methanol was therefore used. Concentrations prepared were 0.02–0.04 g. per liter and all measurements were made at minimum slit width with a Beckman DU spectrophotometer using matched 1-cm. quartz cells.

d. **Isolation and Identification of Lignin Degradation Products from Aspen Wood (i) Investigation of Fraction A.**—Part (8.2 g.) of the "neutral" fraction A obtained by the hydrogenation of aspen wood meal was divided into an ether-insoluble, dark amorphous material (0.43 g.) and an ether-soluble oil (7.7 g.). Of this latter fraction, a portion (4.5 g.) was distilled through a small column (7 in. by 1.6 in. diameter) packed with beads (av. diameter 4 mm.). The distillate (2.03 g.) was thereby divided into eight fractions obtained over a boiling range 75–160° at 1 mm.

4-Hydroxy-3-methoxyphenylethane.—From the fraction boiling 82–87° (1 mm.) (0.56 g.) the *p*-nitrobenzoate derivative was prepared in good yield and after two crystallizations from ethanol–water melted 91.5–93°; mixed m.p. with an authentic sample of 4-hydroxy-3-methoxyphenylethane[13] (m.p. 95.5–96.5°) was 92.5–95°.

Anal. Calcd. for $C_{16}H_{15}O_5N$: OCH_3, 10.3. Found: OCH_3, 10.1.

4-Hydroxy-3,5-dimethoxyphenylethane.—From the fraction boiling 87–111° (1 mm.) (0.70 g.) the *p*-nitrobenzoate derivative was prepared, which, after three crystallizations from ethanol, melted at 150.5–151.5°. A mixed m.p. with an authentic sample[4] from 4-hydroxy-3,5-dimethoxyphenylethane (m.p. 152–153°) was 151–152.5°.

(13) Prepared by the Clemmensen reduction of 4-hydroxy-3-methoxyphenyl methyl ketone.

Anal. Calcd. for $C_{17}H_{17}O_6N$: OCH_3, 18.7. Found: OCH_3, 18.4.

(ii) **Investigation of Fraction B-1. 2-(4-Hydroxy-3,5-dimethoxyphenyl)-ethanol.**—Part (0.64 g.) of the partially crystalline fraction B-1 was washed with ether (5 ml.). This removed the oily fraction and left a white crystalline product, weight 0.065 g., m.p. 103–109°. Twice crystallized from ether, petroleum ether and ethanol this product melted at 112–113°. A mixed m.p. with an authentic sample[4] of 2-(4-hydroxy-3,5-dimethoxyphenyl)-ethanol (m.p. 113–114°) was 112–113°.

Anal. Calcd. for $C_{10}H_{14}O_4$: OCH_3, 31.3. Found: OCH_3, 30.8.

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On the Utilization of Guanine by the Rat[1]

BY M. EARL BALIS, DENIS H. MARRIAN AND GEORGE BOSWORTH BROWN

The administration of guaninine-8-C^{14} to the Sherman rat has now demonstrated an extremely small utilization of this compound, which escaped detection in previous investigations with N^{15}-labeled guanine.

The initial studies of the metabolism of N^{15}-labeled guanine by Plentl and Schoenheimer[2] led to the conclusion that guanine was not utilized by the rat for the synthesis of the tissue polynucleotides. This was repeated and confirmed in this Laboratory with Sherman strain rats, for both orally[3] and intraperitoneally[4] administered N^{15}-labeled guanine. Subsequent studies with this guanine have shown, however, that it may be utilized to a significant extent by the C57 black mouse.[5]

The guanine used in these experiments[2,3,5] contained N^{15} in both the 1- and 3-nitrogens of the ring and in the 2-amino group. Although there is little degradation *in vivo* of the purine ring,[6] the substituent 2-amino group of guanine is lost in the catabolism to allantoin and contributes appreciable isotopic ammonia to the body-pool. This, in turn, leads to a small incorporation of N^{15} into most of the nitrogenous compounds and this obscures any very small specific incorporation of the intact purine. The availability of guanine-8-C^{14} [7] of high specific activity circumvents this difficulty and it also offers an isotope which can be determined at a greater dilution than could the N^{15}.

The study of guanine-8-C^{14} administered at a level of 0.2 mM/kilo./day for three days to the Sherman strain white rat has now led to the detection of a very small specific incorporation of guanine into the polynucleotide guanine (Table I).[7a] In view of the inactivity of the polynucleotide

TABLE I

UTILIZATION OF GUANINE-8-C^{14} BY THE SHERMAN RAT, 0.2 MM/KG./DAY FOR 3 DAYS

	C.p.m./μM.	Relative specific activity[a] × 10^2
Guanine (injected)	17.6×10^3	100
PNA adenine	<1	0.0
PNA guanine	17	0.1
DNA	<1	0.0
Allantoin	3.2×10^3	18.3
Urea nitrate	2.1	0.01
Urea (as $BaCO_3$)	1.7	0.01

[a] Relative activity of isolated compound and administered guanine.

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(7a) NOTE ADDED IN PROOF.—Recently R. Abrams and J. M. Goldinger, *Arch. Biochem.*, **30**, 261 (1951), have mentioned that they have found guanine to be incorporated into rat intestinal PNA.

adenine, it seems unlikely that the incorporation observed is the result of the re-utilization of some degradation product. The dilution factor of 1,000 for the guanine is to be compared with dilution factors of 8 to 20 observed[3,8] for adenine administered under comparable conditions, and it indicates a comparatively insignificant utilization of the administered guanine. However the existence of this small utilization now makes this difference between the Sherman rat and C57 mouse a large quantitative one, rather than the heretofore apparent qualitative one.

The low activity in the urea, which also reflects the activity of expired CO_2,[9] the recovery of over 90% of the radioactivity in the urine and the high activity of the urinary allantoin are compatible with a preferential catabolism of guanine to allantoin which could be at least partially attributed to the abundance of guanase in tissues. It is of interest to note that Greenstein, *et al.*,[10] find no guanase in mouse pancreas and considerable amounts in rat pancreas, although there were no outstanding differences between the appreciable guanase content of other tissues of the two species.

The utilization of adenine by the C57 mouse has also been studied and the results (Table II) show that dietary adenine is incorporated into both the polynucleotide adenine and polynucleotide guanine, but that this occurs to a much smaller extent than in the Sherman rat. The renewal of 1.2% of the polynucleotide adenine of the mixed organs (Table II) is to be compared with the renewals of 4 to 10%[3,8] obtained in the rat when adenine is administered at the same level and for the same period of time. The more rapid BMR of the mouse makes this quantitative difference even more significant.

These quantitative differences between the utilization of either purine by the two mammalian species, and the quantitative differences in the utilization of the purines by two unicellular organisms, yeast[11] and *L. casei*[7] emphasize the species differences to be found in the anabolism of nucleic acids.

TABLE II

UTILIZATION OF ADENINE-1,3-DIN^{15} BY THE C57 MOUSE, 0.2 MM/KG./DAY FOR 3 DAYS

	Atom % excess N^{15}	Calcd. on the basis of 100% in dietary adenine
Adenine (dietary)	4.80	100
Tissue nucleic acids	0.040	0.8
Adenine	.057	1.2
Guanine	.020	0.4
Pyrimidines	.005	0.1

The Administration of Guanine-8-C^{14} to the Sherman Rat.—Two adult Sherman strain rats with a total weight of 820 g. were injected intraperitoneally on each of three successive days with 0.2 mM/kilo. of body weight of guanine-8-C^{14}.[7] A total of 107 mg. of guanine sulfate hydrate containing 8.7×10^6 c.p.m. were given. The rats were maintained on a diet of purina chow to which they were accustomed.

The animals were sacrificed on the fifth day and the organs were removed and the pentose nucleic acid (PNA) and desoxypentose nucleic acid (DNA) were separated from them by the Schmidt and Thannhauser method.[12] The PNA purines were isolated *via* the silver salts following mild acid hydrolysis of the PNA.[11] The adenine and guanine were separated by the procedure of Levene[13] and characterized by their ultraviolet absorption spectra and by filter paper chromatography. The mixed DNA-protein precipitate was extracted with hot 10% sodium chloride solution and the DNA was precipitated with three volumes of ethanol. The urine was collected and from it samples of allantoin[3] and urea nitrate[14] were obtained. The urea retained slight but approximately constant activity after several recrystallizations. Because of the possibility of contamination with traces of the highly active allantoin the urea was further degraded with urease,[15] the evolved CO_2 was collected as $BaCO_3$ and the activity was determined. The samples were prepared as thin films, of less than 250 μg. per sq. cm., on aluminum planchets and the activities were determined with a proportional flow counter (Radiation Counter Laboratories Nucleometer). The barium carbonate was measured at a thickness of 1.5 mg. per sq. cm.

The Administration of Adenine-1,3-N^{15} to the C57 Black Mouse.—Twenty-five C57 black mice (total wt. 450 g.), kept in separate cages, were fed moistened purina chow well-mixed with adenine sulfate (49.7 mg.) containing 4.8 atom % excess N^{15}, over a period of three days. This corresponded to a level of 0.19 mM adenine per kg. per day. On the morning of the 4th day the animals were sacrificed, and the internal organs were removed. The total nucleic acids were extracted and adenine, guanine and silver pyrimidines were prepared[3] from them. The isotopic nitrogen was determined with a Consolidated-Nier Mass Spectrometer, probable error ± 0.001 atom % excess.

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