During an investigation of nitrogen metabolism in birds (1) it was found that, when isotopic ammonia was administered, $\text{N}^{15}$ was incorporated in the nucleic acids. The values obtained suggest that nucleic acids are synthesized even more rapidly than proteins. Since uric acid is a major end-product in avian metabolism, the rapid rate of nucleic acid turnover suggested that it may play an intermediate rôle in the general nitrogen metabolism. The present investigation deals with the interrelationship of purines, pyrimidines, and creatine and their possible function as precursors for nucleoproteins.

If dietary purines can serve in the synthesis of nucleoproteins, administration of isotopic purines must result in their deposition in the nucleic acids as an intermediary step and, finally, in their appearance as nitrogenous end-products. Since the nucleic acids also contain pyrimidines, a similar reasoning applies to the metabolism of these substances. The classical feeding experiments of Cerecedo and coworkers (2–6) indicate that, with the exception of cytosine, which is poorly absorbed, these compounds are oxidized and excreted as urea and oxalic acid.

The nitrogen of isotopic guanine, when administered to rats, is excreted as allantoin and, to an almost insignificant extent, as urea and ammonia (Table I). Practically no isotope could be found in the purines or pyrimidines isolated from nucleic acids. Essentially the same result was obtained with pigeons that had received isotopic guanine, the purine analogue in this case being uric acid. Tissue creatine, isolated as creatinine, contained little or no isotope marker, but the nitrogenous end-product, uric acid, was high. Neither the purines nor the pyrimidines isolated from nucleic acids showed the expected isotope concentration; dietary purines, therefore, do not appear to be incorporated as such into the tissues. When isotopic creatine was fed to rats over a period of 6 days, the allantoin...
isolated from the urine contained no N\(^{15}\). Similarly, uric acid isolated from the droppings of pigeons that had received isotopic creatine (sarcosine as well as amidine N\(^{15}\)) was found to be free of isotope marker. The results of this experiment are given in Table II and show that the only appreciable

\begin{table}
\centering
\caption{N\(^{15}\) Content of Substances Isolated after Administration of Isotopic Guanine}
\begin{tabular}{lccc}
\hline
 & Experiment I, rats & Experiment II, rats & Experiment III, pigeons \\
\hline
Ammonia & 0.74 &  &  \\
Allantoin & 13.9 &  &  \\
Urea & 0.54 &  &  \\
Combined purines & 0.05 & 0.10 & 0.20 \\
Protein hydrolysate & 0.15 &  &  \\
Guanine & 0.02 & 0.20 &  \\
Adenine & 0.25 & 0.12 &  \\
Cytosine & 0.32 &  &  \\
Arginine & 0.22 &  &  \\
Glutamic acid & 0.27 &  &  \\
Tissue creatine & 0.30 & 0.25 & 1.30 \\
Uric acid &  &  &  \\
\hline
\end{tabular}
\end{table}

\begin{table}
\centering
\caption{N\(^{16}\) Content of Substances Isolated after Administration of Isotopic Creatine and Guanidoacetic Acid to Pigeons}
\begin{tabular}{lccc}
\hline
 & Creatine, amidine N\(^{15}\) & Creatine, sarcosine N\(^{16}\) & Guanidoacetic acid \\
\hline
Combined purines & 0.33 & 0.03 & 0.12 \\
Tissue creatinine & 3.95 & 2.40 & 2.64 \\
Uric acid & 0.57 & 0.12 & 0.36 \\
Nucleic acids & 0.21 & 0.15 & 0.03 \\
Average error & 0.10 & 0.10 & 0.10 \\
\hline
\end{tabular}
\end{table}

isotope concentration was found in the tissue creatine. The replacement appears to proceed at approximately the same rate as in mammals (7). Similar results were obtained with isotopic guanidoacetic acid. When this was fed to pigeons, only a negligible amount of N\(^{15}\) appeared in the uric acid isolated from the droppings, but the tissue creatine contained the expected amount.
Upon administration of isotopic uracil and thymine to rats, the only appreciable isotope concentration was found in urinary ammonia and urea. The most significant result of these experiments (Table III) is the low value for allantoin compared to that of urea, which rules out biological conversion of pyrimidines to purines.

**Synthesis of Isotopic Guanine**—Guanine was synthesized according to the procedure of Traube (8) in which, as in his improved synthesis of 2,4-diamino-6-hydroxypyrimidine (9), guanidine was used as starting material. This was regarded as a convenient way of introducing isotopic nitrogen by way of the well established synthesis (7) of cyanamide from isotopic ammonia and normal cyanogen bromide. When cyanamide and ammonium chloride (10) were heated together in ethyl alcoholic solution in bomb tubes at 100° for periods of 5 to 7 hours, no reaction occurred, and, with longer heating or higher temperatures, the cyanamide polymerized to dicyanodiamide. On the other hand, guanidine was readily produced by heating ammonium bromide and cyanamide in the presence of 1 mole of excess ammonia in a bomb tube for 96 hours at 100°. A reasonably pure product and a 96 per cent recovery of the excess isotopic ammonia were secured. Little or no polymerization took place, probably because the excess ammonia raises the pH of the solution sufficiently above that at which polymerization of cyanamide takes most rapidly (11, 12); namely, 9.7. The addition of small amounts of bromine or iodine was found to catalyze the reaction. This addition was later abandoned, as the bromo- or iodoguanidine formed as by-products were difficult to

### Table III

<table>
<thead>
<tr>
<th></th>
<th>Uracil</th>
<th>Thymine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined purines</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td>Ammonia</td>
<td>0.71</td>
<td>1.0</td>
</tr>
<tr>
<td>Allantoin</td>
<td>0.01</td>
<td>0.09</td>
</tr>
<tr>
<td>Urea</td>
<td>1.21</td>
<td>1.49</td>
</tr>
<tr>
<td>Guanine</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>Adenine</td>
<td>0.11</td>
<td>0.20</td>
</tr>
<tr>
<td>Cytosine</td>
<td>0.32</td>
<td>0.28</td>
</tr>
<tr>
<td>Thymine</td>
<td>0.23</td>
<td>0.30</td>
</tr>
<tr>
<td>Creatinine</td>
<td></td>
<td>0.06</td>
</tr>
<tr>
<td>Protein hydrolysate</td>
<td>0.23</td>
<td>0.22</td>
</tr>
<tr>
<td>Feces</td>
<td>0.29</td>
<td>0.41</td>
</tr>
</tbody>
</table>
The guanine finally prepared contained 4.03 atom per cent $N^{15}$ excess with the isotope in the 2-amino group and the 2 pyrimidine nitrogen atoms.

In order to establish the biological origin of substances containing ring systems with more than 1 marked nitrogen atom it becomes necessary to degrade the material and to isolate its split-products. In contrast to similar examples reported previously the amount of starting material in our instance was extremely small. The amount of pure material isolated from nucleic acids (adenoine and guanine) ranged from 50 to 150 mg., depending on the number of animals used. Extensive study of such degradations seemed necessary at the time. Strecker oxidized guanine with chloric acid (13) to guanidine and parabanic acid. We were unable to perfect the technique of this reaction to the point where parabanic acid could be isolated from the oxidation mixture of 50 mg. of guanine or less, but guanidine was isolated as the picrate. When this reaction was carried out with guanine synthesized as described above, the guanidine was found to contain $N^{15}$ in the exact amount demanded by the theory.

Another degradation was the deamination of guanine to xanthine. According to the procedure of Fischer (14) xanthine was obtained in good yield from 50 mg. of guanine as starting material, even after purification through its silver salt. It contained the calculated amount of isotope marker. These two experiments together with the synthesis of guanine give sufficient proof for the correctness of the position of $N^{15}$ in the molecule. At the same time, these experiments show that splitting of the purine ring system with chloric acid occurs between the 1-6 and 3-4 atoms; it also gives added evidence for the existence of cyanamide in its mesomeric form (7, 15).

**Synthesis of Isotopic Thiourea**—The reaction of cyanamide and hydrogen sulfide (16) in acid medium with Sb$_2$S$_3$ as catalyst was used. The final product was recrystallized from n-propyl alcohol until its correct melting point (174°) was obtained.

**Synthesis of Isotopic Thymine**—Thymine was prepared from thiourea and ethyl formylpropionate according to the procedure of Wheeler and Liddle (17). Isotopic thiourea was condensed with the sodium salt of ethyl formylpropionate in methanolic sodium methylate solution to 2-mercapto-5-methyl-6-hydroxypyrimidine which, when treated with concentrated hydrochloric acid, gave thymine in almost quantitative yield.

**Synthesis of Isotopic Uracil**—Although the simplest method for the preparation of uracil seems to be the condensation of urea with malic acid (18), we have synthesized uracil from thiourea and ethyl formylacetate according to Wheeler and Liddle (17), because the resulting compound, 2-mercapto-6-hydroxypyrimidine, could also be used as an intermediate in the synthesis of cytosine. Circumstances, however, prevented us from
preparing cytosine. 2-Mercapto-6-hydroxypyrimidine on treatment with concentrated hydrochloric acid gave uracil, 2,6-dihydroxypyrimidine, in 94 per cent yield.

EXPERIMENTAL

Guanidine Hydrobromide (I)—4.77 gm. (3 moles) of isotopic ammonium chloride, 10.1 atom per cent N\textsuperscript{15} excess, were decomposed according to the procedure previously reported, special care being taken to exclude moisture. The ammonia was carried into a dry ice-cooled bomb tube containing 20 cc. of absolute ethyl alcohol. A gas inlet tube, especially constructed for this purpose, so as to fit the bomb tube, was used. The small amount of ammonia that escaped was caught in a trap containing dilute sulfuric acid. When the absorption of ammonia in the alcohol was complete, the bomb tube was allowed to warm up to 0° and kept at this temperature in ice. A solution of 3.18 gm. of freshly prepared cyanogen bromide in 5 cc. of absolute ethyl alcohol was added, and the tube sealed and heated to 105–110° for 96 hours.

The length of time necessary for this reaction was determined by running a series of experiments with non-isotopic ammonia. The tubes were heated to 110° for various lengths of time and the contents tested with ammoniacal silver nitrate for the presence of cyanamide. At the end of 96 hours the test for cyanamide was consistently negative.

The tube was cooled in ice, opened, cut off as straight as possible, and the open end fire-polished. The gas inlet tube was inserted, and the excess ammonia blown off by a slow stream of nitrogen and collected in an acid trap. 94 to 96 per cent of the excess ammonia (10.1 atom per cent N\textsuperscript{15} excess) could thus be collected. The clear alcoholic solution was slowly evaporated to dryness and the residue dried \textit{in vacuo} at room temperature. 4.02 gm. of guanidine hydrobromide were collected. Although this product was not analytically pure, it was found to be quite satisfactory for the following condensation.

2,4-Diamino-6-hydroxypyrimidine (II)—This compound was prepared according to Traube (9) with methyl alcohol instead of ethyl alcohol and 50 per cent excess cyanoacetic ester; 5.21 gm. of guanidine hydrobromide yielded 4.08 gm. of diaminohydroxyppyrimidine sulfate.

Alloxan-2,4-dimide-5-oxime (III)—This compound was prepared in 99.8 per cent yield as described by Traube (8); 3.45 gm. of the isonitroso compound were obtained from 4.08 gm. of diaminohydroxyppyrimidine sulfate.

2,4,5-Triamino-6-hydroxypyrimidine (IV)—The original procedure of Traube was slightly modified to yield the sulfate directly instead of the free base. 3.45 gm. of the isonitroso compound were finely powdered in a large
agate mortar and slowly added to 50 cc. of boiling water containing 15 cc. of commercial ammonium sulfide. This suspension was kept boiling for 3 hours with occasional stirring and addition of 3 to 5 cc. of ammonium sulfide and the necessary amount of water to keep the volume constant. When all the nitroso compound was reduced, the solution was immediately filtered. On prolonged boiling in the absence of excess sulfide the solution turned pink. This could not be prevented by carrying out the reaction in an atmosphere of nitrogen. The solution was filtered by suction directly into 75 cc. of 3 N H₂SO₄ cooled in ice. 4.68 gm. (82 per cent) of triamino-hydroxypyrimidine sulfate were obtained in the form of light yellow crystals. No further purification was necessary.

\[
3\text{NH}_3 + \text{BrCN} \rightarrow \text{NH} = \text{C} = \text{NH} \xrightarrow{\text{NH}_4\text{Br}} \text{NH}_2 \text{C} = \text{NH} \cdot \text{HBr}
\]

\[
\text{NH}_2 \quad \text{COOC}_2\text{H}_5 \rightarrow \text{NH} = \text{C} \quad \text{CH} \rightarrow \text{NH}_2 \text{C} = \text{NO} \quad \text{NH}_2 \quad \text{C} = \text{NH}_2
\]

\[
\text{NH} = \text{C} \quad \text{C} = \text{NH} \quad \text{NH} = \text{C} \quad \text{C} = \text{NH}_2
\]

\[
\text{NH} = \text{C} \quad \text{C} = \text{NH} \quad \text{NH} = \text{C} \quad \text{C} = \text{NH}_2
\]

\[
\text{NH} = \text{C} \quad \text{C} = \text{NH} \quad \text{NH} = \text{C} \quad \text{C} = \text{NH}_2
\]

\[
\text{NH} = \text{C} \quad \text{C} = \text{NH} \quad \text{NH} = \text{C} \quad \text{C} = \text{NH}_2
\]

\[
\text{NH} = \text{C} \quad \text{C} = \text{NH} \quad \text{NH} = \text{C} \quad \text{C} = \text{NH}_2
\]

\[
\text{NH} = \text{C} \quad \text{C} = \text{NH} \quad \text{NH} = \text{C} \quad \text{C} = \text{NH}_2
\]

\[
\text{NH} = \text{C} \quad \text{C} = \text{NH} \quad \text{NH} = \text{C} \quad \text{C} = \text{NH}_2
\]

\[
\text{NH} = \text{C} \quad \text{C} = \text{NH} \quad \text{NH} = \text{C} \quad \text{C} = \text{NH}_2
\]

\[
\text{NH} = \text{C} \quad \text{C} = \text{NH} \quad \text{NH} = \text{C} \quad \text{C} = \text{NH}_2
\]

\[
\text{NH} = \text{C} \quad \text{C} = \text{NH} \quad \text{NH} = \text{C} \quad \text{C} = \text{NH}_2
\]

\[
\text{NH} = \text{C} \quad \text{C} = \text{NH} \quad \text{NH} = \text{C} \quad \text{C} = \text{NH}_2
\]

\[
\text{NH} = \text{C} \quad \text{C} = \text{NH} \quad \text{NH} = \text{C} \quad \text{C} = \text{NH}_2
\]

\[
\text{NH} = \text{C} \quad \text{C} = \text{NH} \quad \text{NH} = \text{C} \quad \text{C} = \text{NH}_2
\]

\[
\text{NH} = \text{C} \quad \text{C} = \text{NH} \quad \text{NH} = \text{C} \quad \text{C} = \text{NH}_2
\]

\[
\text{NH} = \text{C} \quad \text{C} = \text{NH} \quad \text{NH} = \text{C} \quad \text{C} = \text{NH}_2
\]

\[
\text{NH} = \text{C} \quad \text{C} = \text{NH} \quad \text{NH} = \text{C} \quad \text{C} = \text{NH}_2
\]

\[
\text{NH} = \text{C} \quad \text{C} = \text{NH} \quad \text{NH} = \text{C} \quad \text{C} = \text{NH}_2
\]

\[
\text{NH} = \text{C} \quad \text{C} = \text{NH} \quad \text{NH} = \text{C} \quad \text{C} = \text{NH}_2
\]

Guanine Sulfate (V)—4.62 gm. of triamino-hydroxypyrimidine sulfate and 4.0 gm. of anhydrous sodium formate were refluxed with 50 cc. of 90 per cent formic acid for 8 hours. The solution was filtered and evaporated on the steam bath. The residue was dissolved in dilute sulfuric acid, boiled with charcoal for 5 hours, and the product crystallized. The mother liquors in each case were neutralized to pH 5, the precipitated free base
removed by filtration and redissolved in a smaller volume of dilute sulfuric acid, and the guanine again crystallized as sulfate. On repetition of this procedure, a total of 2.94 gm. of guanine sulfate was obtained.

**Analysis**—(C$_4$H$_6$N$_4$O$_6$)$_2$H$_2$SO$_4$·2H$_2$O. Calculated, N 32.8; found, N 32.8

The compound contained 4.03 atom per cent N$^{15}$ excess; calculated 4.04 atom per cent N$^{15}$ excess.

\[
\text{(V)}
\]

\[
\text{(VI)}
\]

**Degradation of Isotopic Guanine to Guanidine**—50 mg. of free guanine (4.03 atom per cent N$^{15}$ excess) were prepared from the sulfate. The free base was suspended in 4.0 cc. of HCl and 32 mg. of KClO$_3$ added in small amounts over a period of 1 week. At the end of this time, the solution was colorless and all solid material had gone into solution. It was evaporated to dryness under reduced pressure and extracted five times with absolute alcohol. The clear alcoholic solution was concentrated to 1.0 cc. A small amount of semicrystalline material, probably parabanic acid, was removed by filtration. The filtrate was evaporated to dryness, the residue dissolved in 2.0 cc. of water, and 2.0 cc. of saturated picric acid added. After the material had stood in the ice box overnight, 32 mg. of a crystalline picrate precipitated which, when recrystallized from hot water, gave analytical values corresponding to those of guanidine picrate.

**Analysis**—C$_7$H$_6$O$_4$N$_4$. Calculated, N 29.2; found, N 29.4

20 mg. of this picrate were decomposed with dilute hydrochloric acid and the picric acid continuously extracted with ether. Isotope analysis indicated 6.92 atom per cent N$^{15}$ excess; calculated 6.76 atom per cent N$^{15}$ excess.
Degradation of Isotopic Guanine (V) to Xanthine (VI)—32 mg. of guanine (4.03 atom per cent N\textsuperscript{15} excess) were dissolved in 2.0 cc. of 4 N sulfuric acid and heated in a beaker of boiling water, 24 mg. of sodium nitrite were added in very small amounts, and the solution neutralized and cooled in ice. The dark colored xanthine which precipitated was purified by transformation into its silver salt which was decomposed with dilute hydrochloric acid. On neutralization to pH 5 xanthine crystallized in analytically pure form. It contained 3.44 atom per cent N\textsuperscript{15} excess; calculated 3.38 atom per cent N\textsuperscript{15} excess.

\[
\text{C}_2\text{H}_4\text{OCO} + \text{NH}_2 \rightarrow \text{HS} \rightarrow \text{NH}_2 \rightarrow \text{CS} \rightarrow \text{NH}_2
\]

(VII)

\[
\text{C} \rightarrow \text{CH}_3 \rightarrow \text{NH}_2 \rightarrow \text{C} \rightarrow \text{CH}_3 \rightarrow \text{HO} \rightarrow \text{C} \rightarrow \text{CH}_3
\]

(X)

(Thymine)

Isotopic Thiourea (VII)—2.67 gm. of isotopic cyanamide prepared according to the procedure previously reported (7) were dissolved in 70 cc. of water, and 5.34 gm. of antimony pentasulfide and 0.7 cc. of concentrated hydrochloric acid added. The mixture was heated to 90° on the steam bath and a rapid stream of hydrogen sulfide blown through the suspension for 2 hours. After removal of the excess hydrogen sulfide by boiling, the antimony pentasulfide was filtered off and washed with hot water. The filtrate was treated with 20 mg. of potassium carbonate and cleared by filtration through a small amount of infusorial earth. It was then evaporated to dryness under reduced pressure and the residue recrystallized from n-propyl alcohol until its melting point was 174°. The total yield after concentration of mother liquors was 2.14 gm. The material contained 5.28 (calculated 5.25) atom per cent N\textsuperscript{15} excess.

Isotopic Thiouracil (VIII)—2.14 gm. of isotopic thiourea were added to
100 cc. of 95 per cent ethyl alcohol in which 1.5 gm. of sodium had been dissolved. To this mixture 12 gm. of powdered ethyl sodium formyl acetate were added and the solution refluxed for $3\frac{1}{2}$ hours. At the end of this time, 20 cc. of water were added and refluxing was continued for another half hour. The mixture was then brought to dryness under reduced pressure and redissolved in 40 cc. of water, chilled in ice, and acidified with 50 per cent acetic acid. After being left to stand in the ice box overnight, the product was filtered off and recrystallized from 125 cc. of boiling water, charcoal being used to remove colored by-products. 1.57 gm. of thiouracil were obtained.

Isotopic Uracil (IX)—The procedure employed was exactly the same as that described by Wheeler and Liddle (17). Analytically pure uracil was obtained in 96 per cent yield. The final product contained 5.42 atom per cent $N^{15}$ excess; the calculated amount was 5.25 atom per cent $N^{15}$ excess.

Isotopic Thiothymine (X)—2.0 gm. of isotopic thiourea (5.28 atom per cent $N^{15}$ excess) were condensed with 12 gm. of sodium ethyl formylpropionate in the manner described for thiouracil. After two recrystallizations from boiling water 940 mg. of thiothymine were obtained.

Isotopic Thymine (XI)—940 mg. of thiothymine were treated with hydrochloric acid as described by Wheeler and Liddle (17). 760 mg. of thymine were obtained. The product contained 4.88 atom per cent $N^{15}$ excess; the calculated amount was 5.25 atom per cent $N^{15}$ excess. 

Feeding Experiments

The experimental conditions employed in the feeding experiments were identical with those previously reported (7). Rats received the same stock diet. Pigeons received an adequate diet of mixed grains; the isotopic compounds were dissolved in water and administered by stomach tube every 6 hours. Guanine was administered as the hydrochloride, since the free base is insoluble in water. Urinary ammonia, urea, muscle creatine, and amino acids were isolated by the usual procedures. Allantoin was isolated according to Wiechovski (19). The isolation of purines and pyrimidines will be described in the following representative experiment.

Nucleic Acids (General Procedure)—Combined internal organs (liver, kidney, spleen, pancreas, testes, thymus gland) were immediately minced

1 Although somewhat exceeding the experimental error, the discrepancy between these values can be explained as follows: After feeding experiments and elementary analyses had been carried out, an extremely small amount of material was available for spectrographic analysis, necessitating a dilution with a known amount of normal nitrogenous material. The error involved in this particular incidence may well account for the difference of 5.25 and 4.88 atom per cent $N^{15}$ excess.
and suspended in 95 per cent ethyl alcohol overnight. The precipitate was filtered and extracted with absolute alcohol in a Soxhlet apparatus for 6 hours. The alcohol was then replaced with ether and extraction continued for another 6 hours. Adhering solvent was removed in a stream of dry air and the fat-free product powdered in a mortar. 100 gm. of wet organs usually gave about 33 to 35 gm. of dry material. A small sample of this powder was used for the determination of “combined purines” according to Graff and Maculla (20) and the rest extracted with 10 per cent sodium chloride. The mixture was occasionally heated on the steam bath with stirring over a period of 2 to 3 days and finally filtered in a steam-heated Buchner funnel. This extraction was repeated three times and the sodium salts of the nucleic acids precipitated by addition of 2.5 volumes of ethyl alcohol. The salts were centrifuged off and washed several times with absolute alcohol and ether. In this way, 2.4 to 3.0 gm. were obtained from 35 gm. of dry tissue. The sodium salts were dissolved in 100 cc. of water by warming the mixture on the steam bath and stirring at high speed.

The solution was then placed in an ice bath (stirring continued) and 5.0 cc. of concentrated hydrochloric acid added drop by drop. The precipitated nucleic acids were centrifuged, washed with water, alcohol, ether, and finally with absolute ether. The product was almost pure white and practically free of protein; its weight was approximately one-third that of the original sodium salt.

**Nucleic Acid Splitting**—The following representative procedure is a modification of the usual hydrolysis of nucleic acids (21). The procedure was successfully used for the isolation of guanine, adenine, cytosine, and thymine with as little as 450 mg. of nucleic acids. In the original study of the method nucleic acids prepared from beef pancreas were used, which were found to differ appreciably from rat nucleic acids in their relative adenine and guanine content. The latter was found to contain less than one-half the amount of adenine, with a corresponding increase in guanine (Table IV).

1.0 gm. of the finely powdered and carefully dried nucleic acids was suspended in 12 cc. of absolute methyl alcohol and dry hydrochloric acid blown through the solution for exactly 3 hours. The solution turned wine-red, the precipitate dissolved, and finally the purines precipitated as hydrochlorides. The flask was stoppered (glass) and allowed to stand in the ice box for 24 hours. The ice-cold solution was again saturated with dry HCl, and the precipitate centrifuged and washed with methyl alcohol saturated with HCl until the washings were colorless. Combined washings and supernatant were set aside for the isolation of pyrimidines. After being dried at 100°, 300 mg. of purine hydrochlorides were obtained.

The purine hydrochlorides were dissolved in 5.0 cc. of water and heated
with charcoal, filtered, and neutralized with dilute NaOH; when acid to litmus and alkaline to Congo red, the guanine precipitated as an amorphous powder. After having remained in the ice box for 3 days, it was centrifuged and the supernatant solution set aside for the isolation of adenine. The free guanine was dissolved in dilute sulfuric acid and crystallized as sulfate in the manner described in the synthesis of isotopic guanine. Approximately 160 mg. of guanine sulfate were obtained.

The supernatant liquid from the precipitation of guanine was combined with the washings and concentrated to about 3.0 cc. A small amount of insoluble material was removed by filtration and an equal volume of saturated picric acid added. A copious precipitate of adenine picrate formed, which was recrystallized from a minimum amount of 25 per cent acetic acid until the correct analytical figures were obtained (29.2 per cent N). Usually 60 to 70 mg. of adenine picrate were obtained after three recrystallizations; approximately 10 per cent was lost on each recrystallization. N\textsuperscript{15} analyses were carried out in the manner described above for guanidine picrate.

The methanolic solution containing pyrimidine nucleosides was taken to dryness and the dark colored residue suspended in 15 cc. of 20 per cent HCl, sealed in a bomb tube, and heated at 185° for 3 hours. The contents were filtered and the filtrate evaporated to dryness. The residue was digested with 20 cc. of water, filtered, and acidified with 1 drop of concentrated HNO\textsubscript{3}. Undesirable by-products, purines, phosphates, etc., were removed by addition of 5.0 cc. of 10 per cent silver nitrate, the precipitated silver salts carefully washed, and the combined filtrate and washings concentrated to less than 3.0 cc. The bulk of thymine crystallized out in the form of long needles. After repeated recrystallization from water, the yield was 10 to 15 mg.

The filtrate from thymine was neutralized with barium hydroxide and
any precipitate which formed below pH 7 was discarded. On further addition of barium hydroxide pyrimidine silver precipitated together with some Ag₂O. The precipitated silver salts were carefully washed until free from alkali, suspended in 20 cc. of water, and decomposed with hydrogen sulfide. The clear filtrate from Ag₂S was concentrated to 1.0 cc. and 2.0 cc. of saturated picric acid were added. Cytosine picrate precipitated immediately and was recrystallized from hot water until analytically pure. For N₁⁵ analyses the picrate was decomposed as described above.

Guanine Feeding. Experiment I—Four adult male rats with a combined weight of 1273 gm. were fed 158 mg. of guanine hydrochloride (4.03 atom per cent N₁⁵ excess) over a period of 3 days. The amount of guanine was calculated to be approximately equivalent to the amount of allantoin excreted. The results of this experiment are recorded in Table I.

Experiment II—This experiment was identical with Experiment I except that the animals received twice the amount of guanine hydrochloride per kilo per day. The results are recorded in Table I.

Experiment III—Two adult pigeons with a combined weight of 810 gm. were fed 54 mg. of guanine hydrochloride per kilo per day. The animals were killed 6 hours after the last feeding, and purines, creatine, nucleic acids, and uric acid were isolated. The analytical values are recorded in Table I.

Creatine Feeding. Experiment I—Four adult male rats weighing 1215 gm. were fed 44 mg. of creatine hydrate per kilo per day for 6 days. The urines from the first 4 days were pooled and allantoin isolated. The samples of allantoin from the last 2 days of Animals 1 and 2 as well as 3 and 4 were found to have identical isotope content; namely, 0.004 atom per cent N₁⁵ excess. No isotope was found in the combined purines isolated from the internal organs. The creatinine hydrate was prepared as previously reported (15) and contained 3.31 atom per cent N₁⁵ excess in the amidine group only.

Experiment II—Four adult pigeons with a combined weight of 1402 gm. were fed 60 mg. of creatine hydrate per kilo per day for 3 days. The creatine hydrate contained 3.31 atom per cent N₁⁵ excess, with the isotope marker in the amidine group only. Of the substances isolated only the muscle creatine contained isotope, and a degradation with baryta showed that all of it was located in the amidine group, the sarcosine moiety containing none. A protein hydrolysate and arginine isolated from it did not contain any isotope. The analytical values are recorded in Table II.

Experiment III—Two adult pigeons with a combined weight of 872 gm. were fed 44 mg. of creatine hydrate per kilo per day for 3 days. The creatine was prepared from isotopic sarcosine and normal cyanamide and contained 3.34 atom per cent N₁⁵ excess. The combined purines, creatine, and uric acid were isolated. The results are tabulated in Table II.
**Guanidoacetic Acid Feeding**—Two adult pigeons with a combined weight of 710 gm. were fed 47 mg. of guanidoacetic acid (3.34 atom per cent N\textsuperscript{15} excess) per kilo per day for 3 days. The combined purines, uric acid, creatinine, and nucleic acids were isolated. The results are recorded in Table II.

**Uracil Feeding**—Eight adult male rats with a combined weight of 265 gm. were fed 30 mg. of isotopic uracil (5.48 atom per cent N\textsuperscript{16} excess) per rat per day for 3 days. The results of this experiment are given in Table III.

**Thymine Feeding**—Four adult male rats with a combined weight of 1200 gm. were fed 33 mg. of isotopic thymine (4.88 atom per cent N\textsuperscript{15} excess) per rat per day for 3 days. The results are given in Table III.

**DISCUSSION**

In investigations carried out in this laboratory over a period of 6 years, it has been found that the addition to the diet of substances which are normal tissue constituents leads to their deposition as an inseparable mixture of the preformed and dietary material. In contrast to this, the nitrogenous bases of nucleic acids, the purines and pyrimidines, do not enter the structure of the nucleus but are immediately metabolized to their respective end-products. This exception to the rule is difficult to explain. Since these bases are metabolized in a normal fashion, it must be assumed that they enter the cell specific for this transformation and that the metabolic changes take place in the cytoplasm. This may be due either to a certain specificity of the nuclear membrane or the inability of the nucleus to utilize preformed dietary purines and pyrimidines for the synthesis of nucleic acids and nucleoproteins. In view of the fact that there is considerable evidence against the concept of two independent types of catabolisms, the specificity of the nuclear membrane is the more plausible explanation.

The finding (Table II) that creatine and guanidoacetic acid are ineffective as purine precursors and are not metabolized to uric acid illustrates the biological inertia of creatine and its precursors in birds. In the pigeon, as in the rat, the conversion of guanidoacetic acid into creatine appears to be unidirectional.

In contrast, the results of the pyrimidine experiments do not lead to so clear a conclusion. The pyrimidines themselves, although almost quantitatively absorbed when administered in small amounts, are not deposited in the nucleus but are immediately metabolized to urea and ammonia. It is entirely conceivable that the truly nuclear pyrimidines are catabolized in the same way but it is quite possible that they represent intermediates in the biological synthesis of purines in the nucleus if the starting materials are relatively simple substances, such as ammonia and small organic molecules.
The biological changes of purines and pyrimidines here reported represent a "cytoplasmic" catabolism and throw no light upon the "nuclear" synthesis of purines.

The findings here reported, like those of Barnes and Schoenheimer (1) indicate that all amino acids except lysine (22) participate in the synthesis of purines, and that neither histidine nor arginine makes any outstanding contribution to it. The hypothetical conversion may be summarized by the accompanying diagram.

**Diagram:**

```
Diet
Purines --- Purines (allantoin)
Pyrimidines
Amino acids
Proteins ---> guanidoacetic acid ---> creatine ---> creatinine
Nucleoproteins
```

**Summary**

1. Isotopic guanine was prepared which contained isotopic nitrogen in nitrogen atoms 1 and 3 of the ring, as well as in the free amino group at position 2.
2. No connection between the creatine cycle and purine and pyrimidine metabolism could be detected.
3. The conversion of uracil and thymine to urea and ammonia, observed by Cerecedo and coworkers, was confirmed.
4. Evidence was presented that pyrimidines are completely metabolized when administered in small amounts.
5. Guanidoacetic acid was shown to be an effective creatine precursor in birds. It does not act as a purine or pyrimidine precursor.
6. Neither purines nor pyrimidines supplied in the diet are utilized by the body for the synthesis of nucleoproteins. The belief is expressed that they are either synthesized within the nucleus from smaller molecules or they are utilized for such a synthesis if they are supplied as nucleosides, nucleotides, or nucleic acids.

The authors are indebted to Dr. S. Ratner and Dr. D. Rittenberg for their unfailing cooperation.

**Bibliography**

2. Cerecedo, L. R., *J. Biol. Chem.*, **75**, 661 (1927); **88**, 695 (1930); **93**, 283 (1931).
9. Traube, W., German patent 134,984 (1903).
STUDIES IN THE METABOLISM OF PURINES AND PYRIMIDINES BY MEANS OF ISOTOPIC NITROGEN
Albert A. Plentl and Rudolf Schoenheimer


Access the most updated version of this article at http://www.jbc.org/content/153/1/203.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/153/1/203.citation.full.html#ref-list-1