- Dunn, D. B. (1959). *Biochim. biophys. Acta*, **34**, 286.
- Dunn, D. B. & Smith, J. D. (1958). *4th int. Congr. Biochem., Symp. VII*.
- Dunn, D. B. & Smith, J. D. (1959). *Trans. Faraday Soc.* **55**, 490.
- Dunn, D. B., Smith, J. D. & Spahr, P. F. (1960). *J. molec. Biol.* **2**, 113.
- Elion, G. B., Burgi, E. & Hitchings, G. H. (1952). *J. Amer. chem. Soc.* **74**, 411.
- Elion, G. B., Lange, W. H. & Hitchings, G. H. (1956). *J. Amer. chem. Soc.* **78**, 217.
- Gierer, A. & Schramm, G. (1956). *Nature, Lond.*, **177**, 702.
- Greengard, O. & Campbell, P. N. (1959). *Biochem. J.* **72**, 305.
- Hecht, L. I., Zamecnik, P. C., Stephenson, M. L. & Scott, J. F. (1958). *J. biol. Chem.* **233**, 954.
- Hoagland, M. B. (1960). In *The Nucleic Acids*, vol. 3, p. 349. Ed. by Chargaff, E. & Davidson, J. N. New York: Academic Press Inc.
- Hoagland, M. B. & Comly, L. T. (1960). *Proc. nat. Acad. Sci., Wash.*, **46**, 1554.
- Hoagland, M. B., Stephenson, M. L., Scott, J. F., Hecht, L. I. & Zamecnik, P. C. (1958). *J. biol. Chem.* **231**, 241.
- Kalf, C. F. & Simpson, M. V. (1959). *J. biol. Chem.* **234**, 2943.
- Littlefield, J. W. & Dunn, D. B. (1958). *Biochem. J.* **70**, 642.
- Littlefield, J. W. & Keller, E. B. (1957). *J. biol. Chem.* **224**, 13.
- McLean, J. R., Cohn, G. L., Brandt, I. K. & Simpson, M. V. (1958). *J. biol. Chem.* **233**, 657.
- Markham, R. (1955). In *Modern Methods of Plant Analysis*, vol. 4, p. 246. Ed. by Paesch, K. & Tracey, M. V. Berlin: Springer-Verlag.
- Markham, R. & Smith, J. D. (1949). *Biochem. J.* **45**, 294.
- Markham, R. & Smith, J. D. (1951). *Biochem. J.* **49**, 401.
- Markham, R. & Smith, J. D. (1952). *Biochem. J.* **52**, 552.
- Matthews, R. E. F. (1958). *Virology*, **5**, 192.
- Otaka, E. & Osawa, S. (1960). *Nature, Lond.*, **185**, 921.
- Preiss, J., Berg, P., Ofengand, E. J., Bergmann, F. H. & Dieckmann, M. (1959). *Proc. nat. Acad. Sci., Wash.*, **45**, 319.
- Reis, P. J., Coote, J. L. & Work, T. S. (1959). *Nature, Lond.*, **184**, 165.
- Roodyn, D. B., Reis, P. J. & Work, T. S. (1961). *Biochem. J.* **80**, 9.
- Scannell, J. P., Crestfield, A. M. & Allen, F. W. (1959). *Biochim. biophys. Acta*, **32**, 406.
- Sibatani, A., Yamana, K., Kimura, K. & Okagaki, H. (1959). *Biochim. biophys. Acta*, **33**, 590.
- Siekevitz, P. (1959). In *Ciba Found. Symp.: The Regulation of Cell Metabolism*, p. 17. Ed. by Wolstenholme, G. E. W. & O'Connor, C. M. London: J. and A. Churchill Ltd.
- Singer, M. F. & Cantoni, G. L. (1960). *Biochim. biophys. Acta*, **39**, 182.
- Smith, J. D. & Dunn, D. B. (1959). *Biochem. J.* **72**, 294.
- Smith, J. D. & Markham, R. (1950). *Biochem. J.* **46**, 509.
- Tissieres, A. (1959). *J. molec. Biol.* **1**, 365.
- Tyner, E. P., Heidelberg, C. & LePage, G. A. (1953). *Cancer Res.* **13**, 186.
- Weiss, S. B., Acs, G. & Lipmann, F. (1958). *Proc. nat. Acad. Sci., Wash.*, **44**, 189.
- Wyatt, G. R. (1951). *Biochem. J.* **48**, 584.
- Zamecnik, P. C. & Stephenson, M. L. (1960). *Ann. N.Y. Acad. Sci.* **58**, 708.

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Effects of 8-Azaguanine on the Composition of Ribonucleic Acids from Subcellular Fractions

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Several synthetic analogues of purine and pyrimidine bases that are inhibitory for growth are known to be incorporated into the nucleic acids of organisms, replacing some or all of the normal bases. Viruses containing unnatural bases in their nucleic acids may be less infectious than normal virus. For example: 8-azaguanine in the RNA of tobacco mosaic virus (Matthews, 1954) and turnip yellow-mosaic virus (Matthews, 1955), 5-bromouracil in the DNA of T2 phage (Litman & Pardee, 1956) and 2-thiouracil in the RNA of tobacco

mosaic virus (Francki & Matthews, 1959; Francki, 1960). It is, however, by no means established that this incorporation into complete virus particles causes a reduction in the rate of virus multiplication. Even less is known about the relationship between incorporation of analogue and growth inhibition in bacterial, plant and animal cells, although there is indirect evidence that the analogue may inhibit the production of specific proteins through the production of ineffective RNA containing the analogue (e.g. Creaser, 1956; Chantrenne & Devreux, 1960*a, b*; Aronson, 1961*a, b*).

Previous analyses on the incorporation of analogues into RNA have been carried out on preparations from whole cells. In the work described below we have observed substantial differences in

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the amounts of 8-azaguanine incorporated into the RNA of mitochondrial, microsomal and soluble fractions from plant and animal tissues. Treatment with the analogue also brought about changes in the proportions of the normal bases present in some of these fractions.

MATERIALS AND METHODS

Chemicals. The following compounds were gifts of the American Cyanamid Co.: 8-azaguanine; 8-aza-adenine; methylamino-8-azaguanine; dimethylamino-8-azaguanine; 5(4)-amino-1*H*-1,2,3-triazole-4(5)-carboxamide. All these compounds were made up as solutions in aq. 0.1–0.2% NaHCO₃. β -Diethylaminoethylphenylpropyl acetate was obtained from Smith, Kline and French (Philadelphia) and *O*-diazoacetyl-L-serine from Parke, Davis and Co. (New York).

Animals. Albino mice weighing 18 ± 0.5 g. received daily intraperitoneal injections of either 85 mg. of 8-azaguanine/kg. body wt. in 0.4 ml. of 0.1% NaHCO₃, or one of the other azapurines at the dosages described in the text. Control mice were injected with 0.4 ml. of 0.1% NaHCO₃. For most experiments groups of 40 mice were used, the livers being pooled for isolation of the subcellular fractions.

Pea and bean seedlings. Seeds of *Pisum sativum* var. Greenfeast and *Vicia faba* var. Broad Windsor were soaked overnight and then surface-sterilized with 1% 'Cetavlon' (Imperial Chemical Industries Ltd.). The seeds were allowed to germinate in the dark for 2 days on sheets of damp filter paper. The embryos were then sprayed twice a day with either 0.2% NaHCO₃ or 0.2% NaHCO₃ plus 0.01*M*-8-azaguanine for 5 days before the radicles were removed from the seeds.

Subcellular fractions. Mitochondria, microsomes and soluble fractions were isolated from mouse liver, pea and bean seedlings as described by Bergquist & Matthews (1962).

Isolation of nucleotides and estimation of bases in RNA. RNA nucleotides were prepared from each fraction by the procedure described by Bergquist & Matthews (1962), and chromatographed as bands in propan-2-ol–water (Markham & Smith, 1952). Small portions of the chromatographed nucleotides were eluted and hydrolysed in *N*-HCl at 100°. Base compositions were determined according to Markham (1955). The guanylic acid and the mixed adenylic acid, cytidylic acid and uridylic acid bands were eluted separately and rechromatographed in propan-2-ol–water if there was incomplete resolution of the two bands during the first separation.

Methylated guanines were isolated from the mixed adenylic acid, cytidylic acid and uridylic acid band as described by Bergquist & Matthews (1962). Tests with synthetic compounds showed that any methylated 8-azapurines would have been resolved from the methylated guanines in the chromatographic systems employed.

The guanylic acid band from propan-2-ol–water chromatography was rechromatographed in this solvent and the guanine plus 8-azaguanine ribonucleotides were separated from each other by electrophoresis in 0.05*M*-borate buffer, pH 9.0 (Matthews, 1954), and eluted separately. Further purification of the guanylic acid and 8-azaguanilyc acid was achieved as follows. They were chromatographed

again as bands in propan-2-ol–water, eluted and measured amounts were chromatographed in propan-2-ol–water on a 50 cm. $\times$ 40 cm. sheet of Whatman no. 3MM paper. They were then subjected to electrophoresis in the second dimension in 0.05*M*-phosphate buffer, pH 2.0, or were run in the ammonium sulphate–water solvent (Markham & Smith, 1951) to confirm the identification of the 8-azaguanilyc acid as 2'- and 3'-phosphates.

These steps that have been described for the isolation of 8-azaguanilyc acid are necessary not only because of the occurrence of nucleoside diphosphates migrating with guanylic acid and 8-azaguanilyc acid in borate buffer (Smith & Matthews, 1957) but also because of the presence in the alkaline digests of small amounts of dinucleotide-like materials that are only resolved by electrophoresis at pH 2.0. This material may be similar to the compounds described by Smith & Dunn (1959).

8-Aza-adenine, if present, could be detected after elution of the cytidylic acid and adenylic acid areas of electrophoresis strips that had been run in formate buffer, pH 3.5, for the isolation of methylated guanines. The eluate was hydrolysed with *N*-HCl and 8-aza-adenine was resolved by chromatography in propan-2-ol–HCl (Wyatt, 1951) in the first dimension, followed by butan-1-ol–water (Markham & Smith, 1949) in the second dimension.

Because of the quantities of material and number of manipulations involved it was not possible to carry out analyses on duplicate sets of intact starting material. However, the final analyses for ratios of the major bases were carried out in triplicate, those for 8-azaguanine in duplicate or triplicate, and those for methylated purines were duplicates or single values only.

RESULTS

Incorporation of 8-azaguanine into the RNA of mouse-liver fractions. Three groups of 40 albino mice from the same batch were given daily injections of 8-azaguanine (85 mg./kg.). Mice killed 6 hr. after the first injection showed no detectable incorporation of 8-azaguanine. Those killed after 84 hr. gave the following figures for percentage replacement of RNA guanine by 8-azaguanine: mitochondria 0.23%; microsomes 0.1%; soluble fraction 0.52%. At 156 hr. the corresponding figures were 1.90, 0.48 and 2.45%. Thus to obtain enough incorporation for good analytical figures in subsequent tests, animals were dosed daily for 6 days and killed 12 hr. after the final injection. However, different batches of mice varied substantially in the amount of 8-azaguanine incorporated after the same dosing schedule (Table 1).

Polynucleotide origin of the 8-azaguanine measured. In view of these differences a check was made on the origin and identity of the 8-azaguanilyc acid measured after electrophoresis at pH 2.0. Portions of samples used for electrophoresis were run in ammonium sulphate–water to separate the isomeric 8-azaguanosine 2'- and 3'-phosphates. The ratios of amounts of these isomers that were recovered were approximately those expected from

their formation as a result of the alkaline hydrolysis of polynucleotides (Table 1). Incorporation of 8-azaguanine measured on the separated isomers was consistently about 25% lower than when measured on the mixed nucleotides after electrophoresis (Table 1). Nevertheless the data show that measurements made by the latter procedure do reflect differences in the amounts of 8-azaguanine incorporated into the RNA of the different subcellular fractions. A substantially higher percentage of 8-azaguanine was incorporated into soluble and mitochondrial RNA than into microsomal RNA.

Effect of treatment with 8-azaguanine on proportions of normal bases. Table 2 records the proportions of 8-azaguanine, normal major bases and three methylated guanines, for three experiments on different batches of albino mice. The only significant effect of 8-azaguanine treatment on the major bases was in the mitochondrial fraction where there was a substantial drop (approx. 30%) in the content of guanine + 8-azaguanine, compared with guanine from mitochondria of control animals. 8-Azaguanine treatment produced no significant changes in the methylated guanine content of mitochondrial or soluble fraction RNA. However, there was a substantial rise in the amounts of 2-amino-6-hydroxy-1-methylpurine and 6-hydroxy-2-methylaminopurine, and in the microsomal RNA.

A higher proportion of the soluble RNA from livers of mice treated with 8-azaguanine was precipitable at pH 5.2. In the mice used for the three experiments of Table 2 the approximate figures for percentage of total soluble RNA in the pH 5.2 precipitate were: for control mice, 70%, and for 8-azaguanine-treated, 81%.

Other analogues. In view of the established occurrence of methylated guanines in RNA, and particularly their unequal distribution among sub-

cellular fractions, we examined the incorporation of two methylated 8-azaguanines, and of 5(4)-amino-1*H*-1,2,3-triazole-4(5)-carboxamide, a potential precursor of the 8-azapurine nucleus (Table 3). All were incorporated into RNA as 8-azaguaninic acid at about the same concentrations as those expected for 8-azaguanine-dosed animals except that the amount of incorporation into soluble RNA was lower after dosing with dimethyl-amino-8-azaguanine and 5(4)-amino-1*H*-1,2,3-triazole-4(5)-carboxamide. No 8-aza analogues of the methylated guanines were detected but with the quantities of material available we could not have detected a replacement of less than 20–70% of normal methylated purines by their 8-aza analogues.

The only significant alteration in the proportions of the major bases was caused by methylamino-8-azaguanine in mitochondrial RNA, where the total guanine and 8-azaguanine content dropped by an amount comparable with that listed for 8-azaguanine in Table 2. Although incorporation from methylamino-8-azaguanine into RNA was similar to that from 8-azaguanine, methylamino-8-azaguanine was more toxic, causing many deaths at 42.5 mg./kg. whereas 8-azaguanine was only slightly toxic at 85 mg./kg.

Presence of 8-aza-adenine in RNA digests. Smith & Matthews (1957) could not detect 8-aza-adenylic acid in RNA from *Escherichia coli* or *Bacillus cereus* grown in the presence of 8-aza-adenine, 8-azaguanine, 8-azahypoxanthine or 5(4)-amino-1(*H*)-1,2,3-triazole-4(5)-carboxamide, although they found that the presence of 8-aza-adenine may give rise to 8-azaguaninic acid in RNA.

In all experiments on 8-azaguanine incorporation, we searched for the presence of 8-aza-adenine. It was detected on only two occasions—in mouse-liver mitochondrial RNA from one group of animals

Table 1. *Incorporation of 8-azaguanine into ribonucleic acid of subcellular fractions of mouse liver*

Groups of 40 albino mice were used for both experiments. Animals were injected daily with 8-azaguanine for 6 days at the rate of 85 mg./kg. body wt. Recoveries of the isomeric 8-azaguanosine 2'- and 3'-phosphates were made.

Fraction	Expt. no.	Percentage replacement: $\left[\left(\frac{\text{moles of 8-azaguanine}}{\text{moles of guanine + 8-azaguanine}} \right) \times 100 \right]$		Ratio: 8-azaguanosine 3'-phosphate 8-azaguanosine 2'-phosphate
		From mixed 8-azaguanosine 2'- and 3'-phosphates isolated after electrophoresis at pH 2.0	From sum of 8-azaguanosine 2'- and 3'-phosphates isolated in ammonium sulphate-water	
Mitochondria	1	7.05	5.34	1.25
	2	2.80	2.00	1.36
Microsomes	1	2.36	1.87	1.18
	2	0.51	0.38	1.30
Soluble	1	2.78	2.45	1.23
	2	2.47	2.10	1.17

Table 2. *Base composition of ribonucleic acids from subcellular fractions of liver from mice dosed with 8-azaguanine*

Groups of 40 albino mice were injected with 85 mg. of 8-azaguanine/kg. daily for 6 days. Control and treated animals were selected from the same litters for each experiment. Analysis of variance was carried out independently on the figures for the molar ratios of the major bases and on the logarithms of the figures for methylated guanine contents. The following differences were the only ones found to be highly significant: for major bases the mean decrease of 8 in the guanine + 8-azaguanine content of mitochondrial RNA was significant at P 0.01. For the methylated guanines, the increases in microsomal 2-amino-6-hydroxypurine and 6-hydroxy-2-methylaminopurine brought about by treatment with 8-azaguanine were significant at P 0.001.

Subcellular fraction	Treatment	Expt. no.	8-Azaguanine contents:		Molar ratios of major bases					Methylated guanine contents (moles/100 moles of uracil)			
			[$\frac{\text{moles of 8-azaguanine}}{\text{moles of guanine} + \text{8-azaguanine}} \times 100$]		Guanine + 8-aza-guanine					2-Amino-6-hydroxy-1-methylpurine		6-Hydroxy-2-methylaminopurine	
Mitochondria	Control	1	0		23	23	30	24	24	0.27	1.60	0.22	0.22
		2	0		26	19	24	31	31	0.16	0.22	0.02	0.02
		3	0		25	22	32	21	21	0.10	0.24	0.08	0.08
	8-Azaguanine	1	7.05		15	24	33	28	33	0.25	1.36	0.41	0.41
		2	2.80		16	21	29	33	33	0.22	0.23	0.02	0.02
		3	1.02		19	24	30	27	27	0.13	0.22	0.10	0.10
Microsomes	Control	1	0		25	20	31	24	24	0.05	0.08	0.19	0.19
		2	0		26	21	29	24	24	0.08	0.05	0.02	0.02
		3	0		25	22	29	24	24	0.06	0.13	0.10	0.10
	8-Azaguanine	1	2.36		25	22	28	25	25	0.26	0.32	0.03	0.03
		2	0.51		22	20	33	25	25	0.75	0.17	0.02	0.02
		3	0.30		24	23	30	23	23	0.15	0.20	0.04	0.04
Soluble	Control	1	0		27	17	34	22	22	2.23	7.90	1.40	1.40
		2	0		27	19	30	24	24	2.70	2.09	0.38	0.38
		3	0		27	21	29	23	23	1.75	3.53	0.51	0.51
	8-Azaguanine	1	2.78		21	23	30	26	26	3.86	4.26	0.41	0.41
		2	2.47		27	20	31	22	22	3.71	1.9	0.41	0.41
		3	1.64		24	23	32	21	21	1.44	2.98	0.56	0.56

dosed with 8-azaguanine and from another group dosed with dimethylamino-8-azaguanine. The compound, obtained in the acid hydrolysates used in the search for methylated 8-azaguanines, was identified as 8-aza-adenine by comparison of its u.v.-absorption spectrum, fluorescence properties and R_f in three solvents, with those of synthetic 8-aza-adenine. In view of the chromatographic and electrophoretic procedure used before acid hydrolysis it is most likely that this material was present in the original hydrolysates in ribonucleotide form.

Nuclear RNA. We have not yet made any thorough examination of the effects of 8-azapurines on nuclear RNA. However, preliminary tests showed mouse-liver nuclei to have about the same level of incorporation as microsomes after 6 days' dosing. When 8-azaguanine treatment was stopped nuclear RNA continued to accumulate 8-azaguanine over a period of 2 days, whereas in the cytoplasmic fractions, especially the pH 5.2 supernatant, the content of the analogue fell.

Simultaneous administration of other drugs. We have examined the effect of administering two other drugs together with 8-azaguanine on the incorporation of the analogue into RNA: β -diethylaminoethyldiphenylpropyl acetate, which exerts an inhibitory effect on a wide variety of normal detoxification mechanisms (Brodie, 1956), and *O*-diazaoacetyl-L-serine, which may cause a profound suppression of purine synthesis (Levenberg, Melnick & Buchanan, 1957).

Both drugs caused 2.5-fold rise in the amount of incorporation of 8-azaguanine into mitochondrial RNA but had little effect on incorporation into microsomes. *O*-Diazaoacetyl-L-serine doubled the incorporation, whereas β -diethylaminoethyldiphenylpropyl acetate caused a smaller increase in incorporation, into soluble RNA. The figures for (8-azaguanine/guanine + 8-azaguanine) $\times$ 100 for pH 5.2 precipitate RNA and pH 5.2 supernatant

RNA were: treated with 8-azaguanine, 0.98 and 4.30; treated with *O*-diazaoacetyl-L-serine plus 8-azaguanine, 2.00 and 8.10; treated with β -diethylaminoethyldiphenylpropyl acetate plus 8-azaguanine, 1.65 and 5.84.

The drug combinations had no significant effect on the proportions of the major bases except that the guanine + 8-azaguanine content of mitochondrial RNA was lowered as for 8-azaguanine alone (from 25 to 16 for both drug combinations) (cf. Table 2). The amounts of the methylated guanines were little affected by the pairs of drugs except that in pH 5.2 supernatant and precipitate fractions 8-azaguanine + β -diethylaminoethyldiphenylpropyl acetate caused a substantial decrease in the amounts of 2-amino-6-hydroxy-1-methylpurine and 6-hydroxy-2-methylaminopurine and an altered distribution of 6-hydroxy-2-dimethylaminopurine between pH 5.2 precipitate and supernatant RNA (Table 4).

It is not possible to give estimates of error for the analyses in Table 4, since the experiment as a whole was carried out only once. Nevertheless in view of the relatively close agreement in methylated purine figures for control and 8-azaguanine-treated animals we consider the differences for the combined drug treatment to be real.

Incorporation of 8-azaguanine into subcellular fractions of plant tissues. The incorporation of 8-azaguanine into subcellular fractions of pea and bean seedlings showed a similar pattern to that found for mouse-liver RNA (Table 5). Mitochondria and soluble fractions had a higher concentration than microsomes, whereas the concentration in the pH 5.2 supernatant was substantially higher than in the pH 5.2 precipitate.

DISCUSSION

Numerous studies with various radioactive precursor compounds have shown that, among the cytoplasmic cell fractions, the soluble RNA

Table 3. *Incorporation of the 8-azapurine nucleus as 8-azaguanine into ribonucleic acid of subcellular fractions of mouse liver, when various compounds are given interperitoneally*

Batches of 40 mice were dosed daily for 6 days.

Compound	Dose (mg./kg.)	8-Azaguanine content: $\left[\left(\frac{\text{moles of 8-azaguanine}}{\text{moles of 8-azaguanine + guanine}} \right) \times 100 \right]$			
		Mitochondria	Microsomes	pH 5.2 precipitate	pH 5.2 supernatant fluid
8-Azaguanine*	85.0	1.02	0.30	0.98	4.30
Methylamino-8-azaguanine*	42.5	1.53	0.35	0.83	5.06
Dimethylamino-8-azaguanine	28.0	0.41	0.26	0.19	0.3
5(4)-Amino-1 <i>H</i> -1,2,3-triazole- 4(5)-carboxamide	42.5	0.45	0.29	0.24	0.3

* Animals used in these two tests were from the same litters.

Table 4. *Base composition and methylated guanine content of mouse-liver soluble ribonucleic acid from animals dosed with 8-azaguanine and β -diethylaminoethylidiphenylpropyl acetate*

Groups of 40 albino mice from the same batch of litters were used for each treatment. Treatments were given daily for 6 days. The control group received an intravenous injection of 0.4 ml. of 0.1% NaHCO₃. 8-Azaguanine-treated animals received 85 mg. of 8-azaguanine/kg. body wt. (in 0.1% NaHCO₃). The third group received 85 mg. of 8-azaguanine plus 6 mg. of β -diethylaminoethylidiphenylpropyl acetate/kg. body wt. (in 0.1% NaHCO₃).

Treatment	Fraction	8-Azaguanine content: $\left[\frac{\text{moles of 8-azaguanine}}{\text{moles of guanine} + \text{8-azaguanine}} \right] \times 100$	Molar ratios of major bases				Methylated guanine contents (moles/100 moles of uracil)			
			Guanine + 8-aza- guanine	Adenine	Cytosine	Uracil	2-Amino- 6-hydroxy-1- methylpurine	6-Hydroxy- 2-methyl- aminopurine	6-Hydroxy- 2-dimethyl- aminopurine	6-Hydroxy- 2-dimethyl- aminopurine
Control	pH 5.2 precipitate	—	26	21	31	21	0.29	2.04	0.30	0.30
	pH 5.2 supernatant fluid	—	28	19	26	27	5.17	7.30	1.00	1.00
8-Azaguanine	pH 5.2 precipitate	0.98	23	24	33	20	0.30	2.40	0.45	0.45
	pH 5.2 supernatant fluid	4.30	26	20	27	27	6.00	5.30	1.00	1.00
β -Diethylamino- ethylidiphenyl- propyl acetate + 8-azaguanine	pH 5.2 precipitate	1.65	23	25	29	23	0.10	1.06	1.43	1.43
	pH 5.2 supernatant fluid	5.84	28	18	26	27	0.56	0.65	0.25	0.25

Table 5. *Incorporation of 8-azaguanine into ribonucleic acids from subcellular fractions of pea and bean seedlings*

Seedlings were sprayed twice daily with 0.01M-8-azaguanine in 0.2% NaHCO₃ for 5 days.

Fraction	8-Azaguanine content: $\left[\frac{\text{moles of 8-azaguanine}}{\text{moles of guanine} + \text{8-azaguanine}} \right] \times 100$	
	Beans	Peas
Mitochondria	3.02	1.21
Microsomes	0.41	0.47
Whole soluble	2.30	8.50
pH 5.2 precipitate	0.25	3.80
pH 5.2 supernatant fluid	5.20	10.20

becomes labelled most rapidly. It is thus perhaps not surprising that in our experiments soluble RNA generally contained the highest amount of 8-azaguanine. Less expected was the substantially higher incorporation into mitochondrial fraction RNA, as compared with microsomes, which occurred both with mouse liver and the plant materials tested.

The significance of the relatively small amounts of RNA found in the mitochondrial fraction has been the subject of considerable discussion (e.g. Lindberg & Ernster, 1954; Novikoff, 1957; Ernster & Lindberg, 1958). The problem is made difficult by possible contamination with RNA-rich microsomal material or with the endoplasmic reticulum itself, as suggested by Siekevitz (1959). However, some recent evidence indicates an independent role for the RNA isolated with mitochondria (e.g. Rendi, 1959; Kalf & Simpson, 1959; Straub, Ulmann & Venetianer, 1960). Two of our observations on the effects of 8-azaguanine suggest that the RNA isolated with mitochondria from mouse liver is metabolically distinct from microsomal or soluble RNA. First, the proportion of guanine was substantially lowered by 8-azaguanine treatment, whereas there was no significant change in the proportions of the major bases in the other fractions. Secondly mitochondria had a substantially higher incorporation of 8-azaguanine than did microsomes, the most likely contaminant of mitochondrial preparations.

The significance of the methylated purines in RNA is not yet established. There are substantial difficulties in working with these substances (at least with non-radioactive materials) because of the low concentrations at which they occur. Nevertheless, the results reported here show that treatment with 8-azapurines may lead to substantial changes in the amounts and distribution of these bases in certain RNA fractions.

From results reported here there is no indication of what role, if any, incorporation of the analogue into RNA plays in the inhibition of growth by 8-azaguanine. The relatively high amounts of incorporation into the RNA of the soluble fraction do, however, suggest that it would be worthwhile testing *in vitro* the ability of such fractions containing 8-azaguanine to accept and transfer activated amino acids to the ribosomes.

SUMMARY

1. When albino mice were dosed intraperitoneally with 8-azaguanine, a substantially higher percentage of the analogue was incorporated into mitochondrial and soluble ribonucleic acid than into microsomal ribonucleic acid.

2. The 'pH 5.2 supernatant' ribonucleic acid contained a substantially higher proportion of 8-azaguanine than did the ribonucleic acid in the 'pH 5.2 precipitate'. A higher proportion of the total soluble ribonucleic acid was found in the pH 5.2 precipitate fraction in livers from treated animals.

3. Treatment with 8-azaguanine caused a decrease of about 30 % in the total guanine + 8-azaguanine content of mitochondrial ribonucleic acid, but had no significant effects on the proportions of major bases in the other fractions. Substantial changes occurred in the proportions of certain methylated guanines in liver microsomal ribonucleic acid.

4. Traces of 8-aza-adenine were identified in mitochondria from livers of animals dosed with 8-azaguanine.

5. The ribonucleic acid from subcellular fractions of pea and bean seedlings treated with 8-azaguanine showed a pattern of distribution of the analogue similar to that found for mouse liver.

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REFERENCES

- Aronson, A. I. (1961*a*). *Biochim. biophys. Acta*, **49**, 89.
 Aronson, A. I. (1961*b*). *Biochim. biophys. Acta*, **49**, 98.
 Bergquist, P. L. & Matthews, R. E. F. (1962). *Biochem. J.* **85**, 305.
 Brodie, B. B. (1956). *J. Pharmacol.* **8**, 1.
 Chantrenne, H. & Devreux, S. (1960*a*). *Biochim. biophys. Acta*, **39**, 486.
 Chantrenne, H. & Devreux, S. (1960*b*). *Biochim. biophys. Acta*, **41**, 239.
 Creaser, E. H. (1956). *Biochem. J.* **64**, 539.
 Ernster, L. & Lindberg, O. (1958). *Annu. Rev. Physiol.* **20**, 13.
 Francki, R. I. B. (1960). *Virology*, **10**, 374.
 Francki, R. I. B. & Matthews, R. E. F. (1959). *Biochim. biophys. Acta*, **34**, 570.
 Kalf, C. F. & Simpson, M. V. (1959). *J. biol. Chem.* **234**, 2943.
 Levenberg, B., Melnick, I. & Buchanan, J. M. (1957). *J. biol. Chem.* **225**, 163.
 Lindberg, O. & Ernster, L. (1954). *Chemistry and Physiology of Mitochondria and Microsomes, Protoplasmatologia*, vol. 3, p. 18. Vienna: Springer-Verlag.
 Litman, R. M. & Pardee, A. B. (1956). *Nature, Lond.*, **178**, 529.
 Markham, R. (1955). In *Modern Methods of Plant Analysis*, vol. 4, p. 246. Ed. by Paech, K. & Tracey, M. V. Berlin: Springer-Verlag.
 Markham, R. & Smith, J. D. (1949). *Biochem. J.* **45**, 294.
 Markham, R. & Smith, J. D. (1951). *Biochem. J.* **49**, 401.
 Markham, R. & Smith, J. D. (1952). *Biochem. J.* **52**, 552.
 Matthews, R. E. F. (1954). *J. gen. Microbiol.* **10**, 521.
 Matthews, R. E. F. (1955). *Virology*, **1**, 165.
 Novikoff, A. B. (1957). *Symp. Soc. exp. Biol.* **10**, 92.
 Rendi, R. (1959). *Exp. Cell Res.* **17**, 585.
 Siekevitz, P. (1959). In *Ciba Found. Symp.: The Regulation of Cell Metabolism*, p. 17. Ed. by Wolstenholme, G. E. W. & O'Connor, C. M. London: J. and A. Churchill Ltd.
 Smith, J. D. & Dunn, D. B. (1959). *Biochim. biophys. Acta*, **31**, 573.
 Smith, J. D. & Matthews, R. E. F. (1957). *Biochem. J.* **66**, 323.
 Straub, F. B., Ulmann, A. & Venetianer, P. (1960). *Biochim. biophys. Acta*, **43**, 152.
 Wyatt, G. R. (1951). *Biochem. J.* **48**, 584.