Table 1. Glycolic acid oxidase activity (oxygen consumption) in chloroplast fragments from ecotypic populations of *Typha latifolia* L. at three temperatures. The results represent oxygen consumption of two replications, each consisting of six flasks, expressed as milliliters of oxygen per flask per 5 minutes.

Temp. (°C)	Oxidase activity			
	Beaverton		Redmond	
	A	B	A	B
17	31.9	18.8	8.9	8.7
27	30.0	16.3	11.8	9.9
37	19.9	9.7	12.6	9.2

but no data have been provided documenting directly an enzymatic difference between ecotypic populations.

In an investigation designed to determine whether there are definite enzymatic differences between plants adapted to different habitats, four populations of broad-leaved cattail, *Typha latifolia* L., were selected. Two populations were from sites near Beaverton, Oregon, in a semimaritime climate characterized by a long growing season (264 days), cool midsummer high temperatures (about 25°C), and mild midsummer lows (15°C). The other two populations were from Redmond, Oregon, in a continental climate characterized by a short growing season (130 days), warm midsummer highs (35°C), and cool midsummer lows (10°C). Although the mean daily temperatures are similar, diurnal variation is much greater in the Redmond location.

Standard techniques for the isolation of chloroplasts were used (*2*) except that plastids from greenhouse-grown plants were sedimented under conditions between 1000*g* for 1 minute and 37,000*g* for 10 minutes. Chloroplasts were fragmented by suspending the pellet in 0.067*M* phosphate buffer, *p*H 7.8, to a concentration of 0.08 g of plastid fragments per milliliter; 2.5 ml of this suspension was added to each Warburg flask. Enough glycolic acid was in the sidearm to make the suspension 0.008*M*. Standard manometric techniques with KOH were used (*3*).

Data presented are from two experimental sets, each of which consisted of six flasks for each population at each temperature. The data indicate that glycolic acid oxidase activity differed markedly, depending upon the type of climate to which the plant population was adapted (Table 1). Student's *t*-test on paired samples showed that Beaverton A was much more active than all other populations at all temperatures ($P << 0.001$ in all comparisons). The Beaverton B population was more active than either Redmond population at 17° and 27°C ($P << 0.001$) but was less active than Redmond A at 37°C ($P < 0.01$). The two Redmond populations were not significantly different except at 37°C where population A was more active. These data provide direct evidence that enzymatic activity is conspicuously different and that temperature dependence is different in populations occupying distinct habitats.

Although considerable controversy surrounds the role of glycolic acid in the carbohydrate metabolism of higher plants (*4*), there can be little doubt that at normal concentrations of CO_2 a significant proportion of the CO_2 fixed is metabolized by glycolic acid oxidase (*5*). The important function of this enzyme in carbohydrate metabolism suggests that the conspicuous differences in glycolic acid oxidase activity between populations from different habitats probably reflect selective processes that are oriented by climatic adaptation of assimilatory metabolic pathways.

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Succinate: Protective Agent against Hyperbaric Oxygen Toxicity

Abstract. *When succinate is used to protect rats against the toxicity of oxygen at high pressure, 100 percent survive, with normal or above normal concentrations of adenosine triphosphate being present in the cerebral hemisphere, liver, and kidney. In contrast, 90 percent of the nonprotected animals died during exposure. In corresponding tissues of surviving nonprotected animals adenosine triphosphate concentrations are markedly reduced.*

The convulsions and depressed metabolic changes which accompany exposure to oxygen at high pressure were first described by Bert in 1878 (*1*). There has been at least one death attributed to the clinical use of hyperbaric oxygenation (*2*). The problem of toxicity to oxygen at high pressure is an obstacle to more widespread clinical application of hyperbaric oxygenation (*3*).

Studies on cellular metabolism have led us to believe that succinate should offer protection against the toxic action of oxygen at high pressure. A series of experiments was performed on four groups of fasted male Sprague-Dawley rats (160 to 225 g). Group 1 (controls) was given intraperitoneal injection of 7.5 ml of 0.4*M* succinate solution (*p*H 7.4) 2½ hours before they were killed; groups 2, 3, and 4 were given intraperitoneal injections of 7.5 ml isotonic saline, 0.4*M* dextrose solution (*p*H 7.4), and 0.4*M* succinate solution (*p*H 7.4), respectively, 1 hour before exposure (1½ hours) to 100 percent oxygen at 5 atm (absolute pressure).

Nine of the ten animals given isotonic saline (group 2) were dead before the end of the exposure period, and the tenth died before tissues could be removed for analyses. Five of the ten animals receiving dextrose (group 3) died during exposure, three exhibited symptoms of oxygen toxicity (convulsions, loss of consciousness, frothing at the mouth), and two animals appeared normal. All of the 22 animals receiving succinate injections (group 4) were normal in appearance, alert, and active. Six animals were observed for 6 days and showed no aftereffects.

The concentrations of energy stores

Table 1. Relative ATP concentrations, expressed as percentages of control concentration ± standard deviation, in tissues of rats exposed for 1½ hours to 100-percent oxygen at 5 atm. The values for the surviving unprotected rats are from Sanders *et al.* (*4*). The numbers in parentheses indicate the numbers of rats.

Cerebral hemisphere	Liver	Kidney
Controls		
100 ± 12 (9)	100 ± 20 (8)	100 ± 14 (12)
Unprotected		
47 ± 11* (8)	56 ± 14* (5)	37 ± 8* (5)
Succinate protected		
100 ± 25 (9)	138 ± 29* (7)	109 ± 20 (7)

* Significantly different from controls ($P < .01$).

(as indicated by adenosine triphosphate, ATP) of the cerebral hemisphere, liver, and kidney of rats that survived exposure to 5 atm of 100 percent oxygen for 1½ hours were reduced 53, 46, and 62 percent, respectively, as compared to tissues from normal animals (*4*). The ATP concentration was determined on the cerebral hemisphere, liver, and kidney of rats from groups 1, 3, and 4 to see whether succinate would prevent the decrease in ATP. The firefly-luminescence technique (*5*) was used to measure concentration of ATP in the tissue.

Table 1 shows the relative concentrations of ATP in three tissues of animals from the controls, from surviving animals given similar hyperbaric exposures without any protective agent (*4*), and from succinate-injected animals. Tissues from the animals treated with succinate had normal or above normal ATP concentration.

The two dextrose-treated animals that survived the exposure and were normal in appearance had ATP concentrations in the liver and kidney within the normal range. The three dextrose-treated animals that exhibited severe symptoms of oxygen toxicity had an averaged reduction of 58 and 63 percent in ATP concentration in liver and kidney, respectively.

Toxicity of high-pressure oxygen (*6*) is generally attributed to the inhibition of tissue respiration and oxidative phosphorylation (ATP production under aerobic conditions) and inhibition of enzyme activities. Brain-tissue slices exposed to high-pressure oxygen had decreased intracellular potassium and increased intracellular sodium (*7*). This observation led to the hypothesis that high-pressure oxygen exerts its toxic effect on tissue "by reducing energy available" for metabolic functions. We have shown (*4*) that toxicity from high-pressure oxygen is accompanied by marked reduction in ATP concentration in rat tissue. Thus protective efforts against oxygen toxicity should be directed toward restoring ATP concentration and metabolic functions in tissues. Among succinate, glutamate, and α-ketoglutarate, succinate has the highest ATP-production capacity in rat brain, liver, and kidney (*5*). When succinate is the substrate, only two molecules of ATP are formed per atom of oxygen used, as compared to three molecules of ATP per atom of oxygen for the other aerobic substrates which lead to ATP formation by oxidative phosphorylation. Thus succinate not only causes high production of ATP but also uses more oxygen than other oxidative phosphorylation substrates; and both processes counteract the local effects of oxygen. King (*8*) has observed that succinate is necessary for the reconstitution and stabilization of electron-transport particles (the fundamental units of the oxidative phosphorylation process). One effect of high-pressure oxygen is the inhibition of the reduction of nicotinamide-adenine dinucleotide (NAD) (*9*). The reduction of NAD is essential for oxidative phosphorylation with all substrates except succinate. Succinate lessens the effect of high-pressure oxygen on the inhibition of NAD reduction, and there is minimum inhibition of electron flow from succinate into the electron-transport chain (thence ATP production) at an oxygen pressure of 12 atm (*9*). Succinate oxidation may monopolize the respiratory chain and inhibit the oxidation of NADH (*10*). This latter action—if present in high-pressure oxygenation—would tend to counteract the effect of high-pressure oxygen in shifting NADH toward NAD. These observations support our hypothesis that succinate should be an effective protective agent against the toxic effects of high-pressure oxygen.

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Spleen as a Production Site for Erythropoietin

Abstract. *Mice treated with acellular extracts of spleen and liver show a pronounced and significant increase in blood reticulocytes. In control mice treated with saline and extracts of kidney, muscle, and liver of splenectomized mice and rats, the number of reticulocytes remained constant in all the cases.*

In 1926 Krumbhaar (*1*), in his review of the spleen, emphasized the indirect influence of the spleen on blood formation through a stimulating action on bone marrow, possibly after activation of spleen factor by the liver. Later, Gley, Delor, and Laur (*2*) and Ruhenstroth-Bauer (*3*) concluded that the spleen may represent one of the sites of production of a factor that stimulates erythropoiesis.

In 1959, at the conclusion of some research on hypoxilienine (*4*), a suggestion, based on the relationship between the spleen and x-ray mortality, was made by one of us (*5*) that a splenic humoral factor capable of reducing x-ray mortality had a specific