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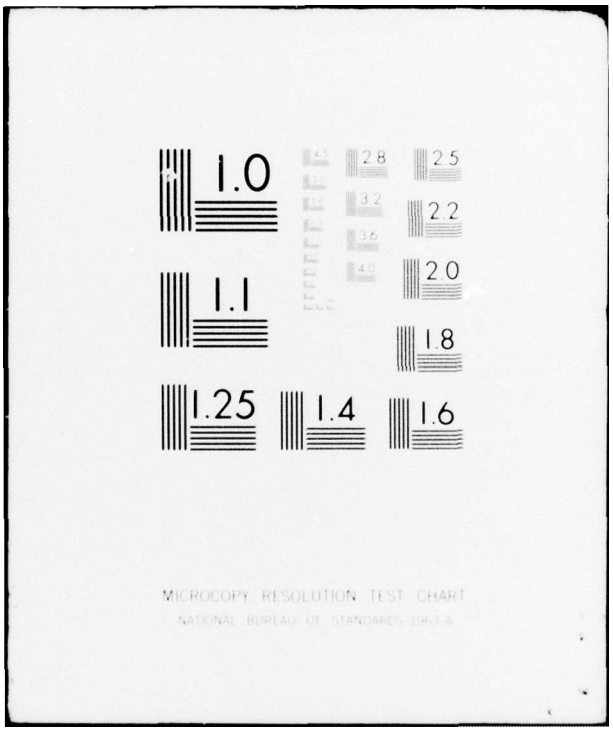
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TECHNICAL REPORT NO. 5

Stored Blood Function

by

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PROGRESS REPORT

1. OBJECTIVES

The overall objective of this research project is to elucidate the physiology and pathophysiology of the retinal circulation in isolated bovine eyes perfused with fresh and stored cow blood collected in citrate phosphate dextrose (CPD). Techniques have been developed to perfuse isolated bovine eyes with whole blood at physiological flow rates and controlled values of pO_2, pCO_2 and pH for extended periods of time.

The specific objectives of this project are (1) to study the effects of variation in red cell affinity for oxygen (RCA) on the retinal function by using electroretinographic (ERG) techniques, and (2) to examine the effects of thrombocytopenic blood on retinal function and on the ultrastructure of the retinal vessels.

2. GOALS FOR 1976

Our goal for this year was (1) to evaluate the integrity of the retina, by using ERG techniques, in isolated eyes perfused with fresh and stored blood, and to study the effects of variations in blood pO_2, pCO_2, pH and RCA on the ERG responses.

(2) To determine the role of the platelets in maintaining the integrity of the retinal microvasculature and retinal function.

3. STUDIES CONDUCTED AND RESULTS OBTAINED.

A. ERG studies

The techniques used to perfuse the isolated eye and to control pO_2, pCO_2, and pH in fresh blood and in stored blood are described in Technical Reports #1, #2, and #3. The experiments were conducted according to the protocol described in Technical Report #4.

Upon arrival in the laboratory, the perfused isolated eyes were prepared for ERG recordings. Each eye was placed into a shielded metal box, specially designed for dark adaptation.

Recording System

Two electrodes were placed on the eye: the active electrode on the cornea and the reference electrode on the sclera near the equator. Wick electrodes made with short lengths of saline-soaked cotton thread were unsatisfactory because they tended to dry out during extended recording procedures. Silver electrodes were specially designed to fit the corneas of the bovine eyes and insure a good scleral contact. These electrodes were chlorided to reduce polarization problems.

Both electrodes were connected to a preamplifier (Grass, P 15) which in turn was connected to an oscilloscope (Tektronix, 5111) and a chart recorder (Hewlett Packard, 7402A) for visualization and recording of the ERG responses.

Light stimulation

A photo stimulator (Grass, PS 22) was installed in front of the cornea, and after adequate dark adaptation the eye was illuminated for 10 μsecond durations at intervals ranging from 10 seconds to 20 minutes. Five different steps of flash intensity with ratios of 1,2,4,8, and 16 were used. The maximum flash intensity was 1,500,000 candlepower.

Blood conditions

During these ERG recordings the eyes were perfused with fresh or stored blood in which pO_2, pCO_2, and pH were maintained at specific levels within the ranges of $\pm$ 10 mm Hg for pO_2, $\pm$ 5 mm Hg for pCO_2, and $\pm$ 0.025 unit for pH. The values of pO_2, pCO_2, and pH are reported in Table I.

TABLE I

pO_2 mm Hg	pCO_2 mm Hg	pH units	Age of blood
39	87	7.01	fresh
32	66	7.07	fresh
80	47	7.09	1 day
74	42	7.10	1 day
75	43	6.97	1 day
86	44	7.16	1 day
59 - 60	46 - 51	7.07 - 7.10	1 day
56 - 61	56 - 57	7.07 - 7.12	1 day
66 - 69	55 - 57	7.10 - 7.14	1 day
45 - 49	56 - 62	7.12 - 7.14	1 day
50 - 52	59 - 61	7.08 - 7.09	1 day
61	54	7.13	1 day
61	57	7.05	3 day
59	92	6.69	20 day
61 - 62	102 - 104	6.69 - 6.70	22 day
160 - 178	28 - 30	7.30 - 7.32	1 day
150 - 158	25 - 32	7.24	1 day
170	47	7.06	1 day
150	23	7.37	1 day
156 - 170	30 - 33	7.20 - 7.22	3 day
125 - 134	40 - 42	7.05 - 7.06	7 day
150	28	7.05	8 day

ERG in a living calf

We recorded ERG in a living calf using our recording system (silver electrodes, amplifier, and recorder). The calf was tranquilized with Sparine (100 mg, IV), and only a topical anesthetic (Ophthaine) was used on the eye.

ERG in isolated eyes

ERG have been recorded in 20 isolated eyes, In these 20 eyes A and C waves were smaller than those obtained in the living calf, and the B waves, when present, were also smaller. The eyes have been dark adapted for 10 to 30 minutes. The duration of dark adaptation, within these limits, did not affect the ERG response.

The intensity of the light stimulus influenced the response: the greater the stimulus intensity, the greater the response, but the amplitude of the waves still remained below normal levels, even when high intensities were used.

The values of pO_2, pCO_2, and pH affected the ERG response: high pO_2 and low pCO_2 values produced A and B waves of greater amplitude than low pO_2 and high pCO_2. These effects were reproducible in the same eye when it was alternately perfused with oxygenated and non-oxygenated blood.

The duration of blood perfusion before the first ERG recording did not alter the responses. The amplitudes of A,B, and C waves were similar after 2 hrs 30 min and after 8 hrs of blood perfusion, as long as the values of pO_2, pCO_2, and pH were held constant.

The shape of the A and B waves appeared to be different when the eye was perfused with stored blood having an increased RCA as compared to fresh blood having a normal RCA.

In summary, our recordings in isolated eyes showed the presence of A and C waves, but the B waves were small or absent. This observation indicates that, in our preparation, the photoreceptors were relatively well preserved, whereas the bipolar cells were deteriorated.

In order to visualize a gross deterioration of the retina (edema, detachment, hemorrhage) during extended recording procedures of a dark adapted eye, the flash of a fundus camera (Kowa) was used instead of the Grass photo stimulator. This procedure allowed concomittant fundus photographs and light stimulation of the retina. The intensities of the camera flashes were varied in steps similar to those of the photo stimulator, and the ERG responses were similar in both cases.

Experiments are now in progress to establish the cause of the retinal deterioration and to determine its occurrence during the course of our experiments. We plan to anesthetize calves in our laboratory to be able to record ERG in vivo and immediately after enucleation.

B. Preparation of thrombocytopenic cow blood

Bovine platelet concentrates have been prepared from fresh whole blood and successfully preserved in both the liquid and the frozen state.

Approximately 450 mls of blood was drawn from the jugular vein into a standard triple pack (Fenwal Laboratories, Deerfield, Illinois) containing 63 mls of citrate-phosphate-dextrose (CPD) anticoagulant. The blood was centrifuged at 4470g x 2.5 minutes to produce platelet rich plasma, this was then expressed into the primary satellite bag and centrifuged at 4470 g x 5 min to concentrate the platelets. Supernatant platelet poor plasma was removed so that 25-30 ml of platelet concentrate remained. The concentrate was left undisturbed at room-temperature for 30-60 minutes after which the platelets were manually resuspended. Sufficient platelet poor plasma was then added to establish a concentrate volume of 50-55 mls. It was necessary to add 6-10% v/v acid-citrate-dextrose (ACD, NIH Formula A) to the platelet rich plasma before centrifuging for platelet concentrate; otherwise resuspension of the concentrate could not be accomplished.

For the liquid preservation studies, 50 ml volumes were stored either at $4^{o}C$ unagitated or at $22^{o}C$ with constant agitation on a rotator revolving eight times per minute.

For the froxen preservation studies, 50 mls of either PPP or 20% PPF/80% 0.9% NaCl, 0.2% dextrose, disodium phosphate solution containing 10 or 12% DMSO was added to an equal volume of platelet concentrate over 5-30 minutes with constant agitation to achieve a final DMSO concentration of 5 or 6%. The platelets were frozen at $-80^{o}C$ in a UCAR-2030-4 polyolefin plastic bag (Union Carbide Corporation, Chicago, Illinois) for 1-484 days. Concentrates were thawed without agitation for 15 minutes at $37^{o}C$; wash solution consisting of either 100 ml autologous PPP and 20 ml ACD, or 20 ml PPF, 80 ml 0.9% NaCl, 0.2% dextrose, disodium phosphate and 20 ml ACD, was added with gentle agitation over a 15-30 minute period; the concentrates plus wash solution were centrifuged at 4470 g x 5 min after which the supernatant was expressed off completely and the platelets resuspended in approximately 25 mls of autologous PPP.

Platelet counts were performed with the Technicon light scattering technique.

The following data were obtained:

1. Anticoagulated whole blood platelet count
   $2.59 \times 10^{5}/mm^{3} \pm 0.91$ (standard deviation of mean) (n=25)

2. Fresh platelet concentrate count
$1.47 \times 10^6/mm^3 \pm 0.60$ (n=22)

3. In vitro recovery whole blood to platelet concentrate
61.6% ± 19.7 (n=22)

4. Fresh concentrate gases post resuspension
pH= 6.50 ± 0.19 (n=23)
pO_2= 69.2 ± 29.3 mms Hg (n=22)
pCO_2= 120.3 ± 44.6 mms Hg (n=21)

Frozen Platelets

5. Fresh platelet concentrate count $1.24 \pm 0.50 \times 10^6/mm^3$ (n=14)

6. Post wash platelet count $0.60 \pm 0.22/mm^3$ (n=10)

7. Freeze-thaw-wash recovery 79.0 ± 16.6% (n=10)
5% DMSO, 5' add 79.0 ± 18.7% (n=7)
6% DMSO, 30' add 79.1 ± 13.8% (n=3)
FZ with 0.9% NaCl, wash with PPP 90.0% (n=1)
FZ with PPP, wash with 0.9% NaCl 67.2 ± 11.1 (n=2)
FZ with PPP, wash with PPP 80.7 ± 17.8 (n=7)

8. Post-wash concentrate pH 6.50 ± 0.12 (n=7)

Liquid Stored Platelets

22°C (n=4)	FRESH	24°	48°
	$x10^6/mm^3$		
Platelet count	1.66±0.55	1.76±0.41	1.41±0.82
pH	6.56±0.06	6.57±0.20	6.38±0.35
pO_2 mms Hg	51.5±23.3	39.5±20.5	54.3±19.5
pCO_2 mms Hg	118.9±12.7	76.2±10.3	79.7±32.2

4°C (n=4)	FRESH	24°	48°
	$x10^6/mm^3$		
Platelet count	1.91±0.80	1.59±0.94	1.72±1.00
pH	6.60±0.12	6.55±0.09	6.30±0.36
pO_2 mms Hg	52.6±9.4	77.6±22.7	91.8±40.0
pCO_2 mms Hg	112.9±20.7	102.6±8.2	87.2±17.2

C. Electron Microscopy Studies

EM's were performed on enucleated perfused bovine eyes by Dr. Shepro and his collaborators. Morphology was found to be poorly maintained and the procedure was therefore suspended until ERG's could be simultaneously performed.

D. Other Studies

In conjunction with the Naval Blood Research Laboratory, Boston, Massachusetts, a study of the ocular effects of administration of the cryoprotectant dimethyl sulfoxide (DMSO) has been conducted. During the last four years, 80 volunteers who have received platelet transfusions containing residual amounts of

DMSO have been examined at periodic intervals. To date, no significant alterations have been noted in the eye. This study involves 5% of the time of the principal investigator.

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

Techniques have been developed to perfuse isolated bovine eyes with whole blood at physiological rates of flow and controlled levels of pO_2, pCO_2, and pH for extended periods of time (up to 24 hours). This experimental eye model has been designed to study the effects of changes in blood parameters on retinal metabolism and circulation. Using fresh and stored blood alternately to perfuse the isolated eye, we are investigating the influence of red cell affinity for oxygen on the retinal function by recording ERG.

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