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STABILITY OF RAT BRAIN GLUTAMINE SYNTHETASE TO OXYGEN 1/
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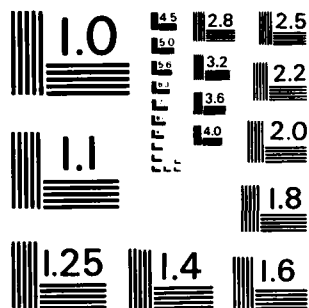
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**STABILITY OF RAT BRAIN GLUTAMINE
SYNTHETASE TO OXYGEN TOXICITY
(OXYGEN AT HIGH PRESSURE)**

MAJOR JAMES T. WEBB

**DEPARTMENT OF BIOLOGY
USAF ACADEMY, COLORADO SPRINGS, CO 80840**

**JULY 1983
FINAL REPORT**

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Editorial Review by Captain Hale
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USAF Academy, Colorado Springs, Colorado 80840

This research report is presented as a competent treatment of the subject, worthy of publication. The United States Air Force Academy vouches for the quality of the research, without necessarily endorsing the opinions and conclusions of the author.

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USAF ACADEMY, COLORADO 80840

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I sincerely appreciate the assistance of Captain Richard C. Postlewaite, DVM, in establishing surgical techniques for rapid acquisition of tissue samples.

INTRODUCTION

The function of glutamate, L-glutamic acid, as an excitatory neurotransmitter in the brain has been well established and documented (1,2,8). The enzymatic action of glutamine synthetase (EC 6.3.1.2) to remove or deactivate glutamate in rat brain glial cells (5) is analogous to the action of acetylcholinesterase on acetylcholine. Inhibition of glutamine synthetase with methionine sulfoximine (4) and oxygen at high pressure (6,12) causes convulsions in mammals. If glutamate removal is dependent upon the activity of glutamine synthetase, the convulsions caused by oxygen at high pressure could be due to inhibition of glutamine synthetase.

The relationship between presence of essential sulfhydryl groups in an enzyme and susceptibility to inactivation of that enzyme by oxygen at high pressure has been studied for numerous enzymes (3,6,7,10,12). The active form of E. coli glutamine synthetase has been shown to be resistant to inactivation by sulfhydryl-binding agents. This indicates that the sulfhydryl groups are protected in some way in the "taut" or active form of the enzyme (9). Effects of oxygen at high pressure on glutamine synthetase activity in mammalian brain have not been previously reported.

Several enzymes from chick and rat brain were analyzed for inhibition by oxygen at high pressure (10,12). The sig-

nificant inhibition of L-glutamic acid decarboxylase by oxygen at high pressure was attributed to oxidation of essential sulfhydryl groups. This inhibition was suggested as a cause of alterations in gamma-aminobutyric acid (GABA) metabolism and of oxygen induced seizures.

This report addresses the question of rat brain glutamine synthetase stability to inhibition by oxygen at high pressure.

MATERIALS AND METHODS

Sprague-Dawley derived, King/Holtzman albino rats (Rattus norvegicus) were bred at the USAF Academy, Colorado and used throughout the experiments. The rats were decapitated and whole brains were homogenized in 49 parts of water in a Waring commercial blender. The homogenate was centrifuged at 4500 rpm for 5 minutes and the supernatant was kept on ice prior to the assays. The supernatant, source of enzyme activity, was assayed for glutamine synthetase activity according to the method of Webb and Brown (11). under the following conditions: Ten minutes of incubation at 25°C; pH 6.8; 2 ml incubation mixture containing 60 mM L-glutamine, 10 mM hydroxylamine-HCl, 0.4 mM Na₂ADP; 20 mM KH₂AsO₄, 3 mM MnCl₂, and 40 mM imidazole. The gamma-glutamyl hydroxamate produced by the transferase enzyme activity was complexed with FeCl₃ (in HCl) and compared to a

gamma-glutamyl hydroxamate standard (Sigma Chemical Co.) at 500nm with a B&L Spectronic 20 Spectrophotometer. A unit of glutamine synthetase activity is defined as the production of one micro-mole of gamma-glutamyl hydroxamate per minute at 25°C.

Flow of pure nitrogen, oxygen, and air from tanks was regulated at 3 liters/minute and directed through 1/4" surgical tubing at the surface of 10 ml of homogenate in 100 ml beakers. The beakers were covered with parafilm to decrease the chance of atmospheric contamination. The beakers were kept in a 25°C. water bath for the duration of the exposure to each gas. This method of maintaining oxygen at high partial pressure was previously used by Cairney (personal communication). Saturation of the medium occurred within 10 minutes and was maintained by constant flow of the gas.

RESULTS AND DISCUSSION

The enzyme activity was linear with respect to aliquot and time over the range of experimental conditions. The pH optimum was 6.8. An Arrhenius plot, Fig. 1, indicated the lack of any significant change in enzyme characteristics from body temperature of 37°C. to the assay temperature of 25°C. The K_m for glutamine was 34mM at 15mM hydroxylamine and 42mM at 10mM hydroxylamine (Figs. 2,4). The K_m for hydroxylamine was 3mM at 60mM glutamine (Fig. 3).

Bubbling the gases through the homogenate produced unrepeatable results. This was probably due to foaming of the mixture and consequent denaturation of the enzyme. The data in Table 1 are based on 220 minutes of gas flow over the homogenate aliquots. There was no significant difference between the enzyme activities in aliquots of homogenate exposed to nitrogen, air, or oxygen. The relatively high enzyme activity of all three homogenate aliquots exposed to gas flow compared to the aliquot kept on ice was probably due to their additional time at 25°C. The mammalian enzyme functions normally at about 37°C. Storage of the homogenate aliquot used to determine maximal activity at about 0°C. could have resulted in less effective aggregation of the quaternary structure necessary for maximum activity than would have been possible at 25°C.

The lack of inhibition of glutamine synthetase activity in the homogenate aliquot exposed to oxygen at high partial pressure indicates that this enzyme is probably not subject to oxygen toxicity. The report by Shapiro and Stadtman (9) on the sulfhydryl groups' stability in glutamine synthetase from E. coli could therefore apply to glutamine synthetase from rat brain.

SUMMARY

These results indicate that rat brain glutamine synthetase is stable under conditions of oxygen at high partial pressure. The convulsions of rats caused by oxygen toxicity are therefore probably not the result of inhibition of glutamine synthetase by oxidation of labile sulfhydryls.

FIGURE 1--Arrhenius Plot; Activity vs Temperature

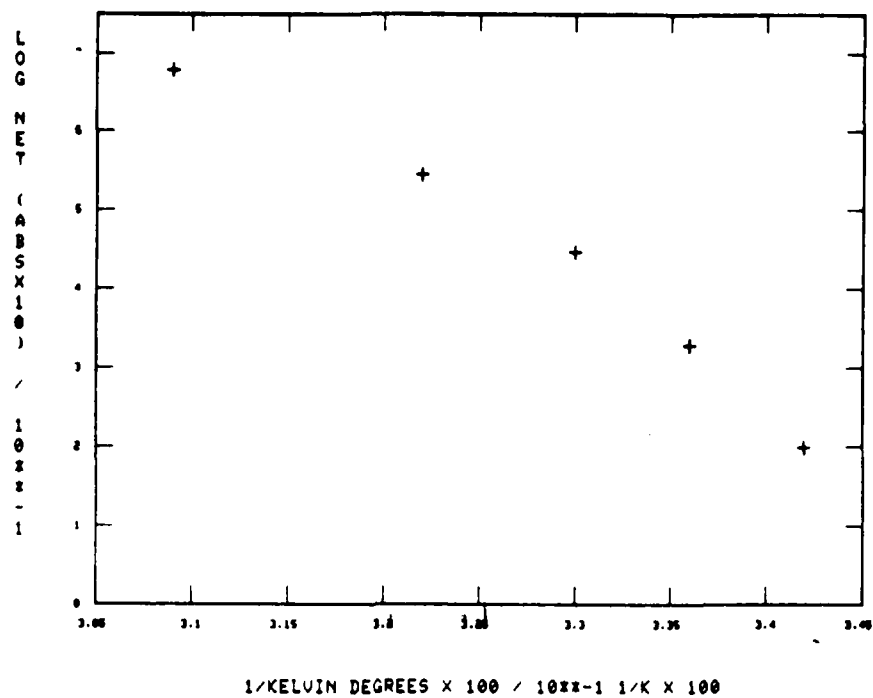


FIGURE 2--Km for Glutamine at 15mM Hydroxylamine

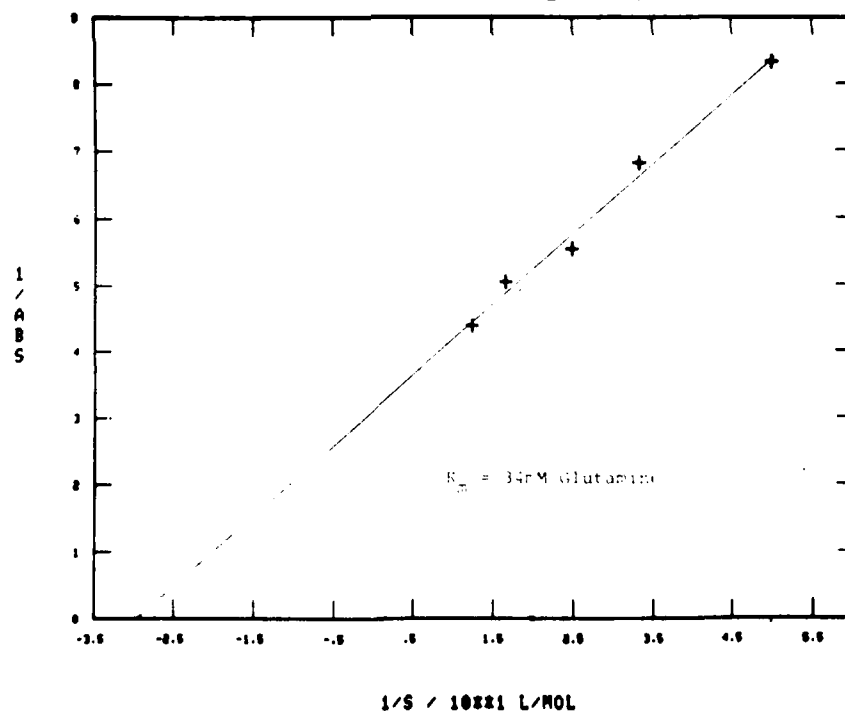


FIGURE 3--Km for Hydroxylamine at 60mM Glutamine

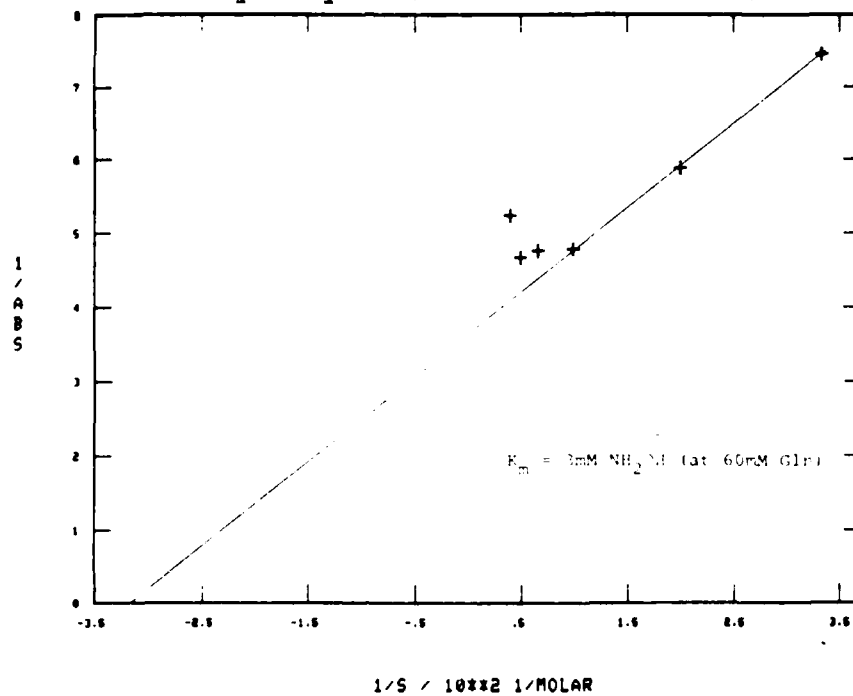


FIGURE 4--Km for Glutamine at 10mM Hydroxylamine

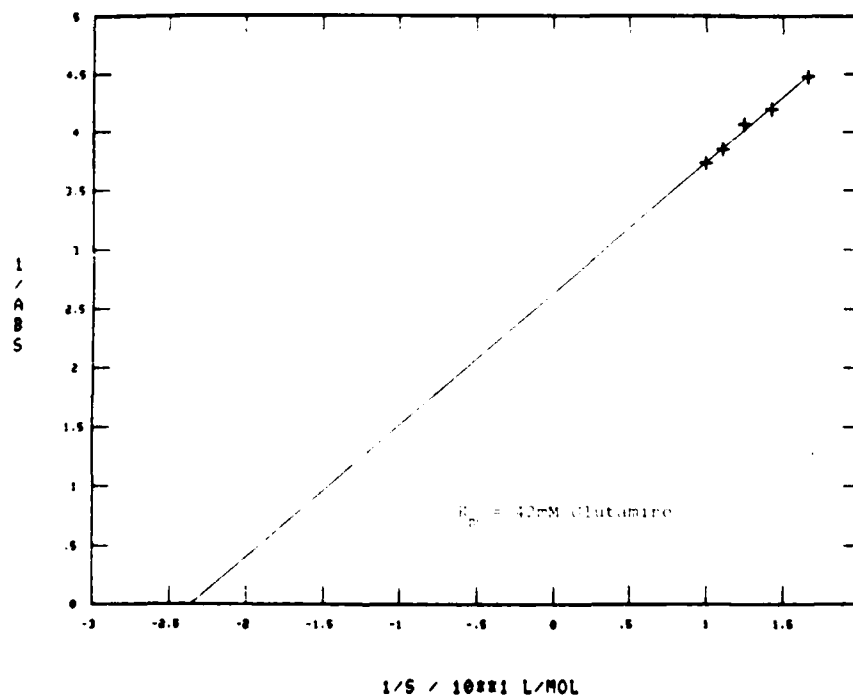


TABLE 1--Effect of pure atmospheric gases on rat brain glutamine synthetase activity.

Exposed to flow of:	Percent of maximal activity
N ₂ (2)	125
Air (2)	138
O ₂ (2)	126

Maximal activity is defined as the activity of an aliquot of the same 2% homogenate supernatant (diluted to 1% with water) which was kept on ice and exposed to the atmosphere with no artificial flow. Mean of all observations was 130±8% of maximal activity.

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