RENEWAL OF NUCLEOTIDES AND NUCLEIC ACIDS IN C_{57} MICE STUDIED WITH ADENINE-4,6-$^{14}C_1$*

by

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INTRODUCTION

Rapid utilization of adenine-4,6-$^{14}C_1$ in C_{57} mice has been shown[1]. Adenine was extensively and rapidly incorporated into derivatives of 5-adenylic acid and into nucleic acids. Results indicated that compounds present in the fraction soluble in cold trichloroacetic acid (TCA) were possible precursors of nucleic acids. Therefore, it seemed desirable to investigate the nature and interconversions of these compounds in more detail, particularly with reference to the equilibration of adenine among soluble nucleotides and the separated ribonucleic acid (RNA) and desoxyribonucleic acid (DNA).

Data presented in this and the following paper[2] confirm the previously reported extensive utilization of adenine for the formation of at least eight nucleotide derivatives in the mouse. Specific activities of cold TCA-soluble 5-adenylic acid derivatives, RNA adenine and DNA adenine in nine tissues of the mouse are compared from 2 hours to 16–29 days after administration of adenine-^{14}C. Apparent half-lives of 5-adenylic acid adenine, RNA adenine and DNA adenine are calculated in these tissues. Evidence is presented that RNA and DNA adenine may be derived from the soluble-nucleotide pool. Results show that nucleic acids and 5-adenylic acid in a given tissue are not in equilibration with those in other tissues.

METHOD

Administration of adenine and fractionation of tissue

Adult male C_{57} mice, weight 28 to 30 g, age 6 to 7 months, were injected intraperitoneally with 1.3 mg of adenine-4,6-$^{14}C_1$[3] ($2.2 \cdot 10^7$ dis/min) dissolved in 0.5 ml of 0.9% saline. Animals were allowed to feed *ad libitum* both before and during the course of the experiment on Purina laboratory chow. Mice were sacrificed by decapitation and the organs were quickly removed and fractionated. The following organs were studied: skinned carcass (including bone)**, liver, small intestine, large intestine (in Series II, the intestines were combined), stomach, kidney, lung, heart, and spleen and testis.

The carcass was homogenized in a Waring blender with cold 10% trichloroacetic acid (TCA) to extract soluble nucleotides; a Potter homogenizer was used to homogenize the other tissues. To ensure complete extraction of cold TCA-soluble material, each tissue was extracted 6 times with

* A preliminary report of this work was presented at the American Chemical Society meeting, September 6–11, 1953: Abstracts of Papers, page 5C.

** The carcass fraction included the muscle and bone of the decapitated, eviscerated, skinned carcass; feet and tail were discarded. It should be emphasized that the carcass fraction used in this study consists of two very dissimilar fractions, muscle and bone marrow. Due to the small amount of tissue available, the spleen and testis were pooled. Intestines and stomach were rinsed with saline.

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10 volumes of cold 10% TCA, but generally only the first two extracts were saved. The TCA was removed by continuous liquid-liquid extraction with ether, and the remaining aqueous phase was analyzed. The cold TCA-extracted tissue was washed twice with ethanol and twice with ether and then treated with 1 *N* KOH at 37° for 24 hours[1]. The RNA was obtained in the supernatant after acidification with 70% $HClO_4$. The DNA-protein-salt precipitate was washed with dilute $HClO_4$ and the washing was added to the main RNA fraction. The precipitate was treated with KOH and $HClO_4$ two additional times, as described above, to free DNA completely of RNA contamination*.

The RNA extracts were neutralized with 4 *N* KOH and the supernatants were analyzed. Generally only the first KOH extract and washing were used, although total activity present and UV absorption in supernatants from reprecipitations of DNA-protein were determined as a check upon the washing procedure.

DNA was extracted from the DNA-protein precipitate with hot 10% TCA, and TCA was removed from this extract by continuous liquid-liquid extraction with ether.

Determination of specific activity of nucleotide and nucleic acid adenine

Total 5-AMP. The specific activity of soluble nucleotides hydrolyzable with $Ca(OH)_2$ to 5'-adenylic acid (5-AMP) in each fraction, referred to as "total 5-AMP" or "acid-soluble 5'-adenylic acid" was obtained as previously described[1]. Generally, duplicate hydrolyses, chromatograms and enzymic analyses were made.

RNA and DNA adenine. To concentrate RNA adenine and to separate it from salt present, the nucleotides were hydrolyzed to free purines by heating a suitable aliquot of the RNA fraction at 100° for 1 h, in 1 *N* HCl. The purines were adsorbed on 0.2 ml of Darco G-60 charcoal and then eluted with two 4-ml portions of 73% ethanol-24% water-3% conc. NH_4OH. The combined eluates were evaporated to dryness and the residue was dissolved in a small amount of 4% acetic acid and chromatographed on Whatman No. 1 filter paper[1]. Adenine was eluted and the specific activity was determined with xanthine oxidase[1].

DNA adenine was adsorbed on Darco G-60 charcoal after acidification of the solution with dilute acetic acid. Thereafter, the procedure was identical to that described for RNA adenine.

Duplicate determinations were usually made for both RNA and DNA adenine. The specific activities determined generally agreed within 5 to 10% except for tissues or time intervals where the specific activity was low (10 dis/min/μg adenine), in which case duplicate values occasionally differed by as much as 25%.

Counting procedures

Direct plating techniques were used for the fractions described. Duplicate samples and duplicate counts were made of all samples. Suitable self-absorption corrections were applied to cold TCA, RNA and DNA fractions. For RNA and DNA samples before chromatography, self-absorption corrections were large (20 to 50%), therefore values for the total amount of radioactivity incorporated are approximate. After chromatography, no self-absorption corrections were necessary.

Radioactivity measurements were made with a Tracerlab SC-16 windowless flow-type counter using platinum dishes approximately 3.7 cm^2 in area. Counting efficiency was approximately 55%; background was equivalent to 35 dis/min.

RESULTS

Two series of mice were used for the experiments described. Series I consisted of 8 mice sacrificed at 2 hours, 6 hours, 1, 2, 3, 7, 16 and 29 days after adenine-4,6-$^{14}C_1$ was administered. Mice in Series II were sacrificed at 2 hours, 1, 3, 7, and 16 days. Data are reported in detail for the first series only, inasmuch as the results of the two series of experiments are in general agreement.

In Table I are recorded the specific activities of the acid-soluble 5'-adenylic acid, RNA adenine and DNA adenine. The per cent of administered adenine incorporated into each of these fractions in individual tissues at the time of maximal incorporation is summarized. "Apparent half-life" of adenine in these fractions is also given for the

* X-irradiation experiments, to be reported in another communication, have indicated that two precipitations and washings of the DNA-protein are barely adequate to free DNA from traces of contaminating RNA, whereas three precipitations remove all, or essentially all, the contaminating RNA from DNA. This is extremely important in a tissue such as liver where the ratio of specific activity of RNA to DNA may be as high as 20 to 30.

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tissues studied. Values for the half-life of adenine in the total 5-AMP, RNA, and DNA have been obtained by plotting specific activity of adenine as a function of time on semilogarithmic graph paper (Fig. 1). Generally two component rates could be obtained. The slower rate component was calculated from data obtained from 7 to 16 days or 7 to 29 days after adenine injection. This curve was graphically extrapolated to zero time, and specific activities of the slow component were subtracted from total specific activities found at early time intervals. The resulting values were plotted and a graphical

TABLE I

SPECIFIC ACTIVITY AND HALF-LIFE OF RNA AND DNA ADENINE AND 5-ADENYLIC ACID IN VARIOUS TISSUE

(dis/min/μg Adenine)

Time after injection	*Small intestine*			*Large intestine*		
	5-Adenylic acid	*RNA adenine*	*DNA adenine*	*5-Adenylic acid*	*RNA adenine*	*DNA adenine*
2 hours	2690	424	109	2120	475	112
6 hours	2280	662	254	1970	697	240
24 hours	1355	883	465	1200	950	342
48 hours	472	495	343	710	650	455
72 hours	278	256	176	376	360	225
7 days	81	79	29	108	97	48
16 days	22	31	12	33	28	14
29 days	4.6	6.5	(7.2)*	—	—	—
Half-life	18 h	17 h	21 h	23 h	19 h	18 h
(Series I)	5.6 days	6.0 days	7.0 days	5.5 days	5.0 days	5.0 days
% "Fast components"	94 %	93 %	89 %	89 %	83 %	79 %
% Incorp. at max.	7.9 %	3.6 %	1.8 %	3.1 %	1.14 %	0.53 %
(Series II)**	23 h	19 h	24 h			
	4.0 days	5.8 days	7.7 days			
% "Fast component"	92 %	82 %	86 %			

Time after injection	*Carcass*			*Liver*		
	5-Adenylic acid	*RNA adenine*	*DNA adenine*	*5-Adenylic acid*	*RNA adenine*	*DNA adenine*
2 hours	278	160	109	1600	133	5.0
6 hours	221	204	196	1540	225	10
24 hours	205	229	162	990	385	18
48 hours	200	210	116	585	306?	15
72 hours	179	193	81	445	387	18
7 days	150	151	24	215	330	15
16 days	87	89	6.5	75	105	11
29 days	(83)*	(65)*	(7.2)*	11.4	26	(10.9)*
Half-life	—	—	35 h	17 h	—	—
(Series I)	12 days	12 days	7.0 days	5.2 days	5.5 days	16 days?
% "Fast component"	—	—	82 %	71 %	—	—
% Incorp. at max.	11 %	1.3 %	0.9 %	8.8 %	1.4 %	0.03 %
(Series II)	22 h	—	27 h	18 h	—	—
	12.7 days	15.3 days	7.3 days	6 days	9 days	—
% "Fast component"	65 %	—	82 %	67 %	—	—

(continued overleaf)

TABLE I (continued)

Time after injection	Lungs			Kidneys		
	5-Adenylic acid	RNA adenine	DNA adenine	5-Adenylic acid	RNA adenine	DNA adenine
2 hours	1400	259	17	1460	172	1.6
6 hours	1295	306	15	1860	332	4.2
24 hours	1075	450	48	1230	549	15.5
48 hours	660	530	46	705	540	36.3
72 hours	440	400	36	525	512	41
7 days	195	305	15	208	343	30
16 days	78	166	11	53	104	24
29 days	—	—		21	35	21
Half-life	24 h	—	~32 h	22 h	—	—
(Series I)	7 days	10.3 days	~20 days	4.8 days	5.5–7.0 days	~40 days
% "Fast component"	80 %	—	—	75 %		—
% Incorp. at max.	0.62 %	0.11 %	0.01 %	3 %	0.44 %	0.03 %

Time after injection	Stomach			Spleen and testes		
	5-Adenylic acid	RNA adenine	DNA adenine	5-Adenylic acid	RNA adenine	DNA adenine
2 hours	1890	287	36	865	64	13
6 hours	1660	530	93	765	80	33
24 hours	965	676	177	645	123	31
48 hours	635	880	272	455	147	37
72 hours	470	438	143	393	135	29
7 days	215	153	52	186	128	33
16 days	82	67	21	76	72	25
Half-life	18–24 h	15 h	16 h	28 h	—	—
(Series I)	6.5 days	7.5 days	6.8 days	7.7 days	11–14 days	—
% "Fast component"	70–80 %	~60 %	68 %	70 %		—
% Incorp. at max.	0.75 %	0.25 %	0.09 %	1.4 %	0.48 %	0.06 %
(Series II)	12–17 h	—	—	26 h	—	—
	8 days	—	~10 days	11.5 days	14–19 days	—
% "Fast component"	83–87 %	—	—	71 %		—

Time after injection	Heart		
	5-Adenylic acid	RNA adenine	DNA adenine
2 hours	257?	—	2.5
6 hours	220	—	8
24 hours	206	—	8
48 hours	195	—	5
72 hours	176	—	12
7 days	117?	—	7
Half-life	~11 days	—	—
(Series I)			
% Incorp. at max.	0.14 %	0.01 %	< 0.01 %

* Values in parentheses not used to calculate "half-life".
** Large and small intestine.

representation of the rapidly metabolized component was obtained. This is illustrated in the inset of Fig. 1. The approximate per cent of the activity incorporated into the "slow" and "rapid" components has been calculated from the "time = 0" values of component curves of the total 5-AMP, and from the ratio of specific activity of the "slow" curve to total specific activity at the time of maximum specific activity for the RNA and DNA. These values are summarized in Table 1 for both series of animals.

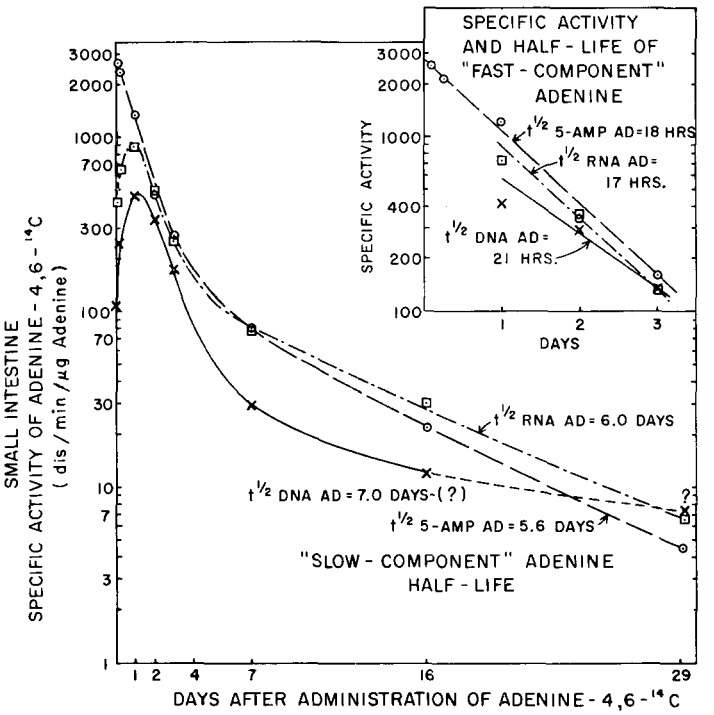

Fig. 1. Specific activity and half-life of 5'-adenylic acid, RNA, and DNA adenine of small intestine of mice from 2 hours to 29 days after administration of adenine-^{14}C. Specific activity and half-life of "fast component" adenine shown in inset.

DISCUSSION

Incorporation of phosphate, glycine, formate, 4-amino-5-imidazole carboxamide, orotic acid, adenine and other precursors into nucleic acids of rats and mice has been studied by numerous investigators. It has been shown that these compounds can be utilized for the formation of nucleic acids, but data for distribution of the administered precursor in separate tissues and in various compounds into which they are incorporated are limited. In many experiments pooled tissues or pooled nucleic acids have been investigated; incorporation of precursor into low molecular weight nucleotides has been essentially neglected. The few kinetic studies have usually been confined to one tissue or to limited time intervals. Extensive kinetic data obtained with ^{32}P-phosphate, particularly in respect to relative incorporation into RNA in cellular fractions of liver[5,6,7,8,9] and carcinoma[9,10] have shown that nuclear RNA incorporates ^{32}P-phosphate more

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rapidly than other cellular fractions. Similar results have been obtained with glycine[9,11], formate[11,12], orotic acid[13,14,15] and adenine[1,16,17].

Experiments from which an "apparent rate of renewal" of nucleic acids can be calculated are limited. The earliest studies of nucleic acid renewal were those of HEVESY and co-workers[18]. A *minimum* apparent daily renewal rate for the DNA ranging from 15% in the mucosa of small intestine to 0.6% in kidney and brain was calculated from the incorporation of ^{32}P-phosphate into DNA of the rat 4 days after administration of ^{32}P. Other experiments compared the incorporation of ^{32}P-phosphate into RNA and DNA of tissues 2 hours after a single administration. The RNA/DNA specific activity ratio ranged from 33 for liver to 2 for intestine[19]. From a detailed analysis of ^{32}P specific activity in the nucleic acid of cellular fractions of mouse carcinoma, BARNUM and co-workers[10] have concluded that phosphorus renewal in the RNA fraction was rapid, from 38% to 150%/h. It was also calculated that phosphorus renewal of DNA was 5%/h, or twice the net increase of DNA.

Estimates of renewal rates of nucleic acid have usually been based upon rate of uptake of ^{32}P-phosphate and assumptions must be made of the nature and specific activity of the immediate precursor. When precursors of nucleic acids such as adenine, orotic acid or glycine are administered, renewal rates based upon their rate of incorporation have several limitations. Assumptions must be made concerning the route by which the precursor is incorporated. Although some adenine may enter RNA directly, orotic acid and glycine must go through intermediates. The specific activity of these intermediates must be known in order to calculate more than the relative renewal rates. Even this calculation is probably misleading, as specific activity of the precursor varies from tissue to tissue, especially as a function of time. A short time after administration of orotic acid or adenine, they are converted into other compounds, primarily uridylic[13,14] or adenylic acid derivatives[1,2], so little or no free orotic acid or adenine is present. Renewal rates based upon uptake must necessarily assume that the exclusive source of the "building blocks" during the time interval studied is the labelled precursor, a situation not likely to be realized with either adenine or orotic acid for longer than several hours after administration[1,2,13]. Experiments in which adenine is administered over a several-day interval, and in which the ratio of specific activity found in the nucleic acid adenine to that administered is reported only for a single time after administration, can give only a minimal value for nucleic acid "turnover"[20,21]. Long-term experiments with adenine[20,21] and formate[22] in which incorporation has been determined at only two time intervals after administration, although permitting a comparison of "apparent retention" to be made in the tissues studied, do not yield sufficient data to calculate a valid "half-life".

From specific activities of adenine and guanine in liver RNA and DNA determined 4, 7 and 12 days after glycine-^{14}C was administered to rats, a half time of about 220 hours was obtained for adenine and guanine of RNA and 150 to 160 hours for that of DNA[23]. The complex nature of specific activity-time relationships in various cellular fractions of rat liver has been shown using orotic acid. A 14-hour half-life was calculated for nuclear RNA from the 2- to 16-hour data[14] and a half-life of the order of 5 to 7 days for the cytoplasmic RNA based on 20- to 91-hour data[13]. In short-term experiments with adenine-^{14}C, FRESCO AND MARSHAK have calculated apparent half-time values of 12 and 24 hours, respectively, for the nuclear RNA and cytoplasmic RNA of mouse liver[16]. Importance of a knowledge of the full course of incorporation and disappearance of

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precursor and the necessity of as complete kinetic data as possible in order to estimate renewal rates was stressed. It was realized that other less rapidly metabolized components may have been present.

In this investigation, specific activities of soluble 5'-adenylic acid derivatives ("total 5-AMP"), RNA adenine and DNA adenine have been determined in 4 tissues of the mouse up to 29 days after the single administration of adenine-4,6-^{14}C and in 5 other tissues up to 16 days. The data presented in Table I show that adenine is rapidly incorporated and renewed in the cold TCA-soluble 5'-adenylic acid derivatives of the mouse. The more numerous time intervals that have been studied have made it possible to estimate that 70 to 94% of the adenine incorporated into the total 5-AMP of all tissues, with the exception of carcass and heart, had an apparent half-life ranging from 18 hours to 28 hours. A small amount of the total 5-AMP in these tissues was renewed at a relatively slow rate with a half-life of 5 to 7 days. A comparison of specific activity in several tissues at two hours to the half-life shows that the tissues with the shortest half-life for 5-AMP also incorporate the most adenine. A comparison of specific activity of the total 5-AMP in tissues at various time intervals indicates that 5-AMP in one tissue is not rapidly equilibrated with that in another.

Present knowledge of the mechanism of nucleotide synthesis suggests that free adenine is not on the normal route of nucleotide biosynthesis from small-molecular-weight precursors. Adenine is rapidly incorporated into soluble nucleotides when it is administered to a rat[24] or a mouse[1,2]. Several mechanisms may be suggested for the incorporation of adenine into adenylic acid nucleotides. SAFFRAN AND SCARANO[25] showed that 5'-adenylic acid is formed when adenine, ribose-5-phosphate and ATP are incubated with a pigeon liver extract. 5'-Phosphoribosylpyrophosphate in the presence of an enzyme isolated from yeast can condense with adenine to form adenylic acid[26]. These mechanisms yield a net synthesis of adenylic acid. The trans-N-glycosidase type of reaction, which has been shown to occur with desoxyribosides, but not as yet with the ribosides or ribotides[27,28], would not result in a net synthesis of nucleotide and could not account for rapid disappearance of free adenine. It would lead, however, to a rapid incorporation of radioactive adenine into soluble nucleotides.

A half-life of 18 hours found for total 5-AMP of the small intestine is equivalent to approximately 4% renewal/hour. The observed renewal based upon uptake of adenine-4,6-^{14}C in 2 hours was 16% or twice the expected renewal based upon its subsequent disappearance. Renewal based upon uptake is a minimum renewal rate as it is assumed that free adenine is present in excess throughout the entire 2 hours and that no renewal of 5-AMP occurs from other sources. It is also assumed that no labelled 5-AMP leaves the pool during this time interval. It is known that considerable free adenine is present ½ hour after administration, but only a very small amount is present at 2 hours[2]. Using the ½ hour total 5-AMP data[2], one can calculate a renewal rate based on uptake of adenine of approximately 20%/hour. The amount of adenine injected is small compared to the amount of adenine derivatives in a mouse, but it is not a true tracer dose as little or no free adenine is normally present. The initial reaction incorporating adenine into nucleotides and perhaps nucleic acids may be dependent on the amount of adenine present, and even the small amount administered may cause an increased formation of 5'-nucleotides. By chromatographic methods[2], greater quantities of inosinic acid were observed ½ hour after administration of adenine than 24 hours later, which probably represents enhanced deamination of 5-AMP to restore tissue levels to normal. In part,

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direct incorporation of adenine may serve merely as a detoxification mechanism for the animal to eliminate adenine by pathways other than by direct oxidation to 2,8-dioxyadenine. This substance is found when adenine is administered in larger amounts than used in these experiments and can cause kidney damage[29]. Inhibition of formate[12,30] and glycine[31] utilization for nucleic acid formation may be due to preferential formation of nucleotides from adenine.

In addition to being rapidly incorporated into nucleotides, adenine is incorporated into RNA and DNA. Maximal specific activity and time of maximal specific activity in RNA and DNA are different for the tissues studied. The decreasing sequence of specific activity in the RNA at 1 day was: large intestine, small intestine, stomach, kidney, lung, liver, carcass, spleen and testis. BROWN, *et al.* found the sequence in rat to be liver, kidney, intestine, spleen and testis[20,32], whereas ABRAMS found, in agreement with our experiments, that the specific activity of intestine was higher than that of liver[31]. The cause of the differences observed is not apparent. Different specific activity-time relationships found for soluble nucleotides, RNA and DNA would indicate that very little exchange occurs between tissues.

A rapid half-life component of 15 to 19 hours, and a slow component with a half-life of 5 to 7.5 days for the disappearance of adenine-^{14}C are evident in small intestine, large intestine and stomach. In intestines, short-half-life fractions comprise 83% to 93% of the radioactivity incorporated into RNA. In liver, sufficient data were not obtained at short time intervals to obtain a fast component comparable to that reported by FRESCO AND MARSHAK[16]. A half-life of 5.5 days was found for liver in Series I based upon the 3- to 20-day data. The RNA-adenine of other tissues had half-lives ranging from 5.5 to 7.0 days for kidney to 10 to 15 days for lung, carcass, spleen and testis.

Calculations of apparent half-life of RNA fractions based upon data for apparent retention values of adenine derived from formate-^{14}C[22] yield similar apparent half-lives for liver (7 days), kidney (7.7 days), testis (11 days), spleen (5.7 days), but since the calculations are based solely on 1- and 24-day values, a significantly different value of 4 days is obtained for small intestine where two component rates appear to exist.

Four tissues (small intestine, large intestine, stomach, carcass) incorporated appreciable amounts of adenine into the DNA fraction. In the first three tissues, a fast-component adenine with a half-life of 16 to 20 hours was found, while carcass had a fast-component adenine with an apparent half-life of 35 hours. Each tissue had a slow-component adenine with a half-life of 5 to 7 days. Seventy to ninety per cent of the radioactivity was incorporated into adenine of the fast DNA component. An apparent half-life of 18 to 23 hours for total 5-AMP, RNA adenine and DNA adenine is to be compared with the estimated life for cells in intestinal mucosa in rat of 40 hours[33]. This may be evidence that little re-utilization of these fractions occurs, but actual physical loss of cells from these tissues should be borne in mind. Other tissues incorporated only a small amount of adenine in DNA and estimates of half-life ranging from 16 to 40 days have a high degree of uncertainty.

It should be reemphasized that half-lives determined by techniques used in this series of experiments are only apparent half-lives, and represent only time required for one-half of the *labelled* adenine molecules which have been *incorporated* into a fraction to be replaced by unlabelled adenine molecules, either synthesized *de novo* or obtained from an exogenous purine source. Molecular fractions whose half-lives are determined in this study are only fractions which have become labelled during the experiment.

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Fractions such as DNA of liver and heart, which are being renewed only very slowly, take up very small amounts of adenine initially, so that a satisfactory estimate of renewal cannot be obtained. In tissues such as intestine, there may be fractions of RNA or DNA that are not being renewed, and which therefore do not become labelled and whose half-life is not evident. Any such fraction in intestine is probably small. No attempt has been made to fractionate DNA or RNA, either on the basis of physical properties or on the basis of cellular fractions. Some evidence has been obtained for the inhomogeneity of DNA after labelling with formate[22] and considerable evidence has been obtained, particularly in liver and tumors, for cellular inhomogeneity of RNA. Evidence has also been obtained for inhomogeneity of RNA of a given cellular fraction at short time intervals after administration of ^{32}P-phosphate, glycine-^{14}C, and orotic acid-^{14}C[34]. Two-component rate curves obtained for the disappearance of adenine from nucleotide and nucleic acid fractions of several tissues clearly indicate the not unexpected overall inhomogeneity in metabolic rates of a tissue.

The RNA/DNA-adenine-^{14}C ratio varies widely from tissue to tissue (Table I), and it also varies as a function of time, so that a comparison of ratios at a single time interval may be misleading and may have only limited validity. In part, the change of this ratio with time may account for the diverse RNA/DNA specific activity ratios found in the literature after administration of a given precursor.

Maximal specific activity of adenine in nucleic acids in all tissues except carcass-DNA occurs 24 to 48 hours after adenine has been administered. Adenine is essentially completely converted to nucleotides or to catabolites of adenine, principally hypoxanthine and allantoin, within two hours after administration[1,2]. Catabolites of adenine have been shown to be poor precursors of nucleic acids[35] and thus it would appear that 5′-adenylic acid nucleotides are serving as precursors of adenine in nucleic acids. If it is assumed that at short time intervals adenine is first incorporated into nucleotides before being incorporated into RNA, and that the mean specific activity of 5-adenylic acid derivatives is 2000 dis/min/μg adenine for the first two hours in intestines, a renewal of over 20% of the RNA would be required to obtain the observed specific activity of 425 to 475 dis/min/μg adenine in RNA of the small and large intestine*. In other words, an equilibration exists between the soluble nucleotides and RNA that is much more rapid than the apparent renewal of RNA. This would be in qualitative accord with results of experiments with ^{32}P-phosphate[10]. The other alternative is a "direct" incorporation of adenine into RNA, or a route of incorporation that does not go through the 5-AMP pool.

If similar calculations are made for the rate of incorporation of adenine into DNA of intestines during the first two hours after administration of adenine-^{14}C, it is found

* It has been assumed that 5-AMP nucleotides have uniform specific activity within cellular fractions. Isolation of the cold-TCA-soluble fractions in cellular fractions by centrifugation in 0.25 *M* sucrose indicated that 90% of the radioactivity in soluble nucleotides was localized in the cytoplasmic supernatant fraction and 10% in the mitochondrial fraction, and the nuclear and microsomal fractions contained only 1 to 2% of the radioactivity[1]. A similar distribution of uridylic acid nucleotides is suggested from experiments with orotic acid[14], except that the microsomal rather than the mitochondrial fraction was found to contain approximately 7% of the acid-soluble radioactivity at 2, 4 and 8 hours. Experiments[36] in which cellular fractions were isolated in non-aqueous media suggest that a large fraction of the ATP of a cell may be localized in the nucleus and in the mitochondria. If this is the case, a study of cellular distribution of nucleotide specific activity as a function of time is desirable. EDMONDS AND LEPAGE have found the nuclear and cytoplasmic nucleotides of Flexner-Jobling carcinoma to have only slightly different specific activities shortly after administering glycine. They found about 10% of the soluble nucleotides in the nuclear fraction[37].

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that the calculated renewal of 7% to 8% based upon the observed half-life of adenine in DNA of intestines is of the same order of magnitude as the incorporation of adenine-^{14}C, assuming that compounds of the 5-AMP pool serve as intermediates. The lag in incorporation of adenine-^{14}C into DNA as compared to RNA[2] is consistent with a mechanism of relatively rapid reversible formation of RNA from soluble nucleotides and slower formation of DNA also from nucleotides.

Additional evidence that 5-AMP nucleotides are direct precursors of RNA adenine, perhaps sole precursors in intestines, is the striking correspondence of specific activity of RNA adenine and 5-AMP adenine in both small and large intestine subsequent to two days after administration. A close correspondence of specific activity of 5-AMP adenine and RNA adenine of carcass was also found, but until these values have been separately determined in muscle and bone marrow the significance is uncertain. In most tissues studied, a close relationship was found between the half-life of 5'-AMP adenine and RNA adenine.

The liver, a tissue in which the most extensive studies of specific activities of nucleotides and nucleic acid fractions have been made[9, 11, 38], does not appear to have the close correspondence of nucleotide and RNA specific activities after adenine-4,6-^{14}C administration. At periods up to 3 days after adenine administration the specific activity of the RNA adenine is less than that of the 5-AMP adenine, while at later periods it is greater.

Considerable indirect evidence has been accumulated in the past several years that soluble nucleotides within a tissue may serve as precursors of nucleic acids[1, 13, 14, 37, 38, 39, 40]. The present concept of RNA structure as a polynucleotide chain in which individual nucleotides are linked by phosphodiester linkages between 3'- and 5'-positions of ribose moieties[41, 42] focuses attention on 5'-nucleotides as logical intermediates for the formation of RNA and perhaps even DNA. The 5'-nucleotides have been found to be normal constituents of tissues[39, 43].

MARRIAN has presented evidence that regenerating rat liver is able to utilize purines, probably acid-soluble, from its environment for formation of DNA[40]. Because of insufficient data on the amount of liver removed, one is unable to calculate whether newly-formed RNA could have also arisen in part from the soluble nucleotide fraction.

Orotic acid-^{14}C was preferentially and rapidly utilized in rat liver[13, 14]. At short-time intervals after administration, orotic acid was incorporated primarily into acid-soluble derivatives of uridylic acid which subsequently were converted into liver RNA. From 2 to 16 hours, the specific activity of the nuclear RNA maintained a specific activity of about one-half of that of acid-soluble uridylic derivatives*. Up to 16 hours cytoplasmic RNA specific activity did not parallel that of acid-soluble uridine phosphates; data enabling a direct comparison to be made at later time intervals were not given. It would be expected, however, that a closer correlation between RNA specific activity and that of soluble uridylic acid derivatives would be found during the 16- to 91-hour period in liver. FRESCO AND MARSHAK[16] have compared acid-soluble adenine, nuclear RNA adenine and cytoplasmic RNA adenine in mouse livers and have obtained rather similar results.

A close correlation of the specific activities of nucleotides obtained from RNA and acid-soluble nucleotides in Flexner-Jobling rat carcinoma after administration of glucose-1-^{14}C has been observed[38]. Nucleotides derived from DNA had consistently

* See footnote, page 511.

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lower specific activities than those derived from RNA and results are not incompatible with the formation of desoxyribotides from ribotides. ROSE AND SCHWEIGERT have also found evidence for DNA formation from nucleotides with cytidine[44].

It would be extremely desirable to have additional experiments in which a comparison of specific activity of acid-soluble nucleotides and nucleic acids is made in several tissues and at several time intervals, particularly after administration of formate or glycine. LEPAGE has compared the incorporation of glycine-2-^{14}C into acid-soluble guanine and adenine derivatives and nucleic acid guanine and adenine in *in vitro* systems[45]. In all cases the specific activity of nucleic acid fractions was less than those of soluble nucleotides. A similar comparison *in vivo* gave corresponding results which have been interpreted as suggesting an interrelationship between acid-soluble nucleotides and nucleic acids[37]. Data from studies that have compared specific acitivity of ATP of muscle with that of RNA and DNA of viscera after formate or glycine[46] or adenine[47] administration are not applicable.

If, as suggested, acid-soluble 5'-nucleotides are intermediates in nucleic acid synthesis, one cannot overemphasize the importance of determining the incorporation of compounds that are being investigated as nucleic acid presursors into the acid-soluble nucleotide fraction as well as into the nucleic acids. Further experiments are obviously necessary to determine the incorporation of nucleotides, particularly 5'-nucleotides such as 5-AMP, into acid-soluble nucleotides as well as into RNA and DNA. 2'- and 3'-nucleotides[48,49] have been administered, but, as discussed elsewhere, emphasis should perhaps be placed on 5'-nucleotides. A more complete knowledge of the mechanisms by which the nucleotides are formed and utilized should aid greatly in our understanding of nucleic acid formation and renewal.

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SUMMARY

Adenine has been shown to be extensively utilized for formation of acid-soluble adenine nucleotides and for formation of RNA and DNA in C_{57} mice. The "apparent half-lives" of adenine in nucleotides, RNA and DNA of the organs of the mouse have been calculated, and a "fast"- and a "slow"-component adenine were found in the adenine-containing fractions in several tissues. Evidence is presented that soluble adenine nucleotides serve as precursors for RNA and DNA, and that the equilibration of nucleotides with RNA is relatively rapid in large and small intestines and probably also in bone marrow. The desirability of more extensive data for nucleotide and nucleic acid specific activity as a function of time with several precursors is emphasized.

RÉSUMÉ

L'adénine est utilisée en grande partie pour la formation des adénine-nucléotides acide-solubles et celle du ARN et du ADN par des souris C_{57}. Les "demi-vies apparentes" de l'adénine dans les nucléotides, le ARN et le ADN des organes des souris ont été calculées, et deux formes d'adénine, une "rapide" et une "lente", ont été mises en évidence dans les fractions de plusieurs tissus qui contiennent de l'adénine. Les résultats montrent que les adénine nucléotides solubles servent de précurseurs du ARN et du ADN et que l'équilibre entre les nucléotides et le ARN est atteint relativement vite dans le gros et dans le petit intestin et sans doute aussi dans la moelle osseuse. Les auteurs insistent sur l'intérêt que présenteraient des déterminations plus nombreuses de l'activité spécifique de nucléotides et des acides nucléiques en fonction du temps avec divers précurseurs.

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ZUSAMMENFASSUNG

Es wurde bewiesen, dass Adenin bei C_{57}-Mäusen weitgehend zur Bildung von säurelöslichen Adeninnukleotiden, sowie von RNA und DNA benützt wird. Die "scheinbare halbe Lebensdauer" von Adenin in Nukleotiden, RNA und DNA der Mausorgane wurde errechnet; mehrere Gewebe wiesen adeninhaltige Fraktionen, mit einer "schnellen" und einer "langsamen" Adeninkomponente auf.

Es werden Beweise dafür angeführt, dass lösliche Adeninnukleotide als Vorgänger für RNA und DNA dienen und dass im Dick- und Dünndarm, sowie im Knochenmark, verhältnismässig schnell ein Gleichgewichtszustand zwischen Nukleotiden und RNA erreicht wird.

Es wird die Wichtigkeit betont, weitere Angaben betreffend die spezifische Aktivität von Nukleotiden und Nukleinsäure, als eine Funktion der Zeit, mit mehreren Vorgängern zu erlangen.

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