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(NASA-TM-79423) EXPERIMENT REQUIREMENTS:  
VITAMIN D METABOLITES AND BONE  
DEMINERALIZATION, SPACELAB 2, EXPERIMENT NO.  
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N78-20757

Unclas  
G3/52 12118

# EXPERIMENT REQUIREMENTS

VITAMIN D METABOLITES  
AND  
BONE DEMINERALIZATION


SPACELAB 2  
EXPERIMENT NO. 1

MARCH 1978

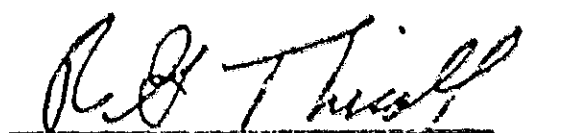
EXPERIMENT REQUIREMENTS DOCUMENT FOR:

EXPERIMENT NO.: 1

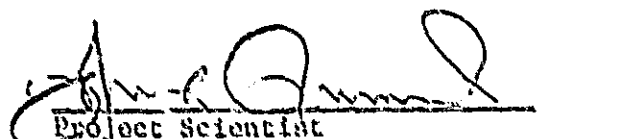
EXPERIMENT TITLE: VITAMIN D METABOLITES AND BONE DEMINERALIZATION

  
Principal Investigator  
Dr. Heinrich K. Schnoes  
Department of Biochemistry  
University of Wisconsin-Madison

March 6, 1978  
Date

  
Project Engineer  
Systems Development Branch  
Life Sciences Project Division  
Space and Life Sciences Directorate  
NASA Johnson Space Center

MARCH 13, 1978  
Date

  
Project Scientist  
Science Support Branch  
Life Sciences Project Division  
Space and Life Sciences Directorate  
NASA Johnson Space Center

March 13, 1978  
Date

NASA Use: Proposal No. \_\_\_\_\_

Mission No. \_\_\_\_\_

Payload No. \_\_\_\_\_

### EXPERIMENT REQUIREMENTS

Experiment  
Title:

VITAMIN D METABOLITES  
AND BONE DEMINERALIZATION

Date:

Prepared  
By:

DR. EMILY M. HOLTON  
Co-Investigator  
NASA ARC

ROBERT G. THIROLF  
Project Engineer  
NASA JSC

Prepared  
For:

SpaceLab Payload Project  
George C. Marshall Space Flight Center  
Huntsville, Alabama 35812

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## ABSTRACT

As a contribution toward an understanding of the molecular basis of bone loss, mineral imbalance, and increasing fecal calcium under conditions of prolonged space flight, the blood levels of biologically active vitamin D metabolites of flight crew members will be quantitatively measured. Prior to the mission, the refinement of existing and the development of new techniques for the assay of all vitamin D metabolites will provide an arsenal of methods suitable for a wide range of metabolite levels. In terms of practical application we envision the analysis of:

a) Animal and human samples: During the development phase, existing methods for the vitamin D metabolites will be refined and new methods developed. Experimental protocols will be tested using samples from animal and human experiments. Plasma from animals, e.g. rats, chickens, and monkeys, maintained on diets differing in vitamin D supplementation, motion restricted (simulated weightlessness), collected at different intervals during the day will be assayed. Human specimens derived from normal, diseased subjects (particularly those with liver, kidney, or bone related problems), and bed-rested patients will complement the animal work. These studies should give a diversity of samples which allow 1) definition of the most effective and appropriate methods for analysis of the vitamin D metabolites in plasma samples of the flight crew, 2) definition of the optimum experimental protocol, and 3) acquisition of an extensive set of baseline data.

b) Spacelab crew samples: Our objective for the second Spacelab mission is the analysis of plasma samples from each member of the crew, as well as ground control specimens collected prior to, during, and postflight. With existing methods, we can obtain data for  $25\text{-OH-D}_3$  and  $1\alpha, 25\text{-(OH)}_2\text{-D}_3$  on a minimum of 5 ml of plasma, but the proposed techniques should allow analysis of all metabolites, require less sample, and maintain at least similar sensitivity. Presently, a 10 ml sample size is preferable for D metabolite assay. A maximum number of samples is, of course, desirable, but in view of the short duration of the Spacelab mission, two premission, two mission, and two postmission samples from each crewmember are required.

c) Flight hardware: The following items shall be required from the Life Sciences inventory of CORE hardware:

- 1)  $-20^\circ\text{C}$  freezer
- 2) Blood collection kit
- 3) Centrifuge

## 1.0 GENERAL INFORMATION

### 1.1 Experiment Purpose and Objective

<u>No.</u>	<u>FO Title</u>	<u>Objective or Purpose</u>
1	Specimen collection	Return plasma specimens for postflight analysis

### 1.2 Equipment Definition

<u>No.</u>	<u>FO Title</u>	<u>Equipment Title/Nomenclature</u>
1	Specimen collection	-20°C Freezer Blood collection kit Centrifuge

### 1.3 Experiment Facility Interface

See Appendix B.

### 1.4 Methods and Procedure

<u>FO Title</u>	<u>Method/Procedure</u>
Specimen collection	Collect 25 ml of anticoagulated whole blood from each of five crewpersons once early in the mission and once late in the mission. Reduce to plasma and cellular fractions; store in freezer for postflight analysis.

### 1.5 Supporting Investigations

The primary experiment objective for this mission is the quantitative analysis of the vitamin D metabolites in plasma samples from members of the crew collected prior, during, and postflight. The supporting investigations include the following:

a) The development of sensitive analytical methods allowing the quantification of all vitamin D metabolites in a single small blood sample. Quantification of  $25\text{-OHD}_3$  and  $1\alpha,25\text{-(OH)}_2\text{D}_3$  is clearly of prime importance, since the latter represents the presumed active target tissue form and the former its obligate precursor. Both compounds can be analyzed by existing methods:  $25\text{-OHD}_3$  by organic extraction, column chromatography for purification and separation from other metabolites, and high pressure liquid chromatography for quantification;  $1\alpha,25\text{-(OH)}_2\text{D}_3$  by preliminary steps similar to that for  $25\text{-OHD}_3$  plus an additional procedure which involves a radioreceptor assay. However, the quantification of all known metabolites (and if possible also unknown ones) could be much more informative since an alteration in vitamin D metabolism under zero-g conditions might not reflect itself dramatically in the absolute level of the active species ( $1\alpha,25\text{-(OH)}_2\text{D}_3$ ), but could find expression in a pronounced variation in the level of another metabolite or even the appearance of a new compound. Since such metabolites could well exhibit unique activity patterns, or could in some fashion, modulate expression of activity by  $1\alpha,25\text{-(OH)}_2\text{D}_3$ --by antivitamin action or because



of competition for blood transport or target tissue receptor proteins, for example--a knowledge of the total metabolite picture could well yield important insights into this complex problem area and would certainly seem an essential prerequisite for focused subsequent endeavors. Furthermore, the known intimate relationship between  $1\alpha,25-(OH)_2D_3$  and  $24R,25-(OH)_2D_3$  production under normal circumstances, points to attempts at complete metabolite analysis as definitely worthwhile, not to say essential, objectives. In the absence of methods for the quantification of metabolites such as  $24R,25-(OH)_2D_3$ ,  $1\alpha,24,25-(OH)_3D_3$ , or  $25,26-(OH)_2D_3$ , we propose the development of techniques capable of analyzing all known (as well as potentially also the unknown) metabolites in one blood specimen as an important aspect of our program. The sample size required for analysis of all vitamin D metabolites should not be greater than that presently required for the analysis of  $25-OHD_3$  and  $1\alpha,25-(OH)_2D_3$  and with extensive testing and refinement of existing and newly developed analytical methods the sample size required should decrease significantly.

b) Extensive testing and refinement of existing and newly developed analytical methods to increase sensitivity and reproducibility as well as to decrease sample size.

c) Testing of experimental protocols from animal and human experiments. Plasma from various animals including rats, chickens, and monkeys will be assayed to define the best experimental protocol. Factors such as vitamin D supplementation, postprandial variations, and/or hypodynamia will be investigated. Human specimens derived from normal, diseased (involving organs known to be involved in vitamin D synthesis or actions), or bed-rested patients will be assayed to compare with the animal work and to suggest shifts in metabolite patterns or potentially new metabolites which might occur in situations somewhat similar to zero-g. Determination of these factors will establish an extensive set of baseline data which should shed light on the influence of vitamin D supplementation on the various vitamin D metabolites, any effect of meal feeding or time-of-day on the D metabolites, and any possible influence of fluid shifts on the production and, hence, concentration of the D metabolites.

## 1.6 State of Development

The flight instruments for this experiment are included in Appendix B. No development is required. The non-flight instruments required for the analysis of the vitamin D metabolites are presently available in our laboratory and require no additional development. However, since rapid advances are being made which impact the versatility and sensitivity of these instruments some alterations may be necessary. Only when such advances impact the experiment significantly in terms of productivity, sensitivity, or selectivity will alterations be made.

## 1.7 N/A

## 1.8 Description of Science to be Achieved

Our proposed program will furnish precise analytical data on vitamin D metabolite levels in subjects exposed to low-gravity environments. This experiment is intended to establish whether the well-known derangements of mineral (specifically calcium) metabolism experienced in long term space missions are reflected in any way in a modulation of vitamin D metabolism to its various metabolites. In addition, extensive data on terrestrial human and animal models will become available. Since research into the biochemistry of vitamin D, during the past decade, has established that vitamin D metabolites, specifically  $1\alpha,25-(OH)_2D_3$ , are key agents in the maintenance of calcium and phosphate homeostasis, monitoring these metabolites under normal and zero-g conditions is thus expected to yield important insights into the molecular aspects of the homeostatic mechanism in both 1-g and gravity-free environments. Metabolite levels are to be determined by established methods such as competitive protein binding, high pressure liquid chromatography and gas liquid chromatography, as well as by new, very general and highly sensitive techniques to be developed as part of our program. The successful development of rapid and convenient analytical methods for metabolites assays will greatly benefit clinical research aimed at understanding and control of a variety of calcium metabolism disorders. We regard this science as an important complement to other on-going researches on the effect of a low-gravity milieu on hormonal and mineral metabolism. In its current limited format, it is designed to furnish analytical technology and good quantitative data required as the experimental and conceptual basis for subsequent detailed biochemical investigations and for any attempts to correct aberrant metabolism by pharmacological or other means.

## 2.0 PHYSICAL REQUIREMENTS

### 2.1 Experiment Equipment

See Appendix B.

#### Experiment Mass (kg)

Freezer	34.1	100% est.
Blood Collection Kits (2)	1.0	100% est.
Centrifuge	<u>11.3</u>	100% est.
Total	46.4	

### 2.2 Equipment Dimensions

See Appendix B.

### 2.3 Experiment Block Diagram, Sketch No. \_\_\_\_\_

N/A

### 2.4 Specimens, Tape, and Film

TABLE 2-4. EXPERIMENT SPECIMENS  
REQUIRED FOR FLIGHT RETURN

ITEM NO.	ITEM <sup>a</sup> (SPECIMEN, TAPE, FILM)	UNIT PACKAGE (CAGE, REEL)	NO. OF PACKAGES REQUIRED	ENVIRONMENTAL LIMITS			
				TEMPERATURE (°C)	PRESSURE (N/m <sup>2</sup> )	HUMIDITY (%)	OTHER (ACCELERATION, SHOCK, RADIATION)
1	Specimens	Container	10	-20	-- Nominal,		habital limits ----

### 3.0 FUNCTIONAL REQUIREMENTS

#### 3.1 Structural Requirements

All hardware to be located in Orbiter cabin at MSFC discretion with PI approval.

See Appendix B.

#### 3.2 Electrical Requirements

See Appendix B.

TABLE 3-2. POWER

THESE DATA APPLY TO EQUIPMENT LOCATED IN/ON ONE (RACK/PALLET) FOR ONE FO. THIS IS 1 OF 1 SET(S) OF TABLES USED TO COMPLETE MY ELECTRICAL REQUIREMENTS.

TOTAL ENERGY FOR THIS EQUIPMENT FOR THIS FO IS .266 kW-h.

PLEASE COMPLETE THE FOLLOWING TABLE: (IF NONE REQUIRED, SO INDICATE.)

FO No. 1

POWER (W)							
EMERGENCY (1)		STAND-BY (2)		OPERATING (3)		PEAK (4)	
dc	ac	dc	ac	dc	ac	dc	ac
					200		

(1) EMERGENCY - MINIMUM POWER REQUIRED TO KEEP EXPERIMENT IN A SAFE CONDITION WHEN ALL MAIN POWER (ac AND dc) IS LOST.  
 (2) STANDBY - ANY POWER REQUIRED BETWEEN THE LAST FO COMPLETION (OR ON-ORBIT POWER-UP) AND THE NEXT FO INITIATION.  
 (3) OPERATING - NORMAL OPERATING LEVEL.  
 (4) PEAK - THE MAXIMUM LEVEL REQUIRED FOR 1 s OR LONGER.

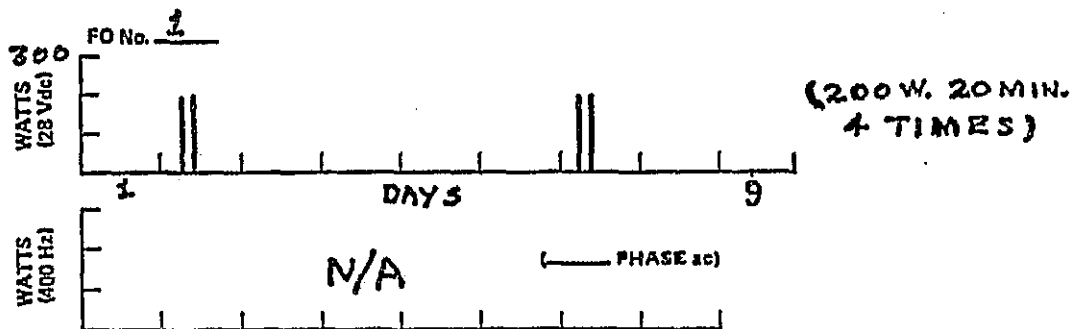


Figure 3-3. Power profile.

### 3.3 Thermal Control Requirements

See Appendix B.

TABLE 3-3a. HEAT TRANSFER CHARACTERISTICS

ITEM NO.	EQUIPMENT NOMENCLATURE	HEAT DISSIPATION (W)		EQUIPMENT COOLING REQUIREMENTS (W)								MAX. TEMP OF COOLING FLUID AT OUTLET (°C)	
				AIR COOLED		LIQUID COOLED (INT. ERT)		SPACECRAFT COLD PLATE		PASSIVE THERMAL CONTROL			
		OPER.	STANDBY	MIN.	MAX.	MIN.	MAX.	MIN.	MAX.	MIN.	MAX.	OPER.	MIN-OPER.
1	Centrifuge	200	-	-	200	-	-	-	-	-	-	-	-

TABLE 3-3b. HEAT TRANSFER CHARACTERISTICS

ITEM NO.	EQUIPMENT NOMENCLATURE	TEMPERATURE LIMITS	SURFACE OPTICAL PROPERTIES	HEATER OPERATIONAL CHARACTERISTICS AND POWER (W)	THERMAL CAPACITANCE (W-HR/°C)	REQUIRED AIR FLOW RATE (L/MIN)	REQUIRED COLD PLATE SURFACE AREA AND LOCATION (m²)	IDENTIFY COOLING CAPACITIES OF EQUIPMENT FROM TO PLATFORM SELECT AN. SEE IN FACT THE MODEL COLLECT
1	Centrifuge	Orbiter Std Atm	NA	NA	TBD	TBD	NA	NA

(3.4, 3.5, 3.6 and 3.7 DELETED.)

### 3.8 Flight Environmental Interfaces

See Appendix B.

(3.9 Deleted)

### 3.10 Safety

- a. All hardware CORE items certified by JSC Safety Office.
- b. No procedures required by this experiment are considered to be hazardous.

#### 4.0 FLIGHT OPERATIONS REQUIREMENTS

(4.1, 4.2 DELETED.)

#### 4.3 Performance Requirements and Constraints for the FO's

Constraints: If the crew members are to receive vitamin D supplementation, it should be in the form of vitamin D<sub>3</sub> (cholecalciferol) rather than vitamin D<sub>2</sub> (ergosterol).

Skills, qualifications, and training for the Payload Specialists are to be determined and outlined by the laboratory technologist specialists at Johnson Space Center.

##### 4.3.2 Description of Events

See Table 4-6.

##### 4.3.3 Power and Thermal Assessments of the Integrated Payload

N/A

##### 4.3.4 POCC Monitoring Requirements

N/A

##### 4.3.5 Training

<u>Sessions Required</u>	<u>Session Duration</u>	<u>Training Hardware Required</u>
1	2 hr/crew	-20°C freezer, blood collection kit, centrifuge, training units arranged in SL-2 flight configured trainer
5	1 hr/man (2)	
4	2 hr/crew	

##### Training Site

JSC  
KSC

##### When Scheduled

1 yr prior to flight  
1 mo prior to flight

#### 4.4 Payload Flight Data File

TABLE 4-10. PAYLOAD FLIGHT DATA FILE CONTENT

NAME: R. Thirolf/JSC

EXPERIMENT: No. 1

PAGE 1 OF 1 1/26/78  
DATE  
REV.

ITEM	RESPONSIBLE INDIVIDUAL	ESTIMATED NO. PAGES	ESTIMATED AVAILABILITY DATE
1	R. Thirolf	5	4/1/80

TABLE 4-6. ON-ORBIT/GROUND OPERATIONS DESCRIPTION

EXPERIMENT/TITLE: Vitamin D Metabolites

NO.	FUNCTIONS (USE STANDARD OR PROVIDE YOUR OWN)	APPLICATION TO FG NO.	TIME DURATION (hr:min)	NO. OF PYLAGEZ CYCLES REQUIRED	AVERAGE POWER (WATT)	AVERAGE POWER (WATT)	ESSENTIAL SALT RATE (M/L)	SALINE SALT RATIO (M/L)	TV SCHEDULE REQUIRED	DETAILED CREW ACTIVITIES		DETAILED PROC-ACTIVITIES		COMMENTS
										DESCRIPTION	TIME (min)	LOC CODE	DESCRIPTION	
1		1	5	1	0	0	0	0	0	Unstow/setup	5	1	---	---
2		1	3	2	0	0	0	0	0	Blood draw	3	1	---	---
3		1	3	1*	0	0	0	0	0	Blood draw	3	1	---	*MS-also
4		1	3	1*	0	0	0	0	0	Blood draw	3	1	---	*Commander also
5		1	20	1	0.2	0	0	0	0	Spin down	1	1	---	PS to other duties after loading centrifuge
6		1	15	1	0	0	0	0	0	Transfer plasma	15	1	---	---
7		1	3	1	0	0	0	0	0	Plasma & cells to freezer	3	1	---	---
8		1	4	1	0	0	0	0	0	Cleanup/stow	4	1	---	---

LOCATION CODES:  
 1. PHYSICALLY POSSIBLE ONLY ON-ORBIT  
 2. PREFER ON-ORBIT OPERATION  
 3. NO LOCATION PREFERENCE FOR OPERATION  
 4. PREFER OPERATION FROM GROUND  
 5. PHYSICALLY POSSIBLE ONLY FROM GROUND

- NOTES:
- 1) Above events typical for specimen collection following sleep cycles for all crewmembers on MD2 and MD7.
  - 2) In Step 2, PS ending sleep cycle is donor.
  - 3) In Step 3, MS replaced by pilot where applicable.
  - 4) Step 4 omitted where applicable.
  - 5) Above assumes commander, MS, PS sleep during same period.

## 5.0 GROUND PROCESSING OPERATIONS

### 5.1 Installation and Assembly Requirements

#### 5.1.1 Special Preparation Prior to Installation of Experiment Equipment into the Spacelab

N/A

#### 5.1.2 Equipment Installation Requirements

<u>Equipment Identification</u>	<u>Installation Requirements</u>	<u>Installation/Interface Instruction</u>
---------------------------------	----------------------------------	---

Freezer	Max. heat removal	(Per below)
---------	-------------------	-------------

Comments: Heat sink to occupy freezer stowage space, then be removed immediately prior to liftoff and replaced with flight heat sink.

#### 5.1.3 Transportation and Handling Requirements

5.1.3.1 Prior to Installation into Spacelab Hardware - Nominal handling adequate.

5.1.3.2 Procedural Requirements/Provisions -

Storage preparation required: YES Procedure No. LSF-1

5.1.3.3 After Installation into Spacelab -

Protective covers, guards: NO

Transport environment: Habital environment adequate

5.1.3.4 Access Requirements -

Preflight: L-5 hours Postflight: L+1 hours

### 5.2 Alignment, Calibration, Servicing and Maintenance Requirements

N/A

### 5.3 Experiment Integration Requirements/Specification

N/A

(5.4, 5.5, 5.6, 5.7 DELETED.)



### 5.8 Off-Line Support Resources Requirements

<u>Lab/Shop Type</u>	<u>Exp. Processing Activity</u>	<u>Special Service</u>
Blood	Plasma	Draw blood, separate plasma and cells, all crew pre- and postflight. Facility to pack specimens with dry ice for 48-hour transit period.

Office space and equipment for 0 personnel are required.

### 5.9 Post-Landing Equipment Disposition Requirements

- a. Return freezer, centrifuge, and blood collection kits to life sciences CORE inventory.
- b. Frozen plasma specimens to be released to PI or PI representative on second day following landing, in conjunction with collection of postflight specimens from crew.
- c. Frozen cells to be released to JSC/Space and Life Sciences Directorate for disposition as required.

## APPENDIX B-1

### CORE Data Sheet

CORE Item: -20°C Freezer

#### ERD Section

##### 1.3 Experiment Facility Interface

The -20°C freezer will mate with the mounting provisions for the standard stowage containers on the Orbiter mid-deck bulkheads. As the program matures and more information becomes available on the air flow and thermal characteristics of the Orbiter cabin, a specific location for the freezer may be determined for most efficient and reliable operation.

##### 2.1 Experiment Equipment

See sketch, p. B-1a

##### 2.2 Equipment Dimensions

See sketch, p. B-1a

##### 3.1 Structural Requirements

Unit to be mounted on forward mid-deck bulkhead; specific location as required by mission.

##### 3.1.4 Controlled Spatial Relationships

Unit to be mounted in location where thermal environment is most acceptable to device, that is convenient to crewmembers to insert and remove during flight, and permits ready access to install and remove items during pre- and post-flight activities.

##### 3.2 Electrical Requirements

NA

##### 3.3 Thermal Control Requirements

None

##### 3.8 Flight Environmental Interfaces

Sensitivity limits, all cases: Pressure (N/m<sup>2</sup>) 0.552 to 1.013 x 10<sup>5</sup>  
Temperature (°C) 0-30

##### 3.10 Safety

Qualified as CORE item.

##### Miscellaneous Information

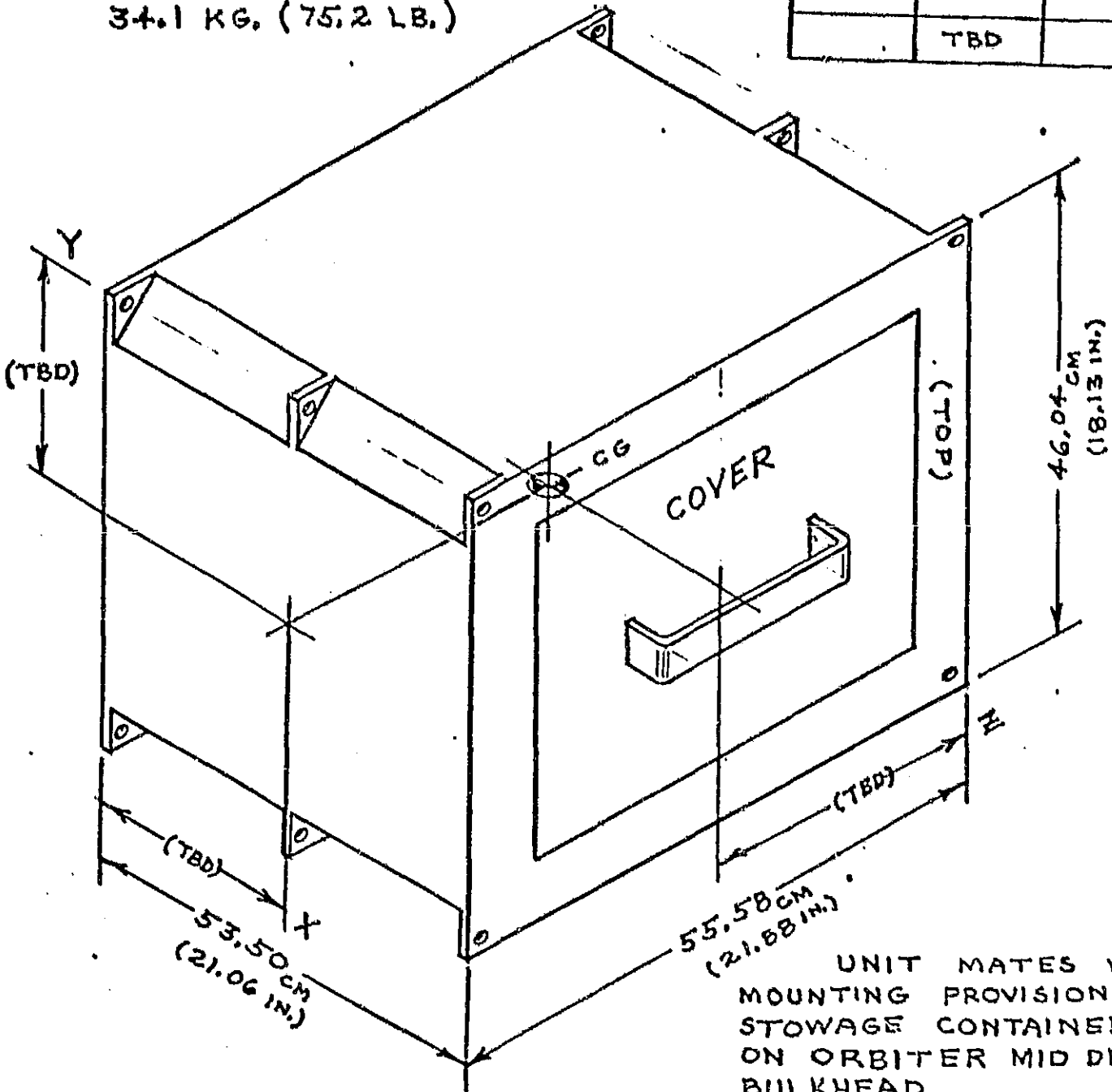
1. Minimum internal temperature: -130°C

PREDOMINANT MATERIAL:  
ETHYLENE GLYCOL

PASSIVE; HEAT SINKS ONLY

34.1 KG. (75.2 LB.)

MOMENT OF INERTIA $\text{kg}\cdot\text{m}^2$		
	TBD	



UNIT MATES WITH  
MOUNTING PROVISIONS FOR  
STORAGE CONTAINERS  
ON ORBITER MID DECK  
BULKHEAD.

-20°C FREEZER

(ERD SEC. 2.2)

rgt 1-26-78

CORE Data SheetCORE Items: Type 2 Blood Collection KitERD Section:1.3 Experiment Facility Interface

The blood collection kit is to be located in a standard stowage locker during non-use periods, then moved to a convenient position to accommodate the blood draw and processing activity.

2.1 Experiment Equipment

See sketch, p. B-2a

2.2 Equipment Dimensions

See sketch, p. B-2a

3.1 Structural Requirements

Units to be restrained at use location by Velcro pads or bungee cords.

3.1.4 Controlled Spatial Relationships

Units to be oriented at use location so that individual items may be readily removed from and returned to the confining recesses in the plastic block.

3.2 Electrical Requirements

None.

3.3 Thermal Control Requirements

None.

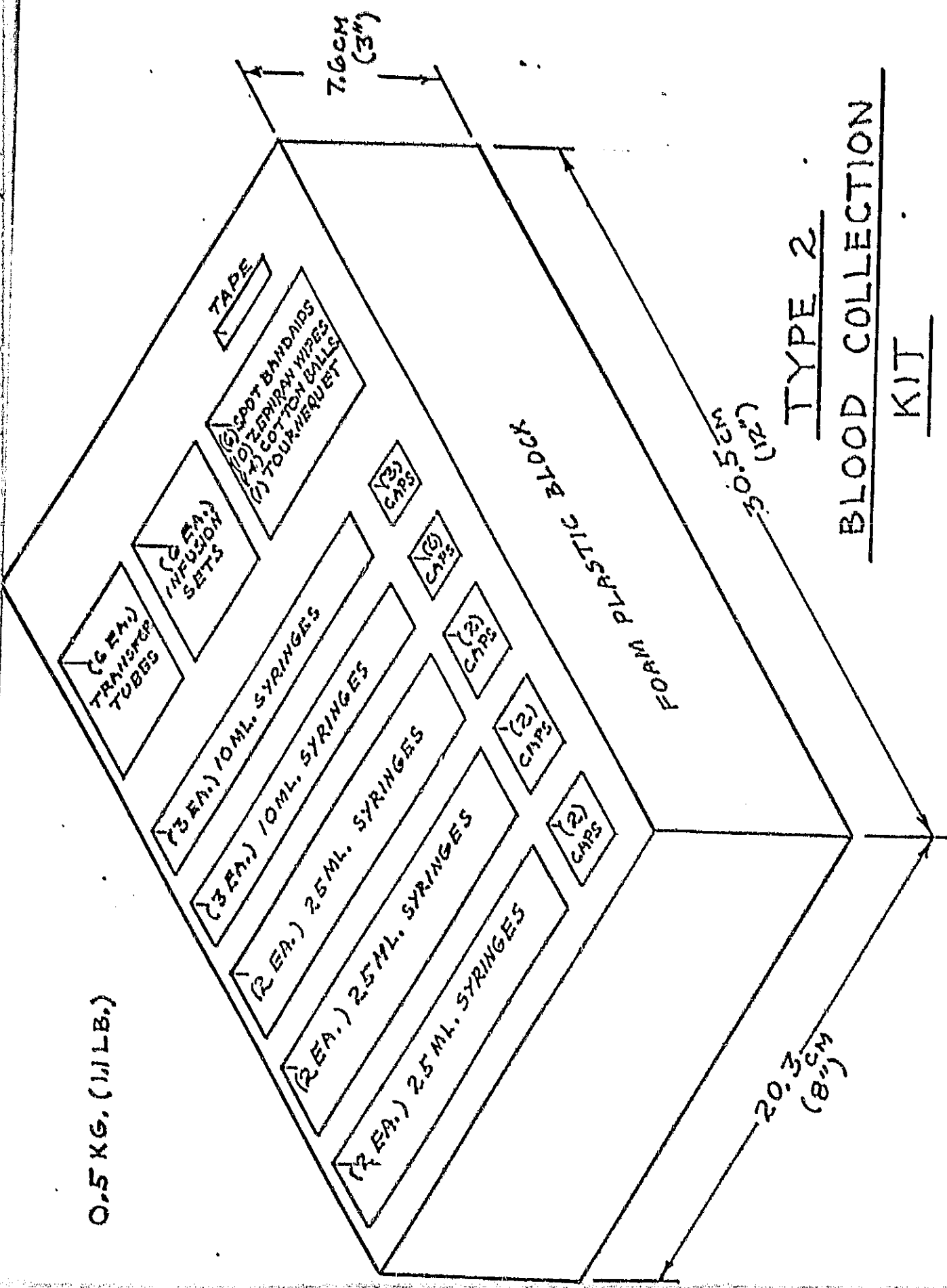
3.8 Flight Environmental Interfaces

Nominal habital limits adequate in all cases.

3.10 Safety

Qualified as CORE item.

0.5 KG. (1.1 LB.)



TYPE 2  
BLOOD COLLECTION  
KIT

(ERD SEC. 2.2)

rgt 12-1-77

CORE Data SheetCORE Item: Type 2 CentrifugeERD Section:1.3 Experiment Facility Interface

The centrifuge is to be located in a standard stowage locker during non-use periods, then moved to a convenient position to accommodate the blood draw and processing activity.

2.1 Experiment Equipment

See sketch, p. B-3a

2.2 Equipment Dimensions

See sketch, p. B-3a

3.1 Structural Requirements

Unit to be mounted on work surface of table in mid-deck area by the use of suction cups.

3.1.4 Controlled Spatial Relationships

Unit to be oriented at use location so as to be convenient in the blood draw and processing activity.

3.2 Electrical Requirements

28 + 4 V DC, 200 W, 1.8m (6 ft.) power cable w/plug to mate with utility outlet.

3.3 Thermal Control Requirements

The centrifuge should not be operated in a location that would be restricted from the ambient air flow in the mid-deck area.

3.8 Flight Environmental Interfaces

TBD

3.10 Safety

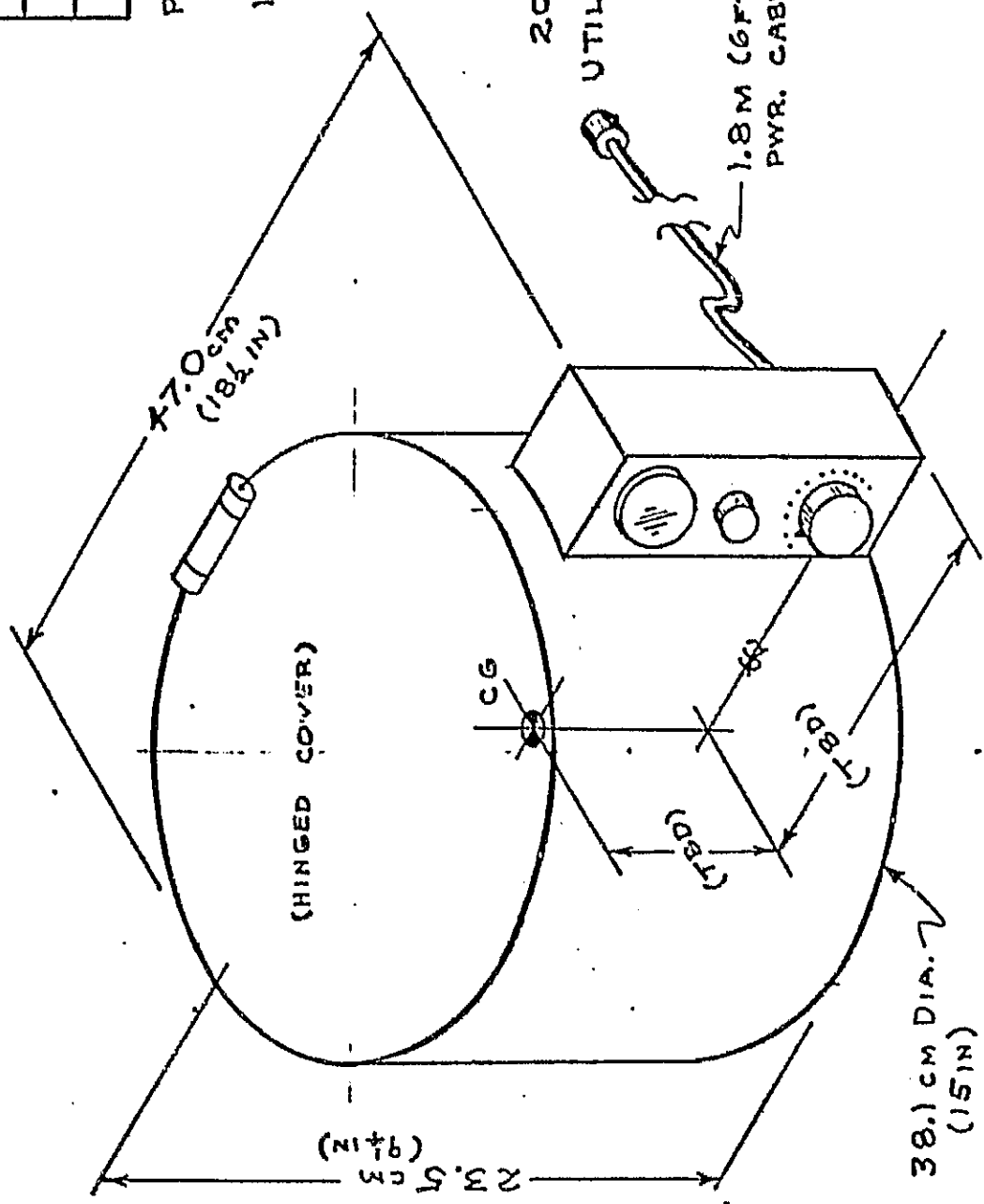
Qualified as CORE item.

MOMENT OF INERTIA kg-m <sup>2</sup>	
	(TBD)

PREDOMINATE MATERIAL:  
METAL  
11.3 kg. (25 lb.)

200W. 28±4V. DC  
UTILITY PLUG

1.8 M (6 FT.)  
PWR. CABLE



TYPE 2 CENTRIFUGE

(ERD SEC. 2.2)