tively, 82 or 89 percent of aldrin and 45 or 60 percent of dieldrin during the 8-day incubation period. To test the hypothesis that aldrin and dieldrin (to a lesser extent) had volatilized, the following experiment was conducted 9 days after the applications.

Strips of filter paper were placed around and atop the remaining petri dishes within each desiccator in order to collect any insecticidal vapors that might escape from the covered dishes; the paper had been immersed in a 0.25-percent solution of corn oil in hexane before placement in the desiccator. After 3 more days of incubation (days 9 to 11), the filter papers were removed and rinsed with hexane. The solvent was then adjusted to 50 ml and analyzed by gas-liquid chromatography (*4*). The hexane was then further concentrated in a flash evaporator and finally placed in a counting planchet, evaporated, and tested for radioactivity with a Geiger-Mueller counter. The residue in the planchet was then redissolved in hexane and tested by thin-layer chromatography (*4*).

Their presence in the filter paper showed that aldrin and dieldrin had volatilized from the covered dishes. The amounts detected by Geiger-Mueller counting and gas-liquid chromatography were nearly identical; only aldrin or dieldrin was detected by thin-layer chromatography. Seven micrograms of aldrin were recovered from the papers kept in a desiccator with four plates of agar originally treated with a total of 82 μg of aldrin.

To obtain more-quantitative data, these experiments were repeated by similar preparation of six plates for each treatment. The contents of two plates of each set were used for initial analyses; the remaining four were placed in a desiccator; oil-impregnated filter papers were placed in each desiccator. After 1 day of incubation at 30°C, the filter papers were replaced by freshly oil-impregnated papers which were removed after the 2nd day of incubation. With dieldrin, similar papers were used for a 3rd day in the desiccators. Immediately after removal of the papers from the desiccators, they were extracted and analyzed.

The contents of dishes treated with aldrin were extracted after 2 days of incubation; those treated with dieldrin, after 3 days. The hexane phases were then analyzed by gas-liquid chromatography, liquid-scintillation counting, and thin-layer chromatography; the water-isopropanol phases, by liquid-scintillation counting only.

The fact that the data from gas-liquid chromatography and liquid-scintillation counting were similar indicated that the total radioactivity in the hexane phases was due to the presence of the original aldrin or dieldrin. Moreover, none or less than 1 percent of the total radioactivity recovered was present in the water-isopropanol phase; the absence of hydrophylic insecticidal metabolites was indicated. Thin-layer chromatography detected only aldrin or dieldrin. Results by gas-liquid chromatography are summarized in Table 1. Forty-eight to 51 percent of the applied aldrin and 4.2 to 7.0 percent of the applied dieldrin had volatilized during the first day of incubation and been trapped in the filter paper outside the dishes; 15 percent of the aldrin and 7 percent of the dieldrin volatilized during the 2nd day of incubation; 15 percent of the dieldrin during the 3rd day of incubation. While half of the applied aldrin volatilized immediately after application, dieldrin was lost at a constant rate during the 3-day incubation period—4.2 to 8 percent daily of the application. At the end of the incubation periods only 3.9 to 11 percent of the applied aldrin and 51 to 56 percent of the applied dieldrin were left on the glass surfaces or on the agar.

In the laboratory, the fate of pesticides in the absence of microorganisms should be determined by control experiments. Volatile compounds low in water solubility may be lost under sterile conditions; a cover of microorganisms to some extent prevents such volatilization. Thus it is more difficult to interpret data regarding the effects of microorganisms on pesticides under laboratory conditions.

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16 January 1968

Autocorrelation Functions of Noisy Electron Micrographs of Stained Polynucleotide Chains

Abstract. *Polyuridylic acid was deposited on carbon films in the presence of the thallous salt of mercury-*p*-dihydroquinone-*O,O*-diacetic acid under conditions leading to attachment of one of these molecules to each nucleotide. In electron micrographs of the stained polynucleotide chain, detail was obscured by the noise resulting from the substrate. Optically obtained autocorrelation functions of the distribution of density along the strands revealed peaks suggesting a structural regularity of 8 angstroms; this was interpreted as the internucleotide spacing.*

In electron-microscopic study of the sequence of bases of nucleic acids it is important to know the internucleotide distance; Highton and Beer (*1*) have inferred this from the lengths of tobacco mosaic virus RNA molecules; their values of approximately 6 Å, however, may be somewhat low since end-to-end distances rather than contour lengths were used. This spacing is now reexamined in an electron-microscopic study of extended polynucleotide chains stained with the dithallous salt of mercury-*p*-dihydroquinone-*O,O*-diacetic acid (Tl_2HgHDA). The chemistry of the staining reaction has been described (*2*).

Electron microscopy of such fine molecular detail falters not so much because of the capabilities of instru-

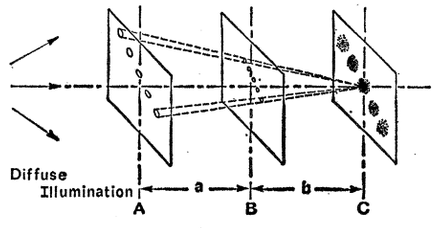
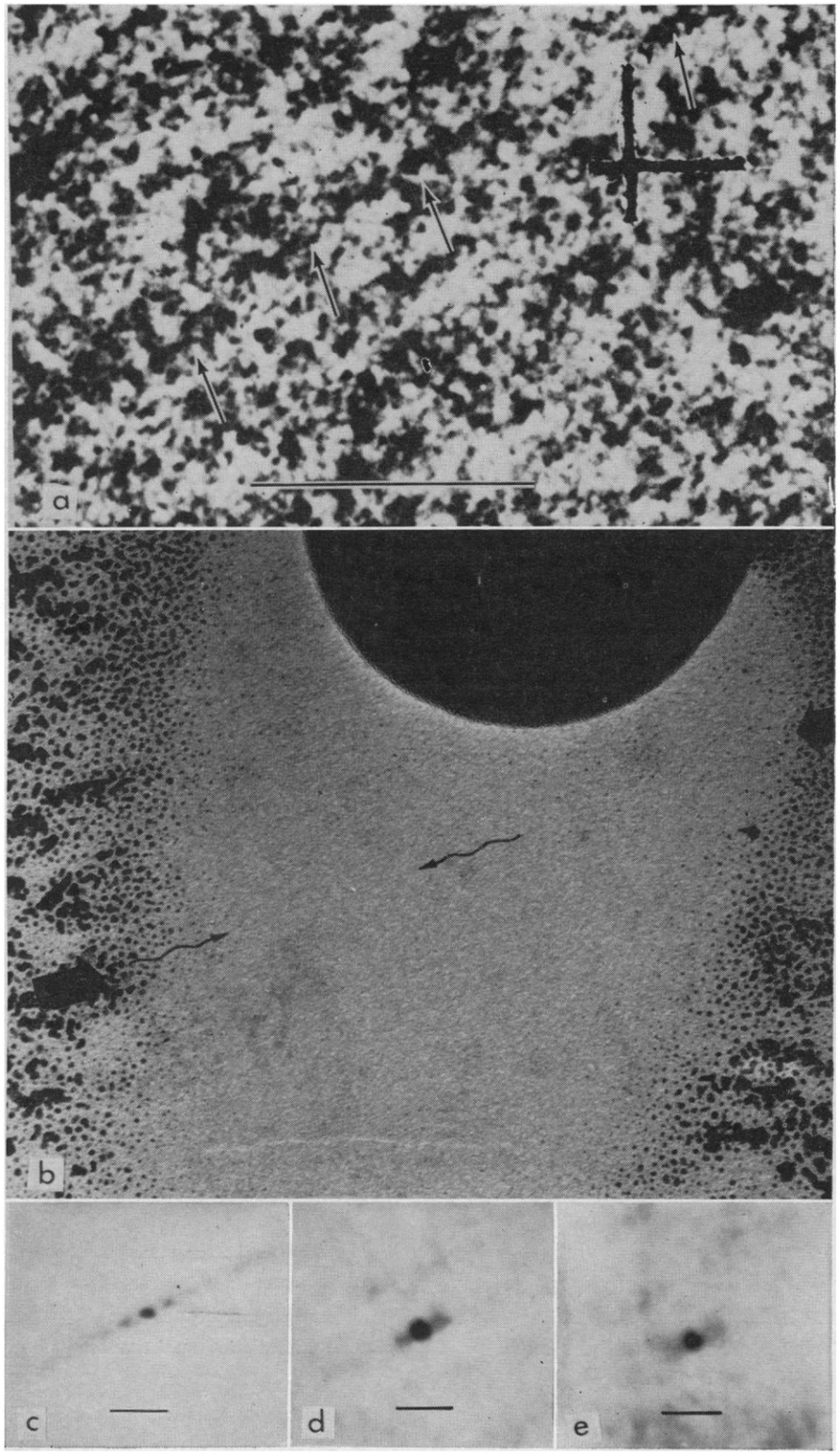


Fig. 1. Optical arrangement for obtaining autocorrelograms of an electron micrograph (*A*). *B* is a copy of *A* at $b/(a+b)$ times the magnification, and oriented parallel to it. *C* is an unexposed photographic emulsion. The system is illuminated from the left with a diffuse source. All rays through corresponding points of *A* and *B* converge on the optical axis at *C*.

ments as because of the "noise" (*3*)—the density variations in the final micrograph that are not related to the structure of the specimen. The noise arises from irregularities in the specimen support, the finite number of electrons contributing to the density of an image point, and photographic grain. Several procedures have been devised for extracting periodic information from noisy micrographs (*4*). In our study we examined the optical autocorrelation functions of the electron micrographs; this method was chosen since it can be readily extended to determination of cross-correlation functions of different noisy micrographs of identical structures. Such a procedure is considered important for molecular microscopy; a discussion will be published elsewhere.



Preparations of extended single polynucleotides were obtained by a modification of the procedures of Highton and Beer (*1*). The polymer was heated in excess ethylenediaminetetraacetate for 30 minutes at 55°C, and then dialyzed exhaustively against Tl_2CO_3 buffer at *p*H 8.5. Then $Tl_2HgHDA(OH)$ was added to a concentration of $5 \times 10^{-3} M$, and the solution was equilibrated for 1 hour at 24°C. Preparations of single extended polynucleotide chains were obtained by streaking the carbon-coated grids over the surface of this solution. The grids were sprayed with 0.188-μ polystyrene spheres in ethanol, and shadowed at an angle of 7:1 in a direction perpendicular to the direction of streaking.

Electron micrographs ($\times$ 160,000) were taken with a Siemens Elmiskop, with astigmatism corrected to less than 0.05 μ. The magnification was calibrated with polystyrene spheres checked against replica gratings; the results were reliable within 5 percent.

Good distributions of strands, presumably single polynucleotide chains with diameters corresponding to 7 to 12 Å, were obtained from solutions of 1.5 mg of polymer per milliliter of reaction solution. Many of these strands could be observed crossing the shadows of the polystyrene spheres, where the visibility must be attributed to the density of the stained molecule (*5*). In these regions the contrast is low and the noise resulting from the background is so high that the structure of the strand cannot be discerned.

A fixed internucleotide distance would lead to structural features that repeat; thus it was of interest to test whether the faintly visible strands had an underlying regularity that was obscure be-

Fig. 2. (a) Electron micrograph of polyuridylic acid stained with Tl_2HgHDA. Arrows indicate stained strand; scale, 50 Å. (b) Original micrograph, with large arrows showing direction of strand inferred from platinum shadow by independent observers. Small arrows indicate area enlarged in (a); sphere diameter, 2500 Å. (c) Autocorrelograph of a strip in the electron micrograph that includes the molecule; scale, 20 Å. (d) Autocorrelograph of whole electron micrograph; scale 20 Å. (e) Autocorrelograph of whole plate except a band containing the molecule; scale, 20 Å.

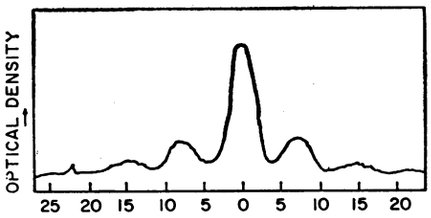


Fig. 3. Trace of autocorrelograph shown in Fig. 2c.

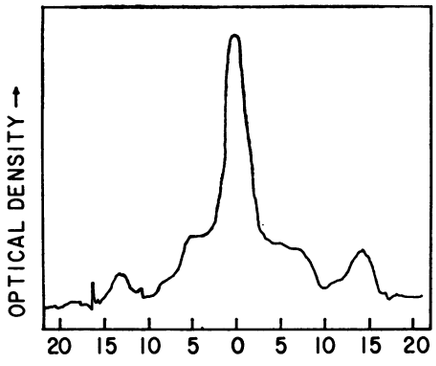


Fig. 4. Trace of autocorrelograph obtained with polyuridylic acid stained with Tl_2CO_3.

cause of the noise. To do this we examined the optical autocorrelation functions of images of the strands.

Formally an autocorrelation function of a two-dimensional transmission function $T(x,y)$ is

$$\Phi(\xi,\eta) = \int_{-\infty}^{\infty}\int_{-\infty}^{\infty} T(x,y)T(x+\xi,y+\eta)\,dxdy$$

Physically it can be considered a measure of the similarity of two patterns, one obtained from the other by a displacement

$$\vec{\xi i} + \vec{\eta j}$$

without rotation. The measure is the average value of the *product of the transmissions* of the displaced and undisplaced pattern. Clearly the autocorrelation of a periodic pattern will peak at displacements corresponding to periodicities in the original pattern. Even if the transmission function is the sum of a periodic and a nonperiodic contribution, the autocorrelation function will have these characteristic peaks, but with lower contrast. This is the basis of the present application, in which autocorrelation functions of noisy micrographs were examined for evidence of underlying periodicity.

The autocorrelation of photographic plates is readily obtained by measurement of the transmission of light through two identical copies, as a function of the displacement of one relative to the other, since *the transmitted intensity is proportional to the product of their respective transmissions.* Here it is obtained by an elegant reported procedure (*6*) in which the plate to be analyzed (Fig. 1, *A*) and an identical one (*B*), at $1/M$ times the magnification, are placed parallel to each other a distance a apart. An unexposed photographic emulsion (*C*) is placed parallel to the others and a distance b from *B* on the side away from *A*, where $(a + b) = bM$. The intensity at the unexposed plate of diffuse light, transmitted first through *A* and then through *B*, is directly proportional to the two-dimensional autocorrelation function of the photograph.

Figure 2a is a portion of an electron micrograph of polyuridylic acid deposited from a solution of the $Tl_2HgHDA(OH)$. The contrast of the granularity of the background is high, and the stained polynucleotide chain (Fig. 2a, arrows) is not easily detected. However, the autocorrelograph (Fig. 2d) clearly shows peaks at displacements of about 8 Å in a direction parallel to the chosen strand. It is easily demonstrated that the spacing is associated with the chosen strand, since the peak is lost when the strand is masked on both transparencies (Fig. 2e). When all of the plate is blocked out except for a narrow strip containing the polynucleotide chain, the autocorrelograph shows remarkably clear peaks, regularly spaced (Fig. 2c); a densitometer trace indicates that the spacing is 8 Å (Fig. 3).

The autocorrelograph of an equivalent area adjacent and parallel to the strand showed no frequent spacing; nor did micrographs of grids streaked on $Tl_2HgHDA(OH)$ without polynucleotide. Strands of polyuridylic acid, deposited in the presence of $10^{-2}M$ Tl_2CO_3 buffer, gave autocorrelation functions that though less distinct had nearly the same periodicity of 7 to 8 Å (Fig. 4).

Thus it appears that polyuridylic acid stained with Tl_2HgHDA, or simply as the Tl salt, when deposited by our procedures exhibits a structural periodicity of about 8 Å. This spacing probably corresponds to the internucleotide distance in the extended polynucleotide chain. In agreement with these results, recent results with yeast alanine transfer-RNA also point to an 8-Å internucleotide spacing in extended polynucleotide chains (*7*).

Finally, molecular models indicate that 8 Å is about the maximum possible internucleotide spacing; the fact that this is also the observed average spacing implies that the strands are fully extended.

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Gallstone of Unusual Composition: Calcite, Aragonite, and Vaterite

Abstract. *A gallstone of almost perfect octahedral symmetry was composed of a mixture of crystallites of the three polymorphous forms of calcium carbonate: calcite, aragonite, and vaterite.*

The crystalline material present in an unusual gallstone (*1*) was determined by sampling different regions of the stone and identifying the substances by the

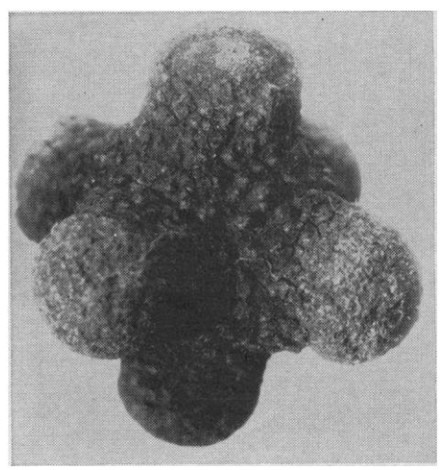

Fig. 1. The gallstone; the greatest dimension is 2 cm.