2*d* on the other can be regarded as cases where either the 6-position is completely occupied by $O_2$ molecules or is not occupied by $O_2$ molecules. The question whether the 6-position is empty or is occupied by $H_2O$ molecules (*4*) is still unanswered. A characteristic feature of the spectra in Figs. 2*c* and 2*d*, corresponding to the hemoglobin without an $O_2$ ligand, is the different intensity of the two lines. Similar behavior has been previously reported and has been attributed to either a preferential orientation of a polycrystalline source or absorber where the crystal *c*-axes and the direction of observation is not random (*5*) or to a directionally dependent Debye-Waller factor (*6*). In blood one does not have a preferential orientation. However, an asymmetry can be expected if the fundamental vibrational frequencies of the iron bound in the ring are directionally dependent. If we assume that the fundamental vibrational frequency is higher "in-plane" than "out-of-plane" and then use the known positive sign of the nuclear quadrupole moment for the 14.4 kev state (*7*) of $Fe^{57}$, we can conclude that the weaker line centered at + 1.7 mm/sec corresponds to the two absorption lines $+1/2 \rightarrow +3/2$, $-1/2 \rightarrow -3/2$ and that the stronger line centered at − 0.6 mm/sec corresponds to the four absorption lines $\pm 1/2 \rightarrow \pm 1/2$; and thus the sign of the electric-field gradient is positive for the spectra in Figs. 2*c* and 2*d*. In summary, the quadrupole splitting, and thus the magnitude of the electric-field gradient in the spectra of Fig. 2, *a–d*, is the same although there is a considerable difference in isomeric shift between the two basically different spectra.

The spectrum of Fig. 2*e* was taken with blood which was exposed to a CO atmosphere. The isomeric shift observed in this case is similar to $O_2$-hemoglobin; however, no quadrupole splitting was observed indicating the absence of a sizable electric-field gradient. The CO-hemoglobin absorption spectrum demonstrates the ease with which a change of the hemoglobin ligand can be detected and points out the fact that recoil-free nuclear resonance absorption could conceivably become an interesting biological tool.

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## Toxic Residues in Soil 9 Years after Treatment with Aldrin and Heptachlor

Abstract. *In silt loam soil heavily infested with European wireworm, a single treatment with aldrin or heptachlor prevented reinfestation for 9 years, even when the soil was under continuous cultivation. Toxic residues were determined by bioassay with* Drosophila melanogaster *Meig. By gas-liquid chromatographic analysis, the residues were found to be mainly dieldrin and heptachlor epoxide.*

In 1953 a long-term experiment was set up on the control of the introduced European wireworm, *Agriotes obscurus* (L.), at Agassiz, British Columbia. The experiment included seven chemical treatments and untreated controls, replicated three times. Each plot was 9 by 18 m. The materials were applied as dusts or sprays to the surface and immediately worked into the soil to a depth of 15 cm by plowing and disking. No further treatments were made. Crops were grown as follows: 1953, potatoes; 1954, oats, peas, and vetch; 1955, oats, peas, and clover; 1956 to 1958, clover; 1959 and 1960, corn; 1961, peas. The wireworm population, determined by sifting untreated soil every year for 7 years, averaged 130 per square meter.

Aldrin and heptachlor at 5.6 kg toxicant per hectare were the most effective treatments. Immediate protection was given to potatoes and complete control was obtained by the 2nd year. No reinfestation occurred. Reinfestation did occur within 5 years in plots treated with aldrin at 2.8 kg, heptachlor at 3.4 kg, and DDT at 16.8 kg per hectare.

In January 1962, 20 laboratory-reared, 7-month-old larvae of *A. obscurus* were put in soil from the plots treated with aldrin dust, aldrin emulsion, and heptachlor dust, at 5.6 kg toxicant per hectare and from the untreated control. The wireworms were immobilized and stopped feeding soon after being placed in the treated soil but remained healthy looking for periods up to 6 weeks. After that time they were unable to burrow into the soil when they were left on the surface and soon became desiccated. The wireworms in the untreated soil burrowed immediately and survived.

The soil treated with aldrin and heptachlor was analyzed further to determine the toxic residue remaining after 9 years of cropping. Thirty 15-cm cores, 10 cm in diameter, were taken, ten from each plot, and were mixed by tumbling and screening. *Drosophila melanogaster* Meig. was the test insect used for bioassay. The method was based on that of Edwards *et al.* (*1*), with modifications that we had developed. Four-gram samples of air-dried soil were weighed into 22-ml bottles, wetted with ¾ ml of a boiled mixture of one part of apple juice to nine parts distilled water. Four or five dosages were used in each run and each dosage was replicated eight times. Untreated soil was included in each assay to correct for natural mortality. Twenty-five flies, from 1 to 3 days old, were aspirated into each jar and left for 24 hours in a climate chamber at 26°C under constant light. Dead and moribund flies were counted.

Since aldrin and heptachlor are con-

Table 1. Recoveries of toxic residues from silt loam soil 9 years after treatment with aldrin or heptachlor, applied at 5.6 kg/hectare in 1953.

| Pesticide (2.5 ppm) | Pesticide residues found in 1962 (ppm) | | |
|---|---|---|---|
| | | GLC* | Bio-assay† |
| Aldrin dust | Aldrin | 0.005 | 0.230 |
| | Dieldrin | .098 | |
| Aldrin emulsion | Aldrin | .006 | .175 |
| | Dieldrin | .153 | |
| Heptachlor dust | Heptachlor | .009 | .317 |
| | Heptachlor epoxide | .169 | |

* Wilkens Hi-FI gas chromatograph with electron capture detector at a potential of 20 volts. Injector temperature 210°C, column 0.318 cm × 0.6 m aluminum packed with 5 percent Dow Silicone II on Chromosorb W60–80 mesh, at a temperature of 137°C for the heptachlor fraction, and 153°C for the other fractions. Inlet pressure 0.7 kg/cm², carrier gas nitrogen. Output sensitivity 10 times; attenuation 2 times; 5-μl injections used throughout. † Against dieldrin and heptachlor expoxide standards.

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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## 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