IMPAIRMENT OF RESPIRATION, ION ACCUMULATION, AND ION RETENTION IN ROOT TISSUE TREATED WITH RIBONUCLEASE AND ETHYLENEDIAMINE TETRAACETIC ACID[1,2]

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Ribonuclease has been reported to impair protein synthesis (1, 2), growth (2), and ion accumulation (15, 24) in plant tissue. Oxidative phosphorylation by plant mitochondria is uncoupled by RNase[3] (8); however, this enzyme has not been found to affect root respiration (1, 8).

The experiments reported here represent a further study of the effects of RNase on respiration and ion accumulation by root tissue. Respiration and ion retention proved to be impaired by exogenous RNase. In addition, EDTA was found to produce similar responses, presumably by removal of divalent ions which normally protect polyribonucleotides from endogenous RNase.

METHODS AND MATERIALS

PLANT MATERIALS: Soybean seed (*Glycine max;* var. Hawkeye) and hybrid corn seed (*Zea mays* L., var. WF9 x M14) were lightly dusted with a fungicide (Spergon; U.S. Rubber Co.) and germinated for 3 days at 28° C in darkness in vermiculite moistened with tap water. Initially, the tip 1.5 cm of the primary roots was used; later the roots were sectioned into three successive 0.5 cm sections. The tissue was rinsed in deionized water, blotted, and rapidly weighed to the nearest milligram before use.

RESPIRATION AND ION ACCUMULATION: Oxygen consumption was measured at 29° C by standard manometric techniques (27). Air was gas phase. The tissue was placed in 2.0 ml of 10^{-3} M potassium phosphate, pH 6.0. Treatments were in duplicate or triplicate. Vessel additives are given in the tables. The EDTA was added as the potassium salt, pH 6.0. Protease-free, five times recrystallised RNase was obtained from the Sigma Chemical Co.

Phosphate accumulation from the Warburg vessel media was measured during the respiratory determinations. Potassium accumulation was largely determined in parallel experiments using 50 ml Erlenmeyer flasks on a shaker at room temperature. In each case the tissue was placed in 10^{-3} M potassium phosphate buffer (pH 6.0) labeled with P^{32} or Rb^{86}. The Rb ion serves as a tracer for K accumulation in short-term experiments with root tissue (14). At the termination of the accumulation period, the exchangeable Rb+K ion was removed from the tissue in two out of four flasks in each treatment by rinsing the tissue with deionized water and placing it in ice-cold 10^{-3} M $CaCl_2$ for 40 minutes. Tissues were rinsed, digested and assayed as previously described (14). Exchangeable Rb+K was calculated from the difference in radioactivity between tissue subjected to exchange with Ca, and tissue which was only rinsed with deionized water.

LEAKAGE OF IONS: Root tip tissue was placed in 125 ml Erlenmeyer flasks containing 10 ml of 1% sucrose with 10^{-3} M potassium phosphate buffer labeled with Rb^{86}. After 2 hours on a shaker at room temperature, the tissue was rinsed and exchangeable ions removed in unlabeled 10^{-3} M phosphate buffer containing 10^{-2} M $CaCl_2$ as above. Next, the tissue was incubated for 1 hour on the shaker in the same sucrose-buffer medium as during the uptake phase but without the Rb^{86} label. Ribonuclease and EDTA were added as indicated in the tables. The tissue was rinsed with water, ashed, and residual radioactivity determined as above.

OXIDATIVE PHOSPHORYLATION OF MITOCHONDRIA: Mitochondria were isolated from 1.5 cm sections of soybean root tips and the oxidative phosphorylation determined as previously described (8, 18). Alpha-ketoglutarate was used as substrate.

ANALYSIS FOR NUCLEOTIDES AND NUCLEIC ACIDS: The analytical procedures were adapted from those previously described for analysis of mitochondria (8). Concentrations were determined from 260 to 290 mμ absorption in the Beckman DU spectrophotometer, using AMP and yeast RNA as standards. Nucleotides diffusing from the tissue were estimated as AMP

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[3] Abbreviations: EDTA, ethylenediamine tetraacetic acid; RNA, ribonucleic acid; RNase, ribonuclease. AMP, CMP, GMP, UMP, monophosphate nucleotides of adenosine, cytidine, guanosine, and uridine.

on the vessel contents at the end of the experiment or preincubation. The weighed root tips from the experiment were homogenized for 2 minutes in 3 ml of water with an ice-jacketed glass homogenizer, and transferred with 3 ml of water to centrifuge tubes. Perchloric acid was added to 0.2 *N* final concentration, and the precipitate sedimented by centrifugation. The pellet was twice resuspended in 0.2 *N* $HClO_4$ and recentrifuged. Cold-acid soluble nucleotides were estimated on the combined supernatants. The absorption spectrum of the cold $HClO_4$ extract from soybean roots showed a smooth increase in optical density from 290 mμ to a pronounced shoulder at 260 mμ; a small increase occurred between 260 to 250 mμ, followed by a decline to 235 mμ. Total nucleic acids were extracted from the washed and defatted precipitate with hot perchloric acid as described for mitochondria (8).

Assays for Enzymatic Degradation of Endogenous and Yeast RNA: About 240 root sections were homogenized for 2 minutes with 15 ml of 0.5 M sucrose in an ice-jacketed glass homogenizer with a Teflon pestle. The homogenate was cleared of cell debris and nuclei by centrifuging at 800 × G for 5 minutes. The supernatant solution was filtered through a small pad of glass wool to catch any fluffy pieces of cell wall debris. The cleared homogenate was adjusted to pH 6.5 with NaOH and used directly as a source of enzyme. The protein concentration was determined by the method of Lowry, et al (17), on aliquots precipitated and washed in 5 % trichloroacetic acid.

Degradation of endogenous and yeast RNA was determined by incubating 1 ml of cleared homogenate with the additives indicated in table XII. Final volume was 2 ml; incubation was for 30 minutes at 30° C; pH was 6.5; determinations were in duplicate. One set of tubes was held in ice to establish the initial level of nucleic acid.

At the termination of incubation, the tubes were placed in ice. Perchloric acid was added to 0.2 *N*. The precipitate was sedimented, washed, and assayed for nucleic acid as above. Experimentation has shown no detectable deoxyribose in comparable cleared homogenates; the endogenous nucleic acid is reported as RNA.

Table I

Inhibitory Effect of Ribonuclease on Root Tip Respiration

Root tips	Buffer	Qo_2(FW) Control	RNase	% Inhibition
Corn	None	552	525	5
	K Phosphate	702	572	19
Soybean	None	639	555	13
	K Phosphate	696	598	14

Twenty root tips were placed in Warburg vessels in 2 ml of deionized water or 10^{-3} M potassium phosphate (pH 6.0) with and without 1 mg RNase/ml. Respiration rates were measured between 70 and 100 minutes after placing roots in the vessels. Qo_2(FW) = μl O_2/hr/gm fresh weight.

Table II

Respiration Rate of Soybean Root Tips as Function of Treatment with Ribonuclease or EDTA

Vessel additive	Qo_2(FW) 0-25 Min	130-190 Min
Buffer	605	735
+ RNase	678	640
+ EDTA	913	493
+ RNase and EDTA	897	372

Twenty root tips were placed in Warburg vessels with 10^{-3} M potassium phosphate buffer (pH 6.0) with indicated additives. Final concentrations: RNase, 1 mg/ml; EDTA, 5×10^{-3} M.

Results

In the previous report on the activity of mitochondria treated with RNase (8), it was noted that corn roots treated with RNase showed no significant impairment of respiration in 2 hours. Table I shows that respiration declines after about one hour if the treatment is made in the presence of potassium phosphate buffer. Soybean root tips were sensitive to RNase in either water or buffer. The increased respiration of tissue in buffer, which is probably a manifestation of the widely described salt respiration, was also obtained with KCl. In subsequent experiments soybean root tips were generally more sensitive to RNase or EDTA treatment than were corn root tips. Experiments were largely done with soybeans. Occasional experiments were done with corn to make certain the results were valid for more than one species.

Table II compares the effects of RNase and EDTA on soybean root tip respiration. The application of EDTA was suggested by the reports of Ts'o (26) and West (30) showing that the RNA of the microsomal fraction of plant tissue is subject to degration by endogenous enzymes if treated with potassium ion or EDTA. Removal of the principal divalent ions of the cell, calcium and magnesium, by substitution or chelation, causes a dissociation of the ribonucleoprotein particles and appears to expose the RNA to degradation. The degradation can be largely halted by Ca or Mg.

In the initial minutes after application, both RNase and EDTA elicited increases in root respiration reminiscent of the increase following RNase application to mitochondria (8). EDTA has previously been reported to increase soybean leaf respiration (29). After 2 hours, respiration declined below the control levels. The EDTA was much more effective than RNase in both the initial respiratory burst and

TABLE III

EFFECT OF KCl ON RESPIRATION AND NUCLEOTIDE CONTENT OF SOYBEAN ROOTS

SOLUTION	Qo_2 (FW)	NUCLEOTIDES			
		DIFFUSIBLE	SOLUBLE	POLY-	TOTAL
			μg/gm fr. wt.		
Buffer (pH 6.0)	603	84	626	3,613	4,323
+ 10^{-2} M KCl	670	107	606	3,654	4,358
+ 10^{-1} M KCl	433	224	520	3,343	4,087

Twenty root tips were placed in 125 ml Erlenmeyer flasks with 10 ml of 10^{-3} phosphate buffer containing KCl as indicated. After 2 hours on a shaker at room temperature duplicate lots were analyzed for nucleotides. In a separate experiment the respiration rates after pretreatment were determined in 10^{-3} M phosphate buffer.

subsequent inhibition; the combination of EDTA and RNase produced the greatest impairment of respiration.

High concentrations of KCl impaired respiration after periods of 2 to 3 hours. Table III shows this effect and provides a balance sheet on the nucleotides. Three arbitrary classes of nucleotides are described: diffusible nucleotides are those which leak from the cells into the medium; soluble nucleotides are those which can be extracted from root homogenates in cold 0.2 *N* $HClO_4$; polynucleotides are those which can be extracted from the residue in hot 0.5 *N* $HClO_4$. The latter classification includes DNA (20). The 0.1 M KCl induced a degradation of polynucleotides and caused a loss of soluble nucleotides. Part of the lost nucleotides diffused from the tissue, and part disappeared (presumably through degradation of purine and pyrimidine bases, since nucleotides were measured by ultraviolet absorption).

Experiments (table IV) with corn root tips show that EDTA and RNase induced the same type of change in nucleotide content as did high concentrations of KCl. KCl was added to control treatments to prevent confounding the EDTA effect with the effect of K ion used in neutralization.

Table V shows that low concentrations of Ca ion would partially reverse the deleterious effects of EDTA on respiration and nucleotide content. Magnesium would also protect respiration.

The addition of monophosphate nucleotides with Ca and Mg gave essentially the same respiration response as Ca or Mg alone; both the KCl and EDTA treated tissue showed increased respiration (table VI).

TABLE V

EFFECT OF CALCIUM ION ON RESPIRATION AND POLYNUCLEOTIDE CONTENT OF SOYBEAN ROOT TIPS

VESSEL ADDITIVES	Qo_2 (FW)	NUCLEOTIDES	
		DIFFUSIBLE	POLY-
	90-150 min	*μg/gm fr. wt*	
KCl, 5×10^{-3} M	672	115	2,770
+ $CaCl_2$, 10^{-3} M	728	107	2,952
EDTA, 2×10^{-3} M	250	896	1,905
+ $CaCl_2$, 10^{-3} M	377	615	2,338

Experimental conditions as in table IV.

The decreased tissue respiration attendant on EDTA application was also reflected in the oxidative and phosphorylative capacity of mitochondria isolated from the tissue (table VII). The P/O ratios of mitochondria from the control (KCl-treated) tissue were surprisingly low; they varied from 0.5 to 1.3 in five experiments. Possibly some degradative metabolism in excised tissue during pretreatment was detrimental to the mitochondria.

TABLE IV

INHIBITION OF RESPIRATION AND LOSS OF NUCLEOTIDES FROM CORN ROOT TIPS TREATED WITH EDTA AND KCl

VESSEL ADDITIVES	Qo_2 (FW)	NUCLEOTIDES			
		DIFFUSIBLE	SOLUBLE	POLY-	TOTAL
	90-120 min		*μg/gm fr. wt*		
KCl, 0.0075 M	573	41	1,415	2,230	3,686
EDTA, 0.003 M	520	265	1,250	2,035	3,550
KCl, 0.025 M	566	94	1,173	1,970	3,237
EDTA, 0.01 M	260	553	658	1,720	2,931
KCl, 0.0075 M	645	43	1,440	2,043	3,526
+ RNase, 1 mg/ml	513	258	1,250	2,055	3,563
EDTA, 0.003 M	600	252	1,340	1,980	3,572
+ RNase, 1 mg/ml	529	258	1,275	1,673	3,206

Respiration measured in duplicate on 20 root tips in 10^{-3} M phosphate buffer (pH 6.0) with indicated additives. Nucleotide analyses made on the vessel contents and tissue after 2 hours.

TABLE VI

EFFECT OF DIVALENT IONS AND NUCLEOTIDES ON RESPIRATION OF EDTA-TREATED ROOT TIPS

ROOT TIPS	PRETREATMENT	VESSEL ADDITIVES	Qo_2(FW)
			90-120 min
Soybean	KCl, 0.005 M (60 min) K-EDTA, 0.002 M	Buffer	464
		+ Ca, Mg, nucleotides	555
		Buffer	263
		+ Ca, Mg, nucleotides	408
Corn	KCl, 0.025 M (90 min.) K-EDTA, 0.01 M	Buffer	564
		+ Ca, Mg, nucleotides	675
		Buffer	284
		+ Ca, Mg, nucleotides	366

Respiration determined in 10^{-3} M phosphate buffer, pH 6.0 with indicated additives. For pretreatment roots were placed in indicated solutions with 10^{-3} M phosphate buffer, pH 6.0, on a shaker at room temperature. Calcium and Mg were added as chlorides, 10^{-3} M. One mg each of AMP, CMP, GMP, and UMP was added to each vessel.

TABLE VII

INHIBITED OXIDATION AND PHOSPHORYLATION OF MITOCHONDRIA ISOLATED FROM ROOT TIPS TREATED WITH EDTA

PRETREATMENT SOLUTION	TISSUE Qo_2(FW)	MITOCHONDRIA Qo_2(N)	MITOCHONDRIA P/O
KCl, 2×10^{-2} M	507	431	0.77
EDTA, 10^{-2} M	285	133	0.00

About eight grams of soybean root tips were placed in 50 ml of 10^{-3} M phosphate buffer with indicated additives and agitated on a shaker at room temperature for 1.5 hours. Solutions were renewed after 45 minutes. Tissue respiration determined on 20 root tips. Mitochondria were isolated from the remaining tissue.

TABLE VIII

LOSS OF ACCUMULATED RUBIDIUM FROM SOYBEAN ROOTS DUE TO RIBONUCLEASE TREATMENT

TREATMENT	Rb^{86}	DIFFUSIBLE NUCLEOTIDE
	c/m/20 roots	*μg/20 roots*
Initial	962	...
Control	895	16
RNase	601	106

Root tips were incubated for 2 hours in 0.001 M potassium phosphate (pH 6.0) labeled with Rb^{86}. Exchangeable ions were removed with Ca and the root tips incubated in phosphate buffer with and without 1 mg/ml RNase for 2 hours.

TABLE IX

LOSS OF NUCLEOTIDES FROM SOYBEAN ROOT SECTIONS DUE TO EDTA TREATMENT

SOLUTION	ROOT SECTION CM FROM TIP	NUCLEOTIDES DIFFUSIBLE	SOLUBLE	POLY-	TOTAL
		μG/GM FR. WT			
Buffer	0.0-0.5	36	796	9,960	10,792
	0.5-1.0	57	319	1,675	2,051
	1.0-1.5	70	189	828	1,087
+ 3×10^{-3} M EDTA	0.0-0.5	206	640	9,570	10,416
	0.5-1.0	311	160	1,320	1,791
	1.0-1.5	225	117	645	987
		EDTA treatment as % of control			
	0.0-0.5	572	80	96	97
	0.5-1.0	546	50	79	87
	1.0-1.5	322	62	78	91

Duplicate samples of 30 root sections were incubated in 10^{-3} M phosphate buffer (pH 6.0) with and without EDTA for 1.5 hours at room temperature on a shaker, followed by analysis for nucleotides as described in the text.

TABLE X

EFFECT OF EDTA ON Rb^{86} RETENTION BY SECTIONS OF SOYBEAN ROOT

SOLUTION	ROOT SECTION	RADIOACTIVITY	EDTA AS % OF CONTROL
	cm from tip	*c/m/gm fr. wt*	
Buffer 10^{-3} M	0.0-0.5	12,600	
	0.5-1.0	6,000	
	1.0-1.5	3,200	
+ EDTA, 2×10^{-3} M	0.0-0.5	2,200	17
	0.5-1.0	1,900	32
	1.0-1.5	1,300	41

Root sections incubated on a shaker for 2 hours in potassium phosphate buffer, pH 6.0, labeled with Rb^{86}. This tissue was rinsed and exchangeable ion removed with 10^{-2} M $CaCl_2$ in unlabeled buffer. The tissue was next placed in the indicated solutions for 1 hour on a shaker at room temperature, rinsed, ashed, and counted.

The increase in diffusible nucleotides subsequent to treating the roots with RNase, EDTA or high concentrations of KCl suggested that the capacity of cell membranes to retain solutes had been impaired. This was directly confirmed by studying the loss of previously accumulated Rb^{86} (table VIII). RNase altered the cell membranes such that the ion leaked from the tissue. As will be shown later, EDTA produced the same effect.

Studies were made with successive 0.5 cm sections of soybean roots to relate the effect of EDTA to mean cell ontogeny. The 0.5 to 1.0 cm section encompasses the main region of cell expansion, and corresponds to the region where ion accumulation per cell, or per unit protein (4, 9) and mitochondrial activity (18) are greatest. The expanding zone of the root is also a region where the mitochondria and other membranous materials are rapidly increasing, and the amount of microsomal RNA is rapidly decreasing (18).

Table IX gives the nucleotide content as a function of EDTA treatment. The greatest leakage occurred in the tip section; the greatest loss of soluble nucleotide in the second (0.5-1.0 cm) section; the greatest degradation of polynucleotide in the 0.5 to 1.5 cm region. Since there appears to be some catabolism of nucleotides capable of diffusing from the tissue, (thus masking the relative loss of membrane semi-permeability) observations were made of the loss of accumulated Rb^{86} as a function of tissue maturity (table X). The meristematic tip region was the most sensitive.

Respiration and K accumulation were most sensitive to EDTA in the 0.5 to 1.0 cm sections (table XI). Phosphate accumulation was most affected in the tip. There was a pronounced increase in exchangeable cation with EDTA pretreatment; the greatest increase was in the expanding zone. The increase can be largely ascribed to loss of exchangeable divalent ions by chelation, but may in part result from increased negative valence at the protoplast surface due to cleavage of phosphate linkages in nucleic acid. A similar increase in exchangeable cation can be obtained with RNase application (data to be published separately).

The capacity of homogenates to digest endogenous and yeast RNA is given in table XII. The addition of EDTA accelerates the degradation of endogenous RNA over the KCl control. The greatest acceleration over control occurred in the tip, but as shown by the homogenate analyses, the higher substrate levels of the tip section may have been responsible for this result. If yeast RNA was added, the highest specific rate was in the 0.5 to 1.0 cm section, followed closely by the 1.0 to 1.5 cm section.

The declining RNA/protein ratio with distance from the tip suggests that the endogenous RNase is partially active during cell expansion and maturation.

TABLE XI

EFFECT OF EDTA PRETREATMENT ON RESPIRATION AND PHOSPHATE AND POTASSIUM ABSORPTION BY SECTIONS OF SOYBEAN ROOT TIPS

PRETREATMENT ADDITIVE	ROOT SECTION	Qo_2(FW)	P ABSORPTION	Rb+K ABSORBED	
				EXCHANGEABLE	ACCUMULATED
	cm from tip	*0-120 min*	*μ moles/gm/hr*	*μ moles/gm*	*μ moles/gm/hr*
KCl, 5×10^{-3} M	0.0-0.5	1,110	0.38	1.66	4.40
	0.5-1.0	399	0.41	0.64	2.16
	1.0-1.5	348	0.34	0.89	1.34
EDTA, 2×10^{-3} M	0.0-0.5	733	0.11	4.67	2.27
	0.5-1.0	101	0.18	2.06	0.19
	1.0-1.5	181	0.19	2.04	0.36
			EDTA treatment as % of control		
	0.0-0.5	66	29	281	52
	0.5-1.0	25	44	322	9
	1.0-1.5	52	56	229	27

Pretreatment for 90 minutes on shaker at room temperature with KCl or EDTA in 10^{-3} M potassium phosphate. Respiration and phosphate absorption determined for 2 hours in Warburg vessels. Uptake of K labeled with Rb^{86} was for 2 hours on shaker at room temperature.

TABLE XII

DEGRADATION OF ENDOGENOUS AND YEAST RNA DURING INCUBATION OF HOMOGENATES FROM SUCCESSIVE SECTIONS OF SOYBEAN ROOT TIPS

ROOT SECTION	HOMOGENATE		RNA	RNA DEGRADATION BY HOMOGENATE		
	PROTEIN	RNA	PROTEIN	+ KCL	+ EDTA	+ RNA
cm from tip	*mg/gm fr. wt*	*mg/gm fr. wt*		*μg RNA/mg protein/hr*		
0.0-0.5	37.20	8.31	0.22	99	229	936
0.5-1.0	7.36	1.35	0.18	100	177	1,861
1.0-1.5	5.76	0.89	0.15	66	187	1,649

Homogenates cleared of cell debris and nuclei as described in methods, and incubated with 5×10^{-3} M KCl, 2×10^{-3} M EDTA or 1 mg/ml yeast RNA. Activity is expressed on the basis of homogenate protein.

but a concurrent synthesis of protein known to occur with root cell expansion (3, 28) would produce the same result.

The dependence of the EDTA effect on temperature is shown in table XIII. The sensitive tissue between 0.5 to 1.5 cm was used in these experiments. Pretreatment of the tissue with EDTA in the cold produced an increase in Rb^{86} uptake, most of which was erased by Ca+Mg resupplied by a rinse period. This result of increased Rb^{86} accumulation which can be reversed by divalent ion is similar to that reported by Tanada (24) with RNase. The EDTA pretreatment at room temperature decreased ion accumulation which was further depressed by Ca+Mg. The results can be interpreted as showing an endogenous enzymatic response to EDTA, which over a short time or with temperature-limited rates increases metabolic activity (cf. table II). Higher rates of enzymatic activity result in eventual inhibition of both respiration and ion accumulation.

TABLE XIII

EFFECT OF TEMPERATURE AND Ca+Mg ON IMPAIRMENT OF K ACCUMULATION BY EDTA

PRETREATMENT		RINSE SOLUTION	Rb^{86} ACCUMULATED
SOLUTION	TEMPERATURE		
			c/m/20 sections
Buffer	Ice	H_2O	2,285
		Ca + Mg	1,475
	Room	H_2O	2,163
		Ca + Mg	1,430
+ EDTA 2×10^{-3} M	Ice	H_2O	3,755
		Ca + Mg	1,793
	Room	H_2O	1,888
		Ca + Mg	607

Tissue was 1 cm sections of soybean root taken 0.5 to 1.5 cm from the tip. Pretreatment was for 1 hour on a shaker in 10^{-3} M potassium phosphate buffer (pH 6.0). Roots were next rinsed for 10 minutes on a shaker in water or 5×10^{-3} M $CaCl_2$ + 5×10^{-3} M Mg SO_4. Accumulation was for 2 hours in 2×10^{-3} M phosphate buffer + Rb^{86}. Exchangeable Rb^{86} was removed in phosphate buffer + 10^{-2} M $CaCl_2$.

DISCUSSION

If it is assumed that the sole action of RNase is to degrade polyribonucleotides, the results reported here and previously (8, 15, 24) provide strong evidence for the existence of RNA essential to respiration, ion accumulation and solute retention. The cytological site of this RNA is probably in the mitochondrial membranes and the limiting cytoplasmic membranes (plasmalemma and/or tonoplast). Ribonucleic acid is believed to occur in the cell membrane of yeast where it contributes to cation binding (21). The enzyme complex responsible for oxidative phosphorylation is considered to be in the mitochondrial membranes (16). Pinchot (22) has demonstrated that the polynucleotide required for oxidative phosphorylation in the bacterium *Alcaligenes faecalis* serves to bind electron transporting and phosphorylating components. An analagous binding role for RNA in mitochondria would explain the effect of RNase on mitochondrial activity and root respiration. In addition, polynucleotide in the limiting cytoplasmic membranes may bind enzymatic components essential to ion accumulation. Loss of such a structural moiety would also explain the noted leakage of solutes (table VIII).

The experiments reported here show that EDTA produces physiological responses similar to those elicited by RNase. The common responses are undoubtedly related through the common destruction of polynucleotides and/or the subsequent loss of solutes. The EDTA (and high concentrations of KCl) must act, then, to expose the RNA to endogenous RNase. This is directly demonstrated with root homogenates in table XII. Higuchi and Uemura (12) offer a similar explanation for the action of chelate ions in releasing nucleotides from yeast. It is possible that Ca and/or Mg (the principal divalent ions) are electrostatically bound to adjacent phosphate valences of RNA, and interfere with cleavage of the polymer. One equivalent of divalent ion can be bound per mole of polynucleotide phosphate (6). Substitution of monovalent K for the divalent ions would then permit degradative metabolism of the RNA. The application of potassium-EDTA would be particularly effective as noted here.

It is of interest in this connection that K will accelerate auxin-induced growth (5, 25), and low concentrations of EDTA will promote root growth (19, 29) and coleoptile elongation (10). (High concentrations of EDTA are growth inhibitory (19)). Perhaps some metabolism of RNA essential to growth is normally controlled by K/Ca + Mg ratio. Increasing the ratio by adding K or by chelation of divalent ions would favour RNA metabolism. Such a metabolism in roots is to be found in the decreasing amount of microsomal RNA and the increasing amount of RNA associated with mitochondrial and membranous material during cell growth (18). Part of this latter RNA may be that which is essential to respiration and ion accumulation, and may contribute to the increase in respiration and ion accumulation per unit protein known to occur with root cell expansion (3, 4, 14, 18, 28). In developing pea leaf tissue, Smillie & Krotkov (23) have described a correlation between RNA and photosynthetic and respiratory energy producing mechanisms.

The experiments with successive 0.5 cm root sections were undertaken to determine if changes in cell size and maturity would be reflected in altered sensitivity to EDTA. Although the affect of EDTA varies with cell maturity, no single pattern of change is evident. Previous observations of an increase in respiration and ion accumulation with cell growth are confirmed (respiration and ion accumulation, table XI, divided by protein content, tableXII). There is an increase in RNase activity with growth; the maximum activity occurs in the second section (0.5-1.0 cm) where the loss of soluble nucleotides and the impairment of respiration and K absorption are maximal. The loss of polynucleotides in this section is quite large. This correlation between loss of physiological function and RNase activity supports the hypothesis outlined above: that is, part of the RNA of the cell is functional with membrane-associated enzymes which carry out respiration and ion accumulation. Loss of protective divalent ions leads to hydrolysis of this RNA by endogenous RNase, with subsequent impairment of physiological function. The hypothesis, however, is not compatible with the maximum impairment of phosphate uptake and ion retention in the tip section where RNase activity is minimal.

In this respect it must be recognized that the removal of divalent cations by chelation (or by displacement with high concentrations of K) could impair root activity in other ways than by induction of RNA degradation. The loss of cations essential to oxidative metabolism could reduce respiration. Honda (13) has described such an effect in ascorbate oxidation by barley roots, but he did not report that EDTA, which he used as a buffer, impaired endogenous respiration. The loss of acid soluble nucleotides with EDTA, RNase or high concentrations of KCl is quite marked; it is likely that the loss of adenine nucleotides and coenzymes is a factor in the decline in respiration. However, the impaired activity of mitochondria isolated from EDTA treated roots (table VII) shows that some change beyond the loss of dissociable cofactors is involved; Mg, AMP, and dissociable respiratory cofactors were supplied to these mitochondria. Furthermore, direct application of RNase to mitochondria produces a similar impairment of activity (8).

Ion accumulation is dependent upon aerobic respiration and semi-permeable membranes. It is not clear in these experiments whether the diminished ion accumulation which follows EDTA application is due to degradation of ion accumulation sites, diminished respiration, "leaky" membranes, or all of these. Again it is possible that chelation of divalent cations has some direct effect on ion uptake and retention. The loss of Mg might well be of primary importance in phosphate accumulation since the process is believed related to oxidative phosphorylation (7), where Mg is required. Helder (11) has reported a leakage of Rb^{86} from barley roots in the absence of exogenous Ca or in the presence of sulphate ion. (The effect of the sulphate could be through the removal of Ca as the insoluble salt.) It is possible that the simple adsorption of Ca to the membrane serves to maintain semi-permeability. Helder's experiments, however, may have also involved degradation of RNA induced by removal of the Ca. Certainly the effect of RNase in causing a leakage of accumulated ion (table VIII) similar to that induced by EDTA suggests that the basic cause of leakage lies in RNA degradation. The temperature dependence of the EDTA effect on K accumulation (table XIII) implies that an enzymatic process is induced by the chelation of Ca and Mg.

The transitory respiratory burst which is obtained shortly after applying RNase or EDTA to the root tissue is not readily explained. The same effect can be obtained by applying RNase to mitochondria (8), but it is accompanied by lowered phosphorylation. Evidently, a partial hydrolysis of the functional RNA leads to an uncoupling of phosphorylation. It is assumed here that EDTA produces the same effect in vivo by induction of RNA degradation. The respiratory burst might also be explained by additional adenine nucleotides being made available, or by mitochondrial membranes becoming more permeable to substrate penetration, but not yet irreparably damaged.

The increased respiration found with K phosphate or chloride (salt-respiration) could be related to that induced by EDTA or RNase. Substitution of K for some divalent ion could permit a partial degradation of mitochondrial RNA by endogenous RNase, leading in turn to the respiratory increase as discussed above. Of course, if the K concentration were too high, and too much divalent ion were substituted, the RNA degradation would ultimately lead to respiratory inhibition (table III).

Summary

Treatment of soybean or corn root tips with ribonuclease, ethylenediaminetetraacetic acid or high concentrations of KCl impaired respiration after one to three hours. Concurrently, cold-acid soluble nucleotides were lost, partly to the medium; polynucleotides

began to degrade. The lowered respiration was related to loss of mitochondrial activity. Cell membranes lost their semi-permeability. The expanding root zone showed the greatest loss of soluble nucleotides and polynucleotides, respiration and K accumulation when treated with EDTA. This zone had the highest RNase activity. Phosphate accumulation and ion retention were most impaired in the meristematic and early cell expansion zones.

It is believed that RNA in membranes is implicated in ion accumulation, solute retention, and oxidative phosphorylation. It is thought that divalent ions normally protect this RNA from degradation by endogenous enzymes. Substitution of monovalent ions for divalent ions leads to the degradation of membranous RNA and the loss of physiological and biochemical functions. Normal metabolism of RNA could be governed by K/Ca + Mg ratios. Salt respiration may result from partial degradation of mitochondrial RNA.

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