livers of both rats and monkeys seem to become adapted quickly to the new circumstances and the structural integrity is restored. The changes in monkeys seem not only less striking than in rats but adaptation appears to occur more quickly even in the presence of oxygen inhaled under higher pressure. Presumably this might also apply to man but only study of human material can answer this with certainty. The major unanswered question is whether the liver during the period of becoming adapted is more sensitive to the damaging effects of volatile solvents which may enter the atmosphere in closed systems such as space capsules or of irradiation which may be encountered during open space flights.

Summary. Livers of monkeys exposed to pure oxygen at 380 mm Hg were examined electron microscopically. Only minimal nonspecific changes were observed; the most significant was an increase in smooth endoplasmic reticulum at the expense of the rough form and glycogen. The changes, which appeared as early as 24 hours and were gone by 2 weeks, are considered an adaptive response rather than a sign of toxicity.

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Effects of Hyperbaric Oxygenation on Metabolism III. Succinic Dehydrogenase, Acid Phosphatase, Cathepsin and Soluble Nitrogen.* (31005)

I. H. HALL,† AND AARON P. SANDERS (Introduced by Barnes Woodhall)

Department of Radiology, Duke University Medical Center, Durham, N. C.

The concentration of adenosine-triphosphate (ATP)(1) and succinic dehydrogenase activity(2) have been demonstrated to decrease significantly in rat brain, liver and kidney after exposure to 5 atmospheres of 100% O_2 for 1½ hours. These losses could be due to oxygen poisoning of the respective enzyme systems, *i.e.*, oxidation of SH groups, or to a general injury process to cells. Since it has been observed that free acid hydrolytic activity increases during cellular injury(3-9), it was important to determine the extent of release of lysosomal enzymes during oxygen toxicity. Cellular injury to protein structure, as indicated by an increase in soluble nitrogen, has been demonstrated in autolytic liver by Bradley(10). Similar injury to cellular protein would be expected in HPO toxicity if energy stores fall below critical levels. The following study was undertaken to determine the effects of high pressure oxygen (HPO) on succinic dehydrogenase, acid phosphatase and cathepsin activities, and acid soluble nitrogen.

Methods and materials. Male rats of the Sprague-Dawley strain (160-225 g) were fasted for 18-24 hours with water *ad libitum* before any experiment. The control group consisted of animals exposed to air at 1 atmosphere. The animals were subjected to 1 and 3 atmospheres (absolute pressure) of 100% O_2 for 2 hours and 5 atmospheres for 1½ hours using the Bethlehem Steel Hyperbaric Chamber. After slow decompression (5 min) the animals were killed and the cerebral hemisphere, liver and kidney cortex were excised. A 0.25 M sucrose + 0.001 M EDTA homogenate was prepared. The following determinations were made on the homogenate: 1. succinic dehydrogenase by the method of

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† Post Doctoral Fellow, U.S.P.H.S. Training Grant, NIH 5-T-1-MH-8394-02.

TABLE I. Succinic Dehydrogenase Activity.*

Tissue	Exp	N	$\overline{X}$	s	P
Brain	Normal	7	286	83	—
	1 atm	6	192	64	.025
	3 "	6	157	41	.008
	5 "	6	122	48	.001
Liver	Normal	7	529	48	—
	1 atm	6	496	60	N.S.
	3 "	6	302	29	.001
	5 "	6	166	78	.001
Kidney	Normal	7	506	74	—
	1 atm	6	385	87	.010
	3 "	6	283	35	.001
	5 "	6	186	44	.001

* Change in log cytochrome C per mg wet weight per min measured at 550 mμ.

TABLE II. % Free Cathepsin Activity.*

Tissue	Exp	N	$\overline{X}$	s	P
Brain	Normal	6	11	2	—
	1 atm	6	26	4	.001
	3 "	6	50	5	.001
	5 "	6	52	7	.001
Liver	Normal	6	25	6	—
	1 atm	6	44	10	.001
	3 "	6	64	11	.001
	5 "	6	64	10	.001
Kidney	Normal	6	34	4	—
	1 atm	6	24	4	.001
	3 "	6	86	6	.001
	5 "	6	40	4	.018

* Expressed as amount of tyrosine released from hemoglobin in 10 min (which is coupled with cysteine-HCl) per gram wet weight measured at 280 mμ.

Cooperstein, Lazarow, and Kurfess(11), 2. free and total acid phosphatase by the method of Gianetto and deDuve(12); the inorganic phosphorus was determined by the method of Chen, Toribara, and Warner(13); the total enzymatic activity was released by Triton-X-100 (0.1%) in the homogenate(14), 3. free and total cathepsin by the method of Anson (15) as modified by Press, Porter & Cebra (24), and 4. total and acid soluble nitrogen were determined by the micro-Kjeldahl digestion followed by the coupling with Nessler reagent.

Results. The number of animals (N), the mean of the sample ($\overline{X}$), the standard deviation (s), and the probable significance level (P) derived from the Student "t" test are shown in Tables I-IV for succinic dehydrogenase activity, % free cathepsin activity, % free acid phosphatase activity, and acid soluble nitrogen respectively. There was a gradual decrease in succinic dehydrogenase activity with increasing pressure (Table I). High pressure oxygen resulted in an increase in the % free activity of cathepsin, and acid phosphatase in rat brain, liver and kidney. The % free cathepsin in brain and liver increased at 1 and 3 atmospheres, but there was no further rise at 5 atmospheres (Table II). In kidney there was a significant reduction in % free cathepsin at 1 atmosphere followed by a sharp rise at 3 atmospheres, and subsequently a reduction at 5 atmospheres. In all 3 tissues the bound cathepsin activity was reduced at 5 atmospheres. The % free acid phosphatase in liver increased with increasing pressure (Table III). In kidney it increased at 1 and 5 atmospheres, but was not significantly changed from normal

TABLE III. % Free Acid Phosphatase Activity.*

Tissue	Exp	N	$\overline{X}$	s	P
Brain	Normal	8	62	10	—
	1 atm	6	31	4	.001
	3 "	6	36	5	.001
	5 "	5	46	5	.010
Liver	Normal	8	31	4	—
	1 atm	6	38	6	.012
	3 "	6	55	6	.001
	5 "	7	53	5	.001
Kidney	Normal	5	33	2	—
	1 atm	6	41	4	.005
	3 "	8	35	5	N.S.
	5 "	7	44	4	.005

* Expressed as amount of phosphorus released from beta-glycerophosphate in 10 min per gram wet weight measured at 700 mμ.

TABLE IV. Acid Soluble Nitrogen.

Tissue	Exp	N	$\overline{X}$	s	P	% of total
Brain	Normal	6	3.3	.5	.012	13
	1 atm	6	2.7	.2	.008	11
	3 "	5	2.2	.8	.001	11
	5 "	9	16.2	1.7	.001	74
Liver	Normal	6	3.6	.3	—	10
	1 atm	6	3.4	.3	N.S.	9
	3 "	5	2.4	.8	.005	7
	5 "	9	16.3	1.8	.001	52
Kidney	Normal	6	4.3	.5	—	13
	1 atm	6	5.2	.7	.0015	16
	3 "	5	3.7	.7	N.S.	11
	5 "	9	16.5	2.0	.001	59

Total and soluble nitrogen were expressed as mg nitrogen per gram wet weight of tissue.

at 3 atmospheres. An interesting observation was the high % free activity of acid phosphatase in normal brain (62%). This could be due to: 1. an increased permeability of the substrate through the lysosomal membrane, 2. a different affinity or type of bonding of the enzyme to the lysosomal membrane which resulted in a higher amount of the enzyme in the free state, or 3. acid phosphatase may play a larger role in normal brain metabolism than in other tissues. It has been proposed that acid phosphatase functions in protein synthesis(23). The particular protein synthesis may occur at a higher rate in brain than in other tissues.

High pressure oxygen produced no significant change in total nitrogen at 1, 3 and 5 atmospheres in rat brain, liver and kidney. The acid soluble nitrogen decreased slightly at 1 and 3 atmospheres in all 3 tissues, and was markedly increased at 5 atmospheres. The increase at 5 atmospheres was larger than 50% of the total nitrogen in all 3 tissues. These data indicate that a large amount of protein degradation has occurred at 5 atmospheres.

Discussion. Cellular injury resulting from high pressure oxygen is quite apparent in these studies. The large increase in acid soluble nitrogen and significant increase in percent free acid phosphatase and percent free cathepsin indicated drastic changes in protein structure and an increase in the hydrolytic system within rat brain, liver and kidney cells. It is well known that lysosomal enzymes are capable of digesting or lysing other subcellular particles, *i.e.,* mitochondria and microsomes(16), thus bringing about further subcellular damage.

The loss in succinic dehydrogenase activity in rat brain, liver and kidney is in agreement with Dickens' observation that succinic dehydrogenase was irreversibly poisoned by high pressure oxygen in rat brain homogenates (17). There was a linear loss in succinic dehydrogenase activity with increasing oxygen pressure. This could be due to direct enzyme inactivation by HPO or possibly a result of proteolytic activity due to the increased levels of % free cathepsin activity, which could act upon mitochondrial membranes with subsequent loss of oxidative enzymes.

The observed increase in lysosomal enzymatic activity is in agreement with reports in the literature. deDuve and Beaufay(4) observed a rise in percent free lysosomal activity of acid phosphatase in liver slices incubated in 95% O_2 and 5% CO_2. They observed a greater percent free activity when isolated mitochondria were used, suggesting that HPO acted directly on the lysosomes to release the enzymes. Sledge and Dingle(18) and Allison(19) observed increased lysosomal activity in chick embryo cells exposed to increased oxygen pressures. These authors hypothesize that the increase in free lysosomal enzymes was due to HPO causing lipid peroxidation which increased permeability of the lysosomal membranes. However, this increase in percent free activity could be due to disturbances in acid-base equilibrium within the cell. Increased intracellular acidity causes increased lysosomal activity and could be one factor in HPO. Bean(20) has claimed that increased hydrogen ions and carbon dioxide pressures contribute to oxygen toxicity. Bean(20) and Gottlieb *et al*(21) have observed that a hydrogen ion buffer, THAM, protects against convulsions caused by HPO. Succinate, which has been used as a buffer, protects against ATP loss during HPO(22). It is possible that the hydrogen ion may be activating lysosomal enzymes during HPO. Dickens has shown that the cathepsin enzyme contains an SH group(17). SH groups are oxidized by HPO and supposedly inactivated. This inactivation of enzymes by HPO could account for the loss in percent of free cathepsin in kidney at 5 atmospheres, and the leveling out of percent of free activity of liver and brain between 3 and 5 atmospheres. If this assumption is correct, then injury due to oxidation of SH groups is relatively late in the HPO toxicity response. This raises the question as to whether the reduction to succinic dehydrogenase activity is due to 1. inactivation of SH groups, or 2. the result of increase of free hydrogen ions, or 3. to lysosomal enzymatic digestion of mitochondria.

The acid soluble nitrogen dropped slightly at 1 and 3 atmospheres in brain, liver and kidney. It has been observed 1. that HPO

inactivates d-amino oxidase and urease(17), 2. that the production of urea requires ATP (which is lost during HPO exposure(1)), and 3. that enzymes of the ornithine cycle contain SH groups which are inactivated by HPO. Thus it appears that the early reduction in soluble nitrogen observed at 1 and 3 atmospheres may be due to the decrease in the deamination processes and in urea production. The high acid soluble nitrogen at 5 atmospheres indicates a considerable amount of protein destruction. The soluble nitrogen was greater than 50% of the total nitrogen. This would result in serious impairment of cellular function as evidenced by the decrease in mitochondrial and lysosomal enzyme activities. Since the rise in soluble nitrogen at 5 atmospheres occurred after the rise in percent of free cathepsin at 3 atmospheres, it would appear that lysosomal activity was responsible for the degradation of proteins.

These studies indicate that increased free lysosomal enzymatic activity and protein destruction play a major role in the cellular damage resulting from oxygen toxicity. Cellular damage caused by 5 atmospheres of HPO is more severe than cellular damage seen after anoxia of 12 hours duration and acidosis at pH 5 for 1 hour(3), as indicated by solubilization of proteins and release of lysosomal enzymes.

Summary. 1. Succinic dehydrogenase, cathepsin and acid phosphatase activities and soluble and total nitrogen were determined in homogenates of the cerebral hemispheres, liver and kidney from rats previously exposed to normal air, and 100% oxygen for 2 hours at 1 and 3 atmospheres, and for 1½ hours at 5 atmospheres. 2. HPO caused a decrease of succinic dehydrogenase activity and an increase in free lysosomal enzymatic activities with increasing pressure. 3. There was a slight decrease in percent soluble nitrogen at 1 and 3 atmospheres, and a marked increase at 5 atmospheres. 4. Increased free lysosomal enzyme activities and subsequent solubilization of protein structures are major destructive factors in high pressure oxygen toxicity.

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