waves about 50 percent of the time of sampling; "below water line," lowest area on the beach exposed to the air by the receding wash of breaking waves at time of sampling.

centration with respect to position on the beach relative to water level on either falling or rising tides could be determined at Gonzaga Bay. The lowest concentration measured was 0.4 cm^3/lit. at a depth of 3 cm at the water line on a falling tide. Pockets of relatively stagnant water retained in depressions in the surface of the underlying rock layer probably account for the variability of results here.

Surface water in the cove on the south shore of Punta Banda had 5.4 to 5.5 cm^3 of oxygen per liter (2 samples). Interstitial oxygen 3 to 5 cm into the beach at the water line on a falling tide was 0.9 to 1.4 cm^3/lit. (4 samples taken within 20 cm of one another).

Surface water at Rincon had 4.8 to 4.9 cm^3 of oxygen per liter (2 samples). The beach here is flat, and the sand is fine, so that capillary forces keep the interstices between the sand grains filled with water even near the high-tide line at low water. Two sets of duplicate samples from two places on the beach about 1 mile apart, both sets taken somewhat above midtide level just before the rising tide covered the sampling spots, showed 0.5 to 0.6 cm^3/lit. at 2 to 3 cm, 0.1 to 0.2 cm^3/lit. at 5 to 7 cm, and 0.0 to 0.1 cm^3/lit. at 10 to 12 cm. The sand was black and gave off an odor of H_2S below about 12 cm.

The more complete study of the beach at Corona del Mar demonstrates another point in addition to the occurrence of anaerobiosis (Fig. 1). This is that whatever oxygen enters the beach disappears rapidly. All observations were made in an area about 5 cm long at about the two-thirds tide level on the beach. Independent series of analyses were made on two different days for each situation. Single samples were taken from each depth in each series. Points plotted in Fig. 1 are means for the replicated series, which averaged agreement within 0.3 cm^3/lit. for samples from depths of 5 cm or more. In each situation air-saturated water percolated into the beach from the waves washing up on its surface. On the rising tide, when the interstices were full of air to start with, percolation was very rapid. This resulted in uniform oxygen saturation of the water filling the interstices, at least down to 20 cm. This oxygen was completely gone, however, at depths of 15 cm and more, within 15 to 20 minutes (the time required for the water line to move up the beach the distance between the "below water line" and "water line" sites).

The situation on the falling tide was even more extreme, anaerobiosis occurring at 5 cm at and below the water line. Part, at least, of the rapid drop in oxygen concentrations between the "above water line" and "water line" sites in this situation was due to slowing of percolation of surface water by the anaerobic water already present between the sand grains.

Some, but not all, of the samples from 10 cm or more into the beaches contained larger quantities of dissolved inert gases (N_2,Ar, perhaps CH_4) than did surface water samples.

The organisms inhabiting fine-grained intertidal sandy beaches at depths greater than 5 to 10 cm must, therefore, be capable of surviving periods of complete anaerobiosis which are very nearly equal in duration to the periods when these particular portions of the beach are submerged by the tide. If the beach involved is very fine-grained, with little slope, anaerobic conditions may be continuous. Organisms living in sands below low tide line are almost surely continuously anaerobic.

The biochemical and physiological adaptations which allow metazoan animals to survive under these extreme conditions must be very striking. The existence of such adaptations is strongly indicated by the fact that several types of sand-dwelling mollusks and nematodes can survive complete anaerobiosis in the laboratory for periods of months and that many other forms survive up to at least 1 week (*7*, *8*).

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References and Notes

1. A. S. Pearse, H. J. Humm, G. W. Wharton, *Ecol. Monographs* **12**, 135 (1942).
2. J. Senez, *Ann. inst. Pasteur* **77**, 512 (1949); *Année biol.* **27**, 425 (1951).
3. R. W. Pennak, *Année Biol.* **27**, 449 (1951).
4. W. Wieser, *Limnol. Oceanog.* **4**, 181 (1959); *Free-Living Nematodes and Other Small Invertebrates of Puget Sound Beaches* (Univ. of Washington Press, Seattle, 1959).
5. M. A. Borden, *J. Marine Biol. Assoc. United Kingdom* **17**, 709 (1931); R. W. Pennak, *Ecology* **23**, 446 (1942).
6. P. F. Scholander, L. van Dam, C. L. Claff, J. W. Kanwisher, *Biol. Bull.* **109**, 328 (1955).
7. T. von Brand, *Biodynamica* **4**, 185 (1944); H. B. Moore, *J. Marine Biol. Assoc. United Kingdom* **17**, 325 (1931).
8. I gratefully acknowledge the assistance at various stages of this investigation of Dr. Diane Gordon and Mr. Michael Levy. I also wish to thank the Scripps Institution of Oceanography, La Jolla, and the Kerckhoff Marine Laboratory, Corona del Mar, for the use of their facilities.

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Sequential Deoxyribonucleic Acid Replication

The deoxyribonucleic acid (DNA)–synthesizing system of Kornberg and co-workers (*1*) provides a basis for refinement of the commonly held mechanical schemata of DNA replication. Diagrams to illustrate replication of the Watson-Crick structure generally show the simultaneous formation of two double structures at each growing point, as in Fig. 1. Since the unfinished chains are antiparallel, such diagrams require that the chains grow by different mechanisms: one by addition to the 3′-

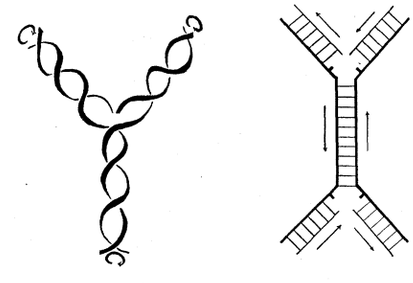

Fig. 1. Replication of Watson-Crick structure, showing simultaneous formation of two double structures at each growing point.

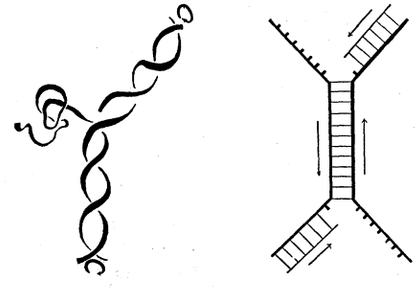

Fig. 2. Undirectional growth of DNA chain, showing sequential (left) and semisequential (right) growth.

position of the nucleoside end, the other to the 5′-phosphate end. The latter chain would have to end with a triphosphate.

Both possibilities of chain growth were at one time considered by Kornberg (*2*), but later the "limited reaction" experiments (*3*) showed that new units are added only to the nucleoside ends of the primer chains. Thus the growth of DNA chains may well be unidirectional, in which case the diagrams in Fig. 1 are erroneous, and Fig. 2 more nearly represents the process.

In consequence of unidirectional chain growth, the formation of replicate double structures is sequential (Fig. 2, left) or semisequential (Fig. 2, right); that is, the formation of one of the new chains, or a different half-length of each new chain, does not begin until the initial double structure has been completely separated. In either case the addition of nucleotides proceeds at twice the rate per chain that would be surmised from the corresponding simultaneous model. Unlike simultaneous models, sequential models predict that up to one-third of the unit DNA is in the single-stranded form at some time in the replication cycle, the observable amount depending on the degree of synchrony among units. If this is true, it may relate to changes in function and in sensitivity to certain agents during the division cycle. If separation of the chains always begins at the same end (for example, where the other end is attached to another structure), then the single chain exposed in successive divisions is always the same member of the complementary pair.

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References and Notes

1. A. Kornberg, *Science* **131**, 1503 (1960).
2. A. Kornberg, in *The Chemical Basis of Heredity*, W. D. McElroy and B. Glass, Eds. (Johns Hopkins Press, Baltimore, 1957).
3. J. Adler *et al.*, *Proc. Natl. Acad. Sci. U.S.* **44**, 641 (1958).

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Seleno-Amino Acid Found in Astragalus bisulcatus

Abstract. Ion-exchange and filter-paper columns were used in a separation of amino acids from an extract of *Astragalus bisulcatus*. Two amino acids were identified, S-methylcysteine and Se-methylselenocysteine.

The identification of naturally occurring selenium compounds has been a subject of investigation since the discovery of selenium in plant material (*1*). Horn and Jones (*2*) reported the isolation from *Astragalus pectinatus* of a crystalline substance containing sulfur and selenium, which analyzed as a complex of 2 parts of selenocystathionine and 1 part of cystathionine.

Table 1. R_F values of S-amino acids, with solvent mixtures A (*3*) (ethanol, 1-butanol, water, and dicyclohexylamine, 10:10:5:2 by volume) and B (formic acid, *tert*-butyl alcohol, and water, 15:70:15 by volume).

Amino acid	$R_F \times 100$	
	Solvent A	Solvent B
Cystine	31.3	9.4
Homocystine	30.5	22
Lanthionine	22.2	8.0
Cystathionine	20.6	12.3
Djenkolic acid	26.6	10.4
Methionine	67.1	67.3
Methionine sulfoxide	37.7	47
S-methylcysteine sulfoxide	41.2	52.3
S-methylcysteine, synthetic	68.2	57.6
Se-compound from *A. bisulcatus*	67.8	58

It was our aim to isolate the selenium compound in *Astragalus bisulcatus* (two-grooved milk vetch) in a manner which would avoid any rearrangements. We used ion-exchange and filter-paper columns for the separation.

The gross separation of the amino acids was made with Amberlite resins. About 80 percent of the selenium was found in the neutral amino acid fraction. Two-dimensional paper chromatograms were made of this fraction of the *A. bisulcatus* extract. By analyzing these paper chromatograms we found that the selenium appeared in only one spot. The spot did not coincide with the sulfur amino acids cystine, homocystine, lanthionine, cystathionine, djenkolic acid, methionine, methionine sulfoxide, and S-methylcysteine sulfoxide (see Table 1). The unknown selenium spot from *A. bisulcatus* had the same R_F values as S-methylcysteine and its selenium analog. In fact, when *A. bisulcatus* extract was chromatographed with either of these acids the same amino acid map was obtained.

The *A. bisulcatus* used for this work was collected in Wyoming and Montana. Dried leaves and stems were ground in a Wiley mill; they analyzed 0.1- to 0.3-percent selenium. A 10-percent water extract was made with isopropyl alcohol as preservative. The neutral amino acids were separated from most of the other compounds by the use of the Amberlite resins IR-4B, buffered IRC-50, and IR-120 (acid form). Absorbing these neutral amino acids on Dowex 50×4 (200 to 400 mesh) and eluting with $0.01M$ NH_3 gave a partial separation into a sulfur and a selenium fraction. The sulfur fraction contained proline and small amounts of other amino acids, but no selenium. The selenium fraction contained some sulfur amino acid, alanine, valine, proline, glycine, and all of the selenium compound. These two fractions were treated separately to isolate the S- and Se-amino acids.

The sulfur fraction was passed through filter-paper columns, with solvent mixtures A (*3*) (see Table 1) and C (formic acid, *tert*-butyl alcohol, acetone, and water, 5:40:40:15 by volume). The portions containing only the sulfur compound were pooled, flash evaporated, and crystallized several times from ethanol. The infrared spectrum for this crystalline sulfur amino acid was identical with that of synthetic S-methylcysteine (*4*). The analysis: calc. for $C_4H_9O_2NS$: C, 35.54; H, 6.71; N, 10.36; S, 23.72. Found: C, 35.80; H, 6.42; N, 10.10; S, 23.55. The agreement of analysis, infrared spectra, and R_F values indicates that the sulfur amino acid in *A. bisulcatus* is S-methylcysteine.

The selenium amino acid fraction, when subjected to the same procedures, yielded a crystalline solid which analyzed for 38.5 percent selenium (calc. for Se-methylselenocysteine: 43.37). The infrared spectrum of the crystalline selenium compound was very close to but not exactly the same as that of pure synthetic Se-methylselenocysteine and seemed to indicate the presence of both S-methylcysteine and Se-methylselenocysteine (*4*). The R_F values with solvent mixtures A and B of synthetic Se-methylselenocysteine and of the natural seleno-amino acid are identical and are also identical with those of S-methylcysteine; they differ from those of methionine and selenomethionine. The results seem to indicate that the seleno-amino acid found in *A. bisulcatus* is Se-methylselenocysteine, at this stage of the work not yet completely separated from S-methylcysteine (*5*).

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References and Notes

1. S. F. Trelease and O. A. Beath, *Selenium*, (published by the authors, New York, 1949); W. O. Robinson, *J. Assoc. Offic. Agr. Chemists* **16**, 423 (1933); K. W. Franke, *J. Nutrition* **8**, 597, 609 (1934); O. A. Beath *et al.*, *J. Am. Pharm. Assoc. Sci. Ed.* **23**, 94 (1934); O. A. Beath, J. H. Draize, C. S. Gilbert, *Wyoming Agr. Expt. Sta. Bull 200* (1934), pp. 1-84.
2. M. J. Horn and D. B. Jones, *J. Am. Chem. Soc.* **62**, 234 (1940); *J. Biol. Chem.* **139**, 649 (1941).
3. T. L. Hardy, D. O. Holland, J. H. C. Nayler, *Anal. Chem.* **27**, 971 (1955).
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* Deceased.

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