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THE METABOLISM IN THE RAT OF GUANYLIC ACID LABELLED WITH ^{32}P*

by

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It has been established that, in the rat, guanine and guanosine are very poor precursors but that guanylic acids serve as effective precursors of pentose nucleic acid (PNA). For example, no incorporation of guanine[1,2] or guanosine[3], labelled with ^{15}N, into the PNA guanine of the combined organs of the viscera of the rat could be detected, although subsequent investigations with these compounds, labelled with the more sensitive tracer ^{14}C, revealed that each was incorporated to a slight extent[4,5]. In contrast, it was found that the PNA guanine of the rat viscera was extensively derived from yeast guanylic acid, which was presumably a mixture of guanylic acids "a" and "b"[6] (currently considered to be the 2' and 3'-isomers) and later it was shown that the "b" isomer was well incorporated[7]. It must be noted that in all of the above studies only the guanine moiety of the administered nucleosides and nucleotides had been labelled so that only this portion of the molecule was traced. However, the results of the experiments do demonstrate that guanine in nucleotide linkage is much more extensively utilized than is the purine, and it may be concluded that the mechanism for the incorporation of guanylic acids does not involve a simple liberation of either the nucleoside or the free purine prior to the incorporation. A possible mechanism would involve the incorporation of the intact nucleotides during the biosynthesis of the PNA. To test this possibility, guanylic acids "a" and "b" were prepared which were labelled uniformly with ^{15}N, uniformly with ^{14}C and with ^{32}P and were administered to rats. The results of the utilization of the phosphorus of these nucleotides are presented here.

The preparation of the multiply labelled guanylic acids was carried out by isolating the nucleotides by ion exchange on Dowex-1[8] (National Aluminate Corporation, U.S.A.) from hydrolysates of nucleic acids labelled with ^{14}C**, ^{15}N[9] and ^{32}P. The ^{32}P labelled nucleic acid was isolated by sodium chloride extraction from yeast which had been grown in a medium containing ^{32}P*** and was purified by precipitation from aqueous solution with glacial acetic acid. The three separately labelled samples of each nucleotide were combined and were administered to male Sherman strain rats of 250–300 g by intraperitoneal injection. Each rat received 0.4 m*M* per kilogram of body weight in three equal doses at about two-hour intervals, and the animals were sacrificed approximately twenty-four hours after the first injection. The spleen, liver, kidneys, testes and small intestine were removed and were frozen on dry-ice. The tissues were combined and were homogenized in ice-cold 10% trichloroacetic acid (TCA). The residue was dehydrated with alcohol and ether to yield a dry tissue powder from which ribonucleotides were obtained by a modification of the SCHMIDT-THANNHAUSER procedure[10] and were separated by ion exchange on Dowex-1.

Samples of the solutions of the nucleotides were plated on aluminum planchets and the activity due to ^{32}P was determined in an internal Geiger-Müller flow counter (Radiation Counter Laboratories, mark 12, model 1, helium-*iso*butane gas), with the use of an aluminum shield which had been found

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** Obtained from Schwarz Laboratories, Inc., New York, U.S.A.

*** Obtained from Oak Ridge National Laboratory, Tennessee, U.S.A., as inorganic phosphate.

to prevent completely the passage of radiation due to ^{14}C, but to allow the passage of about 50% of the ^{32}P radiation. The concentrations of the nucleotide solutions were determined spectrophotometrically. For the determination of the specific activity of inorganic phosphorus, magnesium ammonium phosphate was isolated from the TCA extract[11] and was dissolved in very dilute HCl. The activity was measured, using the same technique and the same shield which was used for the determination of the activity of the nucleotides. On the day on which the specific activity of any of the isolated compounds was determined, the specific activity of the injected nucleotide was also measured so that corrections for ^{32}P decay were not involved. From these specific activities the relative specific activity (RSA) was calculated ($RSA = \frac{\text{specific activity of isolated compound}}{\text{specific activity of injected nucleotide}} \times 100$).

The results are reported in Table I. The results of experiments in which similarly labelled adenylic acids "a" and "b" were used are included for comparison. It is apparent that in each experiment the RSA of each of the isolated nucleotides was essentially the same and that the RSA of the inorganic phosphorus was considerably higher than that of the nucleotides. This random distribution of labelled phosphorus among all the nucleotides*, the greater activity of the inorganic phosphorus and the general similarity in the results of the four separate experiments strongly suggests that the injected nucleotides served as non-specific sources of inorganic phosphorus, or of ribose phosphate, which was then used by the rat in the biosynthesis of PNA. Certainly there is no clear evidence for the utilization of an intact nucleotide, although the random incorporation of phosphorus would tend to mask a small amount of such a utilization. The mechanism for the utilization of the guanine** of guanylic acids for nucleic acid biosynthesis remains obscure.

TABLE I

RSA OF THE PHOSPHORUS OF ISOLATED COMPOUNDS FOLLOWING THE INJECTION OF GUANYLIC ACIDS AND ADENYLIC ACIDS

Isolated compound	*Injected nucleotides*			
	Guanylic acid "a"	*Guanylic acid "b"*	*Adenylic acid "a"*	*Adenylic acid "b"*
Adenylic acid "a"	0.46	0.45	0.36	0.48
Adenylic acid "b"	0.44	0.40	0.32	0.45
Guanylic acid "a"	0.44	0.41	0.30	0.41
Guanylic acid "b"	0.42	0.41	0.31	0.40
Cytidylic acid	0.48	0.40	0.29	0.40
Uridylic acid	0.39	0.39	0.32	0.40
Inorganic P	0.76	0.60	0.48	0.73

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* If the "a" and "b" isomers had been the units incorporated, randomization of the phosphate during alkaline hydrolysis of the PNA would not be expected, since the currently accepted scheme of such a hydrolysis depicts the 2′ or 3′-phosphate of a nucleotide of PNA as remaining attached to that nucleotide when it is liberated from the polynucleotide.

** The incorporation of which has been demonstrated for the guanylic acid "a" sample used here. Complete data on the incorporation of the purine and ribose moieties is being accumulated for future presentation.