INCORPORATION OF 2,6-DIAMINOPURINE INTO THE NUCLEOSIDE PHOSPHATES OF THE MOUSE*

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It has been shown (a) that the inhibition of Lactobacillus casei by high concentrations of 2,6-diaminopurine (2,6-DAP) in a folic acid-containing medium is reversible only by adenine, while in a thymine-containing medium adenine is more effective than other purines in restoring growth (1), (b) that 2,6-DAP is extensively utilized as a precursor of both polynucleotide adenine and polynucleotide guanine by L. casei (2), (c) that an L. casei mutant can utilize 2,6-DAP riboside as the sole purine source while the wild strain cannot (3, 4), and (d) that 2,6-DAP is incorporated into the pentose nucleic acid (PNA) guanine of the rat (5). On the basis of these findings it was suggested that the inhibitory activity of 2,6-DAP is probably due to competition with adenine for incorporation into some specific adenine-containing product, such as adenosinetriphosphate (ATP) or certain coenzymes, rather than to an interference with PNA synthesis or purine interconversion. The fact that Kornberg and Pricer demonstrated (6) that 2,6-DAP riboside can be enzymatically phosphorylated and can be converted to 2-aminoadenosinetriphosphate lends support to this suggestion.

As part of a program to investigate the mechanisms of action of certain known anti-cancer agents, the trichloroacetic acid-extractable nucleoside phosphates of mice which had received injections of radioactive 2,6-DAP were examined in an effort to see whether this purine as such can be incorporated into nucleotides.

Methods and Results

Each of four male Swiss mice was given five intraperitoneal injections of 2,6-diaminopurine-2-C\textsuperscript{14} sulfate monohydrate at a level of 37.5 mg. per kilo per injection in the course of 2½ days, and the mice were sacrificed 4 hours after the last injection. The activity of the injected material was 15.6 µc. per mg. On this schedule a 25 gm. mouse would receive a total

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of 73 μc. of radioactivity. The whole animals, minus tails, skins, hair, and feet, were pooled, and trichloroacetic acid-extractable nucleoside phosphates were isolated by the method described by LePage (7) for the isolation of ATP, except that the final precipitation as the barium salt was not performed. The solution containing the nucleoside phosphates as the sodium salts was subjected to freeze-drying, and the residue thus obtained was used in these experiments. Examination with a laboratory monitor showed this residue to be radioactive.

In an effort to determine whether the radioactivity of the residue was due to free 2,6-DAP which might have been carried along during the isolation, a sample of the residue was subjected to one-dimensional chromatography with one of the ammonium citrate-isopropanol-tetrahydrofurfuryl alcohol solvent systems of Carpenter (8). The resulting chromatogram is shown in Fig. 1.

Figs. 1, 4, and 6 represent composites of chromatograms and radioautograms. The circled spots indicate regions which absorbed ultraviolet light when the paper was scanned with a Mineralight, and the dark spots indicate the positions of radioactive materials.

Fig. 1 indicates the absence of free 2,6-DAP and of 2,6-DAP riboside, but it will be noted that there is considerable activity in the region where one would expect to find riboside phosphates.

Another portion (50 mg.) of the residue was digested with 50 per cent aqueous pyridine (0.6 ml.) at 90–95° for 72 hours. This treatment was chosen because Bredereck and coworkers showed (9) that the hydrolysis of

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1 The 2,6-DAP riboside used throughout the study was synthetic material which was kindly furnished by Dr. G. B. Brown of the Sloan-Kettering Institute.
yeast nucleic acid under these conditions yields nucleosides. This hydrolysate was then used for chromatography.

It was found that satisfactory resolution of a mixture of nucleosides was accomplished by means of two-dimensional chromatography with the urea-butanol and the disodium phosphate-isoamyl alcohol systems of Carter (10), as shown in Fig. 2. It was also found that this technique accomplished the resolution of a mixture of 2,6-DAP and 2,6-DAP riboside, as shown in Fig. 3.

**Fig. 2.** Chromatogram of a synthetic mixture of ribosides. A-R, adenosine; D-R, 2,6-diaminopurine riboside; U-R, uridine; C-R, cytidine; G-R, guanosine.

**Fig. 3.** Chromatogram of a synthetic mixture of 2,6-diaminopurine and 2,6-diaminopurine riboside.
Because of the proximity of the spots for 2,6-DAP and 2,6-DAP riboside and because of the possible lack of reproducibility of RF values in duplicate experiments, it was considered desirable to have both materials present on the chromatogram of the pyridine hydrolysate and then to determine

![Diagram](image)

**Fig. 5.** Sectioning of spot in preparation for rechromatographing 2,6-diaminopurine and 2,6-diaminopurine riboside.

![Image](image)

**Fig. 6.** Chromatogram-radioautogram of the eluates of the sections (1, 2, and 3) of Fig. 5 and of 2,6-diaminopurine and 2,6-diaminopurine riboside.

the presence or absence of radioactive material in each spot. Hence, a solution of non-radioactive 2,6-DAP was placed at the origin of the next chromatogram and, after this had dried, a portion of the pyridine hydrolysate was placed on the same spot. By use of two-dimensional chromatography as described above, the chromatogram shown in Fig. 4 was obtained.

By means of chromatograms of known compounds and of mixtures of known composition, the compounds were identified as shown. One might
speculate that the larger shadowy area is the locus of 2,6-DAP riboside phosphates and the unlabeled encircled area is probably the locus of adenosinephosphates, but no efforts were made to identify the materials positively. It is noted that the preponderant portion of the radioactivity is at the position of 2,6-DAP riboside. On the original radioautogram a faint spot was visible at the position of 2,6-DAP. Evidently a considerable amount of the 2,6-DAP was converted into the riboside, because the riboside could be detected in the absence of carrier material by scanning the chromatogram with ultraviolet light. Although it appeared rather evident that the highly active material was the riboside, it was desirable to establish its identity further. Therefore this active area was cut from the chromatogram and treated as follows:

The paper was cut into three pieces as shown in Fig. 5. To Section 1 was added a solution containing 3 μ of 2,6-DAP, to Section 2 nothing was added, and to Section 3 was added a solution containing 3 μ of 2,6-DAP riboside. Each section was then eluted by draping it across a microscope slide in such a way that the square end of the strip was immersed in N ammonium hydroxide and that advantage could be taken of capillary and siphoning action. The eluate was transferred to the origin of the new chromatogram by repeatedly touching the point of the section to the origin. One-dimensional chromatography with the disodium phosphate-isoamyl alcohol system yielded the chromatogram shown in Fig. 6.

The results are those expected for the riboside. It is felt that this rather conclusively establishes the identity of the compound as the riboside. These findings possibly shed some light upon the mechanism of action of this compound and lay open the possibility that it also competes with adenine for incorporation into other nucleotides and coenzymes.

SUMMARY

When 2,6-DAP is injected intraperitoneally into the mouse, a portion of the material is converted into a substance, probably a nucleoside phosphate, which can be isolated along with ATP and which upon subsequent hydrolysis with 50 per cent aqueous pyridine yields 2,6-DAP riboside.

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BIBLIOGRAPHY

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