ON THE NITROGEN TURNOVER IN PURINES
FROM POLYNUCLEOTIDES DETERMINED
WITH GLYCINE N\textsuperscript{15}

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The work of Brown et al. (1) with N\textsuperscript{15}-containing purines has shown that, of the naturally occurring purines, only adenine can be utilized directly for the synthesis of polynucleotides. When isotopic adenine was fed to the rat, part of it was recovered in rather low dilution in the adenine and in a somewhat higher dilution in the guanine of the polynucleotides from the mixed internal organs. By separating ribonucleic acid (PNA) from deoxyribonucleic acid (DNA), Brown, Petermann, and Furst (2) showed that the adenine had almost entirely entered the PNA fraction. Though these experiments prove that adenine administered in the food can act as a precursor not only of PNA adenine but also of PNA guanine, it does not follow that PNA guanine is formed ordinarily from adenine. Barnes and Schoenheimer (3), in their experiments with N\textsuperscript{14}-containing ammonium citrate, found in the pigeon a higher content of isotope in guanine than in adenine. They were, however, able to show that this was due to a higher N\textsuperscript{15} content in the amino group of guanine than in that of adenine. In experiments carried out in this laboratory (4) with N\textsuperscript{15}-glycine as a precursor for nucleic acid purines and pyrimidines in the hepatectomized and normal rat, it was found that the guanine in both the PNA and DNA fractions had about twice the isotope content of adenine. This finding makes it somewhat improbable that guanine could have been synthesized by way of adenine. The present investigation was carried out in order to settle this question. The experiments were performed with cytoplasma from regenerating liver and intestine from normal rat which had been left over from a previous experiment (4). The aminopurines were isolated from the PNA of cytoplasma and the PNA and DNA from intestine. Part of them was analyzed for N\textsuperscript{15} and the rest subjected to degradation to the corresponding hydroxypurines and these, too, were analyzed for their isotope content. A new method for the preparation of xanthine and hypoxanthine from the aminopurines with chromatography on a starch column was developed for that purpose. With this method as little as 2 mg. of aminopurine can be degraded to the corresponding hydroxypurine. The results of the isotope analyses for the different purines are summarized in Tables I and II.
The figures demonstrate that the isotope content is always higher in guanine than in adenine. The amino group of adenine always has a low isotope content, as indicated by the higher value of hypoxanthine compared with the corresponding adenine. The amino group of guanine in the PNA fractions has a relatively high isotope content, while in the DNA from intestine this is low. It can be seen that a reversal takes place for the two

**Table I**

Aminopurines and Corresponding Hydroxypurines from Intestine of Rat

The ratio $E_{max}/\gamma N$ per ml., which is a measure of purity with respect to foreign nitrogen, is for the pure compounds, adenine 0.180, hypoxanthine 0.197, guanine 0.161, and xanthine 0.170.

<table>
<thead>
<tr>
<th></th>
<th>PNA</th>
<th>DNA</th>
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<tbody>
<tr>
<td></td>
<td>Excess $N^{15}$</td>
<td>$E_{max}$</td>
</tr>
<tr>
<td>Adenine</td>
<td>0.46</td>
<td>0.171</td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>0.54</td>
<td>0.191</td>
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<tr>
<td>NH$_2$ group (calculated)</td>
<td>0.12</td>
<td>0.06</td>
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<tr>
<td>Guanine</td>
<td>0.51</td>
<td>0.165</td>
</tr>
<tr>
<td>Xanthine</td>
<td>0.43</td>
<td>0.165</td>
</tr>
<tr>
<td>NH$_2$ group (calculated)</td>
<td>0.83</td>
<td>0.04</td>
</tr>
</tbody>
</table>

**Table II**

Aminopurines and Corresponding Hydroxypurines from Regenerating Liver Cytoplasm of Rat

<table>
<thead>
<tr>
<th></th>
<th>Excess $N^{15}$</th>
<th>$E_{max}$</th>
<th>$\gamma N$ per ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenine</td>
<td>0.43</td>
<td>0.178</td>
<td></td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>0.51</td>
<td>0.190</td>
<td></td>
</tr>
<tr>
<td>NH$_2$ group (calculated)</td>
<td>0.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guanine</td>
<td>0.97</td>
<td>0.158</td>
<td></td>
</tr>
<tr>
<td>Xanthine</td>
<td>0.97</td>
<td>0.167</td>
<td></td>
</tr>
<tr>
<td>NH$_2$ group (calculated)</td>
<td>0.97</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

purines from the PNA of intestine after deamination; though guanine has a higher isotope content than adenine, the guanine nucleus, as indicated by the results for xanthine, has a lower isotope content than the adenine nucleus, as indicated by the data for hypoxanthine. This is, however, not the case for PNA purines from regenerating liver cytoplasm or for DNA purines from intestine.
EXPERIMENTAL

Administration of N\textsuperscript{15}-Glycine—Organs from a previous experiment (4) were used. The rats had been given two subcutaneous injections of 50 mg. of glycine per 100 gm. of body weight with an interval of 6 hours between doses. The glycine contained 32 atom per cent excess of N\textsuperscript{15}. 6 hours after the last injection the rats were killed and the various organs were immediately put into a large excess of absolute alcohol. They were homogenized with alcohol in the Waring blender, filtered, and dried with alcohol and ether. The hepatectomized rats received the same treatment starting 16 hours after the operation. In this case the livers were put into cold citric acid, and cell nuclei and cytoplasm were separated immediately after death 28 hours from the time of operation. The details are described in a previous publication (4).

Isolation of Adenine and Guanine—The procedure was mainly that of Edman, Hammarsten, Löw, and Reichard (5), though with some modification. The purines were obtained, after separation by the method of Hammarsten (6) of the PNA and DNA, by hydrolysis with \( \text{H}_2\text{SO}_4 \) for 1 hour at 100°. The excess sulfate was precipitated by adding freshly prepared hot solution of barium hydroxide, saturated at boiling temperature to pH 2. The precipitate of barium sulfate was centrifuged immediately and extracted twice with 5 ml. of hot water. The combined supernatants were precipitated with 0.2 volume of a 1 M solution of silver nitrate, as described by Kerr and Seraidarian (7). The precipitate of the silver purines was centrifuged, washed twice with 10 ml. of 0.05 M silver nitrate, twice with alcohol, and once with ether. The dry silver purines were decomposed with 5 ml. + 3 ml. + 3 ml. of 0.5 M \( \text{HCl} \), according to the method of Kerr and Seraidarian (7). The \( \text{HCl} \) solution was evaporated to dryness \textit{in vacuo} and the rest of the \( \text{HCl} \) carefully removed by repeated evaporation.

The residue was dissolved by warming in 1.0 ml. of water containing 0.1 m.eq. of sodium hydroxide. To the hot water solution, 10 ml. of dry butanol were added and the aqueous solution of the purines dissolved in the butanol by warming and gentle shaking. The butanol-water solution was added to the top of a starch column and the chromatogram was run and analyzed as previously described (5), the only difference being that the solvent used for developing the chromatogram was butanol-water (135 ml. of water + 865 ml. of butanol) and did not contain any glycol methyl ether.

Deamination of Adenine or Guanine to Corresponding Hydroxypurines—The method is a modification of the first steps of those of Strecker (8) and Kossel (9) to suit the degradation of small amounts of aminopurines. The
aminopurine (2 to 8 mg.) was obtained in dry form after chromatography by evaporating in vacuo the butanol of the combined fractions containing the same purine base. It was transferred to a 40 ml. centrifuge tube with about 2.5 ml. of N H₂SO₄. About 0.5 ml. of this solution was set aside for establishing the presence or absence of foreign nitrogen by the ratio \( E_{\text{max.}} / \gamma N \) per ml. (5) after proper dilution with N HCl and for isotope analysis. The volume of the rest of the solution was reduced to one-fourth, so that the sulfuric acid became 4 N. To this solution the necessary amount of sodium nitrite (8 mg. of nitrite per 1 mg. of aminopurine) was added, dissolved in 0.2 ml. of water. This was carried out slowly over a period of 10 minutes with continuous stirring. During this time the centrifuge tube containing the solution was placed in a boiling water bath and allowed to stand there for another 5 minutes after the last addition of the nitrite. Since the solution becomes more concentrated by evaporation during the whole procedure, 1 drop of water should be added now and then in order to keep the volume constant. The solution was then diluted with 15 to 20 ml. of water and precipitated hot with barium hydroxide to pH 2. The further procedure was the same as that described for the preparation of adenine and guanine; i.e., precipitation of the supernatant with silver, washing of the precipitate, decomposition with HCl, and evaporation of the acid. It was found adequate to reprecipitate the hydroxypurine once with silver nitrate. This was done in the usual way after dissolving the residue after the careful removal of the HCl in about 10 ml. of 0.01 N sulfuric acid. The free hydroxypurines were prepared from the silver purines as described above.

The hydroxypurines prepared in this way usually contain very little, if any, aminopurines. The highest contamination found was about 10 per cent. In order to remove the aminopurines, and as a further step of purification, the hydroxypurines were subjected to chromatography on a starch column.

**Chromatographic Model Experiment with Adenine and Hypoxanthine—**

About 2 mg. of each of the purines were dissolved in butanol-water with the aid of NaOH in the same way as described for the adenine-guanine chromatography. They were put onto a starch column (length 100 mm., diameter 36 mm.), the chromatogram was run, and the purines localized in the effluent by measuring their light absorption in the ultraviolet (Fig. 1). Fractions containing the same purine were combined, the butanol evaporated in vacuo, and the residue dissolved in 10 ml. of N HCl. By taking an aliquot of the solution for nitrogen determination and determining the light absorption after proper dilution with N HCl, the ratio \( E_{\text{max.}} / \gamma N \) per ml. was determined for each purine and compared with the same ratio for the pure purine. This ratio was, for pure hypoxanthine, 0.197 \( (E_{\text{max.}} \text{ at } 2480 \text{ A}) \) and for adenine, 0.180 \( (E_{\text{max.}} \text{ at } 2620 \text{ A}) \).
Chromatographic Model Experiment with Guanine and Xanthine—Fig. 2 shows a chromatogram for these two bases run under about the same conditions as described above for adenine and hypoxanthine. The length of column in this experiment was 122 mm., however. Another difference is that in chromatographing solutions containing xanthine, as little alkali as possible should be used to dissolve the purines in butanol-water. If excess alkali is used, the resolving power of the column declines and, furthermore, the yield of xanthine drops. In the experiment of Fig. 2, 0.03 m.eq. of NaOH was used for a total of 4 mg. of the bases.

As can be seen from Fig. 2, a complete separation of xanthine from guanine has not been achieved in this experiment. The separation would, however, have been practically complete if the amount of guanine had been small as compared with that of xanthine, as is the case of a degradation experiment. Complete separation can also be achieved with larger amounts of guanine if a longer column is used for the chromatogram. However, this does not usually improve the total yield of pure xanthine. The reason is that part of the xanthine is lost during the chromatography by sticking in the column. These losses, which for a column of 120 mm. are not

![Graph showing chromatogram of adenine and hypoxanthine.](image-url)
more than at the most 20 per cent, increase rather rapidly with the length of the column.

If part of the guanine appears in the same fractions as part of the xanthine, these mixed fractions can easily be distinguished by determining the ratio of the light absorption at 2620 and 2480 A for every fraction. This is indicated by the dash line in Fig. 2. At first, when only xanthine is emerging from the column, the ratio is constant and lies at about 2.20. In the mixed fractions the ratio rapidly declines, the more the xanthine is contaminated with guanine, and finally reaches a constant value of about 0.75 when the fractions consist of pure guanine. A good yield of the bases is still obtained even when the mixed fractions are discarded. Their purity with respect to foreign nitrogen is established in the usual way. The ratio $E_{\text{max.}}/\gamma N$ per ml. is, for guanine, 0.161 ($E_{\text{max.}}$ at 2480 A), and for xanthine 0.170 ($E_{\text{max.}}$ at 2600 A).

The over-all yield for the deamination of the aminopurines and chroma-
tography of the hydroxypurines was established with pure adenine and guanine. For both cases it was found to be 60 to 70 per cent when starting with 2 to 8 mg. of aminopurine.

DISCUSSION

Glycine has been shown by Abrams, Hammarsten, and Shemin (11) to act as a precursor for the nitrogen in the 7 position of nucleic acid purines. According to the views expressed by Brown et al. (1) adenine would be synthesized before guanine, which would subsequently be synthesized from the adenine nucleus. That this can be the case has been shown by Brown’s feeding experiments with isotopic adenine. If this theory corresponds to a general pathway for the synthesis of nucleic acid purines, one would expect it to hold true irrespective of the nature of the isotopic precursor for the purines. One would expect furthermore that the isotope content of adenine would not be lower than that of guanine. This was also the case in Brown’s experiments. The experiments of Hammarsten and coworkers (4) on normal and regenerating liver show, however, that in both PNA and DNA the isotope content of guanine is much higher than that of adenine. These findings could be brought into line with Brown’s theory if it is assumed that the amino group in guanine has a much higher N16 content than the rest of the molecule. At this stage no definite answer could be given to that question as no method for degradation of small amounts of aminopurines was available to us.

The present experiments show that in regenerating liver cytoplasm the main pathway for the synthesis of PNA guanine, with glycine as a precursor, does not proceed via adenine. In accordance with the finding of Barnes and Schoenheimer (3), who used ammonia as a precursor, it was found that the amino group of guanine has a higher turnover rate than that of adenine. Its isotope content, however, is not high enough to suggest that the remaining guanine residue could have been formed from adenine.

The findings with respect to the PNA from intestine would agree rather well with Brown’s theory. There, the difference between the isotope content of guanine and adenine is much smaller and after deamination the relations are inversed, so that the isotope content in hypoxanthine is higher than that of xanthine.

The DNA purines from normal intestine differ from the PNA purines from the same organ in that the amino groups of both adenine and guanine have a relatively low isotope content. As the guanine has a much higher isotope content than the adenine, this proves that in this case the synthesis of guanine has not proceeded via adenine. A difference between PNA purines and DNA purines has been noted earlier by Brown et al. (2).
In feeding experiments on rats with $N^{15}$-adenine, the adenine from the PNA of the mixed internal organs contained about 30 times as much isotope as the adenine from the corresponding DNA. The authors thought the reason for this to be a much lower turnover of the purines in DNA as compared with the purines from PNA. Furthermore, it was assumed that the difference in the turnover ratio DNA to PNA between adenine and phosphorus (12) might indicate that "some portion of the phosphate moieties of a nucleic acid may be exchanged without the purines of the C—N skeleton being affected." In the present investigation, the values for the turnover rates of the purines match fairly well with those obtained by Hammarsten and Hevesy (12) for phosphorus. In their experiment on intestine of the rat, the turnover ratio DNA to PNA for phosphorus was 1.7 as compared with the value 1.7 for adenine and 1.1 for guanine in the present investigation. Because of this, the finding of Brown et al. is explained better perhaps on the hypothesis that adenine cannot be utilized for the synthesis of purines in DNA.

The results obtained in the present investigation clearly indicate the necessity for separating the polynucleotides into DNA and PNA in isotope experiments. Furthermore, they show that there might be a difference in the purine turnover in different organs.

**SUMMARY**

A method for obtaining the corresponding hydroxypurines from small amounts (2 to 8 mg.) of adenine and guanine has been developed.

Intestine and regenerating liver cytoplasm from a previous experiment (4) in which $N^{15}$-glycine had been injected into rats has been worked up for aminopurines of PNA and DNA. These were degraded to the corresponding hydroxypurines.

Evidence has been obtained that the main part of guanine in PNA from regenerating liver cytoplasm and from DNA in intestine is not synthesized via adenine. In the case of PNA from intestine, this possibility could not be excluded.

**BIBLIOGRAPHY**

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