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Incorporation of a Kinin, *N*,6-Benzyladenine into Soluble RNA[1]

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Summary. Kinin requiring tobacco and soybean tissues incubated on a medium containing *N*,6-benzyladenine-8-C^{14} incorporated C^{14} into several RNA components including adenylic and guanylic acids. About 15 % of the label taken up by the tissues appeared in RNA while the remainder was distributed among several metabolites in the soluble, non-polynucleotide fraction. Tissue grown on a kinin labeled in the side chain (*N*,6-benzyladenine-benzyl-C^{14}) also incorporated a small, but nevertheless repeatable, amount of radioactivity into minor RNA components.

Ultracentrifugation studies and methylated albumin chromatography indicated that the bulk of the label from benzyladenine-benzyl-C^{14} is in soluble RNA. Approximately 50 % of the C^{14} in soluble RNA is in a component which has chromatographic properties like that of benzyladenine.

It is suggested that the biological action of the kinins may hinge on their providing substituted bases in RNA in tissues which through differentiation no longer synthesize RNA-methylating enzymes. As an alternative it was hypothesized that a small amount of benzyladenine was incorporated into a *m*-RNA, acting there as a derepressing agent, perhaps by preventing its normal repressing function.

The broad spectrum of biological activity exhibited by kinetin (6-furfurylaminopurine) and its analogues raises the possibility that certain 6-substituted purines have an important role in the control of growth and development of higher plants. The fact that many plant tissues require a kinin for in vitro growth (15), that kinins can exhibit profound growth stimulating effects in concentrations as low as 0.004 mg per liter (17), and that natural kinins have been partially purified from several plant sources lend support to this hypothesis.

Despite the potential significance of this group of plant growth regulators, only a single published study exists concerning the metabolic fate of a kinin in plant tissues. McCalla, Mooré, and Osborne (14) in 1962 demonstrated that senescing cocklebur and bean leaves converted *N*,6-benzyladenine-8-C^{14} into a number of low molecular weight substances which included adenylic, guanylic, and inosinic acids, benzyladenosine, and probably benzyladenylic acid. In addition they reported that although there seemed to be a tiny amount of a labeled compound in alkaline hydrolysates of cocklebur leaf RNA which was chromatographically similar to benzyladenylic acid, the amount was too small for confirmation, and it was concluded that benzyladenine is not incorporated into RNA to any significant extent.

If, however, one desires information dealing with the role of kinins in cell division and growth, it would seem appropriate to investigate a system in which kinins are limiting for these processes. Accordingly tobacco and soybean tissue cultures which have an absolute kinin requirement for in vitro proliferation were chosen for this study; evidence is presented here that a kinin, *N*,6-benzyladenine (BA)[2] is incorporated into the soluble RNA of these cultures. A preliminary report of these findings has been made (9).

Materials and Methods

Synthesis of C^{14} Labeled N,6-Benzyladenine. The general synthetic method is essentially that of Daly and Christensen (6) in which 6-chloropurine is refluxed with the appropriate amine. In the present study, however, the reaction was carried out in water instead of *n*-butanol because of the relative insolubility of the end product compared to the reactants and possible impurities and the consequent ease of purification.

The synthesis of *N*,6-benzyladenine-8-C^{14} (BA-8-C^{14}) was achieved by refluxing 2.15 mg of 6-chloropurine-8-C^{14} (Calbiochem, specific activity 3.6 mc/mM) for 8 hours in 50 ml water containing a like amount of unlabeled 6-chloropurine and 1.0 ml benzylamine. The reaction mixture was taken to dryness in a rotary evaporator, the crystalline material washed twice with 2.0 ml of ice cold water, and the residue taken up in 10 ml hot 95 % ethanol. This preparation was further purified by chromatography on acid washed Whatman No. 1 paper.

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[2] Abbreviations: BA, *N*,6-benzyladenine; *t*-RNA, transfer RNA; *m*-RNA, messenger RNA.

Preliminary studies with unlabeled material indicated that such a procedure resulted in better than 80 % yields of pure white, crystalline material, mp 231 to 232°, presumed to be *N*,6-benzyladenine and having ultraviolet spectra (determined with a Bausch and Lomb model 505 recording spectrophotometer) as follows: λ max 0.1 N NH_4OH 275 mμ, λ max 0.1 N HCl 274.5 mμ, λ max H_2O pH 6.0 269.5 mμ. The melting point and ultraviolet spectra are in good agreement with published values for *N*,6-benzyladenine (2). In addition this material proved to have excellent kinin activity in both tobacco and soybean test systems, inducing detectable proliferation in the latter at concentrations as low as 0.1 μg/liter.

Benzyl labeled *N*,6-benzyladenine (BA-benzyl-C^{14}) was synthesized in a similar manner by reacting 14.92 μM of benzylamine-7-C^{14} (Volk, specific activity 6.7 mc/mM) with 16.18 μM (2.5 mg) 6-chloropurine. For the preparation of some batches, benzylamine-7-C^{14} • HCl (Calbiochem, specific activity 4.1 mc/mM) was used.

Preparation of Tissue Extracts. The origin of the soybean and tobacco tissue cultures used here, their absolute dependence upon a kinin for in vitro cultivation, and their growth on various levels of kinin and auxin have previously been described (8, 10).

For the preparation of nucleotides for anion exchange chromatography, tissues were ground in a Waring blendor with sufficient boiling 95 % ethanol to achieve a final concentration of 70 % ethanol. The extract was centrifuged at 3000 × *g* for 20 minutes, the clear supernatant fluid decanted, and the pellet reextracted with successive washes of hot 95 % ethanol, absolute acetone, acetone-diethyl ether 1:1, v/v, and 70 % ethanol. A final hot 95 % ethanol wash was essentially free of detectable C^{14}. The pellet was then stirred into 10 times its volume of 1 N KOH and incubated at 28° for 18 hours. Potassium was removed as the perchlorate by centrifuging at pH 7.5. DNA polynucleotides were then precipitated at pH 3.5 (HCl) in the presence of magnesium ions by adding 3 volumes of ice-cold 95 % ethanol and allowing the whole to stand at 0° for 6 hours. Precipitated material was centrifuged off, washed twice with cold 70 % ethanol, and taken up in a small volume of water adjusted to pH 8.0 with NaOH. The supernatant fraction, containing RNA nucleotides, as well as the ethanol, acetone and ether extracts were taken to small volumes in a rotary evaporator.

RNA nucleotides were fractionated on Dowex-1 anion exchange resin by a method differing only in minor respects from that of Cohn and Volkin (4), and described in the legend to figure 2.

In other experiments tissues were homogenized at 2° in 0.2 M Tris-HCl buffer, pH 7.6, containing 0.5 M sucrose. The homogenate was centrifuged in a Spinco Model L ultracentrifuge at 12,000 × *g* for 15 minutes. The sediment was discarded and the supernatants centrifuged at 105,000 × *g* for 180 minutes. The particulate fraction thus obtained was washed by resuspension in the homogenizing medium and a second sedimentation. The centrifugate (assumed to contain ribosomal RNA) and the supernatant fraction (soluble RNA) were treated by the method of Bergquist and Matthews (1) which involves extensive dialysis and reprecipitation of the RNA and which is designed to minimize contamination of RNA by low molecular weight, nonpolynucleotide materials.

Total nucleic acids were extracted for methylated albumin chromatography by a method essentially that described by Cherry (3). Preparation of the methylated albumin and fractionation of nucleic acids on columns of this material followed the procedures of Mandell and Hershey (13).

Estimation of Radioactivity. Distribution of radioactivity on paper chromatograms was determined either by autoradiography on Kodak no-screen x-ray film or by scanning with a Nuclear-Chicago Actigraph II paper strip counter. For an estimation of the amount of radioactivity on chromatograms, strips of paper approximately 1.5 × 3 cm were immersed in 15 ml of a solution for scintillation counting [50 mg of 1,4-*bis*-2-(4-methyl-5-phenyloxazolyl) benzene and 3 g of *p*-terphenyl per liter of toluene] in standard counting vials. Samples were assayed for radioac-

Table I. *Distribution of Radioactivity from Benzyladenine in Soybean and Tobacco Tissues*

The figures represent a percent of the C^{14} taken up by the tissues as benzyladenine and are averages of 2 to 5 experiments. Each experiment involved 30 pieces of tissue, incubated from 20 to 40 days, on 250 ml of a medium containing an average of 6.763 × 10^6 cpm as C^{14} labeled benzyladenine. Of this amount an average of 2.75 × 10^6 cpm (or about 40 % of that administered) were recovered by extraction from each batch of 30 pieces of tissue.

Fraction	*N*,6-Benzyladenine-8-C^{14}		*N*,6-Benzyladenine-banzyl-C^{14}	
	Tobacco	Soybean	Tobacco	Soybean
	% of total		% of total	
*Nonpolynucleotide, low molecular weight materials	84.5	84.3	98.0	98.3
Total RNA	14.7	15.0	2.0	1.7
Soluble RNA	...	...	1.6	1.5
Ribosomal RNA	...	...	0.4	0.2
DNA	0.8	0.7	<0.001	<0.001

* See text for details of extraction procedure.

tivity in a Packard tri-carb model 334 liquid scintillation spectrometer.

Fractions from anion exchange or methylated albumin chromatography were dried in a stream of air at 60° onto Whatman No. 1 paper strips which were counted by liquid scintillation. Alternatively in some experiments fractions were pooled, taken to dryness, made up to a small volume in water and counted in duplicate or triplicate by liquid scintillation spectrometry (50 μl portions of the sample were added to a counting vial which contained 5 ml absolute ethanol and 10 ml of the counting solution previously described).

Results

Metabolism of N,6-Benzyladenine-8-C^{14}. About 41 % of the C^{14} supplied in the medium as BA-8-C^{14} (1.0–2.0 mg/liter) was recovered by extraction from tobacco tissue harvested after 35 days culture (avg yield: 31 mg tissue per ml medium). No attempts were made to recover possible excretion products in the medium or the atmosphere or residual radioactivity in the material insoluble after alkaline hydrolysis. From soybeans grown for 40 days (avg yield 38 mg tissue per ml medium) a like amount (40 %) of the C^{14} available in the medium was obtained in extracts. The bulk of the radioactivity was in the nonpolynucleotide, soluble fraction although a substantial amount of labeling in RNA also occurred (table I).

Chromatography of the nonpolynucleotide fractions extracted in ethanol and other organic solvents revealed extensive metabolism of the purine moiety of the kinin. At least 9 radioactive metabolites were separated by paper chromatography of extracts of both soybean and tobacco (fig 1) although the bulk of the C^{14} is confined to 2 or 3 products.

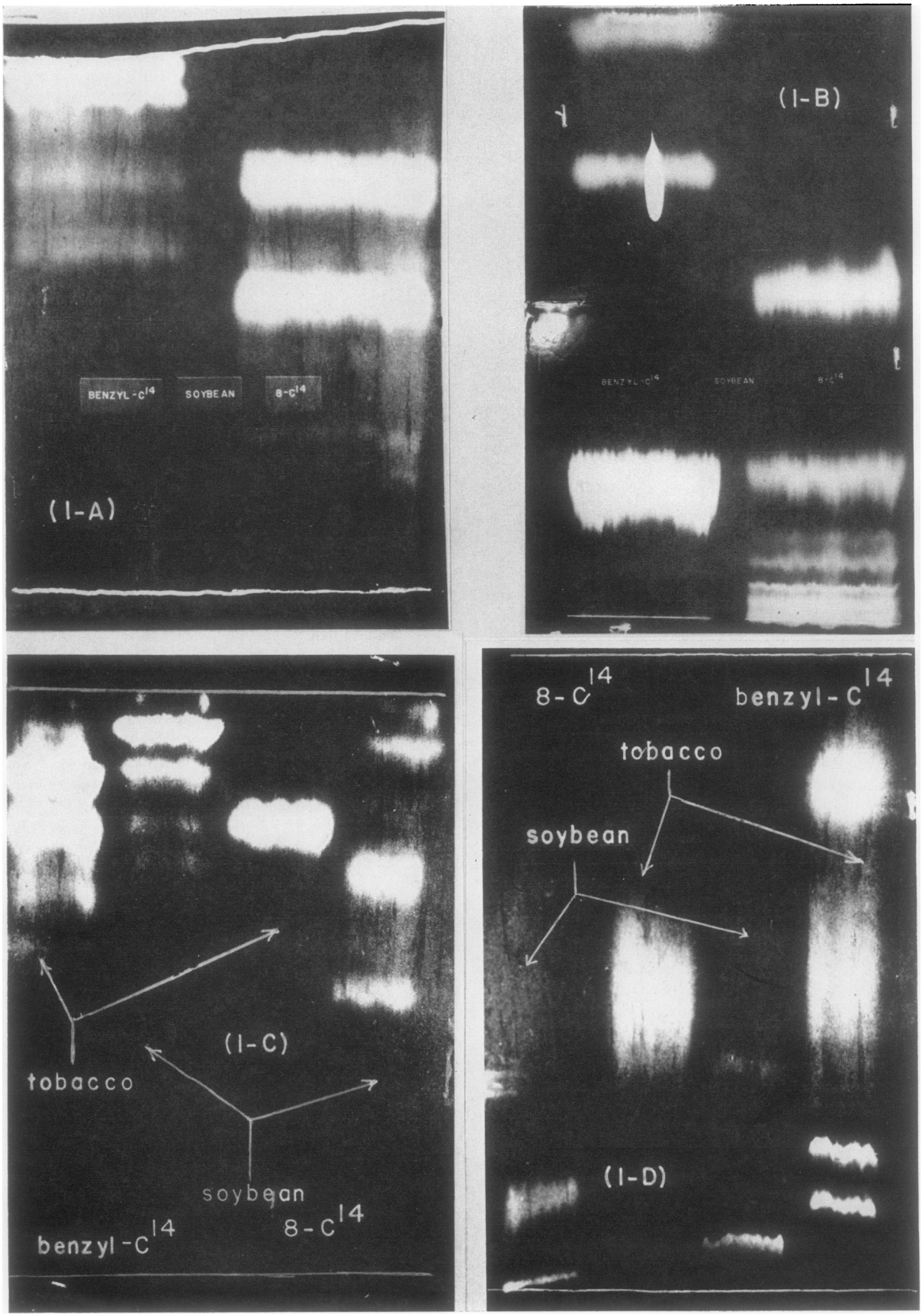
It is clear (table I) that the purine moiety of *N*,6-benzyladenine is incorporated to a fairly large extent into the RNA of soybean and tobacco tissue. Anion exchange chromatography of RNA nucleotides shows that both major and minor components of the RNA are labeled with C^{14} (fig 2). The purine moiety of benzyladenine appeared to be incorporated into guanylic acid preferentially to adenylic acid (fig 2). To check this point further, KOH hydrolysates of tobacco RNA were chromatographed on Whatman No. 3 MM paper and assayed for radioactivity in a paper strip counter; again the bulk of the C^{14} was associated with guanylic acid (fig 3).

Metabolism of N,6-Benzyladenine-Benzyl-C^{14}. Figure 1 demonstrates that the side chain of benzyladenine is likewise extensively metabolized. An even more striking finding is that most of the radioactive metabolites of benzyl labeled benzyladenine do not correspond in R_F values to radioactive metabolites of purine ring labeled benzyladenine. Such a finding indicates that the bulk of the kinin taken up by the tissue has been degraded. Liquid scintillation counting of paper chromatograms run in several solvent systems shows that the benzyl side chain was cleaved from the adenine moiety in more than 95 % of the benzyladenine taken up by the tissue.

Since the purine nucleus of benzyladenine was incorporated into both major and minor RNA components (fig 2), it is important to know whether or not the benzyl side chain accompanied the entry of any of the purine into polynucleotides. Accordingly, KOH hydrolysates were made of exhaustively extracted tissue which had been growing on benzyladenine-benzyl-C^{14}, and these showed a small, but definite and repeatable, amount of radioactivity (table I). The distribution of C^{14} in nucleotides from the KOH hydrolysate fractioned on Dowex-1 anion exchange resin is shown in figure 4. This procedure has been repeated several times and each fraction counted by both liquid scintillation techniques described in Materials and Methods; with the exception of minor differences due to subtle variations in technique, the general pattern of labeling shown in figure 4 was always obtained.

Radioactive material eluted from the column just prior to adenosine-2′-phosphate (fraction a, fig 4) is of particular importance since it occurs as a major radioactive peak in the RNA of both kinin requiring tissues. This fraction was taken to dryness, hydrolyzed at 100° in 1 N HCl for 90 minutes, the HCl removed with the aid of an ion-retardation resin (AG11A8, Calbiochem) and the hydrolysate cochromatographed on Whatman No. 1 paper with benzyladenine. About 75 % of the radioactivity was associated with the benzyladenine control spot in the following solvent systems: (a) water (b) *t*-butyl-alcohol, glacial acetic acid, water (3 : 1 : 1, v/v) (c) isopropanol, HCl, water (670 : 176 : 154, v/v) (d) isopropanol, NH_4OH, water (16 : 1 : 3, v/v). The bulk of the remainder of the C^{14} moved with R_F values in these solvent systems similar to *N*,6-methyladenine. Radioactive components b and c, especially prominent in tobacco, occur in amounts too small even to obtain ultraviolet spectra and have not been identified.

Separation of Labeled RNA into Soluble and Ribosomal Fractions. In order to determine if the observed incorporation of benzyladenine occurred preferentially into any RNA species, soluble and ribosomal fractions were obtained by differential centrifugation and purified by the method of Bergquist and Matthews (1). In a typical experiment RNA was isolated from 29 g of tobacco tissue which had been growing for 30 days on a medium containing *N*,6-benzyladenine-benzyl-C^{14}. The combined soluble nonpolynucleotide fractions contained approximately 10^6 cpm, while the purified soluble RNA fraction contained 15,660 cpm and the ribosomal RNA 3658 cpm. A similar excess of radioactivity in the soluble RNA as compared to the ribosomal fraction was obtained in repeat runs using the differential centrifugation technique. In general the amount of C^{14} incorporated into soluble RNA varied from 0.5


BENZYL-C14
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tobacco
soybean
(1-D)

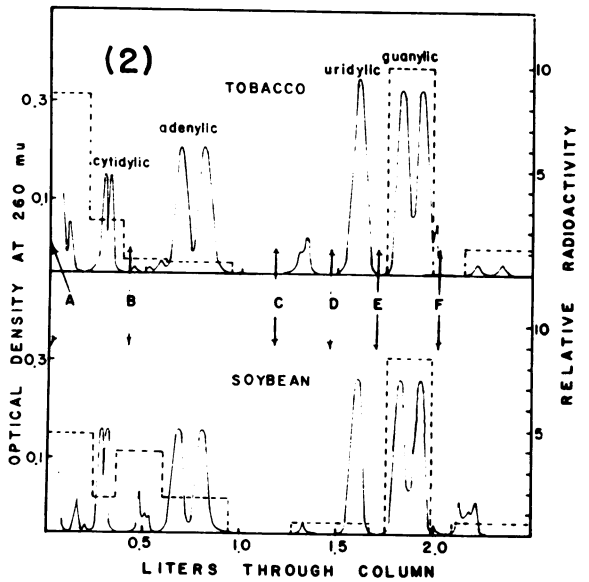

FIG. 2. Anion exchange chromatogram of KOH hydrolyzed RNA from tobacco and soybean tissues grown on *N*,6-benzyladenine-8-C^{14}. Nucleotides from about 15 g (fr wt) tissue were put on a column 1 × 12 cm of Dowex-1-× 8, 200 to 400 mesh in formate form. The column was eluted as follows: A) 0.02 M formic acid, B) 0.15 M formic acid, C) 0.01 M formic acid + 0.05 M ammonium formate, D) 0.1 M formic acid + 0.1 M ammonium formate, E) convex gradient 0.1 M to 1.0 M formic acid + ammonium formate, F) 1.0 M formic acid + 1.0 M ammonium formate. In some runs F) was omitted without changing the results. See text for description of assay for radioactivity.

to 2.5 % of the C^{14} present in the nonpolynucleotide material.

As a further check on these results total cellular nucleic acids from soybean and tobacco tissues grown for 30 days on a medium containing *N*,6-benzyladenine-benzyl-C^{14} were subjected to methylated albumin chromatography. Again it is clear (fig 5) that the great bulk of the radioactivity is in soluble RNA although small amounts appeared in other RNA fractions. The identity of radioactive component d is not clear although a substance having similar chromatographic properties has been characterized as precursor transfer RNA (5). The fractionation shown in figure 5 has been repeated 4 times with similar results.

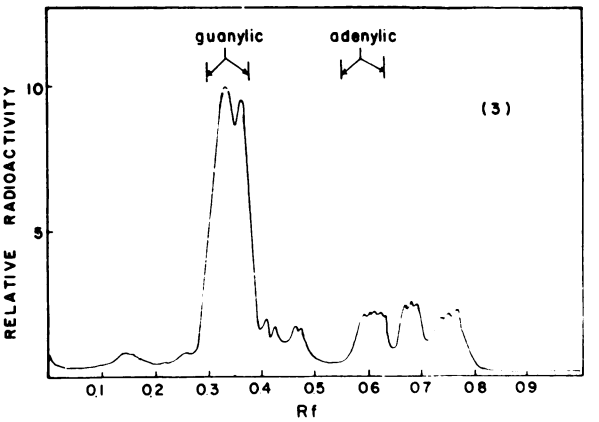

FIG. 3. Distribution of C^{14} in RNA nucleotides from tobacco tissue grown on *N*,6-benzyladenine-8-C^{14}. Nucleotides were separated by chromatography on Whatman No. 3 MM paper in isobutyric acid, water, NH_4OH, 0.1 M disodium ethylenediaminetetraacetate (100 : 55.8 : 4.2 : 1.6, v/v). Radioactivity was assayed in a Nuclear-Chicago Actigraph II paper strip counter.

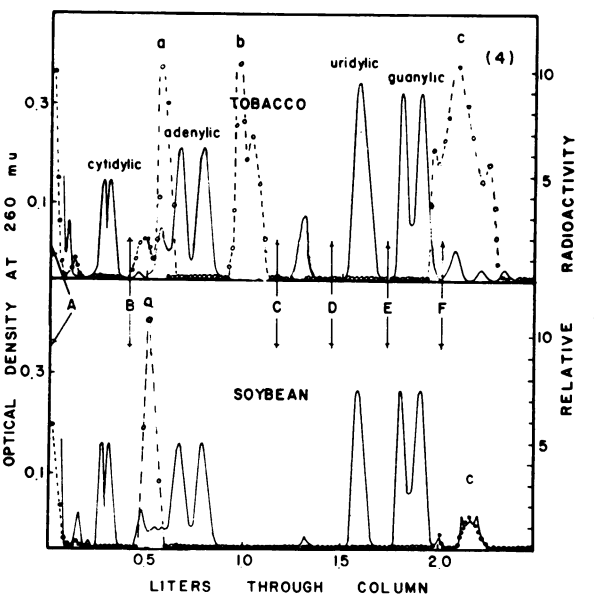

FIG. 4. Anion exchange chromatogram of KOH hydrolyzed RNA from tobacco and soybean tissues grown on *N*,6-benzyladenine-benzyl-C^{14}. See legend to figure 2 for fractionation procedure. See text for description of assay for radioactivity.

FIG. 1. Radiochromatograms of the soluble, nonpolynucleotide fraction of tobacco and soybean tissues, grown on media containing *N*,6-benzyladenine-8-C^{14} or *N*,6-benzyladenine-benzyl-C^{14}. Several of the minor radioactive spots were too faint for photographic reproduction. Solvent systems: *t*-butyl alcohol, glacial acetic acid, water 3 : 1 : 1, v/v (A and C); isopropanol, HCl, water (670 : 176 : 154, v/v) (B); isopropanol, NH_4OH, water (16 : 1 : 3, v/v) (D).

Soluble RNA isolated on methylated albumin columns was precipitated along with unlabeled carrier RNA by adding 3 volumes of 95 % ethanol in the presence of magnesium ions to the elute at pH 4.0 and allowing the mixture to stand overnight at 0°. The precipitate was collected by centrifugation, washed twice with 70 % ethanol, and hydrolyzed in HCl at 100° for 90 minutes. About 50 % of the radioactive material in the hydrolysates corresponded in R_F values on paper chromatograms to benzyladenine in 3 solvent systems (see for example fig 6). One other major radioactive component was present in both soybean and tobacco soluble RNA and was clearly separated from the benzyladenine area in each of the 3 solvent systems used. The second labeled components are not the same substance in the 2 tissues, however, since their R_F values clearly differed.

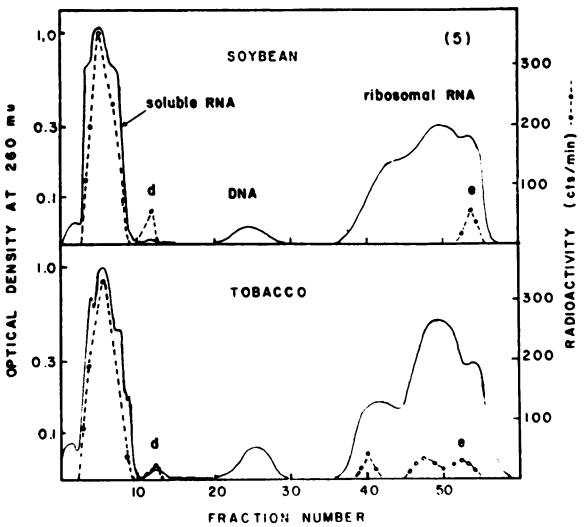

FIG. 5. Fractionation of nucleic acids from tissues growing on media containing *N*,6-benzyladenine-benzyl-C^{14} on methylated albumin-coated kieselguhr (13). Nucleic acid (in 0.05 M sodium phosphate buffer, pH 6.7) was extracted by a phenol-sodium lauryl sulfate method (3) from about 10 g (fr wt) tissue, and separated on a column 2 cm in diameter having a 50 ml bed volume. The column was eluted with an NaCl gradient from 0.3 M to 1.2 M. See text for details of radioactivity assay.

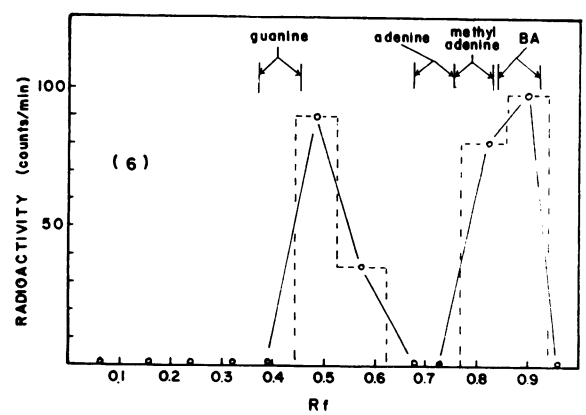

FIG. 6. Chromatographic distribution of radioactivity in an HCl hydrolysate of soluble RNA isolated from tobacco tissue grown on *N*,6-benzyladenine-benzyl-C^{14}. RNA was isolated by methylated albumin chromatography as shown in figure 5, and hydrolyzed in 1 N HCl at 100° for 90 minutes. The hydrolysate was co-chromatographed on Whatman No. 1 paper with unlabeled BA in *t*-butyl alcohol, glacial acetic acid, water (3 : 1 : 1, v/v). Strips were cut out and assayed for radioactivity by liquid scintillation spectrometry as described in the text. Each point is the midpoint of a strip (area of strip is designated by dotted lines).

Discussion

The data presented in this paper suggest that a small amount of the benzyladenine supplied to kinin requiring tissues is incorporated into soluble RNA. The most convincing evidence for this incorporation is that radioactivity appeared in RNA even when the kinin was supplied with C^{14} in the nonpurine portion (methylene carbon of the side chain) and that up to 50 % of the radioactive material in soluble RNA had R_F values on paper chromatograms similar to that of benzyladenine in 3 solvent systems.

Our data do not, however rule out the possibility that the benzyl side chain is first removed from benzyladenine and then reattached to adenine already in RNA in a manner analogous to the enzymatic methylation of RNA components, a process which is known to occur at the polynucleotide level (7). Furthermore, the metabolism of BA-benzyl-C^{14} leading to entry of the labeled methylene carbon into the active methyl pool could account for the presence of C^{14} in the other soluble RNA components. Our studies make it appear probable that both intact BA and some of its metabolites are incorporated into soluble RNA.

The incorporation of unnatural bases presumably intact into the nucleic acids of various organisms is well established (e.g. 12) and it is not surprising to find a similar incorporation of the adenine analog studied here. Whether or not the presence of benzyladenine in RNA is related to its biological function cannot be answered with the present study. It is instructive, however, to note that nearly all of the benzyladenine taken up is degraded by both soybean and tobacco tissues; the benzyladenine found in RNA apparently constitutes a substantial portion of the kinin remaining intact in the tissue after 20 days. It is, of course, possible that benzyladenine having fulfilled its biological role is then immediately degraded; equally likely is that one of the minor metabolic products found in the soluble nonpolynucleotide fraction is an active kinin. On the other hand the ability of kinins to promote RNA and protein synthesis in several systems (19) and the high degree of

localization of kinin effects to their site of application (18, 23) suggest that kinins are rapidly incorporated into larger molecules such as RNA, a proposal first made by Thimann and Laloraya (23). Furthermore the effectiveness at very low concentrations of the kinin, *N*,6-(γ,γ-dimethylallyl amino)-purine (21) and the activity of low levels of kinetin (*N*,6-furfurfurylaminopurine) itself in a kinin-requiring strain of *Clostridium thermocellum* (20) raise the possibility that the relatively high BA requirement exhibited by the tissue cultures used in this study is related to their ability to degrade much of the kinin taken up. Presumably then, only a small amount of the BA entering soybean and tobacco tissue is actually functional, perhaps as a part of soluble RNA.

There is an obvious relationship between the 6-substituted adenines which act as kinins and the methylated bases known to exist naturally in RNA. One of these, *N*,6-methyladenine, a naturally occurring constituent of the RNA of several organisms, has, in fact, distinct kinetin-like properties under certain circumstances (16). Studies with *N*,6-methyladenine-methyl-C^{14} indicate that this kinin is likewise incorporated into the RNA of kinin-requiring soybean and tobacco tissues (unpublished data). The recent discovery that certain 1-substituted adenines are also kinins (11) bolsters the possibility that kinin action is related in some manner to that of the unusual bases in RNA.

The function of the minor components of RNA is still obscure. Recent studies of Comb and Katz (5) indicate, however, that precursor transfer RNA (*t*-RNA), which has an overall base composition similar to functional *t*-RNA but lacks methylated bases and pseudouridine, is unable to form amino acyl RNA; on the other hand it should be pointed out that earlier results by Starr (22) with methyl poor *t*-RNA contradict this finding. Whatever the specific function of methylated bases in RNA, it is tempting to think that the incorporation of BA into *t*-RNA confers amino acid transfer competency on the molecule, much as methylation may do. An interesting consequence of this line of reasoning is that those plant tissues which require kinins for in vitro growth may have lost the ability to methylate RNA; the kinin by virtue of its incorporation into RNA provides the biological equivalent of an RNA methylating enzyme. It could be visualized, therefore, that the control for the differentiation of a meristematic plant cell into a nondividing one is the switching off of genetic information for the synthesis of RNA methylating enzymes. Such an idea implies that kinins have no normal biological role in the intact plant but simply furnish an alternate method for providing substituted bases in RNA, thus stimulating in vitro growth of differentiated cells.

Although it is clear that no C^{14} from BA-benzyl-C^{14} is incorporated into tobacco and soybean DNA, the small amount of radioactivity in RNA fractions other than soluble RNA might have significance. Especially interesting is C^{14} labeled component e (fig 5) eluted from the methylated albumin column near the end of the ribosomal RNA fraction from both soybean and tobacco tissues; similar areas on chromatograms of peanut cotyledon nucleic acid have been described by Cherry (3) as messenger RNA. An attractive hypothesis is that kinins act as derepressing agents, perhaps by being incorporated into a particular *m*-RNA thereby preventing its normal repressing function. Further work is needed to discriminate between this and other equally likely possibilities.

Acknowledgments

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Literature Cited

1. Bergquist, P. L. and R. E. F. Matthews. 1962. Occurrence and distribution of methylated purines in the ribonucleic acids of subcellular fractions. Biochem. J. 85: 305–13.
2. Bullock, M. N., J. J. Hand, and E. L. R. Stokstad. 1956. Synthesis of 6-substituted purines. J. Am. Chem. Soc. 78: 3693–96.
3. Cherry, J. H. 1964. Association of rapidly metabolized DNA and RNA. Science 146: 1066–69.
4. Cohn, W. E. and E. Volkin. 1951. Nucleoside-5′-phosphates from ribonucleic acid. Nature 167: 483–84.
5. Comb, D. G. and S. Katz. 1964. Studies on the biosynthesis and methylation of transfer RNA. J. Mol. Biol. 8: 790–800.
6. Daly, J. W. and B. E. Christensen. 1956. Purines. VI. The preparation of certain 6-substituted and 6,9-disubstituted purines. J. Org. Chem. 21: 177–79.
7. Fleissner, E. and E. Borek. 1962. A new enzyme of RNA synthesis: RNA methylase. Proc. Natl. Acad. Sci. U. S. 48: 1199–1203.
8. Fox, J. E. 1963. Growth factor requirements and chromosome number in tobacco tissue cultures. Physiol. Plantarum 16: 793–803.
9. Fox, J. E. 1964. Incorporation of kinins into the RNA of plant tissue cultures. Plant Physiol. 39: xxxi.
10. Fox, J. E. 1964. Indoleacetic acid-kinetin antagonism in certain tissue culture systems. Plant Cell Physiol. 5: 251–54.
11. Hamzi, Q. H. and F. Skoog. 1964. Kinetin-like growth-promoting activity of 1-substituted adenines [1-benzyl-6-amino purine and 1-(γ,γ-dimethylallyl)-6-aminopurine]. Proc. Natl. Acad. Sci. U. S. 51: 76–83.
12. Mandel, H. G., P. E. Carlo, and P. R. Smith. 1954. The incorporation of 8-azaguanine into nucleic acids of tumor-bearing mice. J. Biol. Chem. 206: 181–89.
13. Mandel, J. D. and A. D. Hershey. 1960. A fractionating column for analysis of nucleic acids. Analyt. Biochem. 1: 66–77.

14. McCalla, D. R., D. J. Morré, and D. Osborne. 1962. The metabolism of a kinin, benzyladenine. Biochem. Biophys. Acta 55: 522–28.
15. Miller, C. O. 1961. Kinetin and related compounds in plant growth. Ann. Rev. Plant Physiol. 12: 395–408.
16. Miller, C. O. 1962. Interaction of 6-methylaminopurine and adenine in division of soybean cells. Nature 194: 787–88.
17. Miller, C. O. 1961. Kinetin and kinetin-like compounds. Modern Methods of Plant Analysis. Vol VI: Springer-Verlag, Berlin. p 194–202.
18. Mothes, K., L. Engelbrecht, and O. Kulajewa. 1959. Über die Wirkung des Kinetins auf Stickstoffverteilung und Eiweiss-synthese in isolierte Blättern. Flora (Jena). 147: 445–64.
19. Oota, Y. 1964. RNA in developing plant cells. Ann. Rev. Plant Physiol. 15: 17–36.
20. Quinn, L. Y., R. P. Oates, and T. S. Beers. 1963. Support of cellulose digestion by *Clostridium thermocellum* in a kinetin-supplemented basal medium. J. Bacteriol. 86: 1359.
21. Rogozinska, J. H., J. P. Helgeson, and F. Skoog. 1964. Tests for kinetin-like growth promoting activities of triacanthine and its isomer, 6-(γ,γ-dimethylallylamino)-purine. Physiol. Plantarum 17: 165–76.
22. Starr, J. L. 1963. The incorporation of amino acids into methyl-poor amino acid transfer ribonucleic acid. Biochem. Biophys. Res. Commun. 10: 181–85.
23. Thimann, K. V. and M. M. Laloraya. 1960. Changes in nitrogen in pea stem sections under the action of kinetin. Physiol. Plantarum 13: 165–78.