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Effect of Neoplastic Tissue on the Turnover of Desoxypentose Nucleic Acid[1]

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In the course of a general investigation of desoxypentose nucleic acid turnover rates, a striking difference was found between the tissues of normal mice and those bearing transplants of mammary carcinoma.

The general plan of these experiments was similar to that used by Hevesy (*1*). The relative nucleic acid turnover rate was measured by giving a tracer dose of radioactive sodium phosphate, sacrificing the animals after 2 hr, and isolating the desoxypentose nucleic acid from the tissues to be investigated. The animals used were female A strain mice bearing bilateral transplants of Strong's mammary carcinoma.

From Table 1, it is evident that there is a very significant increase in the specific activity of the nucleic acid in the livers, spleens, and kidneys of tumor-bearing animals. The increase is proportional to the tumor size, but is not linear. For the small intestines, there is a significant lowering of nucleic acid specific activity of host animals. Tissue examinations showed no metastases.

Sodium phosphate was given intraperitoneally. The mice were sacrificed 2 hr later, the tissues were dissected out as rapidly as possible, and the isolation of the nucleic acid was begun. In order to obtain enough purified desoxypentose nucleic acid, the tissues from three animals had to be pooled.

The method used for isolation of the desoxypentose nucleic acid was essentially Levene's, as modified by Klein and Beck (*2*). Some changes were necessary to make the method suitable for a tracer experiment.

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Five to 10 g of tissue was ground with sand in mortar and pestle and mixed with 10 ml of 5% NaCl solution. The tissue was then boiled in a water bath for a few minutes, 0.25 ml glacial acetic acid was added and then made basic with 0.5 g NaOH and 0.1 g sodium acetate. The basic mixture was boiled for about 1 hr, or until the organs were almost completely dissolved. One ml of glacial acetic acid and 0.7 ml of a 5% 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 crude nucleic acid was centrifuged off.

In order to purify the desoxypentose nucleic acid, it was dissolved in 5 ml of N-NaOH solution, and the following solutions were added: 0.2 ml of a saturated solution of disodium phosphate, and an equal volume of methyl alcohol. After heating in a water bath at 65° C for 15 min, the impurities were centrifuged off. The supernatant solution was placed in an ice bath, acidified with 3 molal HCl, and diluted with an equal volume of methyl alcohol. The nucleic acid was then centrifuged off. The purification was repeated six times.

TABLE 1

AVERAGES OF DESOXYPENTOSE NUCLEIC ACID SPECIFIC ACTIVITIES $\times 10^4$

Tumor weight per animal	No. of animals	Liver*	Spleen*	Kidney*	Intestine*	Tumor*
0 (controls)	24	0.745 ± 0.06	36.0 ± 1.9	0.315 ± 0.027	15.1 ± 0.87	—
0.084 g	24	2.10 ± 0.32	57.8 ± 3.8	0.927 ± 0.132	17.5 ± 0.46	—
1.2 g	188	3.83 ± 0.19	—	—	—	25.0 ± 0.45
2.8 g	24	4.16 ± 0.49	67.2 ± 1.4	0.930 ± 0.11	13.0 ± 0.8	—

* 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 $1\sigma_M$.

After this the nucleic acid was dissolved in NaOH solution and reprecipitated with HCl and methyl alcohol four more times, and finally dissolved in about 5 ml of 0.1 *N* NaOH solution.

Klein and Beck found that the nucleic acid was pure by chemical criteria after only three repurifications. However, as can be seen from the values given here, eight to ten precipitations were found necessary in this experiment in order to attain constant specific activity of the nucleic acid upon successive reprecipitation. In a typical liver sample, the following values were obtained on aliquots taken after the stated number of precipitations.

No. of precipitations	Specific activity
3	27.1×10^{-4}
6	4.9×10^{-4}
8	3.7×10^{-4}
10	3.9×10^{-4}

Individual specific activities of tissues measured in this manner after ten precipitations show considerable reproducibility, as can be seen from the relatively small

standard errors in Table 1. Partial purification of desoxypentose nucleic acid may include a variable amount of some phosphate contaminant of high specific activity, which would account for the variability of several orders of magnitude found in earlier studies.

The presence of mammary carcinoma transplants significantly alters the turnover of desoxypentose nucleic acid in the liver, spleen and kidneys of mice. Work now in progress indicates that the same effect occurs in Slonaker rats bearing transplants of lymphosarcoma and in pregnant rats and mice. It is suggested that the presence of a rapidly dividing tissue mass may influence the rate of cell division (as evidenced by the nucleic acid turnover rate) in the other body tissues.

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Presence of a Phosphatase in the Human Aortic Wall

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Previous failure to demonstrate a phosphatase in the human arterial wall has been a subject of some importance in the discussion of the pathogenesis of arterial calcification. A perusal of the literature shows that phosphatase activity of arterial tissue has been examined only at an alkaline reaction. For this reason a more extensive investigation covering a wider pH range was considered of interest. As the result of such studies on human aortic tissue a definite phosphatase activity with an optimum at pH 5.7–5.8 was demonstrated.

Human aortas obtained at autopsy were carefully wiped free of blood, and the adventitia and external part of the media were stripped off. The intima and residual part of the media were then cut into small pieces, and the tissue was finely ground with glass-distilled water in a Pyrex grinder. The tissue suspension was centrifuged and the supernatant siphoned off. The supernatant solution used in the experiments contained about 3 mg of protein per ml.

For the study of the phosphatase activity, citric acid sodium hydroxide (pH 3.1–7.0) and veronal sodium carbonate (pH 7.55–10.1) buffers of 0.1 molar concentration were prepared. Analytical reagents and glass-distilled water were employed in the preparation, and the pH was checked in a Coleman pH-meter. For each pH level studied, two samples, two controls, and two blanks were run at 37° C. Each sample and control tube contained 5 ml of buffer solution and 0.3 ml of supernatant solution (enzyme) whereas the blanks contained 5 ml of the buffer and 0.3 ml of water. Disodium phenylphosphate (15 mg) in aqueous solution was added at the onset of the experiments. The enzyme activity was stopped by the addition of 2.5 ml of 25% trichloroacetic acid. In the case of the controls the substrate was added after the addition of trichloroacetic acid.

The samples and controls were subsequently filtered. For color development 4 ml of the filtrates and of the blanks was pipetted off, after which 3.2 ml of 0.62 *N* sodium hydroxide, 0.8 ml of Folin and Ciocalteus' reagent, and 2 ml of 20% sodium carbonate were added. The blue color was compared in a Duboseq colorimeter with that of a phenol standard. The enzyme activity was calculated by subtracting the color value of the controls from that of the samples.

Results of studies on phosphatase activity at various pH levels are given in Fig. 1, which shows a maximum

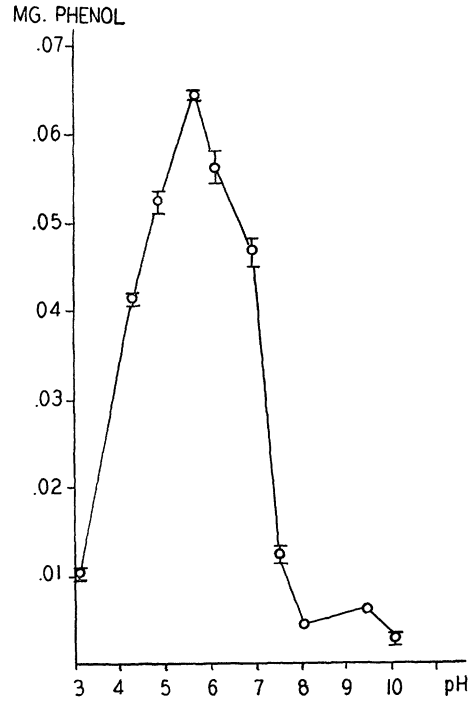

FIG. 1. The cleavage of disodium phenylphosphate at various pH levels by the supernatant of a suspension of human aortic tissue. Protein in sample, 0.9 mg; substrate in sample, 15 mg; reaction time, 30 min; temperature 37° C.

at pH 5.7–5.8. In addition to the major peak, a small peak at about pH 9.5 will be noted. This small elevation probably represents activity caused by traces of alkaline serum phosphatase, since the activity at this pH range was found to be increased by addition of magnesium chloride.

In further experiments conducted at the optimal pH (5.75), a fair proportionality was observed between the amount of supernatant fluid used (0.2, 0.75, and 1.5 mg of protein, 20-min reaction period) and the phosphatase activity, and between reaction time and cleavage of the substrate.

A comparison at pH 5.7 of the activity of aliquot samples of the original aortic suspension and of the supernatant fluid obtained after centrifugation showed a higher phosphatase activity per volume in the supernatant.