

substances cause misreading in vitro and phenotypic suppression in vivo (1, 3, 10). Furthermore, resistance to these drugs of the Drug^D mutants is converted into sensitivity when combinations of drugs are used, unless the genome carries another mutation to resistance in addition to the Drug^D mutation. Indicative of this is the contrast between Sm^SDrug^D and Sm^R-Drug^D strains, the first being sensitive and the second resistant to the mixture of streptomycin and paromomycin. Thus Drug^D mutants possess an altered ribosome too restricted to be functional per se, but responsive to the antirestrictive action of drugs that introduce ambiguity. This responsiveness is a delicate matter, however, and may result in killing instead of permitting growth, if limits in the amount or quality of the introduced ambiguity are surpassed. This specificity of required ambiguity is further illustrated by the fact that kanamycin, which is able to produce misreading in vitro (3) and to which the cells are resistant, is unable to support growth of these Drug^D mutants.

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15. This work was supported by an American Cancer Society grant E-226E and USPHS-National Institute of Allergy and Infectious Diseases grant 5 RO1 A102011-10. For skillful technical help we thank Mrs. C. Herbert and Miss C. B. Steinkuller. We thank Dr. H. E. Machamer of the Parke, Davis Co. for paromomycin and Dr. H. L. Dickison of the Bristol Laboratories for kanamycin sulfate.

24 July 1967

Electron Spin Resonance of Gamma-Irradiated Oriented DNA Prepared by Wet Spinning

Abstract. When moist, oriented DNA is gamma-irradiated and its electron spin resonance spectrum is recorded at 77°K, an unresolved spectrum is obtained, the amplitude of which is strongly dependent on the angle between the direction of the DNA helices and the field. Annealing at 199°K gives an eight-line thymine-like spectrum which also has a marked angular dependence. For dry, oriented DNA, the unresolved spectrum dominates even at room temperature, and the spectra exhibit lower degrees of anisotropy.

The recent development of a new, wet-spinning method for the preparation of oriented DNA (1) has made possible physicochemical studies of oriented DNA with various techniques. Studies on the semiconductivity of dried, oriented DNA (2) and the hydration of moist, oriented DNA with nuclear magnetic resonance (3) have been made. We now report some results from electron spin resonance (ESR) measurements on oriented calf-thymus DNA irradiated with γ -rays.

Our DNA samples were prepared in a humid atmosphere as follows. A 10-mm-wide film of oriented DNA was folded back and forth (perpendicularly to the molecular orientation) to form a concertina-like pack which was slightly compressed into a package of oriented DNA having a parallelepipedal form with the approximate dimensions 2 by 2 by 10 mm. After the sample was equilibrated with a desired relative humidity, it was instantly frozen in liquid nitrogen and irradiated with cobalt-60 γ -rays to a dose of 0.45 megarad. The sample was thereafter mounted between two parallel, thin Teflon plates of a long forklike holder which was inserted into a 3-mm quartz tube fitting the Dewar flask of the ESR spectrometer. These and subsequent manipulations were performed in liquid nitrogen. The quartz tube was repeatedly evacuated and flushed with nitrogen to remove oxygen from the sample and finally evacuated. The tube was connected to an angular scale, and it could be rotated in the Dewar to obtain any desired angle between the DNA helices and the magnetic field. The ESR spectra (first derivative) were recorded with a Varian, X-band spec-

trometer having a modulation unit of 100 kc/sec. The microwave power was only about 0.3 mw; nevertheless, it was sufficient to cause some saturation of the absorption studied.

Moist lithium DNA and sodium DNA samples (Worthington, batch 6HE) at 77°K gave an unresolved spectrum, the amplitude of which was strongly dependent on the angle between the direction of the DNA helices and the field. Annealing of the samples at 199°K for 5 minutes and recording at 77°K gave an eight-line thymine radical-like spectrum which also showed a marked angular dependence and indicated an anisotropy in the hyperfine interaction. The g -values (spectroscopic splitting factors) of the spectra also varied slightly with angle. Figures 1 and 2 give some results for a lithium DNA sample stored at 66 percent relative humidity before irradiation. The corresponding curves for a sodium DNA sample stored at 75 percent relative humidity indicated a slightly smaller anisotropy. The angular dependence observed is real and not due to experimental artifacts. This was tested by studying a polycrystalline isotropic sample of γ -irradiated sucrose under the same experimental conditions; no angular dependence was observed. The ESR signals from oriented sodium DNA, dried in a vacuum at 70°C for 4 days, exhibited a smaller angular dependence than those of the wet samples, and the annealing transition to a thymine-like spectrum was almost completely inhibited (Fig. 3). Later annealing and recording at room temperature seemed to increase slightly the size of the satellites, but the anisotropy was further diminished. A less extensively dried sample gave a higher portion of satellite structure in the spectra. The shape and transformation of the ESR absorption described is in general agreement with several ESR studies of unoriented DNA (4-8), but the angular dependence now observed is a new feature of the absorption.

Little is known about the sites of the unpaired electrons giving the unresolved absorption at 77°K. Comparisons with spectra of the constituents of DNA have not been helpful (4, 5). This technique has, however, been more successful for interpreting part of the satellite spectra which was found to exhibit similarities with the spectra of thymine and thymidine (5-8). Salovey *et al.*, (6) suggested the radical $-\text{CH}_2-\dot{\text{C}}(\text{CH}_3)-$ as giving rise to the

satellite lines of DNA; this was supposed to be formed by reduction of a double bond in thymine on addition of a hydrogen atom. Simultaneously, Eisinger and Shulman (9) reported that ultraviolet irradiation of DNA gave a high yield of satellite structure even at 77°K and suggested the same radical as the main product of ultraviolet irradiation. They gave further evidence for the proposed radical (10), and the hydrogen addition was proved by the use of a sample containing D₂O.

Our spectra obtained from moist, oriented DNA after annealing at 199°K are in good agreement with the thymine-like spectra reported for moist DNA ultraviolet-irradiated at 195°K (10). The low yield of the thymine radical in our dry samples indicates

that, also in γ -irradiated DNA, water plays an essential role in the transformation to this radical. A study of oriented DNA containing D₂O might elucidate the mechanism of formation of the thymine radical.

The moist lithium DNA existed in the B form with the base pairs stacked perpendicularly to the helix axis (11), whereas the moist sodium DNA was in the A form where the base pairs are tilted about 20° from perpendicular position (12). The thymine-like spectrum of lithium DNA showed a slightly higher degree of anisotropy than the corresponding sodium DNA spectrum, indicating that these configurations are essentially preserved on cooling to 77°K.

The unresolved spectrum at 77°K

also showed a higher degree of anisotropy for lithium DNA. This suggests that the unpaired electrons giving the unresolved spectrum also are located mainly on DNA bases. The *g*-values observed give further support for this suggestion. They are slightly smaller for the spectra with helices parallel to the field, as expected for radicals with the unpaired electron in a *p*-orbital on DNA bases (13). The smaller amplitude change with angle observed for dry DNA is also in agreement, taking into account the great structural changes within the helices which have been reported to occur on drying of DNA, probably involving breakage of the interbase hydrogen bonds (14). These changes are reversible even after drying in a vacuum below 80°C (15),

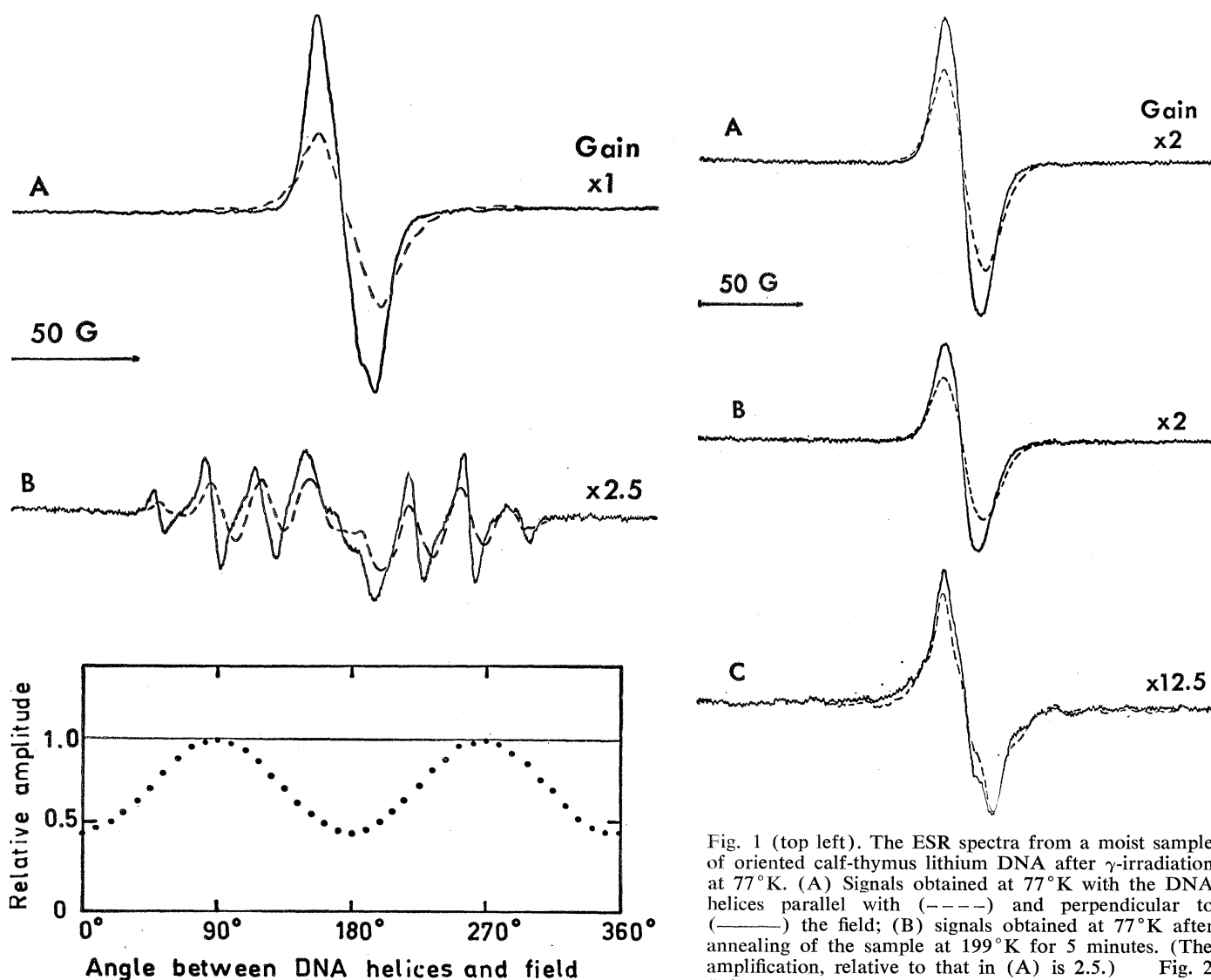


Fig. 1 (top left). The ESR spectra from a moist sample of oriented calf-thymus lithium DNA after γ -irradiation at 77°K. (A) Signals obtained at 77°K with the DNA helices parallel with (----) and perpendicular to (—) the field; (B) signals obtained at 77°K after annealing of the sample at 199°K for 5 minutes. (The amplification, relative to that in (A) is 2.5.) Fig. 2 (left). Relative peak-to-peak amplitude of the ESR

spectrum shown in Fig. 1A as a function of the angle between the direction of the DNA helices and the magnetic field. Fig. 3 (right). The ESR spectra from a dry sample of oriented calf-thymus sodium DNA after γ -irradiation at 77°K. (A) Signals obtained at 77°K with the DNA helices parallel with (----) and perpendicular to (—) the field; (B) signals obtained at 77°K after annealing of the sample at 199°K for 5 minutes; (C) signals obtained after further annealing and recording at room temperature. [The amplifications in (A), (B), and (C), relative to that in Fig. 1A, are 2, 2, and 12.5, respectively.]

and the orientation of the helices is preserved (16). The exact nature of the amplitude variation (Fig. 2) is, however, not quite clear at present. Measurements of the unresolved spectrum with oriented DNA containing D₂O have given greatly altered spectra with indication of partly resolved hyperfine structure; a detailed study will be helpful for the characterization of the primary radicals.

The spectra of dry oriented DNA at room temperature (Fig. 3C) differ from those reported by Elliott and Wyard (17). They studied dry, oriented calf-thymus DNA fibers which were prepared by drawing at 66 percent relative humidity and wound on a quartz plate which could be rotated and moved vertically in an ESR quartz cell (18). After x-irradiation in dry nitrogen at room temperature in the upper part of the cell, the quartz plate with sample was transferred to the lower part of the cell which was placed in the ESR cavity. The reported spectra were much narrower than ours (4 gauss rather than approximately 24 gauss), exhibited a lower amount of fine structure, and showed a great *g*-value displacement in a direction opposite to that of our spectra. For un-oriented DNA, however, they reported a thymine-like spectrum. Elliott pointed out some drawbacks with the quartz-plate holder (18) which might explain part of the differences between their results and ours. However, the disturbance from radicals formed in the quartz plate and the influence of the quartz plate on cavity-Q (quality factor) and frequency might be of greater importance, but these possible sources of error were not discussed by Elliott and Wyard (17). Gamma-irradiation in dry nitrogen at room temperature of one of our extensively dried, oriented sodium DNA samples gave spectra identical to those of Fig. 3C.

Our measurements show that the ESR signals from moist, oriented DNA, which was γ -irradiated and studied at low temperature, exhibit a strong angular dependence. Use of highly oriented DNA seems therefore to offer excellent opportunities for identifying radicals formed or annihilated when the temperature and water content are varied (5, 8). A very promising extension is suggested by the excellent work by Pershan *et al.* (10) on ultraviolet-irradiated DNA. Their measurements at various humidities and annealing temperatures indicated that at least five different radicals can be

formed in this irradiated DNA, but only the thymine radical was identified. The use of oriented DNA possibly could facilitate the identification of the other radicals. Measurements are being made on oriented DNA samples with added paramagnetic ions (19) and on complexes of oriented DNA and substances such as polyaromatic dyes (20).

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11 July 1967

Warm Fog Suppression in Large-Scale Laboratory Experiments

Abstract. *Visibility in warm fog produced in a 600-cubic-meter chamber was increased by factors of 3 to 10 by seeding with carefully sized sodium chloride particles. As little as 1.7 milligrams of salt per cubic meter was effective. Extrapolation of these results indicates that clearing a suitable landing zone for aircraft would not involve prohibitive amounts of seeding material.*

Recent experiments in a 600-m³ cloud chamber have demonstrated that visibility in warm fog can be improved by a factor of up to 10 by seeding with NaCl particles of carefully controlled size distribution. Previous investigations, notably the classic work of Houghton and Radford (1), were aimed at desiccating the atmosphere to substantially less than 100 percent relative humidity by causing condensation (and subsequent precipitation) of water on large saline droplets introduced into the atmosphere. The principal goals of our experiments were (i) to modify the drop size distribution of the fog in such a way as to reduce the Mie scattering coefficient and hence improve the visibility without necessarily altering the liquid water content, and (ii) to determine the minimum amount of dry salt particles of prescribed sizes that could significantly alter laboratory fogs.

Improved visibility results from modification of the drop size distribution in fog from the normal large number of small droplets to a few large droplets containing about the same

liquid water. When a few suitable hygroscopic nuclei per cubic centimeter are introduced into a fog they immediately deliquesce into solution droplets. The equilibrium water vapor pressure over the growing solution drops is lower than saturation relative to pure water. If the concentration of artificial nuclei is sufficient to cause the average relative humidity of the treated region to fall only a fraction of a percent, some desired redistribution of water is accomplished and visibility improved.

Our calculations based on seeding only the lowest 100 m of fog (which would apply to the airport situation) indicated that the most effective NaCl nuclei sizes are between 4 and 10 μ in diameter. Much smaller particles would not grow to sizes significantly larger than natural droplets, while larger particles would fall out of the fog before condensing sufficient water to become "dilute" solution droplets. Approximately 80 percent of the particles were between 2 and 10 μ in diameter; 65 percent were between 4 and 10 μ . The mode of the distribution was consistently between 4 and 5 μ .