SUMMARY

1. The effects of butazolidin, chloroquine diphosphate, cortisol and dexamethasone on the incorporation of ^{14}C from labelled substrates into the soluble intermediates of chopped preparations of rat liver and brain have been studied.

2. All the drugs increased the total incorporation of radioactivity from ^{14}C glucose in brain and decreased the total incorporation of the isotope from $[2\text{-}^{14}\text{C}]\text{acetate}$ in liver and brain. The total incorporation of ^{14}C from ^{14}C glucose and $[3\text{-}^{14}\text{C}]\text{pyruvate}$ in liver was not materially altered by the presence of the drugs.

3. The distribution of ^{14}C from ^{14}C glucose in the soluble intermediates of liver was affected by all the drugs. The main changes were a greatly reduced incorporation of the isotope into alanine and an increased accumulation of radioactivity in maltose, phosphoglycerate, glycerate, glutamate and lactate. None of the drugs affected the distribution of ^{14}C from ^{14}C glucose in brain, from $[3\text{-}^{14}\text{C}]\text{pyruvate}$ in liver or from $[2\text{-}^{14}\text{C}]\text{acetate}$ in both liver and brain.

4. Some implications of these results are discussed.

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Biosynthesis of Polynucleotides

4. THE UTILIZATION OF EXOGENOUS PRECURSORS BY *CANDIDA UTILIS**

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The presence of the enzyme polynucleotide phosphorylase in bacteria and in plant and mammalian tissues (Grunberg-Manago, Ortiz & Ochoa, 1956; Beers, 1957; Brummond, Staehelin & Ochoa, 1957; Littauer & Kornberg, 1957; Hilmo & Heppel, 1957; Hakim, 1959) has made it appear likely that nucleoside 5'-diphosphates are the immediate precursors in the biosynthesis of ribonucleic acid. Cell-free preparations have also been obtained which catalyse the incorporation of nucleoside 5'-triphosphates into ribonucleic acid (Cannellakis, 1957; Edmonds & Abrams, 1957; Hecht, Zamecnik, Stephenson & Scott, 1958; Chung & Mahler, 1958). Since nucleoside diphosphates and triphosphates are readily interconvertible in most tissues, it is difficult to distinguish between them as precursors of ribonucleic acid *in vivo*, or in tissue homogenates. However, it seems probable that incorporation of nucleoside 5'-monophosphates into ribonucleic acid

(Goldwasser, 1955; Heidelberger, Harbers, Leibman, Takagi & Potter, 1956) takes place through either the corresponding 5'-diphosphates or 5'-triphosphates. In part 1 of this series (Barker, Montague, Moss & Parsons, 1957) it was shown that, *in vitro*, pancreatic ribonuclease, besides catalysing the formation of ester linkages between simple nucleotides (Heppel, Whitfield & Markham, 1955), can also bring about the attachment of nucleotides to ribonucleic acid. Such a synthetic reaction taking place *in vivo* might manifest itself in the incorporation of nucleoside 3'- (or 2'-)phosphates into ribonucleic acid through the corresponding cyclic phosphates. It is clearly desirable to establish whether this biosynthetic route does in fact occur in tissues in any circumstances, and experiments are in progress to test this possibility. The work reported here is an attempt to determine the extent to which new internucleotide bonds of ribonucleic acid are formed by polymerization of nucleoside 5'-phosphates. In the present experi-

* Part 3: Barker & Foll (1957).

ments incorporation of exogenous precursors into the ribonucleic acid of *Candida utilis* growing exponentially has been studied.

METHODS

Measurement of radioactivity. Samples of aqueous solutions of materials under investigation were evaporated on aluminium planchets and counted at infinite thinness with a thin end-window counter (window thickness, 1.7 mg./cm.²). Counting was continued until the statistical error was between 5 and 10%. Samples containing both ¹⁴C and ³²P were also counted with an aluminium shield of thickness 12.6 mg./cm.² which absorbed 97% of ¹⁴C-emission and 6% of ³²P-emission. Corrections were applied for decay of ³²P and for absorption by the aluminium shield. Concentrations of solutions for assay were determined by measurement of extinction at 260 mμ where appropriate; concentrations of solutions of ribose were determined by Euler & Hahn's (1946) method.

Electrophoresis. Paper electrophoresis was carried out by the method of Markham & Smith (1952) with citrate or acetate buffer at pH 3.5 and a voltage gradient of 20 v/cm. Authentic materials were examined simultaneously with experimental samples.

Paper chromatography. The following solvent systems were used: solvent 1, 5% Na₂HPO₄-isopentanol (ascending) (Carter, 1950); solvent 2, propan-2-ol-water-aq. 28% NH₃ soln. (85:15:1.3, by vol.) (descending) (Hershey, Dixon & Chase, 1953); solvent 3, butan-1-ol-water-formic acid (77:13:10, by vol.) (descending) (Markham & Smith, 1949); solvent 4, propan-2-ol-water-saturated (NH₄)₂SO₄ (2:19:79) (Deutsch & Nilsson, 1953). Phosphate was detected by the method of Wade & Morgan (1953) and ribose by the method of Buchanan, Dekker & Long (1950). Authentic materials were chromatographed on the same paper strips as the experimental samples.

Ion-exchange-resin chromatography. Mixtures of nucleotides were chromatographed by a modification of the method of Cohn (1950) with columns (15 cm. × 0.5 cm. or 15 cm. × 1 cm.) of Dowex-2 resin (formate form, 8% cross-linked, 200-400 mesh). The material to be chromatographed (approx. 5 mg. of total nucleotides for the small columns) was dissolved in 20 ml. of water, and the pH was adjusted to 8.5 with aq. NH₃ soln. Nucleotides were washed from the column by gradient elution (flow rate 1 ml./min. and 0.5 ml./min. for the larger and smaller columns respectively) by using 4N-formic acid and a 500 ml. mixing flask containing water initially. Fractions (5 ml.) were collected and their extinctions at 260 mμ were measured.

Growth of organism. *C. utilis* (National Collection of Industrial Bacteria; IMI. 23311) was grown in a medium containing (g./l.): glucose, 20; (NH₄)₂SO₄, 4.5; KH₂PO₄, 2.0; MgSO₄·7H₂O, 0.5; CaCl₂, 0.25; the pH was 4.8. In experiments involving incorporation of [³²P]orthophosphate, the concentration of KH₂PO₄ was decreased to 10 mg./l. Cultures were maintained on agar slopes and were subcultured every 3 weeks. Incorporation experiments were carried out with exponentially growing cells as follows. The liquid medium (150 ml.) was inoculated from a slope and incubated at 30° overnight with vigorous aeration. The cells were harvested by centrifuging and suspended in fresh medium to which the material to be

incorporated had been added, such that the cell suspension gave a reading of 20 in the EEL (Evans Electroselenium Ltd., Harlow, Essex) colorimeter used with a red filter. In preparative incorporation experiments, incubation with the fresh medium was continued until growth ceased; in other experiments incorporation was allowed to proceed either until the colorimeter reading was 40 (approx. 90 min.) or, in some experiments as indicated, for 15 min. In all experiments, the cells were harvested by centrifuging and were washed once with a volume of 0.14M-NaCl equal to that of the medium. In experiments in which the radioactivity of the acid-soluble fraction was measured, the cells were washed with three further quantities of 0.14M-NaCl, when a sample (1 ml.) of the supernatant was found to have no measurable activity.

Extraction of sodium nucleates. Experiments in which Kirby's (1956) method was used resulted in extraction of only traces of ribonucleic acid (RNA) from the yeast. A modification of the method of Schmidt & Thannhauser (1945) was therefore used. Since it was intended to hydrolyse the material to nucleoside 5'-phosphates, treatment with alkali was omitted and it was not possible to remove all deoxyribonucleic acid (DNA). However, experiments showed that the sodium nucleates extracted as described below were contaminated with not more than small traces of DNA, which were ignored (cf. Mitchison & Walker, 1958).

The washed yeast was extracted at 0° overnight with 5 vol. of 7.5% (w/v) trichloroacetic acid. The acid-soluble material was obtained as a supernatant after centrifuging, and the residue was washed twice with 7.5% (w/v) trichloroacetic acid. For radioactive measurements trichloroacetic acid was removed by extraction with ether. The residue from which the acid-soluble fraction had been removed was boiled for 2 hr. under reflux with 5 vol. of ethanol-ether (3:1, v/v) and once similarly with methanol-CHCl₃ (1:1, v/v). The residue from this treatment was heated twice at 90° for 1 hr. with 10 vol. of 10% (w/v) NaCl. To the combined supernatants obtained by centrifuging, ethanol (3 vol.) was added, and the precipitate was collected by centrifuging again, washed with ethanol and dried over CaCl₂ (RNA fraction). The yield was approx. 30 mg./g. of dry yeast.

Preparation of nucleoside 5'-phosphates from the ribonucleic acid fraction. A solution of sodium nucleate (100 mg.) in water (100 ml.) was brought to pH 9.3 with aq. 2N-NH₃ soln. and incubated at 37° for 18 hr. with phosphodiesterase prepared from Russell's viper's venom (Ross Allen's Reptile Institute, Silver Springs, Florida, U.S.A.) (20 mg. of venom) by the method of Hurst & Butler (1951). Perchloric acid (30%, w/v) was added to give pH 1, and, after centrifuging, the supernatant was adjusted to pH 8.5 with 0.3N-KOH and chromatographed on a column (1 cm. diameter) of Dowex-2 resin. Appropriate fractions were combined, evaporated below 35°, and finally freeze-dried. Four components were obtained which were characterized respectively as cytidine 5'-phosphate, adenosine 5'-phosphate (AMP), guanosine 5'-phosphate and uridine 5'-phosphate spectroscopically, by paper electrophoresis and by paper chromatography before and after treatment with the 5'-nucleotidase of crude Russell's viper's venom.

Preparation of nucleoside 2'- and 3'-phosphate from the ribonucleic acid fraction. These materials were prepared by

alkaline hydrolysis and ion-exchange chromatography by the method of Cohn (1950).

Preparation of [8-¹⁴C]adenosine. [8-¹⁴C]Adenine (The Radiochemical Centre, Amersham, Bucks.) was converted into *N*⁶-acetyladenine-9-mercurichloride, which was condensed with 2:3:5-tri-*O*-benzoyl-D-ribofuranosyl chloride by a method analogous to that of Fox, Yung, Davoll & Brown (1956). After removal of protecting groups, the material was purified by crystallization from water. The material gave single spots on paper chromatograms run in solvents 1 and 2.

Preparation of [8-¹⁴C]adenosine phosphates

Isotopically-labelled nucleotides were obtained either synthetically from [8-¹⁴C]adenosine, or biosynthetically from [8-¹⁴C]adenine.

Synthesis. Owing to the small scale on which experiments were carried out, it was found more economical to phosphorylate adenosine itself rather than its isopropylidene derivative. This has the disadvantage that some 2'- and 3'-phosphates are also formed, but these were decreased to a minimum by phosphorylating with dibenzyl phosphorochloridate under scrupulously anhydrous conditions (cf. Barker & Foll, 1957), about two-thirds of the total yield of nucleotides consisting of the 5'-phosphate.

Dibenzyl phosphorochloridate prepared from dibenzyl phosphite (0.18 ml.) was added to a solution of [8-¹⁴C]-adenosine (20 mg.) in 4 ml. of pyridine (dried immediately before use by distillation from P₂O₅) at -40°. The mixture was maintained at -40° in a stoppered flask for 3 hr., and the temperature was then allowed to rise to room temperature overnight. Saturated aq. Na₂CO₃ (1.5 ml.) was added with shaking, and solvents were then removed under reduced pressure below 35°. The residue was extracted with CHCl₃, the extract was washed with aq. 1% (w/v) NaHCO₃ (0.5 ml.) and with water (0.5 ml.), dried over Na₂SO₄ and evaporated to dryness under reduced pressure with five successive portions of ethanol (10 ml.). The residue was dissolved in aq. 50% (w/v) ethanol (12 ml.) and hydrogenated at just above atmospheric pressure in the presence of 10% of palladium-charcoal catalyst. After removal of solvent and catalyst, the residue was dissolved in water (10 ml.), the pH was adjusted to 8.5 with aq. NH₃ soln. and the solution was chromatographed on a column (diameter 1 cm.) of Dowex-2 resin. Three fractions were obtained which, after evaporation under reduced pressure followed by freeze-drying, yielded respectively AMP, adenosine 2'- and adenosine 3'-phosphate, identified by comparison with authentic samples on chromatograms run in solvent 4. A sample of the AMP was also converted into adenosine by Russell's viper's venom as described above. Before use, the labelled nucleotides were rechromatographed, and the purity was checked by comparison of the area of the paper containing radioactive and ultraviolet-absorbing materials.

Biosynthesis. *C. utilis* was grown in a medium containing a supplement of 0.1 mc of adenine in each 150 ml. of stock medium. The sodium nucleates were extracted and hydrolysed as described above. The AMP obtained was shown to contain not less than 97.2% of its radioactivity in the adenine residue as follows. A sample (approx. 1 mg.; 2880 counts/min.) of the material was dissolved in water (5 ml.) and 5 ml. of damp Dowex-50 resin (H⁺ form) was added. After 5 min., the resin was collected by filtration,

washed with water and heated at 100° for 3 min. with water (5 ml.). After cooling, the resin was collected by filtration and washed with water. The filtrate was chromatographed in solvent 1 and shown to contain D-ribose 5-phosphate but no adenine or adenine-containing material. The solution containing the ribose phosphate was transferred to a planchet and gave 80 counts/min. The resin was eluted with aq. 2N-NH₃ soln., and the filtrate was chromatographed in solvent 1. It gave no test for ribose-containing material and showed only one spot of ultraviolet-absorbing material, corresponding to adenine. The solution was evaporated and transferred to a planchet and gave 2800 counts/min. The residual resin was not radioactive.

Generally labelled [¹⁴C]adenosine 5'-phosphate. *C. utilis* was grown in 150 ml. of normal medium containing 0.1 mc of generally labelled [¹⁴C]glucose (The Radiochemical Centre), and AMP was prepared as described above. The radioactivity in the adenine and ribosyl residues was determined by the method of Roll, Weinfeld, Carroll & Brown (1956); 12.1% of the activity was present in the adenine residue.

[³²P]Adenosine 5'-phosphate. *C. utilis* was grown in normal medium containing 10⁶ counts/min./l. of KH₂³²PO₄. The AMP was prepared and characterized chromatographically as described above.

[³²P^α]Adenosine 5'-diphosphate. [³²P]AMP was condensed with non-radioactive orthophosphate by the method of Lowenstein (1957). The product was chromatographed on Dowex-2 resin, fractions being obtained which corresponded with AMP and adenosine 5'-diphosphate (ADP); a small fraction containing adenosine 5'-triphosphate (ATP) was also obtained. These materials were identified by comparison with authentic samples on electrophoresis in acetate buffer. Samples of the solutions containing the diphosphate and triphosphate were made 0.1N with respect to HCl and heated at 90° for 15 min. The hydrolysed material was then again submitted to electrophoresis and gave spots corresponding with AMP. The ADP and ATP were purified before use by electrophoresis. The materials were eluted with water from appropriate areas of the paper and freeze-dried.

RESULTS

Extent of utilization of exogenous precursors. The results are presented in Table 1. It is seen that, of the compounds examined, only adenine is efficiently utilized for RNA synthesis, approx. 60% of the label being incorporated into the RNA fraction. ADP and ATP were incorporated to a small but significant extent. In all three cases, incorporation into the RNA fraction was greater than that into the acid-soluble fraction. No detectable activity was found in either fraction of cells grown for 90 min. in a medium supplemented with [8-¹⁴C]-adenosine or its three isomeric monophosphates. No measurable activity was detected in whole, washed cells grown under these conditions, suggesting that, after the cells had been washed, not more than approx. 0.005% of the adenosine, or 0.035% of the monophosphates, remained in the

cells. It is believed that the radioactivity of the RNA fraction properly represents incorporation into the RNA molecule since, after incorporation of [8-¹⁴C]adenine for preparative purposes, radio-

active nucleoside 5'-phosphates were obtained after enzymic hydrolysis of the RNA, whereas alkaline hydrolysis yielded radioactive nucleoside 3'-phosphates. Furthermore, after incorporation of the isotope of labelled ADP, radioactive AMP was recovered from the RNA, whereas no activity was found in the AMP fraction of RNA after incubating the yeast with isotopically labelled AMP.

Table 1. *Incorporation of [8-¹⁴C]adenine and derivatives into the acid-soluble and ribonucleic acid fractions of exponentially growing Candida utilis*

C. utilis was grown for 90 min. in normal medium (150 ml.) to which the appropriate radioactive supplement had been added (approx. 50 µg.). Acid-soluble and RNA fractions were isolated as described in the Methods section, the whole of each fraction was transferred to planchets and the radioactivity measured.

Supplement	Radioactivity (counts/min.)		
	Total added	Acid-soluble fraction	RNA
Adenine	1000	62	645
Adenosine	6420	> 0.35	> 0.35
Mixed adenosine 2'- and 3'-phosphates	1000	> 0.35	> 0.35
AMP	1000	> 0.35	> 0.35
ADP	832	1.3	4.5
ATP	591	2.7	5.5

Equilibration of isotopic labels with metabolic pools. Experiments were carried out over a period of one generation (90 min.). Tables 2 and 3 show the results obtained with [³²P]ADP mixed with [8-¹⁴C]ADP and [³²P]ADP mixed with generally labelled [¹⁴C]ADP. It is seen that, under these conditions of growth, the phosphorus of all four nucleoside 5'-phosphates derived from the RNA becomes labelled. ¹⁴C appears in both adenine and guanine residues. No measurable activity was found in the ribosyl residues of any of the nucleotides with either generally or specifically labelled precursors.

Initial utilization of adenosine 5'-di- and tri-phosphate for ribonucleic acid synthesis. In order to investigate the extent to which added ADP and ATP are used as such for RNA synthesis, incorpora-

Table 2. *Incorporation of doubly labelled adenosine 5'-diphosphate into ribonucleic acid of exponentially growing Candida utilis*

C. utilis was grown for 90 min. on normal medium (150 ml.) to which a supplement consisting of a mixture (approx. 50 µg.) of [8-¹⁴C]ADP and [³²P]ADP had been added. RNA was isolated and hydrolysed as described in the Methods section to nucleoside 5'-phosphates, which were examined for radioactivity due to ¹⁴C and ³²P. GMP, Guanosine monophosphate; UMP, uridine monophosphate; CMP, cytosine monophosphate.

Nucleotide examined	Radioactivity (counts/min./µmole)		Ratio of activities ¹⁴ C/ ³² P
	¹⁴ C	³² P	
ADP (supplement)	493 ± 0.1	164 ± 0.1	3.0 ± 0.002
AMP	36.9 ± 0.37	1.9 ± 0.19	19.4 ± 1.9
GMP	28.4 ± 0.37	1.36 ± 0.16	20.8 ± 2.5
UMP	> 0.35	3.5 ± 0.98	> 0.35
CMP	> 0.35	0.7 ± 0.3	> 0.35

Table 3. *Incorporation into ribonucleic acid of exponentially growing Candida utilis of [³²P]- and generally labelled [¹⁴C]-adenosine 5'-diphosphate*

C. utilis was grown for 90 min. on normal medium (150 ml.) to which a mixture (approx. 50 µg.) of [³²P]ADP and generally labelled [¹⁴C]ADP had been added. RNA was isolated and hydrolysed as described in the Methods section to nucleoside 5'-phosphates, which were examined for radioactivity due to ¹⁴C and ³²P. The purine nucleotides were also hydrolysed and the radioactivity of the purine base was determined as described in the Methods section. Abbreviations are as in Table 2.

Nucleotide examined	Radioactivity (counts/min./μmole)				Ratio of activities ¹⁴ C(purine)/ ³² P
	¹⁴ C			³² P	
	Total	Purine	Ribose		
ADP (supplement)	700.8 ± 0.1	166.6 ± 0.1	543.2 ± 0.1	450 ± 0.1	0.37 ± 0.0002
AMP	0.92 ± 0.037	0.79 ± 0.048	> 0.35	0.3 ± 0.019	2.64 ± 0.2
GMP	0.49 ± 0.059	0.47 ± 0.1	> 0.35	0.166 ± 0.05	2.94 ± 0.89
CMP	0.11 ± 0.098	—	—	0.2 ± 0.07	—
UMP	0.084 ± 0.05	—	—	0.23 ± 0.04	—

Table 4. *Suppression of incorporation of [³²P]orthophosphate into nucleotides derived from ribonucleic acid of Candida utilis by adenosine 5'-di- and -tri-phosphate*

C. utilis was grown for 15 min. in normal medium (150 ml.) to which supplements of either [³²P]orthophosphate (approx. 150 μ c, carrier-free) alone or together with the nucleotide under investigation (0.1 m-mole) had been added. RNA was isolated and hydrolysed either by snake-venom diesterase or by alkali, the nucleotides were separated as described in the Methods section, and the radioactivity was measured. The specific activities of the nucleotides relative to the guanine nucleotides are recorded. The results are the mean of three experiments in each case.

Supplement other than [³² P]orthophosphate	Relative specific activity (activity of guanine nucleotide taken as unity)					
	Nucleoside 5'-phosphate derived from			Nucleoside 2'- and 3'-phosphate derived from		
	Adenine	Cytosine	Uracil	Adenine	Cytosine	Uracil
None	1.23 $\pm$ 0.03	0.92 $\pm$ 0.03	1.46 $\pm$ 0.03	1.28 $\pm$ 0.02	1.15 $\pm$ 0.03	1.14 $\pm$ 0.04
Adenine	1.26 $\pm$ 0.03	0.95 $\pm$ 0.03	1.47 $\pm$ 0.03	—	—	—
Adenosine	1.23 $\pm$ 0.03	0.90 $\pm$ 0.03	1.45 $\pm$ 0.03	—	—	—
AMP	1.23 $\pm$ 0.03	0.93 $\pm$ 0.03	1.46 $\pm$ 0.01	1.27 $\pm$ 0.03	1.21 $\pm$ 0.03	1.17 $\pm$ 0.03
ADP	0.52 $\pm$ 0.03	0.91 $\pm$ 0.03	1.45 $\pm$ 0.03	0.85 $\pm$ 0.03	0.96 $\pm$ 0.03	1.00 $\pm$ 0.03
ATP	0.33 $\pm$ 0.03	0.90 $\pm$ 0.01	1.43 $\pm$ 0.02	0.79 $\pm$ 0.01	0.90 $\pm$ 0.02	0.96 $\pm$ 0.01

tion was investigated over a brief period of time (15 min.). Owing to the low incorporation of ADP and ATP, it was not possible to use precursors of sufficiently high specific activity to give reliable results. Experiments were therefore conducted in the following way. Yeast was grown in the presence of [³²P]orthophosphate for 15 min., the RNA was extracted, hydrolysed with snake-venom diesterase, and the radioactivity of the four nucleoside 5'-phosphates was measured. This gave a reasonably reproducible pattern of labelling (see Table 4). The yeast was then grown in the presence of both [³²P]-orthophosphate and the inactive precursor under investigation. The nucleoside 5'-phosphates from the RNA were again examined. The results are shown in Table 4. It is seen that addition of AMP to the medium has no significant effect on the pattern of labelling of the nucleoside 5'-phosphates. On the other hand, addition of ADP or ATP results in a decreased activity of the AMP relative to the other nucleosides, whereas the activities of the other three nucleotides relative to each other remain unchanged. Similar experiments in which the RNA was hydrolysed with alkali showed that the relative activities of the uracil and cytosine nucleotides were decreased to some extent as well as that of the adenine nucleotide.

DISCUSSION

Relative efficiency of adenine and its derivatives as precursors of ribonucleic acid

Previous studies of the utilization of exogenous precursors of RNA by micro-organisms have demonstrated that adenine is incorporated into the RNA of *C. utilis* (Kerr, Seraidarian & Brown, 1951; Kerr & Chernigoy, 1953), *Tetrahymena gelii* (Flavin & Graff, 1951) and *Lactobacillus casei* (Elion &

Hitchings, 1950). In the last organism and in a *Neurospora* mutant (McNutt, 1958), adenosine is also utilized for RNA synthesis, and in *Escherichia coli* (Balis, Lark & Luzzati, 1955) adenosine 3'-phosphate is also efficiently utilized. On the other hand, in *C. utilis*, adenosine and derived nucleotides are utilized only to a very small extent (Kerr *et al.* 1951). It is seen that the results now presented are in general agreement with previous work so far as adenine is concerned. As was observed by Cowie & Bolton (1957), we also find that incorporation into RNA is greater than into the acid-soluble fraction. It should be noted that in the experiments of Kerr *et al.* (1951), in which a limited utilization of adenosine and its monophosphates was observed, incorporation was allowed to proceed for a very much longer period of time than in our experiments. Under our conditions it is possible to study the utilization of ADP since any independent utilization of adenosine or its monophosphates for RNA formation is not likely to amount to more than 7% of the utilization of ADP.

Metabolism of exogenous adenosine 5'-diphosphate

The experiments recorded in the experimental section, in which incorporation of labelled ADP was examined after 90 min. when the number of cells had doubled, give some indication of the utilization of this precursor in the complete cycle of reactions involved in RNA synthesis. [³²P]ADP, labelled only in the α -phosphate residue was used and the results therefore relate only to the possible utilization of the AMP unit of the ADP molecule.

Since the phosphate residues of all four nucleoside 5'-phosphates obtained from the RNA were labelled, and the ratio of the specific activity of adenine to that of the phosphorus of the AMP residue of the RNA differed from that in the

exogenous ADP, it follows that the AMP residue of ADP does not appear as an intact unit in the RNA after duplication of the cellular material is complete. Moreover, the isotopic label of the ribosyl residue of the ADP supplied was so diluted that no activity could be detected. This indicates that the α -phosphate residue is brought into equilibrium with the orthophosphate (and/or pyrophosphate) pool and that there is no metabolically stable reserve of phosphorylated ribose compounds such as 5-phosphoribosyl pyrophosphate. It also follows that fission of the glycosidic linkage of the ADP is complete.

Previous workers have observed the conversion of exogenous adenine into RNA guanine in *C. utilis* (Kerr *et al.* 1951). The present results show that a similar conversion occurs in this organism when ADP is supplied. Although the ratio of the specific activity of the RNA adenine to that of RNA guanine varies from one experiment to another, which is in accord with the results of Cowie & Bolton (1957), there is no significant difference between the ratio specific activity of base/specific activity of phosphate for AMP and that for guanosine monophosphate derived from the same RNA. This is in agreement with the existence of an equilibrium between the AMP and guanosine monophosphate units of RNA and a common intermediate as suggested by Tomlinson & Zbarsky (1957). Such an intermediate might be inosinic acid since this compound is known to be convertible into both AMP and guanosine monophosphate and is common to known routes for the synthesis of these compounds. Thus the present results are in agreement with the conversion of adenine into guanine at the nucleotide level, but the same result could also arise if free adenine were converted into free guanine, provided that both the corresponding nucleotides are in equilibrium with the same phosphate pool. This point is discussed further below.

Immediate precursors of ribonucleic acid

The experiments discussed above give no evidence as to the nature of the immediate precursors of RNA. However, in experiments with a shorter period of incorporation, fewer reactions appear to be involved. Suppression of the incorporation of orthophosphate into only the AMP fraction of RNA by exogenous ADP or ATP strongly suggests that the AMP units of these compounds are rapidly incorporated as such into the RNA molecule. An alternative explanation needs consideration, namely that the exogenous nucleotides are broken down to yield adenine and orthophosphate, which would dilute the activity of the orthophosphate of the medium to approximately one-tenth of its original value. In this case,

it would be necessary to postulate that the presence of adenine or adenosine so formed had a specific effect on the incorporation of orthophosphate from the medium into the adenine nucleotide fraction of the RNA. No such effect has been observed, as shown in Table 4, and it is concluded that in *C. utilis* under conditions of rapid growth, polymerization of nucleoside 5'-phosphates is a primary reaction in the formation of RNA from ADP or ATP.

It is seen from Table 4 that the activities of the 2'- and 3'-phosphate of cytidine and uridine are affected by added ADP or ATP as much as those of adenosine 2'- and 3'-phosphate. This is consistent with the view that the AMP unit is incorporated into the RNA molecule, since, after alkaline hydrolysis a phosphorus atom originally part of such a unit would remain attached, not to the original nucleoside, but to the nucleoside residue to which the AMP became joined in the process of incorporation (cf. Goldwasser, 1955).

Finally, incorporation of the phosphate residue of the precursors studied results in either all the nucleoside 5'-phosphates or only the AMP being affected, according to the period of incorporation. The absence of decreased activity in the guanosine monophosphate fraction after a brief period of incorporation may be due to polymerization of ADP or ATP being more rapid than the reactions involved in the conversion of adenine nucleotides into guanine nucleotides. On the other hand, the fact that conversion of adenine into guanine appears not to take place in the absence of equilibration of the phosphate residue with the pool strongly suggests that the process involves direct interconversion of the free bases rather than conversion at the nucleotide level.

SUMMARY

1. The incorporation of [8-¹⁴C]adenine, [8-¹⁴C]adenosine, [8-¹⁴C]adenosine 2'- and 3'-phosphate, [8-¹⁴C]adenosine 5'-phosphate, [8-¹⁴C]adenosine 5'-diphosphate and [8-¹⁴C]adenosine 5'-triphosphate into the acid-soluble and ribonucleic acid fractions of rapidly growing *C. utilis* has been studied.

2. Adenosine and its monophosphates were not taken up into either fraction. The other three compounds resulted in a higher degree of labelling in the ribonucleic acid than the acid-soluble fraction.

3. Adenine was efficiently utilized in ribonucleic acid synthesis; adenosine 5'-diphosphate and 5'-triphosphate were utilized to a small but significant extent.

4. Incorporation of adenosine 5'-diphosphate, labelled in the adenine, ribosyl and phosphate residue, over a period of a generation, results in the utilization of all three residues independently.

5. In a period of 15 min., adenosine 5'-di- and -tri-phosphate, but not adenosine 5'-monophosphate, suppress the incorporation of [32 P] orthophosphate into only the adenosine 5'-monophosphate residues of ribonucleic acid.

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Further Observations on the Paraffins and Primary Alcohols of Plant Waxes

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In a series of papers dealing with researches carried out between 1930 and 1938 on the long-chain constituents of plant and insect waxes it was inferred, from evidence based largely on melting-point and long-crystal-spacing data, that all the saturated ketonic and hydroxy acids, as also the corresponding primary alcohols, contained an even number of carbon atoms, whereas the paraffins, ketones and secondary alcohols, which are probably formed from their respective acids by de-

carboxylation, contained an odd number of carbon atoms (Chibnall, Piper, Pollard, Williams & Sahai, 1934). Such findings, in so far as they related to the saturated acids and primary alcohols, seemed to bring the wax constituents into line with the views then current about the constituents of shorter chain-length present in glycerides and similar natural products. There was much less evidence at the time with respect to the *n*-paraffins. The above-mentioned workers examined the meagre