THE METABOLISM OF YEAST NUCLEIC ACID IN THE RAT

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(Received for publication, April 14, 1949)

Recent studies on the fate of labeled dietary purines in the rat have shown that dietary adenine is a precursor of the adenine and of the guanine of tissue nucleic acids (1), but that guanine (2), hypoxanthine (3), and xanthine (3) are not utilized for nucleic acid synthesis. Similar experiments have shown that the pyrimidines, uracil, thymine (2), and cytosine (4) are not incorporated into the nucleic acids of the rat. However, orotic acid (4-carboxyuracil), while not a nucleic acid component, is utilized as a precursor of the uracil and cytosine of nucleic acids (5). Since none of the pyrimidines occurring in nucleic acids are utilized when they are present in the diet, it seems probable that derivatives of these compounds are first synthesized in a way which does not involve the participation of these free pyrimidines, but which may involve orotic acid (6), and that these derivatives are then incorporated into the nucleic acids. Such derivatives could be the pyrimidine nucleosides or nucleotides. Because free guanine is not a precursor of nucleic acid guanine, it has been suggested (1) that similar derivatives of the purines may be involved in the conversion of adenine to nucleic acid guanine. Up to now, no nucleic acid component more complex than the purines or pyrimidines has been studied by the tracer technique. The availability of ribonucleic acid labeled with isotopic nitrogen (7) now enables us to extend these studies to nucleic acids.

The fate of exogenous nucleic acids, nucleotides, and nucleosides has been studied in the past by the method of feeding or injecting these compounds into animals in nitrogen balance and determining what changes occur in the quantities of the various constituents of the excreta. In this way it has been found in several species that, following the administration of nucleic acids, the nitrogen of the purines could be largely, but not always completely, accounted for in the extra allantoin, uric acid, or other free purines (8-11), but that pyrimidines were absent from the urine and that their nitrogen probably had been converted to urea (12, 13). The metabolism

* These authors gratefully acknowledge the assistance of the Office of Naval Research, the National Cancer Institute of the United States Public Health Service, and the James Foundation of New York, Inc.
of the nucleotides and the nucleosides has been studied in the same way. The nitrogen of purine nucleosides has been found to be extensively excreted as uric acid or allantoin (14, 15), although Cerecedo and Allen found that dogs apparently excrete about one-third of the nitrogen of guanosine as urea (16). It has been shown that the nitrogen of the pyrimidine nucleotides and nucleosides is almost completely excreted as urea, with the appearance of no nucleotides or nucleosides and only small amounts of free pyrimidines in the urine (13, 17). While such experiments are of value in demonstrating the end-products of nucleic acid metabolism, they have yielded no information concerning the pathways of tissue nucleic acid synthesis. This present work is the first of a series of investigations designed to gain further information about nucleic acid metabolism in the rat by the use of labeled nucleic acid and some of its hydrolytic products.

In Experiment I a sample of isotopic yeast (pentose) nucleic acid was fed to two male rats over a period of 3 days. In Experiment II a solution of nucleotides, produced by the alkaline hydrolysis of a sample of the isotopic nucleic acid, was administered to two male rats by intraperitoneal injections which were given three times a day over a 3 day period. In each experiment the compounds were furnished at a level of about 0.4 mM of nucleic acid per kilo of body weight per day. During the experiments the urine was collected and was used for the isolation of allantoin, urea, and ammonia. After the administration of the isotopic compounds was completed, 1 day was allowed to elapse before the animals were sacrificed in order that the compounds might be more completely utilized. Nucleic acids were prepared from the viscera and from them guanine, adenine, and mixed pyrimidines were isolated. A sample of nucleic acid-free muscle protein was prepared. The per cent excess N\textsuperscript{15} in the isolated compounds was determined and these values are shown in Table I.

When the nucleic acid was fed, it was extensively absorbed and metabolized, as indicated by the fact that about 27 per cent of the allantoin excreted during the experiment was formed from the isotopic compound and that appreciable degradation to urea and ammonia occurred. There was a small but definite incorporation of the dietary material into the nucleic acids of the viscera. Because the per cent excess N\textsuperscript{15} in the dietary nucleic acids is about 60 times greater than that in the nucleic acids from the viscera, it would require only about 1 to 2 per cent contamination by the former to account for all of the isotope found in the isolated nucleic acids. However, contamination of such a magnitude does not seem probable, because the time that elapsed between the last feeding and the sacrifice of the animals was sufficient to allow essentially complete digestion and absorption of the dietary material and because the intestines were opened and thoroughly washed before they were added to the other organs.
Although the purines and pyrimidines of the dietary yeast nucleic acid contained equal concentrations of isotopic nitrogen, only 1.0 per cent of both the adenine and the guanine of the nucleic acids from the viscera was derived from the dietary material, while 2.2 per cent of the pyrimidines was derived from it. It seems likely, therefore, that the isotope found in both the purines and pyrimidines of the isolated nucleic acids represents a true incorporation of the dietary material and not a contamination by dietary nucleic acid retained in the gut.

**Table I**

*Administration of Nucleic Acid*

<table>
<thead>
<tr>
<th></th>
<th>Nucleic acid feeding, Experiment I</th>
<th>Nucleotide injection, Experiment II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Atom per cent excess N\textsubscript{15}</td>
<td>Atom per cent excess, calculated on basis of 100 per cent in administered compound</td>
</tr>
<tr>
<td>Nucleic acid (dietary)</td>
<td>3.34*</td>
<td>100</td>
</tr>
<tr>
<td>Nucleotides (injected)</td>
<td>0.036</td>
<td>0.037</td>
</tr>
<tr>
<td>Sodium nucleic acids (viscera)</td>
<td>0.037</td>
<td>1.7</td>
</tr>
<tr>
<td>Nucleic acids (viscera)</td>
<td>0.037</td>
<td>1.7</td>
</tr>
<tr>
<td>Purine hydrochlorides</td>
<td>0.037</td>
<td>1.7</td>
</tr>
<tr>
<td>Adenine</td>
<td>0.031</td>
<td>1.0</td>
</tr>
<tr>
<td>Guanine</td>
<td>0.032</td>
<td>1.0</td>
</tr>
<tr>
<td>Silver pyrimidines</td>
<td>0.074</td>
<td>2.2</td>
</tr>
<tr>
<td>Ammonia</td>
<td>0.033</td>
<td>1.0</td>
</tr>
<tr>
<td>Urea</td>
<td>0.058</td>
<td>1.7</td>
</tr>
<tr>
<td>Allantoin</td>
<td>0.086</td>
<td>26.6</td>
</tr>
<tr>
<td>Muscle protein</td>
<td>0.005</td>
<td>0.1</td>
</tr>
</tbody>
</table>

* The value for the nucleic acid preparation was 3.43; that for the purines and pyrimidines isolated from it was 3.34 ± 0.02.

In Experiment II, in which a solution of nucleotides prepared from isotopic nucleic acid was given by intraperitoneal injection, it was found that this mixture of nucleotides was more efficiently utilized for the synthesis of nucleic acids than was the dietary nucleic acid. Although equivalent quantities were administered in the two experiments, the nucleic acids isolated after injection of the nucleotides contained 2.7 times more isotope than was present in the nucleic acids obtained in Experiment I. Again, both purines contained the same atom per cent excess N\textsubscript{15}, but in this case nearly 4 times as much of each was derived from the injected nucleotides. In Experiment II, just as in Experiment I, the incorporation of the iso-
topic material into the pyrimidines of the tissue nucleic acids was greater than the incorporation into the purines, although here the difference in uptake was smaller than it was in Experiment I. The allantoin and urac were derived from the injected compounds to about the same extent as in Experiment I. The level of \( \text{N}^{15} \) in the urinary ammonia was twice as high as it was in Experiment I and was even higher than the \( \text{N}^{15} \) level in the urea isolated from the same urine. This may have been due, partly, to a breakdown of other isotopic compounds in the urine to ammonia, because the ammonia was not isolated from this sample of urine until 2 months after it had been collected, and, although it had been stored under toluene in the refrigerator, it is possible that a small amount of allantoin, for example, had been degraded to ammonia.

**EXPERIMENTAL**

*Materials and Methods*—The labeled nucleic acid was isolated from yeast which had been cultured in a medium containing isotopic ammonium sulfate as the main source of inorganic nitrogen (7), and was found to contain 3.43 atom per cent excess \( \text{N}^{15} \). Adenine, guanine, and mixed pyrimidines were isolated from this nucleic acid and were found to be labeled to the same extent (7). For the preparation of a solution of mixed nucleotides a sample of the isotopic nucleic acid was hydrolyzed by the method described by Buell (18). 1.0 gm. of nucleic acid was suspended in 20 cc. of water and was brought into solution at pH 7 by the gradual addition of 3 N NaOH. After 0.3 gm. of solid sodium hydroxide was added, the solution was diluted to 25 cc. and allowed to stand at room temperature for 24 hours. It was then neutralized to pH 7.5 with 1.0 N HCl, diluted to 50 cc., and stored in the refrigerator until used. This solution contained 0.020 gm. or 0.0153 mm of nucleic acid per cc. as a mixture of nucleotides.

Adult, male, Sherman strain rats were used. They were fed 60 gm. of Rockland Purina chow per kilo of body weight per day, and the nucleic acid preparations were administered at the rate of 0.4 mm per kilo per day, assuming for purposes of calculation a “statistical” tetranucleotide (19) of molecular weight 1300. The maintenance and the method of sacrifice of the animals, the preparation of nucleic acids from the viscera, the isolations of guanine, adenine, and mixed silver pyrimidines, the isolation of the urinary constituents, and the preparation of the muscle protein have been described previously (1).

*Nucleic Acid Feeding; Experiment I*—Two rats weighing 242 and 258 gm. were used. The lighter animal received 126 mg. of the isotopic nucleic acid and 14.5 gm. of food per day for 3 days, while the other one received 134 mg. of the nucleic acid and 15.5 gm. of food. The food was ground into a paste with water, and the nucleic acid was dissolved in water with the aid
of a minimum of 3 N NaOH and was added to the food. This ration was prepared at the start of the experiment and was kept in the refrigerator. Aliquots were removed each day. The animals ate all of the food, but each rat lost about 10 gm. in body weight during the experiment.

**Nucleotide Injection, Experiment II**—Two rats weighing 310 and 260 gm. were used. They received 8.1 and 6.8 cc. of the nucleotide solution respectively per day for 3 days. The daily quota of solution was given in three doses of approximately equal volumes by intraperitoneal injection. The animals ate well, but the heavier one suffered a loss of weight of 9 gm., while the lighter animal lost 15 gm. during the experiment. Both rats developed diarrhea. This condition has been noticed before in rabbits, dogs, and cats following the injection of pyrimidine nucleotides and sodium nucleic acid (9, 13). At sacrifice the lighter animal was found to be suffering from peritonitis. The intestines from this animal, which were quite inflamed, were not added to the organs utilized for the preparation of nucleic acids.

**DISCUSSION**

The extent to which the sample of dietary nucleic acid was digested prior to absorption cannot be ascertained, but it is probable that a mixture of nucleotides, nucleosides, and perhaps purines was produced. It would seem, therefore, that in both of the experiments described here nucleotides or nucleosides were the compounds available for cellular metabolism. If the results of these experiments are compared with the results of the experiments in which adenine was fed (1), it can be seen that the adenine in adenylic acid or adenosine is apparently less efficiently utilized by the rat for nucleic acid synthesis than is the free purine. In the earlier experiment adenine was fed to rats at a level of 0.2 mm (27 mg.) per kilo per day, and it was found that 5.4 per cent of the adenine and 3.2 per cent of the guanine in the nucleic acids of the viscera had been formed from the dietary compound and that these values were increased to 13.7 and 8.2 per cent, respectively, when the level of administration was increased to 1.5 mm (200 mg.) per kilo per day. In these experiments the animals received approximately 0.4 mm of adenine per kilo per day in the form of nucleic acid or adenylic acid, but only 1.0 per cent of both the nucleic acid adenine and guanine was derived from the dietary nucleic acid and only 3.8 per cent of each was formed from the injected nucleotides. This less efficient incorporation of adenine into nucleic acids when administered to rats as nucleotide or nucleoside rather than as free purine cannot be due to a failure of these compounds to be metabolized, since the high isotope levels in the urinary allantoins gives evidence that extensive absorption and metabolism of the purine moieties had occurred in each case. It is more likely that
partial enzymatic deamination of the adenylic acid or adenosine had occurred before these compounds could be utilized for nucleic acid synthesis. It has been demonstrated that an adenosine deaminase present in intestinal secretions can cause extensive conversion of adenosine to inosine (20-22). This same enzyme has been detected in a number of other tissues (23); so that the parenterally administered compound could also have been partially deaminated. It is significant that the injected nucleotides were nearly 4 times more effective as purine precursors than was the ingested nucleic acid.

In both of these cases, it was found that, in the nucleic acids which had been isolated from the viscera, the ratio of adenine to guanine which had been derived from the isotopic compounds was 1:1. This is in sharp contrast to the results that have been obtained in earlier experiments. When free adenine was the labeled precursor and the nucleic acids of the combined viscera were investigated, this guanine to adenine ratio was invariably close to 0.6:1.0 (1). The significance of this observation is not immediately apparent, but it may indicate that nucleic acid guanine need not necessarily be formed from adenine but can also be derived from other precursors.

All of the purines and pyrimidines present in ribonucleic acid have been tested in the rat and none have been found to be precursors of nucleic acid pyrimidines. Therefore the finding that the nucleic acid pyrimidines are derived from orally administered nucleic acid and from a mixture of injected nucleotides probably implies that the pyrimidine nucleotides or nucleosides are utilized by the rat for the synthesis of nucleic acids. The biological synthesis of nucleic acids in the absence of preformed pyrimidine nucleotides or nucleosides probably involves a synthesis of these units in a way which does not involve the free pyrimidines at any step. The role of orotic acid in such syntheses can only be surmised at this time, but it may be that a carboxyl group in position 4 of the pyrimidines is necessary for the attachment of a ribose molecule at position 3.

In both of the experiments described here the nucleic acid pyrimidines were derived from the isotopic compounds to a greater extent than were the purines. However, the difference in uptake that was found following the injection of the nucleotides was smaller than that which was observed after the feeding of nucleic acid. This difference in uptake of the purines and the pyrimidines is not necessarily indicative of any difference in the rates of replacement of the purines and the pyrimidines in the tissue nucleic acids but is probably due to a greater enzymatic degradation of the purine nucleotides or nucleosides than of the corresponding pyrimidine compounds.

1 Furst, S. S., Roll, P. M., and Brown, G. B., unpublished work.
which allows more of the latter to be available for nucleic acid synthesis. Purine nucleosidase, adenosine deaminase, and guanosine deaminase are known to be present in a number of tissues, but pyrimidine nucleosidase has not been found to be as widely distributed (24). Orally administered nucleic acid would probably be subjected to a greater enzymatic attack than would the injected compounds, and the difference in the extent of uptake of the purines and the pyrimidines was found to be greater following this route of administration.

In Experiment I the level of isotope in the urinary urea is nearly twice as great as in the urinary ammonia, which indicates that all of the urea had not been synthesized from body pool of ammonia but that some of it had been formed by direct metabolism of one of the nucleic acid fragments. This result was not noted in Experiment II, but the uncertainty concerning the reliability of the isotope value for this ammonia makes any interpretation of this difference impossible.

In the case of a mutant of Neurospora crassa, Pierce and Loring (25) have observed marked antagonistic effects of certain nucleosides or nucleotides on the utilization of others, notably an inhibition of the utilization of cytosine derivatives by adenosine and the reversal of that inhibition by uridine. In view of such interrelationships the results obtained with the mixture of nucleotides must be interpreted with caution and studies of the individual derivatives are desirable.

The authors wish to thank Helen Getler for assistance, Alice Angelos for aid with the isotope analyses, and Dr. Harold Beyer for cooperation in the maintenance of the mass spectrometer.

SUMMARY

Yeast nucleic acid, in which the purines and pyrimidines were equally labeled with N¹⁵, has been fed to rats and has been found to be a precursor of both the purines and the pyrimidines of the nucleic acids isolated from the viscera. A sample of this nucleic acid, after hydrolysis to nucleotides, was administered to rats by intraperitoneal injection and was found to serve to a somewhat greater extent as a precursor of the purines and pyrimidines of the tissue nucleic acids.

A material present in yeast nucleic acid, presumably pyrimidine nucleotides or nucleosides, in contrast to free pyrimidines, may serve as a precursor of the pyrimidines of tissue nucleic acids.

The purines of dietary nucleic acid or of injected mixed nucleotides are less extensively utilized for nucleic acid formation than is the free purine adenine.
BIBLIOGRAPHY

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NUCLEIC ACID IN THE RAT
Paul M. Roll, George Bosworth Brown,
Frederick J. Di Carlo and Alfred S. Schultz