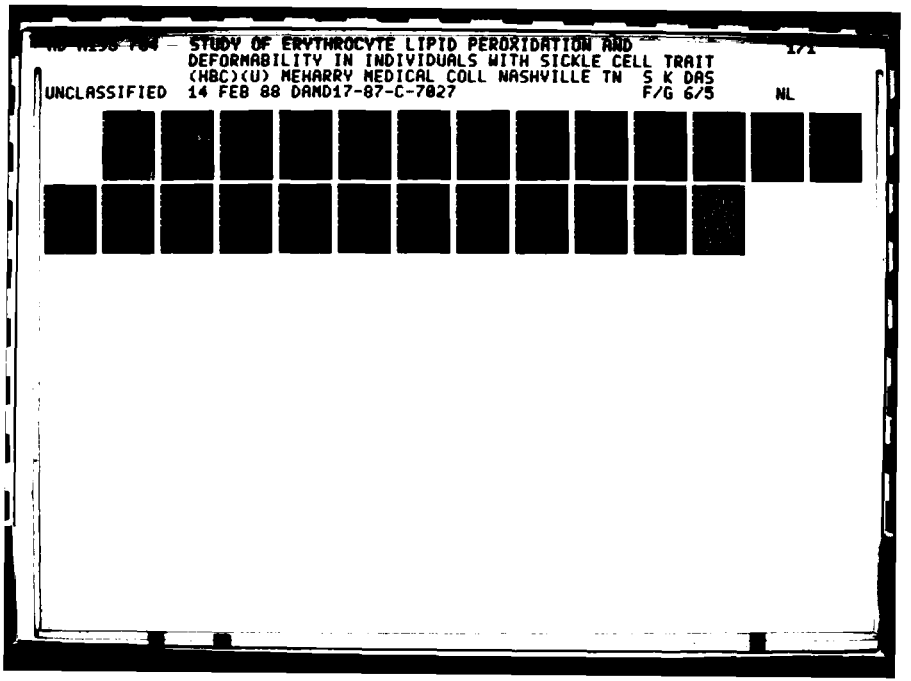
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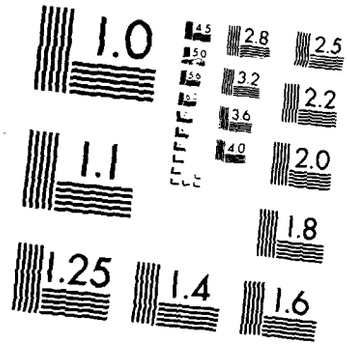
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STUDY OF ERYTHROCYTE LIPID PEROXIDATION AND DEFORMABILITY IN INDIVIDUALS WITH SICKLE CELL TRAIT (HBC)

ANNUAL REPORT

Salil K. Das

February 14, 1988

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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Tread mill exercise causes an increase in the activity of SOD, GSHPx and 6-PGA dehydrogenase without any change in the activity of catalase and G-6-P dehydrogenase in SCT RBC. However, it did not affect the activity of any of the peroxide scavengers, but increased the activity of both NADPH generating enzymes in normal. Exercise did not have any significant effect on lipid peroxidation potential of either normal or SCT RBC. However, it caused an increase in the Ca^{++} content in SCT which was associated with an increase in the activity of Na^{+}, K^{+} - Ca^{++}-ATPases of RBC membranes. Furthermore, exercise caused an increase in the amount of light membranes in SCT RBC.

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SUMMARY

Adverse effects of physical stress in SCT individuals may be associated with biochemical changes in RBC. In this study, we have monitored the effects of tread mill exercise on several biochemical parameters of RBC in 18-40 years old normal and SCT male subjects. Basal activities of catalase and G-6-P dehydrogenase were similar in normal and SCT; however the basal activities of SOD, GSHPx and 6-PGA dehydrogenase were lower in SCT than normal. Exercise caused an increase in the activity of SOD, GSHPx and 6-PGA dehydrogenase without any change in the activity of catalase and G-6-P dehydrogenase in SCT. On the other hand, exercise did not affect the activity of any of the peroxide scavengers, but increased the activity of both NADPH generating enzymes in normal. Even though exercise increased the activity of some of the peroxide scavengers in SCT, this increase was not sufficient to bring the values to the basal levels of the control.Exercise did not have any significant effect on the lipid peroxidation potential of either normal or SCT RBC. However,it caused an increase in the Ca^{++} content in SCT which was associated with an increase in the activity of Na^{+},K^{+}, and Ca^{++} ATPases of RBC membranes. Not only did physical stress increase the density of cell membranes as indicated by an increase in the amount of heavy membranes and a corresponding decrease in the amount of light membranes, but it also brought about changes in the activity of some important enzymes.

TABLE OF CONTENTS

LIST OF TABLES

BODY OF THE REPORT

1. INTRODUCTION

Controversy exists concerning the risks assumed by individuals with sickle cell trait (SCT) while engaged in military activities that involve exposure to hypoxic environments and other stress situations. Some claim that individuals with SCT are relatively asymptomatic, have normal life span (1,2) and their responses to exercise are not impaired (2-4). However, several other reports indicate that physical stress is hazardous to individuals with SCT and cause several problems, such as renal failure (5,6), splenic infarction (7-10), intravascular coagulation (5,6), rhabdomyolysis (5,6,11), sicklemia (12), lowering of exercise values for heart rate and work load (13) and sudden death (14- 17).

It is known that high morbidity and mortality in individuals with sickle cell disease are associated with red blood cell abnormality in terms of hemoglobin polymerization and membrane property changes (18). Furthermore, we have earlier reported that the sickled erythrocyte membrane is more susceptible to peroxidative damage due to some intracellular protective defect (19). It is therefore important that we know whether the adverse effects of physical activity on SCT individuals is related to RBC membrane changes.

2. OBJECTIVE

The long range objective of this project is to test the HYPOTHESIS that individuals with SCT undergo some changes in specific clinical, biochemical and physical properties of their red blood cells under conditions of (a) physical stress and/or (b) deoxygenation.

3. METHODOLOGY

18-40 yrs old male subjects with or without SCT are subjected to continuous graded exercise on a Tread Mill according to Bruce protocol, with electrocardiographic monitoring, followed by 15 minutes of rest. Blood is drawn before exercise, immediately after exercise, and after 15 minutes of rest, for analysis of clinical , biochemicals and physical parameters. During the first year of the project, we have subjected 8 normal and 5 sickle cell trait subjects to continuous graded exercise on the Tread Mill. Furthermore, we have used blood from 6 AA and 3 SS male subjects for standardization of several experimental conditions. Data are treated statistically and

the differences between AA and SCT groups are determined by calculating the t statistic for two means, i.e., the nonpaired t-test (20). It should be pointed out that additional data will be collected on both AA and SCT subjects during the second year of the project. Therefore, we are reporting here only the mean data for both groups. Statistical interpretation will be made after collecting additional data.

For clinical parameters, we record CBC and blood gas profile as well as cardiac function (EKG, blood pressure, heart rate). For biochemical parameters, we record (a) the protein content of RBC and ghosts, (b) the peroxide scavenger status of RBC by measuring the activity of SOD, GSHPx and catalase, (c) the activity of NADPH generating enzymes, such as G-6-P and 6-PGA dehydrogenase, (d) the activity of Na^+,K^+, and Ca^{++} - ATPases, (e) the intracellular and membrane Ca concentration, and (f) the peroxidation potential of RBC lipids. For physical parameters, we record the filtrability and deformability of RBC under oxygenated and deoxygenated conditions. These parameters are recorded by following the procedures referred in Table 1 (see Appendix).

In this study, we prepare two types of RBC membranes, differing in density and they are referred as heavy and light. Since the preparation of membrane is not parallel to current scientific technique, a schematic representation of the membrane preparation is shown in Fig. 1 (see Appendix).

4. SIGNIFICANT FINDINGS

A. Effect of Physical Stress on Complete Blood Cell Count and Blood Gas Profile of Normal and Sickle Cell Trait Individuals (see Table 2).

1. The WBC count is increased during exercise and returns to the basal level after resting in both normal and SCT. It is noteworthy that the basal WBC count is less in SCT than normal.

2. % O_2Hb is less in SCT than normal. Exercise increases the level in both. However, during resting, it is brought back to the basal level in normal, but not in SCT.

3. % CO Hb level in normal is decreased remarkably during exercise and comes back to the basal level

after resting. However, in SCT, there is no remarkable change due to exercise. The basal level is less in SCT than that in normal.

4. % met Hb value is decreased in normal, but increased in SCT during exercise and resting. The basal level is less in SCT than normal.

5. The basal value of O_2 ct is same in AA and SCT. Exercise causes an increase in both. After resting, the value returns to the basal level in AA, but not in SCT.

6. pH becomes slightly acidic during exercise, but returns to the basal level during resting in both cases.

7. pO_2 value is lower in SCT than normal. The value is increased during exercise in both, returning to the basal level in normal after resting. However, in SCT, the value remained increased during resting.

8. The value for BE (base excess) is decreased from a positive to a negative number in AA due to exercise. After resting, this value returns to the basal level. In SCT, the value is also decreased during exercise, but the change is minimal in comparison to that in AA.

9. Other parameters (RBC count, Hb, Hct %, mcv, pCO_2, HCO_3^- and CO_2 ct) did not change by exercise in either normal or SCT.

B. Effect of Physical Stress on Protein Content of RBC and Ghost of Normal and SCT Individuals (see Table 3).

1. There is no remarkable change in the protein content of RBC during exercise in either AA or SCT.

2. 3 K pellet from SCT contains more protein than that of AA during exercise and resting, suggesting that exercise causes some change in SCT RBC whereby it becomes more resistant to osmotic shock.

3. Protein content is increased in heavy membrane but decreased in light membrane in SCT during exercise. No such change occurs in AA. It indicates that RBC membrane becomes more rigid in SCT due to exercise.

4. Light membrane has mor proteins than heavy membranes in both groups.

C. Effect of Physical Stress on Peroxide Scavengers of RBC and Ghost of Normal and Sickle Cell Trait Individuals (see Table 4).

1. SCT RBC hemolyzate has less superoxide dismutase (SOD) than normal. SOD activity is continually increased in SCT during exercise and resting; however the level is still lower than the basal level of AA. Exercise does not have any remarkable effect on AA RBC.

2. SCT membranes (both light and heavy) have lower SOD level than normal. Exercise does not have any remarkable effect on either AA or SCT.

3. RBC hemolyzate and membranes (both light and heavy) have lower GSHPx activity in SCT than normal. Exercise increases the activity in RBC of both normal and SCT. Between peak exercise and resting, the value decreases in normal, but continually increases in SCT.

4. There is no remarkable effect of exercise on catalase activity in either normal or SCT RBC. However, in heavy membrane of SCT, there is an increase in catalase activity between peak exercise and resting.

D. Effect of Physical Stress on Na^+, K^+, and Ca^{++} -ATPase Activities in Erythrocyte Membranes of Normal and Sickle Cell Trait Individuals (see Table 5).

1. Both heavy and light membranes contain less Na^+, K^+-ATPase activitiy in SCT than AA. During exercise, the activity decreases to some extent in AA. But in SCT, the value is increased remarkably beyond the basal level of AA.

3. No significant difference is observed in Ca^{++} -ATPase activity in light membrane between normal and SCT. The activity is increased during exercise and resting in SCT but not in AA.

4. Heavy membrane has higher Ca^{++} -ATPase activity in SCT than normal. The activity in heavy membrane is not affected by exercise in AA, but is decreased in SCT. Between peak exercise and resting, the activity in SCT is increased remarkably.

E. Effect of Physical Stress on G-6-P and 6-PGA Dehydrogenase Activity in Normal and Sickle Cell Trait Individuals (see Table 6).

1. There is no remarkable difference in the basal G-6-P dehydrogenase activity between AA and SCT. However, the basal 6-PGA dehydrogenase activity is lower in SCT than AA. Exercise causes an increase in the activity of both enzymes in AA, whereas it causes a decrease in SCT. However, between peak exercise and resting, the activity in SCT is increased, whereas in AA, it is decreased.

F. Effect of Physical Stress on Intracellular and Membrane Ca^{++} Ion Concentration of RBC of Normal and SCT Individuals (see Table 7).

1. There is no significant change in normal intracellular RBC, 3 K pellet and heavy membrane Ca^{++} ion concentration during exercise and resting. However, in normal light membrane, Ca^{++} ion concentration is decreased during exercise.

2. SCT has higher intracellular and membrane Ca^{++} ion concentration than normal. During exercise, the value is increased in all fractions except in light membrane in which a decrease is observed. After resting, the calcium level is returned to the basal level in all fractions.

G. Effect of Physical Stress on In Vitro Lipid Peroxidation of RBC of Normal and SCT Individuals (see Table 8).

1. RBC has higher lipid peroxidation potential in SCT than AA.

2. Exercise causes an increase in the MDA production in AA, but not in SCT.

3. The endogeneous level of MDA is higher in SCT than AA.

H. Effect of Physical Stress on Deformability and Calcium Accumulation of RBC of Normal and SCT Individuals.

Currently, we are standardizing experimental conditions for filtering RBC drawn from normal, SCT and SS subjects through a 5 μ nucleopore membrane filter under both oxygenated and deoxygenated conditions, to monitor the rise in pO_2 by a Grass Polygraph Recorder through a statham P-23 XL Pressure Transducer.

5. CONCLUSIONS

Physical stress causes an increase in the density of RBC membrane of SCT subjects as evident by an increase in the amount of heavy membrane and a corresponding decrease in the amount of light membrane. This observation needs to be confirmed by additional data which will be gathered from the second year of the project. After establishing this important finding, we will proceed to study the mechanism of this event. In order to achieve this goal, we would like to propose the following future studies (January 1989 - December 1991).

Aim = 1. Whether any alteration of lipid/protein ratio due to physical stress is the cause for a change in the RBC membrane density in SCT ?

Aim = 2. Whether any alteration of membrane fluidity due to physical stress is associated with RBC membrane density in SCT ?

Aim = 3. Whether the heavy membrane comes from the heavy cells (high density) and the light membrane comes from the light cells (low density). Does exercise cause part of the light cells to be converted to heavy cells. If so, is this event related to the accumulation of calcium in RBC ?

Aim = 4. Is their any relationship between loss of deformability and increase in RBC membrane density due to physical stress in SCT ?

Aim = 5. Is their any association between calcium accumulation in RBC and loss of filtrability due to exercise in SCT ?

Aim = 6. Is lectin binding property of RBC membrane protein altered due to physical stress in SCT ?

Aim = 7. Is there any ultrastructural change in RBC membrane due to physical stress in SCT ?

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TABLE 1

ASSAY SYSTEMS

Protein	Lowry et al., J. Biol. Chem. 193 : 265, 1951
Superoxide Dismutase	Hyland et al., Anal. Biochem. 135: 280, 1983
Glutathione Peroxidase	Flohe and Gunzler, Methods in Enzymol. 105 : 114, 1984
Catalase	Sinha, Anal. Biochem. 47: 389, 1972
6-6-PD and 6-PGD	Beutler, in Methods in Hematol: Red Cell Metab. 16 : 57, 1986
Na^+, K^+, and Ca^{++}- ATPases	Sen & Ray, Arch. Biochem. Biophys. 198 : 548, 1979
Calcium	Olson, J. Membrane Biol. 48: 265, 1979
Peroxidation Potential	Stocks & Dormandy, Br. J. Haematol. 20 : 95, 1971

TABLE 2

Effect of Physical Stress on Complete Blood Count (CBC) and Blood Gas Profile of Normal and Sickle Cell Trait Individuals

Parameter	Before Exercise		At Peak Exercise		After Exercise	
	Normal	Sickle	Normal	Sickle	Normal	Sickle
RBC ($\times 10^9$/ml)	5.06	5.40	5.14	5.61	4.83	5.57
WBC ($\times 10^9$/ml)	5.34	4.05	7.22	5.10	5.38	4.70
Hgb (g/dl)	15.27	15.50	15.97	16.18	14.86	15.57
Hct (%)	44.83	45.30	46.14	47.25	43.73	45.60
mcv (fl)	89.83	84.00	90.25	84.50	90.50	84.50
% O_2 Hb	57.67	44.00	80.58	59.30	55.83	70.25
% CO Hb	1.70	0.80	0.23	0.85	1.43	0.95
%met Hb	0.28	0.11	0.23	0.15	0.22	0.35
O_2 ct	9.81	9.20	16.67	13.05	12.05	14.85
p H	7.336	7.320	7.200	7.284	7.285	7.354
p CO_2(mmHg)	53.38	54.35	52.02	52.30	47.83	55.05
p O_2 (mmHg)	42.89	29.05	52.77	37.50	35.15	43.35
BE (mEQ/L)	+ 7.84	+ 7.35	- 3.09	+ 2.40	+ 4.80	+ 4.55
HCO_3^-(mEQ/L)	27.58	27.25	20.03	23.70	22.80	24.35
CO_2ct (mEQ/L)	29.21	28.95	21.45	25.30	24.53	25.70

TABLE 3

Effect of Physical Stress on Protein Content of RBC and Ghost of Normal and Sickle Cell Trait Individuals

Sample	Before Exercise		AT Peak Exercise		After Exercise	
	Normal	Sickle	Normal	Sickle	Normal	Sickle
RBC Hemolysate	43.52*	38.48	41.71	34.41	43.02	44.13
Unhemolyzed cells (3k pellet)	0.22	0.25	0.18	0.42	0.21	0.42
Heavy Membrane	0.45	0.51	0.41	0.87	0.45	0.62
Light Membrane	0.80	0.92	0.89	0.67	0.84	0.64

* Values are mean pg/cell

TABLE 4

Effect of Physical Stress on Peroxide Scavengers of RBC and Ghost of Normal and Sickle Cell Trait Individuals

Sample	Before Exercise		At Peak Exercise		After Exercise	
	Normal	Sickle	Normal	Sickle	Normal	Sickle
A. Superoxide Dismutase (unit $x10^{-9}$/ cell)						
a.Hemolysate	60.96	16.87	62.48	22.70	60.42	44.28
b.Heavy Mem -brane	2.79	1.19	3.18	1.99	3.96	2.10
c.Light Mem -brane	5.06	1.34	4.81	1.57	5.91	1.66
B.Glutathione Peroxidase (μ moles x 10^{-10} NADPH oxidized /cell /min)						
a.Hemolysate	30.89	18.15	45.44	19.01	38.42	28.75
b.Heavy Mem -brane	0.60	0.19	0.31	0.95	0.51	0.50
c.Light Mem -brane	0.63	0.35	0.48	0.21	0.40	0.67
C.Catalase (μ moles x 10^{-7} of H_2O_2 decomposed / cell /min)						
a.Hemolysate	26.38	23.97	28.31	24.49	23.34	25.69
b.Heavy Mem -brane	0.12	0.11	0.17	0.27	0.16	0.41
c.Light Mem -brane	0.44	0.57	0.41	0.40	0.48	0.41

TABLE 5

Effect of Physical Stress on Na^+, K^+- and Ca^{2+} -ATPase Activities in Erythrocyte Membranes of Normal and Sickle Cell Trait Individuals

Sample	Before Exercise		At Peak Exercise		After Exercise	
	Normal	Sickle	Normal	Sickle	Normal	Sickle
A. Na^+ , K^+ - ATPase						
Heavy Membrane	44.61*	52.86	34.79	74.81	39.51	66.99
Light Membrane	100.57	50.03	75.51	177.71	85.05	142.94
B. Ca^{2+}- ATPase						
Heavy Membrane	70.51	100.71	69.47	60.27	53.49	162.94
Light Membrane	71.03	71.73	49.82	130.47	64.46	172.75

* Values are mean n moles $x10^{-9}$ P_i formed /cell /hr.

TABLE 6

Effect of Physical Stress on Glucose-6-Phosphate and 6-Phosphogluconate Dehydrogenase Activity in Normal and Sickle Cell Trait Individuals

Sample	Before EXercise		At Peak Exercise		After Exercise	
	Normal	Sickle	Normal	Sickle	Normal	Sickle
A. Glucose-6-PO_4 Dehydrogenase						
a. Hemolysate	103.22*	98.81	184.01	49.63	118.40	77.67
b. Heavy Mem -brane	3.33	4.66	1.39	1.69	2.41	0.92
c. Light Mem -brane	3.84	9.40	20.67	1.29	4.80	2.30
B. 6- Phosphogluconate Dehydrogenase						
a. Hemolysate	82.90	37.58	204.62	35.80	125.40	86.34
b.Heavy Mem -brane	1.14	0.86	1.04	0.85	1.08	1.10
c. Light Mem -brane	4.00	0.78	24.10	1.19	6.30	1.72

* Value are mean μ moles x 10^{-9} NADP reduced /cell /min.

TABLE 7

The Effect of Physical Stress on Intracellular and Membrane Ca^{2+}-ion concentration of RBC of Normal and Sickle Cell Trait Individuals

Sample	Before Exercise		At Peak Exercise		After Exercise	
	Normal	Sickle	Normal	Sickle	Normal	Sickle
a. RBC	34.10 *	68.30	41.60	100.0	35.20	51.60
b. Unhemolyzed cell (3K pellet)	2.30	6.50	3.20	15.5	2.50	7.30
c. Heavy Membrane	8.60	9.40	9.85	18.3	8.90	10.10
d. Light Membrane	16.34	24.70	8.80	17.6	10.2	25.10

* Values are mean f moles x 10^{-9} / cell

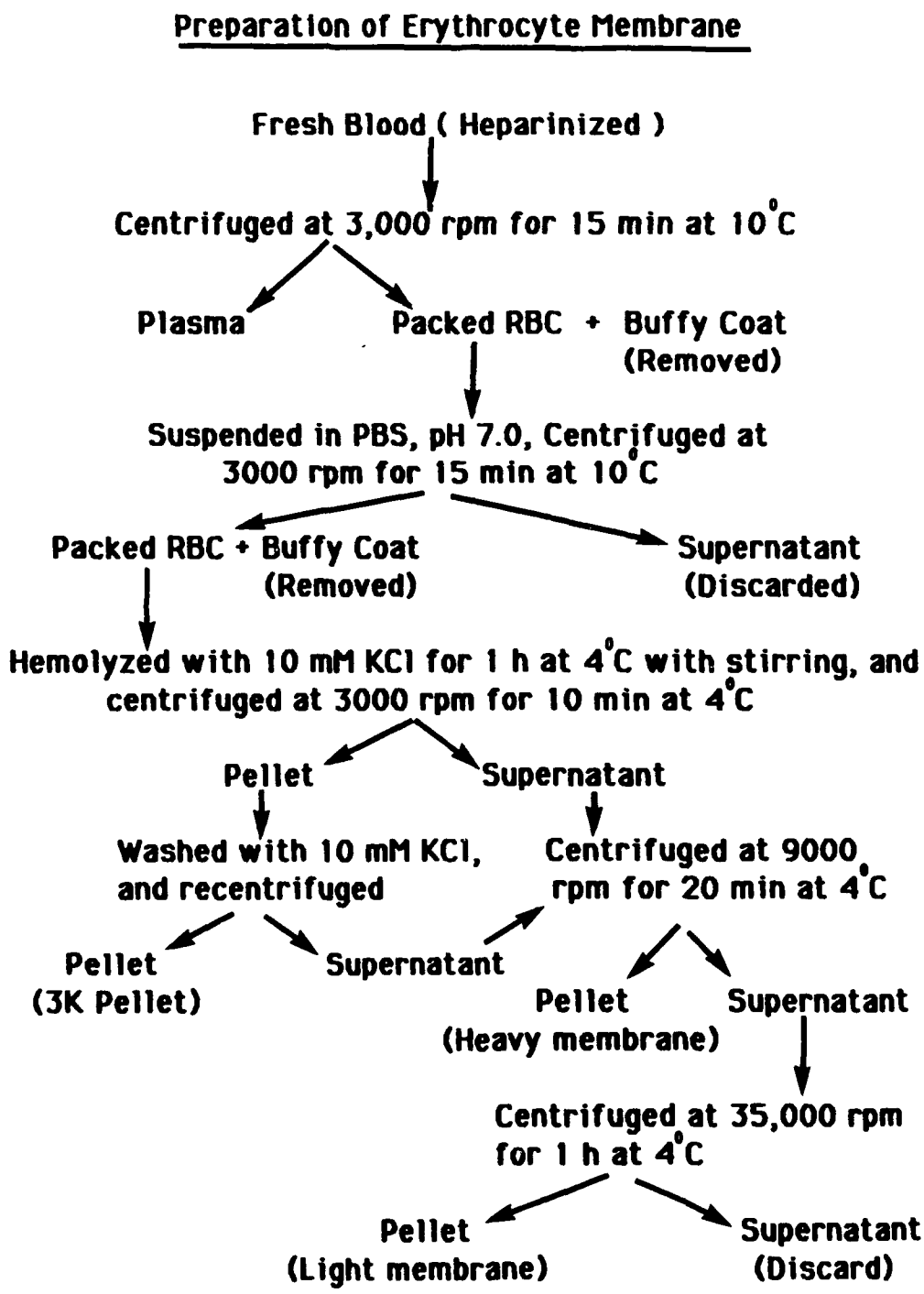
TABLE 8

Effect of Physical Stress on *in vitro* Lipid Peroxidation of RBC Of Normal and Sickle Cell Trait Individuals

Sample	Before Exercise		At Peak Exercise		After Exercise	
	Normal	Sickle	Normal	Sickle	Normal	Sickle
a. RBC	5.66 *	8.65	7.62	11.24	9.76	9.98
b. RBC + H_2O_2	16.56	26.53	20.07	23.02	27.18	25.59
c. RBC + H_2O_2 + Na-azide	267.28	52.94	238.93	49.04	207.44	50.66

* Values are mean n moles $x10^{-9}$ MDA formed /cell
N.D = Not detected.

FIG. 1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Plasma
Packed RBC + Buffy Coat (Removed)
Suspended in PBS, pH 7.0, Centrifuged at 3000 rpm for 15 min at 10°C
Packed RBC + Buffy Coat (Removed)
Supernatant (Discarded)
Hemolyzed with 10 mM KCl for 1 h at 4°C with stirring, and centrifuged at 3000 rpm for 10 min at 4°C
Pellet
Supernatant
Washed with 10 mM KCl, and recentrifuged
Centrifuged at 9000 rpm for 20 min at 4°C
Pellet (3K Pellet)
Supernatant
Pellet (Heavy membrane)
Supernatant
Centrifuged at 35,000 rpm for 1 h at 4°C
Pellet (Light membrane)
Supernatant (Discard)

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