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CHANGES IN CNS METABOLISM IN HIGH PRESSURE NERVOUS SYNDROME, HI--ETC(U)
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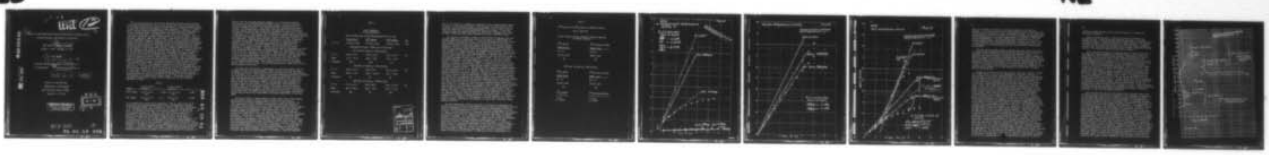

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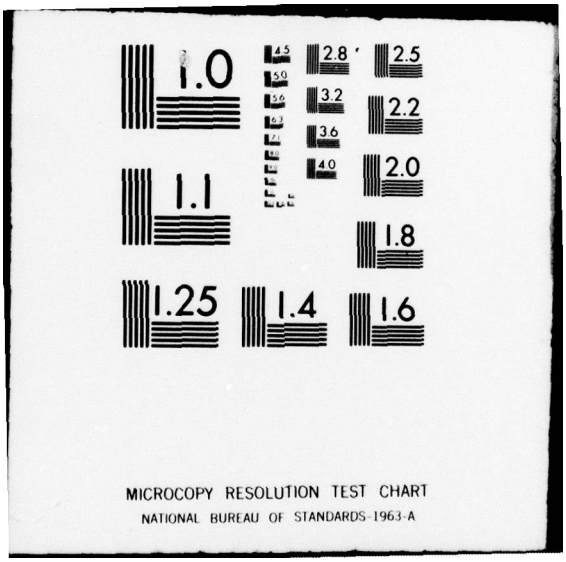
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(6) Changes in CNS Metabolism in High Pressure Nervous Syndrome, High Pressure Narcosis, and Methods of Protection.

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Our initial approach to the study of mechanisms of acute central nervous system high pressure toxicity was to study enzyme activities and concentrations of the series of metabolites (which have been shown to provide protection against O_2 toxicity) during air and hyperbaric oxygen exposures - 5 ATA O_2 - 11 ATA O_2, with or without exogenous administration of the particular protective agent. We have previously shown in Sprague-Dawley rats which have been exposed to 5 ATA O_2 for 60 minutes - the threshold of the onset of convulsions - that there were significant decreased concentrations of succinate, malate, lactate, glutamate, aspartate, GABA, and glutations; a significant increase in glutamine and no significant change in citrate in the brain. We have observed decreased enzyme activities in succinic dehydrogenase and alpha-ketoglutarate dehydrogenase. Neptune has shown significant decreased activity of alpha-ketoglutarate dehydrogenase and pyruvate oxidase. Chance has shown that at oxygen exposures which are sufficient to cause convulsions there is a marked inhibition in the reduction of NAD leading to high levels of oxidized NAD in the cell. It appears that in the early stages of acute CNS O_2 toxicity, the primary factor is an inability of the cell to sustain ATP production due to inhibition of enzyme systems associated with the electron transport chain production of ATP and decreased levels of substrates related to ATP production. However, when an exogenous substrate which stimulates the ATP production (succinate, alpha-glycerolphosphate, GABA and glutamate) is used, convulsions occurred at 5 ATA O_2, much later than non-protected Sprague-Dawley rats when protective substrate levels are still elevated in the brain. This implied an inability to utilize ATP could be a major factor. We examined the ATPase levels in the brain of Sprague-Dawley rats which had been exposed to 5 ATA O_2 for 60 minutes, or to 25 minutes at 7 ATA O_2. At these time intervals for the 2 pressures, the animals are near time of onset of convulsions. The levels of the sodium-potassium ATPase activity and the magnesium ATPase activity were determined in the brain of the rat subjected to such exposures and compared with control animals. The enzyme activities expressed as a percent of control levels is shown for the two different exposures on Table 1.

Table 1

Enzyme	60 min 5 ATA O_2 % Control	P	25 min 7 ATA O_2 % Control	P
Na^+K^+ ATPase	(75.7 ± 15.2) ($\bar{25}$)	< 0.005	(72.1 ± 14.7) ($\bar{15}$)	< 0.005
Mg^{++} ATPase	(91.8 ± 11.3) ($\bar{25}$)	N. S.	(80.2 ± 9.5) ($\bar{15}$)	< 0.005

It is significant to note that at 60 minutes, 5 ATA O_2, there was a 24.3% decrease in the sodium-potassium ATPase, with a non-significant decrease in the magnesium ATPase. At 25 minutes of 7 ATA O_2 there was a 28% decrease in the sodium-potassium ATPase in the brain of these male Sprague-Dawley rats. (It should be noted that the 25 minutes exposures at 7 ATA O_2 and the 60 minutes exposure at 5 ATA O_2 is the time at which animals are convulsing.) When the same pressures were used with 1 ATA O_2 and 4 or 6 ATA N_2 in the exposures, there was no change in either of the ATPase activities - thus it is not a simple pressure effect at these exposures. It is interesting to note that the decrease in the sodium potassium ATPase is essentially the same for the 2 different exposures. At the 25 minute 7 ATA O_2 exposure, we observed a decrease in the magnesium ATPase activity as indicated on Table 1. We added exogenous succinate to another group of rats which were exposed to 60 minutes 5 ATA O_2 and found no significant difference in the decreased enzyme activity between the non-protected animals (as indicated on

Table 1) and the succinate-protected animals, i.e., both groups showed a 25% decrease in the sodium-potassium ATPase activity. Thus, the succinate-protective agent which protected the animals against convulsions did not protect the ATPase activity. This implies that the sodium-potassium ATPase activity of the membranes was sufficient even when only 75% of the control activity remains and was able to maintain the membrane integrity and prevent convulsions. (With the succinate dose (12 mM/kg) the average time to convulsions is 208 minutes.) It thus appears that in the early stages of CNS oxygen toxicity, a decrease in ATP production was the basic cause for the defect which led to the convulsions. However, when protective agents were utilized, we observed a decrease in the Na^+K^+ ATPase which was not enough to cause the animal to convulse at 60 minutes. This implies an excess of the sodium-potassium ATPase activity in the cell under normally functioning conditions, e.g. a decrease of 25% after 60 minutes 5 ATA O_2, does not inhibit the normal maintenance of membrane activity. This was obvious when the exogenous succinate, which stimulates ATP production, was administered. The convulsions did not occur in the 60 to 90 minute time interval. These data also suggest that after a period of time, regardless of the level of ATP production, that the inhibition of the sodium-potassium ATPase eventually reaches the point where membrane integrity cannot be maintained, simply due to the fact that there is not sufficient sodium-potassium ATPase to hydrolize ATP and provide the energy for the membrane sodium pumps. This explains the fact that with protective agents, after a period of time, you have convulsions when a normal level of ATP may be present in the brain.

The work with the kinetics of glutathione as a protective agent, and the pathways involved in protecting against oxygen toxicity, has been concluded. In summary, it has been shown with the use of C-14 glucose for in vivo labeling of the glutamate pool - this includes the glutamate-GABA-succinate shunt components - that with the use of protective agents (succinate or glutathione) that this pool remained essentially unchanged during the HPO exposure time interval in which the animal was protected. However, without the use of the protective agents, there were significant changes in the glutmate, GABA, glutamine and succinate levels in the animals. It thus appears that the maintenance of glutamic, GABA, and succinate substrates at the normal levels was essential to give protection to the animal. Without these protective agents present, there were significant changes in the level of these substrates. Thus evidence was provided that the primary protective pathway of the glutamate and GABA in oxygen toxicity was by way of the GABA to succinate semialdehyde to succinate shunt.

During the course of these studies it became obious that we must examine whether pressure alone is playing a major factor in the activation or inactivation of different enzymes which are related to ATP production. We first examined the effect on ATPase at pressures of 1800 psig, 1340 psig, 1115 psig. These gas mixtures in our initial determinations contained 1 ATA O_2 and the balance of pressure was nitrogen gas. These studies were performed in vitro using brain homogenates, and a modification of the Post and Sen method of ATPase determinations. The results shown on Table 2 indicated a preferential inhibition of the sodium potassium ATPase activity with increasing pressures. At 1340 psig a significant decrease in sodium-potassium ATPase was observed. This was at a pressure where approximately 50% of the rats would experience convulsions. By 1800 psig (all animals would have convulsed) the sodium-potassium ATPase showed a decrease to 38% of control levels. Thus with increasing pressure from 1115 psig to 1800 psig, there was a decrease in sodium-potassium ATPase of 7.4% and 61.7% respectively. It is interesting to note that the average decrease of sodium-potassium ATPase at 1325 psig N_2 (2500 feet sea water equivalent) was not significant, thus implying that at the short times used for these exposures - 15 minutes - there was inadequate

Table 2

Brain Homogenate
(15 min Exposures)

Control ATPase Data μM ATP Hydrolyzed/gm (wet wt.) min.

	Total ATPase	Mg^{++} ATPase	$Na^{+}K^{+}$ ATPase	(n)
($\bar{\chi} \pm \sigma$)	(22.92 ± 2.00)	(11.90 ± 1.65)	(11.02 ± 1.28)	(16)

Experimental Groups - ATPase as % of Controls

1115 psig (15 psig O_2 + 1100 psig N_2)

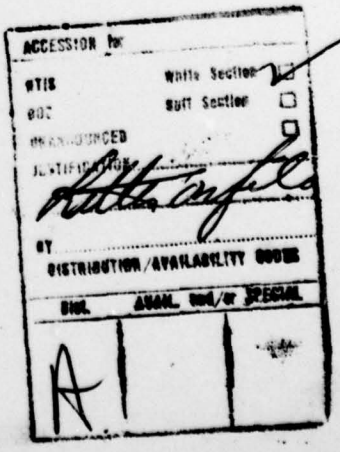
($\bar{\chi} \pm \sigma$)	(85.6 ± 8.2)	(79.0 ± 18.6)	(92.6 ± 15.7)	(9)
Range	(71.9 - 93.5)	(48.9 - 110)	(61.5 - 110)	
% Abnormal	88.9	88.9	77.7	

1340 psig (15 psig O_2 + 1325 psig N_2)

($\bar{\chi} \pm \sigma$)	(98.8 ± 13.4)	(110 ± 8.2)	(86.5 ± 26.4)	(5)
Range	(80.8 - 113)	(104.3 - 122.2)	(53.7 - 118)	
% Abnormal	20	20	80	

1800 psig (15 psig O_2 + 1785 psig N_2)

($\bar{\chi} \pm \sigma$)	(71.5 ± 8.2)	(102 ± 10.1)	(38.3 ± 11.0)	(5)
Range	(66.9 - 82.5)	(92.9 - 115)	(25.5 - 47.5)	
% Abnormal	100	20	100	



time at 1115 psig for a significant depression of the sodium-potassium ATPase to occur. It thus appears (Table 1) that there was an oxygen effect at low pressures which inactivated sodium-potassium ATPase, and at higher pressures there was inhibition of sodium-potassium ATPase by nitrogen. In the high pressure studies the magnesium ATPase was essentially unchanged over a range of 1100 to 1800 psig while using nitrogen as the basic compression agent.

The results of the ATPase studies at these pressures suggested that such pressure might have a significant effect on the uptake or incorporation of specific materials into the tissue. A study was performed where tissue slices were exposed to 1500 psig for a period of 10 minutes prior to injecting a mixture containing C-14 uniformly labeled leucine and P-32 labeled inorganic phosphate. After a 15 minute incubation, the pressure was released rapidly, tissue slices removed, rinsed, blotted and rapidly homogenized in perchloric acid to quench all enzyme activity. The homogenates were assayed for C-14 and P-32 activity in a liquid scintillation counter. These data were compared with control tissue subjected to the same methodology at ambient pressure for the same periods of time (see Table 3). It should be noted that the P-32 uptake in the organic phosphate ion was reduced 24% ($P < 0.001$). The C-14 uniformly labeled leucine had an increased uptake by a factor of 35.4% above control levels ($P < 0.001\%$). Thus active transport of leucine into the cell was not impaired at pressures of 1500 psig, rather it increased and followed a pattern consistent with cell injury as observed with hypoxia, hyperbaric oxygen, and radiation at ambient pressure. The decrease in the negatively charged phosphate ion can be explained due to an exchange taking place between intra- and extracellular materials, e.g. Na, K, and PO_4. The major point is that the magnesium ATPase activity was relatively unaffected through the pressures measured, and the active transport could be maintained at normal levels. These data support the concept that cell membrane will maintain normal active transport functions provided there is adequate ATP and adequate magnesium ATPase to transport material from the extracellular spaces into the cell. We believe these data support the concept that if there is interference with sodium-potassium ATPase activity there would be a corresponding loss of membrane function leading to depolarization hyperexcitability and eventually progressing to where a slight stimulus could cause a grand mal seizure. Such could be the case in high pressure oxygen studies when there is insufficient sodium-potassium ATPase activity to utilize ATP that is present, or when ATP production has been diminished to the point that membrane integrity cannot be maintained. We believe there is a common phenomenon that exists in convulsions due to very high pressure or to hyperbaric oxygen, i.e., an inability for the membrane to obtain sufficient ATP, due to a drop in ATPase resulting in the inability to utilize ATP to maintain normal membrane function.

We have subsequently looked at the effect of pressure on alpha-glycerol-phosphate activity, isocitric dehydrogenase activity, malic dehydrogenase activity, lactate dehydrogenase activity, glucose-6-phosphate dehydrogenase, hexokinase activity, creatine phosphokinase activity, and glutamic dehydrogenase activity. On Figure 1 is shown the results of exposing alpha-glycerolphosphate dehydrogenase to 4 different N_2 pressures, ranging from 445 psig to 1800 psig, for exposure periods of 5 minutes. It is interesting to note that this enzyme was highly sensitive to pressure over this range. (In other studies succinic dehydrogenase activity parallels alpha-glycerolphosphate dehydrogenase activity when subjected to stress.) We expect that succinic dehydrogenase - a protective agent, similar to alpha-glycerolphosphate, which enters the electron transport chain by way of flavoprotein - will show the same pattern of inhibition with pressure. Figure 2 shows the effects of exposures of isocitric dehydrogenase to N_2 pressures of 1340 psig and 1800 psig, with resultant inhibition. On Figure 3 the effects of N_2 pressure at 1800 psig on malic dehydrogenase are

Table 3

C^{14}UL-Leucine and $P^{32}O_4$ Uptake at Ambient Pressure, and at 1500 psig

Control (Tissue slices incubated $\bar{c}$ labeled compounds at ambient pressure)

$P^{32}O_4$ Uptake	C^{14}-UL-Leucine Uptake
CPM/g tissue	CMP/g tissue
($\bar{\chi} \pm \sigma$)	($\bar{\chi} \pm \sigma$)
(4,427 $\pm$ 243)	(5867 $\pm$ 725)
(6)	(6)

1500 psig (15 psig O_2 + 1485 psig N_2)

$P^{32}O_4$ Uptake	C^{14}-UL-Leucine Uptake
CPM/g tissue	CPM/g tissue
($\bar{\chi} \pm \sigma$)	($\bar{\chi} \pm \sigma$)
(3,364 $\pm$ 180)	(7,634 $\pm$ 561)
(6)	(6)
$P^{32}O_4$ Uptake	C^{14}-UL-Leucine Uptake
↓ 24%	↑ 35.4%
$P < 0.001$	$P < 0.005$

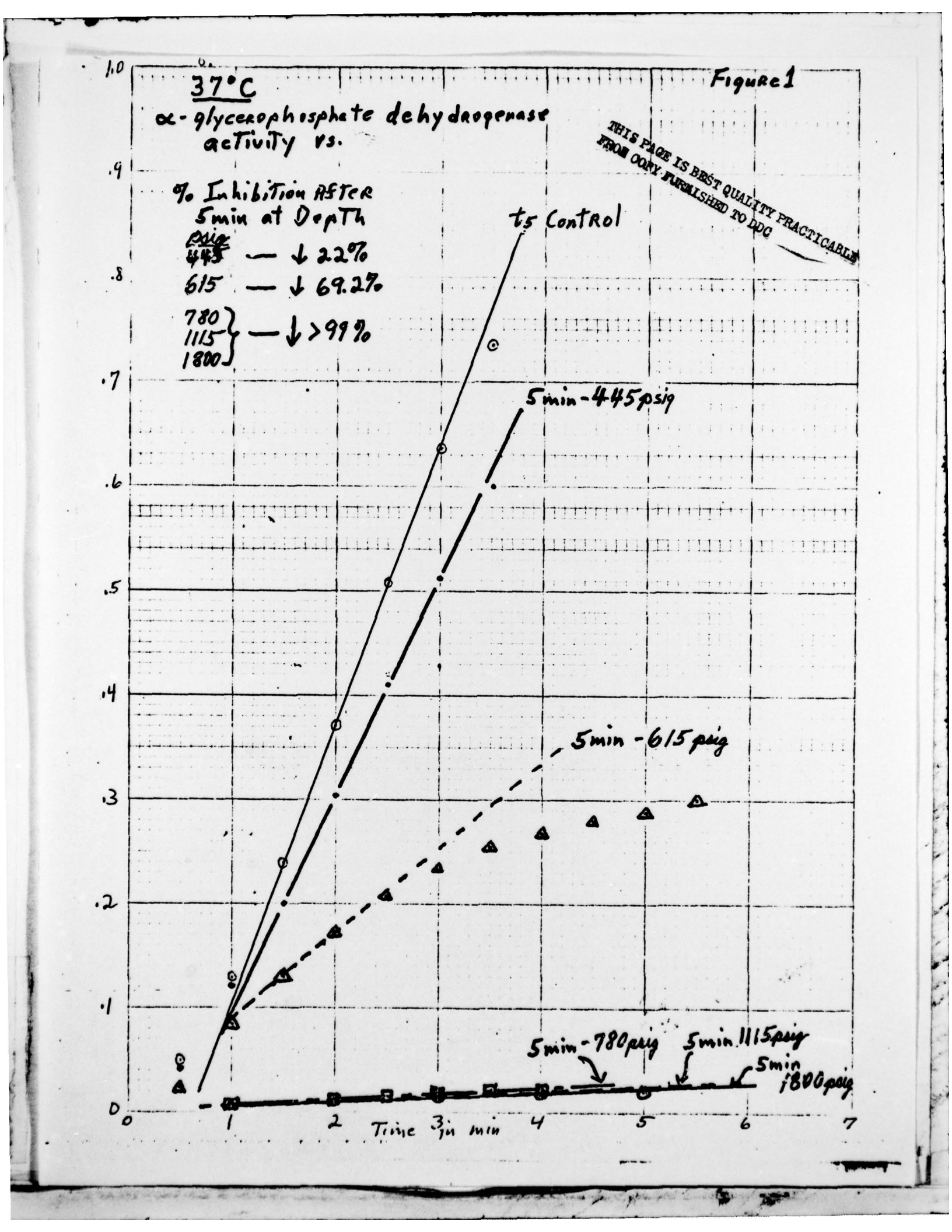
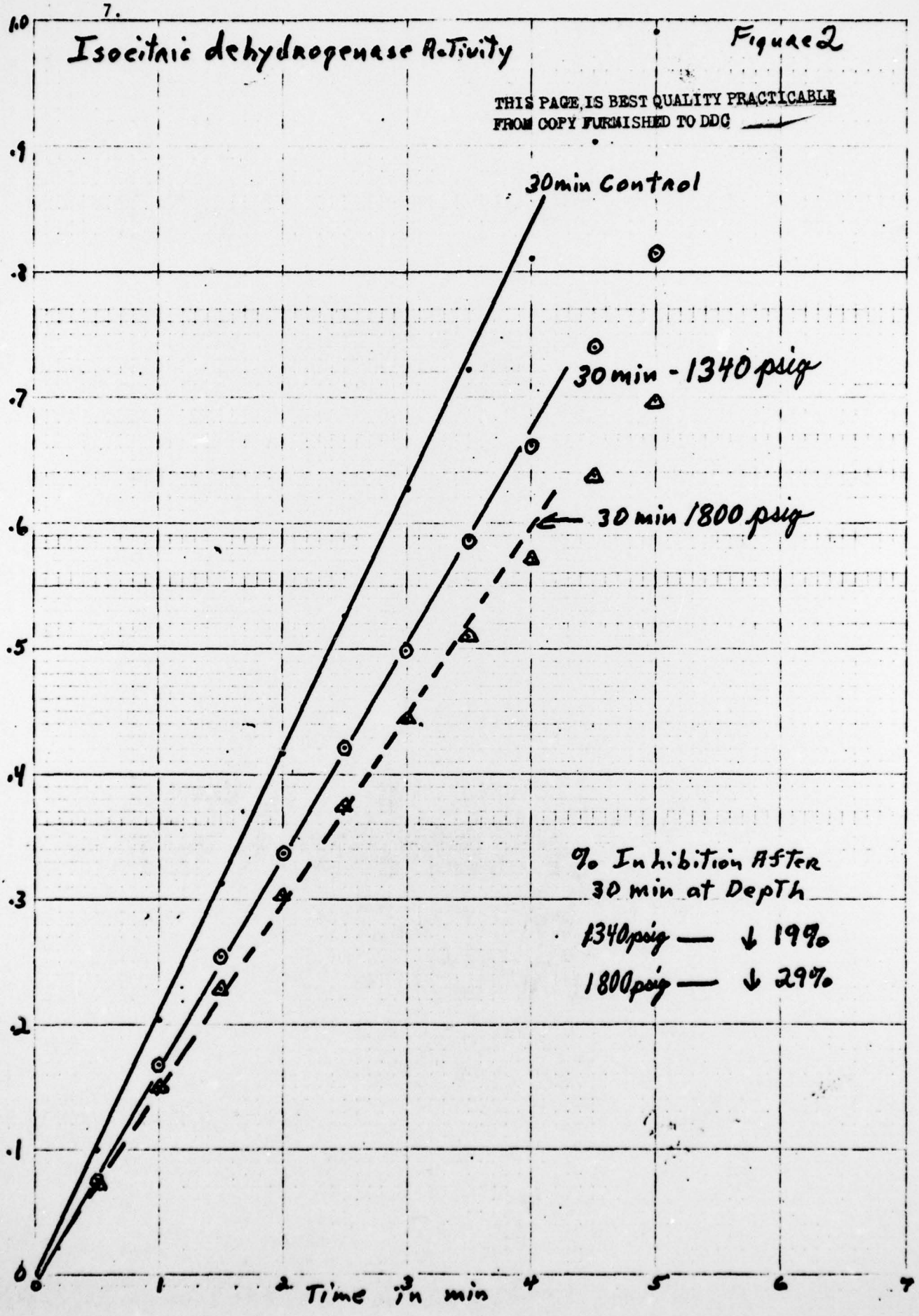


Figure 1


Isocitric dehydrogenase Activity
Figure 2
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30min Control
30 min - 1340 psig
30 min 1800 psig
% Inhibition After
30 min at Depth
1340psig — ↓ 19%
1800psig — ↓ 29%
1.0
.9
.8
.7
.6
.5
.4
.3
.2
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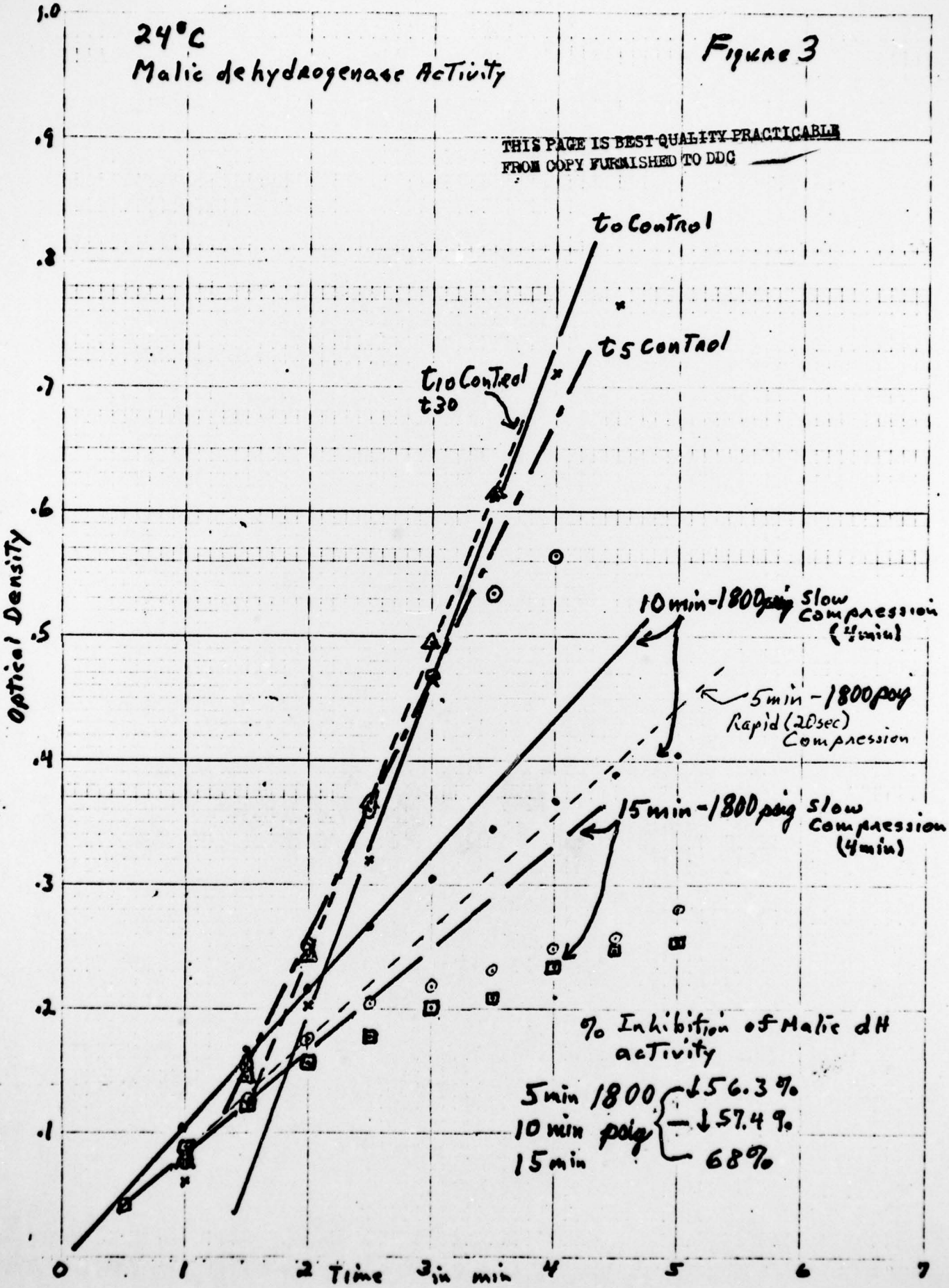
0
1
2
3
4
5
6
7
Time in min


24°C
Malic dehydrogenase Activity
Figure 3
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to Control
t5 Control
t10 Control
t30
10 min-1800 poig Slow compression (4 min)
5 min - 1800 poig
Rapid (20 sec) Compression
15 min-1800 poig Slow compression (4 min)
% Inhibition of Malic dH activity
5 min 1800 poig
10 min
15 min
56.3%
57.4%
68%
Optical Density
Time in min
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
0
1
2
3
4
5
6
7

shown for 5 minutes, 10 minutes, 15 minutes exposures to the 1800 feet pressure. It is obvious that several enzyme systems related to ATP production are inhibited by N_2 pressure as well as hyperbaric oxygen. In the other enzyme systems studied, there was no inhibition of glucose-6-phosphate dehydrogenase, lactic dehydrogenase, and glutamic dehydrogenase at pressures of 1800 psig. Creatine phosphokinase - the enzyme which plays an important role in the central nervous system in maintaining ATP levels constant, was found to have a 34.3% inhibition after 30 minutes at 1800 psig. Considering the key role this enzyme plays in phosphorylating ADP back to ATP further studies should be performed.

Thus key enzyme systems related to energy production, which are related to the maintenance of membrane functions and the maintainence of internal cell functions, can be effected both by pressure and hyperbaric oxygen. It is essential that time be devoted to ascertain what are the critical changes for these key enzymes in the range of pressures where oxygen toxicity are observed, and in the range of pressures where nitrogen narcosis and high pressure nervous syndrome are observed. Determination of the specific enzyme changes and the possibility of using substrate protection in preventing such changes may be of value in assisting in the protection of people who must be subjected to high pressure oxygen exposures or to high pressures of helium and/or nitrogeon under varied conditions.

Studies on the effects of nitrogen, heliox, and trimix on Na^+K^+ - ATPase and the Mg^{++} ATPase activities were subsequently initiated. Whenever we used nitrogen as the primary compressing gas, the cuvette which contained the reaction mixture had 1 ATA O_2 with the balance of gas pressure being nitrogen. Similarly when a heliox was used, there was 1 ATA O_2 in the chamber with further compression accomplished with helium gas. (The effect of nitrogen pressure was a significant depression of the Na^+K^+-ATPase at pressures of 1,000, 2,000, 2,500, 3,000 and 4,000 feet seawater equivalency. The Mg^{++}-ATPase activity for these nitrogen pressures was essentially normal or only slightly depressed but not statistically significant.) The heliox exposures gave a striking contrast in that we found an activation of Mg^{++}-ATPase at 1,000, 2,000, 2,500, 3,000 and 4,000 feet seawater pressure equivalent. The Na^+K^+-ATPase was not significantly different from controls. Thus we found an opposing effect of helium and nitrogen in terms of the particular ATPase which was affected by the gas. In the case of the nitrogen pressure, at the depths indicated, the Na^+K^+-ATPase was inhibited significantly. Under these conditions there would eventually be insufficient Na^+K^+-ATPase activity to sustain the normal membrane functions of the cell. The Mg^{++}-ATPase activity remained normal during this interval. In contrast with the heliox mixture, the Mg^{++}-ATPase activity was significantly increased - the magnitude being dependent upon the time of exposure and the pressure. Under these conditions the Mg^{++}-ATPase activity would be stimulated to the point where the ATP, which is produced at the mitochondria and must migrate to the membrane, would have a much lower probability of ever reaching the membrane, since the increased Mg^{++}-ATPase activity would hydrolyze the ATP in the cytoplasm. Under these conditions - though the Na^+K^+-ATPase at the membrane would remain essentially normal with the heliox exposures - there would be effectively a lack of functional ATP since it would be hydrolyzed by Mg^{++}-ATPase in the cytoplasm. This would result in the same conditions as if there was a decrease in ATP, or as if there were a decrease in the Na^+K^+-ATPase activity. Thus we observed two opposing effects, one where nitrogen inhibits Na^+K^+-ATPase without effecting the Mg^{++}-ATPase. (In this instance the membrane would lose its ability to utilize ATP, could not function properly and would effectively lead to tremors and eventually convulsions). In the heliox exposures a Mg^{++}-ATPase hydrolysis of ATP in the cytoplasm would result in insufficient quantities of the ATP

reaching the membrane which would progress eventually to tremors and eventually convulsions.

When the trimix was used, 1 ATA O_2 was attained, followed by N_2 compression equal to 10% of the total pressure, and taken to the final pressure with He_2. Both Na^+K^+-ATPase and the Mg^{++}-ATPase activities remained essentially normal at pressures equivalent to 4000' sea water. It appears as though the 10% N_2 gas was sufficient to sustain Mg^{++}-ATPase activity at normal levels, and the He_2 gas was sufficient to maintain normal Na^+K^+-ATPase activity. The results of these data are shown in Figure 4.

Prior to these experiments we completed studies of ATPase activities of the lung under oxygen pressures. In striking contrast to the brain, the lung ATPase has very low Na^+K^+-ATPase activity. For example, in brain of fasted rats, the values for total activity, Mg^{++}-ATPase activity, and Na^+K^+-ATPase activity were (29.67 $\pm$ .274), (13.39 $\pm$ 2.74) and (16.37 $\pm$ 0.31) µm ATP hydrolyzed/g min respectively (n = 10). In contrast the fasted lung had total ATPase activity of (25.67 $\pm$ 2.45), Mg^{++}-ATPase activity of (21.92 $\pm$ 2.70) and the Na^+K^+-ATPase activity of (25.67 $\pm$ 2.45), Mg^{++}-ATPase activity of (21.92 $\pm$ 2.70), and the Na^+K^+-ATPase activity of (3.74 $\pm$ 1.23) µm ATP hydrolyzed/g min (n = 10). In a check of the effects of low oxygen exposures on lung ATPase, a group of animals was exposed to 100% O_2 for a period of 3 days. The Mg^{++}-ATPase activity of the lung was decreased 10% ($P < 0.025$) immediately following 3 days of 100% O_2. A similar group of animals was returned to room air for one day and the Mg^{++}-ATPase activity was decreased even further by 37% ($P < 0.01$). This effect on the lung ATPase activities must be pursued further. The Na^+K^+-ATPase activity was variable as indicated by the mean $\pm$ standard deviation shown above, and was not significantly changed following 100% O_2 exposure of 3 days. These preliminary findings indicate that we now must go to higher pressures to ascertain what are the effects of HBO on Na^+K^+-ATPase and Mg^{++}-ATPase activity of lung when subjected to 2 ATA and 3 ATA 100% O_2 exposures.

Since we have noted the fact that oxygen toxicity grossly reduces ATP production and ATP concentration in tissues, we have examined the possibility of utilizing oxygen toxicity as a therapeutic agent in malignant tissues. Our early work has shown us that we can successfully use the combination of high pressure oxygen toxicity as a therapeutic agent in malignancy combined with x-ray doses which together gave successful treatment of fibrosarcoma, but individually would not cause complete tumor regression. The primary effects of O_2 toxicity appears to be depression of ATP in tissues. Malignant tissues have only approximately 60% of the number of mitochondria per cell as the normal tissue. We then looked at microwave exposures as another agent which, when utilized with oxygen, might be additive in depressing energy metabolism and ATP concnetrations in cells and might effectively be used in the treatment of malignancies. The effects of microwaves on the energy levels of the normal brain were studied. We found that microwaves at 591 MHz, 5 to 18 mW/cm^2 power density, would decrease the brain ATP level by 25% and the brain CP level by 40% as rapidly as 30 seconds after turning on the microwaves. A subsequent study was carried out over a 5 minute interval which showed that the microwaves definitely interact with mitochondria to inhibit mitochondrial function with a consequent decrease in the ATP concentration of tissue. We intend to combine microwave exposures with O_2 toxicity therapy in the treatment of some animals tumors to ascertain if these two modalities are synergistic in their actions upon tumor tissue and could serve as combined modalities in the treatment of malignant tumors. (It should be recognized that the normal tissues will be protected from oxygen toxicity inhibition of ATP production by the succinate

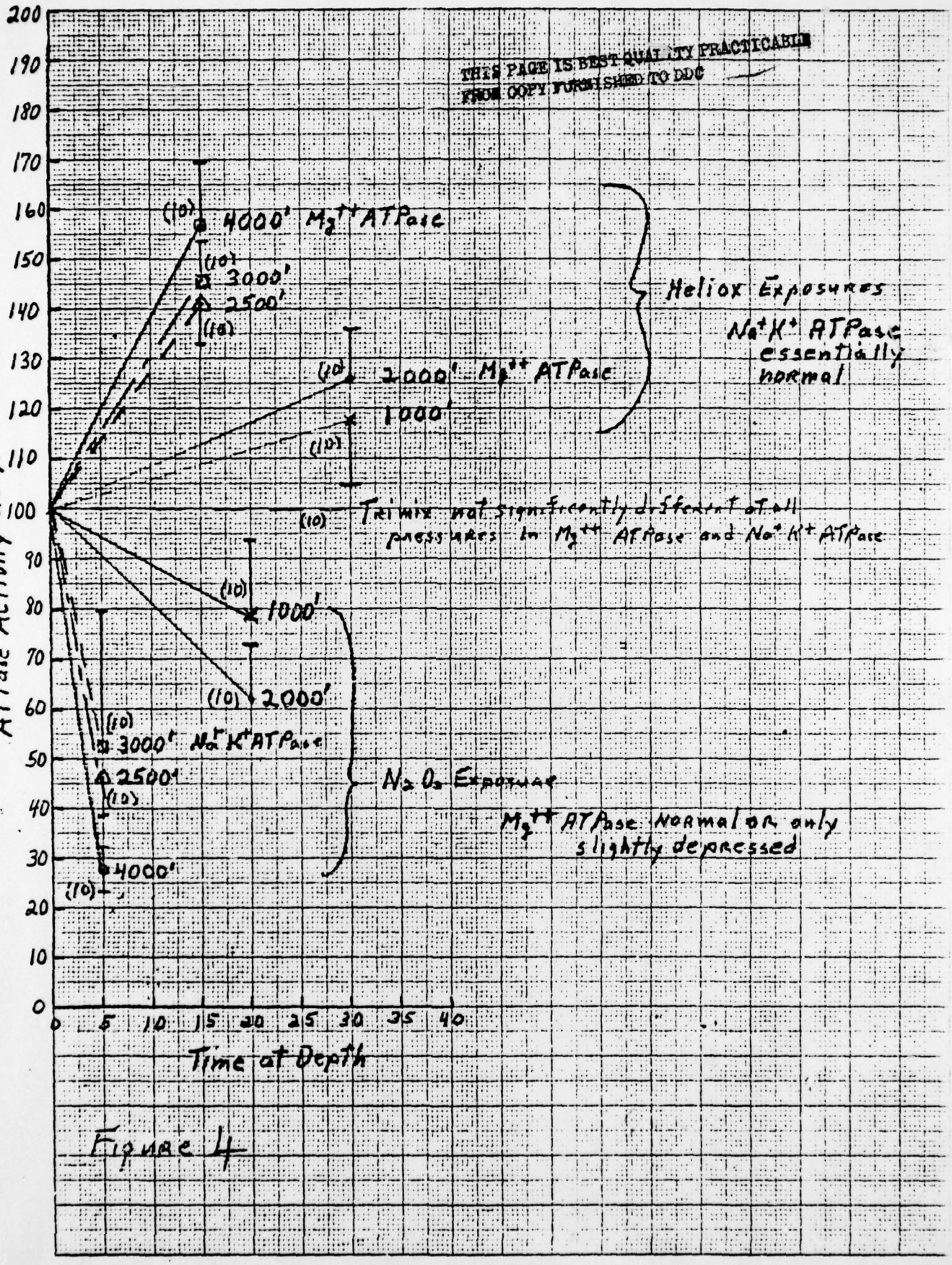

Figure 4

which the malignant tissue cannot use. The fact that the malignant tumor is rapidly reproducing indicates the great need for energy in its own cell reproduction, yet such cells only have approximately 60% of the mitochondrial count per cells as normal tissues. Thus the combined effects of oxygen toxicity inhibition of ATP production by the mitochondria and the microwave inhibition of mitochondrial ATP production should be an additive phenomena to the malignant cells without having a significant damage to the normal tissues which are being protected by succinate and which have approximately 166% of the mitochondria per cell as compared to malignant tissues.)

Publications:*

1. Sanders, A. P. and W. D. Currie: The Effects of Hyperbaric Oxygenation on Metabolism of Lung. Underwater Physiology V, ed. C. J. Lambertsen, FASEB, pp. 483-492, 1976.

Manuscripts submitted:

1. Sanders, A. P., P. C. Pratt and W. D. Currie: The effect of hyperbaric oxygenation on metabolism. VIII. Pulmonary oxygen toxicity. Submitted to Proc. Soc. Exp. Biol. & Med.

2. Sanders, A. P., P. C. Pratt, and W. D. Currie: The effect of hyperbaric oxygenation on metabolism. IX. Adrenalectomy and pulmonary O_2 toxicity. Submitted to Proc. Soc. Exp. Biol. and Med.

3. Sanders, A. P., D. J. Schaefer and W. T. Joines: Microwave Effects on Energy Levels of Brain and Malignant Brain Tumor. Submitted to Journal of Bioelectromagnetics Society.

Manuscripts in preparation:

1. Sanders, A. P.: Enzyme inhibition or Activation by High Pressure

2. Sanders, A. P. and Currie, W. D.: On the Mechanism of Oxygen Toxicity

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