THE CONVERSION OF $1,3-N^{15},2-C^{14}$-ADENINE 
TO 
POLYNUCLEOTIDE ADENINE AND GUANINE 
IN THE ADULT MALE RAT

by
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the requirements for the degree of 
Master of Science 
in the Department 
of 
Biochemistry.

We accept this thesis as conforming to the 
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Date **Sept 28, 56**
ABSTRACT.

1,3-\textsuperscript{15}N,2-\textsuperscript{14}C-adenine was synthesized and administered to rats by intraperitoneal injection. The compound was incorporated as both adenine and guanine into the visceral nucleic acids. Comparison of the \textsuperscript{14}C/\textsuperscript{15}N ratios found in the adenine and guanine isolated from the visceral nucleic acids with that in the injected adenine indicated that for both compounds there had been a loss of \textsuperscript{14}C. The decrease in the \textsuperscript{14}C/\textsuperscript{15}N ratio of the polynucleotide guanine was much greater than that in the polynucleotide adenine. The inference drawn from this marked difference was that on at least one pathway for the conversion of adenine to guanine the purine nucleus does not remain intact. The labile 2-\textsuperscript{14}C was found as \textsuperscript{14}CO\textsubscript{2} in the animals' expired air. Evidence that part of the \textsuperscript{14}CO\textsubscript{2} resulted from purine interconversion was afforded by examining the allantoin excreted in the animals' urine. This compound had a \textsuperscript{14}C/\textsuperscript{15}N ratio consistent with the source from which it was derived, the nucleic acid purines. The deficit of \textsuperscript{14}C, apparent from the \textsuperscript{14}C/\textsuperscript{15}N ratio, in this excretory product accounted for 47\% of the \textsuperscript{14}CO\textsubscript{2} expired by the animals.
ACKNOWLEDGEMENTS

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INTRODUCTION

The question of how purine bases are interconverted is a problem that has occupied investigators for the past ten years. Its origin is to be found in the work of Schoenheimer (1). When isotopes became readily available in the early war years Schoenheimer, Buchanan, Hevesy and others began experimentation on the biosynthesis of nucleic acids and their component purine and pyrimidine bases. These investigations have clarified many of the questions concerning possible precursor molecules for the various atoms in the purine nucleus during the course of 'de novo' synthesis within the body.

In 1944, Schoenheimer and Plentl (3) published the results of investigations which had extended the work beyond the search for precursor compounds to the utilization of the free bases themselves. Although these investigators were aware of Miescher's early observation that preformed purines or pyrimidines were not essential for the synthesis of nucleic acids (2), it seemed worthwhile to them to determine whether the free bases would be utilized for the synthesis of nucleic acids when fed to an animal. Their experiments included the feeding of 1,3-N^15-, 2 amino-N^15-guanine as well as the labelled pyrimidines, thymine and uracil, to rats. On the basis of the results obtained from these experiments, Schoenheimer and Plentl stated: "Neither purines nor pyrimidines supplied in the diet are utilized by the body for the synthesis of nucleoprotein" (3).
Because this appeared to be an exception to the dynamic concept of metabolism, Brown and his co-workers (4), in 1947, reinvestigated the possible utilization of free bases fed to an animal. While they were checking the results obtained when rats were fed labelled guanine they decided to investigate the results of feeding labelled adenine. It was felt that adenine, since it occupied a more central position in an animal's metabolism, might show results different from those of guanine. Therefore, $1,3-N^{15}$-adenine was synthesized and included in one of the diets fed to the rats. The results of this experiment were a startling contrast to those obtained when guanine was fed. It was found that the free base adenine was incorporated into the mixed nucleic acids of the viscera and also that it had been converted to guanine, which had likewise been incorporated into the mixed visceral nucleic acids. Approximately thirteen percent of the adenine and eight percent of the guanine extracted from the mixed visceral nucleic acids had been derived from the $1,3-N^{15}$-adenine which had been fed. Figures of this magnitude left no possibility for the postulation of experimental error. The data of Brown and his co-workers led to two conclusions: first; that the free purine bases could be used by an animal for the synthesis of tissue nucleic acids, second; that they were interconvertible (4).

The experiments mentioned had all been carried out using compounds synthesized from the stable isotope of nitrogen, $N^{15}$. When stable isotopes are used to label compounds the
difficulty of excessive dilution must be avoided. The degree of dilution encountered in these biological experiments resulted in trace incorporation of the labelled bases into the nucleic acids being overlooked. When C\textsubscript{14}, a radioactive isotope, was employed to label the test compound as was done by Balis et al. (5), using 8 labelled guanine, or by Abrams (6), using 2 labelled guanine, it was easily demonstrated that free guanine when fed to an animal was metabolised in a manner similar to free adenine. The degree of incorporation of guanine into the nucleic acids was so slight that it had been masked in the earlier experiments. This masking had occurred as a result of the liberation of the labelled 2-amino group into the general amino group pool on the catabolism of 1,3-\textsubscript{N\textsuperscript{15}},2 amino-\textsubscript{N\textsuperscript{15}}-guanine to allantoin. The free \textsuperscript{15}N, i.e. the \textsuperscript{15}N\textsubscript{2} of the free amino pool, labelled all the purines to an extent sufficient to conceal the slight incorporation of guanine into the nucleic acids (2). It was also discovered, with the aid of C\textsubscript{14} labelled guanine, that when guanine was fed as the free base, in addition to being incorporated into the nucleic acids, a small portion of it was converted to adenine (6).

The experimental evidence from the previously mentioned investigations reinforced the concept that at some point in their anabolic pathways all purines arose from a common intermediate. This theory had been tested by feeding various common purine bases in their free state to see if one could be found which would be converted to either adenine or
guanine in the course of its metabolism. Iso- 
guanine was 
investigated by Bendich and his colleagues (7), hypoxanthine 
and xanthine by Getler et al. (8), and uric acid by Brown and 
his group (9). None of these compounds were converted to 
tissue nucleic acid adenine or guanine. 2,6-diamino purine, 
tested by Bendich et al. (10), was found to contribute 
appreciably to polynucleotide guanine. As much as four per­
cent of the guanine extracted from the nucleic acids had come 
from this compound when it was included in an animal's diet. 
There was, however, no conversion of the compound into adenine 
by the rat.

In 1950 Brown and his co-workers (11) published the 
following metabolic map showing the various substantiated 
metabolic conversions which might conceivably be related to 
the interconversion of adenine and guanine:
On this map three types of arrows are used to designate one of the three different relationships an individual reaction might have to the pathway which free adenine follows during its conversion to nucleic acid guanine. The conventional arrows denote reactions which had been demonstrated and which presumably could lead to polynucleotide guanine. The conventional arrows which have been crossed out denote reactions which would lead to nucleic acid guanine but which had been demonstrated as not occurring within the body. The dotted arrows show possible relationships, proven and unproven, amongst the various purine bases which had been tested as precursors of nucleic acid guanine.

Brown and his co-workers, when analysing this metabolic map, argue that since neither free hypoxanthine nor free xanthine function as a precursor of nucleic acid purines in the rat (8), a preliminary hydrolysis at the 6-amino group of adenine is excluded as the initial step in its conversion to polynucleotide guanine. Whether the recently demonstrated interconvertibility of adenylic acid, inosinic acid, xanthosine monophosphate and guanylic acid will force a reconsideration of this route for the production of nucleic acid guanine from free adenine remains to be seen (12) (13). Although isoguanine occurs in nature, it is not utilized for nucleic acid purine synthesis when it is fed in the free state (7). This fact, Brown and his co-workers feel, renders an initial oxidation at the 2-position of adenine also improbable as the starting point on its conversion to polynucleotide guanine. A direct reaction between adenine and ammonia is unlikely on a chemical
basis (10) and even more so in view of Reichard's (14) evidence that the 2-amino group of guanine is derived for the most part from glycine. Brown and his colleagues conclude that the most probable initial step is a reaction between adenine and glycine.

Brown et al. (11) make no mention of the possible role that an incomplete purine might play as an intermediate in this interconversion of adenine and guanine. This fact is in agreement with the view they held on the essential integrity of the purine skeleton during the interconversion. Work of Buchanan and his group (15) or that of Greenberg and his group (16) on 'de novo' synthesis of ribotides from incomplete purine ring compounds would make one suspect that a reaction between some carboxamide ribotide and glycine is a plausible intermediate reaction. This suspicion would require that the initial step in the interconversion be a rupture of the purine skeleton.

In 1954 Roll and Brown (2) published another metabolic plan. This map took cognizance of new experimental evidence which had accumulated on possible pathways for the interconversion of purines.
It has been inferred from experimental evidence that even in the course of 'de novo' synthesis at least part of one purine may arise via the other (2). This fact is difficult to rationalize if the only path for interconversion presented is that through a common intermediate, consequently, an alternative direct interconversion is also offered. As yet there is no irrefutable evidence indicating at which stage, i.e. free base, riboside or ribotide, the interconversion of adenine and guanine takes place. Current work on the biosynthesis of purines favor the probability of the interconversion taking place as the ribotides. There is, however, other evidence indicating that the interconversion may take place as the free bases or as the ribosides (17). Considering recent work of Ochoa et al. (18), it would appear that the nature of active adenine or guanine in the case of Pentose Nucleic Acid synthesis is that of a diphosphate riboside.

The problem under investigation in this thesis involves the possible lability of the 2-C- atom of adenine on its conversion to polynucleotide guanine. While this phenomenon is in all probability due to a complex series of reactions, there are two possible overall processes:

(1) \[
\begin{array}{c}
\text{HN} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\end{array}
\rightarrow
\begin{array}{c}
\text{HN} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\end{array}
\]

(2) \[
\begin{array}{c}
\text{HN} \\
\text{C} \\
\text{CO}_{2} \\
\text{HN} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\end{array}
\rightarrow
\begin{array}{c}
\text{HN} \\
\text{C} \\
\text{NH} \\
\text{HN} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\end{array}
\rightarrow
\begin{array}{c}
\text{HN} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \\
\end{array}
\]
(1). This involves no disruption of the basic purine structure and a direct amination at the 2 position of adenine. This, as stated earlier, Brown and his colleagues (10) consider to be unlikely.

(2). This involves a rupture of the purine nucleus at the 2 position with subsequent amination and reclosure of the ring. This second route permits the possibility of exchange or oxidation reactions taking place at the 2 position.

In 1953 Paterson and Zbarsky (19) synthesized 2-\textsuperscript{14}C-adenine. This compound allowed a direct investigation of the fate of the 2-C-atom of adenine during the course of its metabolism and catabolism. There was no previous knowledge of this particular atom although it had been the subject of conjecture regarding its possible lability (41) (42). The results obtained by Paterson (20) on feeding 2-\textsuperscript{14}C-adenine to rats would indicate that this particular atom does possess a greater lability than the other C atoms of adenine. However, no direct correlation was possible between this evidence for lability and the interconversion of purine bases. It was felt, by the author, that a metabolic study involving 1,3-N\textsuperscript{15}, 2-\textsuperscript{14}C-adenine might yield the desired information. It would be possible to correlate the relative percentage incorporation of free adenine into polynucleotide guanine in terms of both C\textsuperscript{14} and N\textsuperscript{15} on feeding such a compound. If the two percentages are identical this would furnish direct proof of the integrity of the purine skeleton during the interconversion process. If the two percentages vary to the extent that there is virtually no incorporation in terms of C\textsuperscript{14}, a rupture of the
purine skeleton and subsequent exchange reaction would be indicated. Finally, if the two percentages vary such that the incorporation expressed as $^{14}$C is significantly lower than the incorporation expressed as $^{15}$N the interpretation could be that several routes exist, at least one of them requiring a rupture of the purine nucleus.

The experiments presented in this thesis were undertaken to resolve the problem of whether the purine nucleus remained intact on the conversion of exogenous adenine to polynucleotide guanine. This was accomplished by comparing the $^{15}$N and $^{14}$C incorporation values to discover which of the three above mentioned possibilities was to be found on injecting $1,3-{^{15}}N^{15},2-{^{14}}C^{14}$-adenine into rats. The results indicated that both in the case of the incorporation of exogenous adenine as adenine and in the case of its conversion to guanine and incorporation as that compound the percentage incorporation into the nucleic acids was less when measured as a function of the $^{14}$C content of the injected $1,3-{^{15}}N^{15},2-{^{14}}C^{14}$-adenine than when measured as a function of its $^{15}$N content. Otherwise stated, the injected $1,3-{^{15}}N^{15},2-{^{14}}C^{14}$-adenine appears to lose $^{14}$C both on its incorporation into the visceral nucleic acids as adenine and also on its conversion and incorporation as guanine. The degree of this loss is such as to indicate that the third possibility is the probable one, i.e. more than one route exists for the conversion of adenine to guanine. The tacit assumption arising from this choice out of the three possibilities is that on at least one route the purine nucleus is not maintained intact during the interconversion.
EXPERIMENTAL

A. Chemical

Synthesis

The proposed synthesis of doubly labelled adenine involved in practice the synthesis of two separate compounds. First; \(2-\text{C}^{14}\)-adenine was synthesized by the method of Paterson and Zbarsky (19) adapted from Shaw (21), second; \(1,3-\text{N}^{15}\)-adenine was produced by the method of Bendich et al. (22) modified from that of Todd and his co-workers (23).

It was essential that the \(2-\text{C}^{14}\)-adenine be of high specific activity otherwise on mixing the two compounds, \(2-\text{C}^{14}\)-adenine and \(1,3-\text{N}^{15}\)-adenine, the atom \% excess \text{N}^{15} would have been excessively diluted. Furthermore, the higher the \(2-\text{C}^{14}\)-adenine was in specific activity, the easier it would be to trace the byproducts by means of their \text{C}^{14} content upon the administration of a minimum amount of the test compound.

1) \(2-\text{C}^{14}\)-adenine

The preliminary step in the synthesis of \(2-\text{C}^{14}\)-adenine was the synthesis of \(\text{C}^{14}\)-formate. This was achieved by a combination of the method of Jeanes (32) and that of Abrams and his co-workers (24). \(\text{Na}_{2}\text{C}^{14}\text{O}_{3}\) was converted to \(\text{BaC}^{14}\text{O}_{3}\). The \(\text{BaC}^{14}\text{O}_{3}\) was reduced to \(\text{Ba(\text{C}^{14};\text{N})}_{2}\) by heating in a furnace in the presence of Na-Zn amalgam while at the same time dry ammonia gas was passed over the hot melt. The \(\text{Ba(\text{C}^{14};\text{N})}_{2}\) was converted to \(\text{C}^{14}\)-formic acid by means of acid hydrolysis. The \(\text{C}^{14}\)-formate was condensed with 4-amino, 5-imidazole carboximidine hydrochloride by the method of
Paterson and Zbarsky (19) to give \(2-C^{14}\)-adenine.

\[
\begin{align*}
\text{(1)} \quad & \quad \text{Na}_2C^{14}O_3 \quad \rightarrow \quad \text{Ba}_2C^{14}O_3 \quad \rightarrow \quad \text{Ba}(\text{C}^{14}N) \quad \rightarrow \quad \text{HCl}^{14}\text{OOH} \\
\text{(2)} \quad & \quad \text{HCl}^{14}\text{OOH} \quad + \quad \text{HCN} \quad \rightarrow \quad \text{HCN}
\end{align*}
\]

ii) \(1,3-N^{15}\)-adenine

\(1,3-N^{15}\)-adenine was synthesized by the method of Bendich et al. (22). \(N^{15}\)-Formamidine hydrochloride was prepared and condensed with phenyl-azomalononitrile, the product, 4,6-diamino-5-phenyl-azopyrimidine, was reduced to give 4,5,6-triamino-pyrimidine. Formylation, followed by ring closure of this compound yielded \(1,3-N^{15}\)-adenine.

\[
\begin{align*}
\text{(3)} \quad & \quad \text{HC}^{15}\text{NCHO} \quad + \quad \text{H}^{15}\text{CN} \quad \rightarrow \quad \text{HC}^{15}\text{NCHO} \quad \rightarrow \quad \text{HC}^{15}\text{NCHO} \quad \rightarrow \quad \text{HC}^{15}\text{NCHO}
\end{align*}
\]

iii) \(1,3-N^{15},2-C^{14}\)-adenine

The two compounds, \(2-C^{14}\)-adenine and \(1,3-N^{15}\)-adenine were dissolved with a calculated equivalent of HCl. This solution could then be looked upon as consisting of \(1,3-N^{15},2-C^{14}\)-adenine hydrochloride, a doubly labelled molecule.

**Proof of Identity, Criteria of Purity**

i) Chromatography

A comparison was made between the \(R_f\) values of the synthetic product and a sample of authentic adenine which had both been run simultaneously on a paper chromatogram employing
a solvent of isopropanol, HCl, H₂O as described by Wyatt (31). The Rₐ value quoted for adenine in this solvent is 0.32 (31). Figure 1 shows a contact print of the chromatogram as developed under ultraviolet light (34). The synthetic product shows the same Rₐ value as the authentic adenine. The appearance of but a single spot is evidence of the essentially uncontaminated nature of the synthetic product.

ii) Spectrophotometry

The purines, pyrimidines and their derivatives have very well defined absorption maxima and minima in the ultraviolet region. The method of establishing the wavelength at which the maximum absorption occurs, the wavelength at which the minimum absorption occurs and the ratio of the optical densities obtained at any two arbitrary wavelengths is a valuable aid in characterizing these compounds. When this method of analysis was applied to the synthesized product it was observed that the point of maximum absorption was recorded at 262.5 mÅ and the point of minimum absorption at 229.0 mÅ. These data agree with the values recorded for adenine (43). Further, the ratio of the optical densities recorded at 260 mÅ and 280 mÅ, i.e. \( \frac{A_{280}}{A_{260}} \) was 0.375. This value is also in agreement with the value quoted for adenine. Figure 2 shows the absorption curves from which these values were calculated.

iii) Derivative and its melting point

The picrate salt of adenine was prepared as a derivative. It was recrystallized three times from 25% acetic acid, washed with distilled water, and its melting point
Contact print of a Chromatogram showing a comparison of the $R_f$ values of the synthesized adenine (right) and an authentic sample (left).

The homogeneous nature of the spot verifies the absence of any significant amount of contamination of the synthetic product.
A comparison of the U.V. absorption curves of authentic adenine and the synthesized product. These readings were taken with a Beckman quartz spectrophotometer Model DK 2.
determined. This value is recorded as 285-286°C (with decomposition) (33). The melting point range obtained was 283-287°C (with decomposition).

Measurement of Isotope Concentration

i) $^{14}C$ determination

Measurement of the specific activity of the synthesized adenine was accomplished by the following procedure. An aliquot was treated by the wet oxidation technique of Van Slyke and Folch (28). The CO$_2$ evolved was trapped in carbonate-free alkali and precipitated with Ba(Cl)$_2$. The radioactivity of the thick sample of BaCO$_3$ was determined by counting in a gas flow windowless counter. Both the technique and the counter employed in this laboratory have been described by Paterson (20) and Wright (26). The specific activity of the synthetic adenine was found to be $1.66 \times 10^4$ c.p.m./mg. The specific activity expressed as c.p.m./mM. of adenine was $2.24 \times 10^6$.

The synthesized adenine was shown to be free of any significant amount of contaminating radioactive material by means of an autoradiogram. This was done using the method of Zbarsky and Wright (44). A developed paper chromatogram was placed in contact with X-ray film in a dark room for one week. When the film was developed radioactivity appeared as a single spot, indicating only one radioactive component was present. The $R_f$ value of 0.32 corresponded to the quoted $R_f$ value of adenine (31).
ii) $N^{15}$ determination

The atom % excess $N^{15}$ of the synthetic product was estimated on samples of $N_2$ prepared by the method of Rittenberg (29). An aliquot of the compound was converted to $(NH_4)_2SO_4$ by the Kjeldahl procedure. This $(NH_4)_2SO_4$ was then treated with NaOBr in vacuo yielding $N_2$ which was trapped and stored in breakseal containers. The $N_2$ was analysed on a mass spectrometer for the atom % excess $N^{15}$ present. The concentration of $N^{15}$ in the synthetic adenine as determined by this method was 2.997 atom % excess.

B. Metabolic

Technique of Metabolism Experiments

The $1,3-N^{15}$,$2-C^{14}$-adenine was dissolved in a phosphate buffer (pH 3.8). This solution was administered at twelve hour intervals by means of intraperitoneal injections. While an experiment was in progress the rats were kept in a metabolism cage similar to that described by Wright (26) and Paterson (20). The arrangement of this metabolism cage is such as to permit separate collection of either or both, expired CO$_2$ and, urine and faeces, for any given time period, with a minimum of contamination.

In a preliminary experiment three rats were used. They were injected with adenine at a level of 100 mg./kilo. body weight/day. This experiment was terminated at the end of the first twenty-four hours by technical difficulties.

In a second experiment two rats were used. They were injected with adenine at a level of 50 mg./kilo. body
weight/day for a period of ninety-six hours. The samples collected for analysis from these two experiments are tabulated below.

**TABLE I**
Number and Nature of the Samples Saved in Experiments I and II

<table>
<thead>
<tr>
<th>Nature of Sample</th>
<th>Number of Samples</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experiment I</td>
<td>Experiment II</td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Viscera</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>12 hour urine specimen</td>
<td>1</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>24 hour faeces specimen</td>
<td>0</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>12 hour expired air specimen</td>
<td>1</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

**Treatment of Samples**

At the termination of an experiment each rat was killed by a blow on its head. It was then decapitated and bled. The viscera were immediately removed and frozen in a dry ice-alcohol mixture. They were then homogenized with 10 cc. of cold water in a Waring Blender. This was followed by three successive extractions with 100 cc. portions of cold 5% trichloroacetic acid. After each extraction the tissue was centrifuged at 0°C. and the supernatant was decanted. It was presumed that this 300 cc. of cold yellowish trichloroacetic acid extract contained all the free nucleotides as well as other acid-soluble factors.

i) Tissue

The extracted tissue was treated with 95% ethanol,
100% ethanol, 100% ethanol and ether and finally with ether alone. This treatment removed all of the residual water and fatty material. After this treatment the tissue was air dried. The dry tissue was next extracted for eight hours at 80°C with 150 cc. of 10% NaCl, this treatment was repeated twice, then the filtrates were combined and the tissue discarded. Two and one-half volumes of 95% ethanol were added to the combined filtrate and the solution was allowed to stand overnight. A fine white precipitate of crude sodium nucleate settled out during this period. This precipitate was recovered by centrifugation and, after washing, was immediately resuspended in a small volume of hot 10% NaCl. It was once again reprecipitated by the addition of alcohol, washed, and this time dried.

The pure sodium nucleate was then hydrolysed for one hour in 5 cc. of 1 N. HCl at 100°C. The resultant slightly brownish hydrolysate was adsorbed on Dowex-50 ion exchange resin and elution was carried out with 1 N. HCl using an automatic fraction collector. This eluate was divided into three main fractions on the basis of optical density readings taken at 260 m/ with a model DU Beckman Spectrophotometer. These fractions were reduced to 10 cc. volume each. At this stage paper chromatograms and absorption spectra were done to verify the suspected identity of the components of each fraction. The three fractions were shown to consist of adenine, guanine and a mixture of pyrimidines. The ultraviolet absorption measurements permitted calculation of the concentration of each component from its molar extinction
coefficient. Carbon oxidations were carried out on aliquots of these fractions to obtain the total and specific activities with respect to C\textsuperscript{14}. Further aliquots were treated by the method of Rittenberg (29) to determine N\textsuperscript{15} values in terms of atom % excess N\textsuperscript{15}.

ii) Acid-soluble fraction

The 300 cc. of acid-soluble extract was successively extracted with ether until pH determinations showed the major portion of the trichloroacetic acid to have been removed. The solution was still yellow at this stage. It was now reduced to a volume of 10 cc. and 1 cc. of this was removed for paper chromatography and autoradiography. The chromatography proved to be unsuccessful. If sufficient solution was applied to the chromatogram to detect the radioactivity, separation of the components became impossible; on the other hand with less solution per chromatogram separation was possible, but the radioactivity could not be detected.

The remaining 9 cc. of extract was hydrolysed for one hour in 1 N. HCl at 100\textdegree C. then adsorbed on Dowex-50 ion exchange resin and eluted with 1 N. HCl. This eluate was divided into three main fractions. One tube was removed from each of these fractions concentrated to a volume of approximately \(\frac{1}{2}\)cc. and applied to a paper chromatogram. R\textsubscript{f} values revealed that these three fractions contained respectively, adenine, guanine and a mixture consisting of pyrimidines and hypoxanthine. The radioactive compounds
were located by scanning the chromatograms with an automatic windowless gas flow chromatogram counter. Those compounds showing $^{14}C$ activity by this method were then punched out of the chromatogram and counts were recorded for the paper discs with the conventional counter. Following determination of their activity, these paper discs were extracted for eight hours with 0.1 N HCl. Absorption spectra were then recorded for these extracts to verify the identity and to establish the concentration of the component present. These findings checked with the identities established by means of $R_f$ values on the chromatograms. With these data it was possible to calculate specific and total activities for $^{14}C$ but no attempt was made to correlate the results with counts obtained by the carbon oxidation technique.

The remainder of each fraction, after the removal of the single tube, was concentrated to 10 cc. volume. Absorption spectra were taken from which the concentration of the component in each fraction was established. Aliquots were then oxidized to determine the radioactivity present. In the first experiment only the total activity present was estimated. This value then provided a guide for the treatment to be accorded the acid-soluble fraction of the second experiment. In the case of the second experiment both the total radioactivity and the specific activity of each fraction were determined.

iii) Carbon dioxide

The twelve hour samples of expired $CO_2$ had been
collected in 10% NaOH. After making the solution up to a specific volume, 1 liter, 2 cc. aliquots were removed and the \( \text{Na}_2\text{CO}_3 \) was precipitated from these aliquots as \( \text{BaCO}_3 \). The radioactivity of these thick samples of \( \text{BaCO}_3 \) was determined in the manner described by Paterson (20).

iv) Urine

Each twelve hour sample of urine was made up to a volume of 50 cc. with distilled water and 3 cc. aliquots were withdrawn for determination of the total \( ^{14}\text{C} \) by the conventional carbon oxidation technique (28). Further 5 cc. aliquots of urine were treated with urease in the manner described by Wright (26) for the determination of the radioactivity present in the urea. When this procedure was complete the digested samples were made alkaline after the method of Rittenberg (29) and the evolved \( \text{NH}_3 \) was trapped and converted to \( \text{N}_2 \) for \( ^{15}\text{N} \) determination. Allantoin was measured on each twelve hour sample of urine by the method of Larson (27). Crystalline allantoin was isolated from an aliquot of pooled urine according to the method of Brown and his colleagues (4). Aliquot samples of pooled urine were used for this isolation because it was desired that the \( ^{14}\text{C} \) and \( ^{15}\text{N} \) values from the analysis of the compound be representative of the excretion over the ninety-six hour period. Realizing that the probability existed that a considerable portion of the injected adenine might be excreted unaltered in the urine, analysis to determine if this was so was carried out by means of carrier technique.
pooled samples of urine. The carrier adenine was re-
isolated as the picrate salt. This picrate salt was oxi-
dized and its radioactivity was determined. The paper
chromatograms which had been developed with the urine were
treated in a variety of ways to locate and identify the
sources of $^{14}C$ activity present. Amongst the treatments
 accorded these chromatograms were photography under ultra-
violet irradiation (34), autoradiography (44), and spraying
with para-dimethylaminobenzaldehyde (30), diazotized sulfa-
nilic acid (30) and ammoniacal silver nitrate (30).

v) Faeces

The faeces, after dehydration, were treated by
the conventional oxidation technique and the radioactivity
present was measured as thick sample counts on the BaCO$_3$
produced (28).

vi) Blood

The blood samples were treated by homogenizing
clotted specimens and withdrawing aliquots for oxidation
and subsequent $^{14}C$ determination (28).

C. Experiment I

In experiment I, three male rats of the Wistar
strain, having a combined weight of 515 gms., were injected
intraperitoneally with the buffered adenine solution. The
individual dose per rat was 0.91 cc. which contained 8.62
mgs. of adenine. This dosage was administered twice daily,
thus the rats were being injected at a level of 100 mg./Kilo./day. Since the specific activity of the administered adenine was $1.66 \times 10^4$ c.p.m./mg., the rats received a total dosage of $8.77 \times 10^5$ c.p.m. During the course of the experiment the rats were allowed free access to food and water. This experiment was terminated at the end of twenty-four hours.

Results

The results of the analysis of the tissue nucleic acid purines and pyrimidine mixture from this experiment are presented in Table II.

| TABLE II |
|-----------------|-----------------|-----------------|-----------------|
| **Compound**    | **Radioactivity Found** | **15N15**     |
|                 | **Total c.p.m.**  | **Specific activity c.p.m./mg. Cpd.** | **Atom % excess** |
| Adenine         | 17,200           | 745             | 0.163           |
| Guanine         | 8,030            | 175             | 0.106           |
| Pyrimidine mixture (from ion exchange) | 1,750 | - | - |

The significance of these values is elaborated upon in the discussion. There, for the purpose of clarity, they are converted to values representing percentage incorporation into the visceral nucleic acids and $^{14}C/N^{15}$.
ratios in the individual purine bases.

The specific activities of the three purines separated from the acid-soluble fraction were adenine=1, hypoxanthine=0.97, and guanine=0.29. These values are expressed as relative specific activities because they were derived from counts obtained by a technique of counting which was not correlated with the standard BaCO₃ method of counting.

Examination of the expired air for the first twelve hours of this experiment showed that a total of 60,000 c.p.m. were present. This represents 14% of the activity injected during this period.

The results of examining the urine showed that 49.5% of the total injected activity was eliminated from the animals by this route. The only further subdivision made of the total C¹⁴ activity present in the urine was that present as urea. Urea was shown to contribute only 1% of the total of 49.5%. The N¹⁵ content of the urea was also shown to be negligible, only 0.009 N¹⁵ atom % excess.

Examination of the blood showed a specific activity of 320 c.p.m./cc.

D. Experiment II

In experiment II, two male rats of the Wistar strain having a combined weight of 462 gms. were injected intraperitoneally with the test solution. The individual dose per rat was 0.62 cc. which contained 5.80 mgms. of adenine. This dosage was administered twice daily, thus the rats were being injected at a level of 50 mg./kilo./day.
The specific activity of the administered adenine being $1.66 \times 10^4$ c.p.m./mg. adenine, the two rats received a total of $1.57 \times 10^6$ c.p.m. During the course of the experiment the rats received their normal rations and had free access to water. This experiment was carried on for a total of ninety-six hours.

**Results**

The values obtained from the $^{14}C$ and $^{15}N$ analysis of the purine and pyrimidine bases recovered from the nucleic acids are presented in Table III

**TABLE III**

$^{14}C$ and $^{15}N$ Values of the Adenine, Guanine and Mixed Pyrimidines of the Visceral Nucleic Acids after Intraperitoneal Injection of $1,3-^{15}N$, $2-^{14}C$-adenine (92.8 mgm., $1.57 \times 10^6$ c.p.m.)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Radioactivity Found</th>
<th>$^{15}N$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total c.p.m.</td>
<td>Specific activity c.p.m./mg. Cpd.</td>
</tr>
<tr>
<td>Adenine</td>
<td>12,800</td>
<td>1,575</td>
</tr>
<tr>
<td>Guanine</td>
<td>10,300</td>
<td>398</td>
</tr>
<tr>
<td>Pyrimidine mixture</td>
<td>2,000</td>
<td>-</td>
</tr>
<tr>
<td>(from ion exchange)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

These values are combined with those obtained in experiment I and are recorded again in a more significant form in Table VII.

In this experiment it was possible to calculate both the relative and the absolute specific activities for the three purines recovered from the acid-soluble fraction.
The difference between this experiment and the first being that the counts on these bases were obtained from both paper disc and thick sample BaCO₃ counts. It was explained earlier that the paper disc counts bear no direct relationship to any of the other counts recorded in the data, all of those counts having been done on BaCO₃ precipitates. The relative specific activities obtained were adenine=1, hypoxanthine=0.92 and guanine=0.24. The absolute specific activities were for adenine 4040 c.p.m./mg. cpd. and for guanine 931 c.p.m./mg. cpd. There is no absolute specific activity recorded for hypoxanthine since it was present in a mixture of pyrimidines and was only separated by paper chromatography.

The results of examining the expired air are presented in Table IV.

**TABLE IV**

C¹⁴ expired by Rats after Receiving, by Intraperitoneal Injection, 92.8 mgm. of 1,3-N¹⁵, 2-C¹⁴-adenine (1.57 x 10⁸ c.p.m.)

<table>
<thead>
<tr>
<th>Period of Collection hr.</th>
<th>Radioactivity Found</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total c.p.m.</td>
</tr>
<tr>
<td>0 - 12</td>
<td>44,000</td>
</tr>
<tr>
<td>12 - 24</td>
<td>33,666</td>
</tr>
<tr>
<td>24 - 36</td>
<td>43,666</td>
</tr>
<tr>
<td>36 - 48</td>
<td>70,300</td>
</tr>
<tr>
<td>48 - 60</td>
<td>32,666</td>
</tr>
<tr>
<td>60 - 72</td>
<td>44,666</td>
</tr>
<tr>
<td>72 - 84</td>
<td>34,000</td>
</tr>
<tr>
<td>84 - 96</td>
<td>41,666</td>
</tr>
<tr>
<td>Total</td>
<td>344,630</td>
</tr>
</tbody>
</table>
The possible source of these 344,630 c.p.m. found in the expired air is discussed later. A relationship will be drawn in the discussion between the results in Table IV and those in Tables II and III.

The result of examining the urine for its total \( ^{14} \text{C} \) content is presented in Table V.

**TABLE V**

Excretion of \( ^{14} \text{C} \) in the Urine of the Rats after Intraperitoneal Injection of 92.8 mgm. of \( ^{15} \text{N} \cdot ^{14} \text{C} \)-adenine (1.57 \( \times 10^6 \) c.p.m.).

<table>
<thead>
<tr>
<th>Period of Collection hr.</th>
<th>Volume cc.</th>
<th>Radioactivity Excreted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total c.p.m.</td>
</tr>
<tr>
<td>0 - 12</td>
<td>20</td>
<td>69,400</td>
</tr>
<tr>
<td>12 - 24</td>
<td>16</td>
<td>67,100</td>
</tr>
<tr>
<td>24 - 36</td>
<td>18</td>
<td>109,900</td>
</tr>
<tr>
<td>36 - 48</td>
<td>28</td>
<td>115,700</td>
</tr>
<tr>
<td>48 - 60</td>
<td>18</td>
<td>129,100</td>
</tr>
<tr>
<td>60 - 72</td>
<td>25</td>
<td>131,600</td>
</tr>
<tr>
<td>72 - 84</td>
<td>18</td>
<td>116,700</td>
</tr>
<tr>
<td>84 - 96</td>
<td>20</td>
<td>93,700</td>
</tr>
<tr>
<td>Total</td>
<td>163</td>
<td>833,200</td>
</tr>
</tbody>
</table>

The total of 833,200 c.p.m. recorded represents 53% of the total injected activity. Both the total excretion and the specific activities rise slowly over the first three days then decline somewhat on the fourth day.

The results of urea analysis on the urine are presented in Table VI.
TABLE VI

Excretion of C\(^{14}\) and N\(^{15}\) in the Urea of the Rats' Urine after Intraperitoneal Injection of 92.8 mgm. of 1,3-N\(^{15}\)-,2-C\(^{14}\)-adenine (1.57 x 10\(^6\) c.p.m.)

<table>
<thead>
<tr>
<th>Period of Collection hr.</th>
<th>Radioactivity Excreted</th>
<th>N(^{15})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total c.p.m.</td>
<td>Specific Activity c.p.m./mgm. C</td>
</tr>
<tr>
<td>0 - 24</td>
<td>5,440</td>
<td>35.05</td>
</tr>
<tr>
<td>24 - 48</td>
<td>1,020</td>
<td>17.20</td>
</tr>
<tr>
<td>48 - 72</td>
<td>1,720</td>
<td>10.20</td>
</tr>
<tr>
<td>72 - 96</td>
<td>1,200</td>
<td>8.80</td>
</tr>
<tr>
<td>Total</td>
<td>9,480</td>
<td>-</td>
</tr>
</tbody>
</table>

With the exception of the first day the total counts excreted per day were of a similar order of magnitude. This table shows a steady decline in the specific activity of the urea. This decline in specific activity is comparable to that found for the expired C\(^{14}\)O\(_2\).

Estimation of the allantoin content of the urine revealed that there were 340.4 mgm. of this compound present in the total urine volume. These 340.4 mgm. contained a total of 258,200 c.p.m. The specific activity was calculated to be 758 c.p.m./mg. Cpd. The specific activity in terms of atom % excess N\(^{15}\) was 0.223. It will be discussed later that these figures represent a deficit of 159,800 c.p.m. if the allantoin is considered as arising solely from purines of the same isotope content as that of the injected 1,3-N\(^{15}\)-,2-C\(^{14}\)-adenine.
The addition of carrier adenine to an aliquot of the urine and its subsequent re-isolation led to the recovery of adenine with a specific activity of 55 c.p.m./mg. Cpd. If all the carrier adenine had been recovered from the aliquot, the total counts recorded for all the urine excreted would have been 100,625 c.p.m. This represents 6.02 mg. of the adenine injected, i.e. adenine of specific activity $1.66 \times 10^4$ c.p.m./mg. Cpd.

The paper chromatograms which had been developed on the urine, showed ultraviolet absorption over their entire length, consequently no conclusions could be drawn from this approach concerning purines which might or might not be present. Scanning for radioactivity by means of the automatic chromatogram counter indicated that the major portion of $^{14}C$ activity lay between the $R_f$ values 0.2 - 0.5. This result was verified by means of an autoradiogram. Spraying the chromatograms with para-dimethylaminobenzaldehyde, a reagent which reacts with urea and allantoin, indicated that these substances were both present on the chromatograms. The $R_f$ value determined for urea was 0.71, for allantoin 0.57. The allantoin tailed into the radioactive region of the chromatograms, $R_f$ 0.2 - 0.5. Spraying with diazotized sulphanilic acid, a reagent reacting with the purine bases, was unsuccessful because the reagent was coupling with other compounds to give a complete streaking of the chromatograms, thus no clue was afforded as to the presence or quantity of purines on the chromatograms, which might be contributing to
their radioactivity. Use of ammoniacal silver nitrate as a spray reagent gave negative or inappreciable reaction for the presence of uric acid.

The faeces were analysed only for their $^{14}\text{C}$ content. This value was reasonably constant over each 24 hour period being approximately 9000 c.p.m. The total $^{14}\text{C}$ activity excreted in the faeces over the four day period was 32,930 c.p.m. This value represents 2% of the total activity injected into the animals.

The blood when examined for its $^{14}\text{C}$ content had an activity of 1,280 c.p.m./cc.
DISCUSSION

When adenine is administered to a rat it becomes incorporated into the tissue nucleic acids, appearing both as adenine and as guanine. This fact has been demonstrated for $1,3-N^{15}$-labelled adenine by Brown and his colleagues (4), and for $2-C^{14}$-labelled adenine by Paterson (20). Two possibilities suggest themselves for this conversion of adenine to guanine, first; it is accomplished by a direct amination process with no disruption of the purine nucleus, second; the purine nucleus opens at the 2 position to give an incomplete ring compound which is converted to guanine by amination and subsequent ring closure.

The project undertaken in this thesis was to inject $1,3-N^{15},2-C^{14}$-adenine into rats and to examine the purines recovered from the visceral nucleic acids to determine if any change had occurred in the $C^{14}/N^{15}$ ratio from that of the injected compound. The only rational by which such a change in the isotope ratio could be explained is that during the incorporation of adenine as adenine or during its conversion to guanine and its subsequent incorporation into the visceral nucleic acids as that compound the purine nucleus must be broken at the 2 position. If this event occurred then $C^{14}$ would be liberated and should appear at some other point in the animal's metabolism. This line of reasoning would be substantiated by finding $C^{14}O_2$ in the expired air of the animal. To further relate a change in the isotope ratio of the nucleic acid purines and the
appearance of $^{14}O_2$ in the animal's expired air to one another, a deficit of $^{14}C$ should be observed in the excreted allantoin. The $^{14}C/^{15}N$ ratio in this urinary by-product of purine catabolism should reflect the fact that it is derived from both the adenine and the guanine of the nucleic acids. The data relating to the $^{14}C/^{15}N$ ratios are presented in Table VII.

**TABLE VII**

The Percentage of the Injected $1,3-{^{15}N},2-{^{14}C}$-adenine Incorporated into the Purines of the Visceral Nucleic Acids of the Rats expressed in terms of its $^{14}C$ and $^{15}N$ content.

<table>
<thead>
<tr>
<th>Compound</th>
<th>% Incorporation $^{14}C$</th>
<th>% Incorporation $^{15}N$</th>
<th>$^{14}C/^{15}N$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthetic Adenine</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nucleic Acid Adenine</td>
<td>4.48</td>
<td>5.49</td>
<td>0.826</td>
</tr>
<tr>
<td>Nucleic Acid Guanine</td>
<td>1.05</td>
<td>3.54</td>
<td>0.298</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nucleic Acid Adenine</td>
<td>9.50</td>
<td>11.22</td>
<td>0.852</td>
</tr>
<tr>
<td>Nucleic Acid Guanine</td>
<td>2.39</td>
<td>5.61</td>
<td>0.427</td>
</tr>
</tbody>
</table>

a = % incorporation $^{14}C = \frac{c.p.m./mg.Cpd.}{c.p.m./mg.Cpd. \text{ (injected adenine)}} \times 100$

b = % incorporation $^{15}N = \frac{\text{atom} \% \text{ excess } ^{15}N}{\text{atom} \% \text{ excess } ^{15}N \text{ (injected adenine)}} \times 100$

$a/b = \frac{c.p.m./mg.Cpd. \text{ (injected adenine)}}{\text{atom} \% \text{ excess } ^{15}N} / \frac{c.p.m./mg.Cpd. \text{ (injected adenine)}}{\text{atom} \% \text{ excess } ^{15}N}$
The $^{14}C/N^{15}$ ratio in the injected $L,3-N^{15}-,2-C^{14}$-adenine was established as unity and all other ratio estimations were referred to this value. In both experiments all the purines isolated from the nucleic acids showed a $^{14}C/N^{15}$ ratio less than 1. The adenine recovered from the visceral nucleic acids had, in both experiments, a value of approximately 0.83 for its $^{14}C/N^{15}$ ratio. In other words at least 17% of the injected adenine which was incorporated into the nucleic acids as adenine had passed through an intermediary stage which permitted the $2-C^{14}$-atom to be exchanged for a non-isotopic atom. The guanine recovered from the nucleic acids showed an average value of 0.36 for its $^{14}C/N^{15}$ ratio. This would indicate some 64% of the injected adenine which had been converted to guanine and then been incorporated into the visceral nucleic acids had failed to maintain its purine nucleus intact during the process. Although a critical comparison of data cannot be made between independent biological experiments, the incorporation values compare with those obtained by the two previously mentioned experimentors (4) (20). There is a similarity in order of magnitude for the incorporation as adenine but the conversion to and incorporation as guanine is less marked.

Mention was made earlier of the three possible values the $^{14}C/N^{15}$ ratio for guanine might take in an experiment such as this one.

1) No $^{14}C$ appears in the polynucleotide guanine, i.e. the $^{14}C/N^{15}$ ratio becomes 0.
2) The C\textsuperscript{14} content of the polynucleotide guanine is the same as that of the adenine, i.e. the C\textsuperscript{14}/N\textsuperscript{15} ratio remains at 1.

3) The C\textsuperscript{14} content of the guanine is lower than that of the adenine but still represents a significant fraction of the polynucleotide guanine isolated, i.e. the C\textsuperscript{14}/N\textsuperscript{15} ratio becomes some fraction between 0 and 1.

The assumption is made in all these postulated ratios that there is no loss of N\textsuperscript{15} on the conversion of adenine to guanine. This assumption is made on the basis of the work of Brown and his colleagues (4), and would be valid whether or not the transformation involved a rupture of the purine nucleus.

A plausible explanation for the first possibility, C\textsuperscript{14}/N\textsuperscript{15} = 0, is that an exchange reaction takes place involving an open chain aliphatic compound and some precursor molecule which serves both as a source of the 2-amino group and the 2-C- atom of guanine. Glycine is such a precursor molecule. In 1949 Reichard (14) showed that the 2-amino group of guanine was derived largely from this source. More recent experiments of Van Potter et al. (35) would indicate that the 2-C-atom of guanine is also derived from glycine. The results of both these experiments represent 'de novo' anabolism, consequently this path may be postulated for the synthesis of guanine, but it is not necessarily followed for the interconversion of the two bases and certainly is not the sole path for their transformation.
The second possible ratio mentioned, $\text{C}^{14}/\text{N}^{15} = 1$, is that in which the $\text{C}^{14}$ content of the adenine and guanine separated from the mixed nucleic acids is of the same order of magnitude in both compounds. This result would indicate no loss of $\text{C}^{14}$ during the conversion, a virtual impossibility unless the purine nucleus remains intact and the conversion takes place by a direct amination at the 2 position, preceded by a prior oxidation at this point.

The third possibility, that the $\text{C}^{14}$ content of the guanine is lower than that of the adenine but still represents a significant fraction of the polynucleotide guanine isolated, i.e., $\text{C}^{14}/\text{N}^{15} < 1$, could be explained in several ways. A rupture of the purine nucleus at the 2 position to yield a 4-formamido-, compound wouldn't leave the 2-C-atom exposed, a state in which oxidation and amination become more plausible, but in which exchange also becomes more likely. Either exchange or oxidation would result in a liberation of $\text{C}^{14}$ with a resultant drop in the $\text{C}^{14}/\text{N}^{15}$ ratio.

Two compounds are the possible result of such a splitting of the purine nucleus of adenine in its conversion to guanine, 4-formamido-, 5-imidazole carboxamide or 4-formamido-, 5-imidazole carboximidine, depending on whether deamination at the 6 position precedes or follows splitting of the ring. Compounds of this nature are not unknown both as biological and chemical entities. 4 amino-5-imidazole carboximidine is the product resulting from acid hydrolysis of adenine (36), thus showing a lability of the 2 position
in a chemical sense. 4 amino-5-imidazole carboxamide has been the subject of experimentation by Buchanan (37) and Greenberg (38). The experiments of both men have demonstrated the formation of inosinic acid and hypoxanthine as the products when this compound is incubated with formate.

Any theory involving an open chain intermediate must of necessity explain the evidence of Brown et al. (4) for the retention of N\(^{15}\) in both the 1 and 3 positions of the purine nucleus. If a carboximididine structure is considered and no restrictions are put upon it, resonance immediately dilutes the isotope concentration of the 1 position to one-half its original concentration. Two arguments might be put forward to defeat this suggestion. First; that deamination precedes the ring opening, i.e. the proposed aliphatic intermediate is not a carboximididine compound, but rather a carboxamide compound. This situation would dispose of the necessity for considering resonance effect. Second; the carboximididine structure may not be free, it may have a steric limitation placed upon its behavior by an enzyme involved in the over-all conversion of adenine to guanine. This suggested limitation would be of the same nature as that described by Ogaton (39) for the decarboxylation and hydration reactions in the Krebs Citric Acid Cycle.

Another explanation for a significant fall in the C\(^{14}/N\(^{15}\) ratio upon the conversion of adenine to guanine, lies in the possibility that this transformation takes place not by a single route but by a variety of routes. In some
of these paths a liberation of the 2-C-atom, might occur while in others the transformation might be accomplished with the ring intact. This theory was suggested in the introduction by reference to the second metabolic map published by Roll and Brown (2). In this map the authors show two routes for the conversion of active adenine to active guanine. One path denotes a direct conversion of the two bases, while the other involves a backtracking towards more general precursor molecules. This region of precursor intermediates is one in which incomplete purine nuclei are known to play a prominent role.

Either of those suggested possibilities, a single reaction or a variety of reactions for accomplishing the conversion of adenine to guanine, could be used to explain the observed fact that the incorporation of 1,3-N\textsuperscript{15},2-C\textsuperscript{14}-adenine into the tissue nucleic acids as guanine was less when measured in terms of C\textsuperscript{14} than when measured as N\textsuperscript{15}.

Other results observed from the injection of 1,3-N\textsuperscript{15},2-C\textsuperscript{14}-adenine into a rat are:

1) A significant amount of C\textsuperscript{14} appears in the expired air of the animal.
2) Large amounts of C\textsuperscript{14} activity are found in the animal's urine.
3) An acid-soluble extract of the tissues contains purine nucleotides that are of higher specific activity than the polynucleotide purines.
4) No appreciable quantity of radioactivity is found in the animal's blood.
5) Radioactivity excreted in the faeces is negligible compared to the total injected amount of C$^{14}$.

A technical flaw must be the first possibility considered with regard to the large amounts of C$^{14}$O$_2$ found in the expired air of the animals. Because a gross amount of radioactivity is excreted in the form of by-products in the animal's urine, the possibility exists that these by-products are undergoing bacterial decomposition resulting in the liberation of C$^{14}$O$_2$. If this were the case, then the C$^{14}$O$_2$ in the expired air samples would bear no relationship to the rat's metabolism. Three features of this C$^{14}$O$_2$ production might be cited to relate it to the rat's metabolism rather than to bacterial decomposition. The C$^{14}$O$_2$ shows a marked diurnal variation, which is in agreement with the rat's nocturnal habits (Figure 3). The specific activity of the urea excreted in the urine shows a parallelism to that of the expired C$^{14}$O$_2$ (25). This would follow from the Krebs-Henseleit theory for the formation of urea. Finally the quantity of radioactivity found in the expired air, 25% of the total injected activity, would argue against this 'technical flaw' explanation for the presence of C$^{14}$O$_2$ in the expired air. If the C$^{14}$O$_2$ is a product of the rat's metabolism then it becomes direct evidence for a lability of the 2-C-atom of adenine. As such the inference must be drawn that adenine undergoes some major reaction involving this 2-C-atom. The reaction might be a total degradation of the adenine molecule. This possibility would not be in
FIGURE 3

Histogram showing the Relative Excretion of $\text{C}^{14}_2\text{O}_2$ and $\text{C}^{14}_4$-Urea.

SPECIFIC ACTIVITY $\text{C}^{14}_2\text{O}_2$
c.p.m./mg.C.

RADIOACTIVITY

c.p.m.

TIME (hours)

$\text{C}^{14}_4$-Urea

$\text{C}^{14}_2\text{O}_2$
agreement with the theory for the main pathway of purine catabolism, which is thought to lead to the end product, allantoin, by means of the following steps:

\[
\begin{align*}
\text{Adenine} & \rightarrow \text{hypoxanthine} \\
\text{Guanine} & \rightarrow \text{xanthine} \\
& \rightarrow \text{uric acid} \\
& \rightarrow \text{allantoin}
\end{align*}
\]

At no point on this path would there be a liberation of \( ^{14}C_2 \) from 1,3-N\(^{15} \)-,2-C\(^{14} \)-adenine. Since the reaction sought involves some 25\% of the adenine injected it does not seem likely that a degradation path of this magnitude would have escaped notice. A substantiated reaction that adenine is known to undergo, involving this site, is its transformation to guanine. Moreover this reaction is of sufficient biological importance to be considered as the source for this large amount of radioactivity. Opening of the purine nucleus at the 2- position to yield a 4-formamido-compound exposes the 2-C-atom of adenine to either chance oxidation or exchange or to planned substitution, either possibility resulting in the appearance of \( ^{14}C_2 \) in the expired air.

The source of a large fraction of the \( ^{14}C \) activity in the urine is unidentified after substracting the compounds in which one would expect to find radioactivity. That is, the most logical compounds which one would expect to contain radioactivity after administering 1,3-N\(^{15} \)-,2-C\(^{14} \)-adenine amount for only 42\% of the activity present. The total
urine $^{14}C$ content can be apportioned thus:

1) allantoin 28.0%
2) adenine 11.6%
3) urea 1.4%
4) unknown 58.0%

The unknown 58% of the activity could not be accounted for by any major component that could be detected by chromatographic, autoradiographic or $^{14}C$ chromatographic scanning techniques. The activity which was accounted for appears as the compounds one would expect to arise from the normal metabolism of the free base adenine. Allantoin is the major end-product of purine catabolism in the rat and as such it appears as the major $^{14}C$ containing component which could be identified in the urine. Since adenine is not a required metabolite in the diet of the rat, therefore, one would expect it might be excreted either unchanged or in some conjugated form if it is fed in excess. This suspicion is verified by the recovery of adenine containing $^{14}C$ by using the technique of carrier addition. Urea, the third component investigated, although it contributed very little to the over-all accounting for radioactivity does help to identify the source of the $^{14}C_{02}$ in the expired air as being the rat itself. A further examination of the allantoin excreted shows that its $^{14}C/N^{15}$ ratio is 0.618. If it had been derived from the purines formed from the injected adenine and their $^{14}C/N^{15}$ ratios had remained at 1 then the allantoin should also have had a $^{14}C/N^{15}$ ratio of 1. In this event for allantoin with a specific activity of 0.223 atom
% excess N\textsuperscript{15}, 340.4 mgm. should have contained 418,000 c.p.m. Only 258,200 c.p.m. are present. This value represents a deficit of 159,800 c.p.m., a deficit that can be used to account for approximately 50\% of the C\textsuperscript{14}O\textsubscript{2} found in the animals' expired air. This, furthermore, directly relates the C\textsuperscript{14}O\textsubscript{2} found in the expired air to the drop in C\textsuperscript{14}/N\textsuperscript{15} ratio found in the nucleic acid purines.

In view of the recent work of Ochoa et al. (18) and that of Kornberg and his group (40) the high specific activity shown by the free nucleotides is not surprising. The experiments of both men have shown that the phosphorylated ribotides and deoxyribotides would appear to be the units incorporated into Ribose Nucleic acid and Desoxyribose Nucleic acid. The importance of 2-C\textsuperscript{14}-hypoxanthine ribotide as an intermediary product concerned with the conversion of adenine to guanine cannot be estimated. If it does figure in this role, it is also well known as a breakdown product of adenine in the course of its degradation to allantoin, and the respective contribution of either reaction to the overall total of C\textsuperscript{14}-hypoxanthine present cannot be measured in this experiment. However, Abrams and Bentley (13) have shown that the route leading from adenylic acid through inosinic acid to xanthosine ribotide phosphate and from there to guanylic acid can be considered as a valid series of reactions which could lead from adenine to guanine. A blocked reaction or saturation technique might be used to solve the question of the importance of this route. If
labelled adenine and unlabelled inosinic acid were administered simultaneously, the degree of incorporation of adenine as guanine into the tissue nucleic acids should decrease if inosinic acid is a direct intermediate upon the pathway of this conversion.

Very little can be discussed about any role the blood might play in this transformation phenomenon since the only analysis carried out was an estimation of the total C\(^{14}\) activity. This, in the case of both experiments, turned out to be negligible with respect to the total activity injected and considering the results obtained in the expired air was probably mainly in the form of bicarbonate.

The faeces, likewise, showed very little of the total activity injected, indicating, as might be expected, that faecal excretion plays a very minor role in the disposal of the injected 1,3-N\(^{15}\)-,2-C\(^{14}\)-adenine.

Figure IV presents an overall accounting of the total radioactivity injected each day in terms of percentages contributed by each of the three excretory routes plus a fourth division labelled unknown. This unknown fraction is that portion of the injected dose which presumably remains within the animal and enters the various pathways of the animal's metabolic processes.
FIGURE 4

Daily accountable Radioactivity found in the Three Excretory Products expressed as a Percentage of the Rats' 24 hourly dose of 1,3-N15-,2-C14-adenine (392,500 c.p.m.)
SUMMARY

A. Doubly labelled adenine, $1,3-N^{15},2-C^{14}$-adenine, was synthesized and administered to rats by means of intraperitoneal injections. At the end of an experiment the mixed visceral nucleic acids were isolated and hydrolysed. The adenine and guanine obtained from this source were then examined for their isotope content in terms of their $C^{14}/N^{15}$ ratio relative to the $C^{14}/N^{15}$ ratio in the adenine injected.

B. The ratio of $C^{14}/N^{15}$ in the adenine recovered from the visceral nucleic acids was 0.326 in the first experiment and 0.352 in the second experiment. Therefore, approximately 17% of the injected, $1,3-N^{15},2-C^{14}$-adenine which was incorporated into the nucleic acids as adenine had exchanged its $2-C^{14}$-atom for a non-isotopic atom.

C. The ratio of $C^{14}/N^{15}$ in the guanine recovered from the visceral nucleic acids was 0.298 in experiment I and 0.427 in experiment II. This indicates that approximately 64% of the injected $1,3-N^{15},2-C^{14}$-adenine which was incorporated into the visceral nucleic acids in the form of guanine had exchanged its $2-C^{14}$-atom for a non-isotopic atom.

D. In the purines of the visceral nucleic acids there was 47% less $C^{14}$ found in the guanine than in the adenine despite the fact that they were both derived from the same source, $1,3-N^{15},2-C^{14}$-adenine which had a $C^{14}$ content considered to be 100%. 
E. Large amounts of $^{14}$C activity were found in the expired air upon injection of $l,3-N^{15},2-C^{14}$-adenine into a rat. The amount of radioactivity found as $^{14}CO_2$ represented 25% of the total injected activity. The total activity of the $^{14}CO_2$ was 344,630 c.p.m.

F. The radioactivity found in the urine accounted for 58% of the injected activity. Allantoin, the final degradation product of purine catabolism, represented the largest identifiable pool of $^{14}$C activity contributing to the total radioactivity present in the urine.

G. The $^{14}/N^{15}$ ratio in the allantoin was 0.618. This ratio indicated a total $^{14}$C deficit of 159,800 c.p.m.

H. This deficit of counts in the allantoin was used to account for 47% of the $^{14}CO_2$ found in the animals' expired air.

I. The relative specific activities found for the purines of the acid-soluble fraction were adenine=1, hypoxanthine=0.92 and guanine=0.24 indicating that hypoxanthine in some form represents a possible intermediate on the route of interconversion of adenine to polynucleotide guanine. The absolute specific activities found in the acid-soluble fraction were much higher than those found for the purines of the nucleic acids. Therefore, it could be inferred that interconversion took place at this stage rather than after incorporation into the nucleic acids had taken place.
J. The discrepancy between the specific activities of the $^{14}\text{CO}_2$ and the $^{14}$-urea, (the specific activity of the urea is higher than that of the $\text{CO}_2$), indicated that there is possibly another source for this urea in addition to the Krebs-Henseleit cycle.
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