SG 1095R-3

AUTOMATION OF A TECHNIQUE FOR DETERMINING BACTERIAL SENSITIVITY TO ANTIBIOTICS

October 1967

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FOREWORD

This is the second Quarterly Formal Progress Report, designated SG 1095R-3, on Phase II of a Program for Automation of a Technique for Determining Bacterial Sensitivity to Antibiotics. It covers work performed under Project-Task Number 775401, Contract Number F41609-67-C-0007, by Space-General, a Division of Aerojet-General Corporation, 9200 East Flair Drive, El Monte, California 91734. The Air Force Technical Monitor is

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The period of work covered by this report is 16 July 1967 through 15 October 1967.

The following personnel contributed to the program during this reporting period:

Technical (Space-General)

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Mr. C. C. Vafiades

Consulting

Mr. Garst Reese (University of Southern California)
Dr. John Platt (Private Medical Practice)

This work was performed in the Chemical and Biological Systems, of which Dr. E. Mishuck is Director. Mr. L. T. Carleton is Program Manager.

ABSTRACT

An automated antibiotic sensitivity test breadboard based on the tube dilution principle was fabricated according to designs prepared in the preceding quarter. The completed instrument is housed in two units:

- An incubator and tube transport unit, in which samples are temperature-conditioned and their turbidities measured periodically by light scattering, and
- A console, which contains in separate compartments (a) the tube - transport control unit and a program selector for recording turbidities, (b) a tape printer, (c) logic for processing and interpretation of information, (d) a core memory, and (e) power supplies.

The instrument and requisite biologicals are available for scheduled operations with pure and mixed cultures and chemical specimens.

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ILLUSTRATIONS

Section 1

INTRODUCTION

The purpose of this program is to develop an automated breadboard instrument for determining the sensitivity of bacteria to antibiotics. The instrument, like the manual tube dilution test, operates on liquid samples in test tubes, but provides for more precise and informative data, as well as multiple simultaneous tests and many other advantages of automation.

The program for accomplishing this objective is divided into two phases. Component tasks of these phases are shown in Figure 1. Phase I, Laboratory Studies, was completed on schedule at the end of the fifth month and a Final Research Report issued (Reference 1). Phase II, Breadboard Development, began immediately following completion of Phase I.

Earlier reports of this series (References 1 and 2) recorded the completion of Phase I and of Task 1 (Design) of Phase II. These reports described in detail the laboratory investigations on monitoring numerous bacterial growth curves by light scattering in the Lindberg-Reese automated research instrument, as well as indicating the inhibitory effects of antibiotics in many systems. In addition, the reports discussed the design concepts for the new breadboard, based on the earlier instruments, and presented engineering drawings of mechanical components and electrical circuitry.

Accomplishments during the present report period, extending from the third through the sixth month of Phase II, include the completion of Task 2 which covered the construction and assembly of the breadboard. With respect to Task 3, Laboratory Studies on Pure and Mixed Cultures, only planning and preliminary experiments were possible while the breadboard instrument remained uncompleted. This task will be projected further and will to some extent overlap Task 4, Laboratory Studies on Clinical Specimens. No difficulties are anticipated in fully completing Phase II within the originally scheduled time.

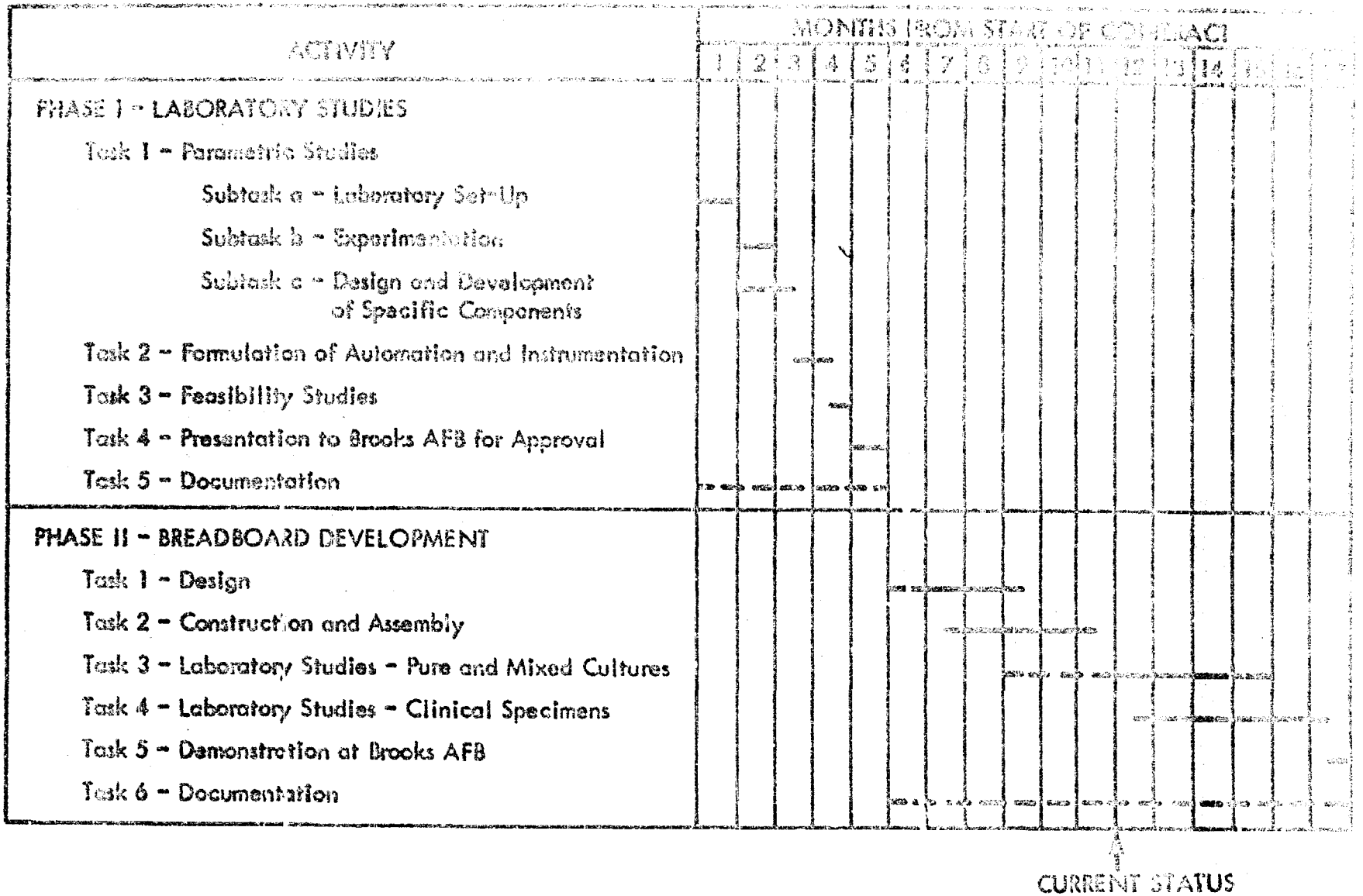

Figure 1. Program Schedule for Automation of Antibiotic Sensitivity Testing

Section 2

TECHNICAL STATUS

2.1 DESIGN, CONSTRUCTION AND ASSEMBLY OF BREADBOARD

Following completion of the mechanical and electrical design described in the preceding quarterly report (Reference 2), the breadboard instrument was constructed and assembled.

Like its predecessor, the Lindberg-Reese research instrument utilized in Phase I, the breadboard consists essentially of two units:

- An incubator containing a sample transport mechanism which positions each tube in turn in an optical beam for measurement of turbidity.
- An enclosure housing the electrical circuits which amplify and interpret the resulting signals from the detecting photomultiplier tube, and provide all necessary controls.

2.1.1 INCUBATOR/TUBE TRANSPORT MECHANISM

The incubator/tube transport mechanism was fabricated and finished on outside subcontracts. The mechanical and structural parts were first fabricated according to engineering drawings shown in the preceding quarterly report (Reference 2) and were then assembled and fitted. The mechanism was then disassembled, and surfaces of the box and other aluminum components were sent out to be hard-anodized, prior to final assembly.

The completed unit is assembled in a small, separate box, as shown in Figure 2. The top of the box holds 20 aluminum modules, each with a capacity of two test tube samples (Figure 3). During a 15-minute cycle, the mechanism shifts each test tube by mechanical motion of the modules in such a manner that the contents are stirred by a stirring bar enclosed in the tube and, subsequently, viewed by a light beam to determine the light scatter or turbidity. The beam enters and leaves the tube through accurately aligned slits in the sides of the module. Figure 4 illustrates the pattern of motion of the modules which permits

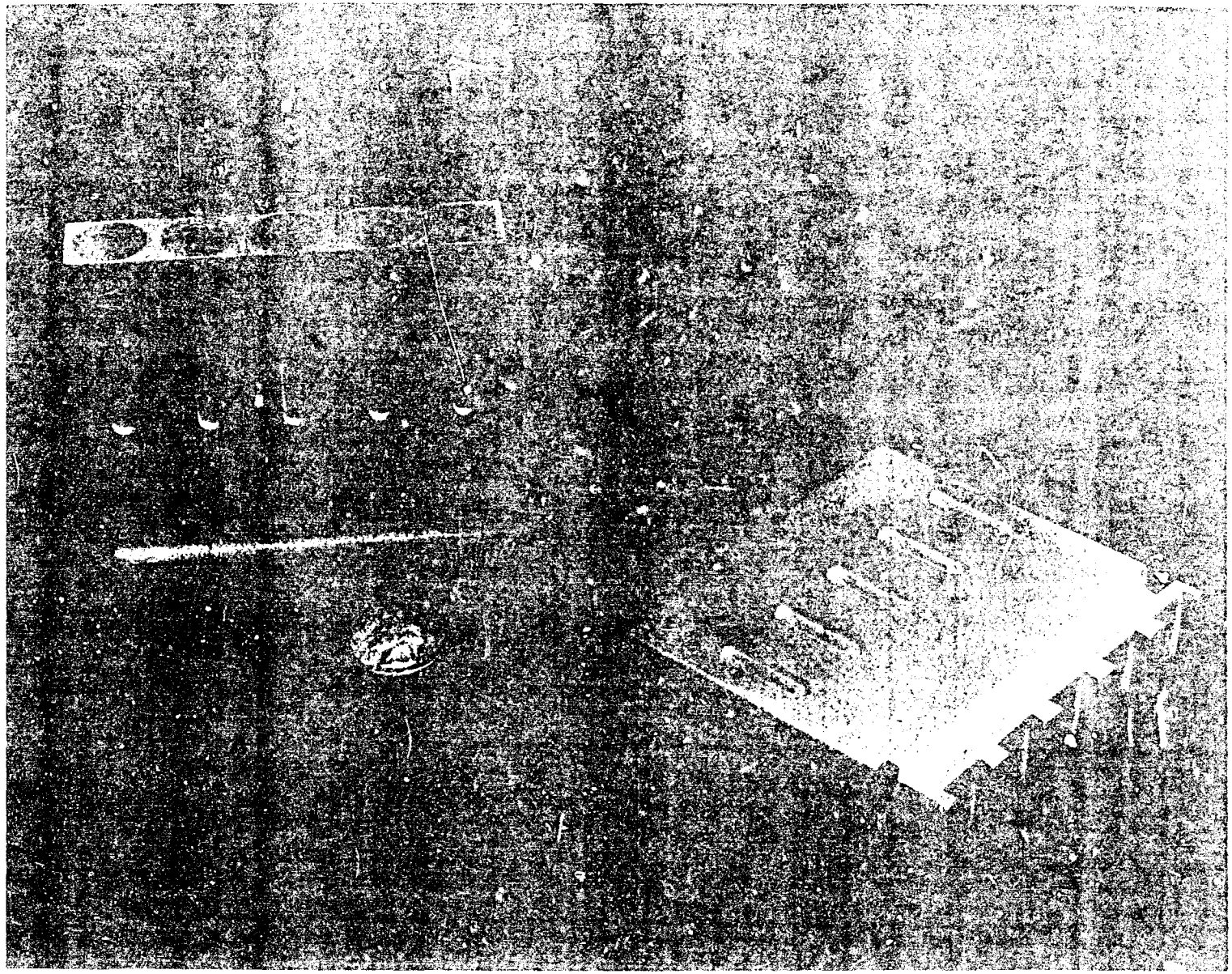

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Figure 3. Modules for Holding Sample Tubes

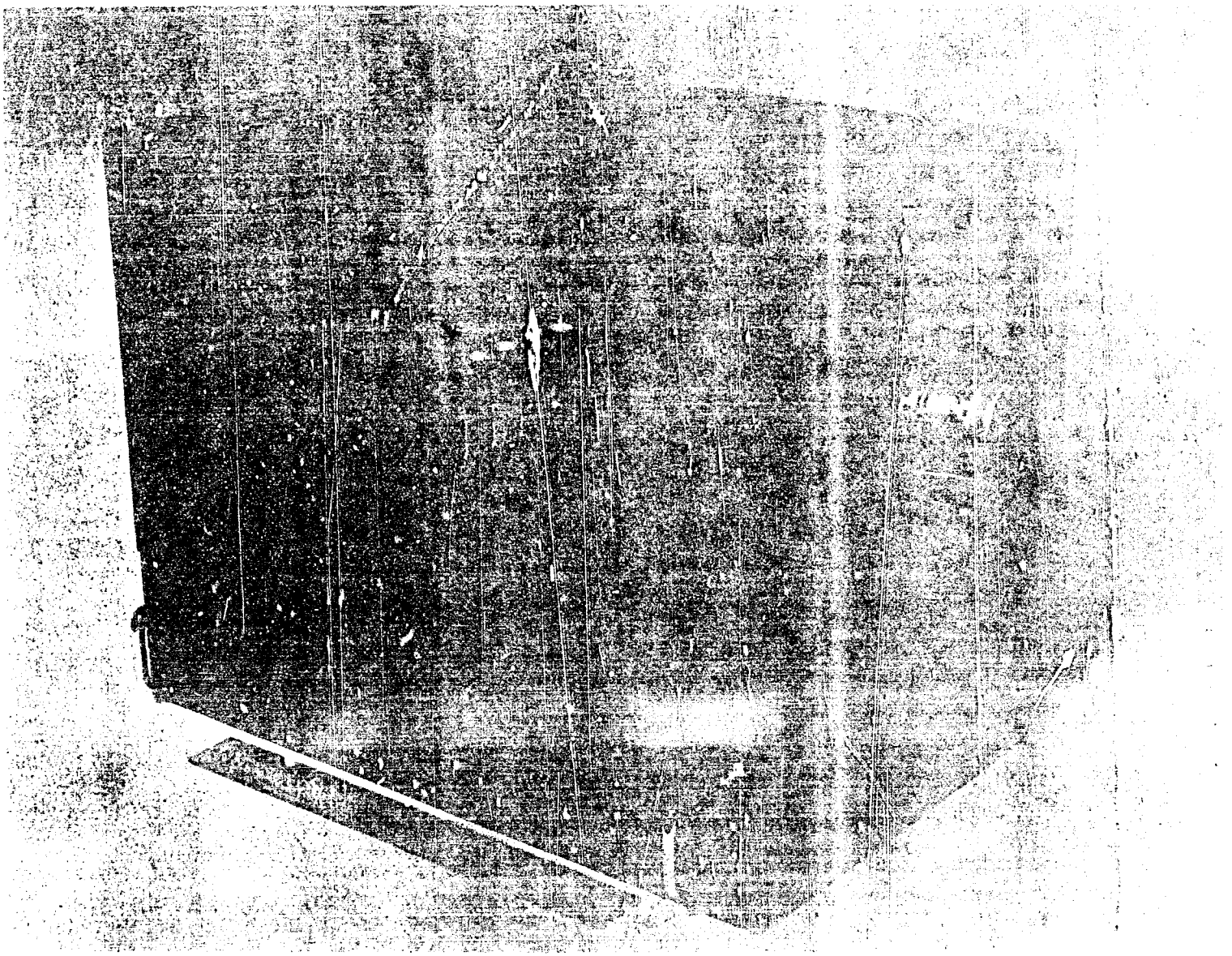

Figure 2. Assembled Incubator/Tube-Transport Mechanism for Antibiotic Sensitivity Test Breadboard

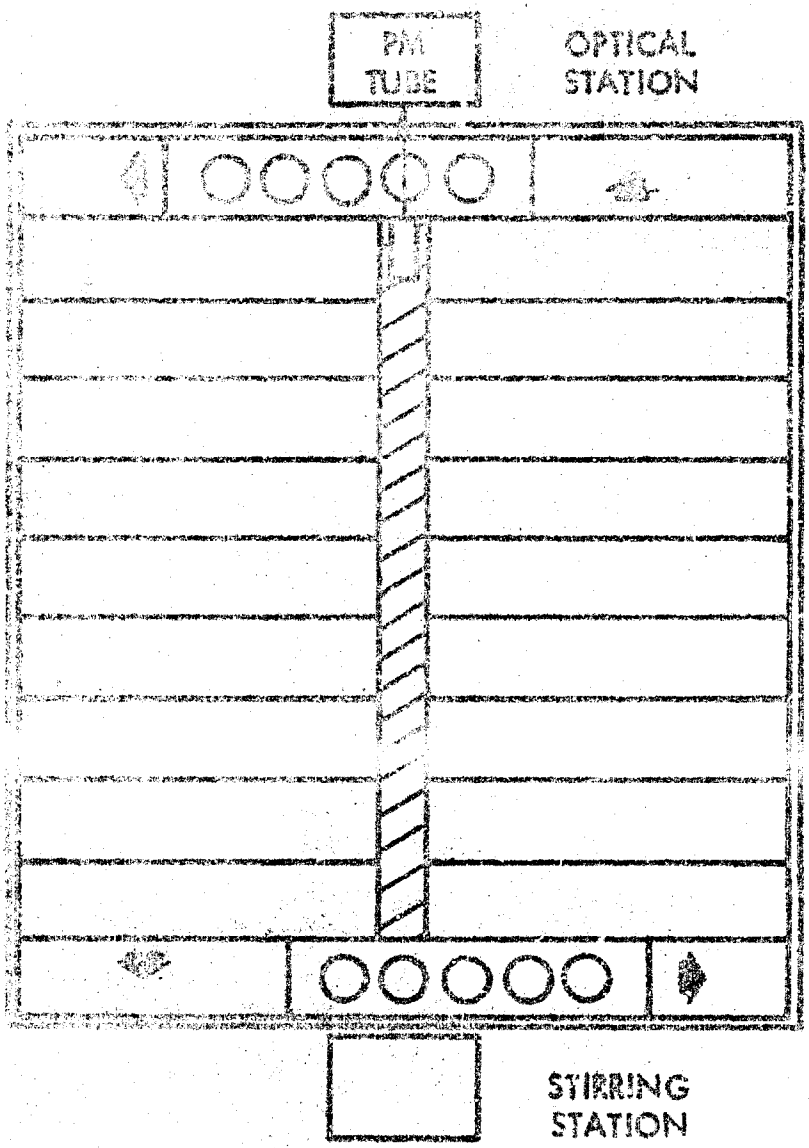

Figure 4. Pattern of Motion of Modules in Incubator

all test tubes to pass through the cycle in sequence. The modules are moved transversely by pusher rigging through the floor, and lengthwise by push rods inserted through the walls, as described earlier (Reference 2).

Under the horizontal floor on which the modules rest is a compartment for the temperature regulator. This consists of heating coils controlled by a thermistor in a resistance bridge circuit. The normal temperature setting is 37°C for the sample compartment, controllable within ± 0.1°C.

2.1.2 CIRCUITRY IN CONSOLE

An Electronic Enclosure console, 57 inches high, 23 inches wide and 31 inches deep (Figure 5), comprises the second major enclosed unit of the system. It houses electrical circuitry and equipment for control and for processing, storage and output of information. The means of operation is illustrated in the generalized block diagram, Figure 6, while detailed circuit diagrams are given in the preceding quarterly report (Reference 2). These circuits and electrical components were connected and installed in the console in the Space-General shops.

With respect to utilization of space in the console, as the figure shows there are five separate drawers and rack compartments.

The tube-transport control unit and the card drum assembly occupy the top compartment. They are shown removed in Figure 7. The tube-transport control unit responds to indications from microswitches in the incubator box. When these switches are depressed by completion of the transverse movement of the two modules at opposite ends (across the stirring station and optical path, respectively), then all modules are aligned in two parallel rows of 10 each. At this point, the control unit actuates the air-driven pushers to shift the rows in opposite directions, bringing new modules into position for transverse indexing. The card drum assembly provides the program for selecting the scale of turbidities and deciding the intervals at which changes are printed out. Instructions are punched in code on a standard Hollerith computer card, which is fastened around the Lucite drum. An elongated incandescent lamp is positioned on a line outside the drum and parallel to its axis. As the drum rotates, a line

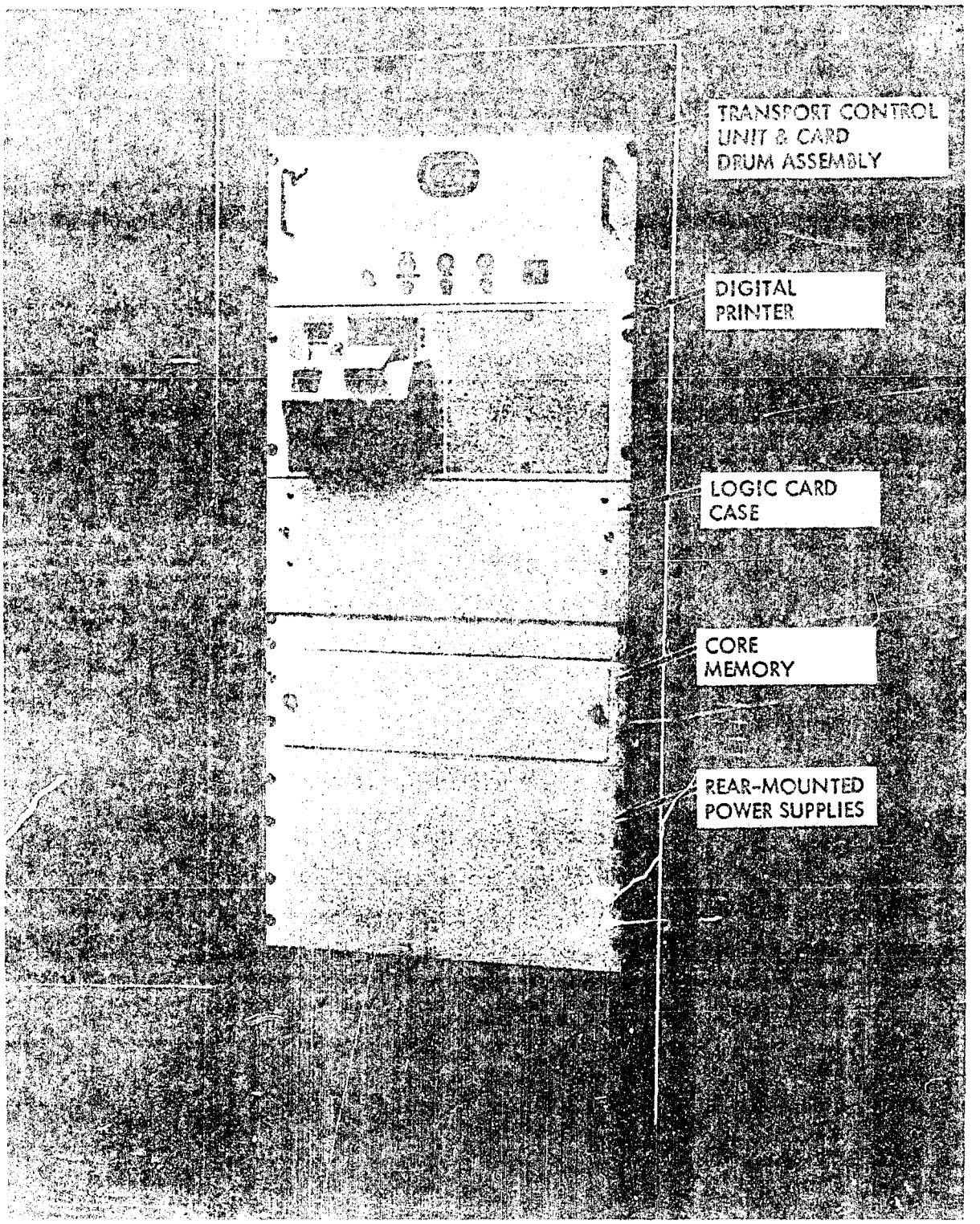

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Figure 5. Console of Antibiotic Sensitivity Test Breadboard

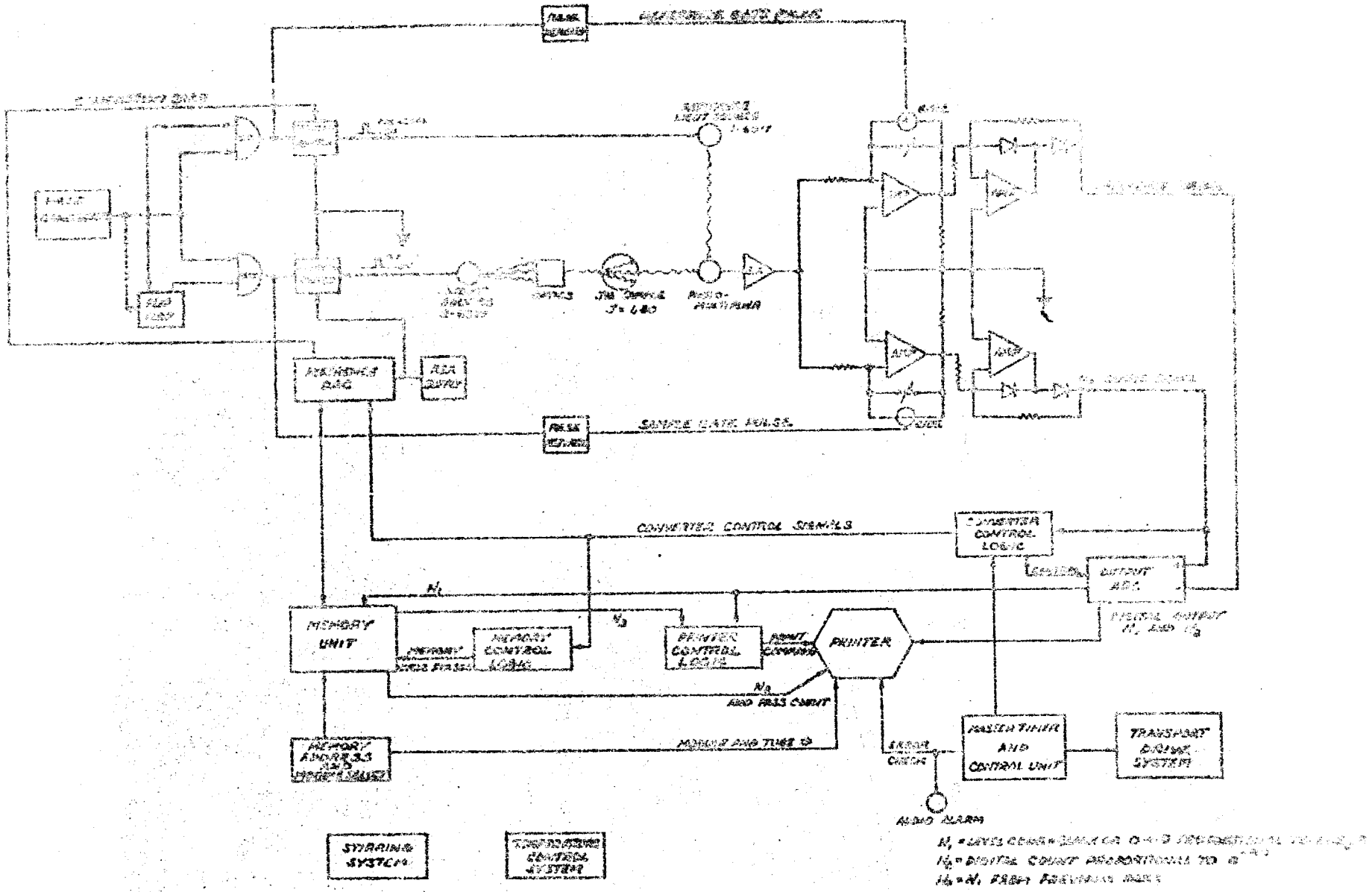

Figure 6. Generalized Circuit Diagram for Antibiotic Sensitivity Test Breadboard

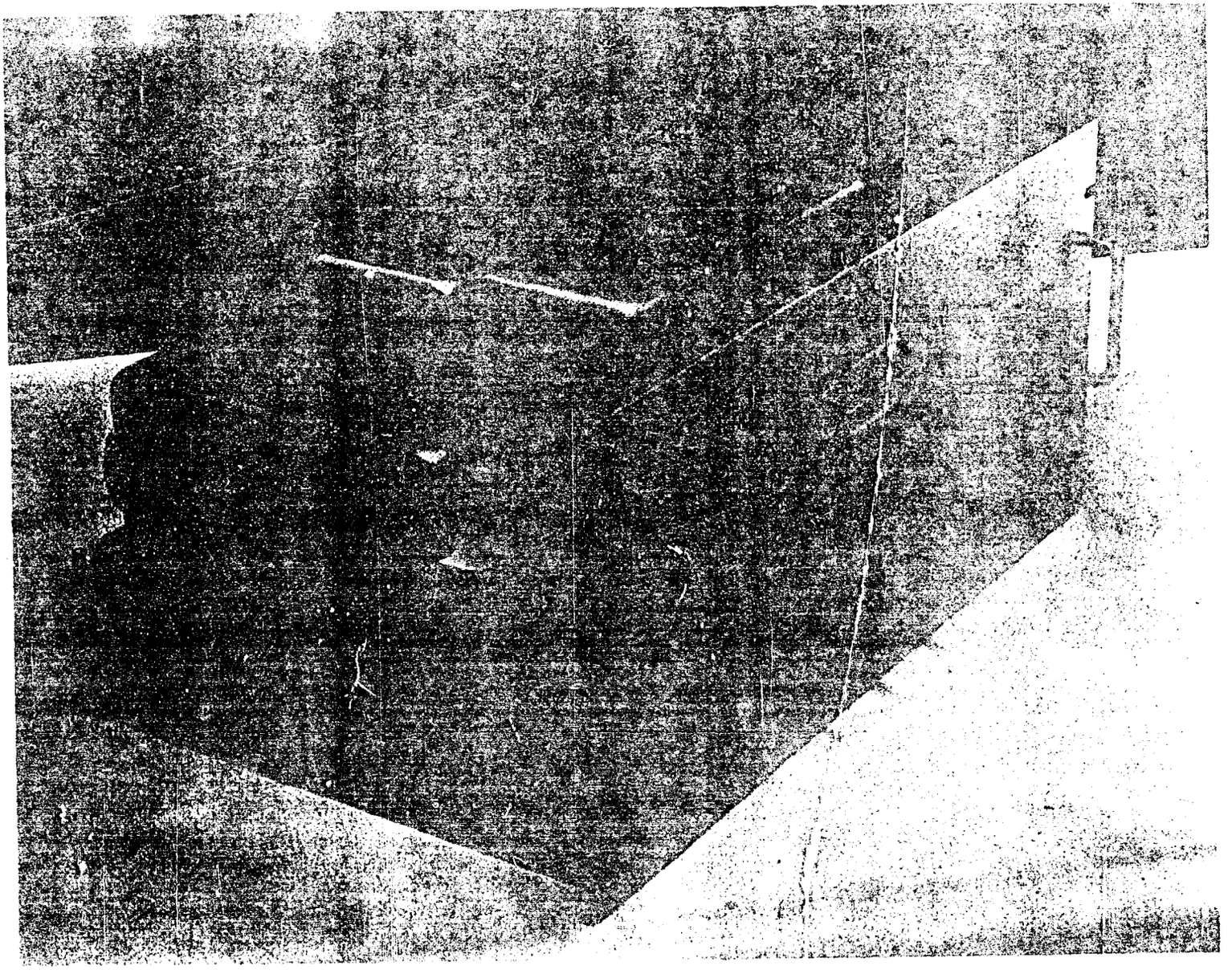

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Figure 7. Tube-Transport Control Unit and Card Drum Assembly (Removed from Console)

[illegible] from the scan of rotation picks up and relays the signals from [illegible] holes.

[illegible] 10-line digital printer, Model 1653, occupies the second compartment from the top. It is shown, removed from the console, in Figure 8. The printer prints information on (1) the module number, (2) the number of the tube within the module, (3) the previous level count, (4) the current level count, (5) the pass number for the module, and (6) the voltage, expressed as the digital count in the output ADC in octal format. For example

> 13 02 15 1665 would indicate that module 13, tube number 4, had gone from level 5 to level 6 during the 15th pass, and the ADC reading was 1665_8.

The third compartment from the top houses the logic unit in the form of a card case. It is shown opened (and with most of the plug-in cards removed) in Figure 9. This grouping of components includes the clock pulse timer which regulates the power input to the reference and sample light sources. It also includes circuitry for amplifying and comparing photomultiplier signals, and the digital-to-analog converter which converts a digital voltage setting stored in the memory to the proper bias for the reference light source. The printer control logic and memory control logic are also located here.

Underneath the logic unit, in the fourth compartment, is the core memory. This is a Ferroxcube FX 12[F], 512-word memory. It is shown in Figure 10. As directed by the logic unit, the calibration settings, level counts, and module pass numbers are written into or read from this memory.

Power supplies for the circuits and for heating the incubator and controlling the tube-transport mechanism occupy the lowest compartment. The space is cooled by an electric fan. These components are visible, in part, in the rear view of the opened console, Figure 11.

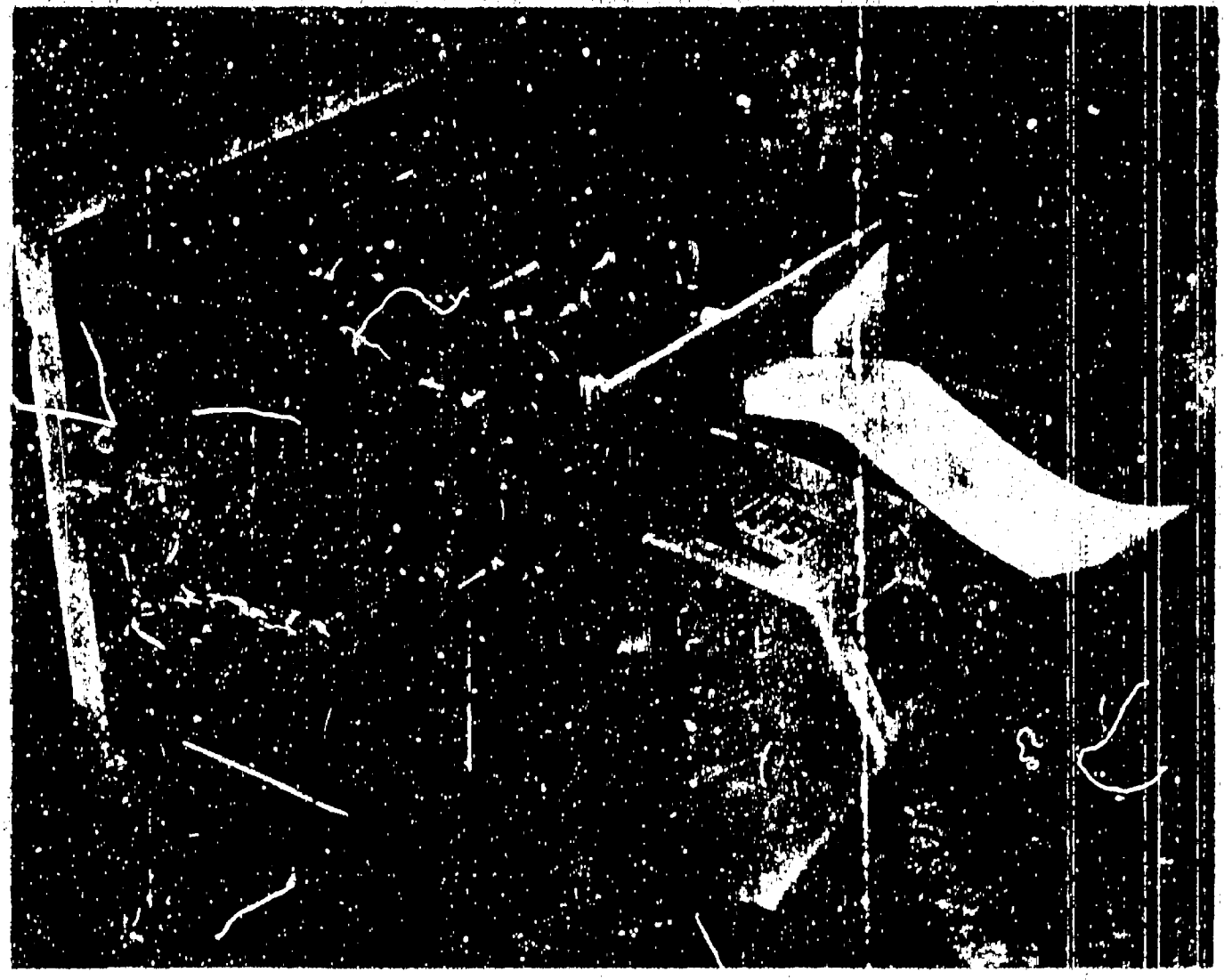

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Figure 8. Beckman Printer Extended from Console

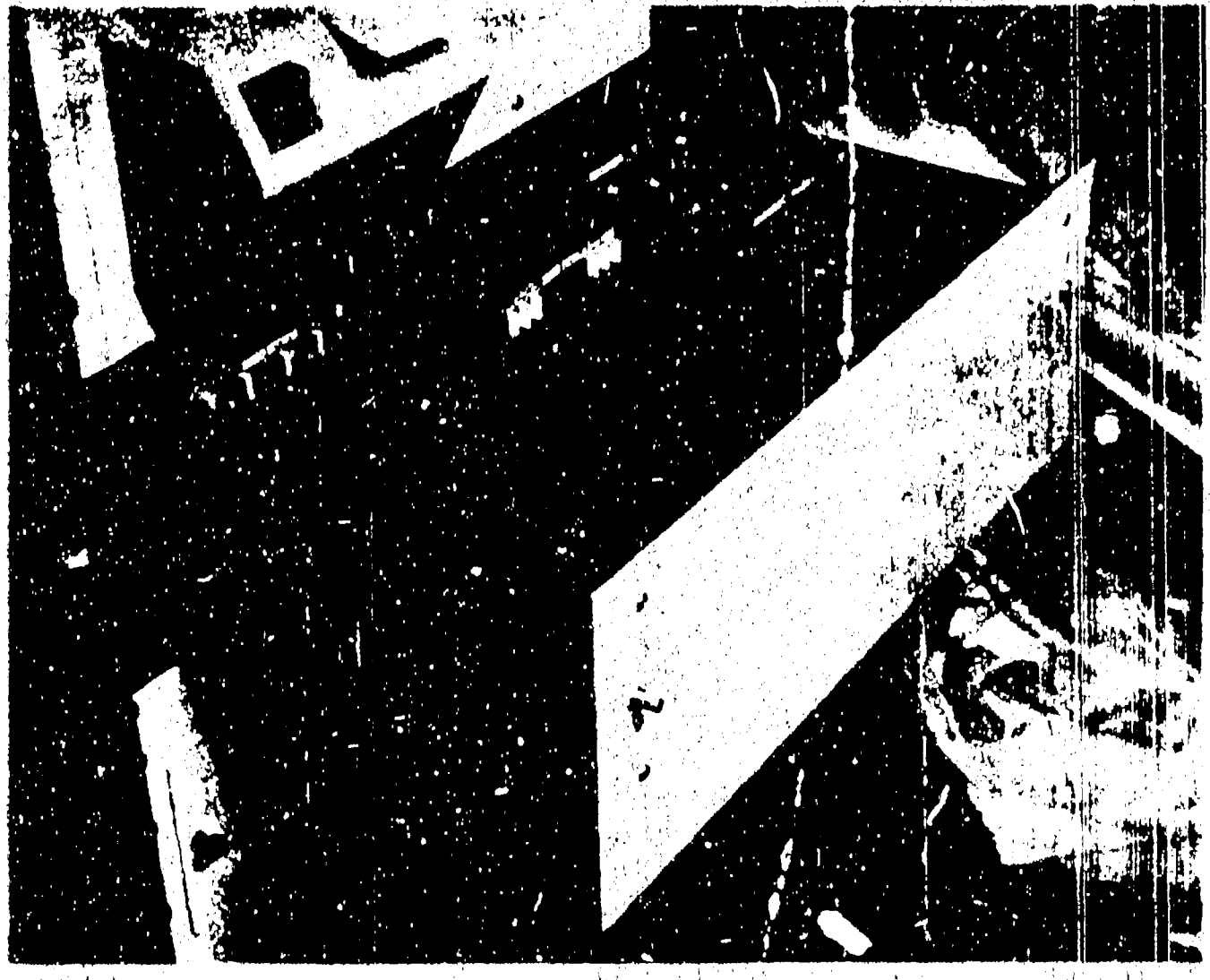

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Figure 9. Logic Card Case Extended from Console (with Most Cards Removed)

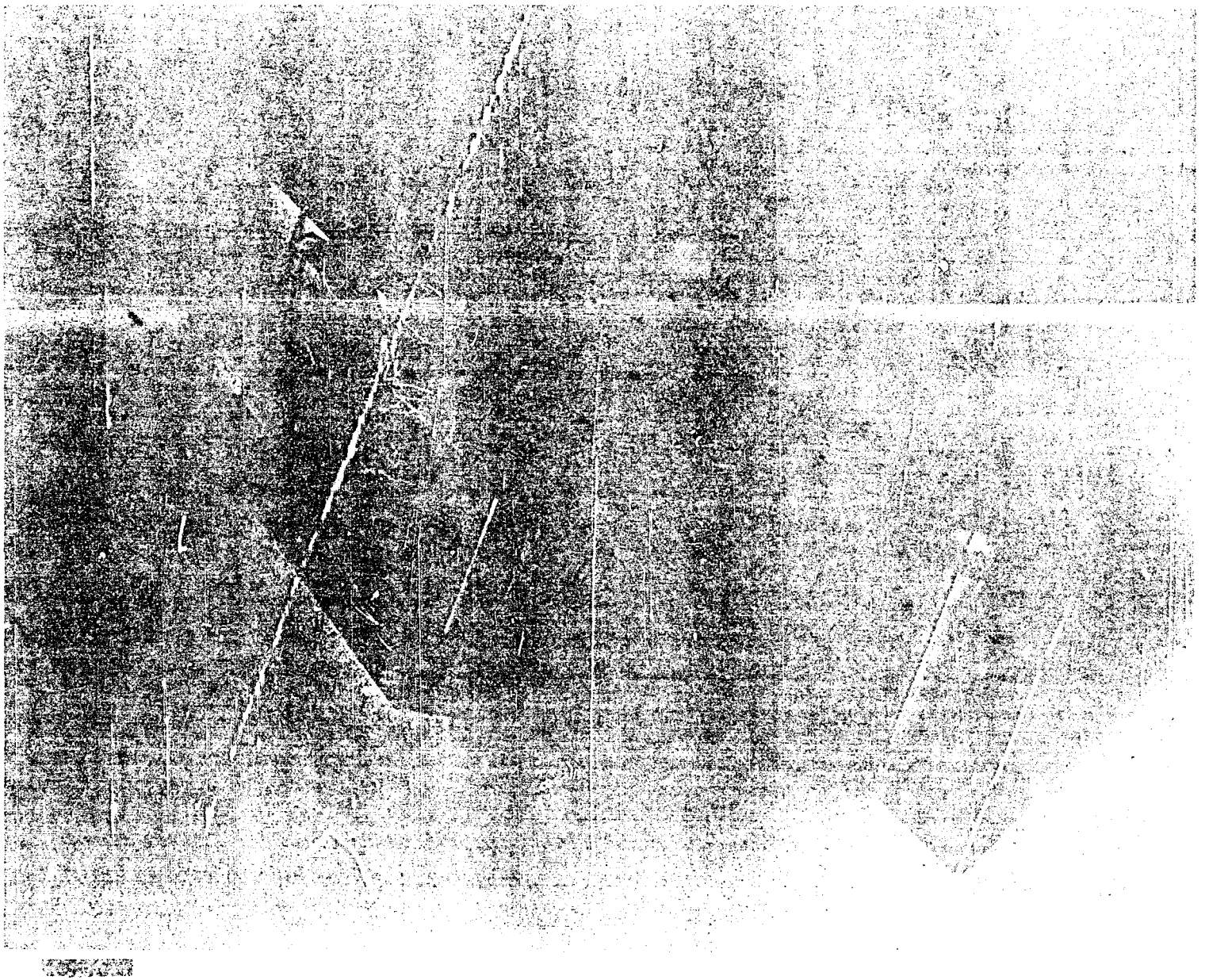

Figure 10. Ferroxcube Memory Removed from Console

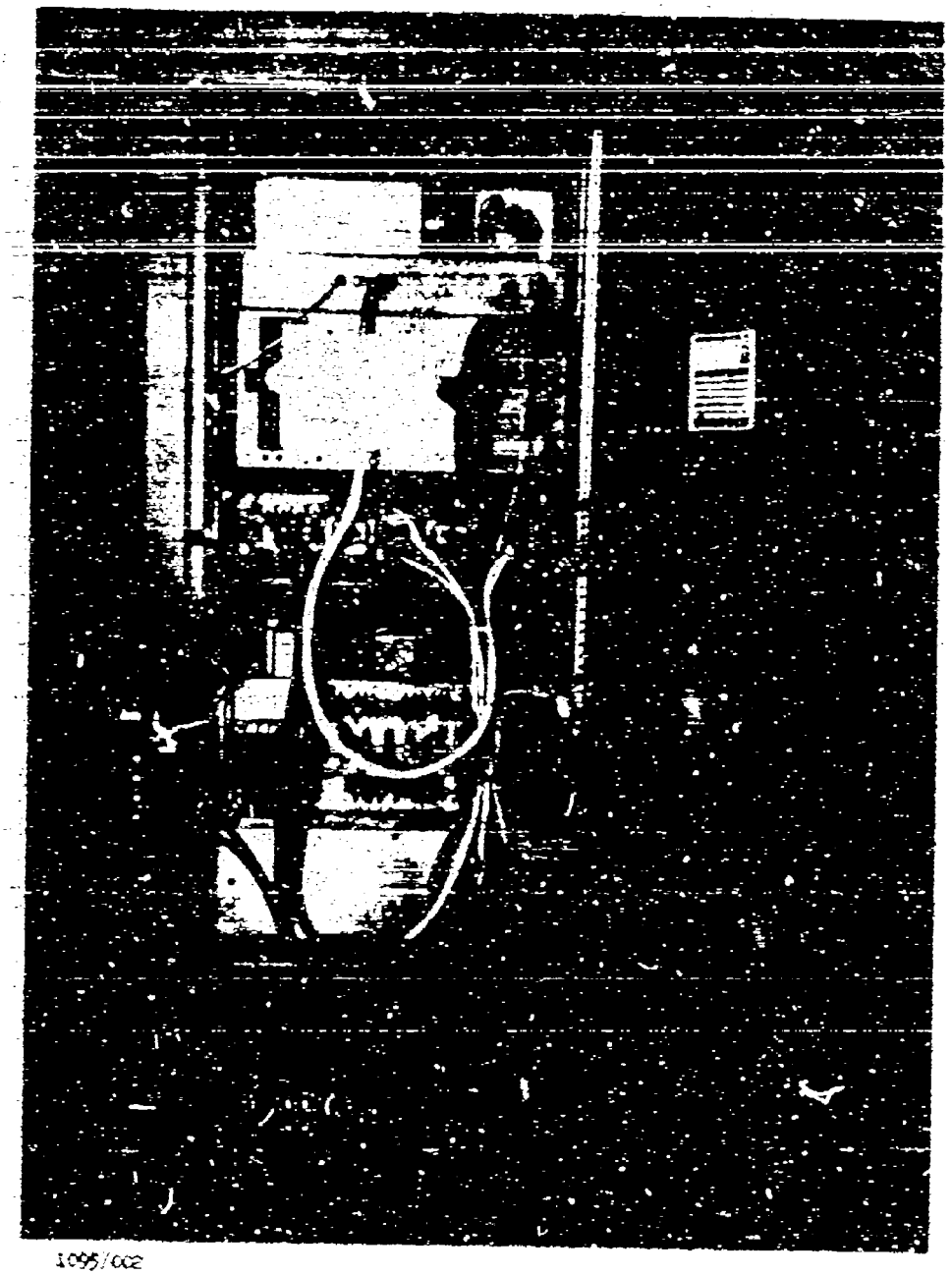

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Figure 11. Rear View of Opened Console Showing Power Supplies in Lower Portion

2.7 LABORATORY STUDIES

Laboratory work has been confined to support efforts in preparation for operation of the breadboard. Supplies of chemicals and biologicals have been maintained in anticipation of this need. These include the following growth media and antibiotics:

Media

- Chapman Stone Medium
- Bacto Dextrose
- Trypticase Soy Broth
- Trypose Phosphate Broth
- Lung Extract

Antibiotics

- Streptomycin sulfate
- Madribon (sulfadimethoxine)
- Chloramphenicol
- Sodium Cephalothin
- Polymyxin-B sulfate
- Penicillin G, potassium
- Penbritin (Ampicillin)
- Gantrisin (sulfisoxazole)
- Kanamycin (II-10) hydrochloride
- Erythromycin

Many strains of bacteria are being continuously cultured and will be available for the studies of pure and mixed cultures. These include the species Escherichia coli, Staphylococcus aureus, Proteus americanus, Streptococcus faecalis and Vibrio metschnikovii, which were studied in Phase I, and many others. The Space-General medical consultant will arrange for local procurement of clinical specimens when these are needed.

A brief laboratory study was performed on sterilization of trypticase soy broth growth medium. Autoclaving (15 minutes at 15 psi and 121°C) gave a product with slightly higher optical absorbance than filtration through a 0.22 μ membrane filter. The difference is not large; however, the filtered medium is preferred as being more reproducible.

Confirmatory studies of antibiotic sensitivity in the Lindberg-Reese experimental device at the University of Missouri, which were originally planned (Reference 2), have now been postponed.

Section 3

CONCLUSIONS

A breadboard instrument has been fabricated for automating the determination of bacterial sensitivity to antibiotics by the tube dilution method. It provides a complete engineering treatment of the basic concepts of the original Lindberg-Reese research device, with significant improvements. No unusual problems were encountered and completion of the program on schedule is anticipated.

Section 8

REFERENCES

1. Space-General, "Final Research Report on Automation of a Technique for Determining Bacterial Sensitivity to Antibiotics," SG 1095 R-1, March 1967.

2. Space-General, "First Quarterly Formal Progress Report on Automation of a Technique for Determining Bacterial Sensitivity to Antibiotics," SG 1095 R-2, July 1967.