Incorporation of the Carbon Skeleton of Adenosine into the Purine Nucleosides of Ribonucleic Acid and Deoxyribonucleic Acid by *Neurospora*

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In a previous study (1), evidence was presented to show that in a mutant of *Neurospora* the whole carbon skeleton of cytidine is incorporated with equal effectiveness into the pyrimidine nucleosides of ribonucleic acid and deoxyribonucleic acid. In this article a comparable experiment is described in which uniformly labeled C\(^{14}\)-adenosine was fed to an adenine-requiring mutant grown in the presence of sucrose. The purine nucleosides of RNA\(^1\) and DNA were all equally labeled. Although the purine nucleosides recovered from the RNA had a distribution of labeling between the purine and the ribose which differed from that of the adenosine fed, the same pattern of labeling was found in the purine deoxyribonucleosides from the DNA. This finding is interpreted to mean that, although some splitting of the administered purine ribonucleoside occurs in its conversion into a ribonucleosyl precursor of the purine nucleosides of RNA, the same ribonucleosyl precursor serves as the intermediate for the formation of the purine deoxyribonucleosides of the DNA. Inasmuch as the purine nucleosides of RNA and DNA have the same specific activities with regard to both their purine and sugar portions, it is inferred that the conversion of a ribonucleosyl to a deoxyribonucleosyl intermediate is the principal mechanism involved in this organism for the biogenesis of the purine nucleosides of DNA.

**EXPERIMENTAL RESULTS**

**Culture of Organism—**Mutant 28610 of *Neurospora crassa* was a gift from Dr. H. K. Mitchell. This organism will not grow in the absence of a purine source. Adenine, adenosine, and hypoxanthine in equimolar amounts support equal growth of the organism (2). Guanine and guanosine, although inactive by themselves, support growth in the presence of an adenine source (3). The organism was grown in 280 ml. of medium (2) containing 4.2 mg. of uniformly labeled C\(^{14}\)-adenosine-1\(\frac{1}{2}\) H\(_2\)O (1,620,000 c.p.m.) (Schwarz). This concentration of adenosine suffices to give almost one-half maximum growth. The medium was divided equally among 11 flasks of 125 ml. capacity. Each was inoculated with 1 drop of a light suspension of conidia and incubated at 25\(^\circ\) for 5 days.

**Separation of RNA and DNA Products—**The mycelia (weight, 0.255 gm.) from the pooled cultures were separated, washed and dried as described previously (1). Of the radioactivity in the filtrate and washings from the mycelia (415,000 c.p.m.) little if any was accounted for as adenosine or adenine. The RNA and DNA were extracted from the ground mycelium as before, discarding the residue (81 mg., 20,000 c.p.m.). The ribonucleotides from the first dialysis had 303,000 c.p.m.; the deoxyribonucleotide fraction, grossly contaminated with ribonucleotides, had 233,000 c.p.m.; and the contents remaining inside the bag (168,000 c.p.m.) had further amounts of nucleotides. The amount of radioactivity recovered in the various fractions (1,140,000 c.p.m.) accounts for 70 per cent of that administered.

Adenylic acid was chromatographed in the isobutyric acid-water-ammonia systems of Magasanik *et al.* (4) and Löfgren (5). Cytidylic acid was chromatographed in these two solvents plus the acid system of Wyatt (6). The band containing uridylic and guanylic acids after chromatography in the system of Magasanik *et al.* (4) was resolved into uridylic acid ($R_f = 0.30$) and guanylic acid ($R_f = 0.20$) by chromatography in the morpholine system (1). The guanylic acid was redchromatographed in the same solvent, whereas the uridylic acid was redchromatographed in Wyatt's system (6), although this is a poor solvent to use after the morpholine system, due to the formation of fluorescent substances from residues left on the paper. Each nucleotide was dephosphorylated and chromatographed (1). The specific activities of the nucleotides and nucleosides were determined from the optical densities of solutions of the substances in both acid and base (except the adenine derivatives which were measured in water) and from their radioactivities. The amounts of the nucleotides purified by chromatography to constant specific activity were of the order of 600 to 700 \(\mu\)g., except for guanosine (390 \(\mu\)g.).

As it has already been shown that the enzymatic degradation of DNA to nucleotides with DNAase and moccasin venom was accomplished without splitting of the deoxyribonucleotides (1), these substances could be separated from one another and from ribonucleotides quite readily with but one solvent (butanol-water-ammonia) (7). In actual practice each deoxyribonucleoside was chromatographed twice, the second time serving merely to concentrate the limited amount of material on a small area of paper.

As an example, the determination of the specific activity of deoxyguanosine is described. The nucleoside spot and the base characteristic of deoxyguanosine. It analyzed 9.16...
 Table I
Comparative specific activities of RNA and DNA constituents

c.p.m. per 10⁻⁸ mole

<table>
<thead>
<tr>
<th>RNA constituents</th>
<th>DNA constituents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adenyl acid</td>
</tr>
<tr>
<td>Adenine</td>
<td>Adenosine</td>
</tr>
<tr>
<td>Guanine</td>
<td>Guanosine</td>
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<tr>
<td>Cytidine</td>
<td>Deoxyadenosine</td>
</tr>
<tr>
<td>Thymidine</td>
<td>Guanosine</td>
</tr>
<tr>
<td>Uridine</td>
<td>Thymidine</td>
</tr>
</tbody>
</table>

 Table II
Distribution of labeling in purine nucleosides

c.p.m. per 10⁻⁸ mole

<table>
<thead>
<tr>
<th>RNA constituents</th>
<th>DNA constituents</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Adenine</td>
</tr>
<tr>
<td></td>
<td>Adenosine</td>
</tr>
<tr>
<td>Guanine</td>
<td>Guanosine</td>
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</tbody>
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 DISCUSSION
The point of principal interest disclosed from the experiments described here is the close biochemical relationship existing between the purine ribonucleosides of RNA and the purine deoxyribonucleosides of DNA. The origin of the purine nucleosides of the DNA almost certainly involves the reduction of a ribonucleosyl intermediate.

Mitchell anticipated this possibility in 1942. He observed
the accumulation of ribonucleotides in the cytoplasm of incompletely differentiated cells from humans given therapeutic doses of γ- and x-radiations, although no comparable increase occurred in the nucleic acid content of the nuclei (11). The explanation offered for these findings (12) was that the probable effect of these radiations upon proliferating cells is one of inhibiting the biological reduction of adenine ribonucleotides (and perhaps other ribonucleotides) to deoxyribonucleotides, a process normally occurring in the nucleus.

Also there is the suggestion that adenylic acid may be converted into deoxyadenosine in the metabolism of the rat, although the result seems not very decisive (13).

Lanning and Cohen (14) remark upon the fact that in Escherichia coli the deoxyribose from the DNA and the ribose from RNA attain comparable specific activities when the organism is grown in glucose-1-C14. Interpreted in the light of these experiments, this suggests that in E. coli, as in Neurospora, both the purine ribonucleosyl and pyrimidine ribonucleosyl intermediates are normally converted into the deoxyribonucleotides. After E. coli has been infected with phage the metabolism of deoxyribose is altered, as shown by its increased specific activity, but whether this effect is specifically on deoxyribose and not on ribose was not explicitly stated (14). Herriott (15) has submitted evidence also to show that DNA synthesis is changed in this organism after infection with phage. Quite contrary to the findings with Neurospora reported here are those of Wacker and Pfahl (16) showing that uniformly labeled uridine-C14 was incorporated as a unit into the uridine of RNA in E. coli 113-3, yet no radioactivity was found in the deoxyribose of thymidine.

As the foregoing experiments show, there is a remarkably similar distribution of labeling within the analogous elements making up the carbon skeletons of RNA and DNA. This is true of the pyrimidine nucleosides in the nucleic acids of the pyrimidine requiring mutant fed with cytidine-C14. It is also true of the purine nucleosides in the nucleic acids of the purine requiring mutant fed with adenosine-C14. This circumstance is so extraordinary that it raises the question of whether the DNA arose from a polynucleotide. Brachet (17) concluded from his experiments upon the developing egg of the sea urchin that the DNA formed after fertilization arises from an RNA pre-existing in the unfertilized egg. He found the amount of DNA in the unfertilized egg to be very small and that the amount of pentose in the egg decreased after fertilization with a corresponding increase in the DNA. More recently Pelc (18) has reported that adenine-8-C14 injected into the mouse appears quite rapidly in the DNA of the spermatogonia and that this RNA, at the proper stage in the cycle of the cell, is used for the synthesis of DNA without, he believes, extensive breakdown.

Volkin and Astrachan (19) have observed a striking coincidence in the relative amounts of P32 incorporated into the different nucleotides in the RNA of E. coli, after infection with phage, and the base composition of the phage-DNA. Furthermore, the P32 associated with this RNA “fraction” decreased as the P32 in the phage DNA increased. These findings are not inconsistent with the view that the DNA arose from a polynucleotide.

If, however, DNA were imagined to arise from a polynucleotide through “deoxygenation” of each ribose residue and methylation or hydroxymethylation of pyrimidine rings without rupture of the polynucleotide chain, there would be no reason to expect the incorporation of deoxyribonucleosides into DNA. Yet Reichard and Estborn (20) have shown that the N15 of deoxycytidine and thymidine is incorporated into the pyrimidines of DNA in amounts comparable to that from cytidine. The RNA, moreover, was not significantly labeled. Similar findings have been reported from experiments involving the administration of thymidine or deoxyuridine to various animals and tissues (21–24). Also, uniformly labeled C14-thymidine upon administration to Lactobacillus leichmannii resulted in extensive labeling of the deoxyribose in DNA, without detectable incorporation into RNA (25). It is therefore evident that in these examples the biogenesis of DNA from an RNA, if it occurs at all, must involve an RNA which constitutes only an extremely minute proportion of the total RNA present. The very existence of enzymes which catalyze the net synthesis of “DNA” from deoxyribonucleoside triphosphates (26) focuses attention on these simpler substances as possible intermediates in the biogenesis of DNA by living organisms.

SUMMARY

1. Uniformly labeled C14-adenosine, when fed to a mutant of Neurospora, was incorporated equally into the purine nucleosides of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) with but little dilution.

2. The distribution of labeling between the aglycone and the glycosyl group in the purine nucleosides of both RNA and DNA was very similar. It is probable that about half the ribose and deoxyribose present in the purine nucleosides of RNA and DNA was derived from intact adenosine.

3. The pyrimidine nucleosides of RNA and DNA, although only 2 per cent as radioactive as the purine nucleosides, all have very similar specific activities, a fact suggesting their common biochemical origin in this mutant also.

4. It is concluded that in this mutant the principal pathway for the biogenesis of the carbon skeleton of the purine nucleosides of DNA involves a ribonucleosyl intermediate.

REFERENCES

Incorporation of the Carbon Skeleton of Adenosine into the Purine Nucleosides of Ribonucleic Acid and Deoxyribonucleic Acid by *Neurospora*
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