RED CELL METABOLISM STUDIES ON SKYLAB

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ABSTRACT

In previous laboratory experiments on animals exposed to levels of hyperoxia, it was demonstrated that several metabolic effects occurred in red cells during such exposure. The most dramatic of these effects was the occurrence of lipid peroxidation in red blood cells and subsequent hemolysis. Other metabolic alterations noted were some changes of glycolytic intermediates (including increases of adenosine triphosphate and decreases of 2, 3 diphosphoglyceric acid and inhibition of phosphofructokinase. On the basis of these background data, similar metabolic studies were performed on humans involved in space flight. These studies included the Skylab experiences. The primary purpose of the investigations was to study red cells for (1) evidences of lipid peroxidation or (2) changes at various points in the glycolytic pathway. The Skylab missions were an opportunity to study blood samples before, during, and after flight and to compare results with simultaneous controls. No direct evidence that lipid peroxidation had occurred in the red blood cells was apparent in the studies.

Nevertheless, certain metabolic changes in red cells were noted.

- Skylab 2: Increases of hexokinase, glyceraldehyde 3-phosphate dehydrogenase, and pyruvate kinase during flight, and decreases of phosphofructokinase after flight.

- Skylab 3: Decreases of hexokinase, phosphoglyceric kinase, acetylcholinesterase, and increases of pyruvate kinase during flight; and decreases in glutathione, glyceraldehyde phosphate dehydrogenase, and phosphoglyceric kinase, and increased adenosine triphosphate, and pyruvate kinase after flight.

- Skylab 4: Decreases of glutathione, glucose-phosphate dehydrogenase, and phosphofructokinase, glyceraldehyde 3-phosphate dehydrogenase during flight.

The most consistent changes (including laboratory high oxygen pressure studies, Skylab Medical Experiments Altitude Test, and Skylab) have included:

- increased adenosine triphosphate
- decreased phosphofructokinase
- decreased phosphoglyceric kinase
- increased pyruvate kinase
- decreased acetylcholinesterase

The data are consistent with the hypothesis that these conditions of space flight do not produce metabolic changes (lipid peroxidation) that are known to result in hemolysis but do result in significant alterations of glycolytic intermediates and enzymes.

INTRODUCTION

The untoward effects of high oxygen tension for many years had been largely of academic and *in vitro* interest only. Development of the use of oxygen under high pressure for medical purposes, and the use of a hyperoxic environment in the cabins of space vehicles for United States manned space flights increased the practical implications of the potential untoward effects. These situations also provided a special opportunity for study of varying aspects of red cell metabolism. It had been demonstrated that susceptible animals exposed to oxygen under high pressure developed hemolysis due to peroxidation of unsaturated fatty acids in red cell membrane (1). It was also demonstrated that a similar event could occur in humans (2). Subsequent studies under simulated and actual space flight conditions demonstrated variable decreases of the red cell mass.

A major limiting factor in the interpretation of data obtained during the Gemini and Apollo series, however, was that blood samples were analyzed before and after flight. There was no information as to what, if any, changes occurred *during* space flight itself. The Skylab program thus offered a unique opportunity for the study of the possible effects of that environment and flight on red cell metabolism.

The studies carried out included an analysis of red cell componets involved with

- peroxidation of red cell lipids,
- enzymes of red cell metabolism,
- levels of 2, 3-diphosphoglyceric acid and adenosine triphosphate.

MATERIALS AND METHODS

The details and schedules of sampling appear elsewhere.

Blood was kept frozen for transport of samples from the Johnson Space Center to the investigator's laboratory. Samples there remained frozen at -70° F (-39° C) until the time of determination. In all procedures, samples of blood drawn concomitantly from controls were run simultaneously with astronaut specimens.

Details of most analytic procedures used have been previously described (3 through 8).

The procedures employed for 2, 3 diphosphoglyceric acid were basically those described by Oski (9) and Krimsky (10).

A hemoglobin determination as made on blood samples using the cyanmethemoglobin method. The final readings were thus expressed as micromoles of red cell 2, 3 diphosphoglyceric acid per gram of hemoglobin. Simultaneous standards were performed with runs, and also checked against a prepared standard curve. The range used on the standard curve was between 0.1 and 0.4 micromoles (μM).

Abbreviations used:

GSH	Reduced glutathione
ATP	Adenosine triphosphate
2, 3-DPG	2, 3-diphosphoglyceric acid
G6PD	Glucose-6-phosphate dehydrogenase
HK	Hexokinase
PFK	Phosphofructokinase

G3PD	Glyceraldehyde phosphate dehydrogenase
PGK	Phosphoglyceric kinase
PK	Pyruvate kinase
AChE	Acetylcholinesterase

RESULTS

The data obtained from Skylab 2 are shown in table I. Preflight differences between astronauts and controls were not noted.

With the in-flight samples, there were increases of hexokinase, pyruvate kinase, and glyceraldehyde phosphate dehydrogenase. The changes of adenosine triphosphate and 2, 3-diphosphoglyceric acid were not significant.

Postflight there was a significant decrease of phosphofructokinase.

The data obtained from Skylab 3 are summarized in tables II and III. Astronauts and controls were identical preflight.

During flight there were significant decreases of hexokinase, phosphoglyceric kinase, and acetylcholinesterase, and increases of pyruvate kinase.

Postflight the changes noted on recovery day and days 1 and 14 varied.

The results of Skylab 4 studies are shown in table IV through VI.

They show that the only significant change occurred in phosphofructokinase during the early stage of the in-flight samples.

DISCUSSION

The advent of the use of medical hyperoxia and the use of increased oxygen tensions in space capsules prompted the need for further study into changes induced by variable environments and the potential untoward effects on many tissues including red cells. Previous studies in our laboratory had indicated that a mechanism, in fact the only mechanism, responsible for destruction of red cells by hyperoxia was peroxidation of the unsaturated fatty acids in red cell membranes.

TABLE I. SKYLAB 2

Determination †	PREFLIGHT Controls Mean ± 1 SD	PREFLIGHT Astronauts Mean ± 1 SD	IN-FLIGHT* Controls Mean ± 1 SD	IN-FLIGHT* Astronauts Mean ± 1 SD	Sig.	POSTFLIGHT Controls Mean ± 1 SD	POSTFLIGHT Astronauts Mean ± 1 SD	Sig.
GSH (mg %)	195.0 ±30.0	183.0 ±56.0	162.0 ±23.0	169.0 ±24.0		130.0 ± 9.0	148.0 ±19.0	
ATP (μM/g Hb)	5.7 ± 1.3	5.7 ± 0.8	3.7 ± 0.7	4.5 ± 1.7		3.6 ± 0.6	3.5 ± 0.5	
Lipid Peroxides	0	0	0	0		0	0	
2,3-DPG (μM/g Hb)	9.6 ± 2.9	8.1 ± 2.7	9.2 ± 2.7	7.8 ± 3.5		14.4 ± 3.0	14.7 ± 4.6	
G6PD (Eμ/g Hb)	7.0 ± 1.4	7.1 ± 1.6	3.9 ± 1.3	3.9 ± 1.1		4.9 ± 0.7	5.3 ± 0.4	
HK (Eμ/g Hb)	0.50± 0.13	0.46± 0.13	0.65± 0.17	2.4 ± 1.6	$P<0.005$	0.18± 0.09	0.48± 0.4	
PFK (Eμ/g Hb)	26.2 ± 7.5	22.7 ± 5.7	30.4 ± 8.5	28.8 ±11.3		37.0 ± 7.0	25.0 ± 6.0	$P<0.025$
G3PD (Eμ/g Hb)	59.0 ±12.0	65.0 ± 8.0	44.0 ± 8.0	53.0 ± 9.0	$P<0.05$	56.0 ±11.0	53.0 ± 4.0	
PGK (Eμ/g Hb)	28.4 ± 4.0	26.7 ± 8.4	18.5 ± 7.0	20.4 ± 6.3		17.8 ± 3.6	19.5 ± 2.5	
PK (Eμ/g Hb)	12.6 ± 4.0	10.7 ± 5.2	4.0 ± 1.2	5.7 ± 1.4	$P<0.01$	8.6 ± 1.8	7.6 ± 1.8	
AChE (Eμ/g Hb)	62.0 ± 6.0	64.0 ± 8.0	53.0 ± 6.0	55.0 ± 6.0		60.0 ± 6.0	65.0 ± 4.0	

*Data given includes *all* samples. No differences between controls and astronauts were observed at each individual sampling time.

†See pages 3 and 4 for translation of these abbreviations.

TABLE II. SKYLAB 3

Determination*	PREFLIGHT Controls Mean ± 1 SD	PREFLIGHT Astronauts Mean ± 1 SD	IN-FLIGHT Controls Mean ± 1 SD	IN-FLIGHT Astronauts Mean ± 1 SD	Sig
GSH (mg %)	167.0 ±20.0	163.0 ±21.0	119.0 ±74.0	121.0 ±50.0	
ATP (μM/g Hb)	5.9 ± 0.9	5.9 ± 0.5	5.3 ± 0.9	5.9 ± 0.9	
Lipid Peroxides	0	0	0	0	
2,3-DPG (μM/g Hb)	6.1 ± 2.4	7.1 ± 1.3	6.1 ± 3.6	7.9 ± 4.3	
G6PD (*E*μ/g Hb)	9.1 ± 1.0	9.6 ± 1.9	4.7 ± 1.1	4.3 ± 1.3	
HK (*E*μ/g Hb)	0.38± 0.11	0.37± 0.18	0.51± 0.17	0.28± 0.13	$P<0.005$
PFK (*E*μ/g Hb)	25.0 ± 2.0	27.0 ± 1.0	28.1 ± 9.5	28.0 ± 3.7	
G3PD (*E*μ/g Hb)	62.0 ±14.0	61.0 ±14.0	39.0 ± 9.0	42.0 ± 5.0	
PGK (*E*μ/g Hb)	36.0 ± 7.0	33.0 ± 2.0	24.6 ± 5.2	20.1 ± 8.3	$P<0.05$
PK (*E*μ/g Hb)	5.2 ± 1.6	5.9 ± 1.0	5.1 ± 1.6	6.5 ± 2.2	$P<0.05$
AChE (*E*μ/g Hb)	43.0 ± 4.0	45.0 ± 7.0	34.0 ±10.0	27.0 ± 7.0	$P<0.05$

*See pages 3 and 4 for translation of these abbreviations.

TABLE III. SKYLAB 3

POST FLIGHT (DAY)

	R + 0			R + 1			R + 14			R + 0, R + 1, R + 14		
Determination*	Controls	Astronauts	Sig.	Controls	Astronauts	Sig.	Controls	Astronauts	Sig.	Controls	Astronauts	Sig.
GSH (mg %)	70.0 ± 6.0	37.0 ±13.0	$P<0.01$	104.0 ± 3.0	64.0 ±10.0	$P<0.01$	181.0 ±56.0	183.0 ±12.0		118.0 ±56.0	94.0 ±64.0	
ATP (μM/g Hb)	4.3 ± 0.8	6.1 ± 0.4	$P<0.01$	4.3 ± 6.0	5.4 ± 0.3	$P<0.025$	7.0 ± 0.7	9.8 ± 1.4	$P<0.025$	5.21± 1.5	6.6 ± 1.9	
Lipid Peroxides	0	0		0	0		0	0		0	0	
2,3-DPG (μM/g Hb)	5.6 ± 1.9	6.5 ± 1.8		8.3 ± 4.0	9.1 ± 1.4		4.5 ± 2.0	4.3 ± 1.7		6.1 ± 3.1	6.8 ± 2.2	
G6PD (Eμ/g Hb)	6.0 ± 1.8	6.1 ± 0.6		5.3 ± 1.2	5.5 ± 1.5		5.8 ± 0.5	7.8 ± 0.2	$P<0.01$	5.7 ± 1.2	6.5 ± 1.3	
HK (Eμ/g Hb)	0.49± 0.13	0.67 ± 0.31		0.48± 0.11	0.50± 0.08		0.38± 0.09	0.39± 0.03		0.45± 0.12	0.52± 0.22	
PFK (Eμ/g Hb)	35.0 ±13.0	29.0 ± 4.0		27.0 ± 7.0	27.0 ± 6.0		28.0 ± 2.0	32.0 ± 4.0		30.0 ±10.0	29.0 ± 5.0	
G3PD (Eμ/g Hb)	63.0 ± 4.0	52.0 ± 6.0	$P<0.05$	69.0 ±20.0	54.0 ± 6.0		45.0 ± 1.0	43.0 ± 5.0		59.0 ±16.0	50.0 ± 7.0	
PGK (Eμ/g Hb)	27.0 ± 1.0	19.0 ± 4.0	$P<0.01$	28.0 ± 3.0	28.0 ± 5.0		21.0 ± 3.0	27.0 ± 2.0	$P<0.025$	25.0 ± 4.0	24.0 ± 6.0	
PK (Eμ/g Hb)	6.1 ± 0.8	7.8 ± 2.0		5.5 ± 0.7	7.8 ± 1.2	$P<0.025$	17.0 ± 2.0	18.0 ± 5.0		9.6 ± 5.0	11.4 ± 6.0	
AChE (Eμ/g Hb)	33.0 ± 2.0	29.0 ± 4.0		29.0 ± 3.0	35.0 ± 8.0		29.0 ± 3.0	26.0 ± 3.0		30.0 ± 3.0	30.0 ± 6.0	

*See pages 3 and 4 for translation of these abbreviations.

TABLE IV. SKYLAB 4

PREFLIGHT

Determination*	Controls Mean ± 1 SD	Astronauts Mean ± 1 SD	Sig.
Met Hgb (%)	0.90± 1.08	4.22± 3.67	$P<0.005$
GSH (mg %)	139.55±28.19	128.37±27.90	
ATP (μM/g Hb)	7.11± 0.54	8.29± 1.89	
Lipid Peroxides	0	0	
2,3-DPG (μM/g Hb)	9.24± 3.09	9.18± 4.99	
G6PD ($E\mu$/g Hb)	7.29± 1.12	6.84± 1.32	
HK ($E\mu$/g Hb)	0.72± 0.10	0.73± 0.11	
PFK ($E\mu$/g Hb)	36.04± 9.36	33.80± 9.56	
G3PD ($E\mu$/g Hb)	54.57± 8.64	56.45±10.79	
PGK ($E\mu$/g Hb)	29.74± 6.17	27.76± 3.76	
PK ($E\mu$/g Hb)	10.25± 4.24	10.06± 2.57	
AChE ($E\mu$/g Hb)	54.68±10.89	60.40±10.04	
ATPase	11.17± 1.36	10.35± 0.79	

*See pages 3 and 4 for translation of theses abbreviations.

TABLE V. SKYLAB 4

Determination*	(INF 1-4) IN-FLIGHT Controls Mean ± 1 SD	Astronauts Mean ± 1 SD	Sig.	(INF 5-8) Controls Mean ± 1 SD	Astronauts Mean ± 1 SD	Sig.
Met Hgb (%)	28.45± 5.92	27.07± 6.13		20.54± 8.20	28.71± 3.16	$P<0.005$
GSH (mg %)	107.77±38.99	88.33±28.08		110.09±38.92	100.86±25.60	
ATP (μM/g Hb)	8.34± 1.83	7.64± 1.48		6.69± 2.13	6.52± 2.39	
Lipid Peroxides	0	0		0	0	
2,3-DPG (μM/g Hb)	3.47± 1.68	3.35± 0.89		4.15± 1.21	4.22± 1.66	
G6PD (Eμ/g Hb)	6.42± 1.62	4.80± 1.29	$P<0.025$	4.92± 1.12	4.77± 0.87	
HK (Eμ/g Hb)	0.22± 0.10	0.20± 0.09		0.39± 0.09	0.38± 0.07	
PFK (Eμ/g Hb)	35.03± 7.75	25.89± 6.68	$P<0.01$	26.30± 9.73	24.92± 4.58	
G3PD (Eμ/g Hb)	55.25± 9.31	49.85± 7.02		63.79±17.86	58.32±17.15	
PGK (Eμ/g Hb)	14.90± 4.10	15.47± 5.52		18.87± 5.58	18.22± 7.79	
PK (Eμ/g Hb)	8.92± 3.63	7.76± 2.67		5.08± 1.20	4.87± 1.00	
AChE (Eμ/g Hb)	55.62± 8.67	51.06± 9.45		50.48± 7.37	43.73± 7.86	

*See pages 3 and 4 for translation of these abbreviations.

TABLE VI. SKYLAB 4

POST FLIGHT (DAY)

	R + 0			R + 1			R + 14			R + 0, R + 1, R + 14		
Determination*	Controls	Astronauts	Sig.	Controls	Astronauts	Sig.	Controls	Astronauts	Sig.	Controls	Astronauts	Sig.
Met Hgb (%)	2.96± 2.58	9.33± 5.62		7.05± 4.61	8.04± 4.68		11.35± 3.93	13.39± 3.74		7.12± 5.11	10.25± 5.26	
GSH (mg %)	75.33±17.20	71.42± 6.87		70.16±11.44	56.51±10.12		78.58±12.11	90.96± 2.02		74.69±14.25	72.96±15.82	
ATP (μM/g Hb)	6.60± 0.36	7.68± 1.00		5.25± 0.22	5.30± 0.14		4.47± 0.00	5.53± 0.37		5.53± 0.81	6.17± 1.23	
Lipid Peroxides	0	0		0	0		0	0		0	0	
2,3-DPG (μM/g Hb)	5.17± 1.00	8.86± 2.49		3.14± 0.10	4.27± 1.45		2.31± 0.32	2.57± 0.46		3.54± 1.35	5.23± 3.14	
G6PD (Eμ/g Hb)	7.46± 1.60	8.85± 1.89		3.88± 0.40	4.81± 1.40		4.21± 0.60	4.27± 0.97		5.18± 1.90	5.97± 2.51	
HK (Eμ/g Hb)	0.61± 0.20	0.49± 0.00		0.21± 0.00	0.19± 0.00		0.19± 0.00	0.27± 0.00		0.34± 0.22	0.32± 0.10	
PFK (Eμ/g Hb)	38.26± 2.41	35.64± 7.05		19.38± 5.10	16.40± 8.55		19.31±10.01	18.26± 4.38		25.65±11.11	23.43±11.07	
G3PD (Eμ/g Hb)	79.14± 9.64	82.43± 4.68		39.27± 5.49	39.94± 7.13		46.04± 4.52	42.19± 5.78		54.81±18.74	54.85±20.40	
PGK (Eμ/g Hb)	25.21± 7.63	24.56± 7.37		9.80± 1.12	11.13± 2.91		9.23± 0.83	11.76± 2.36		14.75± 8.65	15.81± 7.81	
PK (Eμ/g Hb)	12.81± 3.10	14.01± 0.55		4.54± 1.46	4.66± 1.48		3.81± 0.30	6.14± 1.13	$P<0.05$	7.08± 4.52	8.27± 4.25	
AChE (Eμ/g Hb)	72.22± 8.48	60.60± 5.41		28.93± 1.01	29.55± 3.83		32.03± 2.65	35.64± 3.16		44.39±20.38	41.93±14.08	

*See pages 3 and 4 for translation of these abbreviations.

In addition to these changes, other studies also demonstrated alterations of glycolytic intermediates and enzymes which, however, could not be linked to concrete evidence of cell damage.

Previous space flight studies were limited by the fact that samples could only be obtained before and after flight, and frequently inappropriate controls existed. The major contribution allowed by the Skylab series of studies was the availability of simultaneous control samples as well as in-flight samples from astronauts. It should be noted, however, that there were progressive gas composition changes as the varied series of Gemini, Apollo, and Skylab flights occurred.

In our present studies, there was no evidence of lipid peroxidation in any of the samples. This may be taken as evidence that the likelihood of overt red cell damage would be slim. There were, however, certain changes observed in glycolytic intermediates and enzymes. For perspective, these may be summarized in Table VII. Included in this table are summary data from the Skylab Medical Experiments Altitude Test (SMEAT) and our own laboratory (OHP) studies using oxygen under pressure. It is apparent that the most consistent change noted, a decrease of phosphofructokinase, had been verified a number of times. It is this enzyme step which is thought to be at the center of the so-called Pasteur effect, and which is susceptible to the effects of oxygen. Other changes have been less consistent and the significance of all of these changes is not understood.

SUMMARY

In summary therefore, it is possible to conclude that there are no evidences of lipid peroxidation, that biochemical effect known to be associated with irreversible red cell damage, and the changes observed in glycolytic intermediates and enzymes cannot be directly implicated as indicating evidence of red cell damage.

TABLE VII. SUMMARY OF RBC METABOLISM CHANGES

DETERMINATIONS*	OHP STUDIES	SMEAT	SKYLAB 2	SKYLAB 3	SKYLAB 4
GSH		+		+	
ATP	+			+	
Lipid Peroxides					
2,3-DPG	+				
G6PD		+		+	
HK			+	+	
PFK	+	+	+	+	
G3PD			+	+	
PGK		+		+	
PK			+	+	
AChE		+			

*See pages 3 and 4 for translation of these abbreviations.

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