mean that considerable amounts of both DNA and RNA polymerases with masked activities were in fact present in the hybrid polymerase preparation. But this seems unlikely since the three polymerase activities are obtained separately at different points in the preparation procedure. The presence of inhibitors in the purified preparation of hybrid polymerase, and their instability to storage and to the various treatments, would require rather unlikely properties for the inhibitory factors. Also, no inhibition is observed when DNA and RNA polymerase preparations are mixed. At present, therefore, we feel that the evidence points to a subunit structure.

Other observations can be explained readily by the subunit hypothesis. Since RNA polymerase is capable of copying only one chain of a DNA template, both in vivo (*9*) and in vitro (*10*), the native form would have just one active site. In contrast, DNA polymerase always synthesizes two chains simultaneously (*4*). One could, therefore, speculate that only monomeric RNA polymerase-1 and dimeric DNA polymerase are found inside the cell, the other polymerase activities being produced by dissociation and reassociation during or after isolation. The two subunits of the DNA polymerase would probably be oppositely oriented, like the polarities of the two template chains. Both DNA and RNA polymerase interact with the same template, but different sites of interaction have been suggested (*11*) by the differential action of actinomycin. The different sites may be related to the mono- or bifunctional character of the enzymes. Thus, one groove of the DNA helix may accommodate the dimeric DNA polymerase, which interacts with both chains, whereas monomeric RNA polymerase lies in the other groove.

SYLVIA LEE-HUANG
LIEBE F. CAVALIERI
Sloan-Kettering Division,
Graduate School of Medical Sciences,
Cornell University, New York 10021

References and Notes

1. M. Chamberlin and P. Berg, *Proc. Natl. Acad. Sci. U.S.* **48**, 81 (1962).
2. S. Lee-Huang and L. F. Cavalieri, *ibid.* **51**, 826 (1964).
3. Abbreviations: RNA, ribonucleic acid; DNA, deoxyribonucleic acid; poly(C+I) is a complex of polycytidylate and polyinosinate; poly(dG+C) and poly(dC+G) are complexes of polydeoxyguanylate and polycytidylate, and polydeoxycytidylate and polyguanylate, respectively; dGTP and dCTP are 5′-triphosphates of deoxyguanosine and deoxycytidine; GTP and CTP are the 5′-triphosphates of guanosine and cytidine.
4. S. Lee-Huang and L. F. Cavalieri, *Proc. Natl. Acad. Sci. U.S.* **50**, 1116 (1963).
5. L. F. Cavalieri and S. Lee-Huang, unpublished.
6. T. Nakamoto and S. Weiss, *Proc. Natl. Acad. Sci. U.S.* **48**, 880 (1962); J. S. Krakow and S. Ochoa, *ibid.* **49**, 88 (1963).
7. The existence of two forms of RNA polymerase in purified fresh preparations can be deduced from similar data (*5*); see also Nakamoto and Weiss (*6*) and Krakow and Ochoa (*6*).
8. C. Markert, *Science* **140**, 1329 (1963).
9. V. Allfrey and A. Mirsky, *Proc. Natl. Acad. Sci. U.S.* **48**, 1590 (1962); M. Hayashi, M. N. Hayashi, S. Spiegelman, *ibid.* **50**, 664 (1963); G. Tocchini-Valentini, M. Stodolsky, A. Aurisicchio, M. Sarnot, F. Graziosi, S. Weiss, E. P. Geiduschek, *ibid.* **50**, 935 (1963).
10. M. Hayashi, M. N. Hayashi, S. Spiegelman, *ibid.* **51**, 351 (1964); E. P. Geiduschek, G. P. Tocchini-Valentini, M. Sarnot, *ibid.* **52**, 486 (1964).
11. L. F. Cavalieri and B. H. Rosenberg, *Progr. Nucleic Acid Res.* **2**, 7 (1963); L. F. Cavalieri and R. G. Nemchin, *Biochim. Biophys. Acta* **87**, 641 (1964).
12. Supported in part by funds from the NCI, NIH, PHS (grant CY-3190) and the AEC [contract No. AT(30-1)-910].

5 February 1965

DNA Synthesis in Aluminum-Treated Roots of Barley

Abstract. *When cell division in barley roots is halted by treatment with aluminum, DNA synthesis continues. However, the type of DNA synthesized has an unusual base composition and is metabolically labile. A part of this labile DNA was found in the form of a hybrid composed of genetic DNA and labile DNA.*

The damaging effect of aluminum in the cationic form on root growth of many plant species (*1*) may be partly explained by an inhibition of cell division (*2*). The roots of barley are particularly sensitive to aluminum (*3*). We have treated the roots of barley, *Hordeum vulgare* var. Proctor, with aluminum sulfate ($10^{-3}M$) in water culture at *p*H 4.5. Examination of "squash" preparations of root apices stained with acetocarmine revealed that during this treatment there was a steady decline in the number of cells entering prophase, and after 24 hours mitotic figures were no longer seen (Fig. 1). The rate of cell division was not markedly affected in roots raised on a similar medium at *p*H 4.5 when no aluminum was added. The effects on the root growth of many species at this *p*H are only slight (*4*).

In the roots of wheat there are two physiologically distinct fractions of double-stranded DNA (*5*). One has a low molecular weight (2 to 3 $\times$ 10^5), and a mole percentage of guanine plus cytosine equal to 51; it accounts for some 20 percent of the total DNA in root and is metabolically labile. The other form, which we consider to be genetic DNA, has a high molecular weight (4 to 6 $\times$ 10^6) and a mole percentage of guanine plus cytosine equal to 41. This form of DNA is metabolically stable. Two such fractions have been characterized from the roots of *Zea mays* with orthophosphate-^{32}P and thymidine-^{14}C as DNA precursors (*6*).

The two forms of DNA can be separated by chromatography on a column containing methylated-albumin-kieselguhr (MAK) (*7*). The metabolically labile, low-molecular-weight DNA of wheat root can be eluted step by step with NaCl (0.5*M*) in phosphate buffer (*p*H 6.8, 0.05*M*). After these steps the metabolically stable high-molecular-weight DNA of wheat root can be eluted with NaCl (1.0*M*) in the same phosphate buffer.

We have studied the effect of aluminum treatment on the synthesis of these two DNA fractions with orthophosphate-^{32}P as a DNA precursor. Four batches of barley plants were grown on plastic mesh frames in water culture at *p*H 4.5, the medium containing the following in millimolar concentrations: KCl, 0.5; CaCl, 2.5; NaH_2PO_4, 0.05; $MgSO_4$,0.25; NH_4NO_3, 1.0; ferric citrate, 0.04; trace elements (*8*). When the roots had grown to approximately 10 cm in length, two batches of plants were transferred to a similar medium without phosphate and containing aluminum sulfate ($10^{-3}M$). The other two batches were transferred to this phosphate-free medium without the addition of aluminum sulfate.

After 48 hours there were no mitotic stages in the roots of the aluminum-treated plants although active division was taking place in the roots of the untreated control plants. After washing the roots, the four batches of plants were labeled for 4 hours with carrier-free $^{32}P_i$ (inorganic P). The roots from one control and one aluminum-treated batch of plants were then harvested. The two remaining batches of plants were given a "chase" treatment (48 hours) with sodium dihydrogen phosphate ($10^{-4}M$). Aluminum sulfate ($10^{-3}M$) was added to that batch of plants which had been aluminum-treated prior to the addition of isotope. The roots were then harvested, and DNA was extracted (*5*) and fractionated on a MAK column as described.

In the untreated roots of barley there are two DNA fractions corresponding to those found in wheat roots (*5*)

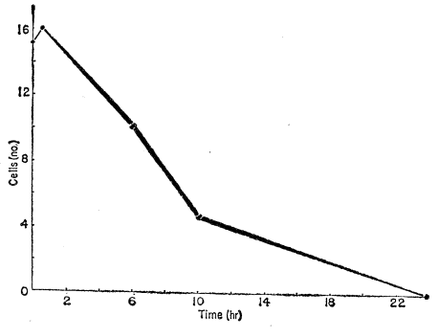

Fig. 1. Effect of aluminum sulfate ($10^{-3}M$) on the number (ordinate) of cells entering prophase in barley roots. Mean values from three "squash" preparations stained with acetocarmine.

(Table 1). In the aluminum-treated roots there are two apparent anomalies. The specific activity of the high-molecular-weight DNA is similar to that of the high-molecular-weight DNA from the actively dividing control roots. In the absence of cell division only slight incorporation of $^{32}P_i$ might have been expected. Also the newly synthesized DNA of this fraction is metabolically labile, resembling in this respect the low-molecular-weight DNA fraction. Thus it appears that the DNA synthesized during the period in which isotope was added is in fact of the low-molecular-weight form and that a part of this exists hybridized with the high-molecular-weight DNA. If this is so two requirements must be met. The base composition of newly synthesized DNA associated with the high-molecular-weight DNA must resemble that of the low-molecular-weight DNA in aluminum-treated roots, but not in untreated ones. This we have verified experimentally by determining base compositions by distribution of ^{32}P in the deoxynucleotides. The percentages of guanine plus cytosine in the control of low- and high-molecular-weight DNA's were 52 and 42, respectively; the corresponding percentages in the treated low- and high-molecular-weight DNA's were 51 and 53, respectively. For these determinations the barley DNA fractions were mixed with calf-thymus DNA as a carrier and treated with NaOH (0.3 *M*, 18 hours at 37°C, and 10 minutes at 70°C) to hydrolyze RNA. After acid precipitation, the DNA was digested with crystalline deoxyribonuclease (*p*H 5.0, 25°C) and then with purified snake-venom phosphodiesterase (*p*H 8.8, 37°C) (*9*). The deoxynucleotides were fractionated on Dowex-formate column (*10*), with a linear gradient of ammonium formate (*p*H 4.2, 300 ml of 0.1*M* to 300 ml of 1.0*M*). Five ml of 1*N* formic acid was added to the mixing vessel after the elution of deoxyadenylic acid. The distribution of ^{32}P followed that of deoxynucleotides, thus the radioactivity measured was derived from DNA.

Table 1. Specific activity of DNA fractions from aluminum-treated and untreated barley roots after a 4-hour label with carrier-free $^{32}P_i$ and again after a 48-hour "chase."

DNA fraction	Radioactivity (count/sec) per μg DNA-P	
	Label	Chase
LMW control*	107	10
HMW control†	24	22
LMW treated	71	18
HMW treated	26	7

* LMW, low-molecular-weight DNA having a base composition and chromatographic behavior on a MAK column similar to that of a wheat-root DNA fraction with a molecular weight of 2 to 3 × 10^5. † HMW, high-molecular-weight DNA which, by the same two criteria, resembles a wheat-root DNA fraction with a molecular weight of 4 to 6 × 10^6 (*5*).

Table 2. Refractionation of heat denatured DNA fractions labeled with ^{32}P from aluminum-treated and untreated barley roots. G, guanine; C, cytosine.

DNA fraction	Al treatment	Amount μg DNA-P	Radioactivity per μg DNA-P (count/sec)	G+C (mole %)
		Eluted by NaCl		
LMW	—	2.9	22	52
HMW	—	1.6	5.6	
LMW	+	4.8	15	51
HMW	+	5.3	28	52
		Eluted by NaOH		
LMW	—	0.5	24	
HMW	—	11.2	6	42
LMW	+	0.5	16	
HMW	+	18.5	1.2	51

The second requirement is that after separation of the hybrid into its components, these parts should behave chromatographically as two distinct molecular species. One should contain most of the total radioactivity and behave like low-molecular-weight DNA, the other should contain most of the total DNA, and behave like high-molecular-weight DNA. That this is in fact so has been verified. DNA may be rendered single-stranded by heating and subsequent cooling (*11*). We have heated DNA (3 μg DNA-P per ml) in distilled water for 2 minutes at 100°C. After rapid cooling the solution was stirred with MAK in a chromatography column containing one volume of NaCl (0.1*M*) in phosphate buffer (0.05*M*, *p*H 6.7). The column outlet was closed while the DNA adsorbed to the MAK. After 5 minutes the column was allowed to drain and it was then used in the normal manner. In this way the tendency of the DNA to become renatured is reduced. Low-molecular-weight DNA treated in this manner can be eluted with NaCl (2.5*M*) in phosphate buffer (0.05*M*, *p*H 6.8) whereas the high-molecular-weight DNA, which is resistant to elution with NaCl, can be eluted with NaOH (0.5*M*). Table 2 shows the results of such refractionation of high- and low-molecular weight DNA extracted from aluminium treated and untreated barley roots and meets the second requirement. Base compositions were determined for all fractions as described above and are given in Table 2.

M. Sampson
D. Clarkson
D. D. Davies
University of East Anglia, School of Biological Sciences, Norwich, Norfolk, England

References and Notes

1. E. Hutchinson, *Soil Sci.* **60**, 29 (1945).
2. D. Clarkson, *Ann. Bot.*, in press.
3. B. L. Hartwell and F. R. Pember, *Soil Sci.* **6**, 259 (1918).
4. D. I. Arnon and C. M. Johnson, *Plant Physiol.* **17**, 525 (1942).
5. M. Sampson, A. Katoh, Y. Hotta, H. Stern, *Proc. Nat. Acad. Sci. U.S.* **50**, 459 (1963).
6. M. Sampson and D. D. Davies, in preparation.
7. J. D. Mandell and A. D. Hershey, *Anal. Biochem.* **1**, 66 (1956).
8. D. I. Arnon, *Amer. J. Bot.* **25**, 322 (1938).
9. R. Sinsheimer, *J. Biol. Chem.* **198**, 293 (1952).
10. E. S. Canellakis and R. Mantsavinos, *Biochim. Biophys. Acta* **27**, 643 (1958).
11. J. Marmur and D. Lane, *Proc. Nat. Acad. Sci. U.S.* **46**, 453 (1960).
12. Aided by fellowships, to M.S. from Shell Chemicals, and to D.C. from the Department of Scientific and Industrial Research.

5 April 1965

Particles Resembling Papova Viruses in Human Cerebral Demyelinating Disease

Abstract. *Degenerated brain tissue obtained from a deceased patient who had progressive multifocal leukoencephalopathy had been fixed in formalin prior to processing for electron microscopy. In ultrathin sections virus-like particles resembling papova virions were frequently observed in glial nuclei. Correlation with light-microscopy findings suggests that demyelination resulted from the cytocidal effect of the virus on oligodendroglia.*

Progressive multifocal leukoencephalopathy (PML) is a rare human demyelinating disease first reported in 1958 by Åström, Mancall, and Richardson (*1*). Only about 35 cases have