Effects of Phosphate Bond Energy on Nucleic Acid Stability in Tumor Homogenates*

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Recently in studies of anaerobic glycolysis in homogenates of a transplantable rat carcinoma (Flexner-Jobling) a medium was developed which is optimal for sustaining the phosphate bond energy (adenosine triphosphate) in the reaction mixtures (3). This should make it possible to study, in these homogenates, synthetic reactions which require energy. As a preliminary to these studies, an effort was made to determine whether nucleic acids were breaking down during incubation of the tumor homogenates and, if so, whether phosphate bond energy had any influence upon the reactions. Maintenance of phosphate bond energy was found to be correlated with maintenance of the nucleic acids.

EXPERIMENTAL

The tumors used were subcutaneous implants of Flexner-Jobling carcinoma in Sprague-Dawley rats of 100 to 200 gms. The implants were used at 8 to 12 days, when they were actively growing and free from any gross evidence of necrosis. The rats were killed by decapitation and tissues removed rapidly to small beakers of isotonic KCl imbedded in ice. After trimming away connective tissue, they were homogenized with Potter-Elvehjem homogenizers (5) in 9 volumes of cold isotonic (1.15 per cent) KCl.

The experiments were carried out in Warburg flasks at 37.6° C. with a 95 per cent nitrogen–5 per cent carbon dioxide atmosphere. Each flask contained the following additions, described as the "complete" medium; omissions were made where noted and water substituted: 0.30 ml. of 0.024M potassium phosphate (pH 7.4), 0.15 ml. of 0.5M potassium bicarbonate, 0.30 ml. of 0.4M nicotinamide, 0.15 ml. of 0.15M potassium pyruvate, 0.10 ml. of 0.01M adenosine triphosphate (ATP) potassium salt, 0.20 ml. of 0.003M diphosphopyridine nucleotide potassium salt, 0.20 ml. of 0.1M magnesium chloride, 0.10 ml. of 0.3M glucose, 0.15 ml. of 0.04M hexosediphosphate potassium salt, 0.15 ml. of 0.2M potassium fluoride, 0.30 ml. of 10 per cent homogenate of the tissue in isotonic potassium chloride,—and water to make the volume to 3.0 ml. Each flask was incubated the specified time, then stopped by addition of 0.25 ml. of 65 per cent trichloracetic acid from the sidearm. The contents were then transferred to a 13 × 100 mm. tube in each case, and the precipitated protein quantitatively transferred from the flask to the tube by means of two washes with small volumes of 5 per cent trichloracetic acid. The protein precipitate was centrifuged and the supernatant fluid discarded. The procedure for determining nucleic acids in these precipitates was that of Schneider (6), with modifications necessary for dealing with such small quantities of tissue (30 mg.). The procedure was as follows. Each precipitate was given two successive washes by suspending it in 3 ml. of 5 per cent

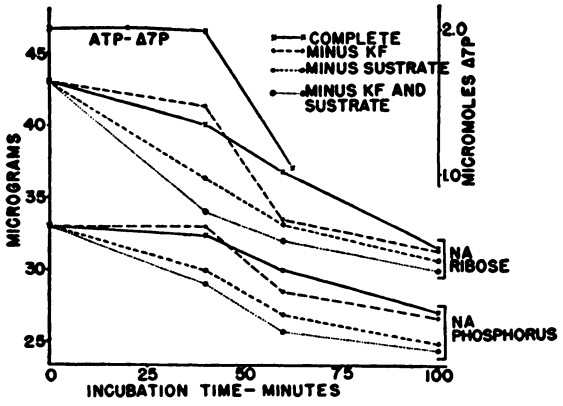

FIG. 1.—Stability of nucleic acids in tumor homogenates (30 mg. wet weight of tissue).

trichloracetic acid, centrifuging and discarding the supernatant fluid each time. Then each was treated with 0.5 ml. of water plus 2.0 ml. of 95 per cent ethanol, centrifuged and the wash discarded. A second such wash was made with 2.0 ml. of ethanol to assure removal of the acid. The precipitate was now suspended in 2.0 ml. of 3:1 alcohol-ether and heated 3 minutes in a water-bath at 40° C. The precipitate was centrifuged and this treatment repeated twice. The alcohol-ether remaining was permitted to evaporate at 37° C. and the dry precipitate treated with 1 ml. of 5 per cent trichloracetic acid. The tube was now heated at 90° C. in a water-bath for 15 minutes. Aliquots of the acid were used to determine phosphorus and ribose by methods previously described (4).

Some 7 experiments were carried out with the tumor tissue. The data are presented in Figure 1. Each experiment included duplicate flasks for each point given on

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each of the curves except for the ATP curve, which was obtained in a separate experiment. The results of these experiments were averaged for the figure. There were variations in the amount of nucleic acid breakdown from one experiment to another but not in the relative positions of the curves.

Two experiments were carried out in a similar manner, with the same medium, using homogenates of brain. The averages of the results for these are given in Table 1.

DISCUSSION

It can be seen in Figure 1 that the ATP was maintained for 40 minutes in the tumor homogenates then declined rapidly. The decline results from the cessation of glycolytic activity due to exhaustion of substrate. The curve given is for a complete medium. Direct measurement of ATP was not made for the other conditions under which nucleic acid studies were made, but it is obvious that ATP will decline rapidly from the start of incubation in the cases where substrate was omitted. Where ATP was maintained, the nucleic acids were apparently also maintained, and the loss of ATP is correlated with loss in the nucleic acids. There was not sufficient material for measurement of desoxyribonucleic acid, but from the measurements of total nucleic acid phosphorus and ribose, it is possible to calculate ribonucleic acid and desoxyribonucleic acid by difference. The initial levels found in these analyses agree well with results obtained by Schneider in his analyses of this same tumor (7). The decline appears to be approximately two-thirds due to ribonucleic acid, and one-third to desoxyribonucleic acid. When brain homogenates were studied, the decline in nucleic acid was negligible whether or not ATP was maintained. Brain appears to lack the potential for carrying out this reaction. Preliminary experiments with liver and kidney without a sustaining glycolytic system showed a loss of approximately 9 per cent of the nucleic acid phosphorus of liver in a 60 minute incubation and 18 per cent in kidney. These are then intermediate between brain with no breakdown, and tumor with approximately 23 per cent breakdown per 60 minutes.

TABLE 1

STABILITY OF NUCLEIC ACIDS IN BRAIN HOMOGENATES DURING ANAEROBIC GLYCOLYSIS

	Nucleic Acid Phosphorus per 30 mg. of Tissue			
Incubation time min.	Complete medium microgm.	Medium −fluoride microgm.	Medium −substrates microgm.	Medium −fluoride −substrates microgm.
0	11.3	11.3	11.3	11.3
40	10.6	10.5	10.0	9.8
60	10.1	10.0	10.0	9.5
100	10.6	10.6	10.5	9.9

The products of this reaction were not determined, but since they are acid-soluble, it is likely the reaction is a depolymerization. It is not possible to ascertain from these data whether the maintenance of nucleic acid by ATP represents prevention of breakdown or concomitant breakdown and resynthesis, nor is it apparent therefore whether the activity concerned is a reverse of normal nucleic acid synthesis. However some correlation seems to exist between ability to grow and possession of this activity on the part of the several tissues examined. Analytical data are available from the work of Greenstein (1, 2) on depolymerases of ribonucleic acid and desoxyribonucleic acid in a variety of tissues. There appears to be little difference between hepatoma and liver with respect to these enzymes. However, no data could be found for the assay of rat brain and Flexner-Jobling carcinoma for the depolymerases.

SUMMARY

Studies of the nucleic acid phosphorus and ribose in homogenates incubated at 37.6° C. demonstrated that when homogenates of Flexner-Jobling rat carcinoma were provided with ATP by means of an active anaerobic glycolysis, the nucleic acids were maintained. In the absence of a system to maintain ATP, the nucleic acid declined at a linear rate of approximately 23 per cent per hour. Brain homogenates maintained their nucleic acid even in the absence of ATP. Homogenates of liver and kidney gave intermediate rates of decomposition for nucleic acid (9 and 18 per cent respectively).

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