NUCLEOTIDE & RIBONUCLEIC ACID METABOLISM OF CORN SEEDLINGS[1]

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The consideration of nucleotides and ribonucleic acid (RNA)[2] in metabolism has become increasingly important in cellular biology during the past few years. The commonly occurring nucleotides of wheat and corn plants have been identified by Bergkvist (1) and Cherry and Hageman (3), respectively. The latter have shown that the nucleoside di- and triphosphates in etiolated corn embryos shift to the nucleoside monophosphates as a function of growth.

Little is known about the metabolism of nucleic acid during seed germination. Oota et al (11, 12) have examined nucleic acid metabolism in germinating seeds, and have found large amounts of RNA stored in the cotyledons of a bean. Germination resulted in a steady decrease in the RNA content of the cotyledons with an increased RNA content in the seedling. The presence of the embryo was essential for breakdown of RNA in the cotyledons. If embryos were detached from the cotyledons, no RNA degradation occurred (13).

Recently, Cherry et al (4) have shown that RNA is synthesized in etiolated corn embryonic axis but degraded in the scutellum with both reactions being greater in hybrid plants than in their inbred parents.

Oota and Takota (12) have proposed that there are at least two types of RNA-s i.e. functional and transportable, in bean germ tissues. Their results appear to illustrate conversion steps from functional RNA to transportable RNA.

Hanson (6) indicates that RNA in membranes is implicated in ion accumulation, solute retention, and oxidative phosphorylation.

The experiments reported here represent studies on the metabolism of nucleotides, RNA, and protein in various cytoplasmic fractions of corn seedlings. Incorporation of P^{32} into various soluble nucleotides and cytoplasmic RNA-s, and ADP-C_8^{14} uptake by excised tissue were studied.

MATERIALS & METHODS

PLANT MATERIAL: Hybrid corn seed[3] (*Zea mays* L. var. WF9XM14) were lightly dusted with a fungicide (Spergon, U. S. Rubber Co.) and approximately 100 were placed embryo down on paper towelling supported by a layer of gravel (400 ml) in a 2 quart pyrex utility dish. The gravel and towelling were saturated with 200 ml of deionized water containing 0.5 mc of P^{32} (5.2 μg) as potassium phosphate. The seeds were germinated in darkness in a humid atmosphere at 29° C for 1, 2, 3, or 4 days. Additional deionized water was added to the dish during the germination period to keep the gravel saturated. Seedlings were harvested and washed several times with deionized water to remove excess P^{32} before use.

Plant material for the ADP-C_8^{14} studies was obtained by the above procedure except that the seeds were moistened with 10^{-4} M $CaCl_2$ instead of the radioactive phosphate solution.

ISOLATION OF CYTOPLASMIC PARTICULATES: The plant tissue (about one gm) was homogenized for 3 minutes in 5 ml of deionized water with an ice-jacketed glass homogenizer with a power-driven Teflon pestle. The homogenate was cleared of cellular debris by centrifugation at 500 *g* for 5 minutes. An aliquot of the debris-free homogenate was subjected to differential centrifugation in a Spinco model L ultracentrifuge, each successive particulate fraction being obtained from the supernate of the preceding fraction. The centrifugal forces, time, and the arbitrary designation of the isolated fractions were as follows: mitochondria, 22,000 *g* for 15 minutes; microsomes, 110,000 *g* for 1 hour; cytoplasmic sap, particulate-free supernate. The terms used to designate the cytoplasmic fractions are operational, as listed above, and do not necessarily mean the presence of pure morphological entities.

An isotonic grinding medium (0.5 M sucrose) was also used in the isolation of particulate fractions. Analysis of the particulates obtained from water and 0.5 M sucrose extractions were comparable; therefore, only the results from water extraction will be reported.

[1] Received August 5, 1960.

[2] The following abbreviations will be used: AMP, adenosine monophosphate; ADP, adenosine diphosphate; ATP, adenosine triphosphate; UMP, uridine monophosphate; UDP, uridine diphosphate; UTP, uridine triphosphate; GTP, guanosine triphosphate; CMP, cytosine monophosphate; CTP, cytosine triphosphate; DPN, diphosphopyridine nucleotide; TCA, trichloroacetic acid; RNase, ribonuclease; and RNA, ribonucleic acid.

[3] We are indebted to Dr. W. J. Mumm, Crow's Hybrid Corn Co., Milford, Ill., for the corn seed used in this work.

ANALYSIS OF CYTOPLASMIC FRACTIONS: The total acid-soluble nucleotides and RNA were extracted and measured by a method (5) similar to that of Ogur and Rosen (10). Aliquots of each of the water suspended particulate fractions (mitochondrial & microsomal) and the cytoplasmic fraction, and debris-free homogenate were acidified with 1.2 M $HClO_4$ to a final molarity of 0.2 which precipitated the RNA. The RNA precipitates were isolated by centrifugation and washed twice with 0.2 M $HClO_4$. The entire extraction procedure for the acid soluble nucleotides was performed at 2° C. The supernatant solution and the two washes were combined, made to volume, and the soluble nucleotides estimated by the difference of absorbancy at 260 and 290 mμ using AMP as a standard.

The precipitated RNA pellets were extracted thrice with 2:2:1 ethanol-ether-chloroform to remove lipids. RNA was hydrolyzed by incubating the lipid-free residue with 0.5 M $HClO_4$ at 70° C for 40 minutes. Absorbancy differences at 260 and 290 mμ of the cleared supernatant solution were referred to a standard curve obtained from similarly treated yeast RNA.

Protein was determined by the method of Lowry et al (8) on ice-cold 10 % TCA precipitates of each of the cytoplasmic fractions.

DETERMINATION OF RADIOACTIVITY: Aliquots of the acid-soluble nucleotides and of the hydrolyzed RNA were neutralized with KOH and the $KClO_4$ was removed by centrifugation. Each of the nucleotide and hydrolyzed RNA samples was dried and the radioactivity counted.

ADP-C_8^{14} UPTAKE: Root tips, 1 cm long, were sectioned from primary and secondary roots, apical mesocotyl sections (1 cm), and scutella were removed from 3-day-old corn seedlings. The tissue was rinsed in deionized water, blotted, and rapidly weighed to the nearest milligram before use. Tissue samples were placed in petri dishes and incubated in the light at room temperature with 30 ml of a solution containing 1 % sucrose, 10^{-3} M KH_2PO_4, and 1 μc of ADP-C_8^{14} (0.8 mg) adjusted to pH 6.3 with NH_4OH. After 8 hours the tissue was removed, rinsed several times with deionized water, weighed, and frozen.

Samples of the fresh tissue were frozen for initial nucleotide and RNA determinations.

NUCLEOTIDE EXTRACTION & ION-EXCHANGE CHROMATOGRAPHY: Scutellum and radicle tissues were removed from the etiolated corn seedlings (3 days old) and the acid-soluble nucleotides extracted from each tissue with ice-cold 0.6 M $HClO_4$. The procedures for extraction and column chromatography of the nucleotides were the same as previously described (3), except that Dowex-1 × 8 was used instead of Dowex-1 × 10. The eluate was collected in 10-ml volumes. The nucleotide content was determined by the absorption of light at 260 mμ and 1-ml aliquots from each fraction were dried and counted for radioactivity.

PAPER CO-CHROMATOGRAPHY OF ORGANIC PHOSPHATES & ORTHOPHOSPHATE: Samples obtained by concentrating the eluate of peak fractions from column chromatography were co-chromatographed on paper with standard phosphate compounds in different solvents. The solvents most used for chromatography were *n*-propanol: NH_4OH: H_2O (60:30:10) and isobutyric acid: NH_4OH: H_2O (57:4:39). After the paper chromatograms were developed and dried, phosphorus-containing compounds were detected with a molybdic acid spray reagent (2). In some instances in which the unknown radioactive samples were too dilute to react with molybdic acid, the paper was cut into 2-cm strips and these were in turn cut into 2-cm squares. Each of the squares was counted for radioactivity, thereby establishing the location of the unknown on the paper.

All results reported in this paper are based on two or more separate experiments. Each assay was made in duplicate.

RESULTS

CHANGES DURING SEED GERMINATION: Typical dry weight changes of corn scutellum and radicle are given in table I. The scutellum decreased in dry weight about 18 % whereas the radicle increased in dry weight 42-fold in the 3-day period (1–4 days).

As corn seed germinate there is a production of nucleotides in both scutellum and radicle tissues (table II). An interesting facet of RNA metabolism during corn seed germination is that 57 % of the scutellum RNA was degraded by the 4th day whereas radicle RNA increased 182 %. Growth (dry wt) of the radicle occurred at the same rate as nucleotide synthesis, but RNA was synthesized at a much slower rate.

Protein metabolism was similar to RNA metabolism in the scutellum and radicle tissues; however, the RNA to protein ratio was not constant for either tissue. The RNA to protein ratios (μg/mg) in the scutella were 67, 79, 57, and 51 from 1 to 4 days, inclusively, whereas the ratios in the radicles were 160, 143, 79 and 96.

Changes in protein content in the designated fractions of radicle and scutellum are represented in

TABLE I

DRY WEIGHT CHANGES OF CORN SCUTELLUM & RADICLE TISSUE WITH GROWTH

AGE, DAYS	DRY WT/SCUTELLUM mg	DRY WT/RADICLE mg
1	24.5	0.6
2	23.1	6.8
3	21.0	18.5
4	20.1	26.1

TABLE II

CHANGES IN CYTOPLASMIC NUCLEOTIDES, RNA & PROTEIN IN CORN SEEDLINGS WITH GROWTH

AGE, DAYS	μg/SCUTELLUM			μg/RADICLE		
	NUCLEOTIDES	RNA	PROTEIN	NUCLEOTIDES	RNA	PROTEIN
1	39	150	2,230	9	51	320
2	51	145	1,830	118	96	670
3	80	99	1,720	268	97	1,230
4	80	65	1,290	369	144	1,500

figure 1. The protein content in all the cytoplasmic fractions of the radicle (fig 1–A) increased. However, the major protein increase was in the mitochondrial and cytoplasmic sap fractions with the microsomal protein increasing very little. In general, the cytoplasmic protein in the radicle was almost evenly divided into mitochondrial and cytoplasmic sap proteins.

Scutellum cytoplasmic proteins decreased in all fractions (fig 1–B). The greatest protein loss was from the cytoplasmic sap. The amount of mitochondrial protein remained essentially the same on the 2nd day and decreased on the 3rd and 4th days.

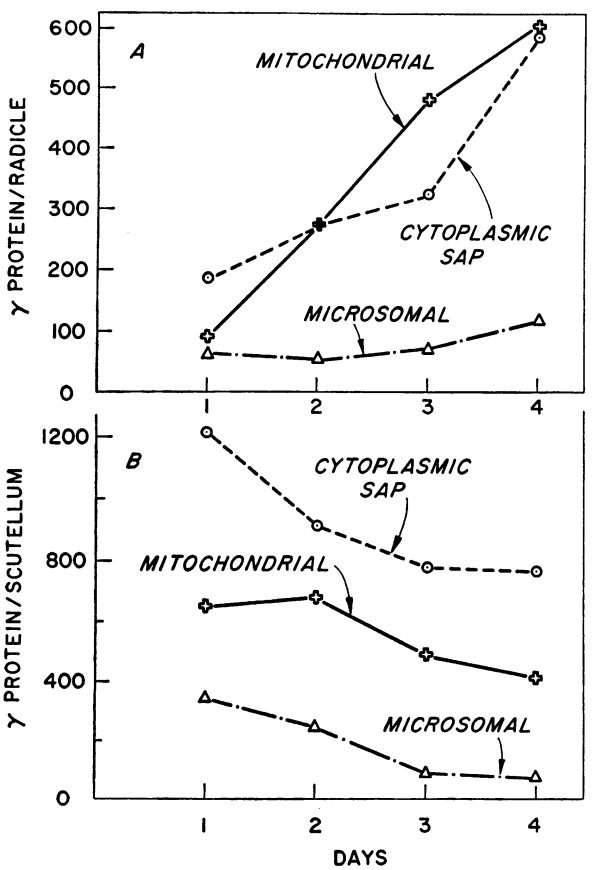


FIG. 1. Changes in the protein content in various cytoplasmic fractions of corn radicles and scutella during seed germination and growth.

Microsomal protein in the scutellum decreased in a linear manner from 1 to 3 days with no essential loss on the 4th day.

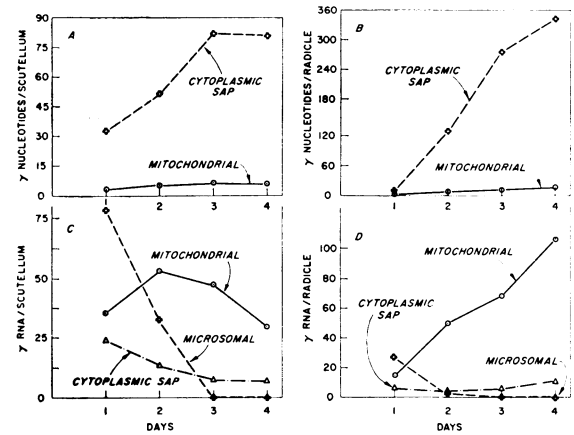
Changes in nucleotide and RNA contents in the cytoplasmic fractions of scutellum and radicle tissues are illustrated in figure 2. No nucleotides were detected from the microsomal fraction with small amounts from the mitochondrial fraction. The scutellum and radicle cytoplasmic sap nucleotides (fig 2–A & 2–B) increased the 2nd and 3rd days at average rates of 24 and 130 μg per day, respectively. There was no change in scutellum nucleotides on the 4th day with the radicle nucleotides increasing slightly.

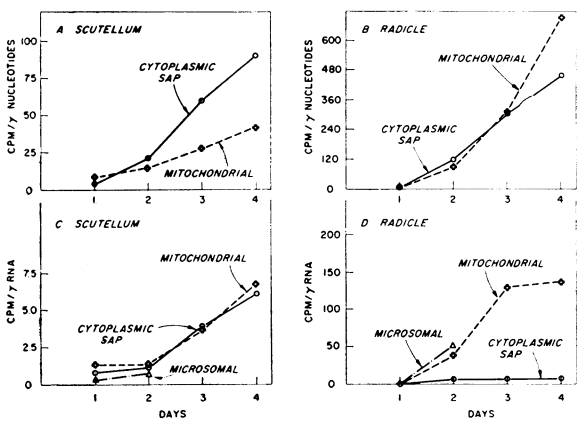
Over 50 % of the RNA content of 1-day old corn scutella was in the microsomes (fig 2–C). However, the greatest RNA degradation in the scutellum occurred in the microsomes with the RNA content decreasing to almost zero on the 3rd day. Mitochondrial RNA increased on the 2nd day (17 μg) and decreased 6 and 18 μg on the 3rd and 4th days, respectively. Cytoplasmic sap RNA of the scutellum decreased at an average rate of 8 μg per day from 1 to 3 days with no change in the RNA content on the 4th day.

About half of the RNA content of 1-day old radicles was in the microsomes with most of the RNA of 2-, 3- and 4-day-old radicles in the mitochondria (fig 2–D). Mitochondrial RNA of the radicle increased from 1 to 4 days at an average rate of 30 μg per day. Microsomal RNA of the radicle decreased to almost zero by the 2nd day with no change thereafter. Cytoplasmic sap RNA remained unchanged on the 2nd and 3rd days with the content slightly increasing on the 4th day.

The relative amounts of radioactive inorganic phosphate incorporated into nucleotides and RNA in the cytoplasmic fraction of scutellum and radicle tissues are illustrated in figure 3. The relative rates of phosphate incorporation into the mitochondrial and cytoplasmic sap nucleotides of the scutellum (fig 3–A) were linear from 1 to 4 days. The cytoplasmic sap nucleotides were labeled with P^{32} at a faster rate and incorporated a larger amount than the mitochondrial nucleotides. The mitochondrial and cytoplasmic sap nucleotides (fig 3–B) of the radicle were labeled at the same rate on the 1st, 2nd, and 3rd days, but the mitochondrial nucleotides were labeled at a faster rate on the 4th day. The rate of phosphate incor-

poration into both the mitochondrial and cytoplasmic sap nucleotides increased with time. No attempt was made to correct for the amount of labeled inorganic phosphate in the acid-soluble (nucleotide) fraction.





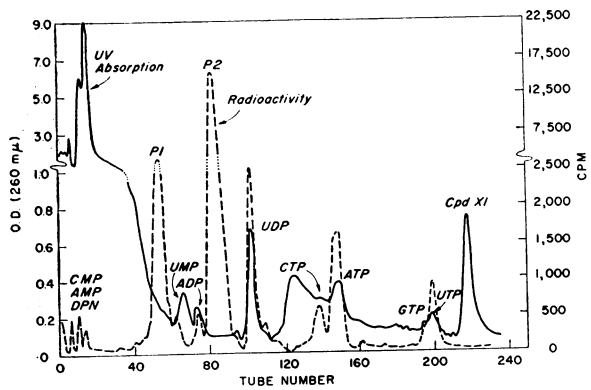


FIG. 2. (*top*). Changes in nucleotide and RNA contents in various cytoplasmic fractions of corn radicles and scutella during seed germination and growth.

FIG. 3. (*center*). The incorporation of radioactive phosphate into cytoplasmic nucleotides and RNA-s during seed germination and growth.

FIG. 4. (*bottom*). An elution-chromatogram of the acid-soluble compounds from 3-day-old corn radicles grown in a radioactive phosphate medium.

No radioactive phosphate was incorporated into the cytoplasmic RNA-s of the scutellum (fig 3-C) on the 1st and 2nd days. The mitochondrial and cytoplasmic sap RNA-s incorporated only small amounts of radioactive phosphate on the 3rd and 4th days. The low amount of phosphate incorporated may be due to exchange reactions rather than de novo synthesis of RNA from nucleosides and inorganic phosphate since mitochondrial and cytoplasmic sap RNA-s in the scutellum are being degraded at the time when small amounts of the label are incorporated.

The relative amount of phosphate incorporated into the microsomal RNA is reported only for 1- and 2-day-old scutella (fig 3-C) and radicles (fig 3-D), because very small amounts or no RNA was detected in the microsomal fractions of older tissue. Even though the amount of RNA in the microsomes of 3- and 4-day-old seedlings was too little to be measured accurately, a fair amount of radioactivity could be measured.

The majority of the radioactive phosphate incorporated into the cytoplasmic RNA-s of the radicle was in the mitochondria (fig 3-D). The rate of phosphate incorporation into the mitochondrial RNA was nearly linear from 1 to 3 days with none being incorporated on the 4th day. The cytoplasmic sap did not incorporate radioactive phosphate into its RNA to a significant extent. Even though the microsomal RNA was decreased nearly to zero by the 2nd day in the radicle, a large amount of the residual microsomal RNA was labeled with radioactive phosphate, suggesting a rapid turnover.

GROWTH & ADP-C_8^{14} UPTAKE BY EXCISED TISSUES: Root and mesocotyl sections incubated under suitable conditions increased in fresh weight 13 and 15 %, respectively, in an 8-hour period (table III). The increased weight of the scutella (3 %) was probably due to hydration. The root and mesocotyl tissues lost soluble nucleotides during the incubation period with no change in the soluble nucleotide content of the scutellum. None of the tissues had a net gain or loss in RNA.

All three of the tissues appeared to accumulate large amounts of ADP-C_8^{14} into the acid-soluble fractions. Root tips accumulated more ADP-C_8^{14} than did either the mesocotyl or scutellum tissues. However, the scutellum tissue accumulated three times more ADP-C_8^{14} than did the mesocotyl sections.

Even though there was no net gain in RNA in any of the tissues, the root tips and scutella incorporated a large amount of ADP-C_8^{14} into their RNA-s. The ratios of ADP-C_8^{14} incorporated to accumulated were 1:7, 1:12, and 1:6 for root tips, mesocotyl sections, and scutella, respectively. Although the mesocotyl tissue accumulated less (67-75 %) ADP-C_8^{14} than either scutellum or radicle tissues (table III), it was even less efficient in incorporating ADP-C_8^{14} into RNA.

SEPARATION OF ORGANIC PHOSPHATES & PHOSPHORIC ACID BY COLUMN CHROMATOGRAPHY: An

elution-chromatogram of the ultraviolet absorbing and P^{32} labeled components from 3-day-old radicle tissue which had been grown in a radioactive phosphate medium is given in figure 4. Similar elution-chromatograms were obtained from 3-day-old scutellum tissue; however, due to the likeness to their radicle elution-chromatograms only the latter will be presented. The identification of the nucleotides was established by the order and position in which they were eluted from the column as previously described (3). All the commonly occurring nucleotides previously identified were labeled with P^{32} by the 3rd day.

In general, the radioactive peaks were much sharper than the ultraviolet peaks (O.D., 260 mμ) which suggests that other ultraviolet absorbing compounds, not nucleotides, are adsorbed to the Dowex and are eluted from it contaminating the nucleotide peaks and thus interfering with quantitative nucleotide measurements. Generally, UDP, ATP, and UTP contained most of the nucleotide P^{32} label. There were two radioactive peaks (P_1 & P_2) which did not correspond to any of the ultraviolet peaks. P_1 and P_2 contained 10 and 42 % of the total radioactivity added to the column, respectively. The composited eluates of P_1 and P_2 were each lypholyzed to dryness and co-chromatographed on paper using two different solvents (Methods). Each of the unknown samples (P_1 & P_2) contained only one component. The Rf values of P_2 in both solvents used were the same as that of phosphoric acid. The identity of P_2 as phosphoric acid was further verified by adsorbing radioactive phosphate on a Dowex–1 × 8 column using ADP as a marker and eluting with the same system as used for column chromatography of the acid-soluble nucleotides (3). Radioactive phosphoric acid was eluted from the column at the same tube number as was P_2.

The identity of P_1 was not established; however, its migration in both solvents was similar to the hexose monophosphates. Due to the small amount of P_1 obtained from the column, a sugar moiety was not determined. The position of P_1 on the elution-chromatogram suggests that it may be a sugar phosphate.

It is interesting that very little of the radioactive phosphate was incorporated into the nucleoside monophosphates. No trace of radioactivity was found in Compound XI which agrees with previous work (3) that Compound XI is not a nucleotide.

Discussion

The evidence gained from these experiments indicates that there is a dynamic change in the particulates of radicles and scutella as corn seedlings grow. The RNA measurements, which may be regarded as an index to particulate mass, show that the microsomes sedimenting at 110,000 *g* rapidly decrease with seedling growth (fig 2). The small microsomal particulates appear to be either completely degraded or attached to larger or similar particulates so that some sediment with mitochondria as the plant material matures. If the microsomes are attached to other particulates so that they have a mass similar to mitochondria or if they are attached to the mitochondria, it is not known whether or not the cytological function of the microsomes is also changed. Although the cytoplasmic particulates undergo a dynamic change, it seems unlikely that the microsomes are completely degraded since protein is synthesized on the 4th day. The rapid decrease in microsomal RNA with growth is probably due to the decline in number of active cells as compared to mature cells.

Investigations by Lund et al (9) clearly show that there are changes in cytoplasmic particulates during growth and maturation of root cells which correlate with changes in the composition and physiological activity of these cells.

The mitochondrial RNA of the scutellum and radicle tissues may be correlated with the changes of the mitochondria. Lund et al (9) reported that immature mitochondria develop cristae and reach maturity as roots grow. Hanson et al (7) reported that the mitochondrial mass of corn scutellum increased with germination for 3 days, then began to decline. During germination of corn seed the respiratory activity of the scutellum (7) increased for 3 to 4 days with a decline in respiration thereafter. There observations on the development of mitochondria in

Table III

Changes in Fresh Weight, Soluble Nucleotides, & RNA, & ADP-C_8^{14} Uptake by Scutellum & Root Tip & Mesocotyl Sections in 8-Hour Period

Tissue	Tissue growth % (increase)	μg/gm Fresh weight				ADP-C_8^{14} Uptake[1] (cpm/gm Fresh wt)	
		Soluble nucleotide		RNA			
		Initial	Final	Initial	Final	Soluble nucleotides	RNA
Root tips (1 cm)	12.8	1,630	1,375	2,290	2,260	11,875	2,033
Mesocotyl section (1 cm)	15.2	1,530	1,235	813	835	3,370	317
Scutella	3.0	959	963	2,170	2,185	9,210	1,980

[1] The sum of radioactivity in the soluble nucleotide and RNA fractions is considered as the amount accumulated.

corn scutellum can be associated with the increase in mitochondrial RNA (fig 2).

As corn seed germinates there is a steady decline in scutellum RNA and a synthesis of radicle RNA (table II). The loss of scutellum RNA is greater than the increase in soluble nucleotides. A possible explanation for the mode of RNA utilization is that the RNA is degraded to nucleotides and used for growth of the embryonic plant or that a transportable RNA is moved to the growing tissue where it is used.

Summary

The change in amounts of nucleotides and RNA in corn seedlings during germination has been studied. Normally, as corn seeds germinate there is a production of nucleotides in the scutellum and in the radicle tissues. Both scutellum and radicle tissues incorporated fairly large amounts of radioactive phosphate into the acid-soluble nucleotides with the radicle accumulating and incorporating radioactive phosphate at a faster rate than the scutellum.

RNA of the scutellum was rapidly degraded apparently at the expense of the microsomes. Mitochondrial RNA was highest on the 2nd and 3rd days with rapid degradation occurring on the 4th day. The amount of radioactive phosphate incorporated into the scutellum RNA-s appears to be due to exchange reactions rather than de novo synthesis.

Generally, all of the RNA of the radicles was in the mitochondria except for 1-day-old radicles which had about half of the RNA content in the microsomes. All of the radioactive phosphate incorporated into the cytoplasmic RNA-s in the radicles was ultimately localized in the mitochondria. The cytoplasmic sap RNA incorporated no radioactive phosphate.

The protein content increased in all the cytoplasmic fractions in the radicle from 1 to 4 days whereas the protein content in the scutellum cytoplasmic fractions decreased with germination.

The nucleotide content of excised root tips and mesocotyls decreased in an 8-hour incubation period with ADP-C_8^{14}. The scutellum nucleotides did not change in the same period. The RNA contents of the root tips, mesocotyl sections, and scutella remained the same throughout the incubation period, however, the root tips and scutella incorporated a fairly large amount of ADP-C_8^{14} into their RNA-s. Mesocotyl sections did not incorporate ADP-C_8^{14} into its RNA as well as root tips or scutella.

The organic phosphates and phosphoric acid labeled with P^{32} from 3-day-old radicle tissue were separated by ion-exchange chromatography. The nucleoside di- and triphosphates contained most of the nucleotide radioactive phosphate. Two radioactive peaks were separated that did not correspond to any nucleotide peaks. The first radioactive peak was not identified by paper co-chromatography but it is believed to be a sugar phosphate. The second peak was identified as phosphoric acid using paper co-chromatography and ion-exchange chromatography.

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