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METABOLISM OF ADENINE-2-C^{14}, ADENINE-4,6-C^{14} AND ADENINE-8-C^{14}

Edward L. Bennett and Hilda Karlsson

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METABOLISM OF ADENINE-2-C^{14}, ADENINE-4,6-C^{14} AND ADENINE-8-C^{14}*

By Edward L. Bennett and Hilda Karlsson

(From the Radiation Laboratory, University of California, Berkeley, California.)

January, 1957

Several contradictory reports (1-3) concerning the possible lability of the adenine ring in mammals prompt us to report the results we have obtained in mice utilizing three carbon-14-labeled adenines: adenine-2-C^{14}, adenine-4,6-C_2^{14}, and adenine-8-C^{14}.

Gordon first suggested that complex paths may be involved in the incorporation of exogenous purines or in the biological interconversions of the purines. Thus utilization of adenine may appear to differ according to the position of the isotopic label (4). Subsequently Gordon reported results supporting this suggestion from feeding experiments in rats in which the metabolism of adenine-8-C^{14} was compared with values reported in the literature for adenine-1,3-N^{15} (1). Abrams has recently compared the incorporation of adenine-2-C^{13} and adenine-8-C^{14} into the ribonucleic acids of rat liver (2) during two time intervals. No significant change in the isotope ratio from that of the administered compound was found. The evidence for purine ring lability in microorganisms, fowl, and mammals has

used to obtain $C^{14}O_2$ and $C^{14}O_2/C^{12}O_2$ (specific activity) excretion patterns (9). The respiration data presented are the average of 2 to 3 individual experiments.

In the first experiment, mice were sacrificed at 1, 8, 15 and 28 days after administration of Ad-C^{14}. In the second experiment, in which the metabolism of Ad-2-C^{14} and Ad-4,6-C^{14} was more critically compared, 3 pairs of littermates — 3 mice injected with Ad-2-C^{14} and 3 mice with Ad-4,6-C^{14} — were sacrificed at 1 day and another 3 pairs at 15 days after the adenine was administered. To determine the effect of folic acid inhibitors, aminopterin (40 mg./kg.) or A-methopterin (180 mg./kg.) (Lederle) was administered 30 minutes prior to the adenine. The animals were sacrificed 24 hours later. The animals were sacrificed and the tissues were separately fractionated as previously described (10, 11).

The specific activities of the 5-adenylic acid derivatives of the soluble nucleotide fraction (5-AMP), RNA-Ad, and DNA-Ad were determined by differential enzymatic spectrophotometric methods combined with direct plate counting (after paper chromatographic purification) (10,11). In addition, the specific activities of the RNA-guanine (RNA-Gu) and DNA guanine (DNA-Gu) were determined by similar methods in which rat liver guanase and xanthine oxidase were used to convert the guanine to uric acid (12-14). All guanine specific activities were converted to μg. equivalent adenine specific activity.

Direct-plating techniques were used to determine the radioactivity in the isolated fractions (10). Radioactivity measurements were made with a Nuclear-Chicago D-47 windowless gas-flow counter and automatic sample changer, with platinum dishes of approximately 3.7 $cm.^2$ area. Counting efficiency was approximately 53 per cent; background was equivalent to 45 dis./min. Samples with low activity were routinely counted for duplicate periods of 30 to 60 minutes, and reliable determinations of activity could be made on samples that counted as little as 25 per cent above background.

RESULTS AND DISCUSSION

Previous studies of adenine incorporation in this laboratory have utilized Ad-4,6-C^{14} (10-12), whereas Ad-8-C^{14} or Ad-1,3-N^{15} has generally been used by other workers (5). Our present knowledge of the biosynthesis of the purine ring results chiefly from the research by Buchanan and co-workers (15-18) and Greenberg and co-workers (19-21), who have shown that formate is the precursor of the C-2 and C-8 positions of the purine ring. On the basis of these findings, it appears that the C-2 and/(or) C-8 position of the purine ring may be labile and able to equilibrate with an "active formate" of the biological system. We considered that these positions might be labile before the adenine had been incorporated into nucleotides or nucleic acids, in which case the $C^{14}O_2$ respiration curves from

Ad-2-C^{14} or Ad-8-C^{14} should indicate a relatively high initial rate of oxidation to $C^{14}O_2$, similar to that obtained with sodium formate.

The $C^{14}O_2$ respiration data obtained after administration of differently labeled adenines and of formate-C^{14} are shown in Fig. 1. $C^{14}O_2$ was obtained from each of the labeled adenine compounds. Marrian et al. (3) reported no radioactive CO_2 from Ad-8-C^{14} fed to rats, but the Ad-8-C^{14} that they fed had an activity of 3.4×10^4 cts./min./μM. compared with the 2.3×10^6 dis/min./μM. for the Ad-8-C^{14} we utilized, so that the small oxidation we observed may not have been detectable in their experiments. $C^{14}O_2$ from Ad-4,6-C^{14} is believed to arise primarily from the C-6 carbon when adenine is converted to allantoin. Thus, the 10 per cent CO_2 obtained in 22 hours from Ad-4,6-C^{14} represents 20 per cent oxidation of the purine ring to allantoin. The metabolic steps involved in the formation of $C^{14}O_2$ from Ad-2-C^{14} or Ad-8-C^{14} are not known. The $C^{14}O_2$ respiratory patterns obtained from Ad-2-C^{14} and Ad-8-C^{14} do not in any way resemble that obtained with formate-C^{14}, and thus a rapid exchange of the C-2 or C-8 position of free adenine with "formate" of the biological system is not indicated. The rate patterns of $C^{14}O_2$ excretion of the isomerically labeled adenines differ; Ad-2-C^{14} and Ad-8-C^{14} are initially oxidized relatively slowly. Since the most rapid excretion of $C^{14}O_2$ occurs from 2 to 6 hours after administration of the Ad-C^{14},

a time when free Ad-C^{14} is present in only trace quantities (12), the oxidation must be through secondary products of adenine metabolism. In humans, further oxidation of uric acid has been shown by recovery experiments using uric acid-N^{15} (22) and more directly using uric acid-2-C^{14} (23), so that purine ring oxidation at positions other than C-6 is not unique to the mouse.

Also, specific-activity curves obtained from Ad-C^{14} are anomalous in that the $C^{14}O_2$ specific activity does not exhibit a uniform decline from an early maximum shown by many compounds (i.e. glycine, formate, glucose); instead periods of increased specific activity are often observed 10 to 20 hours after adenine is injected. This may represent increased catabolism of nucleotides and nucleic acids associated with food intake or with cyclical phases of cell division.

In addition to determining differences in the $C^{14}O_2$ respiration curves after administration of the isomerically labeled adenines, we investigated possible differences in incorporation into nucleotides and nucleic acids during various time intervals after injection. Buchanan and Schulman have demonstrated in pigeon liver homogenates an exchange of formate with C-2 of inosinic acid, presumably due to an interconversion to 4-amino-5-imidazole carboxamide ribotide (17). Abrams and Bentley have presented evidence in rabbit bone marrow preparations that adenylic acid is converted to guanylic acid via inosinic acid and xanthylic

acid (24, 25). We have previously shown that adenine is extensively and rapidly incorporated into adenylic acid nucleotides and more slowly into guanylic acid nucleotides (10-12). After 3 days, the specific activity of the adenine nucleotides of the small intestine, large intestine, and carcass were similar to the specific activity of the corresponding RNA, and it was suggested that the renewal of RNA due to breakdown and resynthesis from nucleotides of the cell is more rapid than indicated by the disappearance of radioactivity from RNA. Therefore, if the postulated exchange of the C-2 or C-8 position of the purine ring occurs in mice, animals injected with Ad-2-C^{14} and (or) Ad-8-C^{14} should have less isotope incorporated into nucleotides and (or) nucleic acids than animals administered Ad-4,6-C^{14}. This difference should exist at 24 hours if the exchange occurs primarily with free purines, and should become larger with time if the exchange occurs at the nucleotide or nucleic acid level. If the reaction

$$\text{inosinic acid} \rightleftharpoons \text{4-amino-5-imidazole carboxamide ribotide} + \text{"formate"}$$

is the principal reaction responsible for ring lability (17), and if inosinic acid is an intermediate in the conversion of adenylic acid to guanylic acid, it may be expected that the guanine specific activity after injection of Ad-2-C^{14} will decrease more rapidly than after injection of Ad-4,6-C^{14}. This effect might be larger in tissues that have more rapid nucleic acid renewal, such as the small

and large intestines.

In our experiments, the specific activity of the soluble nucleotide adenine isolated as 5-AMP, RNA-Ad, RNA-Gu, DNA-Ad, and DNA-Gu have been determined for five tissues after administration of Ad-2-C^{14}, Ad-4,6-C^{14}, and Ad-8-C^{14}. The first experiment was designed to determine if any gross differences existed in the metabolism of these three isomerically labeled compounds, and single animals were utilized for four time intervals up to 28 days. The specific activities of the 5-AMP, RNA-Ad, and RNA-Gu are shown in Table I-A, and the specific activities of the DNA-Ad and DNA-Gu are presented in Table I-B. The percentage of the control (Ad-4,6-C^{14}) specific activity for each tissue fraction is also calculated.

No large consistent differences in the specific activities of the nucleotides or nucleic acids were found after administration of the isomerically labeled adenines. In only nine comparisons out of 82 was the ratio Ad-8-C^{14}/Ad-4,6-C^{14} less than 0.8, and these deviations appeared to be random and not associated with a particular tissue. No decrease of this ratio with time was observed. However, in more than 50 per cent of the comparisons made after 24 hours, the Ad-2-C^{14}/Ad-4,6-C^{14} ratio was less than 0.8; this was particularly true for liver and kidney RNA, and carcass DNA. It should be borne in mind that this first experiment was a pilot experiment with single animals to determine if any large differences existed in the mouse such as those

observed for the rat by Gordon (1), who reported data after feeding experiments from the pooled internal organs at one time interval.

As a result of these first experiments on the respiratory excretion of $C^{14}O_2$ and the incorporation of the isomarically-labeled adenines into nucleotides and nucleic acids, it was apparent that any ring lability and consequent differences in the metabolism of adenine would be small. Accordingly, a second experiment was made, using 6 pairs of littermates, in which the metabolism of Ad-2-C^{14} and Ad-4,6-C^{14} was more critically compared. Three pairs of animals were sacrificed at 24 hours to obtain early differences in incorporation and 3 pairs were sacrificed at 15 days to determine if any change in the Ad-2-C^{14}/Ad-4,6-C^{14} ratio was apparent. The results are presented in Tables II-A, -B and -C for the incorporation into nucleotide adenine, into RNA-Ad and RNA-Gu, and into DNA-Ad and DNA-Gu, respectively. The Ad-2-C^{14}/Ad-4,6-C^{14} ratio for the average specific activity obtained for each tissue fraction ranged from 0.88 to 1.15, with an average value of 1.02. No difference in the average ratio was observed between 1 and 15 days. It is therefore concluded that the C-2 position of the purine ring is not labile relative to the C-4,6 positions, either during the initial incorporation or subsequently, after incorporation into nucleotides and nucleic acids. Once the purine ring is broken, it is catabolized to end products which are excreted. Thus our results confirm and extend those obtained

by Abrams for rat liver RNA and by Marrian for rat visceral nucleic acids. Our results do not confirm those obtained by Gordon for the nucleic acids of the pooled visceral organs of the rat. It should be pointed out that he administered the adenine by feeding. However, a more important uncontrolled variable is believed to be that the comparison was made between data obtained by different workers in different laboratories.

The Ad/Gu ratios have been compared for RNA and DNA fractions of the various tissues at several times after administration of the adenine. In all cases, the ratios showed a large decrease with time, i.e., from 7 to 3 at 1 day to 1.1 to 1.2 at 28 days for the RNA of liver and small intestines. This ratio was similar for a given time interval and tissue fraction after injection of Ad-2-C^{14}, Ad-4,6-C^{14}, or Ad-8-C^{14}. This constitutes additional evidence that the purine ring is not labile during the conversion from adenine to guanine. A decrease in the Ad/Gu ratio has been observed previously in the RNA of regenerating rat liver injected with Ad-N^{15} (17) and in cytoplasmic RNA and nuclear RNA and DNA of mouse liver at periods up to 24 hours after injection of adenine (27). A similar decrease in the Ad/Gu ratio was observed for some but not for all fractions of rat RNA and DNA sacrificed 1 and 3 days after formate injection (28).[2] Swick et al. (30) have also presented evidence that the conversion of adenine to guanine in rat liver RNA is more rapid

than the reverse reaction.

Several processes can be suggested for the decrease in Ad/Gu ratio with time: (a) adenine may be converted to guanine in the intact nucleic acid; (b) guanine may be renewed independently of adenine in nucleic acids and may have a slower renewal; (c) nucleic acids rich in guanine may be renewed at a slower rate than those rich in adenine; and (d) adenine is slowly converted to guanine in the nucleotide pool (11), and this, combined with renewal of nucleic acids from this pool, leads to a decrease in the Ad/Gu ratio. Since the nucleic acids are probably synthesized, at least in part, from components of the acid-soluble nucleotide pool which are radioactive, the renewal of nucleic acids is more rapid than estimated by disappearance of radioactivity from the nucleic acids (10, 12). It is to be noted that the Ad/Gu ratio decreases more rapidly in RNA than it does in DNA, which is consistent with the concept that RNA is in relatively rapid equilibrium with the soluble-nucleotide pool, while DNA synthesis is associated with cell renewal. Suggestions similar to (b) and (c) were made by Bendich _et al._ (23) to explain the unequal retention of formate in DNA and RNA of rats. To us, the last mechanism (d) appears to be more consistent with known processes. It is further to be noted that in no case after adenine administration did the Ad/Gu ratio become less than unity as might be expected if any of the first three mechanisms were operative at a significant rate. Our

observations and interpretation of the mechanism of conversion of adenine to guanine in nucleic acids are not in agreement with the suggestion by Balis et al. (31) that conversion of adenine to guanine must occur within 24 hours after injection.

A preliminary investigation has been made of the effect of dosage upon the incorporation of adenine into nucleotides and nucleic acids. The results (Table III) are expressed as [(specific activity obtained with 0.12 mg. adenine)/(specific activity obtained with 1.2 mg. adenine)] x 100. Thus a value of 10 indicates that the incorporation was proportional to the dosage. The liver and kidney incorporated significantly larger amounts of adenine, and small intestine slightly less adenine, into RNA-Ad than expected on a proportional basis. This effect probably explains the reversal of the relative incorporation into liver and small intestine noted by different investigators (26,29,32,33). At high dosages more adenine is incorporated into intestinal RNA than into liver RNA, whereas at low dosages the converse is true. The strikingly decreased incorporation into carcass DNA (bone marrow) at the lower dosage is also to be noted. Incorporation into guanine was markedly decreased in all tissue fractions at the low dosage. This result is particularly apparent when the Ad/Gu ratios are compared. These observations on the incorporation of adenine at two dosages are consistent with the concept that in part the utilization of exogenous adenine represents in part a

detoxification mechanism. The primary conversion is to adenylic acid, probably by reaction with 5-phosphoribosylpyrophosphate (34). At higher dosages, the abnormally high amount of adenylic acid derivatives present leads to an increased amount of inosinic acid which is subsequently converted to guanylic acid. In addition, it is probable that adenine yields hypoxanthine prior to incorporation into nucleotides, and this free hypoxanthine is subsequently converted into guanylic acid derivatives. These reactions are probably particularly rapid in the kidney, and it should be noted that this organ has the lowest Ad/Gu ratio.

Other factors, in addition to amount of adenine administered, that influence the incorporation and — in particular — the conversion of adenine into guanine (Ad/Gu ratio) are the folic antagonists, aminopterin or A-methopterin. As shown in Table IV, a changed pattern of incorporation of Ad-2-C^{14} was noted in the drug-treated animals, the effect being different in the different tissues. In most tissues, an increased specific activity in RNA-Gu was observed, whereas only a slight effect was noted upon the specific activity of RNA-Ad. Similar effects were noted in the DNA of the intestines. One mg. of aminopterin appeared to be more effective than 4.5 mg. of A-methopterin. Skipper et al. (35) have noted a greatly decreased utilization of formate or CO_2 for nucleic acid synthesis after multiple aminopterin or A-methopterin injections, but obtained no effect upon CO_2 utilization shortly

after a single dose of aminopterin. They suggested that multiple injections of folic acid antagonists may be necessary to produce a folic acid deficiency. Our results were obtained after a single injection, but it should be noted that the dosage we used (40 mg. aminopterin/kg.) was five times the dosage they utilized. Goldthwait and Bendich (36) have observed a greatly decreased incorporation of formate into the nucleic acids of intestines and liver of rats after a single injection of aminopterin. In the rat, as in the mouse, the pattern of incorporation of adenine into nucleic acids was changed by aminopterin. They observed no change or an increased incorporation of adenine into guanine, while a decreased incorporation into adenine was generally noted. It should be pointed out that the changes in specific activity observed after drug administration are not necessarily correlated with increased or decreased rates of nucleic acid biosynthesis. Aminopterin, by depressing formate utilization, causes an increased utilization of adenine and, in particular, an increased conversion to guanine. In addition, increased specific activities are to be noted in the nucleotide adenine of the intestines. If RNA specific activities are related to the nucleotide specific activity, the data we obtained may actually be indicative of decreased nucleic acid synthesis in the intestines.[3]

SUMMARY

Possible lability of the purine ring during or after incorporation into nucleotides or nucleic acids in mice has been investigated with adenine-2-C^{14}, adenine-4,6-C^{14}, and adenine-8-C^{14}. No evidence was found for extensive ring lability. Radioactive CO_2 was obtained from each of the isomerically labeled adenine-C^{14} compounds, indicating oxidation of the purine ring at positions other than C-6.

A marked decrease in the adenine/guanine ratio with time has been noted. These data are in agreement with the concept that nucleic acids are synthesized from components of the acid-soluble nucleotide pool. It is further suggested that there is considerable reutilization of nucleic acid components.

The pattern of utilization of exogenous adenine is influenced by dosage and by aminopterin and A-methopterin.

The authors are greatly indebted to Professor Melvin Calvin for advice and encouragement during this investigation. Aminopterin and A-methopterin were gifts of Lederle Laboratories. The technical assistance of Miss Barbara Krueckel and Mrs. Ruth Deane is gratefully acknowledged. Dr. B. M. Tolbert and Mrs. Martha Kirk generously made available the $C^{14}O_2/C^{12}O_2$ analyzer and assisted greatly in making the measurements.

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FOOTNOTES

1 Adenine-2-C^{14} and adenine-8-C^{14} were purchased from Isotope Specialties Company, Burbank, California.

2 The evidence presented by Brown and Roll (5) for a decrease in the Ad/Gu ratio does not appear to be correct inasmuch as both groups of rats were sacrificed 24 hours after a series of three daily formate injections (28,29).

3 The concept that nucleic acids are broken down to and resynthesized from nucleotides can explain the differential effects of A-methopterin on formate-C^{14} and $P^{32}O_4$ incorporation discussed by Williams et al. (37), and also the differential effects on formate and adenine incorporation (36), without the suggestion that portions of nucleic acids are exchanged in the absence of synthesis.

* A preliminary account of this work has been presented at the International Conference on the Peaceful Uses of Atomic Energy (1955).

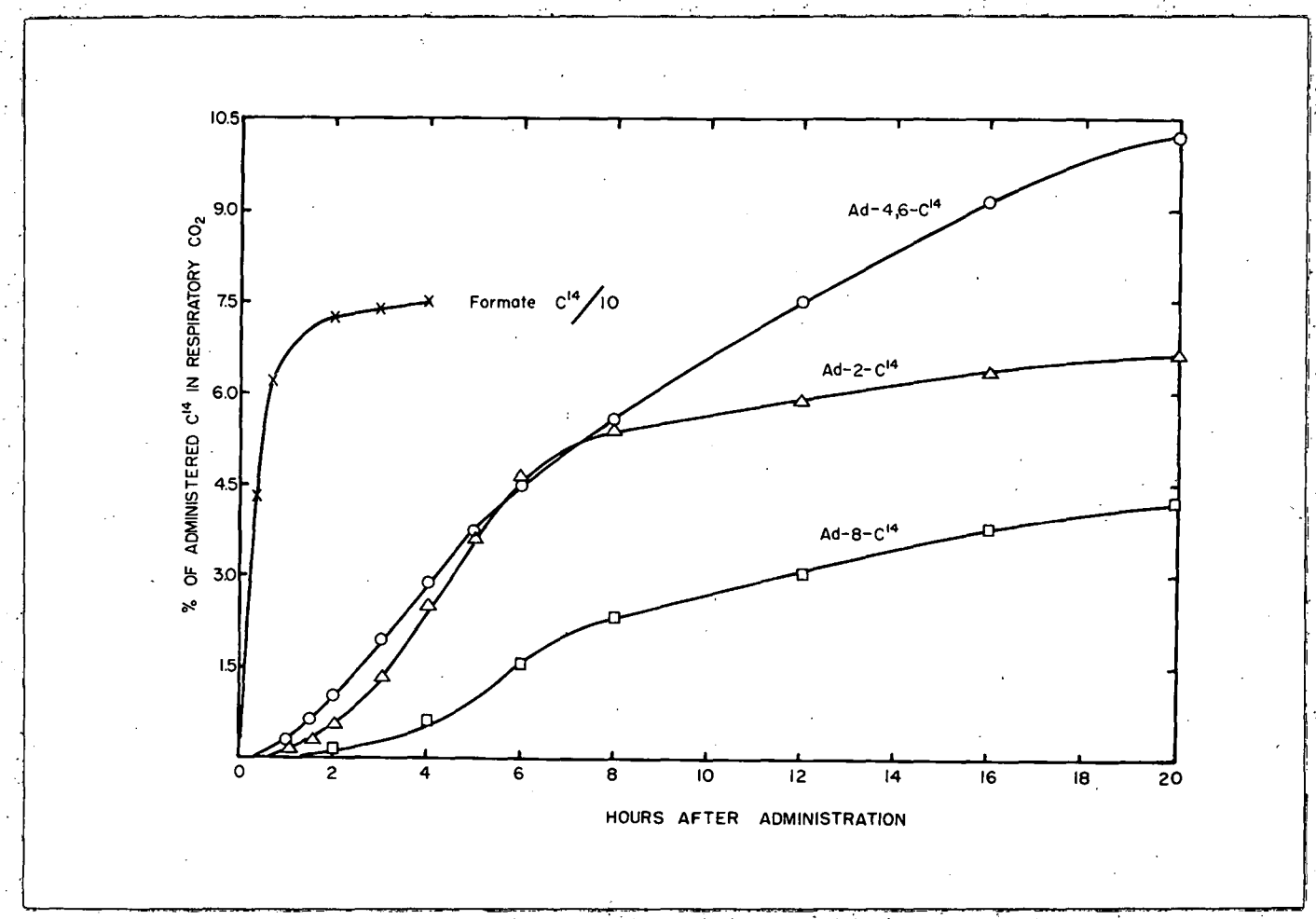

Figure 1. Percentage cumulative respiratory excretion of $C^{14}O_2$ from C_{57} male mice after administration of 1.2 mg. Ad-2-C^{14}, Ad-4,6-C^{14} or Ad-8-C^{14}. The cumulative excretion of formate-C^{14} has been divided by 10.

TABLE IA

Specific Activity of 5-Adenylic Acid and RNA-Adenine and Guanine in C_{57} Mice after Administration of Adenine-2-C^{14}, Adenine-4,6-C_2^{14} and Adenine-8-C^{14}*

Time after Injection (days)	Compound	5-Adenylic Acid s.a.	% Ad-4,6	RNA-Adenine s.a.	% Ad-4,6	RNA-Guanine s.a.	% Ad-4,6	Ratio Ad/Gu
				Small Intestine				
1	Ad-4,6-C_2^{14}	1410		890		112		7.9
	Ad-8-C^{14}	1350	96	755	85	107	96	7.1
	Ad-2-C^{14}	1350	96	790	89	100	89	7.9
8	Ad-4,6-C_2^{14}	60		60		22		2.7
	Ad-8-C^{14}	38	63	40	67	15	68	2.7
	Ad-2-C^{14}	66	110	60	100	20	91	3.0
15	Ad-4,6-C_2^{14}	18		26		13		2.0
	Ad-8-C^{14}	22	122	23	89	12	92	1.9
	Ad-2-C^{14}	22	122	24	92	11	85	2.2
28	Ad-4,6-C_2^{14}	4.7	1	5.8		4.9		1.2
	Ad-8-C^{14}	5.7	121	6.7	116	6.1	124	1.1
				Large Intestine				
1	Ad-4,6-C_2^{14}	1260		990		185		5.3
	Ad-8-C^{14}	1390	110	1160	117	188	102	6.2
	Ad-2-C^{14}	1380	109	945	95	180	97	5.3
8	Ad-4,6-C_2^{14}	92		87		36		2.4
	Ad-8-C^{14}	72	78	68	78	29	81	2.3
	Ad-2-C^{14}	110	120	102	117	38	105	2.7
15	Ad-4,6-C_2^{14}	30		33		24		1.4
	Ad-8-C^{14}							
	Ad-2-C^{14}	36	120	30	91	21	87	1.4
28	Ad-4,6-C_2^{14}	13		11		7.8		1.4
	Ad-8-C^{14}	14	108	13	118	9.0	115	1.4
				Carcass				
1	Ad-4,6-C_2^{14}	205		256		37		6.9
	Ad-8-C^{14}	203	99	236	108	32	86	7.4
	Ad-2-C^{14}	194	94	228	89	33	89	6.9
8	Ad-4,6-C_2^{14}	149		171		50		3.4
	Ad-8-C^{14}	136	91	160	94	49	98	3.3
	Ad-2-C^{14}	159	107	151	89	44	88	3.4
15	Ad-4,6-C_2^{14}	144		158		48		3.3
	Ad-8-C^{14}	135	94	148	93	54	112	2.7
	Ad-2-C^{14}	136	95	117	74	38	79	3.1
28	Ad-4,6-C_2^{14}	100		90		40		2.2
	Ad-8-C^{14}	104	104	92	102	42	105	2.2
				Liver				
1	Ad-4,6-C_2^{14}	1270		445		61		7.3
	Ad-8-C^{14}	1240	98	400	90	51	84	7.8
	Ad-2-C^{14}	1250	98	420	94	45	74	9.3
8	Ad-4,6-C_2^{14}	230		485		138		3.5
	Ad-8-C^{14}	200	87	390	80	114	83	3.4
	Ad-2-C^{14}	162	70	340	70	104	75	3.3
15	Ad-4,6-C_2^{14}	77		240		119		2.0
	Ad-8-C^{14}	81	105	255	107	116	97	2.2
	Ad-2-C^{14}	56	73	166	69	86	72	1.9
28	Ad-4,6-C_2^{14}	14		37		34		1.1
	Ad-8-C^{14}	14	100	41	111	39	115	1.1
				Kidney				
1	Ad-4,6-C_2^{14}	865		480		115		4.2
	Ad-8-C^{14}	985	114	525	109	117	102	4.5
	Ad-2-C^{14}	935	108	370	77	103	90	3.6
8	Ad-4,6-C_2^{14}	195		385		215		1.8
	Ad-8-C^{14}	149	77	425	110	187	87	2.3
	Ad-2-C_{14}	158	81	255	66	151	70	1.7
15	Ad-4,6-C_2^{14}	83		215		140		1.5
	Ad-8-C^{14}	90	108	185	86	107	77	1.7
	Ad-2-C^{14}	59	71	120	56	85	61	1.4
28	Ad-4,6-C_2^{14}	25		58		47		1.2
	Ad-8-C^{14}	25	100	57	99	53	113	1.1

* Male C_{57} mice, age 4 to 5-1/2 months, weight about 25 gm., were administered 1.2 mg. of adenine-4,6-C_2^{14}, or adenine-2-C^{14}, specific activity 1.8×10^4 dis./μg. The specific activities of the guanine have been converted to the gamma equivalent of adenine. The mice were sacrificed at the indicated time after administration of the adenine.

TABLE IB

Specific Activity of DNA-Adenine and Guanine in C_{57} Mice after Administration of Adenine-2-C^{14}, Adenine-4,6-C_2^{14} and Adenine-8-C^{14}*

Time after Injection (days)	Compound	DNA-Adenine	%Ad-4,6	DNA-Guanine	%Ad-4,6	Ratio Ad/Gu
			Small Intestine			
1	Ad-4,6-C_2^{14}	465		85		5.5
	Ad-8-C^{14}	400	86	78	92	5.1
	Ad-2-C^{14}	445	96	71	84	6.3
8	Ad-4,6-C_2^{14}	29		6.8		4.3
	Ad-8-C^{14}	24	83	5.9	87	4.1
	Ad-2-C^{14}	20	69	4.8	71	4.2
15	Ad-4,6-C_2^{14}	14		4.9		2.9
	Ad-8-C^{14}	16	114	4.1	84	3.9
	Ad-2-C^{14}	11	72	4.8	98	2.3
28	Ad-4,6-C_2^{14}	7.9		2.5		3.2
	Ad-8-C^{14}	9.2	116	3.3	132	2.8
			Large Intestine			
1	Ad-4,6-C_2^{14}	420		63		6.7
	Ad-8-C^{14}	405	96	65	103	6.2
	Ad-2-C^{14}	425	101	55	87	7.7
8	Ad-4,6-C_2^{14}	47		9.9		4.7
	Ad-8-C^{14}	30	64	8.4	85	3.6
	Ad-2-C^{14}	59	125	12.6	127	4.7
15	Ad-4,6-C_2^{14}	17		4.9		3.5
	Ad-8-C^{14}	--	--	--	--	--
	Ad-2-C^{14}	11	65	3.3	67	3.3
28	Ad-4,6-C_2^{14}	11		4.3		2.6
	Ad-8-C^{14}	7.3	66	2.7	63	2.7
			Carcass			
1	Ad-4,6-C_2^{14}	178		33		5.4
	Ad-8-C^{14}	149	84	36	109	4.1
	Ad-2-C^{14}	127	71	26	79	4.9
8	Ad-4,6-C_2^{14}	22		9.9		2.2
	Ad-8-C^{14}	15	68	7.6	77	2.0
	Ad-2-C^{14}	16	73	7.2	74	2.2
15	Ad-4,6-C_2^{14}	13		4.1		3.2
	Ad-8-C^{14}	11	85	4.2	102	2.6
	Ad-2-C^{14}	5.5	42	2.0	49	2.7
28	Ad-4,6-C_2^{14}	8.5		4.1		2.1
	Ad-8-C^{14}	7.6	90	3.4	83	2.2

* See footnote to Table IA.

TABLE IIA

Specific Activity of 5-AMP of Mice Injected with Adenine-2-C^{14} or Adenine-4,6-C^{14}*

Time after Injection (Days)	Small Intestine		Large Intestine		Carcass		Liver		Kidney	
	Adenine 4,6-C^{14}	Adenine 2-C^{14}	Adenine 4,6-C^{14}	Adenine 2-C^{14}	Adenine 4,6-C^{14}	Adenine 2,-C^{14}	Adenine 4,6-C^{14}	Adenine 2-C^{14}	Adenine 4,6-C^{14}	Adenine 2-C^{14}
1	1295	1495	1085	1180	270	220	690	890	830	925
	1475	1345	1495	1180	260	235	1095	1210	1140	1095
	1185	1305	905	1230	245	270	1115	930	865	1035
Average	1318	1382	1162	1197	258	242	967	1010	945	1018
Ad-2-C^{14}/Ad-4,6-C^{14}	1.05		1.03		0.94		1.04		1.08	
15	16	21	--	41	146	174	68	57	59	75
	25	19	45	35	155	119	52	70	77	78
	19	17	21	37	102	88	58	76	67	79
Average	20	19	33	38	134	127	59	68	68	77
Ad-2-C^{14}/Ad-4,6-C^{14}	0.95		1.15		0.95		1.15		1.13	

* Male C_{57} mice, age 3-1/2 months, weight about 25 gm. were administered 12 mg. of adenine-4,6-C^{14} or adenine-2-C^{14}, specific activity 1.8×10^4 dis./μg. The specific activities of the guanine have been converted to the gamma equivalent of adenine. The mice were sacrificed at one or fifteen days after administration of the adenine. The RNA-kidney samples were lost.

TABLE IIB

Specific Activity of RNA-Adenine and Guanine of Mice Injected with Adenine-2-C^{14} or Adenine-4,6-C_2^{14} *

Time after Injection	Adenine-4,6-C_2^{14}			Adenine-2-C^{14}			Adenine-4,6-C_2^{14}			Adenine-2-C^{14}		
	Adenine s.a.	Guanine s.a.	Ratio	Adenine s.a.	Guanine s.a.	Ratio	Adenine s.a.	Guanine s.a.	Ratio	Adenine s.a.	Guanine s.a.	Ratio
		Small Intestine						Large Intestine				
1 day	650	104	6.2	660	109	6.0	830	157	5.3	880	189	4.7
	660	101	6.5	680	94	7.2	975	205	4.8	900	166	5.4
	545	86	6.3	605	87	6.9	625	123	5.1	830	141	5.9
Average	618	97	6.4	648	97	6.7	810	162	5.0	870	165	5.3
Ad-2-C^{14}/Ad-4,6-C_2^{14}	1.05	1.00					1.07	1.02				
15 days	21	10	2.1	24	12	2.0	30	22	1.4	32	22	1.5
	29	14	2.1	24	13	1.9	39	29	1.3	27	18	1.5
	28	15	1.9	23	12	2.0	23	18	1.3	31	20	1.6
Average	26.0	13.0	2.0	23.7	12.3	1.9	30.7	23.0	1.3	30.0	20.0	1.5
Ad-2-C^{14}/Ad-4,6-C_2^{14}	0.91	0.95					0.99	0.88				
		Carcass						Liver				
1 day	230	38	6.1	195	41	4.8	255	39	6.5	310	39	8.0
	225	47	4.8	205	39	5.3	350	61	5.7	415	65	6.4
	220	43	5.1	200	40	5.0	375	59	6.4	370	42	8.8
Average	225	42.7	5.3	200	40.0	5.0	327	53.0	6.2	365	48.7	7.5
Ad-2-C^{14}/Ad-4,6-C_2^{14}	0.89	0.94					1.11	0.92				
15 days	146	46	3.2	160	58	2.8	154	93	1.7	140	84	1.7
	142	52	2.7	124	45	2.8	154	88	1.7	182	91	2.0
	106	44	2.4	109	47	2.3	170	76	2.2	191	96	2.0
Average	131	47.3	2.8	131	50.6	2.6	159	85.7	1.9	171	90.3	1.9
Ad-2-C^{14}/Ad-4,6/C_2^{14}	1.00	1.06					1.08	1.05				

* See footnote to Table IIA

TABLE IIC

Specific Activity of DNA Adenine and Guanine of Mice ~~of Mice~~ Injected with Adenine-2-C^{14} or Adenine 4,6-C_2^{14} *

Time after Injection	Adenine-4,6-C_2^{14} Adenine s.a.	Adenine-4,6-C_2^{14} Guanine s.a.	Ratio	Adenine-2-C^{14} Adenine s.a.	Adenine-2-C^{14} Guanine s.a.	Ratio	Adenine-4,6-C_2^{14} Adenine s.a.	Adenine-4,6-C_2^{14} Guanine s.a.	Ratio	Adenine-2-C^{14} Adenine s.a.	Adenine-2-C^{14} Guanine s.a.	Ratio
	Small Intestine						Large Intestine					
1 day	360	62	5.8	280	48	5.8	440	75.5	5.9	410	67	6.1
	305	58	5.3	350	50	7.0	340	67	5.1	390	78	5.0
	255	41	6.2	245	45	5.5	295	42	7.0	310	61	5.1
Average	307	55.7	5.7	292	47.7	6.1	358	61.3	5.8	370	68.7	5.4
Ad-2-C^{14}/Ad-4,6-C_2^{14}	0.95	0.89					1.03	1.12				
15 days	13	4.2	3.1	11	4.1	2.7	11	7.5	1.5	15	7.5	2.0
	11	4.0	2.8	11	3.7	3.0	15	8.0	1.9	16	7.8	2.1
	15	5.4	2.9	15	5.2	2.9	15	6.0	2.5	19	8.7	2.2
Average	13.0	4.5	2.9	12.3	4.3	2.9	13.7	7.2	1.9	16.7	8.0	2.1
Ad-2-C^{14}/Ad-4,6-C_2^{14}	0.95	0.95					1.25	1.11				
	Carcass						Liver					
1 day	138	28	4.9	138	23	6.0	11	2.6	4.3	15	2.8	5.4
	154	33	4.7	148	25	5.9	14	1.9	7.4	11	1.9	5.8
	142	28	5.1	122	25	4.9	14	2.5	5.6	9.4	2.2	4.3
Average	145	29.7	4.9	136	24.3	5.6	13.0	2.3	5.7	11.8	2.3	5.2
Ad-2-C^{14}/Ad-4,6-C_2^{14}	0.94	0.82					0.91	1.00				
15 days	13	8.2	1.6	13	4.8	2.7	21	8.0	2.6	22	7.6	2.9
	10	5.5	1.8	12	4.6	2.6	15	6.5	2.3	23	11	2.1
	13	4.5	2.9	13	6.0	2.2	42	18	2.3	40	17	2.4
Average	12.0	6.4	1.9	12.7	5.1	2.5	26.0	10.8	2.4	28.3	11.8	2.4
Ad-2-C^{14}/Ad-4,6-C_2^{14}	1.06	0.80					1.09	1.09				

* See footnote to Table IIA.

TABLE IIC (continued)*

Time after Injection	Adenine-4,6-C_2^{14} Adenine s.a.	Adenine-4,6-C_2^{14} Guanine s.a.	Adenine-4,6-C_2^{14} Ratio	Adenine-2-C^{14} Adenine s.a.	Adenine-2-C^{14} Guanine s.a.	Adenine-2-C^{14} Ratio
		Kidney				
1 day	11	4.2	2.6	8.2	3.0	2.7
	13	3.6	~~3.6~~	11	3.0	3.7
	8.3	4.1	3.0	8.4	3.4	2.4
Average	10.8	4.0	2.7	9.2	3.1	2.9
Ad-2-C^{14}/Ad-4,6-C^{14}	0.85	0.78				
15 days	26	15	1.7	22	7.3	2.0
	--	11	--	14	7.0	2.1
	17	7.6	2.3	17	9.3	1.8
Average	21.5	11.2	1.9	17.7	7.8	2.3
Ad-2-C^{14}/Ad-4,6-C_2^{14}	0.82	0.69				

* See footnote to Table TIA.

TABLE III

Effect of Dosage upon Incorporation of Adenine-4,6-C_2^{14} into Nucleotides and Nucleic Acids of C_{57} Male Mice*

Tissue	Amount of Adenine-4,6-C_2^{14}	% of Control				% of Control		
		5-Adenylic Acid	RNA Adenine	RNA Guanine	Ad/Gu Ratio	DNA Adenine	DNA Guanine	Ad/Gu Ratio
Small Intestine	1.2 mg.	[illegible]	[illegible]	[illegible]	7.9			5.5
	0.12 mg.	10	8.5	4.1	16	7.1	2.8	14
Large Intestine	1.2 mg.				5.9			6.8
	0.12 mg.	17	12	5.7	13	11	3.2	23
Liver	1.2 mg.				8.3			--
	0.12 mg.	18	17	7.5	20	--	--	--
Carcass	1.2 mg.				8.1			5.2
	0.12 mg.	13	11	6.0	13	2.6	<2	>8
Kidney	1.2 mg.				3.2			6.3
	0.12 mg.	21	28	16	6.0	22	<25	>7

* 1.2 mg. or 0.12 mg. of adenine-4,6-C_2^{14} was administered by intraperitoneal injection to two male C_{57} mice. They were sacrificed 24 hours later. To calculate approximate specific activities, refer to the 24-hour adenine-4,6-C_2^{14} data in Table II-A,B and C.

TABLE IV

Effect of Aminopterin and A-Methopterin upon the Incorporation of Adenine-2-C^{14} into Nucleotides and Nucleic Acids of C_{57} Male Mice*

Tissue	Treatment	5-Adenylic Acid % Control	RNA Adenine % Control	RNA Guanine % Control	Ad/Gu Ratio	DNA Adenine % Control	DNA Guanine % Control	Ad/Gu Ratio
Small Intestine	Control				7.9			6.3
	Aminopterin	175	138	168	6.5	93	138	4.2
	A-Methopterin	129	97	100	7.7	73	106	4.3
Large Intestine	Control				5.3			7.7
	Aminopterin	152	131	191	3.6	83	180	3.5
	A-Methopterin	132	112	139	4.2	98	169	4.5
Liver	Control				9.3			
	Aminopterin	66	86	138	5.8			
	A-Methopterin	84	81	105	[illegible].2			
Carcass	Control				6.9			4.9
	Aminopterin	87	104	90	5.2	91	96	4.6
	A-Methopterin	101	90	80	7.6	94	88	5.1
Kidney	Control				3.6			
	Aminopterin	100	118	154	2.8			
	A-Methopterin	150	134	153	3.2			

* 1.0 mg. of aminopterin or 4.5 mg. of A-methopterin was injected into the mouse 30 min. prior to the intraperitoneal administration of 1.2 mg. of adenine-2-C^{14}. The mice were sacrificed 24 hrs. later. To calculate specific activity, refer to the 24-hour adenine-2-C^{14} data in Table I - A, B and C.

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