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TECHNICAL STATUS REPORT

Contract No. NASw-870

Period Nov. 4, 1965 to Feb. 4, 1966

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.

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I. INTRODUCTION

Under Contract No. NASw-870 with the National Aeronautics and Space Administration (Biosciences Programs), Space/Defense Corporation has been working on the development of two respirometer systems for use in the conduct of circadian rhythm studies in space. These systems are: 1) a system for use with a single specimen of the potato tuber, and 2) a system employing up to twelve specimens of this organism.

During the period covered by this Quarterly Progress Report (November 4, 1965 to February 4, 1966) the effort has been directed to the conduct of functional performance evaluation tests of the single-cell system, and to the design and fabrication of an engineering model of the multi-cell system. The results of these efforts have in the main been extremely encouraging in that the single-cell system appears to be performing well, while providing a resolution of the oxidative metabolism which is potentially much greater than anticipated. The design and development of the multi-cell system has proceeded to the fabrication stage of the engineering model of this unit, which is approximately 50% complete.

II. PROGRAM STATUS

A. General

During the period of November 4, 1965 to February 4, 1966 the efforts under the NASw-870 Program have proceeded in two directions. These are: 1) continuation of the performance evaluation tests of the single-cell respirometer system, and 2) initiation of the design and development of a breadboard multi-cell respirometer system. The effort devoted to development and performance evaluation of the single-cell respirometer has been reduced to a relatively low level, but is being maintained on a minimum effort basis in order that long term performance data may be obtained for application to the developmental effort of the multi-cell system.

B. Technical

1. Single-Cell Respirometer System

The single-cell respirometer system, Figures 1 and 2, developed during the initial phases of this contractual effort, has been undergoing essentially continuous functional operational testing for a period of approximately six months. The first potato specimens were placed on test in mid August of 1965 and a specimen is established in the respirometer at this writing (last week of February 1966). It must be noted that potato testing has not been continuous; interruptions have occurred for equipment adjustment and modification, also for potato replacement.

While maintaining an attitude of guarded optimism (cautious enthusiasm), the results of these system performance tests have been gratifying. There are, however, interrelated problems and external factors which must be examined in order to evaluate the test results and our overall progress to date. In the subsequent paragraphs some of these impinging problems

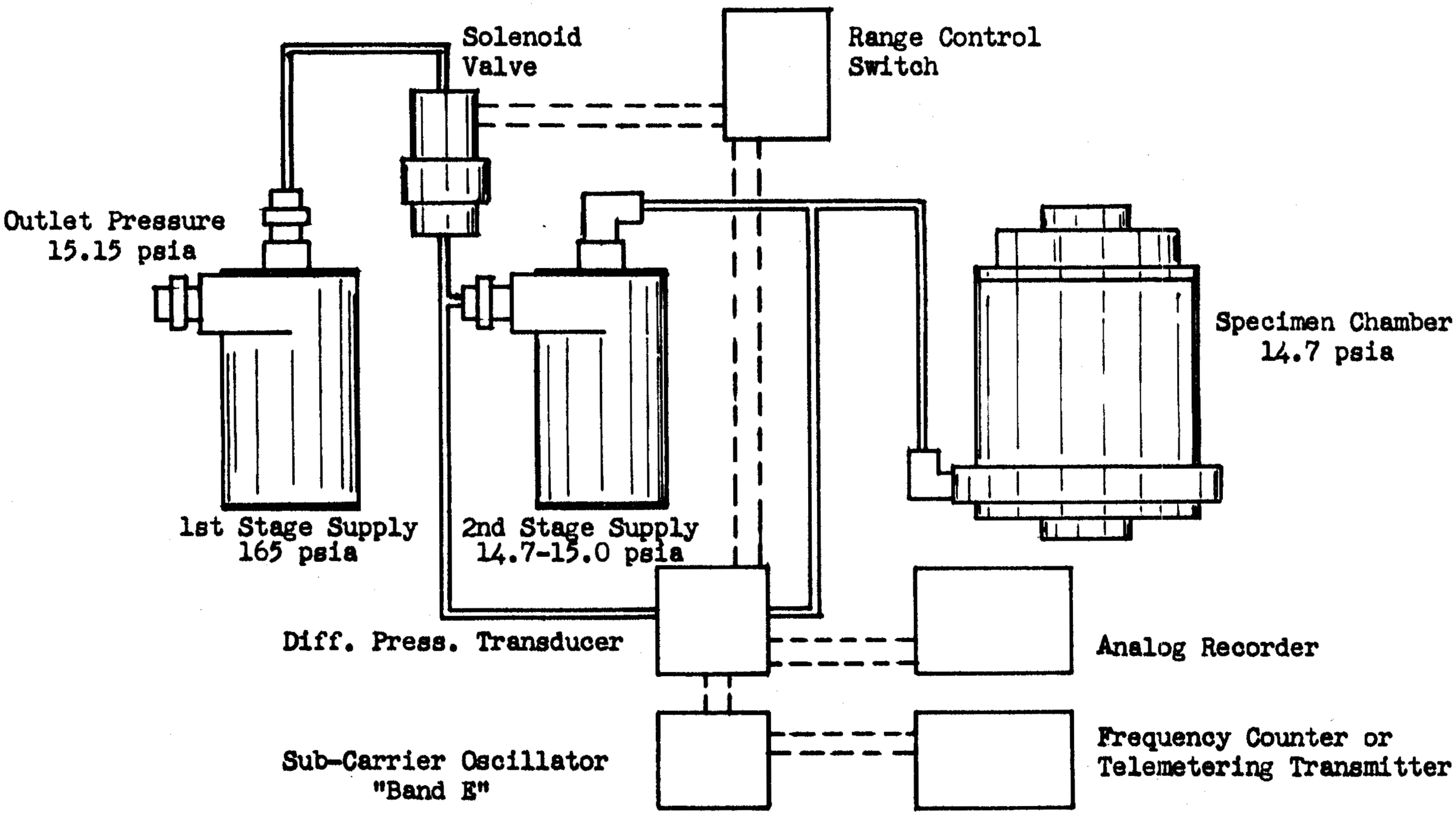

Figure 1. Schematic Diagram Two-Stage Resupply and Monitoring System

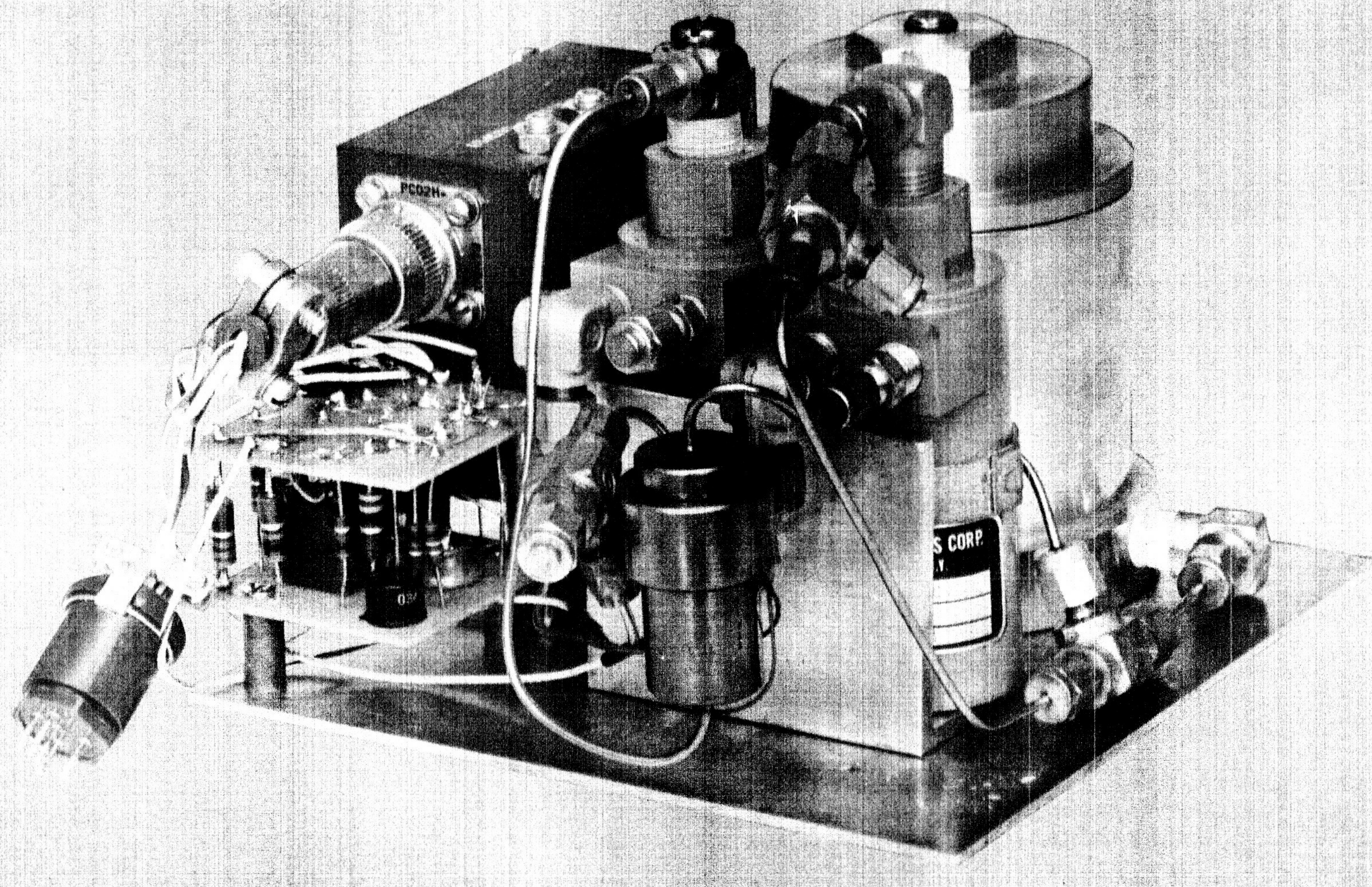

FIGURE 2. SINGLE-CELL RESPIROMETER SYSTEM

are discussed in order to establish our status and describe it as clearly as we can, based on our understanding and conclusions.

First, there is no doubt that the data derived from the tests proves that the respirometer works. Further, it has measured potato oxygen consumption with a resolution not, to our knowledge, elsewhere possible. This is shown by comparisons with respirometer control experiments in which a potato was not present. The results of these control experiments will be discussed later.

Second, there is little doubt that the variation in oxygen consumption does show definite periodicity. Typical data collected are presented in Figures 3 and 4. Figure 3 shows variations in potato oxygen consumption during three different calendar periods for different specimens. The observation periods were August and December 1965 and February 1966. Figure 4 provides a longer duration view of the potato metabolism variations observed in one specimen during a one month period during August and September 1965.

<u>Controls</u>. In the control portion of the test program the respirometer was placed into operation without a potato specimen being used. The results of these tests show the respirometer to be a minor contributor to the total oxygen consumption rate measured with a potato specimen in the chamber. Occasional "recharges" (the measure of O_2 consumption) were noted during "zero load" tests (conducted without an organism). These are attributed to measured ambient temperature

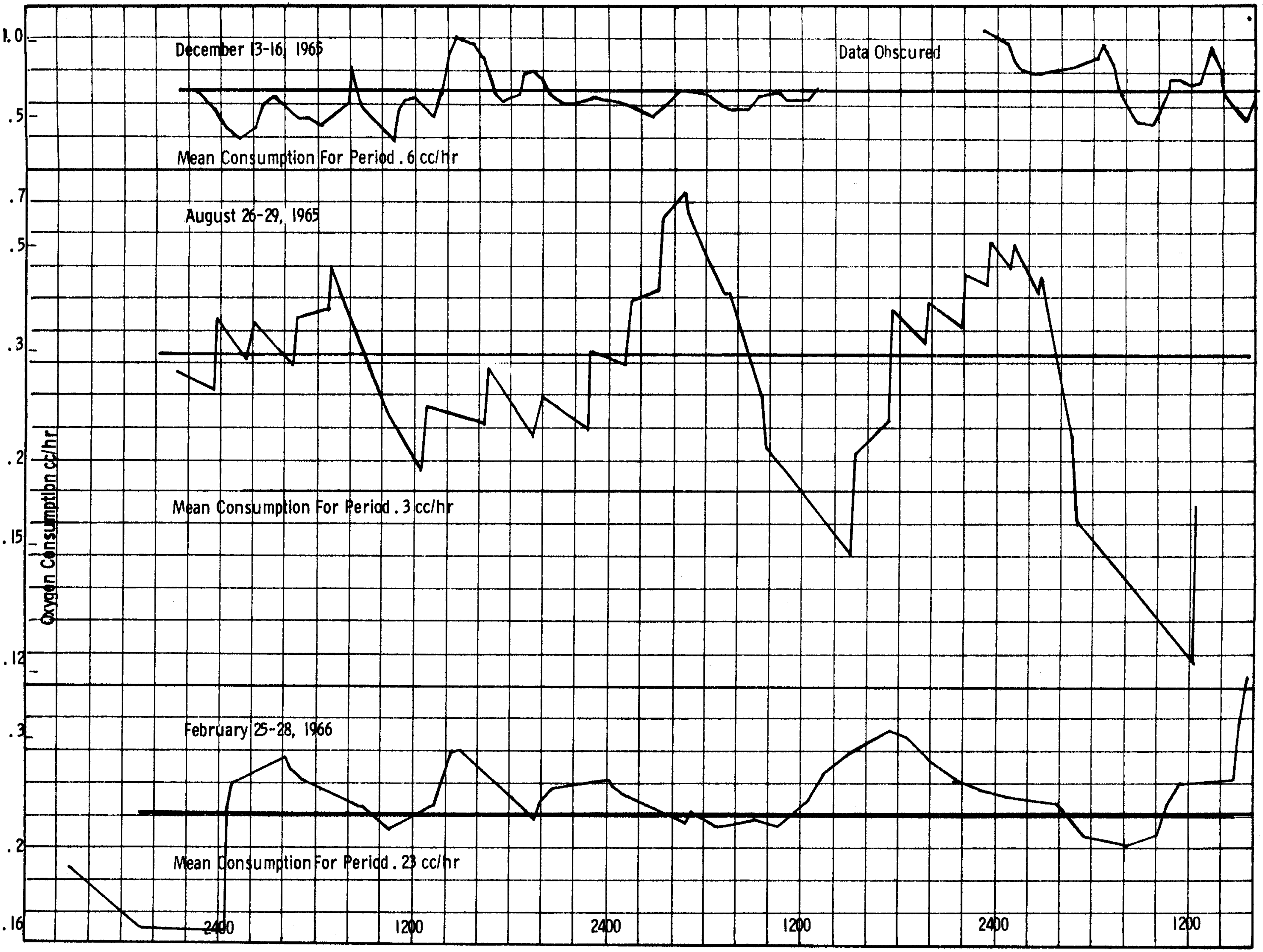

FIGURE 3. OXYGEN CONSUMPTION VS. TIME

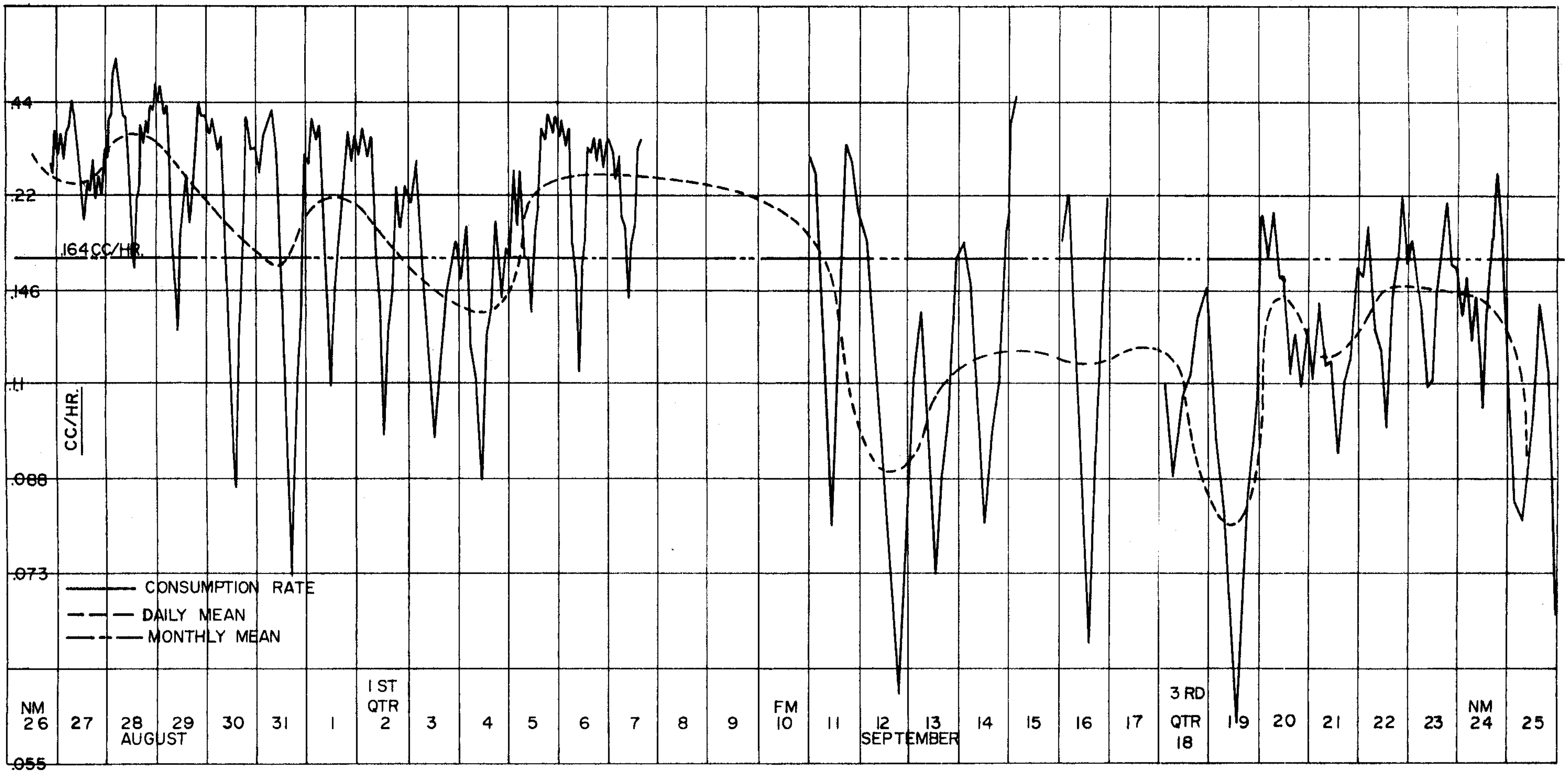

FIGURE 4. TYPICAL OXYGEN CONSUMPTION RATES

variations providing differential O_2 bottle heating because of the different absorptivity/emissivity ratios of the second-stage supply bottle and the specimen chamber. These differences are modest in our present system; for actual flight hardware only minor changes to achieve a better thermal balance will practically obscure this source of instrumental error.

Specimen Tests and Discussion. When a prepared potato sample is placed in the respirometer, respiration (both periodic and nonperiodic) is observed. Since the purpose of this program is to develop a tool which will permit the study of the changes in the metabolism rate of a potato specimen in the space environment, the validity of scientific interpretation of the data obtained is not of prime inportance, except as it has bearing on the credibility of the equipment function. Accordingly, for purposes of this test program, it has been assumed that close correlation of the test data with potato metabolism characteristics (as reported in the literature) would show acceptable functional performance of the respirometer. The fact that the data obtained to date does not quantitatively follow the averaged data presented in published works cannot, however, be interpreted to mean that the respirometer is not functioning as intended; nor can it be assumed that it is providing data of such greater accuracy that all other data may be inaccurate. Inescapably, the performance of the respirometer must be viewed in terms of the performance of the potato. But this presents a problem in data interpretation. Thus, there is a slight enigma.

Our respirometer design criteria were based on published data describing the mean oxygen consumption of the potato; therefore, the single-cell respirometer system was designed to provide a ninety-day supply for a 7 gram specimen using approximately .06 ml of oxygen/hour. The data obtained during performance evaluation tests of the system, however, has varied from approximately one order of magnitude above this averaged rate to less than 1/3 of this rate. This apparently excessive variation can be interpreted either to mean:

(1) that the respirometer is leaking (high use rate) or

(2) not measuring it all (low use rate) or

(3) that changes in the viability of the specimen is responsible (high use = necrosis; zero use = death).

Based on these alternatives, the tests have been interrupted periodically to check these possibilities. The result of these checks is that the assumptions were normally found to be either false or unconfirmable. Therefore, based on our interpretation of the limited data obtained, it is assumed (and our conclusion) that the respirometer is, in fact, <u>measuring variations in single-specimen metabolisms not previously possible</u>.

The discrepancies between our measurements and the published literature may well be attributable to differences in the sample base. The hourly variations recorded by Brown, Barnwell, et al, were based upon the algebraic sum of the activity of at least twelve and usually fifteen specimens. With this population, variations of 35% were seen. With the apparent ability to measure with high resolution the activity of a single potato specimen, it is not difficult to conclude that we are observing the individual excursions around the mean of .06 ml/hr. which was responsible for the 35% variation seen by other workers.

The second variable noted was the substantial difference between the Fall and Winter tests. This is probably attributable to the (published) differences in seasonal O_2 consumption, rather than instrument variabilities. In these studies maximum rates are seen in the Fall (August - November) and minima are observed in late Winter to early Spring (January - March). Since we are working with a single-cell device, it is possible that statistical distribution provided us with specimens in both Fall and Winter that had high and low rates at the extremes of the normal ranges. Now, let us consider the following factors:

(1) statistical distribution;

(2) the greater resolution of the respirometer;

(3) the single-specimen constraint;

(4) the seasonal extremes;

(5) the relative paucity of our data (compared

to the years of data upon which comparison is based); and

(6) the wide variability in consumption rates of the individual specimens, and the still wider variability between different specimens.

When the above factors are all considered and the metabolism data is evaluated only for indications of rhythmicity, there is excellent reason to believe that our data can be correlated with previously published work.

Accordingly, although the performance evaluation tests to date cannot be considered as representing a rigorous and absolute determination of system functional acceptability, we believe that they do provide a reasonable basis for assuming that the single-cell respirometer is a satisfactory instrument for conducting the studies for which it was intended. In our opinion, more data will almost surely substantiate this contention.

Problems. During the performance evaluation tests, we have encountered difficulty in maintaining specimen viability. The degree of success in maintaining the specimen in a living state seems to be a function of the elapsed time following harvest of the potato. The longer the time after harvest, the greater the difficulty in maintaining viability. It is our present belief that a better potato selection procedure will reduce this problem substantially.

Because of the extremely small quantities of oxygen which must be measured to monitor the oxidative metabolism of a single 7 gram potato specimen, it is readily apparent the even very minute leakages, either internal or external, will be of very great significance in the interpretation of data obtained. Our early recognition of the extreme importance in achieving essentially leak free seals and closures of valve seats has resulted in a constant perusal of the data for indications of leakage within, or from the system. Such problems have, on occasion, been found to result in significant perturbations upon the oxygen consumption rates. Since malfunctions of this type are immediately recognized, however, there is little chance of the perturbed data being accepted as valid information. In almost every instance the problem has been associated with either the first- or second-stage regulator seats, or the solenoid valve seat. These seats each utilize Dow Corning Type RTV-881 Silastic compound, selected because of its basic inertness in an oxygen atmosphere and its ease of deformation with very light loads applied. However, the material has been found to develop a memory which causes a permanent deformation at the point of contact. This, in turn, causes seat leakage when minor displacements of the sealing surface occur. The only satisfactory solution to this problem, up to this time, has been to replace the seat material. A continuing effort is being made to identify a material which will provide a positive seal, yet will exhibit (essentially) total resilience when the sealing force is removed.

In summary, there are minor deficiencies with our present single-cell respirometer; these appear correctible for a flight unit. Other minor packaging improvements will also be made to the device, as shown in Figure 2, for flight use. Of greatest significance, however, is the fact that we are measuring metabolism.

2. Multi-Cell Respirometer System

The design and development of the multi-cell respirometer system has progressed to the stage that hardware fabrication is approximately 50% complete. It is expected that the engineering model of this unit will be completed and system checkouts initiated by early May 1966.

The engineering model, as it has been designed, will employ a common primary oxygen supply located in the center of the respirometer package. In this initial configuration, oxygen from this source will be supplied to a single second-stage supply from which oxygen will be furnished to the twelve (12) individual potato specimen chambers which will, in this configuration, be interconnected. However, the multi-cell system package has been designed, and our engineering model fabricated, with cavities for twelve independent second-stage oxygen sources provided in the respirometer body.

The initial configuration of the respirometer (with the specimen chambers interconnected) has been selected primarily to minimize initial costs, because of the relatively high cost of second-stage outlet pressure regulators. While making maximum use of program funds, this decision will permit a reasonable functional evaluation of the multi-cell system while monitoring the rate of oxygen consumption of twelve specimens on an integrated basis. If initial tests are satisfactory, it is planned for the next configuration of this system to incorporate the eleven additional second-stage outlet pressure regulators. In this latter configuration the oxygen consumption rates of the twelve specimens can be monitored individually by use of a commutating valve which will sequentially sample the pressure differential between each second-stage supply and its associated specimen chamber.

As a part of the multi-cell respirometer system design, after a trade-off analysis of the factors, a decision has been made to modify the second-stage refill technique used in the single-cell system. The benefit will be conservation of electrical power.

The single-cell refill technique uses a solenoid valve placed between the first-stage outlet and the second-stage inlet. This valve is energized by a special solid state limit switch which opens the valve when the signal from the differential pressure switch approaches 0 volts and closes the valve when this signal reaches a level of 5 volts. The principal reason for this modification is to reduce the amount of electrical power which will be required for operation of the respirometer system in the twelve independent specimen configuration. The modified design incorporates special pneumatic valves positioned between the primary supply and the second-stage supplies of oxygen. These valves sense the absolute pressure of the second-stage supply and permit regulated pressure from the primary supply to flow into the secondary supply until an absolute pressure of 15.0 psi is present in that bottle. When this pressure level is reached, the pneumatic valve closes and will remain closed until secondary supply pressure drops to 14.75 psia at which time it will again open to refill the second-stage bottle.

The design of this second-stage fill valve has been completed and units are now in the fabrication stage. Because of the relatively small additional cost involved in producing a limited quantity of these valves, rather than merely one, we have decided to fabricate all units which will be required for the twelve individual specimen (subsequent) configuration of the multi-cell system. While it is recognized that the first engineering models of our new valve may not function exactly as expected, the design of this valve is such that problems which might arise will be capable of resolution without major engineering changes. Therefore, this decision is believed to be justified.

III. PLANS FOR THE COMING PERIOD

In the coming period, our efforts will be aimed primarily toward completion of the engineering model of the multi-cell respirometer system, then the initiation of preliminary testing and functional evaluation procedures. Secondary level efforts will continue the functional performance evaluation tests of the single-cell respirometer. As a portion of the effort related to both systems, we expect to locate an improved material for use at the internal seal interfaces.

It is exciting to contemplate --- during the month of June --- the possibility (and our aim) to conduct simultaneous comparative bench tests of the two potato respirometers. The single-cell version (Mark I) will be producing metabolism data descriptive of one specimen; the multi-cell (Mark II) will yield an integrated total reflecting the metabolism of twelve specimens. The comparative results of these tests are eagerly awaited.