TABLE I. Urinary Taurine Excretion, Cottage Placement and I.Q. or S.Q. Scores for 41 Mongoloid Subjects.

Subject	Log (mg taur/ g creat)	Cottage placement	I.Q. or S.Q.
R.M.	1.55	B	40
D.F.	1.47	B	14
M.H.	1.13	B	40
V.S.	1.53	B	8
L.B.	1.92	B	17
R.A.	1.20	B	19
V.F.	.03	B	36
C.S.	.41	B	15
W.M.	.90	B	17
R.C.	1.52	B	20
G.M.	.29	B	12
J.S.	.79	B	8
P.W.	.93	C	3
R.M.	.43	C	7
P.L.	.60	C	14
M.J.	.56	C	14
A.A.	.57	H	34
E.B.	1.67	H	43
C.E.	1.60	H	36
U.S.	1.29	H	24
P.H.	.35	H	24
S.O.	1.71	H	44
M.B.	.99	H	41
R.C.	1.04	H	36
P.B.	1.92	H	17
J.F.	1.25	H	26
S.H.	1.02	H	30
K.W.	1.17	H	20
S.T.	1.42	H	34
W.C.	1.61	H	23
G.B.	1.15	H	18
J.S.	1.73	H	41
R.S.	.65	H	30
A.L.	1.32	H	37
T.S.	1.08	H	21
D.W.	1.17	H	23
W.T.	1.53	H	35
G.S.	.67	H	31
J.K.	1.14	H	20
S.C.	1.35	H	22
C.P.	1.62	H	43

effects(1). Studies now in progress with tracer doses of S^{35} taurine indicate that the lowest excretors among mongoloids convert most administered taurine to at least 2 metabolites other than sulfate. Current experiments also indicate that the low taurine excretion observed among most mongoloids is correctable with pyridoxine hydrochloride.

Summary. Significant positive associations were found between urinary taurine excretion and 2 measures of adaptive behavior, cottage placement and I.Q. scores. The correlation ratio between cottage placement and taurine excretion was $E_{yx} = .41$. I.Q. score regressed on taurine excretion yielded a coefficient of .32. Both associations are significant at the 5% level.

We thank Mr. Richard Humphrey for I.Q. evaluations and repeat tests and Mrs. Alma Davis for technical assistance.

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Received April 13, 1965. P S.E.B.M., 1965, v119.

Effects of *in vivo* Hyperoxia on Erythrocytes IV. Studies in Dogs Exposed to Hyperbaric Oxygenation.* (30314)

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Although the toxic effects of oxygen on erythrocytes have been recognized for several years(1-3), the underlying mechanism of cell damage has not been established. The

* This work was supported in part by U.S.P.H.S. Hematology Training Grant CRTY-5042 and U.S.P.H.S. Research Grants HE-04702-05 and CA-08170.

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practical importance of clarifying this mechanism has increased with the use of 100% oxygen environments in space capsules(4,5) and the increasing medical use of oxygen under high pressure (OHP)(6-11). Evidences of hemolysis in humans have been noted under both of these conditions(4,5,12). Previous studies in this laboratory demonstrated that hemolysis occurring in mice and humans exposed to oxygen under high pressure resulted from cell damage associated with peroxidation of erythrocyte lipid(12-14). The purpose of the present study was to investigate the *in vivo* effects of OHP on canine erythrocytes since preliminary studies showed changes of osmotic fragility in the absence of detectable hemolysis.

Materials and methods. Animals. Twenty dogs whose weight ranged from 20 to 32 lb were studied before and after OHP. They were fed standard vitamin-enriched canine chow and had water *ad libitum*. During the 14 days before exposure to OHP they were de-wormed and treated with oral Neomycin.

Exposure to oxygen under high pressure. The chamber used for hyperbaric oxygenation had a volume of 12.56 cubic feet and a maximum pressure capacity of 60 lb per square inch gauge (psig) or 75 lb per square inch absolute (psia) (5 atmospheres absolute pressure). Inflow and outflow valves allowed a through and through ventilation with oxygen, and gas in the tank was continually circulated by a fan through soda lime USP to absorb carbon dioxide. No CO_2 could be demonstrated in gas samples obtained from the chamber midway through the OHP procedure. Temperature within the tank was approximately that of the surrounding room (21°C). Portholes in the tank allowed observation of the dogs. The average time required to reach maximum pressure was 3 minutes. The dogs were kept at a pressure of either 45 or 58 psig until their first convulsion (generalized tonic, clonic movements) occurred. Time at pressure before the first convulsion ranged from 0.5 to 3.0 hours (average 1.3 hours). The usual decompression time was 15 minutes.

Blood studies. Sixteen ml of venous blood were drawn from the dogs immediately before and after exposure to OHP. Ten ml were defibrinated with glass beads, and the remainder was heparinized.

Microhematocrits were determined, and plasma was examined visually for evidence of hemolysis. Erythrocytes were examined in Wright's stained blood films and reticulocyte percentages were determined using new methylene blue stain(15). Blood methemoglobin content was determined as previously described(14). Erythrocytes were examined for glucose-6-phosphate dehydrogenase activity (16), catalase activity(17), cholinesterase activity(18), reduced glutathione content (19), and Heinz bodies(15). Price Jones curves were plotted immediately before and after OHP exposure using wet, sealed blood films. Defibrinated blood was used for osmotic fragility(15) and autohemolysis studies (4°C, 25°C, and 37°C)(20). Lytic sensitivity of erythrocytes to H_2O_2 was determined as previously described(13) except that concentrations of H_2O_2 used in the present study were 0.01%, 0.025%, 0.05%, and 0.1%.

Lipid peroxides were determined by measuring the chromagen formed by the reaction of 2-thiobarbituric acid with malonylaldehyde(21). The term "lipid peroxide" has been used with the understanding that products of lipid peroxidation and not the lipid peroxides themselves were measured. The formation of lipid peroxides in erythrocytes during incubation with H_2O_2 was determined as follows: one ml of a 10% suspension of washed erythrocytes in physiologic saline was incubated with 1 ml of 0.05% H_2O_2 for 2 hours at 37°C. Two ml of 10% trichloroacetic acid were added, the mixture thoroughly agitated, and then filtered through Whatman #1 paper. One ml of the filtrate was added to 1.2 ml of 0.67% 2-thiobarbituric acid, mixed well, and heated in a boiling water bath for 15 minutes. After cooling to room temperature, the absorbance reading was taken at 535 mμ. A blank containing physiologic saline instead of blood was carried throughout the procedure. Lipid peroxide content in erythrocytes before *in vitro* incubation with H_2O_2 was determined by adding 0.5 ml of packed cells to 1 ml of physiologic saline and mixing well with 1.5 ml of 10%

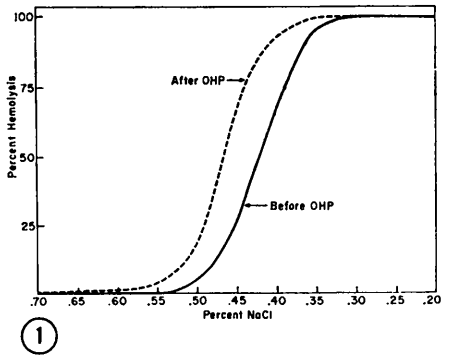
TABLE I. Effect of *in vivo* Hyperoxia on Osmotic Fragility of Canine Erythrocytes in .45% NaCl.

	% hemolysis
Before OHP	21.2 ± 4.5
After OHP	39.3 ± 5.3

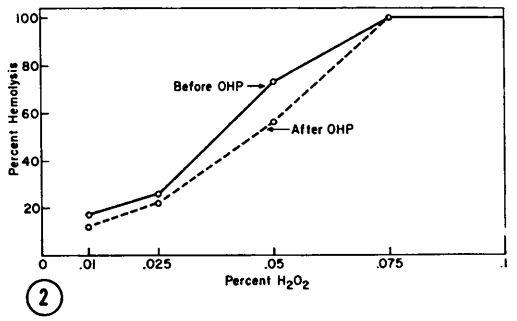
Values given are average of 20 dogs. OHP = oxygen under high pressure.

trichloroacetic acid. After filtering through Whatman #1 paper, the lipid peroxide content was determined as described above.

Results. 1. Nineteen of twenty dogs had an increase of hematocrit after OHP. The hematocrit of one dog was unchanged. Hematocrits before OHP ranged from 30% to 37% (average 34.7%) while hematocrits immediately after OHP ranged from 31% to 52% (average 41.5%). Plasma hemoglobin and reticulocytes (1.5% before OHP) were not increased after OHP. Price Jones curves showed no change in erythrocyte size after OHP. No evidences of hemolysis were noted in dogs 2, 4, and 6 days later.



(1)



(2)

FIG. 1. Effect of *in vivo* hyperoxia on erythrocyte osmotic fragility of a dog in a representative experiment.

FIG. 2. Lytic effect of H_2O_2 on canine erythrocytes. Each point at different concentrations of H_2O_2 represents the mean value of all dogs.

2. Cells exposed to *in vivo* OHP showed increased osmotic fragility (Fig. 1 and Table I). The increases were significant ($p<.02$), and were consistently more pronounced at the higher concentrations of NaCl. No follow-up studies were performed to determine the duration of the osmotic shift.

3. No consistent changes were observed in erythrocyte autohemolysis. There was no change in glucose-6-phosphate dehydrogenase activity or reduced glutathione content, and no Heinz bodies were formed during OHP. No methemoglobin was present before or after OHP.

4. The catalase activity of canine erythrocytes was low (0.037 milliequivalents of perborate decomposed by 1.0 ml of hemolysate with a hemoglobin of 0.02 g per cent) when compared to that of humans (normal average 0.941) and mice (normal average 0.340). No change of catalase activity occurred during OHP.

5. Cholinesterase activity of erythrocytes was decreased after OHP in all but one dog (Table II). The largest decrease of activity

TABLE II. Effect of *in vivo* Hyperoxia on Canine Erythrocytes Cholinesterase.

	Cholinesterase activity
Before OHP	17.1 ± 1.8
After OHP	13.3 ± 1.1

Values given are mean values of duplicate determinations in all dogs and their standard deviation. Values are expressed as enzyme units/μl packed cells/minute and represent means of all dogs. OHP = oxygen under high pressure.

in a dog was from 16.3 enzyme units/μ liter packed cells/minute to 9.1, and the mean decrease of 3.8 was significant ($p<.05$).

6. Canine erythrocytes were quite sensitive to the lytic effect of hydrogen peroxide (Fig. 2). Intact erythrocytes exposed to *in vivo* OHP were slightly but consistently less sensitive to H_2O_2 than erythrocytes before OHP.

No lipid peroxides were demonstrated in erythrocytes immediately after OHP. Lipid peroxides were demonstrated in erythrocytes after incubation with H_2O_2, and significantly higher levels were found in erythrocytes after *in vivo* exposure to hyperoxia ($p<.05$) (Table III).

TABLE III. Lipid Peroxide Formation in Canine Erythrocytes During Incubation with H_2O_2.

	Thiobarbiturate pigment (abs max at 535 mμ)
Before OHP	.292
After OHP	.355

Mean values of each group are given. OHP = oxygen under high pressure.

Discussion. Previous studies of the effects of *in vivo* hyperoxia on erythrocytes demonstrated that hemolysis in mice exposed to OHP was correlated with *in vitro* lytic sensitivity of erythrocytes to H_2O_2 and lipid peroxide formation. *In vivo* and *in vitro* lysis as well as lipid peroxide formation were enhanced by tocopherol deficiency and prevented by treating animals with alpha-tocopherol(13). Similar observations were made in humans exposed to OHP(12). These findings suggested that lipid peroxidation was directly or indirectly responsible for the erythrocyte damage occurring during exposure to *in vivo* OHP.

In the present study of dogs the hematocrit increase probably reflected hemoconcentration. Since Price Jones curves were not shifted and cell size in blood films was not changed, the increased hematocrit did not reflect an increase in cell size. It was also unlikely that the hematocrit change resulted from increased erythropoiesis since hyperoxia would not be expected to suppress erythropoiesis(22), no increase in reticulocytes was noted, and cholinesterase activity (higher in young cells) was consistently lower after OHP.

Although no lipid peroxides were demonstrated in erythrocytes directly after OHP, the fact that after incubation with H_2O_2 there were more lipid peroxides in post-OHP than pre-OHP erythrocytes suggested that processes involved in lipid peroxidation had been initiated *in vivo*. Similar changes were noted in previous studies of mice exposed to OHP(13).

No evidence of *in vivo* hemolysis during the hyperoxic exposure was noted. However, it is possible that a small population of red cells was destroyed *in vivo* as reflected by the decrease in *in vitro* sensitivity of erythrocytes to H_2O_2 after OHP.

A small population of cells which was most susceptible to oxidant stress (molecular oxygen, H_2O_2) could have been hemolyzed *in vivo* and consequently was excluded from the post-OHP *in vitro* test. Another population of susceptible erythrocytes, damaged but not hemolyzed during OHP, was lost during the several manipulations required in the H_2O_2 sensitivity test. The remaining population of cells would then be more resistant to oxidant stresses (in this case H_2O_2) than erythrocytes not exposed to *in vivo* OHP.

Erythrocyte membranes were altered as reflected by the increased osmotic fragility after OHP, suggesting that cell membrane permeability had been changed. Increases in osmotic fragility have also occurred in humans maintained in 100% oxygen environments at psia for 2 weeks(4). Reed and Swisher(23) demonstrated that a loss of erythrocyte lipid due to decreased exchange between plasma and erythrocyte lipids preceded changes in osmotic fragility and measurable hemolysis. It was therefore possible in our studies that rupture of fatty acid double bands in the erythrocyte membrane during OHP directly altered membrane permeability which resulted in increased *in vitro* osmotic fragility.

An additional explanation for the increased osmotic fragility after OHP was suggested by the work of Greig and Holland(24) who demonstrated that *in vitro* inhibition of canine erythrocyte cholinesterase resulted in increased osmotic fragility. It was possible that the same phenomenon occurred in our *in vivo* studies since inhibition of cholinesterase activity and increased osmotic fragility occurred concomitantly. Since cholinesterase activity was expressed as enzyme units per volume of packed cells, changes of activity could have reflected changes in cell size, but this was unlikely since erythrocyte size (determined by Price Jones curves and direct observation of cells in blood films) was not altered by OHP. The cholinesterase molecule has been shown to contain -SH groups(25), and lipid peroxides have been shown to inhibit -SH bearing enzymes(26). It was therefore possible that lipid peroxides generated during OHP re-

sulted in inhibition of cholinesterase activity. Preliminary studies in our laboratory have demonstrated an *in vitro* inhibition of cholinesterase by peroxides, but not by oxygen *per se*(27). Thus, although hyperoxia can indeed result in damage to -SH bearing compounds, as has been noted in the literature, this effect may result from the formation of lipid peroxides rather than from oxygen *per se*.

That cell damage did not result from abnormalities of the usual oxido-reduction transformation systems of the erythrocyte was evidenced by the absence of change in glucose-6-phosphate dehydrogenase activity, reduced glutathione content, methemoglobin content, and absence of Heinz body formation.

It was also unlikely that the low catalase activity was solely responsible for *in vivo* red cell damage since studies by Hochstein and Cohen(28) demonstrated that glutathione peroxidase and not catalase was the primary mechanism for removal of slow steady-state generated H_2O_2 (a system probably more analogous to that occurring *in vivo* during exposure to OHP).

Summary. Erythrocytes of 20 dogs were studied before and after *in vivo* exposure to oxygen under high pressure (OHP). No evidence of *in vivo* hemolysis was noted but erythrocytes from dogs exposed to OHP demonstrated increased osmotic fragility and decreased acetylcholinesterase activity. Additional evidence indicated the *in vivo* formation of lipid peroxides in erythrocytes. No change in glucose-6-phosphate dehydrogenase activity, reduced glutathione content, or methemoglobin content occurred, and no Heinz bodies were formed during OHP. Thus, inadequacy of the pentose-phosphate shunt was probably not responsible for cell damage. It is suggested that altered permeability of erythrocytes resulted directly from peroxidation of lipids or indirectly from inhibition of erythrocyte acetylcholinesterase or a combination of both effects.

The authors gratefully acknowledge the technical assistance of Betty D. Horton, and the aid of Patricia Johnson in preparation of the manuscript.

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Received April 14, 1965. P.S.E.B.M., 1965, v119.