UCRL-1659

UNCLASSIFIED

UNIVERSITY OF CALIFORNIA — BERKELEY

TWO-WEEK LOAN COPY

This is a Library Circulating Copy
which may be borrowed for two weeks.
For a personal retention copy, call
Tech. Info. Division, Ext. 5545

RADIATION LABORATORY

DISCLAIMER

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor the Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or the Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or the Regents of the University of California.

UCRL-1659
Unclassified-Health and
Biology Distribution

UNIVERSITY OF CALIFORNIA

Radiation Laboratory

Contract No. W-7405-eng-48

TURNOVER RATE STUDIES ON NUCLEIC ACIDS

Anita H. Payne, Lola S. Kelly
and Hardin B. Jones

February 6, 1952

Berkeley, California

TURNOVER RATE STUDIES ON NUCLEIC ACIDS

INTRODUCTION

Since the discovery of nucleic acid from salmon sperm by Friedrich Miescher in 1872,[1] nucleic acid has been found in all living cells. Today two types of nucleic acids are recognized. These are differentiated by the sugar they contain, i.e. desoxypentose or pentose. Both of the nucleic acids contain the purine bases, adenine and guanine, and the pyrimidine base cytosine. Only desoxypentose nucleic acid (DNA) contains the pyrimidine base, thymine, and in some instances 5-methyl-cytosine.[2] Pentose nucleic acid (PNA) alone contains the pyrimidine base, uracil. Each purine or pyrimidine is present in nucleic acid as a phosphoriboside or nucleotide. The individual nucleotides are joined by phosphoester linkages. Until quite recently it was believed that the nucleic acid molecule was a tetranucleotide containing equi-molecular amounts of the four different bases as originally postulated by Levene.[3] However, wide variations in the molar proportions of the different bases have been found[4,5,6,7,8] which vitiates the classical tetranucleotide hypothesis.

The two nucleic acids differ in their physio-chemical properties. In cold alkali PNA is hydrolyzed into its individual nucleotides,[9,10] while no such hydrolysis occurs as a result of the action of alkali on DNA. PNA is fairly stable in cold acid, but DNA readily loses its purine bases in this reagent.[11,12] The molecular weight of DNA has been found by Signer, Casperson, and Hammarsten[13] to be between 500,000 - 1,000,000. The molecule of PNA has been found to be much smaller than that of DNA. It varies considerably according to the method of preparation. Cohen and

Stanley[14] found an approximate particle weight of 300,000 for the freshly isolated nucleic acid of tobacco mosaic virus. Fischer, Böttiger and Lehmann-Echternacht[15] recorded a weight of 10,350 for the acid of yeast, while Fletcher, Gulland, Jordan and Dibben[16] found a range between 10,280 and 23,250.

From early work performed on nucleic acids it was believed that DNA was found only in animal cells, while PNA was unique to plant cells. However, it is now known that both types of nucleic acids occur in all cells, whether plant, animal or bacterial. DNA has been found solely in the nucleus associated with the chromosomes.[17] Small amounts of PNA, are also found in the nucleus associated with the nucleoli[18] and the heterochromatin of the chromosomes.[19,20] The evidence to date indicates that the nucleic acid in the cytoplasm is exclusively PNA and it is nearly all associated with particulate matter.[21,22] PNA varies in composition and amount from tissue to tissue. It is found in highest concentrations in tissues where active protein synthesis is taking place.[22] The composition of DNA is characteristic for a given species,[23] and the amount per diploid-nucleus is strikingly constant for all tissues within a species.[23,24,25,26] Both nucleic acids are found as conjugated nucleoproteins in the cell. DNA is associated with basic proteins protamines and histones[27,28] and an acidic protein called chromosomin by Stedman and Stedman,[29] which has also been found my Mirsky and Pollister.[27]

DNA synthesis is believed to be intrinsically related to cell division. Studies determining the turnover rate of DNA in different animal tissues with the use of radioactive phosphorus[30] indicate that the values obtained for the different tissues correlate well with the

known mitotic indices of these tissues.

The role of PNA in protein synthesis has been discussed by several authors. Casperson and his colleagues were the first to suggest this relationship. According to Casperson[31] the histone and PNA, which are formed in the nucleolus, diffuse out from the nucleolus towards the nuclear membrane on the outside of which PNA and cytoplasmic proteins are formed. He has found there is a correlation between cytoplasmic PNA, the rate of protein synthesis, and the presence of a large nucleolus. Further elaborating on the relationship of cell structure to the function of PNA, Brachet[32] proposes that the cytoplasmic pentose nucleoprotein granules are organelles for the synthesis of protein. This theory is based on the observation that these granules consistently contain dipeptidases and cathepsins which synthesize peptide linkages under favorable conditions. Additional evidence is offered by the finding that these granules always contain some of the specific proteins synthesized by the cells and that there is a progressing complexity of these particles as they become larger.

A possible mechanism by which PNA may contribute to protein synthesis has been presented by Spiegelman and Kamen.[33] They suggest that nucleoproteins are "specific energy donors which make possible reactions leading to protein and enzyme synthesis." They have demonstrated with the use of P^{32} that yeast cells grown anaerobically in the presence of glucose, i.e., where no active protein synthesis is taking place, do not exhibit a change in the concentration of radioactive phosphorus in the yeast nucleoprotein. When ammonium salts are added in order to evoke synthesis of new proteins, P^{32} flows out of the

nucleoprotein fraction. They also observed that agents such as sodium azide and dinitrophenol, in concentrations which prevent enzyme formation and protein synthesis without inhibiting fermentation, also stop the flow of phosphate from the nucleoprotein. These results have led Spiegelman[34] to suggest that nucleic acids might act as phosphate donors and "funnel energy into the protein synthesizing mechanism."

The application of radioactive isotopes in the study of biosynthesis of nucleic acid has made possible the elucidation of hitherto inaccessible problems. The isotope most widely used in these studies has been radioactive phosphorus (P^{32}). Hevesy[30] has studied the incorporation of P^{32} into DNA of small intestine, lymphoid tissue, spleen, Jensen sarcoma, testes, muscles, liver, kidney and brain, and the incorporation of P^{32} into PNA of intestine, liver and spleen. Since C^{14} labeled compounds have been made available in the last few years, many investigators have used various C^{14} labeled compounds in the study of nucleic acid turnover rates. The carbon compounds employed are incorporated into the purines and pyrimidines and probably into the sugar moiety. Other compounds which have been employed in the metabolic studies of nucleic acid are glycine and adenine labeled with heavy nitrogen (N^{15}). Results obtained with these different labeling agents vary considerably not only for the different precursors employed, but also from author to author utilizing the same precursor.

The above labeling agents have been applied to nucleic acid studies not only in normal animals, but also in those with several pathological conditions. An example of the latter is the study of Kelly and Jones[35] on the effect of tumor transplants on the nucleic acid

turnover rate in other tissues such as the liver, spleen and intestine. They observed, using P^{32}, that the DNA turnover rates in livers and spleens of mice bearing transplants of mammary carcinoma were significantly higher than in the same tissue of normal mice, while there was no change in the intestinal DNA. In a later paper by Kelly et. al.[36] the same effect was observed in pregnant mice and in rats bearing transplants of lymphosarcoma. Reddy and Cerecedo[37] have reported an increased content of both DNA and PNA in the livers of mice bearing Crocker-sarcoma 180 transplants.

The purpose of the present study was to determine the nucleic acid turnover rates with the following labeling agents, formate-C^{14}, glycine-2-C^{14}, adenine-4,6-C^{14} and inorganic phosphorus (P^{32}) under normal and pathological conditions. These labeling agents were chosen because they are incorporated into different parts of the nucleic acid molecule. Formate and glycine are small-molecule precursors of the purines and of the pyrimidine, thymine. Formate contributes mostly to carbon 2 and 8 of the purine[38] and to the methyl group of thymine[39]. Glycine-2-C^{14} is incorporated to a large extent into carbon atom 5 of the purine[40], the remainder is incorporated into carbon atom 2 and 8 via formate[41], and into the methyl group of thymine[42]. Adenine is incorporated into the polynucleotides as such and is also partially transformed into polynucleotide guanine[43]. The purine skeleton is retained during the transformation of adenine into guanine. Isotopic phosphorus is incorporated into each of the nucleotides. Thus it was hoped that a comparison of the incorporation of each of the precursors into each of the nucleic acids would lead to a better understanding of the biosynthesis of nucleic acids.

The purpose of Experiment I was to study the turnover rates of DNA, cytoplasmic PNA (cPNA) and nuclear PNA (nPNA) employing the above four labeling agents under identical experimental conditions. By comparing the liver PNA:DNA, nPNA:cPNA, spleen DNA:liver DNA, and intestine DNA: liver DNA ratios it was hoped to determine whether the nucleic acid molecule is synthesized *in toto* or whether parts of the molecule turnover independently.

The purpose of Experiment II was to determine whether the increase in turnover rate observed with isotopic phosphorus in liver DNA of mice bearing transplants of mammary carcinoma, as observed by Kelly and Jones, could also be observed in the liver nPNA, and/or the cPNA. Measurements of the turnover of these fractions in mice bearing tumor transplants other than mammary carcinoma, were also measured in order to obtain additional data for comparative purposes. By studying the PNA turnover rates, it could also be determined whether the effect of the tumor tissue was a direct effect on the DNA turnover, or only brought about indirectly through an increase in the PNA turnover.

Experiment III is in elaboration of Experiment II, its purpose was to determine if the change in turnover of nucleic acid phosphorus was due to an over-all change in phosphorus metabolism or if it was a true indication of an increase in DNA turnover in these tissues. For this purpose carbon labeled formate and glycine were employed to study the nucleic acid turnover rate in the liver, spleen, and intestine of mice bearing tumor transplants.

EXPERIMENTAL METHOD

A strain mice were used in all the experiments. The procedure used for the nucleic acid isolations was an adaptation of several published methods.

The method of Barnum et. al.[44] was followed for the separation of the nuclei from the cytoplasm and the subsequent purification of the nuclei and separation of nuclear pentose-nucleoprotein (PNA-protein) from desoxypentose-nucleoprotein (DNA-protein).

The livers were put in an iced beaker, forced through a tissue press with holes about 1 mm in diameter, and homogenized 2-3 minutes in 4 volumes of ice-cold saline (0.85 percent NaCl containing 2 ml 0.1 N NaOH/liter). Homogenization was carried out in a Potter-Elvehjem tube. The homogenate was centrifuged 4 min at 1400 x g in an International centrifuge operated in a cold room. The resultant supernatant fluid was siphoned off and stored in the refrigerator until the following day for the isolation of the cytoplasmic PNA (Flow diagram I-A). The nuclear sediment (Flow diagram I-B) was suspended in 2 percent cold citric acid and centrifuged for 10 minutes in a clinical centrifuge at 500 x g. The sediment was resuspended and centrifuged 14 times at the same speed after which the supernatant was perfectly clear. The centrifugations were carried out as follows: once for 10 min., twice for 5 min., five times for 3 min., and six times for 1 min. The isolated nuclei were then washed twice with saline to remove citric acid and then were extracted in 95 percent methyl alcohol, followed by three extractions with boiling alcohol-ether (3:1), and a final alcohol extraction. The lipid-free nuclei (Flow diagram I-B-e) were then suspended in an ice-cold

buffer at pH 10 prepared by mixing equal volumes of 0.1 M Na_2CO_3 and 0.1 M $NaHCO_3$. The suspensions were kept in an ice-water bath for 30 minutes and then centrifuged in a clinical centrifuge for 3 minutes. The sediments containing the DNA protein were washed twice with the cold buffer solution. The supernatant and washes containing the PNA protein were combined and made 5 percent to trichloroacetic acid (TCA) and centrifuged in an International centrifuge.

The precipitate which represents the nuclear PNA-protein (Flow diagram I-B-1) and residual DNA-protein was washed once in 5 percent TCA. In order to obtain the nucleic acid in a reprecipitable form the method from this point on differs from the method of Barnum et. al. To remove the residual DNA-protein from the PNA-protein the nucleo-protein precipitate was suspended in 0.14 M NaCl and brought to a neutral pH. The DNA-protein was insoluble in this concentration of NaCl and could be removed by centrifugation. The supernatant contained the PNA-protein and was precipitated out by bringing the solution to pH ~5 with glacial acetic acid and centrifugation. The PNA-protein was then suspended in ice-cold 5 percent NaOH and kept at $0^{\circ} \pm 2^{\circ}$ for 2 hours.[45] This splits the protein from the nucleic acid. The protein was precipitated out by adding glacial acetic acid until the solution was just acid to litmus (pH was ~6.5). The solution was centrifuged and the supernatant filtered through tissue paper. The PNA remained in the supernatant and was precipitated by bringing the solution to pH 2 by the addition of HCl and then adding an equal volume of 95 percent methyl alcohol and centrifuging. This precipitate was dissolved in 0.05 M $NaHCO_3$. Several reprecipitations could be carried out at this point until the

nucleic acid maintained a constant specific activity. It was found in the case of P^{32} labeled nucleic acid that the specific activity did not change significantly after reprecipitation. However, it was found necessary in the case of the carbon labeled PNA to reprecipitate the nucleic acid five times further in order to attain a constant specific activity.

The DNA (Flow diagram I-B-2) was obtained from the gelatinous sediment that remained after the pH extraction by following a modified Klein and Beck method.[35] The sediment was suspended in 10 ml of 5 percent NaCl solution and boiled in a water bath for a few minutes, 0.25 ml glacial acetic acid was added, and then the mixture made basic with 0.5 g sodium hydroxide and 0.1 g sodium acetate. The basic mixture was boiled for about one hour. One ml of glacial acetic acid and 0.7 ml of 5 percent dialyzed ferric hydroxide solution were then added. After standing a short time, another milliliter of acetic acid was added and the solution was centrifuged. The supernatant was treated with an equal volume of methyl alcohol, and the precipitate (nucleic acid) was centrifuged off.

In order to attain a constant specific activity, the DNA was dissolved in 5 ml of 0.1 N NaOH and reprecipitated five times with HCl and methyl alcohol. In the case of the P^{32} labeled nucleic acid, 0.2 ml of saturated solution of disodium phosphate was added, the first time the DNA was dissolved in 0.1 NaOH. The final C^{14} labeled nucleic acid was dissolved in 0.05 M $NaHCO_3$ instead of 0.1 N NaOH for the determination of specific activity.

The cytoplasmic PNA (Flow diagram I-A) was precipitated from the cytoplasmic extract which had been obtained earlier. The extract was made 5 percent to TCA, centrifuged, and the supernatant containing the

acid soluble phosphorus was decanted. The sediment was washed once with 5 percent TCA and then suspended in cold 5 percent NaOH. The procedure from here on was the same as for the nuclear PNA.

As is shown in Table I, using either the orcinol or the diphenylamine reagent, the density of the color developed per microgram of phosphorus in the nucleic acids isolated by the above method agreed well with the commercial preparations. The diphenylamine reagent and the orcinol reagent were prepared according to the directions given by Barnum et. al.[44] The density was determined with a Beckman DU spectrophotometer. There was essentially no contamination of cytoplasmic PNA or nuclear PNA with DNA as determined by the Feulgen reaction and by the diphenylamine reagent.

The specific activity of the purified nucleic acid was determined in the following manner. One aliquot of the sodium nucleate solution was used for the determination of the phosphate concentration by the method of Fiske and Subbarow. A second aliquot was used for counting. The specific activity of the nucleic acid is expressed as counts per minute per mg of nucleic acid phosphorus divided by counts per minute injected corrected to a mouse of standard weight, 25 gm.

Flow Diagram of Nucleic Acid Isolation

I Liver Homogenate
1 - centrifuge

A - Supernate Cytoplasm
- a - ppt. protein with TCA
- b - NaOH hydrolysis of cPNA protein
- c - separation of protein from cPNA by ppt. protein with glacial acetic acid

2 - Supernate cPNA
- a - ppt. of cPNA with HCL and methyl alcohol
- b - purification of cPNA

1 - Sediment Protein
2 - discard

B - Sediment Nuclei
- a - citric acid washes
- b - alcohol extraction
- c - alcohol-ether extraction
- d - repeat alcohol extraction
- e - nPNA-protein extraction in bicarbonate buffer

1 - Supernate nPNA-protein plus DNA-protein contamination
- a - protein precipitation with TCA
- b - elimination of DNA protein contamination in 0.14 M NaCl at neutral ph

2 - Sediment DNA-protein
- a - extraction of DNA in NaCl, NaAc, NaOH mixture
- b - ppt. of protein with glacial acetic acid and ferric hydroxide

3 - Supernate nPNA protein
- a - ppt. protein with glacial acetic acid
- b - NaOH hydrolysis of nPNA-protein
- c - separation of protein from cPNA by ppt. protein with glacial acetic acid

4 - Sediment DNA
- a - discard

5 - Sediment Protein
- a - discard

6 - Supernate DNA
- a - ppt. of DNA with HCl and methyl alcohol

7 - Supernate nPNA
- a - ppt. of nPNA with HCl and methyl alcohol
- b - purification of nPNA

8 - Sediment Protein
- a - discard

TABLE I

Tests for the purity of DNA and PNA

	Cyto-plasmic PNA	Nuclear PNA	Commer-cial° PNA	DNA	Commer-cial° DNA
E (diphenylamine reaction)[a]				0.018± 0.007	0.0194± 0.006
E (orcinol reaction)[b]	0.123± 0.018	0.113± 0.013	0.111± 0.011		
N:P	1.68	1.80	1.88	1.90	1.84*
Feulgen test	---	---	---	+	+

a Optical density at 600 mμ wavelength, calculated per μg P in sample.

b Optical density at 660 mμ wavelength, calculated per μg P in sample.

o This material was obtained from the Nutrional Biochemical Corporation, Cleveland, Ohio.

* The Nutrional Biochemical Corporation's N:P ratio is given at 1.71.

EXPERIMENT I

Introduction

In recent years numerous studies have been made on the relative turnover rates of desoxypentose nucleic acid (DNA) and pentose nucleic acids (PNA) employing as labeling precursors such different tracers as sodium phosphate labeled with P^{32}, formate labeled with C^{14} and glycine and adenine labeled either with C^{14} or N^{15}. Considerable controversy has developed as to which one of these tracer compounds gives an accurate measurement of the "relative turnover-rate" of the nucleic acids.

In order to determine the "absolute turnover-rate"(i.e. the percent of nucleic acid that is renewed within a given time) of the nucleic acids it is necessary to know the specific activity of the immediate precursor of the nucleic acids. Since even the identity of the immediate precursor is not known at present it is necessary to find another way to compare the rate of incorporation of such labels as inorganic phosphorus (P^{32}), formate-C^{14}, glycine-2-C^{14} and adenine-4,6-C^{14} into the nucleic acids. Judging from the varied metabolic paths of these labeling agents, the percent of the injected dose eventually made available for the synthesis of nucleic acid is probably not the same for any two labeling agents. Assuming that the labeling agent within any one tissue is equally available to both PNA and DNA, it is reasonable to compare the specific activity of PNA with that of DNA when any one precursor is used. If this specific activity ratio is constant irrespective of the precursor used then, the molecules of PNA and DNA are renewed <u>in toto</u> or at least the labeled part of the molecule is renewed at an equal relative rate in PNA and DNA.

The findings for the PNA:DNA ratios in rat livers are tabulated in Table II. The inconsistency of the data presented in Table II may possibly be due to the lack of duplication of the experimental procedure of one author by the procedure of another. The time interval elapsing between the administration of the labeling agent and the sacrifice of the animal, varies among the different experiments. Since the incorporation of the active precursors into the three nucleic acids - cytoplasmic PNA (cPNA), nuclear PNA (nPNA) and DNA - does not proceed at the same rate, the ratios of the specific activities of these nucleic acids at various time intervals are not the same. Other variable factors which must be considered are the mode of administration and the quantity of the labeling agent, and the degree of utilization of the labeling agent by the different nucleic acids. With respect to the last mentioned factor, it has been suggested by Reichard[46] that adenine cannot be utilized in the synthesis of the purines of DNA.

Hoping to clarify the inconsistency of the findings of the various investigators, it is attempted in this experiment to compare the nucleic acid specific activity using sodium phosphate (P^{32}), formate-C^{14}, glycine-2-C^{14}, adenine-4,6-C^{14} as precursors under identical experimental conditions.

Experimental Work

Male A strain mice weighing between 20-25 gm were used for all the experiments. The mice were fasted 24 hours prior to the experiment. They were injected intraperitoneally with 20 μc of the labeled

precursor unless otherwise indicated about 9 AM and sacrificed with ether precisely 4 hours later, except in the one 24 hour experiment presented in Tables III and IV. Each precursor was in isotonic saline, and 0.1 cc was injected. The specific activities of the precursors are given in the tables. The livers, intestines, and spleens were removed and the isolation of nucleic acid begun as soon as possible. Twelve livers (except where otherwise indicated), two intestines, and four spleens were pooled.

All of the precursors injected were in isotonic saline. The procedure used for the 24-hour experiment differed in some details from the other experiments. The mice were fasted only during the duration of the experiment. Since adenine is known to have toxic properties, it seemed advisable to administer an equal amount of inactive adenine to some of the control mice.

Results and Discussion

In the 24-hour experiment the incorporation of adenine-4,6-C^{14} formate-C^{14} and P^{32} are compared. The results are tabulated in Tables III and IV. Concerning the cPNA:DNA ratio the results obtained are similar to the 4-hour experiments. The cPNA:DNA ratio of the formate labeled nucleic acid is much lower than the ratio for either the adenine or the phosphorus labeled nucleic acids. The cPNA:DNA ratio obtained with adenine is the same as with P^{32} within experimental error. It is interesting to note (Table III) that the availability of exogenous adenine partially inhibits the incorporation of formate into the purines of both DNA and PNA. This same effect has been noted

by Goldthwait and Bendich.[47] The decrease in the specific activity of the phosphorus labeled DNA and cPNA observed with the simultaneous injection of inactive adenine is not significant.

The specific activities obtained with the four precursors in the four-hour experiments are shown in Tables V, VI, VII, and VIII. As is seen in Table IX the specific activity ratios of cPNA to DNA using formate and glycine as precursors are considerably lower than the same ratio for P^{32}. However, the specific activity ratio for nPNA and cPNA using formate is comparable to that using phosphorus. This lower ratio of PNA:DNA utilizing glycine and formate as precursors as compared to inorganic phosphorus possibly indicates an incorporation of phosphorus independent of total nucleotide renewal. However, the findings of several authors may give an explanation as to why there could be a lower ratio of PNA:DNA with glycine and formate as compared to phosphorus, and yet no "independent exchange" of the phosphorus moiety without the purines of the C-N skeleton being affected. Elwyn and Sprinson[42] and Totter, Volkin, and Carter[39] showed that both glycine and formate are incorporated into the pyrimidine, thymine, but not into the pyrimidines, uracil or cytosine. This would mean that there are two bases labeled in PNA while in DNA three bases are labeled. An additional explanation is suggested in the fact that Löw[43] found that the C^{14} of glycine-1-C^{14} is incorporated into the pentoses of PNA. The C^{14} of formate and glycine-2-C^{14} may be incorporated into the pentoses of the nucleic acids, but as yet this has not been demonstrated. However, in support of this possibility is the fact that it was found that the "formate nPNA:cPNA" is not significantly different from the same ratio using P^{32}. If either

the amount of radioactive carbon or the rate at which it is incorporated is greater in the desoxypentose than in the pentose sugar the ratio of PNA:DNA would be lower in the case of the nucleic acid labeled with formate and glycine than with P^{32}.

In another attempt to compare the different precursors the specific activity ratios of spleen DNA:liver DNA and intestine DNA:liver DNA obtained with different precursors were measured. As the data in Tables IX and X shows the intestine DNA:liver DNA ratio does not differ significantly in the case of adenine, formate, and phosphorus, while the glycine ratio is lower. The spleen DNA:liver DNA ratio is the same within experimental error only for formate and adenine.

Employing adenine C^{14}, which is incorporated into nucleic acids both as adenine and as guanine[43], and comparing the liver cPNA:DNA ratio with that of the P^{32} ratio it is seen in Tables IX and X that they are the same within experimental error. This equality does not agree with results obtained by Furst, Roll and Brown[48] employing adenine as the labeling agent. Furst et. al. find a ratio of total liver PNA:DNA of 73:1. Brown[49] attributes this "either to the fact that adenine does not serve as a precursor of the DNA purines, or to the fact that the DNA purines are not in a rapid dynamic equilibrium." In a later paper Furst and Brown[50] compare the incorporation of N^{15} labeled glycine and C^{14} labeled adenine into liver nucleic acids by simultaneous administration of the two labeling agents. They find a total liver PNA:DNA ratio of 50-60:1 with the use of adenine and 2.5-3:1 with the use of glycine. They interpret these results to mean that there exist two mechanisms of DNA synthesis. Reichard[46] believes

that the "difference in the turnover ratio DNA to PNA between adenine and phosphorus might indicate that some portion of the phosphate moieties of a nucleic acid may be exchanged without the purines of C-N skeleton being affected. On the other hand Abrams and Goldinger[51] employing adenine-8-C^{14} and guanine-8-C^{14} in a study on the purine renewal rates in bone marrow preparations find the same renewal rates as Stevens in an identical experiment employing P^{32} as inorganic phosphate. Abrams and Goldinger suggest that "there can be no independent turnover of purines from an intact polynucleotide structure, but rather that entire nucleotides must be incorporated as units." The data presented in this experiment with adenine and phosphorus is in agreement with Abram's and Goldinger's suggestion. The close agreement between adenine-C^{14} and P^{32} labeled nucleic acids both in the 4-hour and the 24-hour experiment is an indication that the nucleic acid molecule is synthesized <u>in toto</u>, and that the turnover rates obtained with the use of P^{32} are a true indication of the rate of synthesis of nucleic acids.

TABLE II

Ratios of Specific Activities of PNA:DNA in Rat Liver Utilizing Various Precursors

Precursor	Mode of Administration	Time after administration of precursor	Ratio of specific activity of PNA:DNA	Author
$Na_2HP^{32}O_4$	Subcutaneous injection	2 hours	33	Hammarsten and Hevesy[54]
$Na_2HP^{32}O_4$	i.p. injection	24 hours	40*	Barnum and Huseby[55]
$Na_2HP^{32}O_4$	i.p. injection	24 hours	100°	Barnum and Huseby[55]
$Na_2HP^{32}O_4$	parenterally	3 days	5	Brues, Tracy, Cohn[56]
Glycine-2-C^{14}	fed by stomach tube	24 hours	1.2	LePage and Heidelberger[40]
Glycine-2-C^{14}	fed by stomach tube	18 hours	2.2+	Heidelberger and LePage[41]
Glycine N^{15}	S.Q.	6 hours	4.1	Bergstrand, Eliason, Hammarsten, Noberg, Reichard, Ubisch[57]
Glycine N^{15}	fed	5 days	2.5-3	Furst and Brown[50]
Adenine 8-C^{14}	fed	5 days	50-60	Furst and Brown[50]
Adenine 1,3 N^{15}	fed	5 days	73-100	Furst, Roll, Brown[48]

* Mouse liver.

\+ Calculations taken from their data of the specific activity of the PNA and DNA purines.

° Nuclear PNA:DNA ratio.

24 hour Experiment

TABLE III

Specific Activity x 10^4 of cPNA and DNA
Utilizing Adenine 4,6-C^{14}, Formate-C^{14}, and $Na_2HP^{32}O_4$
as Precursors

Precursor	cPNA	DNA	cPNA:DNA
Adenine-4,6-C^{14}+	127±4.7	5.13±0.8	24.8±4.0
Formate-C^{14}	9.3±1.7	1.82±0.22	5.11±1.1
Formate-C^{14} + Adenine*	1.96±0.017	0.93±0.075	2.1±0.16
$Na_2HP^{32}O_4$	44.4±1.7	2.72±0.54	16.3±3.1
$Na_2HP^{32}O_4$ + Adenine*	38.6±0.62	1.86±0.26	20.8±2.95

* inactive adenine injected = 0.95 mg.

\+ Specific activity = 4.2 uc/mg - injected 0.95 mg (~4 uc)/mouse.

TABLE IV

Comparisons of Specific Activity Ratios

cPNA:DNA	Probability[1]
Adenine-4,6-C^{14} vs. $Na_2HP^{32}O_4$	<u>0.18</u>
Adenine-4,6-C^{14} vs. $Na_2HP^{32}O_4$ + Inactive Adenine	<u>0.46</u>
Formate-C^{14} vs. $Na_2HP^{32}O_4$	0.034
Formate-C^{14} vs. Formate-C^{14} + Inactive Adenine	0.05
Formate C^{14} vs. $Na_2HP^{32}O_4$ + Adenine	0.01

_____ = no significant difference.

1 - probability found from the distribution of t[53]

$$t = \frac{M_1 - M_2}{\sqrt{(\sigma_{M_1})^2 + (\sigma_{M_2})^2}}$$ where M = mean σ_M = standard error of mean

TABLE V

Specific Activity x 10^4 of Nucleic Acids labeled with Formate C^{14}*

	Tissue	DNA	cPNA	nPNA	No. of mice
	Liver	3.10±0.36	7.70±0.52	29.4±3.7	48
		2.90±0.52	4.60±0.17		12+
		2.20±0.6	9.58±0.72	25.1±3.7	36
Average		2.74±0.24	7.99±0.45	27.6±2.4	
	Intestine	50.6±2.7			48
		70.4±6.1			12
		69.0±3.5			36
Average		60±2.6			
	Spleen	63.4±3.8			48
		69.1±1.8			12
		55.6±1.9			36
Average		61.1±3.1			

\+ 2 livers pooled for DNA and cPNA determinations.

* Specific activity of formate-C^{14} = 19.12 uc/mgm.

TABLE VI

Specific Activity x 10^4 of Nucleic Acids labeled with $Na_2HP^{32}O_4$

	Tissue	DNA	cPNA	nPNA	No. of mice
	Liver	0.92±0.24	19.25±0.3	105.5±4.2	24
		1.10±0.12	12.8±2.1		12+
		1.82±0.26	24.9±1.8		16+
		1.17±0.16	17.2±1.1	63.5±5.1	36
		1.61°	15.8±0.52	54.2±2	24
Average		1.26±0.14	17.9±1.3	71.4±8.5	
	Intestine	22.8±0.9			24
		15.3±0.9			12
		38.6±2.4			16
		32.5±1.0			36
		22.1±1.5			24
Average		26.7±1.2			
	Spleen	53.3±4.6			24
		37.2±4.2			12
		55.3±3.6			16
		47.6±3.0			36
		43.1±2.7			24
Average		48.2±1.7			

\+ 2 livers pooled for DNA and cPNA determinations.

o This value only represents 12 mice.

TABLE VII

Specific Activity x 10^4 of Nucleic Acids labeled with Adenine-4,6-C^{14}*o

Tissue	cPNA	DNA	No. of mice
Liver	49.0±1.4	3.28±0.36	16
Intestine		126± 12	16
Spleen		69± 11	16

* Specific activity of Adenine = 4.2 uc/mg.

o dose injected = 7 uc/mouse.

TABLE VIII

Specific Activity x 10^4 of Nucleic Acids labeled with Glycine-2-C^{14}

	Tissue	cPNA	DNA	No. of mice
	Liver	6.36±0.75	1.82±0.22	10*
		7.66±0.37	2.37±0.38	22+o
Average		7.25±0.36	2.20±0.29	
	Intestine		21.2±1.6	10
			20.7±1.9	22
Average			20.9±1.5	
	Spleen		24.3±2.1	10
			16.9±0.62	22
Average			19.2±1.5	

* Specific activity of glycine = 11.1 uc/mg.

\+ Specific activity of glycine = 12 uc/mg.

o dose injected = 7 uc/mouse.

TABLE IX

Specific Activity Ratios of the Various Nucleic Acids

	Formate-C^{14}	Glycine-2-C^{14}	Adenine-4, 6-C^{14}	$Na_2HP^{32}O_4$
Liver nPNA:cPNA	3.45±0.36			3.99±0.55
Liver nPNA:DNA	10±1.24			56.7±9.2
Liver cPNA:DNA	3.0±0.31	3.23±0.42	15.0±1.7	14.2±1.86
Intestine DNA: Liver DNA	22±2.16	9.5±1.4	30.3±4.4	21.2±2.54
Spleen DNA: Liver DNA	22±2.0	8.7±1.3	16.6±3.1	38.3±4.4

TABLE X

Comparison of the Specific Activity Ratios of the Various Nucleic Acids

	Probability P			
	Formate-C^{14} vs. $Na_2HP^{32}O_4$	Glycine-2-C^{14} vs. $Na_2HP^{32}O_4$	Adenine-4, 6-C^{14} vs. $Na_2HP^{32}O_4$	Glycine-2-C^{14} vs. Formate-C^{14}
Liver nPNA:cPNA	0.44			
Liver nPNA:DNA	0.001			
Liver cPNA:DNA	0.001	0.001	1.00	0.22
Intestine DNA: Liver DNA	1.00	0.001	0.12	
Spleen DNA: Liver DNA	0.01	0.001	0.01	

____ = no significant difference.

EXPERIMENT II

Introduction

An increased rate of P^{32} incorporation into the desoxypentose nucleic acid (DNA) of livers and spleens in mice and rats bearing transplanted tumors and in pregnant mice and rats has been reported by Kelly and Jones[35] and Kelly et al.[36] The present experiment deals with an extension of this work to some other tumors and with measurements of the rate of P^{32} incorporation into the nuclear and cytoplasmic pentose nucleic acid of these tissues.

According to current theories PNA is intimately connected with protein synthesis and so might show a higher turnover in animals undergoing rapid tissue growth. If this were the case and if PNA and DNA had a common precursor, then DNA might show a higher specific activity not due to a change in its turnover but due only to the increased specific activity of its precursor. The present experiment was an attempt to test this possibility.

Experimental Work

All mice were fasted for 24 hours prior to the experiment. They were injected intraperitoneally with approximately 30 μc of P^{32} labeled sodium phosphate in isotonic saline at pH 7 about 9 AM and killed with ether precisely four hours later. The livers were removed and the isolation of the nucleic acids was begun as soon as possible. In order to obtain enough material the livers from eight or more mice were pooled.

Results and Discussion

The results of the measurements are given in Tables XI, XII, and

XIII. Each value represents the specific activity obtained on the pooled livers of at least eight animals. In the cytoplasmic PNA determinations enough material was available so that each sample was divided into two at the beginning of the isolation procedures and the two separate determinations were averaged to give the values in the table. The duplicates were within 10 percent of each other.

As can be seem from the tables the increased liver DNA specific activity observed earlier in female A strain mice occurs also in male A strain mice bearing the transplanted mammary carcinoma, in female A strain mice bearing transplanted sarcoma A274 and in female C57 strain mice bearing a transplanted mammary carcinoma.

Contrary to expectations there was no apparent difference in the rate of P^{32} incorporation into the cytoplasmic PNA of the livers of tumor bearing mice. However, the individual variations are rather large so that a small effect would not necessarily be noticed. Furthermore the cytoplasmic PNA is a mixture of the various cytoplasmic fractions and there might be a considerable change in one of these fractions without it being evident in the values.

The nuclear PNA specific activities of the tumor group show a statistically significant decrease from those of the control group (Table XI); however, these measurements should be viewed with caution. The ratio between the nuclear and cytoplasmic PNA specific activities is much lower than obtained by Barnum et al.[44] indicating that the nuclear PNA may still be contaminated, possibly with cytoplasmic PNA.

It is clear from these experiments, however, that there is no drastic change in the PNA metabolism of the livers of tumor-bearing

hosts. The increase in the DNA specific activity is apparently due to a true change in its turnover rate and not to a secondary effect associated with PNA turnover or a change in overall phosphate metabolism.

In a series of 48 female A strain mice bearing transplants of mammary carcinoma (1.3 gms per mouse) the average liver weight was found to be 7.57 percent ± 0.18 percent of the body weight. Eighteen control animals had an average liver weight of 6.19 percent ± 0.17 percent of the body weight. This indicates a 20 percent greater liver weight in tumor animals. Recently Reddy and Cerecedo[37] have reported an increased content of both DNA and PNA in the livers of mice bearing Crocker sarcoma 180 transplants. Kosterlizz and Campbell[52] have found an increase in the DNA content of livers of pregnant rats. These observations suggest that in the livers of tumor-bearing mice the increased DNA turnover is an indication of increased call proliferation.

TABLE XI

Specific Activities x 10^4 of Liver Nucleic Acids in ♂ A Strain Mice Bearing Mammary Carcinomas

Weight and Age of Tumors	DNA*		Cytoplasmic PNA		Nuclear PNA	
	Tumor	Control	Tumor	Control	Tumor	Control
0.17 gm 7.5 days	1.72 1.96 1.44 1.73 2.02 1.68 1.41	1.09 1.37 0.69 1.06	17.6 16.8 16.0 14.1 11.9 11.4	11.2 11.9 12.4 10.8	55.3 62.6 53.0	53.8 63.8 66.8
1.0 gm 10 days	3.75 4.91 5.45	1.25 0.71	12.5 11.6 11.6	7.2 9.3	39.7 45.2 58.2	45.4 49.3
2.0 gm 15 days	2.22 1.89 2.24 5.08	0.53 0.75	13.6 12.0 4.2 8.2 11.8	11.5 12.1 13.5	43.1 35.7 41.9 36.6 48.5	55.3 48.5
2.4 gm 18 days	1.55 1.70	0.93	7.3 7.4	9.1	37.8 34.7	51.5
Averages	2.55±0.37	0.97±0.11	11.7±1.0	10.9±0.7	45.6±2.7	55.3±2.9
Probability of Significance (P)	0.002		*0.27*		0.013	

* The values given represent the number of P^{32} counts per milligram of phosphorus divided by the number of counts injected, normalized for the weight of the mice. Errors quoted are $1\sigma_M$. No significant differences italicized.

TABLE XII

Specific Activities x 10^4 of Liver Nucleic Acids in ♀ C57 Mice Bearing Mammary Carcinomas

Weight and Age of Tumors	DNA		Cytoplasmic PNA		Nuclear PNA	
	Tumor	Control	Tumor	Control	Tumor	Control
2.4 gm 11 days	3.65 4.09 3.21	0.56 0.63	9.2 9.9 7.8	7.8 7.7	60.0 45.0	
5.2 gm 14 days	13.0 10.5	0.27 0.44	13.2 9.3	10.6 10.2	79.0	50.0
Averages	6.8±2.0	0.48±0.08	9.9±1.0	9.1±0.8		
Probability of Significance (P)	0.028					

TABLE XIII

Specific Activities x 10^4 of Liver Nucleic Acids in ♀ A Strain Mice Bearing Sarcoma A274

Weight and Age of Tumors	DNA		Cytoplasmic PNA		Nuclear PNA	
	Tumor	Control	Tumor	Control	Tumor	Control
0.55 gm 7 days	2.39 2.63 3.00	0.75 0.88	12.0 12.8 13.1	10.6 11.4	56.5	47.0
2.0 gm 12 days	2.28 2.28 2.05	0.55 0.76	12.1 13.6 20.9	12.1 14.1		
Averages	2.44±0.14	0.74±0.09	14.1±1.5	12.1±0.8		
Probability of Significance (P)	0.001		0.14			

EXPERIMENT III

Introduction

The observation made in the previous experiment that the increase in incorporation of P^{32} in the liver and spleen DNA is not noted in liver nuclear pentose-nucleic acid (nPNA) or cytoplasmic pentose nucleic acid (cPNA) is an indication that the increase found in the liver and spleen DNA of tumor-bearing mice as compared to controls is not an indirect effect due to a change in total phosphorus metabolism -- e.g., an increase in cell permeability to phosphorus, but is probably due to an increase in cell proliferation. To further substantiate this conclusion formate-C^{14} and glycine-2-C^{14} were employed as precursors.

Experimenal Work

Male A strain mice were used in all the experiments. The mice were fasted for 24 hours prior to being sacrificed. The relative nucleic acid turnover rate was measured by giving 20 uc (except where otherwise indicated) intraperitoneally of formate-C^{14} or of glycine-2-C^{14} sacrificing the mice after four hours, isolating the DNA from livers, intestines, spleens and tumors and cPNA from livers, and measuring the specific activity of the nucleic acid.

Results and Discussion

As can be seen in Tables XIV and XV, the increase in specific activity observed earlier with the use of P^{32} in liver and spleen DNA is also observed with formate-C^{14} and glycine-2-C^{14}, while the cPNA specific activity in the tumor-bearing mice does not vary significantly

from the control groups, except in the group of mice bearing sarcoma A274 transplants. The cPNA specific activity in this group is lower than the cPNA values for the entire group of control mice. However, the average cPNA value of the control mice which were used the same day as the sarcoma A274 mice was 4.6 which is not significantly different from 5.1, the average cPNA value obtained for the sarcoma 274 mice.

The specific activity of the intestinal DNA is significantly lower in all the tumor bearing mice as compared to the controls.

The results with glycine-2-C^{14} do not agree with those of Le Page and Heidelberger.[40] They find essentially no difference in the specific activity of liver DNA of control rats from that of rats bearing multiple Flexner-Jobling carcinoma transplants.

Their values observed in liver DNA and in tumor DNA are also approximately the same as the values obtained for the liver DNA.

A ratio of tumor DNA:liver DNA of 5.5 was obtained employing glycine-2-C^{14} as the labeling agent, which is considerably lower than the ratio of 70 observed using formate-C^{14} as the tracer. The cPNA:DNA ratios are the same for both glycine and formate, namely 3.1.

Le Page and Heidelberger interpret their results to indicate that there may exist two separate pathways of nucleic acid synthesis. However, the difference in the tumor DNA to liver DNA ratio that was observed using glycine and formate may also be explained by a difference in the availability of the two precursors in the various tissues. This may be partially substantiated by the findings that the intestine DNA: liver DNA and the spleen DNA:liver DNA ratios are also lower with glycine labeled nucleic acid than with the formate labeled nucleic acid, while

the cPNA:DNA ratio in the liver is the same using either precursor.

From the present experiments it can be concluded that the increase in specific activity of liver and spleen DNA in tumor bearing mice as compared to control mice with the use of radioactive phosphorus was due to a true change in the DNA turnover in these two tissues.

TABLE XIV

Specific Activity* x 10^4 of Nucleic Acid labeled with Formate-C^{14} [o] in ♂ A Strain Mice

	Liver[1] DNA	Liver[1] cPNA	Spleen[2] DNA	Intestine[3] DNA
	3.14	8.55	70.4	98.7
	1.62	11.65	70.8	85.3
	3.78	14.22	66.2	88.9
	1.22	8.68	82.6	73.0
	1.88	8.38	99.0	79.1
	2.10	12.85	81.8	74.0
	2.36	8.66	83.8	89.1
	1.15	9.81	108.6	107.0
	2.20	5.43		78.7
	4.57	2.17		47.7
	3.17	2.60		81.6
	2.18	10.32		67.2
	1.63	2.47		65.7
	3.57			85.8
	2.28			74.3
Averages	2.46±0.27	8.13±1.1	82.9±5.5	79.7±3.8

1. Each value represents 2 livers.
2. Each value represents 4 spleens.
3. Each value represents 2 intestines.
4. Each value represents tumor tissue from 2 mice.

* Specific activity expressed as counts per minute per milligram of nucleic acid phosphorus divided by counts per minute injected, normalized for the weight of the mouse.

o Specific activity of formate = 19.12 uc/mg.

TABLE XIV (cont)

Specific Activity* x 10^4 of Nucleic Acid labeled with Formate-C^{14} in ♂ A Strain Mice Bearing Mammary Carcinoma

	Liver[1] DNA	Liver[1] cPNA	Spleen[2] DNA	Intestine[3] DNA
	5.23	6.53	211.0	57.9
	8.22	5.74	168.0	74.0
	11.65	8.58	191.0	55.5
	9.53	9.90	225.0	66.9
	5.34	6.70	160.0	69.6
	4.26	8.84	187.0	61.0
	14.15	7.79	185.0	72.0
	7.40	7.84		59.5
	8.86	6.13		60.0
	6.22	9.01		68.2
	8.23	12.35		63.5
	7.78	11.44		68.4
	6.26	8.37		71.6
	6.19	11.63		73.1
	10.78	8.57		67.2
Averages	8.01±0.7	8.63±0.47	190±9	65.9±1.6

1. Each value represents 2 livers.
2. Each value represents 4 spleens.
3. Each value represents 2 intestines.
4. Each value represents tumor tissue from 2 mice.

Comparison of Formate-C^{14} labeled Nucleic Acid Specific Activities in Control Mice with Mice Bearing Mammary Carcinoma

	Liver DNA	Liver cPNA	Spleen DNA	Intestine DNA
Probability of significance p	0.001	0.7	0.001	0.01

* Specific activity expressed as counts per minute per milligram of nucleic acid phosphorus divided by counts per minute injected, normalized for the weight of the mice.

TABLE XIV (cont)

Specific Activity* x 10^4 of Nucleic Acid labeled with Formate-C^{14} in ♂ A Strain Mice Bearing Sarcoma A274

	Liver[1] DNA	Liver[1] cPNA	Spleen[2] DNA	Intestine[3] DNA	Tumor[4] DNA
	12.75	5.00	142.7	44.0	84.3
	17.38	5.68	143.6	72.7	61.7
	20.49	6.85	178.7	60.1	71.1
	10.34	3.17		67.3	80.0
	19.17	4.74		50.4	74.7
	21.43			45.2	
Averages	16.92±2.2	5.10±0.68	155±14.0	56.6±5.6	74.4±4.4

1. Each value represents 2 livers.
2. Each value represents 4 spleens..
3. Each value represents 2 intestines.
4. Each value represents tumor tissue from 2 mice.

Comparison of Formate-C^{14} labeled Nucleic Acid Specific Activities in Control Mice with Mice Bearing Sarcoma A274

	Liver DNA	Liver cPNA	Spleen DNA	Intestine DNA
Probability of significance p	0.001	0.07	0.03	0.015

* Specific activity expressed as counts per minute per milligram of nucleic acid phosphorus divided by counts per minute injected, normalized for the weight of the mice.

TABLE XV

Specific Activity* x 10^4 of Nucleic Acid labeled with Glycine-2-C^{14}*o
in Normal ♂ A Strain Mice and in ♂ A Strain Mice Bearing Mammary Carcinoma

	Controls				Mammary Carcinoma				
	Liver[1] DNA	Liver[1] cPNA	Spleen[2] DNA	Intestine[3] DNA	Liver[1] DNA	Liver[1] cPNA	Spleen[2] DNA	Intestine[3] DNA	Tumor[4] DNA
	2.18	6.20	19.0	33.6	5.20	15.3	30.5	11.6	11.3
	1.87	9.43	17.2	17.1	4.64	7.8	33.6	15.0	13.2
	1.96	8.61	16.6	31.5	6.48	6.7	36.4	11.9	12.5
	1.63	6.49	16.0	21.3	3.58	7.7	41.3	11.7	12.1
	3.43	6.54	15.8	17.9	3.93	10.9	45.3	12.4	23.4
	2.58	8.23		22.5	4.27	9.1		11.8	11.1
	1.73	8.62		13.7	5.17	9.2		15.6	11.6
	1.62	6.07		18.5	3.30	10.0		16.9	10.9
	1.44	8.12		16.7	4.46	9.1		12.8	11.7
	1.98	8.53		16.4	4.09	8.0		15.5	
	5.63	7.45		18.6					
Averages	2.37±0.38	7.66±0.37	16.9±0.62	20.7±1.9	4.51±0.31	9.4±1.2	37.4±0.3	13.5±0.7	13.1±1.4

1. Each value represents 2 livers.
2. Each value represents 4 spleens.
3. Each value represents 2 intestines.
4. Each value represents tumor tissue from 2 mice.
* dose injected = 7.35 uc/mouse.
o specific activity of glycine = 12 uc/mg.

Comparison of Glycine-C^{14} labeled Nucleic Acid Specific Activity in Control Mice with Mice Bearing Mammary Carcinoma

Probability of significance p	Liver DNA	Liver cPNA	Spleen DNA	Intestine DNA
	0.01	0.2	0.001	0.01

_____ = Significant difference.

SUMMARY AND CONCLUSIONS

The turnover rate of liver nucleic acids (i.e., cytoplasmic PNA, nuclear PNA, and DNA), spleen DNA, and intestine DNA has been studied with the use of formate-C^{14}, glycine-2-C^{14}, adenine-4,6-C^{14}, and $Na_2HP^{32}O_4$ under identical experimental conditions in normal mice. The turnover rate of liver nucleic acids with P^{32}, formate and glycine-2-C^{14} and in spleen DNA, intestine DNA, and tumor DNA with formate and glycine has also been measured in mice bearing mammary carcinoma or sarcoma transplants. The liver cPNA:DNA, nPNA:cPNA, spleen DNA:liverDNA, intestine DNA:liver DNA specific activity ratios obtained with the four labeling agents have been compared in the normal mice. In the mice bearing the tumor transplants, the effect of the tumor tissue on the liver and spleen nucleic acids was studied.

The following results were obtained in male A strain mice.

1. Liver cPNA:DNA ratios are the same for formate and glycine labeled nucleic acids, but considerably lower for P^{32} labeled nucleic acids.

2. Liver cPNA:DNA ratios are the same for adenine and phosphorus labeled nucleic acids.

3. Liver nPNA:cPNA ratios are the same for formate and phosphorus labeled nucleic acids.

4. Intestine DNA:liver DNA ratios are the same for adenine, formate, and phosphorus labeled nucleic acids, but considerably lower for glycine labeled nucleic acids.

5. Spleen DNA:liverDNA ratios are the same for formate and adenine labeled nucleic acids, lower for glycine labeled nucleic acids, and higher

for phosphorus labeled nucleic acids.

The lower cPNA:DNA ratios found with glycine and formate as compared to the ones found with adenine and phosphorus may be explained by the findings of Elwyn and Sprinson,[42] and Totter, Volkin, and Carter[39] that both glycine and formate are incorporated into the pyrimidine, thymine, but not into the pyrimidines, uracil or cytosine, which means that two bases are labeled in PNA while three bases are labeled in the DNA. However, with phosphorus and adenine an equal number of nucleotides are labeled in the PNA and DNA. The above is also substantiated by the observation that nPNA:cPNA ratios are the same for formate and phosphorus labeled nucleic acids. The differences found in the intestine DNA: liver DNA and spleen DNA:liverDNA observed with the four precursors may be explained by a difference in the availability of each precursor in the various tissues owing to differences in their metabolic pathways.

The results obtained with adenine as the labeling agent do not agree with earlier findings by Furst and Brown[50] and Furst, Roll and Brown[48] who found a much higher PNA:DNA ratio in rat liver than was observed in mouse liver in the present experiment.

The results obtained in this experiment indicate that there is not an independent turnover of any one fraction of the nucleic acid molecule, but that the nucleic acid molecule is synthesized <u>in toto</u>.

The results obtained in the study of the effect of tumor transplants on nucleic acid synthesis in the liver, spleen, and intestine when compared to normal are as follows.

1. The rate of incorporation of P^{32}, formate-C^{14}, and glycine-2-C^{14} into liver DNA is increased.

2. There is a decreased rate of P^{32} incorporation into liver nuclear PNA in male A strain mice bearing mammary carcinoma.

3. There is no significant variation from normal in the rate of incorporation of P^{32}, formate-C^{14}, and glycine-2-C^{14} into liver cytoplasmic PNA.

4. There is an increased rate of formate and glycine incorporation into spleen DNA.

5. There is a decreased rate of formate and glycine incorporation into intestine DNA.

6. In A strain mice bearing transplants of mammary carinoma, there is an increase of 20 percent above normal of the average liver weight/ body weight ratio.

The above observations suggest that in the livers of tumor-bearing mice the increased DNA turnover reflects an increased cell proliferation. This concept is supported by the recent report of Reddy and Cerecedo[37] of an increased content of both DNA and PNA in the livers of mice bearing Crocker Sarcoma 180 transplants. Since PNA is believed to be intimately connected with protein synthesis, it is plausible that PNA has a higher turnover in liver tissue undergoing rapid growth. That there is no increase above the normal controls in the specific activity in liver PNA from mice with tumor transplants may be due to PNA being a composite of different cytoplasmic PNA fractions. A study on the PNA obtained separately from the mitochondrial, microsomial, and supernatant fraction might well reveal an effect on any one of the cytoplasmic PNA fractions.

BIBLIOGRAPHY

1. Friedrich Miescher. Die Histochemischen und Physiologischen Arbeiten. Leipzig, 1897.

2. Wyatt, G. R. Occurrence of 5-Methyl-Cytosine in Nucleic Acids. Nature 166, 237 (1950).

3. Levene, P. A. and Bass, L. W., Nucleic Acids. Chemical Catalogue Co., New York, 1931.

4. Gulland, J. M., Jordon, D. O., and Threlfall, C. J., Desoxypentose Nucleic Acids. Part I. Preparation of the Tetrasodium Salt of the Desoxypentose Nucleic Acid of Calf Thymus. J. Chem. Soc. 1129 (1947).

5. Loring, H. S., Symposium on Chemistry and Metabolism of Nucleic Acids and their Constituents - Introduction. Fed. Proc. 6, 487 (1947).

6. Chargaff, E. and Vischer, E., The Composition of the Pentose Nucleic Acids of Yeast and Pancreas. J. Biol. Chem. 176, 715 (1948).

7. Chargaff, E., Lipshitz, R., Green, Charlotte, Hodes, M. E., The Composition of the Desoxyribonucleic Acid of Salmon Sperm. J. Biol. Chem. 192, 233 (1951).

8. Marshak, A., Purine and Pyrimidine Content of the Nucleic Acids of Nuclei and Cytoplasm. J. Biol. Chem. 189, 607 (1951).

9. Calvery, H. O., and Remsen, D. B., The Nucleotides of Triticonucleic Acid. J. Biol. Chem. 73, 593 (1927).

10. Calvery, H. O., Some Chemical Investigation of Embryonic Metabolism. I. The Isolation of Four Pentose Nucleotides from Chicken Embryo. J. Bio. Chem. 77, 489 (1928).

11. (a) Feulgen, R. Z., Darstellung und Eigenschaffen der Thyminsäure. Hoppe-Seyl Z. 101, 296 (1918).

(b) Feulgen, R. Z., Das Verhalten der Thyminsäure der Phenylhydrazin. Hoppe-Seyl Z. 102, 262 (1918).

12. Bredereck, H and Müller G., Über die Thyminsäure (Nucleinsäuren, XII Mitteil) Ber deutsch. Chem. Ges. 72, 115 (1939).

13. Signer, R., Casperson, T. and Hammarsten, E., Molecular Shape and Size of Thymonucleic Acid. Nature 141, 122 (1938).

14. Cohen, S. S. and Stanley, W. M., The Molecular Size and Shape of the Nucleic Acid of Tobacco Mosaic Virus. J. Biol. Chem. 144, 589 (1942).

15. Fischer, F. G., Böttger, I. and Lehmann-Echternacht, H., Über die Thymo-polynucleotidase aus Pankreas Nucleinsäuren. Hoppe-Seyl Z. 271, 246 (1941).

16. Fletcher, W. E., Gulland, J. M., Jordan, D. O., The Constitution of Yeast Ribonucleic Acid. Part VII Diffusion Coefficients and Molecular Weights. J. Chem. Soc. 33 (1944).

17. Greenstein, Jessie P. Nucleoproteins. Advances in Protein Chemistry 1, 209 (1944).

18. Casperson, T. and Schultz, J., Ribonucleic Acids in both Nucleus and Cytoplasm, and the Function of the Nucleolus. Proc. of Natl. Aca. of Science 26, 507 (1940).

19. Schultz, J., Casperson, T. and Aquilonus, L., The Genetic Control of Nucleolar Composition. Proc. of Natl. Aca. Science 26, 515 (1940).

20. Chantrenne, H., Heterogeneite des Granules Cytoplasmiques du Foie de Souris. Biochem. et Biophys. Acta 1, 437 (1947).

21. Schneider, W. D., Nucleic Acids in Normal and Neoplastic Tissues. Cold Spring Harbor Symposia. Quant. Biol. 12, 169 (1947).

22. Brachet, J., Biochemical and Physiological Interrelations between Nucleus and Cytoplasm during Early Development. Growth Symp. 11, 309 (1947).

23. Chargaff, E., Vischer, E. and Doniger, R., Chemical Specificity of Nucleic Acids and Mechanism of their Enzymatic Degradation. Experientia 6, 201 (1950).

24. Boivin, A., Vendrely, R., Vendrely, C., L'acide desoxyribonuclei que du voyen celluaire. C.R. Acad. Sci. 226, 1061 (1948).

25. Mirsky, A. E. and Ris, H., Variable and Constant Components of Chromosomes. Nature 163, 666 (1949).

26. Vendrely, R., and Vendrely, C., La teneus du voyen cellulaire en acide desoxyribonucleique a travers les organes, les individues et les espeses animales. Experientia 5, 327 (1941).

27. Pollister, A. W. and Mirsky, A. E., The Nucleoprotamine of Trout Sperm. Journ. of Gen. Phsio. 30, 101 (1946).

28. Mirsky, A. E. and Pollister, A. W., Chromosin, Desoxyribose Nucleoprotein Complex of the Cell Nucleus. Journ. of Gen. Physiol. 30, 117 (1946).

29. Stedman, E. and Stedman, E., Chromosomin, a Protein Constituent of Chromosomes. Nature 152, 267 (1943).

30. Hevesy, G., Nucleic Acid Metabolism. Adr. Biol. Med. Phys. 1, 409 (1948).

31. Casperson, T., The Relations between Nucleic Acid and Protein Synthesis. Symp. Ex. Biol. 1, 127 (1947).

32. Brachet, J., The Localization and the Role of Ribonucleic Acid in the Cell. Annals New York Acad. Sci. 50, 861 (1950).

33. Spiegelman, S. and Kamen, M. D., Some Basic Problems in the Relation of Nucleic Acid Turnover to Protein Synthesis. Cold Spring Harbon Symp. Quant. Biol. 12, 211 (1947).

34. Spiegelman, S., Nuclear and Cytoplasmic Factors Controlling Enzymatic Constitution. Cold Spring Harbor Symp. Quant. Biol. 11, 256 (1946).

35. Kelly, L. S. and Jones, H. B., Effect of Neoplastic Tissue on the Turnover of Desoxypentose Nucleic Acid. Science 111, 333 (1950).

36. Kelly, L. S., Payne, A. H., White, R. S. and Jones, H. B., The Effect of Neoplasia or Pregnancy on the Tissue Desoxypentose Nucleic Acid. Cancer Research 11, 694 (1951).

37. Reddy, D. V. N. and Cerecedo, L. R., Effect of Transplanted Tumors on the Nucleic Acid Content of Mouse Tissue. Fed. Proc. 10, 236 (1951).

38. Heinrich, M. R. and Wilson, D. W., The Biosynthesis of Nucleic Acid Components Studied with C^{14}. J. Biol. Chem. 186, 447 (1950).

39. Totter, J. R. Volkin, E. and Carter, C. E., Incorporation of Isotopic Formate into the Nucleotides of Ribo-and Desoxyribonucleic Acids. J. Am. Chem. Soc. 73, 1521 (1951).

40. Le Page, G. A. and Heidelberger, C., Incorporation of Glycine-2-C^{14} into Proteins and Nucleic Acids of the Rat. J. Biol. Chem. 188, 593 (1951).

41. Heidelberger, C. and Le Page, G. A., Incorporation of Glycine-2-C^{14} into Purines of Pentose Nucleic Acid and Desoxyribose Nucleic Acid. Proc. Soc. Exp. Biol. and Med. 76, 464 (1951).

42. Elwyn, D. and Sprinson, D. B., The Extensive Synthesis of the Methyl Group of Thymine in the Adult Rat. J. Am. Chem. Soc. 72, 3317 (1950).

43. Löw, B., Preparation of Furoic Acid from Ribonucleic Acid. Acta Chem. Scand. 4, 294 (1950).

44. Barnum, C. P., Nash, C. W., Jennings, E., Nygaard, O., and Vermund, H., The Separation of Pentose and Desoxypentose Nucleic Acids from Isolated Mouse Liver Cell Nuclei. Arch. Biochem. 25, 376 (1950).

45. Johnson, T. B., and Harkins, H. H., Researches on Pyrimidines CV 14. The Examination of Yeast Nucleic Acid for 5-methylcytosine. J. Am. Chem. Soc. 51, 1779 (1929).

46. Reichard, P., On the Nitrogen Turnover in Purines from Polynucleotides Determined with Glycine N^{15}. J. Biol. Chem. 179, 773 (1949).

47. Goldthwait, D. A. and Bendich, A., Effect of Aminopterin on Nucleic Acid Metabolism in the Rat. Fed. Proceedings 10, 190 (1951).

48. Furst, S. S., Roll, P. M. and Brown, G. B., On the Renewal of the Purines of the Desoxypentose and Pentose Nucleic Acids. J. Biol. Chem. 183, 251 (1950).

49. Brown, G. B., Biosynthesis of Nucleic Acids in Mammals. Fed. Proceedings 9, 517 (1950).

50. Furst, S. S. and Brown, G. B., On the Role of Glycine and Adenine as Precursors of Nucleic Acid Purines. J. Biol. Chem. 191, 239 (1951).

51. Abrams, R. and Goldinger, J. M., Utilization of Purines for Nucleic Acid Synthesis in Bone Marrow Slices. Arch. of Biochem. 30, 261 (1951).

52. Kosterlitz, H. W. and Campbell, R. M., Composition and Structure of the Liver Cell in Pregnancy. Nature 160 , 676 (1947).

53. Fisher, R. A. and Yates, F., Statistical Tables for Biological, Agricultural and Medical Research, Oliver and Boyd Lts., Edinghburg V, P. 32 (1948).

54. Hammarsten, E., and Hevesy, G., Rate of Renewal of Ribo and Desoxyribo Nucleic Acids. Acta Physiologica Scandinavica 11, 335 (1946).

55. Barnum, C. P. and Huseby, R. A., The Intracelleular Heterogeneity of Pentose Nucleic Acid as Evidenced by the Incorporation of Radiophosphorus. Arch. of Biochem. 29, 7 (1950).

56. Brues, A. M. Tracy, M. M. and Cohn, W. E., Nucleic Acids of Rat Liver and Hepatoma: Their Metabolic Turnover in Relation to Growth. J. Biol. Chem. 155, 619 (1944).

57. Bergstrand, A., Eliasson, N. A., Hammarsten, E., Norberg, B., Reichard, P. and von Ubisch, H., Experiments with N^{15} on Purines from Nuclei and Cytoplasm of Normal and Regenerating Liver. Cold Spring Harbor Symp. 13, 22 (1948).