CR-105413
TR69-103
6 March 1969

TECHNICAL STATUS REPORT

Contract No. NASw-870

Period 4 November 1968
to 4 February 1969

Support of the work reported herein has been provided by the Biosciences Program Division, Office of Space Sciences and Applications, Headquarters, National Aeronautics and Space Administration

Prepared for: National Aeronautics and Space Administration (SB)
Washington, D.C. 20546

Prepared by: Paul J. Heberlein
Project Manager (Phase I)

Jeffry S. Life
Project Manager (Phase II)

SPACE / DEFENSE CORPORATION
1600 North Woodward Avenue
Birmingham, Michigan 48011
Telephone: (313) 647-1304

TABLE OF CONTENTS

LIST OF FIGURES

1.0 BACKGROUND INFORMATION

This document reports progress on tasks defined under Contract No. NASw-870, Modification No. 6. Under this agreement, Space/Defense Corporation (herein after "the contractor") has continued to develop and evaluate the performance of single and multi-specimen respirometers. The ultimate goal of this program is to provide instruments suitable for performing research concerned with biorhythms and the mechanisms responsible for the establishment and control of observed rhythmic activity.

The configuration of these instruments is specifically oriented toward the conduct of biological rhythmicity studies in the space environment. The potato tuber has been chosen as the experimental subject on the basis of the relative simplicity in the basic requirements for the maintenance of a viable organism over extended periods, and secondly, because of a great mass of baseline data already collected from this organism in the earthbound environment.

The program thus far has been responsible for the successful development of single-specimen respirometric systems selected for flight in the Apollo Applications Program. Currently, development work focuses on multi-specimen systems more suitable for other potential space experiment applications. The progress of this work is described under paragraph 2.0, below.

In addition to these biorhythmic studies, a concurrent, second phase effort has been initiated to establish the feasibility of developing an experimental population of fishes which can be abnormally oriented relative to earth gravity, with the ultimate goal of relating the role of gravity to the regulation of biological systems. The progress of this program is reported in paragraph 3.0, below.

2.0 PROGRAM STATUS: PHASE I

2.1 General

During this reporting period, all respirometric development programs described in the contract are fully active. Further baseline data has been collected from the single specimen units and final assembly of the two multi-specimen has been completed. Continued problems in regulator hysteresis have led to a concerted effort to solve this persistent problem.

2.2 Technical

2.2.1 Single Specimen Respirometer

The single specimen respirometers have operated continuously for more than 10 months without breakdown. The tubers had to be replaced twice and the scrubbing units replaced five times over this period. A new specimen collection scheme has resulted in maintaining an active organism in the respiration units for four months or longer. Mechanical malfunctions have not been observed except for those periods when the scrubbing of CO_2 was appreciably impaired due to depletion of the scrubbing entity. At this time hysteresis in the second stage regulator becomes a major factor which perturbs the data appreciably. Performance of the new solenoid valve has been excellent with no valve malfunction occurring during the reporting period. The systems are at present closed down, not because of malfunction, but because the system is regarded as fully developed.* Furthermore, the amount of data being generated defies hand-analysis, considering the resources of Contract NASw-870. However, data collection and reduction (possibly by EDP modes) will be re-instituted soon.**

2.2.2 Collaboration with Other Experimental Activities

During the reporting period active collaboration with the Ames Research Center BIOSATELLITE program group was maintained. Data and drawings were supplied and hardware was loaned to NASA-ARC and their contractors, TRW Systems, Inc., and Northrup Aviation, Inc. In addition, the contractor participated in a symposium at NASA-ARC sponsored by Headquarters, NASA, OSSA, to discuss configuration of a post-AAP manned biological laboratory and implicating potato respirometric biorhythm experiments. All evidence indicates acceptance of single or multi-specimen units for flight aboard these vehicles.

---

* With the exception of the problem of second stage hysteresis, discussed elsewhere herein.

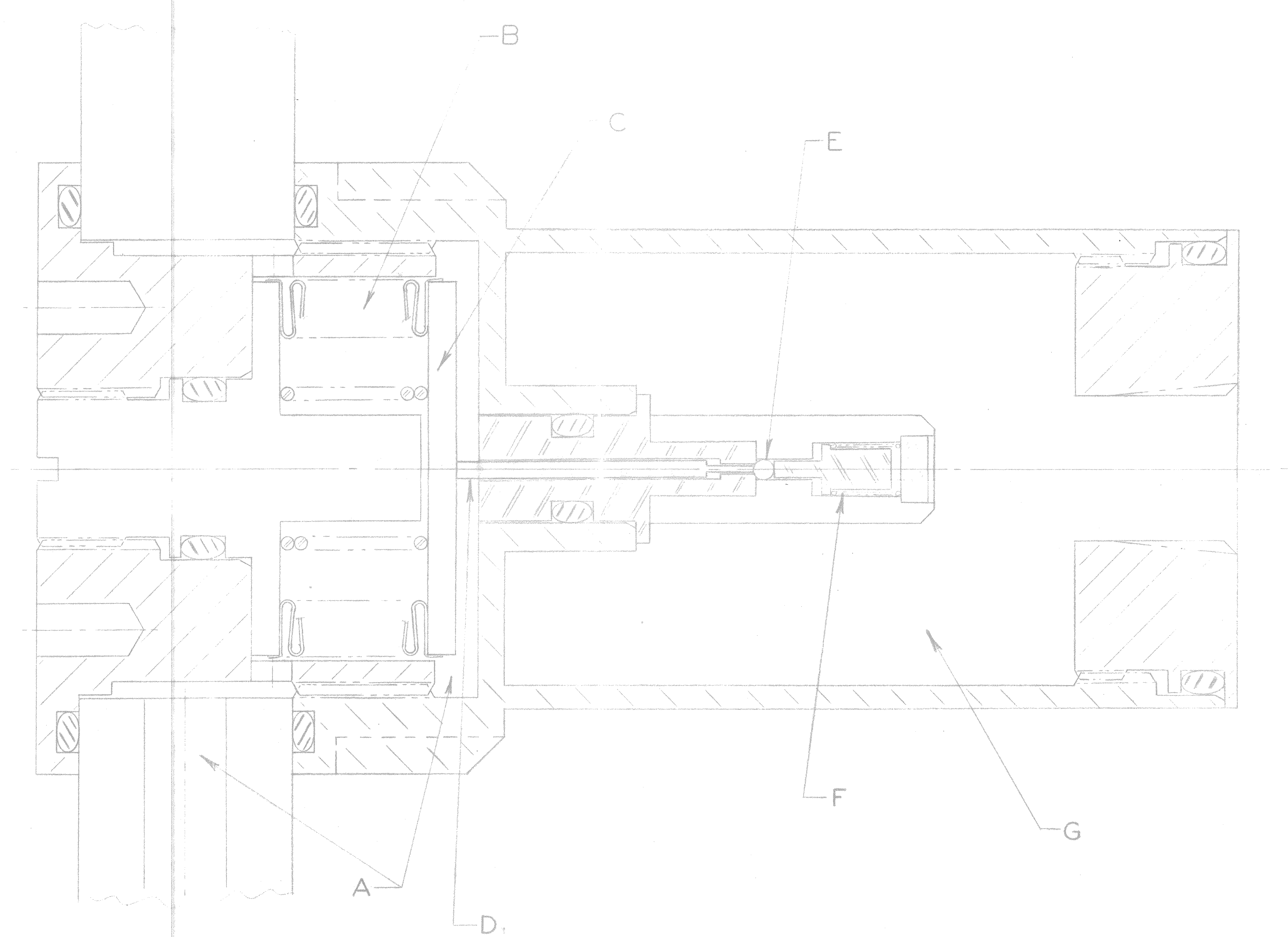
** Under the AAP Flight Hardware Contract, successor to NAS 9-7172.

2.2.3 Multi-Specimen Unit Effort

The second generation unit was assembled and put on bench test. Data from bench tests indicated that all systems were successfully operative, except that the second-stage regulators, during low demand periods, demonstrated the characteristic hysteresic behavior, perturbing the data quality. In the past, our approach to this intractable problem had been to redesign the deformable ("soft seat") seal used to assure the tight closures needed for high accuracy. Changes in seal configuration and seal materials had been helpful, but had not solved the problem to our satisfaction. Accordingly, when a final assault on the problem was planned we decided to abandon the "soft seat" concept and take a "hard seat" (metal to metal) approach. We recognized the problems inherent in this approach, but reasoned that if the regulator orifice could be kept small enough and the surfaces smooth enough, the problems might be avoidable. Accordingly, a design effort was launched, which has resulted in the configuration shown in Drawing S/D 121-300A, as Figure 1. As pressure at "A" decreases due to potato respiration, the constant pressure aneroid bellows "B" expands, pushing plate "C" up, bearing on rod "D", displacing tunsten carbide valve ball "E", (restrained in place by counter spring "F"). This allows oxygen under pressure to flow from reservoir "G", past the valve ball, rod, plate and bellows to "A". The critical interface is the metal-to-metal seal at "H" where the valve ball seats itself. If lapping tolerances at "H" are good enough and ball surface smoothness at "E" are satisfactory, no difficulties with this precision, low-flow

BRUNING 54865

REVISIONS			
LTR	DESCRIPTION	DATE	APP



QTY	PART NO.	DESCRIPTION	ITEM

MATERIAL		SPACE/DEFENSE CORP. 1600 North Woodward Ave. Birmingham, Mich. 48011
SPECIF		
TREATMT		
WT	SCALE 5:1	TITLE: O[illegible] REG[illegible]
NEXT ASSY		
MODEL		
DRAWN B. I	2-15-69	SIZE C / PART NO. 12[illegible] / REV
CHK		
APP		

UNLESS OTHERWISE SPECIFIED
TOLERANCES ARE:
ANGLES ±
TWO PLACE DIM. ±
THREE PLACE DIM. ±

regulator should be encountered. Fabrication of bench models has begun.

2.3 Plans for the Coming Period

Fabrication, assembly and test of the "hard seat" regulator will be accomplished. Performance at low flows will be compared to the soft seat device.

3.0 PROGRAM STATUS: PHASE II

3.1 General

To study the role gravity plays in regulating certain biological systems, teleost fishes were chosen because their orientation, relative to gravity, could be altered for prolonged periods of time. These lower vertebrates orient to gravity through integrating stimuli of external (visual) and internal (vestibular) origin. By surgical elimination of internal stimuli and altering the external cue, we hypothesized that this animal could be made to swim consistently in different orientations relative to the earth's gravitational force field, thereby providing a useful tool in studying the role gravity plays in neurohypophyseal hormone regulation.

Once fish can be made to swim at predetermined axes relative to gravity it is desired to determine the effects these altered orientations may have on the hypothalomo-hypophyseal system. The accurate assay of endogenous arginine-vasotocin in tissue sections taken from the hypothalamus, infundibulum and neurohypophysis of control and experimental fish must therefore, also be undertaken. To do this the following events must occur: arginine vasotocin must be synthesized; antibodies to the exogenous hormone developed; cross-reaction between endogenous hormone and exogenous hormone antibodies shown; and quantitative measurements made of changes in elaboration and/or transport of endogenous hormone in both control and experimental fishes.

As of the start of the reporting period we had successfully synthesized arginine vasotocin and have had limited success in inducing fish to persistently swim at altered angles relative to gravity. During the reporting period we continued work in both biochemical and surgical areas and the considerable progress is described below.

3.2 Technical

3.2.1 Development of the Test Animal

During this reporting period we have continued work with the angel fish (Pterophyllum scalare). Surgical procedural problems have been worked out with the assistance of Dr. Jelle Atema (of the University of Michigan group working under Dr. Rudolf J. vonBaumgarten). The removal of labrinthine structures is performed by making a small incision 3 mm behind each eye in fish anesthetized with MS222, and carefully removing the vestibular structures with dissecting forceps.

The entire procedure is done with the aid of a dissecting microscope. Within minutes the fish is swimming in a tumbling manner, completely disoriented. This behavior continues until the third day, when the fish begins orienting toward the single bright light illuminating the otherwise darkened aquarium. It is at this time the operated fish also begins eating and interacting with other fish in the aquarium. All behavior appears normal except for the orientation of the operated fish. As the light is incrementally moved around to the bottom of the aquarium, the operated fish again demonstrates disoriented swimming behavior, but soon "locks on" the light and swims inverted. The unoperated fish, of course, do not respond. Figure 2 illustrates the behavior of an operated fish continuously orienting to a light cue rather than gravitational stimuli. A labyrinthectomized fish is at center. Note the inverted position, relative to his unoperated fellows at upper left and upper right.

3.2.2 Biochemical Effort

During the reporting period a second synthesis of arginine-vasotocin was successfully performed in our laboratory, using the Merrifield technique. The derived material is highly active on the basis of preliminary assays, showing about 50 times the biologic activity of the oxytocin standard. Purification procedures have been devised and purification of synthesized material continues. A rabbit colony has been established and innoculation of these animals for antibody production has been started. An epidemic of "snuffles,"

Figure 2.

Labyrinthectomized fish swimming inverted in response to photostimulus from below. Note control fish swimming normally.

a lagomorphic adenovirus, caused the loss of the original colony but it appears that the second group is healthy.

We are currently preparing a paper for submission to the _Journal of Biological Chemistry_ describing this synthesis. In addition, a note will be submitted to _Copeia_ which briefly describes the synthesis of this posterior pituitary hormone and the significance of this work in providing material for studies in teleost endocrinology.

3.3 Plans for the Coming Period

(a) Antibody production will be continued by innoculating rabbits with purified synthetic arginine-vasotocin.

(b) Antibody presence in rabbit serum will be determined.

(c) Antibody purification and conjugation with Rhodamine B-200 will be attempted.

(d) Fish orientation studies will continue, attempting the use of rheotropic cues to augment the visual cue thus hastening the development of stabilized inverted swimming behaviors.

(e) Develop experimental plans for future phases of this research effort.

ACCESSION CUTOFF REPORT

ISSUE NO. (CSTAR) 10

ISSUE ASSIGNMENT CUTOFF DATE 4/28/69

ACCESSIONING CUTOFF DATE 5/1/69

TITLES ASSIGNED 400

TITLES TRANSFERRED (Includes pre-accession kills, rejects, & reassignments) 0

ACCESSION NUMBERS USED X69-14201 THROUGH X69-14600

ACCESSIONS NOT USED (At cutoff) 0

TOTAL ACCESSIONS (Including kills) 400

cc: E.E. Baker
W.L. Brown
T.T. Chapman
F.N. Cross
P.F. Eckert
J.W. Everett
B. Gilchrist
M.B. Hanes
M.G. Harris
A.P. Heizer
R.I. Hong
M.G. James
D. Kuny
H.A. Reger
C.W. Rusteburg
L.M. Schreiber
D.D. Thomas
J.J. Waldo
W.E. White