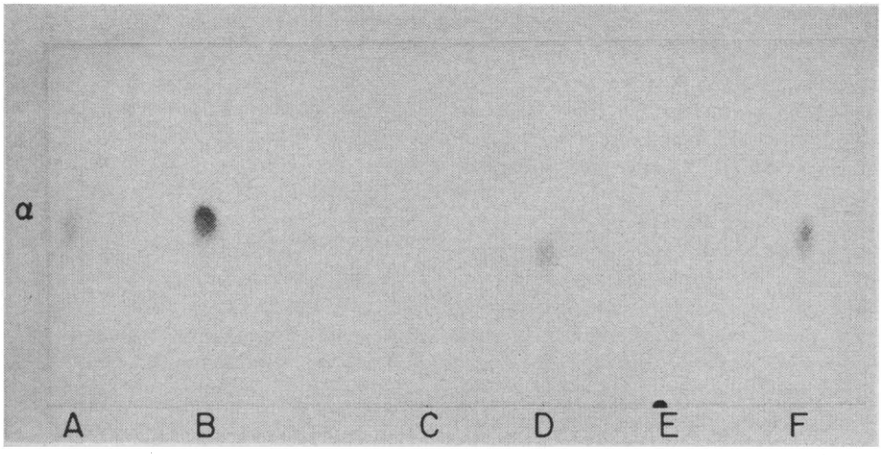

Fig. 2. Action pattern of *B. macerans* amylase and cyclodextrinase. Experimental conditions were identical to those of Fig. 1 except that the chromatogram was sprayed with 1.0*M* phosphate buffer, *p*H 6.7, air dried, and sprayed with an iodine solution (0.0035*M* I_2 and 0.25*M* KI) to detect α-dextrin. (*A*) α-dextrin + crude cyclodextrinase. (*B*) α-dextrin + partially purified *B. macerans* amylase. (*C*) Starch + cyclodextrinase. (*D*) Starch + *B. macerans* amylase. (*E*) Control (starch). (*F*) Control α-dextrin. (α) Refers to α-dextrin.

detected in 18- or 24-hour culture filtrates.

The action patterns of a crude preparation of cyclodextrinase and a preparation of *B. macerans* amylase (partially purified by manganese chloride, ammonium sulfate precipitations, and DEAE chromatography), are shown in Fig. 1. A linear series of malto-oligosaccharides was obtained when the crude dialyzed preparation of cyclodextrinase (which still contained some *B. macerans* amylase) was incubated with either α-dextrin (*9*) or with starch. With the partially purified, highly active preparation of *B. macerans* amylase, only small amounts of oligosaccharides were formed. This is presumably due to traces of cyclodextrinase that remained in the preparation. It should be noted that no cosubstrates were used in these experiments.

Figure 2 shows that α-dextrin was formed from starch by the *B. macerans* amylase preparation, but none was detected when the crude cyclodextrinase preparation was incubated with starch, even though oligosaccharides were formed (*10*).

It thus appears that at least two enzymes participate in the formation and degradation of Schardinger dextrins: (i) *B. macerans* amylase which forms Schardinger dextrins from starch and (ii) cyclodextrinase which degrades the cyclic dextrins to glucose, maltose, and malto-oligosaccharides. In addition, these enzymes are intracellular rather than extracellular. The detection of *B. macerans* amylase in culture filtrates in previous studies (*3, 4*) has probably resulted from cell lysis occurring during the long incubation periods.

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Table 1. Intracellular *Bacillus macerans* amylase and cyclodextrinase. *B. macerans* ATCC 8514 was grown at 38°C on a rotary shaker at 300 rpm in duplicate Fernback flasks, each containing 2 liters of the medium described or in medium containing 0.1 percent $CaCO_3$ in place of the phosphate and $CaCl_2$. The flasks were inoculated with a 1 percent inoculum of *B. macerans* in the same medium. After 18 or 24 hours incubation the cells were harvested by centrifugation, washed once with 20 ml of 0.01*M* phosphate buffer, *p*H 6.7, and suspended in 20 ml of the same buffer. The cells were broken either in a French pressure cell at 10,000 pounds per square inch or by sonic disintegration for 15 minutes in a Raytheon sonic oscillator. Deoxyribonuclease (10 μg/ml) and 1.0 ml of 0.05*M* $MgCl_2$ were added to the extract and, after stirring for 30 minutes at 30°C, cell debris was removed by centrifugation for 30 minutes at 17,500 rpm in a Serv-all centrifuge (SS-34 head, 0°C). The Tilden–Hudson test was positive in all cases.

Time of incubation (hr)	Medium	Cell-free extract	
		Dextrinogenic (units/ml)	Saccharogenic (units/ml)
18*	$CaCO_3$	12.5	122
18*	PO_4	12	28.2
24†	$CaCO_3$	7.7	50
24†	PO_4	4.3	50

* Cells were broken by sonic disintegration.
† Cells were broken in the French pressure cell.

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28 August 1964

Association of Rapidly Metabolized DNA and RNA

Abstract. *An association of rapidly metabolized DNA and RNA labeled with P^{32} has been found in peanut cotyledons by incorporation of either uridine-C^{14} or thymidine-C^{14} by peanut cotyledon slices. Chromatography of the extracted nucleic acids on methylated-albumin columns shows that the rapidly metabolized DNA and RNA are contained only in the first half of the DNA peak where RNA and DNA occur in equal amount. One-fourth of the radioactivity is recovered in ribonucleotides. Density-gradient centrifugation in cesium chloride indicates that the material containing the P^{32} has a higher density than most of the DNA.*

Mead (*1*) has isolated a DNA-RNA complex from *Drosophila melanogaster*. Other natural DNA-RNA complexes have also been described (*2, 3*). It is believed that a DNA-RNA complex is an intermediate in the transcription of the genetic information of the DNA (*4*). Konrad and Stent (*5*) were unable to find a putative DNA-RNA

complex that presumably would arise when bacteriophage T4 infects *Escherichia coli*. One of the two reasons they offer for their failure to find a DNA-RNA complex is that the messenger RNA is removed from the template DNA when the protein is denatured and removed during the extraction of nucleic acids. As shown in this paper RNA associated with DNA, possibly as a native DNA-RNA complex, has been isolated from germinating peanut seeds. Konrad and Stent suggested that, since messenger RNA in bacteria is very rapidly metabolized, only 0.1 percent of the messenger RNA labeled with an isotope is nascent. Because the rates of metabolism are much lower in seeds than in bacteria a higher percentage of nascent messenger RNA would be expected.

Changes in ribonucleic acid fractions in the cotyledons of germinating peanuts (*Arachis hypogaea*) were studied by chromatography (*6*) on methylated-albumin columns. Both RNA and DNA were labeled with P^{32}, and the phenol-extracted nucleic acids were separated into six fractions as follows: two peaks in the soluble RNA region, DNA, two ribosomal RNA's, and messenger RNA (Fig. 1). The DNA peak (fraction II) reacts with diphenylamine and contains up to 11 percent of the total P^{32} incorporated into nucleic acid; only the first half of the peak contained the radioactivity.

Slices of peanut cotyledons were incubated for 2 hours in a solution containing $10^{-4}M$ ammonium citrate, *p*H 6.0, 1 percent sucrose, and carrier-free $NaH_2P^{32}O_4$. Subsequently, the RNA was extracted by homogenizing 10 g of tissue in a solution containing 20 ml of 0.01*M* tris-HCl, *p*H 7.6, 0.06*M* KCl, and 0.01*M* $MgCl_2$; 3 ml of 5.5 percent sodium lauryl sulfate; 1 ml of bentonite (40 mg); and 34 ml of cold phenol. The aqueous solution was removed and treated twice with equal volumes of cold phenol, and the nucleic acids were precipitated by the addition of two volumes of cold ethanol. The extracted nucleic acids were dialyzed for 2 days against 0.05*M* phosphate buffer, *p*H 6.7, in the cold. All extraction procedures were carried out at about 2°C. Comparison of the amount of RNA extracted by the above method with that extracted with phenol-sodium lauryl sulfate at 27°, 37°, and 60°C gave higher RNA yields at 27° and 37°C, but lower yields at 60°C.

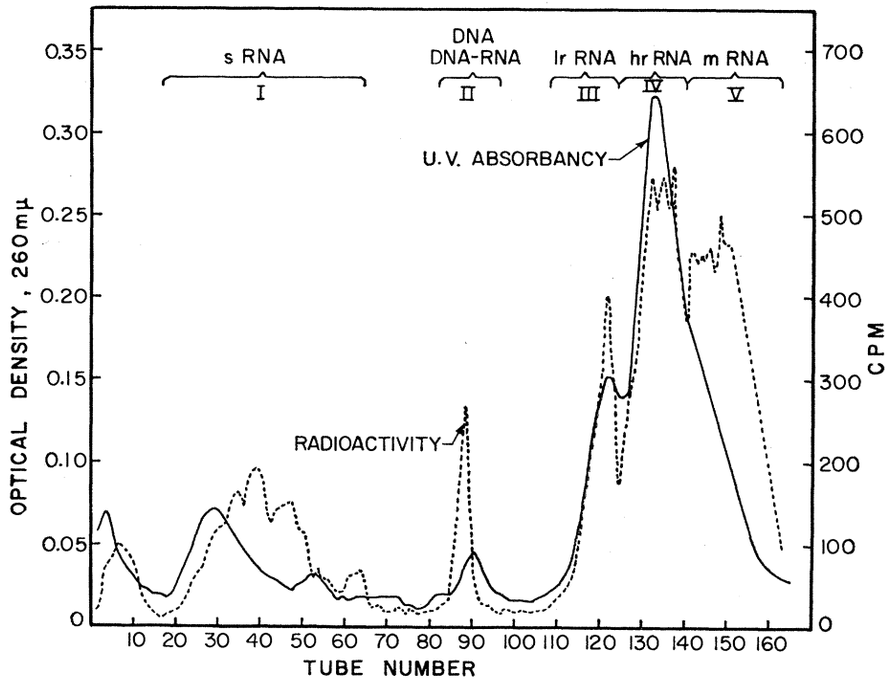

Fig. 1. Separation of an RNA extract from 4-day-old peanut cotyledons on a column of methylated-albumin on kieselguhr. Slices of the peanut cotyledon tissue were incubated for 2 hours with $NaH_2P^{32}O_4$, and then the RNA was extracted in cold phenol. Purified RNA (2 mg) was eluted from the column with a linear gradient of NaCl from 0.3 to 0.9*M* phosphate buffer, *p*H 6.7. Fractions containing 5 ml each were collected.

The DNA fraction (fraction II of Fig. 1) was collected from several methylated-albumin columns, combined, and precipitated in two volumes of cold ethanol. The precipitate was dissolved in 0.05*M* phosphate buffer, *p*H 6.7, and dialyzed against the same phosphate buffer. This concentrated material obtained from the total DNA peaks combined from several individual chromatograms of nucleic acids on methylated-albumin columns will henceforth be referred to as the DNA peak. As will be shown, this peak contains at least three components, one of which is RNA. When this preparation of the DNA peak is rechromatographed on a methylated-albumin column only one peak is found, and the radioactivity is confined to the first half of the peak.

Cells of peanut cotyledons do not divide during seed germination. Therefore, it is generally assumed that the DNA of these nondividing cells is stable and is not metabolized even though there is a slight increase during germination (*7*). In this initial study most of the P^{32} of the DNA peak therefore appeared to be incorporated into RNA. On the other hand, as shown in Table 1, 25 percent of the DNA peak is composed of RNA which contains only 28 percent of the radioactivity. To substantiate this further, RNA and DNA contents were estimated by the orcinol and diphenylamine tests, respectively. The orcinol test showed 89 μg of RNA per milliliter or 27 percent, and the diphenylamine test showed 240 μg of DNA per milliliter or 73 percent, which closely agrees with the foregoing data.

Thus, it is surprising that the DNA has the same specific radioactivity (count/min per μg) as the RNA. As most of the P^{32} is confined to the first half of the DNA peak, this means that this portion of the DNA peak contains about equal amounts of RNA and DNA while one-fourth of the P^{32} is in RNA and three-fourths in DNA. Thus, in the first portion of the DNA peak the rapidly metabolized DNA contains about three times as much P^{32} as an equal amount of RNA contains. To investigate this point further, slices of peanut cotyledons, and for comparison, apical hypocotyl sections, were incubated for 9 hours in solutions containing either uridine-C^{14} or thymidine-C^{14}. The DNA peaks obtained from chromatography of the phenol-extracted nucleic acids on methylated-albumin col-

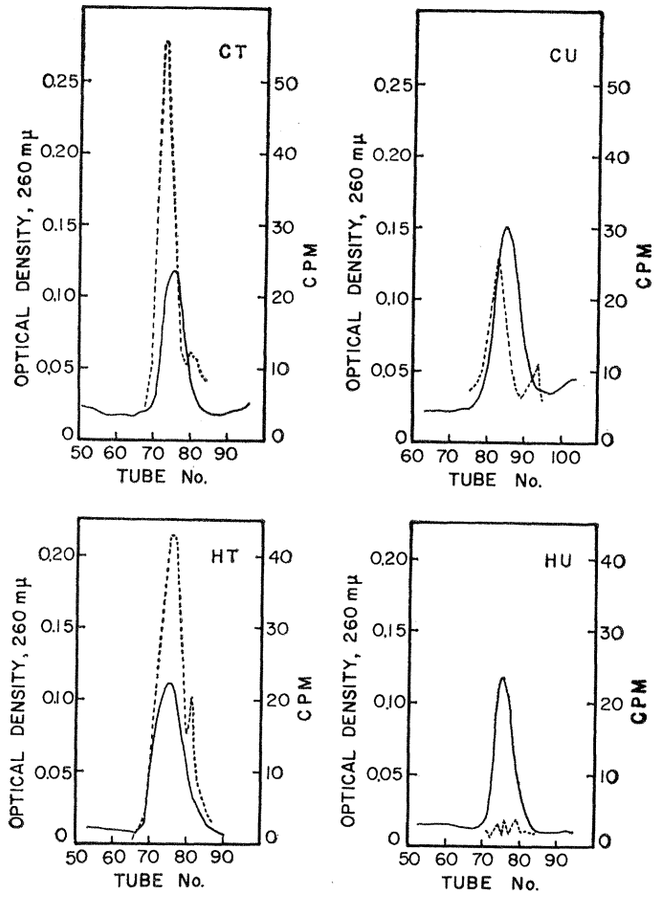

Fig. 2. Comparison of the DNA peak labeled with either uridine-C^{14} or thymidine-C^{14} from peanut cotyledons and hypocotyl tissue. Samples (15 g each) of sliced peanut cotyledons and apical hypocotyl sections (2 cm) from 10-day-old seedlings were incubated in 30 ml of solutions containing $10^{-4}M$ ammonium citrate, 1 percent sucrose, 300 μg streptomycin and 20 μc of uridine-C^{14} or thymidine-C^{14}. Samples were incubated for 9 hours at 30°C. The nucleic acids were extracted and separated on methylated-albumin columns. Abbreviations: CT, cotyledon tissue labeled with thymidine-C^{14}; CU, cotyledon tissue labeled with uridine-C^{14}; HT, hypocotyl tissue labeled with thymidine-C^{14}; HU, hypocotyl tissue labeled with uridine-C^{14}; the solid line indicates the ultraviolet absorbancy; and the broken line indicates the radioactivity.

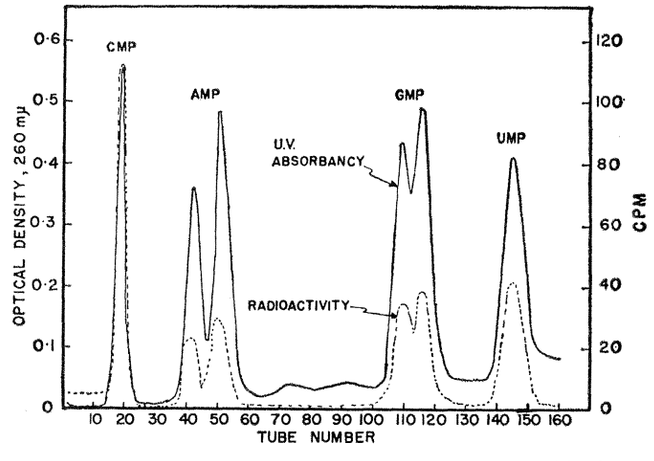

Fig. 3 (bottom left). Elution diagram of ribonucleotide from RNA associated with the DNA peak. Approximately 0.2 mg of the DNA peak containing RNA (50 μg) and P^{32} (100,000 count/min) was incubated with 3 mg of carrier RNA in $0.5M$ KOH for 16 hours at 37°C. The neutralized ribonucleotides were separated on Dowex-1 column by a step gradient of formic acid. The solid line represents the ultraviolet absorbancy from the carrier RNA whereas the broken line represents the P^{32}-labeled ribonucleotides of the RNA associated with the DNA peak.

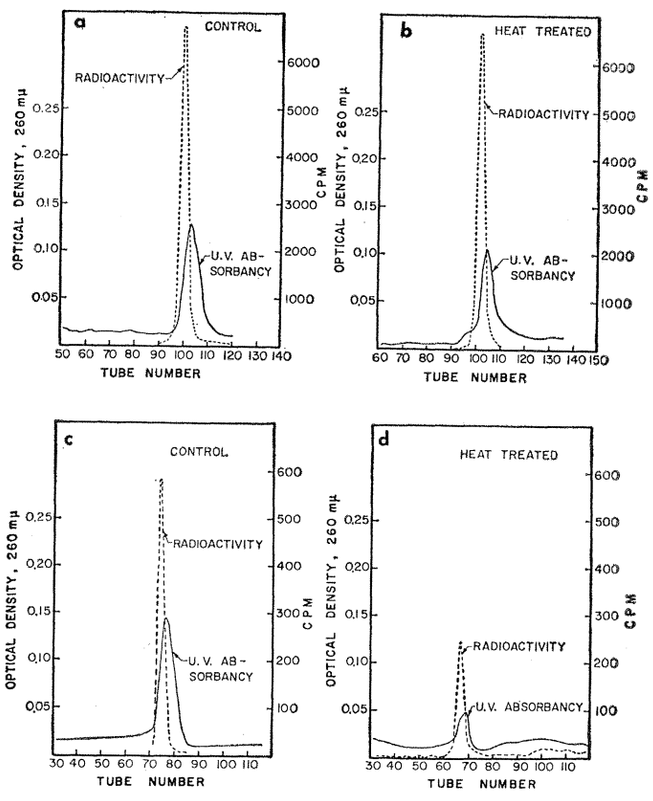

Fig. 4. Effect of heating the dialyzed DNA peak in two concentrations of NaCl. Samples were heated with buffered NaCl at 90°C for 15 minutes, rapidly cooled, and co-chromatographed on columns of methylated-albumin on kieselguhr. Elution was effected with a linear gradient of NaCl from 0.3 to $0.9M$ in $0.05M$ sodium phosphate buffer, pH. 6.7. *a* and *c*, Controls (not treated); *b*, the dialyzed DNA peak after heating in $0.4M$ NaCl–$0.5M$ sodium phosphate buffer, pH 6.7, for 20 minutes at 90°C; *d*, the DNA peak after heating in $0.05M$ NaCl–$0.05M$ sodium phosphate, pH 6.7, for 30 minutes at 90°C.

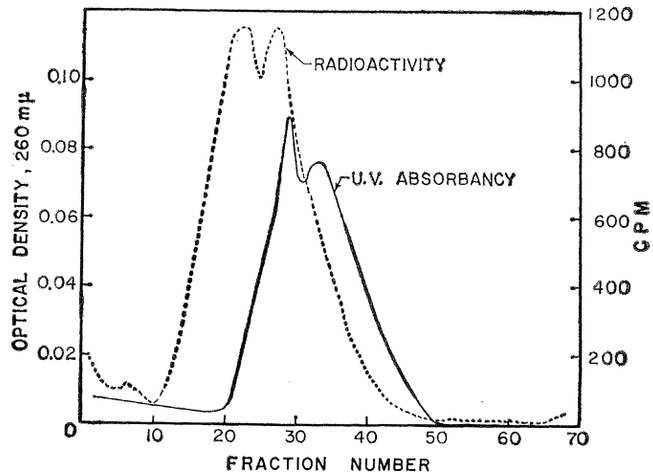

Fig. 5. The CsCl density gradient profile of the DNA peak. A sample of approximately 125 μg of DNA-RNA from the DNA peak in 0.4 ml and 4 ml of CsCl (density of 1.72) was centrifuged (SW-39 rotor) at 32,500 rev/min for 96 hours. After centrifugation, 60 fractions containing 3 drops each were collected from the bottom of the centrifuge tube.

umns are shown in Fig. 2. Thymidine-C^{14} is rapidly incorporated into DNA by both cotyledon and hypocotyl tissue. In the case of the hypocotyl tissue the radioactivity closely coincides with the ultraviolet absorbancy, perhaps because of a rapid metabolism of DNA, while in the case of the cotyledons the first portion of the DNA contained most of the thymidine-C^{14}. Uridine-C^{14} was also confined to the first portion of the DNA peak. Evidently, the DNA peak from hypocotyl tissue contains little or no RNA, as uridine-C^{14} was not incorporated.

To determine the nucleotide composition of the RNA associated with the DNA peak, samples from the dialyzed DNA peak labeled with P^{32} and samples of carrier RNA were incubated with 0.5*M* KOH at 37°C for 16 hours. At the end of the experiment, the extract was neutralized with perchloric acid, and the nucleotides were chromatographed on a Dowex-1 column with a gradient of formic acid (Fig. 3). The radioactive ribonucleotides from the RNA associated with the DNA peak coincided very well with the four mononucleotides of the carrier RNA. As judged from the radioactivity of the four nucleotides separated on the Dowex column, the nucleotide composition of the DNA-associated RNA (percentage molar ratio) is as follows: cytidylic acid, 26.8 percent; adenylic acid, 22.1 percent; guanylic acid, 30.6 percent; and uridylic acid, 20.5 percent.

Hybrids may be produced from purified RNA and DNA from the same species by heating a mixture of the two nucleic acids to 90°C for a few minutes, holding the mixture at 70°C for several hours, and slowly cooling it to allow binding of RNA to the complementary portion of the DNA molecule (*3*). This DNA-RNA complex can, in turn, be broken by heating to 90°C or more for a few minutes and rapidly cooling it. To test whether the RNA is associated with DNA by bonding similar to that described for DNA-RNA hybrids (*3*) we heated a sample of the dialyzed DNA peak in 0.4*M* NaCl–0.5*M* phosphate buffer, *p*H 6.7, to 90°C for 20 minutes and rapidly cooled it to about 10°C. The material was then chromatographed on a methylated-albumin column and compared with an identical sample of the same DNA peak which was not heated. As shown in the top portion of Fig. 4, the DNA-RNA fraction was not affected to a large extent by heating at 90°C. Approximately 20 percent of the ultraviolet absorbancy and 10 percent of the radioactivity were lost by the heat treatment. When another sample of the DNA peak was heated to 90°C in a solution of lower salt concentration (0.05*M*) for 30 minutes and then chromatographed on a methylated-albumin column, 70 percent of the ultraviolet absorbancy was lost from the DNA peak while only 30 percent of the radioactivity was lost (bottom of Fig. 4). This suggests that the DNA peak is composed mostly of two-stranded DNA which melts at a temperature (T_m) lower than the isotopically labeled DNA-RNA fraction. A melting profile of the DNA peak indicates the T_m to be 86° to 87°C.

Table 1. Estimation of the amount of RNA and DNA in the DNA peak by alkaline and acid hydrolysis. The RNA was removed with 0.5*M* KOH at 37°C for 16 hours. Subsequently the DNA was removed with 0.5*M* $HClO_4$ at 70°C for 40 minutes. Complete recovery of radioactivity was obtained.

Method of hydrolysis	Radioactivity (%)	Ultraviolet absorbancy (%)
0.5*M* KOH	27.8	25.1
0.5*M* $HClO_4$	72.2	74.9

Hybrids produced from purified RNA and DNA can be resolved by centrifugation in a CsCl density gradient since the DNA-RNA complex has a density slightly higher than that of DNA. Such density gradients could establish whether the DNA peak contains a mixture of a DNA-RNA complex and DNA, and whether the complex contains the bulk of the P^{32}. Figure 5 shows that when a sample of the DNA peak is placed in CsCl (density of 1.72) and centrifuged (SW-39 rotor) for 4 days at 32,500 rev/min a large amount of the radioactivity is associated with a material having a density greater than the major DNA; this is similar to that for heat-produced DNA-RNA hybrids (*3*). An examination of the density gradient profile shows that 65 percent of the P^{32} is associated with the first 25 percent of the ultraviolet peak. Since both RNA and DNA are found in the DNA peak and the P^{32} is associated with a material having a density greater than DNA, it is concluded that the DNA peak separated by methylated-albumin chromatography contains rapidly metabolized RNA and DNA moieties which have densities greater than the major DNA, whether or not they be complexed. As judged by the profile of the CsCl gradient (Fig. 5) there are at least three components in the DNA peak. There is a component sedimenting nearest the bottom of the tube, as indicated by the first radioactive band, which may be RNA. The second component is indicated by the second radioactive band and the first ultraviolet band in the CsCl. This second component may be the rapidly metabolized DNA. The third component is indicated by the second ultraviolet band and contains little radioactivity. This third component is perhaps the major DNA of the peanut cotyledon.

Thus, the total DNA fraction consists of about 25 percent RNA, 25 percent rapidly metabolized DNA, and 50 percent nonmetabolic DNA. Sampson *et al.* (*8*) showed that the growing regions of root and leaf tissue contain as much as 20 percent of their DNA in a low molecular-weight form which is metabolically labile. This form of DNA is eluted from the methylated-albumin column in a weaker NaCl solution than that in which fibrous DNA is eluted. This elution pattern resembles the separation obtained in this study. Sampson *et al.* do not report any RNA in their DNA; however, they show that only 80 percent of the DNA is composed of deoxyribonucleotides. Therefore, it seems likely that the fibrous and low molecular-weight DNA's reported by them may contain RNA and may be a DNA-RNA mixture similar to the one reported in this paper. Even though these results do not unequivocally prove that the RNA in the DNA peak is in the form of a putative DNA-RNA complex, the RNA and rapidly metabolized DNA appear to have a chemical association.

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