Phosphorylated Intermediates in Tumor Glycolysis

III. Effects of Anoxia and Hyperglycemia*

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Studies of glycolysis in tumors (2) have previously involved the use of extracts, minces, slices or homogenates. While these provide valuable information, each having certain advantages, all suffer from the potential criticism that they represent a considerable modification of the in vivo conditions. Dilution is usually necessary and cofactors must be added with resulting controversy over the relative importance of fragmented systems. In a study of the levels of phosphorylated intermediates in tumors (3,4), it was noted that breakdown of adenosine triphosphate and other phosphorylated intermediates was very rapid in homogenized or frozen-ground tissue and much less rapid in the intact tumors. This made it appear that something could be gained by adding to the information on tumor glycolysis through the study of glycolysis in anoxic and anoxic hyperglycemic animals bearing tumors, by analysis of the intact tumors incubated for various periods. Measurements of the lactic acid and phosphorylated hexoses in these tumors after blood flow is cut off should give some indication as to whether the lactic acid is formed from these hexose esters. Kidneys similarly incubated were used as controls, since kidney is known to possess a phosphorylative system, and since the pattern of phosphorylative intermediates and level of glycogen is approximately the same quantitatively in kidney and tumor (3).

EXPERIMENTAL

Fed rats of Sprague-Dawley strain weighing 100 to 130 gms. were injected subcutaneously with a mince of Flexner-Jobling carcinoma or Jensen sarcoma. They were used for experiment after 8 to 12 days, when the tumors weighed 400 to 800 mgm. The animals were anesthetized by intraperitoneal injection of nembutal, 50 mgm./kgm., to minimize struggling. Some were frozen in liquid air as controls and the tumors dissected out in the frozen state. Others were decapitated and incubated, with the tumors in situ, for the indicated time at 37° C. The tumors were then dissected free and dropped in liquid air. Timing was from decapitation to immersion of the tumor in liquid air. Rats for the hyperglycemic groups were injected intraperitoneally with 20 per cent glucose solution, 10 ml./kgm. of body weight, 20 minutes prior to decapitation. Separate groups of rats were used to obtain kidneys incubated in situ after decapitation, with and without previous glucose injection.

Analyses of these tissues were made as previously described (3), except that pyrophosphate phosphorus measurements of incubated samples, in order to be more accurate, had to be made on barium precipitates which were treated with magnesia mixture and ammonium hydroxide briefly to remove the bulk of the inorganic phosphorus. Lactic acid analyses and "hexose" results are presented in Figs. 1, 2, and 3. The hexose values represent the sum of the hexose available in the tissue as glycogen, glucose-6-phosphate, fructose-6-phosphate and hexosediphosphate. Each point on the curve represents analyses on 3 to 4 separate tumor samples, each taken from a separate animal treated as described above. Fig. 4 presents analyses for the easily hydrolyzable (7 minutes, 100° C. in 1 N HCl) phosphorus of the barium-insoluble fraction. Consequently it is the sum of the "high-energy" phosphorus of adenosine triphosphate and adenosine diphosphate.

DISCUSSION

There is the complication to this experimental setup that both phosphohexose breakdown and formation of further phosphohexose from free glucose can go on simultaneously, and the degradation be masked. This tends to be minimized in the tumors because, as has been demonstrated by analysis of the incoming and outgoing blood supply of tumors (1), the tumor quickly depletes the blood glucose. Consequently, when the blood flow is stopped at any time, there is currently very little free glucose left available. This is not so true of the kidney tissue and there is much more likelihood of this phase of the results being obscured in the kidney.

The figures show pyrophosphate phosphorus falling at much the same rate in all the tissue incubations. Data for the kidney parallel closely those for

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the tumors. Lactic acid production is more rapid in the hyperglycemic animals. The difference here is more pronounced in the tumors, where lactic acid accumulation is more rapid. In the tumors, the hexose available as glycogen and phosphorylated hexose (very little glycogen available in any case; this all disappears in the first 3 minutes of incubation) decreases as lactic acid increases, in general. The decrease appears less rapid in the hyperglycemic animals, presumably due to formation of more hexose phosphate from free glucose. In kidneys the hexose analyses are difficult to interpret. The increase rather than decrease of hexose phosphates in the anoxic sample must represent the preponderance of formation over decomposition. It is not possible to say what factors were involved in the unpredicted shift.

The rapid fall from a high level of adenosine polyphosphate phosphorus in the tumors when blood flow is cut off, without any disruption of the tissue to activate phosphatases would indicate there is a dynamic equilibrium involving much adenosine polyphosphate synthesis in the metabolism of the tumor. This would support the concept that a phosphorylative metabolism plays a large role in tumor metabolism.

SUMMARY

Intact Flexner-Jobling carcinomas and Jensen sarcomas growing on fed rats with and without glucose injections were incubated while anoxic in the decapitated host and analyzed, after 0, 1, 3 and 30 minutes' incubation. Analyses were made for lactic acid and various phosphorylated intermediates. Kidney samples treated in a parallel manner were studied as control tissues. On incubation, lactic acid accumulated in all three tissues and reached higher levels in the hyperglycemic tissues. At the same time phosphorylated hexoses disappeared. A rapid drop in pyrophosphate phosphorus of adenosine di- and triphosphate occurred, indicating a rapid turnover in the tissue in vivo.

REFERENCES

JENSEN SARCOMA

LACTIC ACID

Mg. per 100 gm.

INCUBATION TIME - MINUTES

Fig. 1

FLEXNER-JOBLING CARCINOMA

LACTIC ACID

Mg. per 100 gm.

INCUBATION TIME - MINUTES

Fig. 2

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PYROPHOSPHATE PHOSPHORUS

JENSEN SARGOMA

FLEXNER-JOBLING CARCINOMA

KIDNEY

Fig. 3

KIDNEY

LACTIC ACID

HEXOSE

Fig. 4
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