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Berkeley, California

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ABSTRACT

Intraperitoneally administered adenine-4,6-C^{14} is rapidly and extensively incorporated into nucleotides and nucleic acid by C57 mice. The incorporation rate of adenine into the PNA indicates a pathway involving little or no dilution, therefore not thru the general 5-adenylic acid pool. The "half-life" of the nucleotide fraction was found to be 2.5 days in the liver, 13 hours and 3.5 days in the stomach and intestines, and 12 days in the carcass (including bone), whereas the PNA adenine had a "half-life" of 7-8 days in the liver, 3.5 days in the stomach and intestines, and 8 days in the carcass. The most rapid incorporation of adenine in the PNA of the liver occured in the nuclear fraction and the least rapid in the microsomal fraction.

(*) Presented at the Second International Congress of Biochemistry, Paris, July, 1952.

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The biological synthesis and turnover of nucleic acids has received increasing attention with the ready availability of isotopes, particularly P^{32}, N^{15} and C^{14}. P^{32} has been used to study turnover rates of nucleic acids, or at least the turnover rates of the phosphorus in nucleic acids (4, 19). N^{15} and C^{14} have been utilized to study the possible precursors of nucleic acids and to a lesser extent to study nucleic acid turnover. Of the simpler substances, ammonia (3), glycine (9,38), carbon dioxide and formate (33, 39) have all been shown to be incorporated into the nucleic acid purines. Of the more complex substances, adenine (11), and guanine (2) are incorporated into the nucleic acid purines but not into the pyrimidines. The reported incorporation of adenine is significantly less for the C57 male mouse than for the Sherman rat, while guanine is reported to be used more efficiently by the mouse than by the rat (2).

The whole animal experiments have generally reported the utilization of adenine after several days of feeding, and have usually isolated the nucleic acids from only a limited number of organs. The incorporation of adenine into the soluble nucleotides, with the exception of those in the carcass, generally has been neglected. The amount of the administered adenine incorporated into the nucleotide and nucleic acid fractions in the various tissues has not been

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reported. Although studies have been made of the variation with time of the distribution of administered P^{32} (5, 13, 19, 21, 22, 30), glycine (9, 18), and orotic acid (20, 34) in the cell fractions of the liver, no similar studies have been reported with adenine.

In the studies reported here, adenine-4,6-C^{14} has been utilized to study the turnover rate of the soluble nucleotides as well as the nucleic acids of C57 mice. Examination has been made of the soluble nucleotides for the possible presence of intermediate compounds in the synthesis of nucleic acids from adenine. In addition, the distribution of adenine-C^{14} in the cellular components of the liver as a function of time has been studied.

METHODS

Administration of Adenine and Fractionation of Tissue.

Adult male C57 mice, weight 23-26 gms., age 4-6 months, were injected intraperitoneally with 1.2 mg. of adenine-4,6-C^{14} (6) containing 1.7×10^7 dis/min, dissolved in 0.5 ml. of 0.9% saline. In those experiments in which respiratory CO_2 and urine samples were collected, the animals were placed in metabolism cages, otherwise they were placed in individual cages designed to prevent contamination of food and water by the excreta. The mice were sacrificed by decapitation and the organs were quickly removed and immediately fractionated as described below. Generally the following organs were utilized: the carcass (including bone) after skinning, liver, and stomach and intestines.*** The data represent one to three mice at each time interval.

The entire carcass and the stomach and intestines were homogenized in a Waring blendor with cold 10% trichloroacetic acid to extract the acid soluble nucleotides (36); a Potter homogenizer was used for the liver. To ensure complete extraction, each tissue was extracted 5 times with relatively large amounts of

(***) The stomach and intestine fraction also includes the spleen, pancreas and gonads.

TCA (125 ml./extract for the carcass, 30 ml. for the stomach and intestines, and 10 ml. for the liver). Generally only the first two extracts were saved since they were shown to contain about 97% of the total cold TCA extractable carbon-14. This fraction is subsequently referred to as the nucleotide fraction.

Subsequently the tissue was washed with alcohol and then ether and the total nucleic acid extracted from the residue with hot 10% TCA(36). The TCA was conveniently removed from the extracts by continuous extraction for 1-2 hours with ether in a suitable liquid-liquid extractor.

The total amount of carbon-14 activity in each extract was determined by direct plating techniques. An approximate value for the nucleotide content of each fraction was made by measuring the ultra-violet absorption at 260 mμ.

Separation of Liver Cell Components.

The differential centrifugation technique of Schneider and Hogeboom (37) in 0.25 M sucrose was used to separate the liver cell components. After removal of the connective tissue, the liver was homogenized in 10 ml. of sucrose in a plastic Potter homogenizer, and the homogenate centrifuged at 1500 RMP for 15 minutes (Spinco centrifuge, No. 40 rotor). The nuclear precipitate was rehomogenized and recentrifuged. The combined supernatants were centrifuged at 7000 RPM for 15 minutes and the mitochondrial precipitate obtained was washed by suspension in sucrose solution and recentrifugation. The supernatant was centrifuged at 30,000 RMP for 30 minutes and the microsomal precipitate obtained was washed by suspension in sucrose solution and recentrifugation. The cellular fractions obtained were extracted with cold and then hot TCA. PNA was estimated by the orcinol reaction (31, 32), and DNA was estimated by the diphenylamine reaction (14). In addition, total nucleic acid was estimated by ultra-violet absorption at 260 mμ using the factor 0.025 density units (vol. in ml. x density at 260 mμ)/μ g. nucleic acid. Good agreement was obtained.

Determination of Specific Activity of Nucleotide and Nucleic Acid Adenine.

1 ml. aliquots of the nucleotide fraction containing 25-50 μg. of adenine as nucleotides were allowed to stand 2-3 days at room temperature with a slight excess of calcium hydroxide to hydrolyze ADP and ATP to 5-adenylic acid (28). Subsequently the mononucleotides were precipitated by the addition of 2 volumes of ethanol. The precipitate was dissolved in a small amount of dilute acetic acid and chromatographed two dimensionally on Whatman No. 1 filter paper, first in 40% butanol-25% propionic acid-35% water (wt. %), and subsequently in 60% propanol-30% ammonium hydroxide-10% water (vol. %). The main spot, of 5-adenylic acid, was located with ultra-violet light and eluted with 0.1% formic acid. Several less intense and smaller spots were frequently observed nearer the origin and were eluted in a similar manner but they usually contained less than 5% of the activity. The specific activity of the 5-adenylic acid was determined after estimation of the quantitiy of material present with 5-adenylic acid deaminase(23).

Adenine in the hot TCA extracted nucleic acids was isolated by hydrolyzing the adenylic and guanylic acids with 1 M HCl at 90°, and subsequently chromatographing on Whatman No. 1 filter paper, first in propanol-ammonia-water and then in butanol-propionic acid-water. Adenine and guanine were separately eluted with 0.1% formic acid. The concentration of adenine was determined specrophotometrically at 305 mμ with xanthine oxidase (26), and the carbon-14 in a separate aliquot of the eluate was determined.

The free adenine-C^{14} in the nucleotide extracts of mice at several time intervals after injection was determined by carrier chromatography on Whatman No. 1 filter paper. The adenine spot was eluted and the activity present in the original extract calculated from the percentage of carrier adenine recovered and the carbon-14 present in the eluate.

Counting Procedures.

Direct plating techniques were used for the TCA extracted fractions and for compounds eluted after chromatography. Generally duplicate samples were counted. Suitable self-absorption corrections were applied. Respiratory CO_2 was determined by collection of the CO_2 in sodium hydroxide and precipitation as barium carbonate by standard techniques. The radioactivity measurements were made with a proportional counter (Nucleometer, Radiation Counter Laboratories, Chicago) (counting efficiency 40%, background equivalent to 100 dis/min) or a GM counter (Brüel and Kjaer, Copenhagen) (counting efficiency 16%, background equivalent to 30 dis/min).

RESULTS

The distribution of carbon-14 activity expressed as "% of total injected" in the nucleotide and nucleic acid fractions 2 hours and 24 hours after injection of 1.2 mg. of adenine-4,6-C^{14} into male C57 mice is shown in Table I. In addition, the percentage remaining as free adenine in the nucleotide fraction is indicated. About 50% of the administered adenine has been converted into compounds which are not rapidly excreted. Table II indicates the rate of excretion of radioactive respiratory CO_2 during the first 24 hours following the administration of adenine-4,6-C^{14}.

Paper chromatography of the urine followed by radioautography has indicated 4 to 6 radioactive compounds to be present, including allantoin, probably uric acid, and in some experiments adenine and hypoxanthine. Presumably the radioactive respiratory CO_2 represents the 6 position of the adenine which is converted to allantoin (10). Guanine-4-C^{14} is known to be converted to allantoin but to yield no radioactive respiratory CO_2 (8).

The total carbon-14 activity of the nucleotide fraction of the stomach and intestines from 1/2 hour to 16 days after administration of the adenine is shown in Figure 1. The specific activity of the 5-adenylic acid obtained upon treatment of this fraction with calcium oxide is also presented. Similar data for the specific activity of the carcass 5-adenylic acid and the liver 5-adenylic acid is presented in Table III. Estimation by ultra-violet absorption of the quantity of 5-adenylic acid derivatives extracted in the stomach and intestines, carcass, and liver gave average values of 3.0 mg., 10 mg., and 1.2 mg. (expressed as adenine) respectively. Enzymatic assay of the extracts by muscle 5-adenylic acid deaminase and potato apyrase or by intestinal adenosine deaminase and alkaline phosphatase, nucleoside phosphorylase and xanthine oxidase (24, 25) indicated that the extract of stomach and intestines contained about 50% of the ulta-violet absorbing material at 260 mμ as adenylic acid compounds. About 40% of the nucleotides was 5-adenylic acid and the remainder was ADP and ATP. The corresponding figures for the carcass nucleotide fraction were 75-85% "pure" with 5% as AMP and 95% as ADP-ATP and for the liver 55-60% "pure" with 70% as AMP and remainder as ADP-ATP.

The radioactive compounds in the nucleotide fraction of the stomach and intestines, and the liver 2 hours after administration of the adenine were investigated by paper chromatography followed by radioautography. As shown in Figure II, two radioactive zones identified as 5-adenylic acid and ADP (ATP?) account for over 80% of the radioactivity present as determined by elution and subsequent carbon-14 determination. The radioactive 5-adenylic acid also coincided with carrier 5-adenylic acid upon two dimensional chromatography followed by radioautography. Spot X_1 accounting for 10% of the activity is believed to be uric acid, spot X_2 is probably allantoin, and spot X_3 corresponds with a radioactive compound also found in the urine but as yet unidentified.

Treatment of the nucleotide fraction of the stomach and intestines and the liver obtained two hours after administration of adenine with muscle deaminase and crude potato apyrase containing adenosine deaminase and phosphatase yielded inosine as the main radioactive compound as shown by chromatography in propanol-ammonia-water and subsequent radioautography. This spot was eluted and rechromatographed two dimensionally with carrier inosine, adenosine, and adenine. The radioactivity was associated only with the inosine.

The specific activity of the nucleic acid adenine of the stomach and intestines is shown in Figure III. The decrease of specific activity of the adenine in the nucleic acid of the stomach and intestines follows a first order reaction rate after 24 hours and from the data obtained a "half-life" or "turnover rate" of the nucleic acid can be calculated. Similar data for the specific activity of the carcass and liver nucleic acid adenine is presented in Table III. The values for the liver nucleic acid adenine were calculated from the specific activity of the PNA as determined by the orcinol reaction. It was assumed that the adenine represented 10% of the nucleic acid and the ratio of activity in the adenine to that in the guanine was 3/1. Approximately this ratio was obtained in the carcass and the stomach and intestines. Table III also compares the specific activity of the nucleotide adenine with that of the nucleic acid adenine. Inasmuch as incorporation of adenine into desoxynucleic acid (DNA) is small (16,33), to calculate the specific activity of the PNA adenine in the carcass and viscera, the values in Table III should be multiplied by approximately 2 for carcass and 1.6 for the stomach and intestines although there is considerable uncertainty as to the exact PNA/DNA ratio for these organs as the colorimetric methods used were not sufficiently specific.

The "half-lives" of the adenine in the nucleotides and nucleic acid of the liver, stomach and intestines, and carcass have been calculated from the data for 1 day to 16 days are are presented in Table III.

The distribution of radioactivity in the cellular components of the liver as obtained by fractionation in 0.25 M sucrose at several time intervals after administration of adenine-C^{14} is shown in Table IV. As isolated, the cytoplasmic supernatant fraction contained approximately 90% of the nucleotide activity at all time intervals, the mitochondrial fraction contained 10%, and the nuclear and microsomal fractions contained no more than 1-2% of the nucleotide carbon-14 activity. To what extent this represents the distribution of nucleotides in the normal cell, or to what extent it represents an artifact of the isolation method is not known.

As shown in Table IV, there is a rapid increase in the radioactivity associated with the nuclear nucleic acid while the increase is least rapid in the microsomal fraction. After three days, the specific activity of the nucleic acid is similar in all cell fractions. Qualitatively, the result is similar even when expressed on a total nucleic acid basis, i.e., if it is assumed that the DNA is incorporating the adenine to the same extent as the PNA.

DISCUSSION

The experiments indicate that the utilization of adenine by the C57 male mouse after intraperitoneal injection is rapid and extensive. One half hour after administration, 30% of the adenine remained as free adenine while at 2 hours only 2% of the adenine remained. This utilization is not unique to the C57 mouse, normal and sarcoma bearing A strain mice use adenine to a similar degree (8). Extensive utilization of adenine to form nucleotides and nucleic acid has also been demonstrated in feeding experiments with chickens (35) and in perfused rabbit and cat livers (7) and pigeon liver homogenates (17). In 24 hours, 15% of the adenine has been incorporated into the nucleic acid of the mouse. It has been reported (2) that the utilization of dietary adenine by C57 mice is smaller by a factor of 5 than that which occurs in the Sherman rat. If an extrapolation can be made from injected to dietary adenine, this would indicate that 75% of the adenine would be incorporated into the nucleic acid of the rat. This value would appear to be much too high.

The results obtained by the C57 mouse for the incorporation of adenine into the soluble nucleotides (AMP, ADP, and ATP) are in marked contrast to those which have been reported for the rat after dietary administration of adenine (12) and

and N^{15} ammonia (25). In the mouse, the incorporation into the nucleotide fraction has been extensive; on a specific activity basis, the soluble nucleotides in the liver and stomach and intestines are 10-20 times more active than the PNA adenine 2 to 24 hours after injection of the adenine. The carcass ratio is approximately unity, depending on the time chosen for comparison and the actual DNA/PNA ratio. One experiment has indicated that the major adenine incorporation in the carcass nucleic acid has occured into the bone marrow rather than the muscle nucleic acid.

Several explanations may be offered for the apparent discrepancy in the utilization of adenine for nucleotide and nucleic acid formation in the mouse and rat: 1) the difference may be a species difference; 2) in the experiments reported with mice, the entire carcass including bone marrow was used; and 3) the relative incorporation into nucleic acid and nucleotides may depend on the amount or concentration of adenine administered. However, radioactive nucleotides were shown to be in the muscle of the carcass in a 24 hr. experiment in which the muscle and bone were separated before the extraction. The specific activity of the nucleotide fraction of the muscle was similar to the total carcass value at the same time. There is some evidence that large doses of adenine are incorporated relatively more into the nucleotides of the carcass (12). The amount of adenine administered to the mice in the experiments reported here was comparable to the lower daily dose fed to rats but it was administered intraperitoneally at one time instead of by feeding. A recent preliminary note (29) describing experiments in which adenine-8-C^{14} was intraperitoneally injected into male rats at a comparable dose level used in the above experiments indicates that adenine was extensively incorporated into soluble nucleotides of the liver and viscera. The ratios of injected specific activity of the adenine to the specific activity of the soluble nucleotides and nucleic acid adenine are similar to those found in the mouse.

The extensive incorporation of the adenine into the nucleotides and nucleic acid of the mouse has made it possible to calculate the "half-life" of the adenine component in these fractions in the liver, stomach and intestines, and carcass from the data shown in Table III as shown in Figures I and III. From the values obtained, "half-lives" of 2.5 days and 12 days have been calculated for the nucleotides of liver and carcass. Two components are indicated for the metabolism of adenine in the nucleotide fraction of the stomach and intestines; one with a half life of 13 hrs.; the other 3.5 days. This may be due only to the diversity of tissue represented. Similarly, values of 8 days, 3.5 days and 8 days have been calculated for the nucleic acid adenine of the same tissues. Since the PNA and DNA were not separated in the stomach and intestines and the carcass, the nucleic acid value could possible represent an average value for the two types of nucleic acid. However, data indicate that the DNA/PNA ratio for utilization of adenine in the rat is about 1/70 to 1/100 on a specific activity basis. A ratio of 1/25 has been obtained in the livers of A strain mice injected with adenine (24 hr. experiments) (33). Therefore, unless, as is unlikely, radioactive compounds are subsequently incorporated into the DNA, the figures for the decay rate of the adenine activity in the nucleic acids probably represent the turnover of adenine in the PNA. The adenine/guanine specific activity ratio appeared to be about 3/1 for all tissues and for all time intervals; however a careful study of this ratio has not been made.

The values obtained for the biological "half-life" represent the formation of the nucleotides and the adenine (and probably guanine) of the nucleic acid from non-radioactive, presumably exogenous, material. The actual turnover of nucleic acid in the carcass may be more rapid than determined above if the carcass utilized radioactive compounds from the liver or stomach and intestines.

The "half-life" for the nucleic acid adenine of mouse liver is similar to that in rat liver (16). It is also similar to that calculated from the renewal of nucleic acid in rabbit bone marrow in vitro (1). The value of 13.7% obtained for the renewal of PNA in the internal organs of the rat after feeding adenine for 3 days (11) can only be a very minimal value for the turnover of nucleic acid adenine, since no allowance can be made for the adenine incorporated into the nucleic acid from other precursors, i.e. formate, glycine, etc. which are normally used for nucleic acid synthesis. If, in the rat, as in the mouse, the adenine is rapidly metabolized to other compounds, it will not be present long after administration, subsequently the PNA will be formed from other precursors. The low incorporation of adenine into DNA of non-regenerating tissue does not necessarily indicate that DNA has a much slower renewal rate than PNA, it may indicate that adenine is not efficiently utilized in non-regenerating tissue for DNA synthesis. The relative specific activities of DNA and PNA show wide variation depending upon the nature of the radioactive compound administered and the time after administration (18, 27, 33). This is further indication that the calculation of "turnover rate" from incorporation in not valid.

From the experimentally determined renewal rates of the nucleic acid, the expected turnover of PNA in 2 hrs. would be 0.7% in the liver and carcass and 1.6% in the stomach and intestines. From the renewal rate and estimated value for the PNA/DNA ratio of the tissues, the minimum expected dilution of the incorporated adenine at 2 hours can be calculated. It is found, that the observed dilution of the adenine incorporated into the PNA is actually slightly less than calculated for a "direct" incorporation. This indicates that, unless the turnover of nucleic acid adenine is greatly accelerated by the presence of relatively large amount of adenine, the adenine is being incorporated by means which are either direct or which involve intermediates which dilute the adenine only slightly. Although the 5-adenylic acid of the stomach and intestines and the liver is much more radioactive than the nucleic acid adenine, it is probably not an intermediate in nucleic acid synthesis inasmuch as a turnover of 10-20% of the nucleic acid in

2 hours would be necessary to obtain the observed activity whereas the calculated turnover in this period is 0.7-1.7% in the several tissues. One must not overlook the possibility that the 5-adenylic acid in different cell fractions or cell sites, i.e. the nucleii, might have a different carbon-14 activity than the total or average 5-adenylic acid and be a direct intermediate.

The specific activity of PNA isolated in liver cell components fractionated in sucrose by the differential centrifugation method indicated the most rapid utilization of adenine in the "nuclear PNA" fraction and the least rapid in the microsomal fraction. Similar results have been reported for P^{32}, glycine, and orotic acid (4, 18, 20, 34). The "decay curves" for the total activity of the various cellular fractions indicate that the rate of disappearance of activity in the nuclear fraction from 3 to 16 days is comparable to that in the other cellular fractions. Two explanations may be offered for this: 1) either the estimated activity of the nuclear PNA at 7 to 16 days is too high due to the relatively increased amount of activity in the DNA (although the adenine is incorporated to a much smaller extent, its turnover is certainly slower), or 2) the "nuclear" PNA as isolated contains two types of PNA, one of which is a rapidly metabolized PNA as suggested by the data obtained with adenine for 1/2 and 2 hours and by P^{32} and orotic acid for short time intervals. The extent of "contamination" of the nuclear fraction by mitochondrial or other PNA is not known, but it must be small since our figure of 8% of the total PNA present in the nucleii is in agreement with the amount reported when citric acid is used to separate the nucleii (9). The contamination by mitochondria can also be estimated to be less than 10% of the mitochondrial fraction from the total activity found in the cold TCA fraction.

The "half-life" of 8 days calculated for the liver nucleic acid adenine agrees well with the 7 day value obtained from the data (34) for the specific activity of the supernatant PNA (including microsomes) after administration of orotic acid to the rat. The rapid increase in labeled PNA in the nucleii appears to eliminate the possibility of a "nuclear membrane" impermeable to preformed purines except during growth as has been offered as a tentative suggestion to explain the relative non-utilization of adenine in DNA synthesis during this period (15).

From 2 hours to 24 hours after injection of adenine there is a large increase (7-10% of the injected adenine) in the total amount of C^{14} activity in the nucleic acid. Since at 2 hours, there is less than 2% of the injected adenine-C^{14} still remaining free, it is impossible that the increase can be due solely to further free adenine being incorporated. Thus, there is evidence that other radioactive compounds are present in the nucleotide fraction which subsequently are converted into nucleic acid. The rapid decrease in total activity in the liver and stomach and intestine nucleotide fractions from 2 hours to 1 day followed by a slower first order decline also indicates there is a compound or compounds made more reactive, either by virtue of its structure or location in the cell, which may be utilized for nucleic acid synthesis. By the enzymatic and paper chromatographic methods described above, no direct evidence has been obtained for the existence of an unusual or new nucleotide in these extracts, but further work is in progress. Any such intermediate might be expected to represent as little as 10-20% of the radioactive material present two hours after administration of the adenine, and a considerable smaller percentage of the ultra-violet adsorbing material.

The feasibility of readily obtaining 5-adenylic acid-C^{14} in fair yield for further biological experiments after administration of adenine-C^{14} to mice should also be pointed out.

SUMMARY

Intraperitoneally administered adenine-4,6-C^{14} is rapidly and extensively incorporated into nucleotides and nucleic acid by C57 mice. The incorporation rate of adenine into the PNA indicates a pathway involving little or no dilution,

therefore not thru the general 5-adenylic acid pool. The "half-life" of the nucleotide fraction was found to be 2.5 days in the liver, 13 hours and 3.5 days in the stomach and intestines, and 12 days in the carcass (including bone), whereas the PNA adenine had a "half-life" of 7-8 days in the liver, 3.5 days in the stomach and intestines, and 8 days in the carcass. The most rapid incorporation of adenine in the PNA of the liver occured in the nuclear fraction and the least rapid in the microsomal fraction.

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TABLE I

DISTRIBUTION OF CARBON-14 AFTER INJECTION OF ADENINE-4,6-C^{14} INTO MALE C57 MICE

(% OF ADENINE INJECTED)

TISSUE	2 HOURS AFTER INJECTION			24 HOURS AFTER INJECTION		
	COLD TCA EXTRACT	HOT TCA EXTRACT	FREE ADENINE	COLD TCA EXTRACT	HOT TCA EXTRACT	FREE ADENINE
CARCASS	14	1.9	0.3	12.2	4.4	< 0.1
LIVER	10	0.6	1.0	5.7	2.2	< 0.03
STOMACH AND INTESTINES	18	5.0	0.7	7.8	7.3	< 0.05
KIDNEY	9	0.1		8.1	0.5	
SPLEEN	0.6	0.1		0.4	0.5	
LUNGS	0.4	0.04				
HEART	0.1	0.01				
TOTAL.	52	7.8	2.0	34	14.9	< 0.2
BREATH	2.1			16		
URINE	40			30		
TOTAL.	104			97		

TABLE II

% OF ADMINISTERED ADENINE-4,6-C^{14} RADIOACTIVITY IN RESPIRATORY CO_2

TIME AFTER INJECTION (HOURS)	% ACTIVITY IN CO_2	TIME AFTER INJECTION (HOURS)	% ACTIVITY IN CO_2
0-1	0.6	8-10	1.4
1-2	1.6	10-12	1.3
2-4	2.2	12-16	2.3
4-6	1.8	16-24	3.1
6-8	1.7		
		TOTAL. 0-24	16.0

TABLE III

SPECIFIC ACTIVITY AND HALF-LIFE OF NUCLEIC ACID ADENINE AND 5-ADENYLIC ACID IN VARIOUS TISSUES

(dis/min/μg Adenine)

TIME AFTER INJECTION	LIVER		STOMACH AND INTESTINES		CARCASS	
	5-ADENYLIC ACID	NUCLEIC ACID*	5-ADENYLIC ACID**	NUCLEIC ACID	5-ADENYLIC ACID	NUCLEIC ACID
1/2 hr.	740	95	800	35.5	74	16.8
2 hours	1080 1730	88 65	1930 2300	205 250	190 235	106 142
24 hours	800	—	790	265	166	178
74 hours	428	340	290	188	144	116
7 days	148	290	126	79	113	78
16 days	—	91	23.6	13	68	39
HALF-LIFE (DAYS)	2.2-2.5	7-8	13 hours 3.5	3.5	12	8

* The specific activity of the liver nucleic acid was calculated from the PNA analysis of the liver fractions. It was assumed that adenine represented 10% of the nucleic acid (by weight), and the ratio of activity in the adenine to that in the guanine was 3/1.

** Two "half-lives" are represented in the decay rate of the nucleotide fraction of the stomach and intestines.

TABLE IV

SPECIFIC ACTIVITY OF PNA IN C57 MOUSE LIVER CELL

FRACTIONS AFTER INJECTION OF ADENINE-4,6-C^{14}

(dis/min/μg PNA)

SOURCE OF PNA	% OF TOTAL PNA IN FRACTION*	TIME AFTER INJECTION				
		0.5 hrs.	2 hrs.	3 days	7 days	16 days
Nuclear	7	4.6	41.7	43.0	38.9	9.5
Mitochondrial	19	1.2	7.3	46.0	39.2	14.0
Microsomal	50	0.4	2.7	42.5	38.2	11.2
Supernatant	24	2.6	14.8	52.0	41.4	12.6
Av. dis/min/μg		1.24	8.7	45.0	39.2	16.8

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* The total amount of nucleic acid averaged 12.5 mg/liver. 29% was present as DNA, 71% as PNA. It is assumed that all of the carbon-14 activity is in the PNA. At 0.5 and 2 hrs. this is undoubtedly true, at subsequent times it is possible the DNA may contain a significant fraction of the activity.

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CAPTIONS TO FIGURES

Fig. 1. Total carbon-14 activity of the nucleotides isolated by extraction with cold TCA from the stomach and intestines of mice from 0.5 hours to 16 days after administration of adenine-4,6-C^{14} X-----X

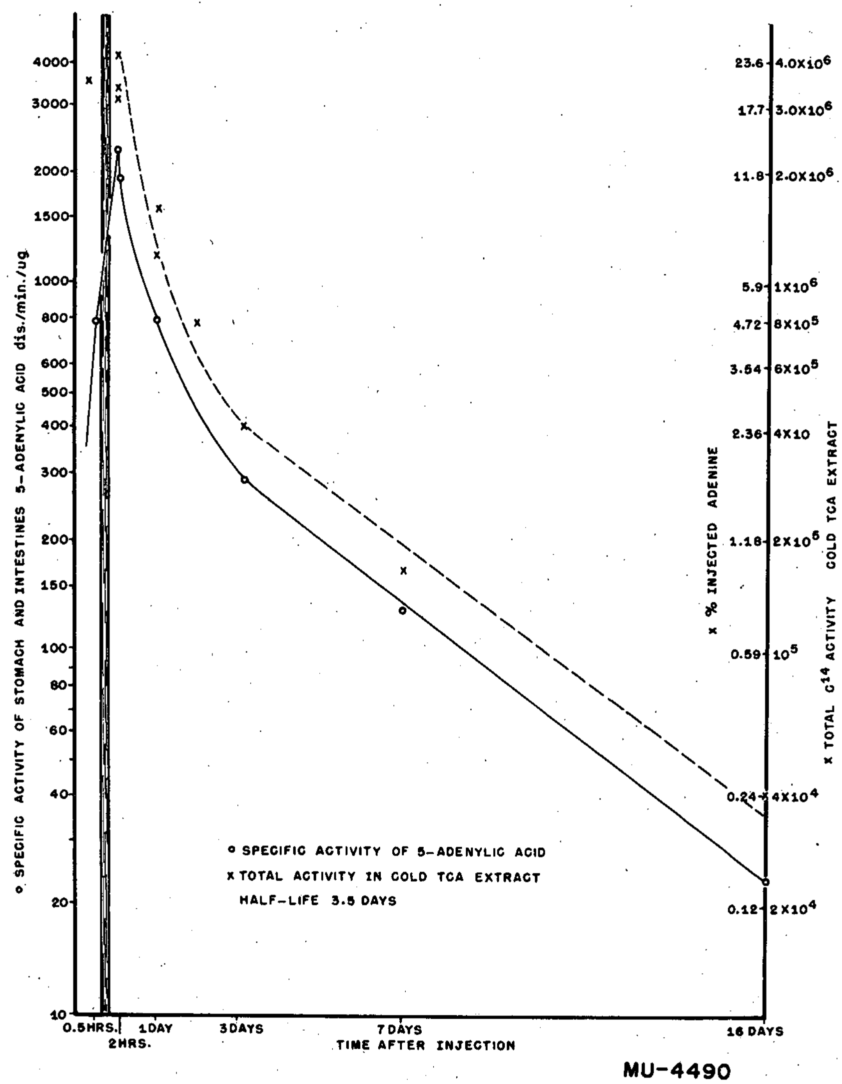
Specific activity of the 5-adenylic acid isolated from the same source

0_____0

Fig. 2. Radioautographs of chromatographed cold TCA extracts of viscera (lower) and liver (upper) obtained two hours after injection of adenine-4,6-C^{14} into C57 mice.

X_1, X_2 and X_3 not identified.

Fig. 3. Carbon-14 activity of the nucleic acid adenine isolated from the stomach and intestines of male C57 mice from 1/2 hr. to 16 days after injection of adenine-4,6-C^{14}.



o SPECIFIC ACTIVITY OF STOMACH AND INTESTINES 5-ADENYLIC ACID dis./min./ug.
x % INJECTED ADENINE
x TOTAL C14 ACTIVITY COLD TCA EXTRACT
o SPECIFIC ACTIVITY OF 5-ADENYLIC ACID
x TOTAL ACTIVITY IN COLD TCA EXTRACT
HALF-LIFE 3.5 DAYS
0.5HRS.
2HRS.
1DAY
3DAYS
7DAYS
16 DAYS
TIME AFTER INJECTION
MU-4490

Fig. 1

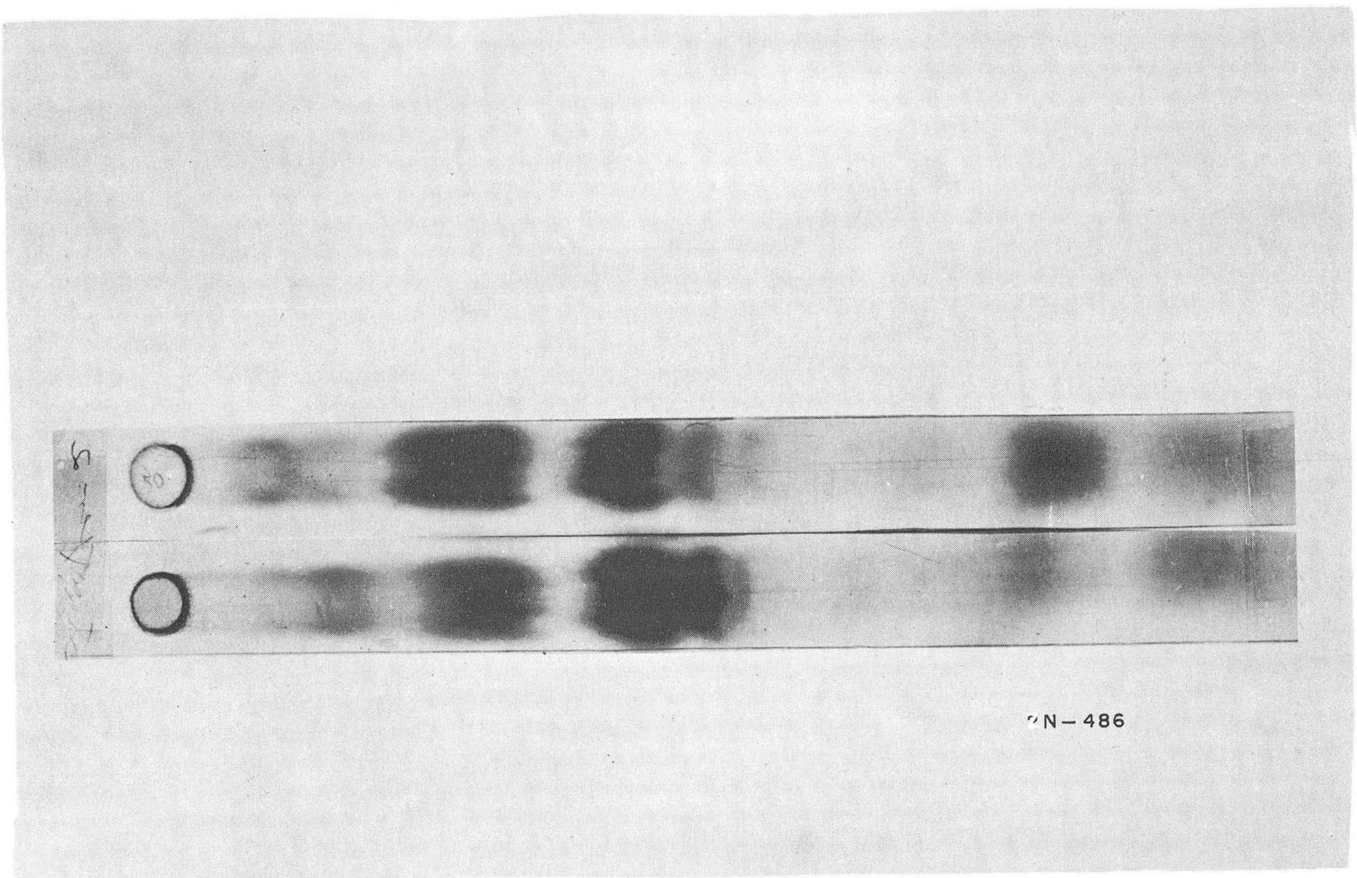
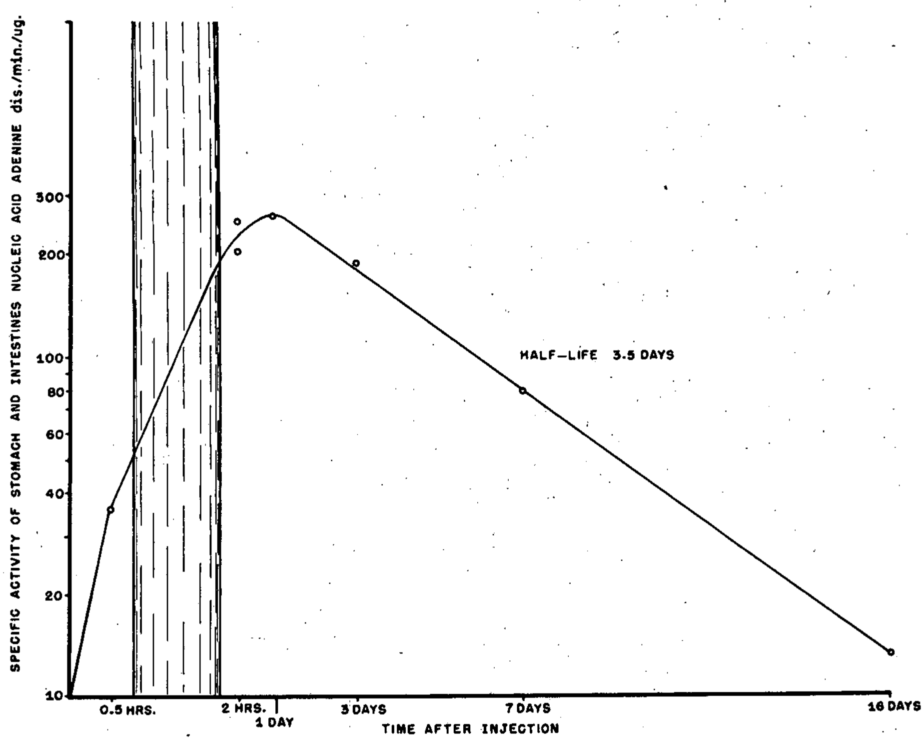


Fig. 2


SPECIFIC ACTIVITY OF STOMACH AND INTESTINES NUCLEIC ACID ADENINE dis./min./ug.
300
200
100
80
60
40
20
10
HALF-LIFE 3.5 DAYS
0.5 HRS.
2 HRS.
1 DAY
3 DAYS
7 DAYS
16 DAYS
TIME AFTER INJECTION

MU-4489

Fig. 3