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*METABOLICALLY LABILE DEOXYRIBONUCLEIC ACID**

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DNA prepared from a variety of growing plant tissues may be resolved on a methylated albumin column into two distinct fractions. A full account of the preparative procedure and physical characterization of the components will be published elsewhere. For the purpose of this communication, the following points are to be noted: tissues are disintegrated in cold 95% ethanol and extracted with 5% sodium lauryl sulfate (w/v) in 0.05 *M* sodium citrate at a pH of 7.4. The nucleic acids are precipitated with two volumes of ethanol, re-extracted with 2.5 *M* sodium chloride, deproteinized with chloroform:amyl alcohol, and digested with ribonuclease.[1] The product is dissolved in 0.1 *M* NaCl:0.05 *M* potassium phosphate (pH, 6.8), and loaded on a methylated albumin column.[2, 3] By stepwise elution with increasing concentrations of NaCl, two DNA fractions are obtained, one eluting at 0.4–0.5 *M* NaCl, the other at approximately 0.65 *M*. Viscometric analyses indicate their respective molecular weights to be of the order of 2–3 $\times 10^5$ and 4–6 $\times 10^6$. Both fractions show melting profiles typical for a double-stranded helix, but the maximum increase in optical density of the low molecular weight form is about 31–33%.

The proportion of the two forms varies with type of tissue and with physiological state. Male germinal tissue contains negligible amounts of the low molecular weight form; ovules have not been analyzed. Growing regions of root and leaf, including zones of differentiation, may have as much as 20% of their total DNA in the low molecular weight form. Dormant embryos in wheat seeds have about 10%, but the value rises sharply on induction of germination. Accurate estimates have not yet been made for mature tissues because of possible complications arising from endogenous breakdown of high molecular weight DNA. It will become apparent from the experiments here reported that low molecular weight DNA isolated from growing tissues is not a breakdown product.

Experimental Results.—Wheat seeds (var. Selkirk) were germinated and grown in Hoagland's solution with the phosphate component at half strength. When the leaves reached 2–3 inches in height, P^{32}-inorganic phosphate was added to the medium. Roots were removed from plants after various periods of exposure to the isotope, and also from plants which, following exposure, were thoroughly washed

with 0.1 *M* phosphate and returned to Hoagland's medium. Leaves were not used in these experiments because of their sluggish response to isotope addition or removal. Root DNA was prepared as described. The fractions removed from the column were concentrated by lyophilization, treated overnight with 0.2 *N* NaOH at 37°C, and again at 70°C for 20 min to remove possible traces of RNA. Neither low nor high molecular weight DNA were degraded under these conditions. Recoveries were virtually quantitative for the high molecular weight form and nearly so for the low molecular weight. In the course of experimentation it was found that losses (10–20%) could be avoided by maintaining the concentration of low molecular weight DNA at relatively high levels (0.25 mg/ml). The DNA content of alkali-treated fractions was determined by ultraviolet absorption and by the diphenylamine reaction.

Typical results of such experiments are given in Figure 1. The principal feature of these results is the contrasting behaviors of the two DNA forms with respect to P^{32} turnover. High molecular weight DNA behaves in the expected fashion; the specific activity of P^{32}-DNA decreases only slightly following exposure of roots to P^{31}-phosphate. By contrast, the specific activity of low molecular weight DNA decreases to the vanishing point following the same exposure to P^{31}. Precise studies of RNA turnover during these treatments were not made, but if ribonuclease treatment prior to column chromatography was omitted, the RNA released by alkaline hydrolysis from the low molecular weight DNA fraction showed no comparable decrease in radioactivity following exposure to P^{31}.

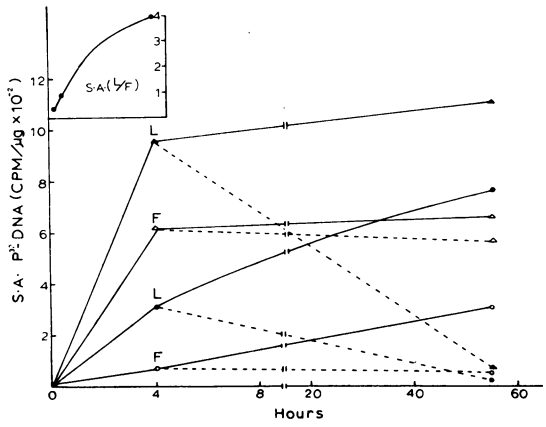

FIG. 1.—Changes in specific activity (S.A.) of fibrous (F) and low molecular weight (L) DNA in growing wheat roots following exposure to P^{32}-phosphate. The terms "fibrous" and "high molecular weight" will be used interchangeably. Dashed lines represent values after "chasing" with P^{31}-phosphate. The inset is discussed in the text; the abscissae are the same as those for the main graph.

The inset in Figure 1 shows the relative specific activities of the two types of DNA following brief exposures of wheat roots to isotope. This particular pattern, however, is not consistently obtained. Physiological conditions influence the pattern, and so too, undoubtedly, the nature of precursor pools. Results such as these are nevertheless difficult to explain in terms of one form of DNA being the precursor of the other. Tentatively at least, one may assume that the two forms of DNA are functionally distinct. Further experiments were undertaken to test this assumption and also to establish more soundly that the material being studied in the low molecular weight fraction was, in fact, DNA.

The two types of P^{32}-DNA were prepared from corn roots, digested with crystalline deoxyribonuclease followed by purified snake venom phosphodiesterase,[4] calf thymus DNA being added to each of the fractions as a carrier. The deoxy-

nucleotides were fractionated on a Dowex-formate column.[5] The plot of radioactivity tracked the plot of ultraviolet absorption as shown in Figure 2. Most of the hydrolytic products from the two plant preparations were the same as those from calf thymus DNA. These results thus further establish that the labeled material being studied is DNA. A second and important characteristic is, however, revealed by this experiment. The total radioactivities in each of the deoxynucleotide fractions is listed in Table 1. It may be seen that, although the composition of high molecular weight DNA falls in the class typical for higher plants, the composition of the low molecular weight form is very different. Assuming a random labeling of the deoxynucleotides, one may conclude that both turnover characteristics and composition mark low molecular weight DNA as functionally distinct from its high molecular weight counterpart.

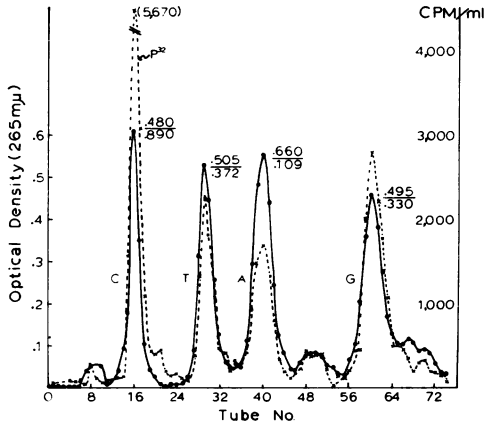

FIG. 2.—Elution pattern of low molecular weight DNA obtained from growing wheat roots. Only a small portion of the roots was labeled; the remainder of the low molecular weight DNA was obtained from wheat grown in a greenhouse. Optical densities at 260/280 mμ are given for the peak tubes. Symbols are the same as in Table 2. The fractionation system used was slightly modified from the original.[5] A 50 ml mixing flask was substituted, and the reservoir first filled with 0.2 *M* ammonium formate; the latter was replaced with 0.4 *M* ammonium formate after elution of thymidylate, and with 0.6 *M* after elution of adenylate. Each tube contained 3 ml of eluate.

To obviate the uncertainties implicit in the assumption of random deoxynucleotide labeling, a relatively large amount of low molecular weight DNA was prepared from wheat roots and added as carrier to a sample of labeled material. The deoxynucleotides were prepared and fractionated in the same way as those from corn roots. The results of such fractionation are shown in Figure 2. The parallel in curves of radioactivity and ultraviolet absorption is clearly apparent; so too is the preponderance of deoxycytidylate and deoxyguanylate residues over those of thymidylate and deoxyadenylate. The low molecular weight forms of DNA derived from corn and wheat roots are therefore similar.

To establish that the ultraviolet absorption was due to deoxyribonucleotides, each of the fractions was concentrated and assayed microbiologically for deoxy-

TABLE 1

RADIOACTIVITY OF DEOXYNUCLEOTIDES IN LOW MOLECULAR WEIGHT AND FIBROUS DNA

Nucleotide	cpm P^{32} × 25 Fibrous	Low m.w.
d-cytidylate	5,973	12,795
d-guanylate	5,645	13,326
d-thymidylate	9,598	10,420
d-adenylate	8,769	10,954
% GC	39	55

Corn seedlings were grown in the presence of P^{32}-phosphate for 24 hr. The two DNA fractions (fibrous and low molecular weight) were digested with deoxyribonuclease and phosphodiesterase using calf thy mus DNA as carrier. The components were resolved as described for Fig. 2. The eluates were adsorbed on a Dowex-1 (Cl^-) column, washed with 1×10^{-3} *M* NH_4Cl (pH, 8), and eluted with 1.0 *M* NaCl.

riboside content.[6] With the exception of deoxycytidylate, a satisfactory correspondence was found between ultraviolet and microbiological data (see Table 2). To eliminate the possibility that traces of ribonucleotides were responsible for the observed radioactivities, a portion of each of the deoxynucleotide fractions was spotted on paper and resolved in an ethanol:ammonium acetate:borate mixture.[7] Only one ultraviolet-absorbing spot was observed for each eluate, and the R_f value was similar to that of a known sample of deoxynucleotide. The ultraviolet-absorbing spot and the remainder of the paper were extracted separately with 0.1 *N* HCl; radioactivity and deoxyriboside content were both determined. The deoxycytidylate and deoxyguanylate fractions, respectively, had 17 and 24% of their counts outside the spots; these values were taken as a measure of radioactive contamination. Thymidylate and deoxyadenylate showed negligible activity outside the spots, but, unlike the other two fractions, showed the presence of some deoxyribosidic material outside the observed spots. The results of these analyses together with various calculations are shown in Table 2.

TABLE 2

COMPOSITION AND RADIOACTIVITY OF DEOXYNUCLEOTIDE FRACTIONS

Fraction	Total cpm (corrected)	Total μmoles (u.v.)	Total μmoles (bioassay)	Total μmoles (corrected) bioassay)	S.A. (eluates)	S.A. (paper)
C	17,450	0.42	0.54	0.54	32,300	37,200
T	13,550	0.56	0.54	0.42	32,300	37,300
A	14,200	0.57	0.57	0.46	30,900	34,400
G	21,600	0.66	0.69	0.66	32,700	34,400
GC content (%)	58	49	51	58		

Total counts per minute in the pooled and concentrated eluates were corrected by multiplying the original counts in each by the percentage of counts localized in the deoxynucleotide spot on the paper chromatogram. The corrections were as follows: d-cytidylate (C), 83%; thymidylate (T), 93%; d-adenylate (A), 96%; d guanylate, 76%. The deoxyriboside content of each of the eluates was similarly corrected; the original and corrected values are recorded in the table. The specific activities (S.A.—cpm per μg DNA) of the pooled eluates and the paper spots were determined using the values of deoxynucleotide content measured by microbiological assay.

The main conclusion to be drawn from the above results is that the inference initially made respecting the presence of a metabolically labile form of DNA is correct. The near coincidence in values obtained by ultraviolet analysis, diphenylamine test, enzymatic digestion, and quantitative determination of the products by ultraviolet and microbiological analysis, leaves little room for alternative interpretations. It would be premature, however, to draw detailed conclusions about the composition of this form of DNA. Although enzymatic digestion of high molecular weight DNA yields the expected complement of deoxynucleotides, the minor irregularities in the elution pattern of low molecular weight DNA digests need not be attributed to experimental error. The possibility remains that distinctive minor components are present, and this may be related to the fact that only 80% of the DNA loaded on the column was recovered in the pooled fractions. Even so, the similarity in specific activities of the deoxynucleotide fractions, whether determined from the column eluates or from the paper spots, indicates that the turnover characteristics manifest in Figure 1 apply to the molecule as a whole.

Discussion.—In studies to be reported elsewhere, high molecular weight DNA was found to have the same composition irrespective of tissue of origin. No evidence for turnover of this DNA was obtained. Thus, with respect to all properties

studied, the high molecular weight form in higher plants behaves typically for genetic material. This does not appear to be true for the low molecular weight form. A number of reports have been published concerning the existence of DNA in chloroplasts[8] and of "nonfibrous" DNA in nuclei of calf thymus.[9] No satisfactory evidence exists for presuming a homology between the form studied here and those reported elsewhere. The fact that label can be almost entirely "chased" out of the molecule argues against its being a genetic determinant in the conventional sense. Its rapid formation in embryos induced to germinate suggests rather a physiological role. If so, the gross composition here reported may not be a constant characteristic, even though in roots of both wheat and corn the guanine-cytosine content appears to be greater than that of the high molecular weight form. At this stage of study the most important conclusion to be drawn is that a form of metabolically labile DNA exists.

Summary.—Growing tissues of higher plants have two forms of DNA. One of these has an average molecular weight of the order of 10^5 and is characterized by a relatively rapid rate of turnover. The composition of this DNA appears to differ from its high molecular weight counterpart.

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*INTERFERENCE WITH GLYCEROKINASE INDUCTION IN MUTANTS OF E. COLI ACCUMULATING GAL-1-P**

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The inhibitory effect of galactose on the growth of mutants of *E. coli* lacking gal-1-P-uridyl transferase has been established by the work of Kurahashi and Wahba[1] and Yarmolinsky *et al.*[2] The inhibition is very pronounced in a synthetic medium containing glycerol as sole carbon source, and the addition of glucose to the medium promptly relieves inhibition.[2] The growth inhibition is accompanied by the accumulation of gal-1-P in the cell.[3] Similar findings have been reported from this laboratory with another mutant of *E. coli* which lacks the enzyme UDPG synthetase.[4]

It is not clear how galactose causes growth inhibition in these mutants. The