Studies on the Biosynthesis of Riboflavin

7. THE INCORPORATION OF ADENINE AND GUANINE INTO RIBOFLAVIN AND INTO NUCLEIC ACID PURINES IN *EREMOTHECIUM ASHBYII* AND *CANDIDA FLARERI**

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It is now well established that, after removal of C-8, purines are actively incorporated into rings B and C of riboflavin (see e.g. Goodwin, 1959; Plaut, 1961*a, b*). Further, the addition of purines to cultures of the flavinogenic fungi *Eremothecium ashbyii* and *Candida flareri* stimulates the synthesis of riboflavin (Goodwin & Pendlington, 1954; Goodwin & McEvoy, 1959). In *E. ashbyii* guanine is more effective than xanthine, the purine structurally most similar to riboflavin, and both are more effective than adenine, whereas hypoxanthine is least effective (Brown, Goodwin & Jones, 1958). In *C. flareri* guanine and xanthine are both actively flavinogenic, but adenine and hypoxanthine, although supporting growth, have no flavinogenic action (Goodwin & McEvoy, 1959).

The work described below was undertaken in an attempt to explain the varying effects of the different purines in the two micro-organisms. Before this could be done it was necessary to prepare biosynthetically uniformly labelled (U) [^{14}C]adenine and [^{14}C]guanine. The only purines available commercially were labelled in C-8, which is lost when the purines are converted into riboflavin.

A short account of part of this work has already appeared (Audley, Goodwin & McEvoy, 1959).

EXPERIMENTAL

Organisms. *Eremothecium ashbyii* used throughout was the original strain of Goodwin & Pendlington (1954). The culture was maintained at room temperature on agar slopes containing (g./100 ml.): agar, 1.5; D-glucose, 1.0; bacteriological peptone, 1.0; yeast extract, 0.1. Subcultures were made every 2 weeks. The liquid medium used was that described by Goodwin & Pendlington (1954), with bacteriological peptone added to a concentration of 0.04%. After adjustment to pH 5.8, the medium was autoclaved at 15 lb./in.² for 15 min.

The strain of *Candida flareri* used throughout was NRRL 245, kindly provided by the North Regional

Research Laboratories, Peoria, Ill., U.S.A. It was maintained by fortnightly transfers on Sabouraud medium-agar slopes. The liquid medium used was that of Goodwin & McEvoy (1959).

Cultural conditions. For both organisms media (15 ml.) were dispensed in 50 or 75 ml. Erlenmeyer flasks, inoculated with 3 drops of a 2 days' culture grown on the basal medium and incubated in a rotary shaker at $28 \pm 1^\circ$.

Determination of riboflavin. Concentrations of riboflavin in the media were determined spectrophotometrically (Goodwin & Pendlington, 1954).

Radioactivity measurements. All activities were assayed on a windowless gas-flow counter (Nuclear Measurements Corp. Inc., Chicago, Ill., U.S.A.) (efficiency 50%) as described by Goodwin (1958), or on a conventional end-window system (efficiency 5%).

Labelled materials. [$8\text{-}^{14}\text{C}$]Adenine and [$8\text{-}^{14}\text{C}$]guanine were purchased from The Radiochemical Centre, Amersham, Bucks. [^{14}C]Adenine and [^{14}C]guanine were isolated from cells of the blue-green alga, *Anabaena cylindrica*, grown in $^{14}\text{CO}_2$.

Preparation of [^{14}C]adenine and [^{14}C]guanine. The procedure described below was adopted after many preliminary experiments to find the conditions best suited to our purpose.

Anabaena cylindrica was grown in bulk in the presence of $^{14}\text{CO}_2$ by Dr J. Glover and Dr P. P. Shah (Glover & Shah, 1957; Shah, 1959). They removed lipid from the cells with boiling ethanol and kindly provided us with the blue-green lipid-free powder for the preparation of [^{14}C]adenine and [^{14}C]guanine.

(a) Preparation and purification of RNA. The method used was an adaptation of that of Chargaff *et al.* (1950) for extraction of RNA from defatted yeast. The powder (1 g.) was placed in a 50 ml. Pyrex centrifuge tube and extracted with 7 ml. of 10% (w/v) NaCl by refluxing on an oil bath for 1 hr. The contents were centrifuged and, after removal of the supernatant, the residue was again extracted with 5 ml. of 10% NaCl. Absolute ethanol (6 vol.) was added to the bulked supernatants and the mixture set aside overnight at 4° . The resulting precipitate of crude RNA was centrifuged off, transferred to a tared 15 ml. centrifuge tube, washed twice with small volumes of absolute ethanol and twice with small volumes of ethyl ether, and weighed.

The crude extract (100 mg.) was suspended in 5 ml. of water in a 15 ml. centrifuge tube and warmed to 40° on a water bath for a few minutes to dissolve the sodium nucleate. The residue was centrifuged off and the supernatant transferred to a 15 ml. centrifuge tube and cooled to 4° ; 0.25 vol. of barium acetate (20%, w/v) and absolute ethanol (1 vol.) were added and the mixture was allowed to stand at 4° for 1 hr. The white precipitate was collected by

* Part 6 (Goodwin & Horton, 1960).

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centrifuging, washed with 5 ml. of barium acetate (5%, w/v) and resuspended in 2 ml. of water. Finely powdered Na_2SO_4 (20 mg.) was added and the mixture stirred for 1 hr. with a fine glass rod driven by an electric motor. The mixture was centrifuged, the supernatant removed and the residue was resuspended in 1 ml. of water; a further portion of Na_2SO_4 (5 mg.) was added and the mixture stirred as before for 1 hr. and then centrifuged. The two supernatants were cooled to 4° and ethanol (2.5 vol.), made 0.05N with respect to HCl, was added; the mixture was allowed to stand overnight at 4°. The white precipitate of purified RNA was collected by centrifuging, washed three times with absolute ethanol, three times with ethyl ether and then weighed. Six batches of labelled cells were prepared; weights of purified RNA obtained from these batches of cells are given in Table 1. It is not possible to state exactly the reason for the varying yields of purified RNA from the various batches; two major possibilities are: (a) varying conditions of culturing the organism, and (b) contamination of the RNA with small amounts of protein.

(b) Hydrolysis of purified RNA. Purified RNA (20 mg.) was hydrolysed with N-HCl (1 ml.) for 1 hr. at 100° in a sealed tube (Smith & Markham, 1950).

(c) Separation and purification of adenine and guanine. The bases were separated by chromatography on Whatman no. 1 paper with the solvent system of Smith & Markham (1950), which consisted of 2-methylpropan-2-ol, 700 ml. (at 26°), HCl (constant b.p.), 132 ml., and water to 1 l. (at 20°). Guanine showed up in ultraviolet light as a blue fluorescent spot and adenine as a dark-absorbing spot, as reported by Smith & Markham (1950); if the dried chromatogram were exposed to NH_3 vapour, guanine was visualized more effectively as a dark-absorbing spot and the intensity of the adenine spot was increased. This solvent system satisfactorily separates guanine (R_F 0.37) from adenine (R_F 0.44) and from cytidylic acid (R_F 0.62) and uridylic acid (R_F 0.76), which are the other products of hydrolysis of RNA.

The purines were eluted with 0.1N-HCl by the method of Dent (1947), which gave essentially quantitative recoveries when the bases were assayed spectrophotometrically. The molecular extinction coefficients used in calculating the amount of bases present were: for guanine, 10.6×10^3 at 250 μ ; for the adenine, 13.0×10^3 at 260 μ (Smith & Markham, 1950). The mean ratio of guanine to adenine in the algal hydrolysates was 1.0:0.82.

With the Smith & Markham solvent system the maximum loading of guanine on one spot that could be used without trailing was 100 μ g., so the amounts of the various hydrolysates which were applied in one spot on the paper were adjusted accordingly.

Carrier unlabelled guanine (7 mg. in N-HCl) was added to the bulked eluates of the guanine spots (4 mg. in approx. 300 ml.), which were then filtered and concentrated *in vacuo* to approx. 7 ml. The concentrate was transferred to a centrifuge tube and the pH made 7.0 by the gradual addition of solid Na_2CO_3 . After standing overnight at 4°, the precipitate was collected by centrifuging, dried at 80° and weighed (27 mg.). Spectrophotometric assay indicated the presence of approx. 7 mg. of guanine, and only 11 mg. was present originally. The contaminant was a brown powder, with solubility properties similar to those of guanine but with no absorption at 250 μ , which had been eluted from the chromatography paper. The guanine was

separated from this contaminant via its silver salt. Crude material (2 mg.) was dissolved in 2 ml. of warm N-perchloric acid in a 15 ml. centrifuge tube; any residue was removed by centrifuging, and the silver salt of guanine precipitated by the addition of 0.5 ml. of ammoniacal AgNO_3 [20% (w/v) AgNO_3]. After standing for 1 hr. at 4°, the white precipitate was centrifuged, washed twice with water, twice with 0.1N- H_2SO_4 and finally twice again with water. Free guanine was recovered by warming the precipitate with 3 ml. of 0.1N-HCl and removing the precipitated AgCl by centrifuging. No attempts were made to purify guanine via its picrate (Hitchings & Fiske, 1941), because in pilot experiments with unlabelled guanine recoveries better than 70% were never obtained.

After addition of unlabelled carrier (6.8 mg.), adenine was purified via its picrate, because in this case preliminary experiments indicated that quantitative recoveries could be obtained. Adenine (approx. 1 mg.) in 0.1N-HCl (10 ml.) was adjusted to pH 3.5–3.7 and saturated sodium picrate solution (1 ml.), prepared according to Benedict (1929), was added. A yellow crystalline precipitate formed immediately, but the mixture was allowed to stand for 30 min. before the picrate was filtered off through a small weighed sintered-glass crucible (porosity 3). The precipitate was washed with 2×1 ml. of 50% saturated sodium picrate, dried overnight at 80° and weighed. The picrate was recrystallized from water. Table 2 indicates the recoveries obtained; the yield of labelled adenine picrate was 24.3 mg. The picrate was easily and efficiently converted into the free base by a method previously used for preparation of adenosine from adenosine picrate (Davoll & Lowy, 1951). Adenine picrate (2–5 mg.) was dissolved in warm 0.01N-HCl and the solution allowed to pass through a small column (2 cm. $\times$ 1 cm.) of Dowex 1 (Cl^- form; X2). Picric acid was retained as a narrow yellow band at the top of the column and adenine passed straight through. The column was washed with water and fractions were collected until $E_{260 \mu}$ fell to zero. The combined fractions were evaporated

Table 1. *Yield of purified ribonucleic acid from various batches of defatted cells of Anabaena cylindrica*

Batch no.	Wt. of defatted powder (mg.)	Wt. of RNA isolated (mg.)
1 + 2	200	37
3	430	8
4	725	46
5	300	13
6	429	13
Total	2084	117

Table 2. *Recovery of adenine as adenine picrate*

See Experimental section for details of picrate formation.

Expt. no.	Wt. of adenine used (mg.)	Wt. of picrate precipitated* (mg.)	Wt. of picrate after crystallization (mg.)
1	10.5	28.4	24.4
2	10.5	28.8	23.6
3	10.5	28.8	23.8

* Theoretical yield 28.3 mg.

to dryness, yielding a white powder; chromatographic and spectrophotometric examination indicated that the specimen was pure adenine.

Guanine (0.1 μ c) and adenine (0.07 μ c) were chromatographed in the 2-methylpropan-2-ol-HCl-water system and the chromatogram was radioautographed for 3 months. The radioautograph showed that the adenine was completely free from radioactive impurities; the sample of guanine did contain trace amounts of fast-running radioactive impurities, but their activity was insignificant compared with that of guanine.

Isolation and purification of xanthine from cells of E. ashbyii and C. flareri. Two methods of isolation were used. In the first method, cells were ground for 10 min. in a mortar with a little silver sand and 20 ml. of aq. 80% (v/v) ethanol. If carrier xanthine was necessary it was added at this point (2 mg. in 20 ml. of water) and the extraction was carried out for a further 5 min. The residue was filtered off, washed and kept at -26° until required. The extracts and washings were combined and concentrated *in vacuo* to approx. 10 ml. In the second extraction method cells were cooled to 4° and transferred to a mortar. An aqueous solution of carrier xanthine was then added if required, and the mixture extracted for 5 min. by grinding with 10 ml. of cold 10% (w/v) trichloroacetic acid. The resulting slurry was centrifuged and the residue re-extracted with 5 ml. of cold 10% trichloroacetic acid. The combined extracts were extracted with ethyl ether until the pH of the aqueous phase rose to 5. Further carrier xanthine could be added at this point if required. All the operations just described, including the harvesting of the cultures, were carried out at 4° . Nucleotides were removed from the extract by passing it through a column (3 cm. $\times$ 0.5 cm.) of Dowex 1 (Cl^{-} form; X2). The column was washed with water (20 ml.) and the combined eluates were evaporated almost to dryness *in vacuo*. The residue was made strongly alkaline with NH_3 and the purines (and pyrimidines but not purine nucleosides, if present) were precipitated with a few drops of 20% (w/v) AgNO_3 . The free purines were regenerated as described above; occasionally the AgCl produced was colloidal and could not be sedimented by centrifuging. It was removed by passing the solution through a small column (1.5 cm. $\times$ 0.5 cm.) of Dowex 50 (H^{+} form). Colloidal AgCl passed through and was discarded; the adsorbed xanthine was eluted with 2N-HCl.

The xanthine was purified by successive chromatography in three solvent systems: (i) propan-2-ol, 170 ml., HCl (sp.gr. 1.16), 41 ml., water, to 150 ml.; (ii) butan-1-ol saturated with water at room temp., 100 ml., aq. 15N- NH_3 soln., 1 ml.; (iii) butan-1-ol, 77 ml., water, 13 ml., formic acid 10 ml. System (i) separates adenine and xanthine effectively; system (ii) separates xanthine from adenine and hypoxanthine; system (iii) separates xanthine from guanine. Any traces of ribosides would be easily separated from xanthine by any of these systems (Wyatt, 1955). Xanthine was eluted with 0.1N-HCl and shown to be spectrophotometrically pure, but, to eliminate any possible radioactive contaminants, it was adsorbed from 0.1N-HCl on a Dowex 50 (H^{+} form) column (1.5 cm. $\times$ 0.7 cm.) and the column was washed with water and then 0.1N-HCl. The xanthine was then eluted with 2N-HCl. This procedure would remove any residual traces of adenine or guanine which are strongly adsorbed from strongly acid solution (e.g. 2N-HCl) (Cohn, 1955). Although assays run before and after this final step indicated that the xanthine was pure before ion-exchange chromatography (Table 3), the step was always carried out as a precaution.

Isolation of riboflavin. Crystalline riboflavin was isolated from culture media by a procedure described in detail by Goodwin & Horton (1960).

RESULTS

Incorporation of labelled purines into xanthine and RNA purines in Eremothecium ashbyii. Two types of experiment were carried out to discover whether xanthine was formed from adenine and guanine by *E. ashbyii*. In the first the labelled purine and unlabelled xanthine were added to 48 hr. cultures and after 24 hr. the cells were harvested, unlabelled xanthine was added and the cells were extracted with aq. 80% (v/v) ethanol as described in the Experimental section. In the second type labelled purine only was added at 48 hr.; the cells were harvested 2 hr. later and, after the addition of carrier xanthine, extracted with cold trichloroacetic acid. The extracts in both cases were purified in the same way. The results obtained are recorded in Table 4. They indicate that in both cases a small but significant amount of activity is incorporated into xanthine.

In both experiments the activity in the RNA bases was also determined (Table 5). This Table indicates that after incubation for 24 hr. with labelled purines the total incorporation of both purines and their interconversion are essentially the same. During the first 2 hr., however, adenine is much more effectively incorporated into RNA purines and converted to a greater extent into guanine, than is guanine into adenine.

Incorporation of labelled purines into riboflavin and RNA purines in Candida flareri. *C. flareri* was grown in shake culture and either [U^{14}C]adenine or [U^{14}C]guanine was added as soon as riboflavin synthesis began, which was usually between 21

Table 3. *Radioactivity of xanthine isolated from Eremothecium ashbyii*

Purification was done as described in the Experimental section.

Expt. no.	Total activity (counts/min.)	
	After chromatography*	After chromatography and ion-exchange chromatography†
1	700	780
2	12 000	12 300
3	1 430	1 290
4	1 570	1 530

* In solvent systems.

† Dowex 50 (H^{+} form).

Table 4. *Production of xanthine from adenine and guanine by Eremothecium ashbyii*

[8-¹⁴C]Purines were added to 48 hr. shake cultures. Xanthine was extracted as described in the Experimental section, either 2 or 24 hr. later. Incubation temp., 28°. Xanthine was added with labelled purines for 24 hr. incubation but not for 2 hr. incubation.

Substrate	Specific activity ($\mu\text{C}/\mu\text{mole}$)	Total counts in xanthine		
		Counts added	After 2 hr. incubation	After 24 hr. incubation
[8- ¹⁴ C]Adenine	4.8	1×10^6	1 360	740
[8- ¹⁴ C]Guanine	2.4	1×10^6	1 550	12 150

Table 5. *Incorporation of [8-¹⁴C]adenine and [8-¹⁴C]guanine into ribonucleic acid by Eremothecium ashbyii*

Experimental conditions were as described in Table 4. RNA was extracted and purines were isolated as described in the Experimental section.

Substrate	Specific activity of nucleic acid purines (counts/min./ μmole)					
	After 2 hr. incubation			After 24 hr. incubation		
	Adenine (A)	Guanine (G)	Ratio A/G	Adenine (A)	Guanine (G)	Ratio A/G
[8- ¹⁴ C]Adenine*	278 000	21 200	13.1:1	142 000	23 600	6.0:1
[8- ¹⁴ C]Guanine*	1 870	45 400	1:24.3	19 400	144 500	1:7.5

* Counts added were as in Table 4.

Table 6. *Incorporation of [U-¹⁴C]adenine and [U-¹⁴C]guanine into riboflavin by Candida flareri*

Labelled purines were added to 21 hr. shake cultures. Riboflavin was isolated 8 hr. later as described in the Experimental section. Incubation temp., 28°. Two experiments were carried out at different times under the same conditions. Specific activity of adenine: 7950 counts/min./ μmole ; specific activity of guanine: 9530 counts/min./ μmole .

Substrate	Total counts added (counts/min.)	Wt. of riboflavin synthesized (mg.)	Total counts in riboflavin (counts/min.)	Percentage incorporation
Expt. 1				
[U- ¹⁴ C]Adenine	35 500	1.80	5 750	16
[U- ¹⁴ C]Guanine	28 800	1.83	19 600	68
Expt. 2				
[U- ¹⁴ C]Adenine	15 700	1.40	2 180	14
[U- ¹⁴ C]Guanine	11 800	1.31	8 010	68

and 24 hr. after inoculation. The cells were harvested 8 hr. later and the RNA purines were isolated from the cells; riboflavin was isolated from the culture medium. Two experiments were carried out; they show that both purines are effectively incorporated into riboflavin (Table 6), and adenine into RNA adenine and RNA guanine; however, guanine is incorporated only into RNA guanine (Table 7). The efficiency of incorporation into riboflavin is even greater than that indicated by the percentage incorporation of added radioactivity. As C-8 of the purines is lost during incorporation into riboflavin, the percentage of counts incorporated must be multiplied by $\frac{5}{4}$ to obtain the efficiency of conversion. Adenine is then incorporated to 19% and guanine to the spectacular value of 85%.

Table 7. *Incorporation of [U-¹⁴C]adenine and [U-¹⁴C]guanine into ribonucleic acid purines by Candida flareri*

Experimental conditions were as given in Table 6.

Substrate	Specific activity of nucleic acid purines (counts/min./ μmole)		
	Adenine (A)	Guanine (G)	Ratio A/G
Expt. 1			
[U- ¹⁴ C]Adenine	1320	850	1.55:1
[U- ¹⁴ C]Guanine	—*	1230	—
Expt. 2			
[U- ¹⁴ C]Adenine	840	350	2.40:1
[U- ¹⁴ C]Guanine	—*	730	—

* No counts detectable.

DISCUSSION

Formation of xanthine from adenine and guanine.

It is clear (Table 4) that xanthine is produced from both adenine and guanine in *E. ashbyii*. As there is no incisive evidence for the existence of xanthine oxidase in fungi, xanthine is probably formed in the following way: adenine and guanine are converted into nucleotides by a nucleotide pyrophosphorylase (reaction 1) (Liebermann, Kornberg & Simms, 1955), and as they are interconvertible in *E. ashbyii* (Table 5) they can both be converted into xanthosine 5'-phosphate (XMP); indeed, XMP is a mandatory intermediate between adenylic acid and guanylic acid (see Goodwin, 1960) (Fig. 1). Xanthine could then be formed from XMP through reaction (1).

A nucleotide pyrophosphorylase specific for xanthine has been found in *Escherichia coli* (Lagerkvist, 1958). This explanation is satisfactory for the 2 hr.-incubation experiment but after 24 hr. (Table 4) one would expect on this basis that the xanthine formed from adenine would be at least as great as that from guanine; the reverse situation is probably due to the fact that a considerable amount of any exogenous adenine is deaminated by *E. ashbyii* to hypoxanthine, which is only slowly metabolized (Brown *et al.* 1958).

Incorporation of [¹⁴C]adenine and [¹⁴C]guanine into RNA in E. ashbyii and C. flarerii. The results presented in Table 5 demonstrate that after incubation for 24 hr. adenine and guanine are both incorporated by *E. ashbyii* into RNA purines and are interconvertible to the same extent; the adenine/guanine ratios were 6.0:1 and 1.7:5 when [8-¹⁴C]-adenine and [8-¹⁴C]guanine were the respective substrates. After short incubation times the interconversion is much less, the corresponding ratios being 13:1 and 1:24. Al Khalidi (1958) reported that incorporation of [¹⁴C]guanine into RNA adenine by his strain of *E. ashbyii* was negligible; he does not state the time of incubation in his experiments, but even if it were only a short period, for example, 2 hr., the incorporation although small should on our evidence have been detected. This discrepancy might be due to strain variation in *E. ashbyii*.

The situation in *C. flarerii* is markedly different from that in *E. ashbyii*; after an incubation for 8 hr., which approximates to incubation for 24 hr. in *E. ashbyii* because *C. flarerii* is a much faster-growing organism, significant amounts of adenine are converted into guanine (Table 7) (adenine/guanine ratio 1.55:1 and 2.4:1 in two similar experiments), but conversion of added guanine into

RNA adenine could not be demonstrated. For the overall conversion of GMP into AMP the key enzyme, GMP deaminase, which reductively deaminates GMP to IMP (Mager & Magasanik, 1958), is required but is probably absent from *C. flarerii* (see Fig. 1). The pattern is similar to that observed in *Mycococcus pyrogenes* var. *aureus* (see Brown 1955) and *Rhodospirillum rubrum* (Goodwin & Passorn, 1961).

C. flarerii will grow on guanine but not adenine as sole nitrogen source (Goodwin & McEvoy, 1959). One explanation is that at some stage during its metabolism by *C. flarerii* guanine is deaminated and the liberated NH_4^+ ion is used for further growth; adenine, on the other hand, is presumably not deaminated. In support of this view Goodwin & McEvoy (1959) found no evidence of adenase in *C. flarerii*; however, nor was the formation of xanthine from guanine apparent (McEvoy, 1959). Indeed this cannot be the complete explanation because xanthine as sole carbon source will also support growth of *C. flarerii*; one is drawn to the conclusion that enzymes degrading guanine and xanthine are quickly induced under appropriate conditions, whereas those dealing with adenine cannot be induced. The presence of NH_4^+ ions must completely inhibit synthesis of any guanine-degrading enzymes because on a medium containing ammonium sulphate 85% of added [U-¹⁴C]guanine appears in riboflavin (Table 6).

Mechanism of incorporation of purines into riboflavin. The view that xanthine or a derivative is the immediate precursor of rings B and C of riboflavin

Fig. 1. Accepted pathways of nucleotide interconversions.

is attractive because xanthine is the purine structurally most closely related to riboflavin. However, although we have shown that [^{14}C]guanine is incorporated into riboflavin, there is still no direct evidence that C-8 is lost at the oxidation level of xanthine.

A further problem that is still not settled is whether C-8 is lost from a free base, nucleoside or nucleotide. The very effective incorporation of, for example, guanine (85%) into riboflavin by *C. flarer*i (Table 6), compared with its incorporation into RNA (Table 7) suggests that the possibility should at least be entertained that the biosynthetic pathway to riboflavin does not share common precursors, in particular nucleoside 5'-phosphates, with the RNA pathway. If the view that a xanthine derivative is the direct precursor of riboflavin is accepted, then in *C. flarer*i, if guanine were converted into GMP and then, conventionally, into XMP, IMP would be an intermediate; IMP would give rise to AMP (Fig. 1), and thus guanine would be incorporated into RNA adenine; this is not the case (Table 7). A possible alternative is that the purines are first ribitylated by a reaction analogous to that concerned in choline biosynthesis (see Goodwin, 1960) as the first step (reaction 2), and that it is a much faster reaction than are the conventional mechanisms available for purine metabolism.

Purine + CDP-ribitol

It is equally possible that the nucleoside 5'-phosphates are riboflavin precursors but that an active feed-back mechanism is functioning in *C. flarer*i which prevents their incorporation into RNA. To be effective, the feed-back mechanism should be more marked with guanine, which cannot be converted into RNA adenine, than with adenine, which can be converted into RNA guanine (Table 7).

SUMMARY

1. The preparation of [^{14}C]adenine and [^{14}C]guanine from cells of the blue-green alga *Anabaena cylindrica* grown in the presence of $^{14}\text{CO}_2$ is described.

2. [^{14}C]Xanthine can be detected in cells of *Eremothecium ashbyi* metabolizing either [$8\text{-}^{14}\text{C}$]adenine or [$8\text{-}^{14}\text{C}$]guanine.

3. *Eremothecium ashbyi* incorporates adenine into ribonucleic acid adenine and guanine; similarly, guanine is incorporated into both bases in ribonucleic acid. The extent of interconversion varies with time of incubation.

4. *Candida flarer*i incorporates adenine into ribonucleic acid, adenine and guanine; guanine is, however, incorporated only into ribonucleic acid guanine.

5. [^{14}C]Adenine and [^{14}C]guanine are effectively incorporated into riboflavin by *Candida flarer*i; under the conditions described the percentage conversion of adenine is 19% and that of guanine 85%.

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