6520/Human Patients

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NAVAL HOSPITAL
CHELSEA, MASSACHUSETTS 02150

NH2/1/TDC:ms
6470
1 May 1963

From: Commanding Officer, Naval Hospital, Chelsea, Massachusetts 02150
To: Secretary of the Navy, Washington, D. C. 20390
Via: Chief, Bureau of Medicine and Surgery (Code 742), Washington, D. C. 20390

Subj: Approval for use of human volunteers in studying effects of freezing platelets and red blood cells; request for

Ref: (a) MANMED Art 20-4
(b) MANMED ltr: BUMED-742:CFT:MS: 6470/CHELSEA: Ser: 470 of 17 April 1963

Encl: (1) Copies (2) of AEC License #20-00074-02 Amendment #17 item 9.C.
(2) Protocol for Evaluation of Frozen Red Blood Cells (2) copies
(3) Protocol for Evaluation of Preserved Platelets (2) copies

1. In accordance with references (a) and (b), it is requested that approval be granted to study the effects of freezing on platelet survival and the survival characteristics of red blood cells in human volunteers as outlined in enclosures (1), (2), and (3).

TRACY D. COTTLE

See # 4-118
2 6511

U. S. ATOMIC ENERGY COMMISSION
BYPRODUCT MATERIAL LICENSE
Supplementary Sheet

License Number 20-00374-02

For Your Files

Amendment No. 17

Department of the Navy
U. S. Naval Hospital
Chelsea, Massachusetts 02150

In accordance with application submitted March 5, 1966, License Number 20-00374-02 is amended as follows:

Item 9. is amended to add the following:

a. Study of the effects of [illegible] on clinical survival in [illegible] adults. This study shall be carried out by, or under the supervision of, LCDR Keenan D. Morrison, M.C.

b. Study of the survival characteristics of human red blood cells in [illegible] adults. This study shall be carried out by, or under the supervision of [illegible] M. Valeri, M.D.

For the U. S. Atomic Energy Commission

FEB 16 1968

[illegible]
Materials Branch

Enclosure (1)

NBRL/A/ms
22 January 1968

EVALUATION OF FROZEN RED BLOOD CELLS

A technique has been developed for freezing red blood cells (RBC's) and thereby allowing blood to be stored for a much greater time interval than is possible with the present liquid preservative (ACD) of blood. The purpose of this study is to clinically evaluate the survival characteristics of these frozen RBC's using isotope techniques.

Two methods will be employed. In one 50-60 cc of the frozen RBC's will be labelled with 10 uc of Cr^{51} chromate and transfused into normal volunteers and patients. The recipient's RBC mass will be measured indirectly using a non-radioactive Evan's blue technique. About 20% of these recipients will receive 5 uc I^{131} labelled human serum albumin (RISA) to determine plasma volume as a check on the accuracy of the Evan's blue technique. The other method of measuring the survival of the frozen RBC's will be done in patients only and will utilize differential agglutination techniques. The patient will receive 5 uc Cr^{51} chromate before a transfusion of frozen RBC's to determine RBC volume. Two subsequent RBC volume determinations over a 3 month period will be done so that this group of patients will receive a maximum of 15 uc Cr^{51}. Again about 20% of these patients will receive 5 uc I^{131} labelled RISA to indirectly measure RBC mass.

As both of these isotope techniques are well established, information concerning the isotopes and radiation dosimetry will be somewhat abbreviated. Many of the Cr^{51} tagged RBC's will eventually be trapped by the spleen. Almost all of the radioactivity freed from the destroyed RBC's will be excreted in the urine. RISA distributes throughout the body in the intra- and extravascular pool. Most of the I^{131} is excreted in the urine with about 2% excretion in the feces. Using an effective half-life for Cr^{51} of 28 days in the spleen and 14 days in the peripheral blood, the total body dose of 15 uc of Cr^{51} would be 5.25 mrads and the spleen dose would be 19.5 mrads. The total body dose from the 5 uc of I^{131} labelled RISA would be 8.5 mrads. The doses selected have been found to be the minimum amount that can be used, consistent with good counting statistics.

The normal volunteers will be over 18 years of age, will not be pregnant and their consent for the administration of the radionuclides will be obtained. It is expected that about 50 normal volunteers will be needed for survival studies over a 2 year period. They will receive 10 uc Cr^{51} which will result in a radiation dosage of 13 mrads to the spleen and 3.5 mrads total body radiation. In addition, about 10 of the 50 normal volunteers will receive 5 uc I^{131} labelled RISA, resulting in a total body dose of 12 mrads to the 10 individuals. The patients participating in the study will be anemic adults over age 18 whose anemia is stable, as is seen in uremia, chronic infection, and with neoplasms. They also will not be pregnant and will consent to the

administration of the radionuclides. About 50 such patients will also be needed over a 2 year period. They will undergo the 3 RBC volume determinations and receive a maximum of 15 uc Cr^{51} resulting in 19.5 mrads radiation to the spleen and 5.25 mrads total body radiation. About 10 of these 50 patients will receive 5 uc I^{131} labelled RISA resulting in a total body dose of 13.75 mrads in these 10 individuals. These figures compare quite favorably to the permissible body burden for occupational exposure of 450mrads/week to the spleen and 100 mrads/week to the total body.

This study will be carried out in the Radioisotope Unit and the Blood Research Building at this hospital. All isotopes will be dispensed and counted in the Radioisotope Unit. The physical facilities and equipment have been described in previous applications. Injection of the normal volunteers and preparation of the samples to be counted will be carried out in the Blood Research Building under the direction of Dr. Valeri and the Radiation Protection Officer (Dr. Meier).

This study will carried out under the direction of Dr. Cesare R. Valeri. His research training and experience are as follows:

1. M.D. - Harvard Medical School, 1958
2. Medical Internship and Junior Resident in Medicine, Boston City Hospital, 1958-1960
3. Senior Resident in Medicine, New England Medical Center, Boston, 1960-1961
4. Fellow in Hematology, Boston City Hospital, 1961-1962
5. Hematology and Blood Research, U. S. Naval Hospital, Chelsea, Mass., 1962-present

His isotope training and experience has been forwarded previously on form AEC 313a.

Results of this study will be reported upon its completion.

FROZEN PLATELET SURVIVAL STUDY

1. Obtain 2 units whole ACD blood.
2. Prepare PRP by differential centrifugation (free of RBCs and WBCs).
3. Combine PRP in 600 ml transfer pack and add 1/10 vol ACDA and air.
4. Spin full speed for 15 minutes - connect to another transfer pack - invert quickly and drain PPP.
5. Place 30 cc of the PPP in a syringe.
6. Return 3 cc of this PPP to the platelet button, add 250-300 uc Cr^{51} and 1 cc saline through tube - incubate fifteen minutes - room temp.
7. Remove the concentrate with a syringe and place a precise amount into another bag through tube - add exactly equal quantity of 20% glycerol in PO_4 buffer, (or 5% DMSO) slowly in 1 cc increments and continue mixing for five minutes after addition.
8. Freeze at 2 /min, and store at -80 C, 24 hours.
9. Thaw by immersion of bag in tap water (less than 30 C).
10. Add 4 cc of special citrate (sterile) (8 grams Na_3 citrate, 0.4 gm citric acid - pH 6.16 - osmol 940) mix 1 minute.
11. Add PPP at about 2 cc/min for 20 min, then 10 cc/min. - add air.
12. Spin full speed 15 min, decant PPP quickly into beaker (measure pH _____ - osmolarity _____).
13. Add the remaining 27 cc of PPP to button, suspend.
14. Infuse precisely measured volume into patient and save about 2 cc for injection sample study.
15. Obtain samples from patient at 20 min, 1 hour, 24 hours and daily for ten days.

INJECTION SAMPLE STUDY

1. Count platelets
2. Make quadruplicate 200 aliquots - label a, b, c, d.

(Enclosure (2))

a. Add 5 cc 1% NH_4oxalate - spin hard 15 min, decant supernatant.
b. Add 5 cc 1% NH_4oxalate - spin hard 15 min, decant supernatant.
c. Add 5 cc saline - spin hard 15 min, decant supernatant.
d. Do not wash.

3. Repeat wash on a, b and c, twice.

4. Cover tubes and save for counting.

PATIENT SAMPLE STUDY

1. 10 cc collected in syringe containing 1 cc of 1% EDTA. Mix well.

2. Place 2 cc of whole blood into counting tube - cover - label - save.

3. Place remainder into large tube - spin at 900 for 15 minutes.

4. Aspirate PRP - count platelets and measure exact volume of PRP.

5. Spin full speed, 15 min, decant PPP into another tube - save 2 cc for counting. Label button and PPP - cover - save.