verted, in soil, to their epoxides (*1, 2*), the soil samples were analyzed by gas-liquid chromatography. The soil sample (25 g) was extracted with 10 percent acetone in redistilled petroleum ether, b.p. 30° to 60°C, in a high-speed blender. The supernatant liquid was removed by filtration and the residue was re-extracted two more times. The acetone was removed from the combined extracts by washing with water, and the hydrocarbon layer was dried over sodium sulfate. Direct anaylsis of this extract was impossible because of the presence of numerous electron-capturing co-extractives. Purification was effected by adsorption chromatography on a mixture of 10 g of Florisil and Celite 545 (5:1 by weight) containing 4.5 percent of water. Aldrin and heptachlor were eluted with petroleum ether (40 ml), further elution with 25 percent benzene petroleum ether (80 ml) removed heptachlor epoxide and dieldrin. Both fractions were adjusted to a final volume of 50 ml (1 ml of extract is equivalent to 0.5 g of soil). Recoveries were established by adding known amounts of pesticides to untreated soil.

Gas-liquid chromatographic analysis with an electron-capture detector showed that conversion of aldrin and heptachlor to their epoxides was virtually complete (Table 1). The results indicate that a single application of aldrin and heptachlor to soil under continuous cultivation and cropping may leave toxic residues for at least 9 years. Considering the amount of toxic residue it is not surprising that there was no reinfestation. In view of these results recommendations for retreatment of land should be critically reviewed.

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References

1. C. A. Edwards, S. D. Beck, E. P. Lichtenstein, *J. Econ. Entomol.* **50**, 622 (1957).
2. N. Gannon and J. H. Bigger, *ibid.* **51**, 1 (1958).

2 December 1963

Actinomycin D as a Probe for Nucleic Acid Secondary Structure

Abstract. *The binding of actinomycin D to DNA results in an increase in the temperature at which thermal dissociation of the DNA occurs. With this enhancement of thermal stability as a criterion for binding, it is shown that complementary RNA and DNA-RNA hybrid do not bind actinomycin D. This result can be interpreted to mean that these polynucleotides have helical conformations which differ from that of DNA.*

There are a number of physical and chemical methods for estimating the degree of order of polynucleotides in solution. These include the measurements of ultraviolet absorbancy and optical rotation (*1*); reactivity toward formaldehyde (*1, 2*), ribonuclease (*3, 4*), and polynucleotide phosphorylase (*1, 5*); and measurement of low-angle x-ray scattering (*6*). None of these has been completely satisfactory in the case of RNA, and the search for independent methods continues.

The drug actinomycin D (AMD) has been shown to bind to DNA (*7, 8*), provided the DNA contains guanine (*9*). Actinomycin interacts in solution with deoxyguanosine and guanosine, to a lesser extent with adenosine, and not at all with other nucleosides (*10*). Hamilton *et al.* have found that, in fibers drawn from AMD-DNA complexes, the conformational transition B → A that normally occurs in DNA fibers as the humidity is lowered is prevented (*11*). They proposed a model for the structure of the complex in which the chromophore of actinomycin D is hydrogen bonded to the amino group of guanine and to the ring oxygen of deoxyribose, and the cyclic peptides of AMD lie along the narrow groove of the DNA helix. One of the features of this model is that the stereo-chemical requirement for binding actinomycin D have the helix in the B conformation, that is, with the plane of the base pairs perpendicular to the helix axis, and the deoxyribose-ring oxygen 1.7Å above the plane of the base pair. The importance of this feature is that the requirement for binding becomes structural as well as chemical; in view of Kersten's finding that AMD interacts with guanosine in solution, it becomes reasonable to look for AMD binding to RNA molecules which might be expected to have helical regions in the B conformation.

In order to detect AMD binding sites in DNA-RNA hybrid in the presence of an unknown amount of denatured and native DNA, it was necessary to find a method of determining AMD binding which measured a property of the polynucleotide, rather than of AMD itself. Binding of AMD has previously been measured by the inhibition of its biological activity against *Staplyococcus aureus* (*7*), by equilibrium dialysis (*7, 8*), by inhibition of DNA-primed enzymic reactions (*9, 12*), and by changes in its absorption spectrum (*7, 8, 13*). In view of the proposed model for the complex, in which AMD essentially locks guanine and deoxyribose in the stereo-chemical relationship characteristic of the B helix, it was reasonable to expect that AMD would increase the stability of the DNA helix in solution, reflected in a higher temperature at which transition to the randomly coiled form occurs. This prediction was independently made and verified by Reich (*14*).

The effect of AMD on the thermal dissociation of DNA, measured at the ambient temperature (*d*-curve, for dissociation) and after quenching in ice and re-equilibration at 25°C (*i*-curve, for irreversible dissociation) (*15*), is shown in Fig. 1 for two different DNA's. First to be noted is that the expected effect of AMD upon the *d*-curves is observed; the mid-point of the thermal dissociation, $T_{1/2,d}$, is raised 4.5°C at a nucleotide/AMD ratio of 25 (Fig. 1*a*) and 7.5°C at a nucleotide/AMD ratio of 10 (Fig. 1*b*). Second, $T_{1/2,i}$ is similarly raised by AMD, but the effect is greater, being 6.0°C and 12.1°C in Fig. 1*a* and 1*b*, respectively. Geiduschek has noted that for a series of DNA's, the difference between $T_{1/2,d}$ and $T_{1/2,i}$ depends upon the guanine-cytosine (G-C) content of the DNA, which is greater the lower the percentage of G-C (*15*). This dependence has been interpreted in terms of G-C nuclei in individual DNA molecules, which persist as small ordered regions when the remainder of the molecule has dissociated, and then serve to keep the strands in register during their reassociation when the temperature is lowered.

If AMD is specific for guanine, then Geiduschek's interpretation of the G-C dependence of the difference between

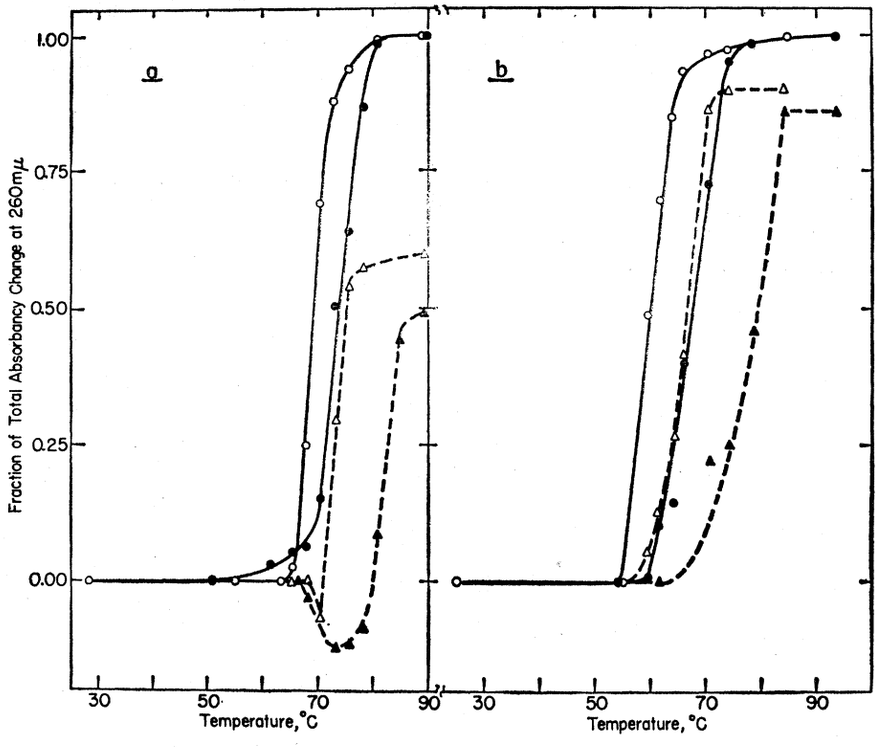

Fig. 1. *a*, Thermal dissociation of phage alpha DNA (G = 21 percent) in 0.01*M* PO_4, *p*H7. Solid lines are *d*-curves and dashed lines *i*-curves (see text). Open symbols are without AMD and filled symbols with AMD, 10 μg/ml. *b*, Thermal dissociation of Osaka phage DNA (G = 21 percent) in 0.01*M* PO_4, *p*H 7; AMD concentration is 25 μg/ml.

$T_{1/2, d}$ and $T_{1/2, i}$ predicts that AMD should enhance this difference. This is precisely what is observed. However, alternative interpretations cannot be excluded; this experiment taken alone merely indicates that sites at which AMD is bound act as nuclei in the sense described.

Actinomycin D has no effect on the thermal dissociation of *Escherichia coli* sRNA (soluble RNA), as shown in Fig. 2. The sRNA was prepared by phenol extraction of the supernatant of an extract of *E. coli* centrifuged for 9 hours at 38,000 rev/min; it is contaminated with a small amount of DNA, as can be seen in the AMD-dependent dissociation at 73°C. This is the expected result, since sRNA has been shown to have the A-helix conformation *(16, 17)*. These experiments establish the desired alternative measure of AMD binding, judged by the dissociation of the secondary structure of nucleic acid. We now turn to the application of this method to the determination of secondary structure in ordered complementary RNA and RNA-DNA hybrids.

The test was first applied to complementary RNA (cRNA) of bacteriophage T7. This RNA is made by the enzyme RNA polymerase from *Micrococcus lysodeikticus*, with DNA from bacteriophage T7 as primer *(17)*. The base composition of this RNA is the same as that of the DNA primer, and after suitable annealing (4 hours at 59°C in 0.3*M* sodium chloride; 0.03*M* sodium citrate; RNA concentration, 120 μg/ml) the structure is mostly helical *(18)*. The thermal dissociation of T7 cRNA in 0.03*M* sodium chloride and 0.003*M* sodium citrate is shown in Fig. 3. It occurs at an extremely high temperature for this solvent ($T_{1/2, d}$ = 80°C) and shows a considerable degree of irreversibility, both characteristic of highly ordered polynucleotides *(4)*. Actinomycin D has no effect on either the *d*- or *i*-curves, and so the cRNA does not contain B-helical regions. In a sense this too is an expected result, since this cRNA may be a model for the hypothetical replicating form of RNA-viruses, and AMD has no effect on their multiplication *(19)*.

Finally, the test has been applied to a DNA-RNA hybrid. For this purpose DNA from bacteriophage alpha was used as primer with RNA polymerase from *M. lysodeikticus* in a reaction mixture which included UTP^{32} (uridine triphosphate). After 30 minutes incubation at 30°C, the reaction mixture was chilled and shaken three times with an equal volume of water-saturated phenol. The final aqueous phase was dialyzed extensively against 0.3*M* so-

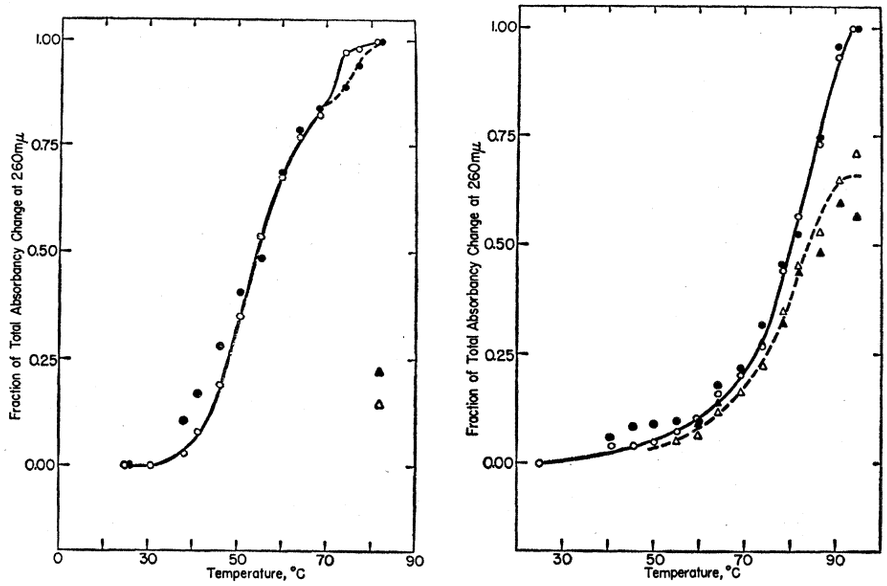

Fig. 2 (left). Thermal dissociation of *E. coli* sRNA in 0.01*M* PO_4, in the absence (○) and the presence (●) of 25 μg of AMD per milliliter. Triangles indicate readings taken at 25°C after quenching the samples heated to 82°C. Note the slight amount of contaminating DNA reflected in AMD-dependent dissociation at 73°C. Fig. 3 (right). Thermal dissociation of cRNA of bacteriophage T7 in 0.03*M* sodium chloride, 0.003*M* sodiom citrate, *p*H 7, in the absence (○, △) and the presence (●, ▲) of 25 μg of AMD per milliliter.

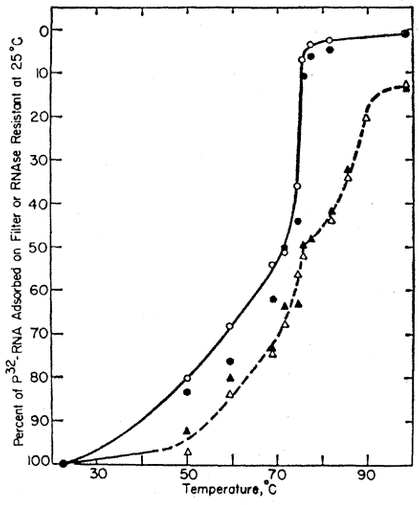

Fig. 4. Thermal dissociation of bacteriophage alpha DNA-RNA hybrid and cRNA, in 0.01*M* PO_4, *p*H 7. The solid line shows the percentage of P^{32}-RNA absorbed by the filter after heating to a given temperature and then cooling rapidly, in the absence (○), and presence (●) of 25 μg of AMD per milliliter. The dashed line shows the percentage of ribonucelase resistant P^{52}-RNA after heating and cooling in the absence (△) and presence (▲) of 25 μg of AMD per milliter.

dium chloride and then against 0.15*M* sodium chloride and 0.015*M* sodium citrate. In the latter solvent, hybrid was made by mixing 26 μg RNA and 160 μg DNA from bacteriophage alpha, and heating for 5 minutes at 100°C; the reaction mixture was rapidly cooled and then annealed at 52°C for 4 hours. Approximately 65 percent of the RNA in the mixture was resistant to ribonuclease; of this resistant fraction, two-thirds was RNA-DNA hybrid and one-third was ordered (helical) RNA. Thermal dissociation of these RNA complexes was measured in two ways. Samples of the annealed mixture were diluted 40-fold by a solution of 0.01*M* phosphate, *p*H 7, with and without AMD, heated to a given temperature for 10 minutes, quenched in ice, and then subjected to the following tests. In the first, the sample is made 0.01*M* in Mg^{++}; then 1 μg of pancreatic ribonuclease per milliliter is added, and the whole is incubated for 10 minutes at 37°C. The sample is chilled, precipitated with 5 percent trichloroacetic acid (TCA), collected on a Millipore filter, and counted. The radioactivity (count/min) in the acid-precipitable fraction represents UMP^{32} (uridylic acid) in the ribonuclease-resistant RNA, which includes both RNA-DNA hybrid and ordered RNA. A second sample, after quenching, is diluted further with 0.5*M* potassium chloride and passed through a coarse paper filter, according to a procedure developed by Hall and Nygaard (*20*). The filter selectively retains RNA-DNA hybrid, which is measured by the radioactivity due to the UMP^{32} in the RNA.

The result of this experiment is shown in Fig. 4. The percentage of P^{32}-RNA trapped by the filter, and consequently a measure of hybrid dissociation, is not affected by AMD. The graph of the percentage of P^{32}-RNA which is ribonuclease-resistant reveals two dissociations, one occurring at the temperature at which hybrid dissociates, and a second about 10°C higher. This second dissociation corresponds to the temperature of irreversible dissociation of cRNA in this solvent. Again, neither dissociation is affected by AMD, as measured by properties of the RNA molecules themselves. The result with DNA-RNA hybrid is of special significance, since it indicates that the DNA strand in hybrid is in a conformation that cannot bind AMD. It is already known that one helical conformation does not bind AMD, the A helix, of which sRNA is a representative. It is tempting to suggest that DNA-RNA hybrid, as well as ordered complementary RNA, are in the A-helical conformation.

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References and Notes

1. P. Doty, H. Boedtker, J. Fresco, R. Haselkorn, M. Litt, *Proc. Natl. Acad. Sci. U.S.* **45**, 482 (1959).
2. R. Haselkorn and P. Doty, *J. Biol. Chem.* **236**, 2738 (1961).
3. G. Kalnitsky, J. P. Hummel, H. Resnick, J. R. Carter, L. B. Barnett, C. Dierkes, *Ann. N.Y. Acad. Sci.* **81**, 542 (1959).
4. E. P. Geiduschek, J. Moohr, S. B. Weiss, *Proc. Natl. Acad. Sci. U.S.* **48**, 1078 (1962).
5. M. Grunberg-Manago, *J. Mol. Biol.* **1**, 240 (1959).
6. S. N. Timasheff, J. Witz, V. Luzzati, *Biophys. J.* **1**, 525 (1961).
7. J. M. Kirk, *Biochim. Biophys. Acta* **42**, 167 (1960).
8. H. M. Rauen, H. Kersten, W. Kersten, *Z. Physiol. Chem.* **321**, 139 (1960).
9. I. H. Goldberg, M. Rabinowitz, E. Reich, *Proc. Natl. Acad. Sci. U.S.* **48**, 2094 (1962). J. Hurwitz, J. J. Furth, M. Malamy, M. Alexander, *ibid.*, p. 1222.
10. W. Kersten, *Biochim. Biophys. Acta* **47**, 610 (1961).
11. L. D. Hamilton, W. Fuller, E. Reich, *Nature* **198**, 538 (1963).
12. I. H. Goldberg, M. Rabinowitz, E. Reich, *Proc. Natl. Acad. Sci. U.S.* **49**, 226 (1963).
13. E. Reich, I. H. Goldberg, M. Rabinowitz, *Nature* **196**, 743 (1962).
14. E. Reich, *Science*, this issue.
15. E. P. Geiduschek, *J. Mol. Biol.* **4**, 467 (1962).
16. M. Spencer, W. Fuller, M. H. F. Wilkins, G. L. Brown, *Nature* **194**, 1014 (1962).
17. T. Nakamoto, F. Fox, S. B. Weiss, *J. Boil. Chem.*, in press.
18. E. P. Geiduschek and S. B. Weiss, personal communication.
19. E. Reich, R. M. Franklin, A. J. Shatkin, E. L. Tatum, *Science* **134**, 556 (1961); ———, *Proc. Natl. Acad. Sci. U.S.* **48**, 1238 (1962).
20. A. Nygaard and B. D. Hall, *Biochem. Biophys. Res. Commun.* **12**, 98 (1963).
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Actinomycin: Correlation of Structure and Function of Its Complexes with Purines and DNA

Abstract. *Actinomycin inhibits strand separation of helical DNA at concentrations corresponding to those which inhibit DNA polymerase activity in vitro. This report proposes first that actinomycin affects DNA polymerase indirectly by way of the changed physical properties of the template and that it functions in the major groove of helical DNA; second, that RNA polymerase functioning in the minor groove is subject to direct steric inhibition by bound actinomycin; and third, that the reaction of actinomycin with free purines is unrelated to its reaction with DNA.*

Actinomycin (AM) (Fig. 1) is a bacteriostatic, tumor-inhibiting chromopeptide antibiotic which inhibits the syntheses of nucleic acids by intact organisms and by enzyme preparations. These effects are of interest in part because they are useful in the study of gene action and related problems, but perhaps more because of the selectivity and specificity of action of this unusual compound. Low concentrations of AM selectively suppress cellular (*1, 2*) and enzymic DNA-directed RNA synthesis (*3, 4, 5*), DNA synthesis being affected only at much higher concentrations (*1, 2, 5*). The inhibition of RNA