Effect of Neoplastic Tissue on the Turnover of Liver Nucleic Acids*

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In two earlier papers (5, 7), the authors reported an increased rate of incorporation of P^{32} into the desoxyribonucleic acid (DNA) of livers and spleens in mice and rats bearing transplanted tumors and in pregnant mice and rats. The present paper 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 pentosenucleic acid (PNA) 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.

METHODS

All mice were fasted for 24 hours prior to the experiment. They received intraperitoneal injections of P^{32}-labeled sodium phosphate in isotonic saline at pH 7 about 9 A.M. and were killed with ether exactly 4 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.

Each animal received approximately 30 μc. of P^{32}-labeled sodium phosphate. The procedure used for the nucleic acid isolations was an adaptation of several published methods and will be given in detail. In view of previous experience with active contaminants, it was felt that it was important to have the final nucleic acids in such a form that they could be easily reprecipitated to constant specific activity.

The method of Barnum *et al.* (2) was followed for the separation of the nuclei from the cytoplasm and the subsequent purification of the nuclei and separation of nuclear pentosenucleoprotein (PNA-protein) from desoxypentosenucleoprotein (DNA-protein).

After being cooled the livers were forced through a tissue press with holes about 1 mm. in diameter and were homogenized 2–3 minutes in 4 volumes of ice-cold saline (0.85 per cent NaCl containing 2 ml. 0.1 N NaOH per liter). Homogenization was carried out in a Potter-Elvehjem tube. The homogenate was centrifuged 4 minutes at 1,400 $\times$ g in a No. 1 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. The nuclear sediment was suspended in 2 per cent cold citric acid and centrifuged for 10 minutes in a clinical centrifuge at 500 $\times$ g. The sediment was resuspended and centrifuged 14 times at the same speed, after which treatment the supernatant fluid was perfectly clear. The centrifugations were carried out as follows: once for 10 min., twice for 5 min., 5 times for 3 min., and 6 times for 1 min. The isolated nuclei were washed twice with saline to remove citric acid and then were extracted in 95 per cent methyl alcohol, followed by three extractions with boiling alcohol-ether (3:1), and a final alcohol extraction. The lipid-free nuclei 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 were 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 per cent to trichloroacetic acid (TCA) and centrifuged in a No. 1 International centrifuge.

The precipitate which represents the nuclear PNA-protein and any possible residual DNA-protein was washed once in 5 per cent TCA. In order to obtain the nucleic acid in a reprecipitable form,

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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 nucleoprotein precipitate was suspended in 0.14 M NaCl and brought to pH 7. DNA-protein is insoluble in this concentration of NaCl and could be removed by centrifugation. The PNA-protein contained in the supernatant fluid was precipitated out by bringing the pH of the solution down to ~3 with glacial acetic acid and separated by centrifugation. The PNA-protein was then suspended in ice-cold 5 per cent NaOH and kept at 0° ± 2° for 2 hours (4). This split the protein from the nucleic acid. Essentially no decomposition of nucleic acid occurs at this temperature. 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 fluid filtered through tissue paper. The PNA remained in the supernatant fluid and was precipitated by bringing the solution to pH 2 by the addition of HCl and then adding an equal volume of 95 per cent 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 that the specific activity did not change significantly after reprecipitation.

The DNA was obtained from the gelatinous sediment that remained after the pH 10 extraction by following a modified Beck and Klein method previously published (6).

The cytoplasmic PNA was precipitated from the cytoplasmic extract which had been obtained earlier. TCA was added to a concentration of 5 per cent, the extract was centrifuged, and the supernatant fluid containing the acid soluble phosphorus was decanted. The sediment was washed once with 5 per cent TCA and then suspended in cold 5 per cent NaOH. The procedure from here on was the same as for the nuclear PNA.

As is shown in Table 1, with the use of 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 that of the commercial preparations. The diphenylamine reagent and the orcinol reagent were prepared according to the directions given by Barnum *et al.* (2). 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 (3).

TABLE 1

ANALYTICAL COMPARISON OF DIFFERENT PREPARATIONS OF NUCLEIC ACIDS

	Cytoplasmic PNA	Nuclear PNA	Commercial* PNA	DNA	Commercial* DNA
E (diphenylamine reaction)†				0.018 ±0.007	0.0194 ±0.006
E (orcinol reaction)‡	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	0	0	0	+	+

* This material was obtained from the Nutritional Biochemical Corporation, Cleveland, Ohio.
† Optical density at 600 mμ wave length, calculated per μg, P in sample.
‡ Optical density at 660 mμ wave length, calculated per μg. P in sample.
§ The Nutritional Biochemical Corporation's N:P ratio is given as 1.71.

A second aliquot was used for counting the P^{32} with a Victoreen geiger tube.

RESULTS AND DISCUSSION

The results of our measurements are given in Tables 2–4. Each value represents the specific activity of the nucleic acid obtained from 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 agreed to better than 10 per cent.

As can be seen from the tables, the increased specific activity of liver DNA, 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 our expectations, there was no significant difference in the rate of incorporation of P^{32} in 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, our cytoplasmic PNA is obtained from a mixture of the

various cytoplasmic fractions, and there might be a considerable change in one of these fractions without its being evident in our values.

The nuclear PNA specific activities of the tumor group show a statistically significant decrease from those of the control group (Table 2). However, it is felt that these measurements are to be viewed with caution. The ratio between the nuclear and cytoplasmic PNA specific activities is much lower than that obtained by Barnum and Huseby (1), indicating that our 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 in-

TABLE 2

SPECIFIC ACTIVITIES × 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.	1.72	1.09	17.6	11.2	55.3	53.8
7.5 days	1.96	1.37	16.8	11.9	62.6	63.8
	1.44	0.69	16.0	12.4	53.0	66.8
	1.73	1.06	14.1	10.8		
	2.02		11.9			
	1.68		11.4			
	1.41					
1.0 gm.	3.75	1.25	12.5	7.2	39.7	45.4
10 days	4.91	0.71	11.6	9.3	45.2	49.3
	5.45		11.6		58.2	
2.0 gm.	2.22	0.53	13.6	11.5	43.1	55.3
15 days	1.89	0.75	12.0	12.1	35.7	48.5
	2.24		4.2	13.5	41.9	
	5.08		8.2		36.6	
			11.8		48.5	
2.4 gm.	1.55	0.93	7.3	9.1	37.8	51.5
18 days	1.70		7.4		34.7	
Av.	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 are expressed as standard deviations of the mean. Significant differences are italicized.

TABLE 3

SPECIFIC ACTIVITIES × 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.	3.65	0.56	9.2	7.8	60.0	
11 days	4.09	0.63	9.9	7.7	45.0	
	3.21		7.8			
5.2 gm.	13.0	0.27	13.2	10.6	79.0	50.0
14 days	10.05	0.44	9.3	10.2		
Av.	6.8±2.0	0.48±0.08	9.9±1.0	9.1±0.8		
Probability of significance (P)	*0.028*					

TABLE 4

SPECIFIC ACTIVITIES × 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.	2.39	0.75	12.0	10.6	56.5	47.0
7 days	2.63	0.88	12.8	11.4		
	3.00		13.1			
2.0 gm.	2.28	0.55	12.1	12.1		
12 days	2.28	0.76	13.6	14.1		
	2.05		20.9			
Av.	2.44±0.14	0.74±0.09	14.1±1.5	12.1±0.8		
Probability of significance (P)	*0.001*		0.14			

crease 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 over-all phosphate metabolism.

In a series of 48 female A strain mice bearing transplants of mammary carcinoma (1.3 gm. per mouse), the average liver weight was found to be 7.57 per cent ± 0.18 per cent of the body weight. In eighteen control animals the average liver weight was 6.19 per cent ± 0.17 per cent of the body weight. This indicates a 20 per cent greater liver weight in tumor-bearing animals. Recently, Reddy and Cerecedo (9) reported an increased content of both DNA and PNA in the livers of mice bearing Crocker sarcoma 180 transplants. Kosterlitz and Campbell (8) 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 cell proliferation.

SUMMARY

The rate of incorporation of P^{32} into DNA, nuclear PNA, and cytoplasmic PNA of liver tissue was measured in male A strain mice bearing transplanted mammary carcinoma, in female A strain mice bearing transplanted sarcoma A274, and in female C57 strain mice bearing a transplanted mammary carcinoma. An increase of P^{32} incorporation into the liver DNA was noted in all three groups of tumor-bearing mice when compared to the controls. A decrease in P^{32} incorporation into nuclear PNA was observed in the male A strain mice bearing mammary carcinoma. No significant difference in the rate of incorporation of P^{32} into the cytoplasmic PNA was observed in the tumor-bearing mice.

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