A Comparison of the Anaerobic Glycolysis of Human Brain and Glioblastoma*

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The purpose of this investigation was to compare the glycolytic response of malignant astrocytomas to that of the human brain (white matter) when incubated under anaerobic conditions. Cerebral tissue rapidly reacts to oxygen deprivation by degrading glucose and glycogen to form an equivalent amount of lactate (two equivalents of lactate per equivalent of hexose expended). Glycolysis in the mammalian brain thus follows a well-defined anaerobic system (the Embden-Meyerhof pathway) to provide a short-term emergency source of energy for the ischemic brain. Glycolysis in itself, however, cannot fulfill the energy demands of an ischemic brain for more than a few minutes.

This general biochemical response of tissue to oxygen want is known as the "Pasteur effect," and has been the subject of intensive investigation in murine brain by Lowry and co-workers. Prolonged ischemic incubation (more than 1 hour) is not associated with any further glycolytic activity in the murine brain, but has not been investigated in the human brain. Neoplastic tissue differs markedly from oxygenated mammalian brain in that it produces lactic acid excessively even during aerobic conditions. The exceptional ability of neoplasms to produce lactate undergoes further augmentation during ischemic incubation. Thus, tumors in general, and human brain tumors in particular, demonstrate a clear-out Pasteur effect.

In this study, two glioblastomas and companion samples of white matter were incubated anaerobically for periods of time ranging from 5 minutes to 4 hours. Direct quantitation of major glycolytic metabolites and co-factors at varying intervals of anaerobic incubation permitted an accurate estimate of the glycolytic flux rate in the two tissues. The observations indicate a fundamental quantitative difference in the mechanism of anaerobic glycolysis between the human white matter and malignant astrocytomas studied.

Experimental Method

Source and Preparation of Tissue. Human white matter and neoplastic tissue were obtained from two patients at the time of craniotomy. The operative procedures were performed under general endotracheal anesthesia. Neither patient had received steroids, dehydrating agents, or intravenous infusions containing carbohydrates immediately before or during surgery. One patient had a glioblastoma of the right parietal lobe (this tissue is designated as tumor I and white matter I), and the other a glioblastoma of the right frontal lobe (the tissue designated as tumor II and white matter II). Care was exercised to resect white matter that did not appear to be edematous or hemorrhagic, without the use of the electric knife. The extreme sensitivity of the analytical techniques enabled the determination of a wide range of glycolytic substrates in portions of white matter 20 to 30 mg in weight. Larger specimens of the tumors were taken for incubation and analysis by cup forcep biopsy. Tumor I was a highly malignant astrocytoma composed of anaplastic cells with marked cellular palisading and focal necrosis. Tumor II was an exceptionally pleomorphic glioblastoma with areas of focal necrosis and vascular hyperplasia. Frozen sections of white matter demonstrated no evidence of edema, tumor, or cellular reaction.

Samples of tumor tissue and companion white matter were frozen after varying intervals of ischemia in Freon-12 (CCl₃F₂) chilled to its freezing point by liquid nitrogen (−150°C). Tissue designated as "zero time" was frozen within a few seconds after separation from its blood supply. Serial portions of resected tissue were incubated

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anaerobically in mineral oil at 37°C for periods up to 4 hours and then frozen. Tissue once frozen was never allowed to thaw, and was stored at −80°C. Gross dissection of the neoplastic tissue and white matter was performed at −20°C to remove obvious hemorrhagic and necrotic areas. Tissue was weighed on a Roller-Smith torsion balance at −20°C, and extracted in 3 molar perchloric acid at −8°C. The explicit details for weighing, extracting, and neutralizing the tissue extracts have been given.7

Analytical Method. The measurements of tissue metabolites were conducted using specific spectrophotometric and fluorometric techniques in conjunction with purified crystalline enzymes.7,8 These techniques, devised by Lowry and co-workers, combine the specificity of enzymatic reactions with the extreme analytical sensitivity inherent in the fluorescent properties of pyridine nucleotides. A direct assay of metabolites with tissue levels as low as 10⁻⁴ moles per kilogram wet weight is attained. This sensitivity allows reliable measurements of substrate levels in exceptionally small quantities of extracted tissue. The specific methods for measuring glucose, glycogen, lactate, glucose-6-phosphate, phosphocreatine, ATP, (adenosine triphosphate), ADP (adenosine diphosphate), and 5'-AMP (adenosine 5'-monophosphate) in milligram quantities of fresh tissue have been given. All values are expressed as millimoles or micromoles per kilogram wet weight.

Results

Both the normal white matter and glioblastomas exhibited a marked Pasteur effect, namely, a significant net increase in lactate production when subjected to conditions of complete ischemia. The two tissues differ, however, in the rate at which lactate is produced during the incubation conditions (Fig. 1). Values for “zero time” lactate in white matter are at expected levels for mammalian brain,8 and for the glioblastomas are within the range reported for a variety of malignant intracerebral tumors (Table 1). The rate of lactate production during the first 5 minutes of ischemic incubation in white matter is several fold greater than in the two glioblastomas (Fig. 1). For white matter I and II, the rates of lactate production during the first 5 minutes of ischemic incubation are 3.5 and 5.3 mM/kg/minute, whereas the rates for the two glioblastomas are 0.5 (T-I) and 0.8

Fig. 1. Rates of lactate production in ischemic white matter (B) and glioblastoma (T). Tissues were taken from two patients; patient I had a glioblastoma of the right parietal lobe, and patient II had a glioblastoma of the right frontal lobe.