

UNCLASSIFIED

AD NUMBER

ADB171111

LIMITATION CHANGES

TO:

Approved for public release; distribution is unlimited.

FROM:

Distribution authorized to DoD only; Critical Technology; APR 1991. Other requests shall be referred to Army Medical Research and Development Command, Fort Detrick, MD 21702-5012. This document contains export-controlled technical data.

AUTHORITY

USAMRDC per memo dtd 24 Sep 1993

THIS PAGE IS UNCLASSIFIED

L ②

AD \_\_\_\_\_

DEVELOPMENT OF DESIGN PARAMETERS AND CONCEPTUAL DRAWING  
FOR A PLASMA ETCHER TO CLEAN AND STERILIZE  
SURGICAL INSTRUMENTS

ANNUAL REPORT

ROBERT W. BARR

APRIL 1, 1991

**S DTIC ELECTE D**  
**E**  
JAN 26 1993

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND  
Fort Detrick, Frederick, Maryland 21702-5012

Contract No. DAMD17-88-C-8190

Anatech Ltd.  
5510 Vine Street  
Alexandria, Virginia 22310

**OPTICAL TECHNOLOGY**

Distribution authorized to DOD Components only, April 1991.  
Other requests shall be referred to Commander, U.S. Army  
Medical Research and Development Command, Fort Detrick,  
Frederick, Maryland 21702-5012.

The findings in this report are not to be construed as an  
official Department of the Army position unless so designated  
by other authorized documents

AD-B171 111



53  
208

93-01266



98 1 25 020

# REPORT DOCUMENTATION PAGE

Form Approved  
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE April 1, 1991	3. REPORT TYPE AND DATES COVERED Annual 1 Mar 90 - 1 Mar 91
----------------------------------	---------------------------------	--

4. TITLE AND SUBTITLE Development of Design Parameters and Conceptual Drawing for a Plasma Etcher to Clean and Sterilize Surgical Instruments	5. FUNDING NUMBERS DAMD17-88-C-8190 65502A 3P665502M802 BA DA315246
--	---

6. AUTHOR(S) Robert W. Barr	
--------------------------------	--

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Anatech, Inc. 5510 Vine Street Alexandria, VA 22310	8. PERFORMING ORGANIZATION REPORT NUMBER
---	--

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research & Development Command Fort Detrick Frederick, MD 21702-5012	10. SPONSORING / MONITORING AGENCY REPORT NUMBER
---	--

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT <del>Distribution authorized to DOD Components only, April 1991.</del>	12b. DISTRIBUTION CODE
--	------------------------

13. ABSTRACT (Maximum 200 words)

DTIC QUALITY INSPECTED 5

Accession For	
NTIS CRA&I	<input type="checkbox"/>
DTIC TAB	<input checked="" type="checkbox"/>
Unannounced	<input type="checkbox"/>
Justification	
By _____	
Distribution / _____	
Availability Codes	
Dist	Avail and/or Special
E-4	

14. SUBJECT TERMS Medical facilities; Equipment and supplies	15. NUMBER OF PAGES
---	---------------------

	16. PRICE CODE
--	----------------

17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited
---	--	---	---

## TABLE OF CONTENTS

I.	Identification of the Problem.....	1
II.	Physical Principles of Operation.....	2
	A. Surface Alteration by Extended Exposure of Implements to Energetic Ions.....	3
	B. DC Discharge. Theoretical Considerations.....	4
	C. Sterilization by Ion Plasmas.....	5
	1. Kill Requirements.....	5
	2. Run-Time Dependence of Sterilization.....	6
	3. Voltage Dependence of Sterilization.....	6
	4. Sterilization vs. Cleaning.....	7
	5. Limitations.....	7
III.	Engineering Considerations and Choices: Process Chamber Design.....	8
	A. Vacuum Vessel.....	8
	B. Electrical Behavior of Various Anode and Cathode Structures.....	9
	C. Implement Support Structure.....	10
IV.	Diagnostics and Assessment.....	10
	A. Plasma and Ion Flux Measurement.....	10
	1. First Large Area Fluence Experiment.....	10
	2. Probe Measurements of Plasma Properties.....	11
	B. Sterilization Testing.....	12
	1. Standard Protocol for Autoclave Sterilization..	14
	2. Sterilization of Nonconductors.....	15
	3. Experimental Device and Procedure.....	15
	4. Summary of Sterilization-test Runs.....	16
	5. Sterilization Tests in the Stainless Steel Clamshell Configuration.....	18
	C. Surface Cleaning Experiments.....	19
V.	Summary of First Year Work and Projections for Second Year.....	20
	A. Executive Summary.....	20
	B. Work Calendar - Gantt Charts.....	21
	C. Administrative.....	21
	D. Second Year Effort Plan.....	21
	Appendix 1 - MicroBioTest Services.....	22

## LIST OF ILLUSTRATIONS

Table 1	- First Set of Sterilization Tests, 10/24-11/2/90. Sterilization go/no go evaluation by MicroBioTest...	25
Fig. 1	Implement and tray as cathode of glow discharge, showing anticipated sheath structure and normally impinging ions.....	26
Fig. 2	Typical straight line semilogarithmic survivor curve for <i>B. subtilis</i> var. <i>niger</i> spores on a stainless steel surface.....	27
Fig. 3	Comparison of radiation, dry-heat and thermo- radiation inactivation of <i>B. subtilis</i> .....	28
Fig. 4	D-value as a function of temperature for <i>B.</i> <i>subtilis</i> spores.....	29
Fig. 5	Two experimentally indistinguishable dependences of sterilization on run time.....	30
Fig. 6	Vacuum chamber.....	31
Fig. 7	Stainless steel "clamshell" drawer (conceptual- test model) with cathode rack.....	32
Fig. 8	Prototype of Pyrex clamshell drawer containing high-voltage cathode tray and stainless steel anode plates on top and bottom (grounded).....	33
Fig. 9	Wire Form for holding hollow cylinder spore- carriers (65% of actual size).....	34
Fig. 10	Interleaving moving cathodes.....	35
Fig. 11	Large-Area discharge cathode plate with thermocouple sensors.....	36
Fig. 12	Cross-section of thermocouple fluence sensor element	37
Fig. 13	Insulator standoff posts for parallel-plate single- sided discharge.....	38
Fig. 14	DC discharge fluence profiles in air and argon at 100 mTorr, 50 mA. Thermocouple temperature changes after 10 minutes.....	39
Fig. 15	Relative temperature rise of thermocouples in DC air discharge (100 mTorr, 50 mA) after 10 minutes, with hemostat on cathode.....	40
Fig. 16	Plasma probe. Probe element is 007" Tungsten wire inside teflon and ceramic insulator tube inside ceramic-coated stainless support tube.....	41
Fig. 17	First probe circuit shown with DC power supply to device.....	42
Fig. 18	Current-Voltage Relation for a Cylindrical Langmuir Probe.....	43
Fig. 19	Experimental device for sterilization by ion plasma.	44
Fig. 20	Pre-sterilization of carrier removal hook.....	44
Fig. 21	Removing spore-carrier cylinders to incubation broth after sterilization run.....	45
Fig. 22	Carriers in broth test tubes labeled with date, run #, position in run.....	45
Fig. 23	Pan cathode with carriers, during run.....	46
Fig. 24	Gantt Chart - First year progress chart.....	47
Fig. 25	Gantt Chart - First year, scientific breakdown.....	48
Fig. 26	Gantt Chart - Second year, projected.....	49

## I. IDENTIFICATION OF THE PROBLEM

This work was proposed in response to the Army's stated need for an instrument for use in field hospitals to clean blood and organic debris from surgical instruments and sterilize the instruments. The device needs to be lightweight, small in volume, and water and manpower efficient. The need for such a device makes sense. Conventional autoclaving requires use of a large, heavy instrument that sterilizes by producing steam at high pressure. Sterilization takes 30 to 60 minutes, and the autoclave consumes considerable electric power to produce steam. A simpler method would be an asset, particularly in a field situation where access to water, power, space and personnel is expected to be limited.

Anatech proposed that ion plasma etching technology be employed to meet the needs described above. Work done in SBIR Phase I demonstrated that plasma etchers sterilized metal samples and effectively removed blood and organic matter from stainless steel surgical instruments, and the device required would clearly be small, lightweight, and able to be operated simply in the absence of water.

It was reasonable that use of plasma etching methodology should occur to Anatech. We currently build and market several types of plasma-based cleaners for use in research and quality assurance. These instruments are used, among other applications, to clean the surfaces of transistors, printed circuits and other micro-electronic components at various stages of their industrial production. The same principles of operation can presumably be incorporated into a plasma-based cleaner/sterilizer that would be effective in decontaminating surgical tools.

Anatech was further motivated to pursue this work because we are certain that the potential market for a plasma-based sterilizer is greater than that created by its use in military field hospitals. Nearly every hospital, doctor's office and dentist's office has a need to sterilize small instruments in an efficient and quick way. We expect that the process we have conceived will prove attractive, particularly when compared to present methodology, for many of these applications.

Upon successful completion of Phase II, Phase III money and resources will be sought to bring the process and instrument to marketing and to production. Phases I through III are steps toward the realization of Anatech's corporate charter of widespread commercialization of plasma processing.

The device is designed to accommodate trays or drawers of metal surgical implements, and processing is expected to require approximately 20 minutes. After operation of the ion plasma,

sealed trays will be removed from the etcher, and can be stored containing sets of the clean, dry, sterile surgical tools.

Operation of the etcher offers the following advantages over existing technology.

- a) The etcher operates with no requirement for water. Organic material is removed from stainless steel surfaces by the action of accelerated ions generated from  $N_2$  and  $O_2$  molecules.
- b) The etcher is lighter (perhaps 300 lbs compared to 400-800 lbs for current devices) and more portable than autoclaves in current use.
- c) Sterilization is quicker than a typical present-day autoclaving cycle, and significantly less electric power is consumed.
- d) Surgical tools are not heated to very high temperatures in the etcher, and there is no significant damage to tool surfaces such as that observed after many cycles of autoclaving in steam or ethylene oxide.

## II. PHYSICAL PRINCIPLES OF OPERATION

The operation of the etcher depends on the use of ion plasmas (also called glow discharges) to clean and sterilize stainless steel surgical tools. Accelerated ions present in ion plasmas are able to remove organic material from metallic surfaces in two ways: 1) by physically desorbing (sputtering) small molecular fragments broken off larger structures such as proteins, carbohydrates or nucleic acids, and 2) by reacting chemically with organic matter to produce volatile, non-toxic gases such as  $CO_2$ ,  $NO$ ,  $NH_3$ , and others. The process is performed at a low enough ion energy (less than 2keV) that damage to stainless steel is not observed. Organic matter can be removed because it is composed of smaller, more sputterable and more reactive atoms. Ion plasmas are developed in air and oxygen at reduced pressure ( $N_2^+$  and  $O_2^+$  are, therefore, the principal accelerated ions present) between electrodes in a sturdy chamber.

The device itself is quite simple both conceptually and operationally. Etchers and cleaners that operate in the same manner are employed widely in industry to strip photoresist and other materials from the surfaces of semiconductor devices during their fabrication, to deposit metal on the surfaces of objects to be observed in the scanning electron microscope, and to clean printed circuit boards in the final stages of their production. Our initiative in the present work, therefore, is to adapt plasma etching technology to the task of cleaning and sterilizing medical implements.

## A. SURFACE ALTERATION BY EXTENDED EXPOSURE OF IMPLEMENTS TO ENERGETIC IONS.

### Ion Source Experiments

Partly because Anatech manufactures and has available an ion beam source capable of generating 100mA of ions (of any gas) at several hundred eV energy over  $\sim 35 \text{ cm}^2$ , in the beginning of Phase Two we looked at the effect of such beam currents on stainless steel medical implements and on plastics. Although we had felt that the uni-directionality of such a source might make great complications for cleaning all sides of a medical implement (or several implements), the ion energies were comparable to what we envisioned using, and the current densities were somewhat higher than those used in the discharges of Phase One. We felt it was important at the outset to evaluate whether this ion bombardment would noticeably discolor the implements, or melt or cloud plastics.

Thus as a first step in assessing the commercial viability of cleaning implements by immersion in an ion plasma, and the long-term wear or surface modification of the implements brought about by repeated cleaning, we observed the surface properties of different targets after 30 minutes exposure to various ion currents and voltages. In the first such experiments beams of Argon ions with mean energies of 130 V to 400 V were used, in low pressure ( $10^{-4}$  Torr) backgrounds. Beam currents were of order 100mA, at current densities of order  $5\text{mA/cm}^2$ . The targets were (a) stainless steel plates larger than the 2" diameter of the impinging ion beam (b) 3/8" chrome ball-bearings placed on stainless steel shim stock holders, and (c) clear Lucite 3/8" diameter beads so placed. Distances to the beam source were 15cm or 25cm.

- a) The 3" X 3" stainless square plates showed surface polishing and cleaning inside the beam area and some slight yellowing outside the beam area. The visible differences were most noticeable at 135 Volts and least at 400 Volts, where the surface appeared uniformly cleaned.
- b) The chrome ball bearings showed significant darkening on the cathode side at 134 V (118mA total beam current), even after only 15 minutes exposure. The ambient pressure was  $2.6 \times 10^{-4}$  Torr. The sheet metal on which the ball bearings rested had a light brown burn mark, the diameter of which is about 70% of the ball bearing diameter. At 400 Volts (10.6mA total beam current), in addition to the cathode-side darkening, the anode became slightly discolored.
- c) The Lucite beads showed significant darkening on the cathode side at 134V (6.2mA total beam current) after 30 minutes. At 400V (12.1mA total beam current), the bead surface melted. The bead was sputter coated from the cathode side up covering about 65% of the bead.

## B. DC DISCHARGE. THEORETICAL CONSIDERATIONS:

We use the collection of implements and a partially-transmitting tray floor as the cathode of a glow discharge at ~100 mTorr pressure and with 5-10 eV electron energies. This provides a flux of ions accelerated across the plasma sheath impinging normal to the implement surfaces in all but the most "shadowed" crevices. The plasma sheath, i.e. the ion acceleration layer, would have a thickness somewhat larger than one Debye length,

$$d_p \text{ (cm)} \approx 740\sqrt{T_{ev}/n_i}$$

where  $T_{ev}$  is the mean electron energy in eV (a function of discharge electric field and pressure) and  $n_i$  is the ionization density in ions/cm<sup>3</sup> just outside the plasma sheath. For a 100 mTorr discharge with 50V/cm bulk electric field, the mean electron energy in an air plasma would be around 30 eV and the ionization density of order 10<sup>10</sup> cm<sup>-3</sup>. This gives sheath thickness on the order of 400 μm, through which one finds accelerating voltages of order 4 $T_{ev}$  or greater. Thus the conducting utensil surfaces accessible to the ion plasma are bombarded by a flux of ions accelerated to hundreds of eV energies. The flux is approximately the ion thermal flux

$$F \text{ (#/cm}^2\text{/s)} = \frac{1}{4}n_i v_{ith}$$

where the thermal speed of the ions (which remain near room temperature in such a discharge) is of order

$$v_{ith} \sim 0.5 \times 10^5 \text{ cm/s}$$

This flux is then expected to be of order 10<sup>15</sup> ions/cm<sup>2</sup>/sec or 60 μA/cm<sup>2</sup>, for an ion density of 10<sup>11</sup>cm<sup>-3</sup> in the ion plasma. Sharp points (of dimension  $\approx r_n$ ) receive somewhat higher fluxes as a result of geometric focusing (Fig. 1).

This flux of ~40eV - 400 eV ions into bacteria disrupts their structure and evaporates molecular fragments from the surface, thus cleaning organic material from the metal surfaces as well as killing living organisms made up of such bacteria.

In addition to this kinetic ionization and dissociation, however, if the discharge contains oxygen, various oxygen ions, atomic oxygen, and ozone are formed and these chemically oxidize the biomolecules. This advantage in cleaning may be partially offset by the tendency of oxygen to attach electrons, reducing the electron density in the discharge and reducing the sheath voltage that accelerates positive ions onto the surface. Water liberated from the surface contamination also attaches electrons, with the same consequences. As a result of these theoretical observations, we compared ion plasma sterilization in nitrogen, air, and oxygen-enriched air at the same pressures and similar discharge

currents. Oxygen-enhanced air seemed to do a slightly more reliable sterilization.

### C. STERILIZATION BY ION PLASMAS

#### 1. Kill Requirements

Sterilization of medical operating implements requires reliable, verifiable killing of all organisms on the implements, and a capability to maintain the sterility during storage. The organisms may be of a number of types and sizes. Some are more resistant to chemicals, others to heat or steam. One of the types most resistant to dry sterilization is bacteria *Subtilis* spores, and as a result this organism has become a standard challenge organism for testing and verifying sterilization in the industry. Considerable information on its resistance has been acquired. Fig. 2, for example, shows survivors vs. heating time at 125° C for dry heat sterilization of stainless steel objects coated with these spores [NASA Report SP-5105, *Advances in Sterilization...*, Bionetics Corp., 1978]. Fig. 3 shows survivors vs time for a combination of dry heat and ionizing (nuclear) radiation.

The ion plasma sterilization resembles this latter combination, in that (1) ionizing charged particles are bombarding the organism (although they are generated by the ion plasma and not by nuclear sources), and (2) the temperature of the implement rises during the ion bombardment. Both of these are quantified elsewhere in this report.

Because the culturing method provides only binary (yes/no) answers (and has some statistical variability) it has not yet been practical to amass the data necessary for drawing reliable sterilization boundaries for the various experimental configurations in the parameter space of current, electric field, gas pressure and type, and run time. We know, however, that for the typical operating parameters in our double-sided planar and clamshell devices (150-300 mA at 400-1200 V, 5 cm anode-to-cathode, 100 mTorr, O<sub>2</sub>, 10-30 min) surface sterilization is achieved, as evaluated by culturing.

Readers and potential users should be aware that organisms not on the immediate surface, e.g. those imbedded in unremoved flecks of tissue, suture, or those otherwise encapsulated at depths of a few micrometers or more, will generally not be sterilized by ion bombardment or surface chemistry (though they may be killed by the attendant heating). Even the thermal killing of such encapsulated organisms can be about one order of magnitude less for a given temperature, and similar kill percentages can require higher temperatures (e.g. 20° higher for the encapsulation referred to in Fig 4.)

## 2. Run-Time Dependence of Sterilization

Because chemical etching is relatively independent of current density and voltage, the etch rate of biological materials by oxygen should depend less steeply on voltage at higher substrate temperature and more steeply at lower temperature where chemical etching is weaker. In our device, however, the temperature rises with time at a rate proportional to the ion energy deposition rate, i.e. proportional to  $IE$ , where  $I$  is the current and where the ion bombarding energy  $E$  is proportional to the applied voltage or the electron temperature  $T_e$ , which depends on  $E/P$ . The glow discharge is operated in a parameter range where the current  $I$  is only weakly dependent on voltage.

Sterilization primarily by neutral oxygen chemistry might be expected, then, to occur mostly near the end of a run, after the samples have become warm, whereas sterilization dependent on ion flux would occur uniformly in time throughout a run. The neutral chemistry component of sterilization would thus be expected to be more sensitive to run time (like  $t^2$ ) than the ion component, which should vary as  $t$ , for fixed  $I$  and  $E/P$ . If sterilization by thermally enhanced neutral chemistry were the dominant mechanism, one would expect long runs to be much more successful than short ones, as long as one was below the threshold for complete sterilization.

In addition, however, sterilization by excited neutral oxygen molecules can depend on discharge current and voltage, because the number density of the various excited states depends on these, and not just on substrate temperature. Because neutrals outnumber ions by a factor of  $10^5$  or so in the discharge, the number density of excited neutrals may be comparable with the number density of ions near the implement surfaces.

Statistical variability of the analysis protocol for testing sterilization also makes it difficult to do such quantitative analysis of run-time dependence without a very large number of runs. Our preliminary indications are that run time is not such a strong influence, at values between 10 min and 30 min, but statistical scatter on the order of 20% of the saturation-value of the number of organisms killed can easily mask any distinction between  $t$  and  $t^2$  dependence below saturation (Fig. 5)

## 3. Voltage Dependence of Sterilization

It is characteristic of discharge behavior that the voltage and current cannot really be varied independently. But over a wide range of voltages, the current rises only slightly with voltage, so ion-energy dependence of sterilization effectiveness can in principle be observed semi-quantitatively by logging the minimum run time to sterilize for different voltages. It is likely that the sterilization time exhibits a threshold behavior with voltage, such that it becomes quite long below a critical voltage.

However, this may not be observable either, because of the critical voltage lying well below the feasible operating voltages for the discharge.

#### 4. Sterilization vs. Cleaning

Both sterilization and cleaning would appear to proceed a few Angstroms at a time, because the ion energies do not allow deep penetration of ions into the material. But sterilization requires only disruption of the complex information-bearing chemical, secondary, and tertiary structure of macromolecules. Rearranged radicals and high molecular weight fragments will often be positively charged in the removal process, and these will have a strong tendency to redeposit immediately on the cathode, with perhaps new chemical reactions occurring on redeposition. This seems to be the case with lipids, which appear to polymerize on the cathodic implements rather than being cleaned by fragmentation and evaporation.

If every impact of a sheath-accelerated ion caused one irreversible bond disruption (probably not a bad assumption), then the  $\sim 10^{15}/\text{cm}^2/\text{sec}$  impacts on the discharge cathode would break, in 1 sec., many of the  $\sim 2 \times 10^{16}$  bonds/cm<sup>2</sup> exposed on the "surface" of an adhering cell or virus. If all the unbonded atoms left the surface without redepositing (perhaps a bad assumption), one could expect an etch rate of about 0.5 Å/s or 30 Å/min. This is roughly the etch rate of graphite by nonreactive ions at a few hundred eV energy. A cell wall 0.1 μm (1000 Å) would be worn through in about 30 min., and the cell would lyse. These are roughly (within a factor of 2) the thicknesses and times we anticipate and observe.

However, microscopic amounts of lipid coating (say 1 μm thick), or a multicell cluster, might take considerably longer. If redeposition of molecular fragments is accompanied by a high probability of "sticking" by hydrogen (or other) bonding, one might not practically be able to clean the surface. Using potentially-reactive oxygen, which forms CO<sub>2</sub> and H<sub>2</sub>O as well as CHO radicals on reacting with hydrocarbons, the probability of redeposition is probably reduced below what would be expected for, say, argon-ion bombardment of the same hydrocarbons. Nonetheless, it might be expected to take several hours to fully remove a 1 μm film.

This observation leads us to emphasize the need for pre-cleaning of implements, e.g. in a dishwasher, to remove most of the biological material from them, before ion-sterilizing them.

#### 5. Limitations

Ion etching of surface coatings, whether reactive or not, is limited to microscopic cleaning depths. For removal of carbon-containing material, an etch rate of 40 Å/min is typical. This

means that in an hour's time, only 0.24  $\mu\text{m}$  of surface contamination can be eroded off. Macroscopic amounts of material, e.g. .5mm thickness of tissue, skin, blood, etc. cannot be cleaned off an implement by ion etching, or by low-pressure chemistry, in a reasonable amount of time. Wrapped implements cannot be cleaned or sterilized through the paper or porous cloth wrapping. Implements to be sterilized must be metal (or conducting) and must be unwrapped.

### III. ENGINEERING CONSIDERATIONS AND CHOICES: PROCESS CHAMBER DESIGN

#### A. VACUUM VESSEL

The first experiments at Anatech under this contract were done in an existing vacuum chamber, which had several disadvantages:

1. It was not a dedicated facility
2. It had limited viewing access (2 small ports with only one viewing the discharge proper)
3. Sheets of insulating plastic had to be placed at the vacuum chamber walls to keep the discharge in the space between the anode and cathode plates. Positioning of these sheets was fairly critical in order to avoid arcs or enhanced local discharges at the insulator joints.
4. Diagnostics leads fed through the removable top, and removal of the top required careful re-coupling of the diagnostic pin connectors and careful re-positioning of the insulating plastics. Thermocouple leads also had to be handled carefully.
5. Access to the cathode for placing and removing implements was either through the top (a nuisance) or through the small viewing ports (also a nuisance).

A transparent, nonconducting vacuum vessel with easier access, better diagnostic-wire feedthroughs, and more convenient orientation and design was required. A dedicated system incorporating such a vacuum vessel was soon built; a design view of it shown in Fig. 6. The system exceeded our expectations and solved very nicely the access problems described. Stable, reproducible discharges, both AC and DC are normal in the device.

This experimental system was originally built with an available small vacuum pump. Pumpdown to 50 mTorr took about 15 minutes after the samples were inserted. A larger pump and vacuum hose reduced this pumpdown time to about 7 minutes.

This vacuum vessel serves very well for our experimental work, allowing excellent viewing and diagnostics. For a production sterilizer, however, a more compact and rugged module is appropriate, with provision for sterile removal and/or storage of implements.

We have begun work on the engineering and testing of a fieldable prototype device, a removable sealed sterilizer drawer. This idea that a sterilized set of implements, which cannot have been wrapped, can be maintained sterile and sealed for long times after sterilization, is very appealing. The drawer module would contain both electrodes, and would plug into electrical, vacuum, and gas feed connections on insertion into the device, and on withdrawal would disconnect from these in such a way as to maintain partial vacuum until deliberately opened at a later time. Because plasma sterilization does not penetrate wrapping as steam does, this solves the problem of sterile storage.

As first steps toward testing this idea, we acquired inexpensively both stainless steel and Pyrex "clamshell" drawers. The stainless version has the entire outside as (grounded) anode, and a mesh cathode, supported on insulators, inside. This has been tested, and although it allows only limited visual diagnosis, it appears to have appropriate current, voltage, and luminosity characteristics and to successfully sterilize stainless steel spore carriers. The device is shown in Fig. 7.

The Pyrex version (Fig. 8) has stainless steel plates on the inside of the largest-area surfaces, as a double anode, with the mesh cathode supported equidistant between them. This configuration has insulating, rather than conducting, side walls, obviating the possibility of concentrating the discharge at the cathode edges if implements should slide or roll too close to the edges.

These clamshell drawers are being tested first in the experimental vacuum chamber of Fig. 6.

#### B. ELECTRICAL BEHAVIOR OF VARIOUS ANODE AND CATHODE STRUCTURES

The discharge power supply and metering were constructed so as to be able to run convertibly as either DC or 60 Hz AC. The DC (and AC) discharges were easily ignited and did not need a special initializing voltage spike generator. This is convenient both for Phase II experiments and for the simplicity of the eventual field equipment. The operating characteristics of the discharge were observed, and the required operating conditions for the discharge are easily maintained. The required voltages are 300 - 1000 V at 100 - 200 mTorr.

The current drawn by an open cathode structure was lower (at the same voltage and pressure) than that to a plate cathode. (In practice, the voltage had to be higher to get the same current.) This is as expected. When a solid pan cathode was placed just beneath the open cathode and electrically connected to it, the plasma luminosity was, surprisingly, highest between the two cathodes, and formed there first as the voltage was raised above threshold; and the current was more consistent with the magnitudes measured between solid anode and cathode. Next, the open mesh cathode was placed approximately equidistant between two

anode plates. A large ink-marked piece of sheet stainless steel shim stock was laid on the open mesh cathode and run for 30 minutes in 120 mTorr of  $>50\%$   $O_2$ , at relatively low current (150 mA total). The result was nearly total removal of the ink on the top side and moderately good removal from the bottom side, with some faint mesh markings on the bottom, perhaps from sputtered material from the ceramic cathode-support posts.

When the open cathode was placed equidistant between two anode plates, the power supply voltage could at first not be raised enough to provide the desired current at 100 mTorr pressure, so a new power supply was constructed to supply up to 0.5 A at up to 1.5 KV, DC or AC.

This double-sided (anode-cathode-anode) configuration provides high electric field (the voltage, about 1.3 KV, occurs with an anode-cathode distance half as much as in single-sided operation). The current in a typical run is 250 mA total, about 125 mA from each side.

Stainless steel cylinder spore carriers impregnated by MicroBiotest were run in this double-sided version of the device. To prevent the small cylinders from falling through the cathode mesh openings, the cylinders were skewered on a 2 mm dia. stainless wire bent into a form as in Fig. 9. This scheme of holding the cylinders was later improved, as described on page 17.

#### C. IMPLEMENT SUPPORT STRUCTURE

In order that certain places on the implements, where they contact the supporting cathode, do not escape cleaning (or become overly discolored by local plasma processes), we arrived at a design for a periodically-shifting support mechanism. Such a mechanism must not result in excessive drift or bunching of implements on the cathode tray; to avoid this we made a (proprietary) design of two interleaved support racks which would alternatively support the implements, exchanging roles every few seconds (Fig. 10). The effectiveness of such a structure as a discharge cathode was tested in a preliminary way; further testing is underway.

Fabricated interleaving cathodes were delivered, assembled, and tested (on a test-stand outside of the experiment) with various drivers. One driver scheme is pneumatic. Parts for this are simple and progress has so far been encouraging.

#### IV. DIAGNOSTICS AND ASSESSMENT

##### A. PLASMA AND ION FLUX MEASUREMENT

###### 1. First large area fluence experiment

In order to determine how nonuniform such a large-area discharge

would be, and how close to the center we would need to keep the implements to be sterilized, we designed an experiment in which an array of thermocouple energy-flux-density sensors on the cathode would provide us at least relative intensities of the ion bombardment. The thermocouples needed to be electrically connected to but thermally somewhat insulated from the cathode; electrically at cathode potential so that they would collect ion flux as would the cathode, and thermally insulated from the cathode as large heat sink so their temperature rise could be measured. For each thermocouple, a copper washer (and screw head) served as the energy-gathering area.

This experiment was designed, built and leak-tested; at first a vacuum leak found in the thermocouple leads feed-through could not be remedied with the existing hardware, so a new feedthrough flange was obtained. The large-area test cathode, pictured in Fig. 11, has roughly the area of the standard medical implement tray; holes like the one marked A hold the energy deposition sensors; those like the one marked B allow the thermocouple leads through to the back of the cathode, while the eight holes marked C hold the support posts that separate the anode and cathode of the discharge; the holes marked D supply the electrical connection from the copper washer to the cathode.

Detail of the calorimetry sensors in holes A is shown in Fig. 12, in a side-on view. The material shown shaded is Omega type 200 Mullite ceramic insulator. The impinging particle energy on the copper washer W and the stainless steel screw head S heats the thermocouple T. The ceramic mitigates the heat loss to the large-area stainless cathode plate K. A rough calculation was made of the heat loss through the leads, but the principal objective of the experiment was a comparison of the relative intensities at the different locations on the cathode.

The anode-cathode standoff posts are shown in Fig. 13, where again the shaded material is the mullite insulator mentioned above.

Fluence uniformity, as measured by thermocouples beneath copper washers at 13 locations on the cathode, was only fair, and certain corners of the cathode appear to have received very low energy fluxes. Two typical fluence patterns are shown in Fig. 14, for air discharges at 50mA and 100 mTorr.

To correct for individual differences in the thermocouples, calibration temperature readings were taken before the start of each run, and the temperature rises of all 13 were calculated from the final temperatures measured (quickly) when the discharge was turned off after 10 minutes of operation. Because of plasma electrical effects on the microampere-carrying thermocouple leads, the thermocouple readings were stabilized only after the discharge was turned off.

During the sequence of experiment runs, we rotated the cathode of the discharge device (with its thermocouples attached) by 180° and observed changes in the fluence pattern. These changes were not simple 180° rotational changes, indicating that thermocouple peculiarities were not biasing the fluence pattern in any obvious way.

However, this rotation and the repeated runs with slightly different insulator sheet positioning just inside the vacuum chamber walls did show, at least qualitatively, that the fluence pattern can depend quite a bit on surrounding conditions near the walls, and that it has only moderate reproducibility. With implements on the cathode, the fluence pattern changed further (Fig. 15), which was not unexpected. Some dependence of the discharge intensity pattern on wall conditions is qualitatively understandable, and any lack of reproducibility resulting from repositioning of insulator sheets near the wall in the initial test chamber was done away with when the experiment was set up in the nonconducting vacuum vessel.

Placing a hemostat and a pair of sharp-pointed tweezers on the cathode allowed us to assess whether points on the implements would selectively act as the active cathode, leaving the rest of the cathode un-bombarded by ions. This did not occur, as was confirmed by the thermocouples and visible observation. Implements do not disrupt the functioning of the DC discharge. A small amount of intermittent pinpoint arcing occurred at the tips, and also near the hinge of the hemostat, but did not affect thermocouple readings in any major way and did not leave noticeable burn marks on the implement tips. The hemostat showed some discoloration very near the hinge gap, which may perhaps be attributable to silicone or grease from the hinge, reacting and/or being sputtered on the nearby implement surface. Ink marked on the hemostat (in a band around one handle) was mostly removed, but ink marked on the inside surface of the tweezers was not polished off after 10 minutes of exposure on the cathode of the DC discharge.

To assess the effect of plastics (e.g. on implement handles) on the discharge - and effects of the discharge on the plastics - we placed on the cathode a wire hook passing through a Lucite bead. No significant effects were seen during or after 10 min. of operation.

Based on some early discharge work, low current densities anticipated in the discharge,

$J (\mu\text{A}/\text{cm}^2) \sim 400 P_{\text{Torr}}^2$

would have made the anticipated thermocouple temperature changes quite small unless the discharge operating pressure were raised above the previously anticipated 100's of mTorr. At 1 Torr, sheath deposition of 100 eV ions would give, according to the early work, only about 0.04 W/cm or 0.7° C heating.

Fortunately, the current densities observed in our discharge (and others) are much higher than those predicted by the early discharge work; at 100 mTorr, rather than the  $\sim 4 \mu\text{A}/\text{cm}$  predicted by VonEngel [Ionized Gases, 1955], we infer current densities on the order of  $0.2 \text{ mA}/\text{cm}$ , and this is consistent with observed temperature rises of  $1^\circ \text{C}$  to  $30^\circ \text{C}$  in the thermocouple sensors on the cathode. Observing the temperature changes (to infer energy flux densities in different areas of the cathode) was thus not difficult at 100 mTorr and we did not need to raise the pressure to  $\sim 1$  Torr. This was fortunate also because raising the pressure above  $\sim 300$  mTorr tended, in our first runs, to cause arcing at the edges of the insulator sheets near the vacuum tank wall.

An important question to be answered about ion plasma sterilization and cleaning is the importance of various oxygen ions and radicals as hot chemical cleaning agents, as compared with the physical disruption of cells and evaporation of material by relatively nonreactive ions, e.g.  $\text{N}_2^+$ . Ozone concentration in a discharge for example, is optimized at values of E/P (electric field/pressure) near  $10\text{V}/\text{cm}/\text{Torr}$ . Operating our experimental DC discharge at 100 mTorr and 850 V, we now estimate typical E/P values in the positive column around  $20 \text{ V}/\text{cm}/\text{Torr}$ . (By contrast, the RF discharge of the PA 200 device [see Phase I report #5] had AC values of E/P around  $40 \text{ V}/\text{cm}/\text{torr}$ ). If ozone specifically were most important for cleaning, the RF discharge would not be preferable. At the higher E/P values,  $\text{O}_3^+$  is dissociated and  $\text{O}^+$  is the dominant oxygen form. One would expect  $\text{O}^+$  to be just as effective at initiating oxidation cleaning, however.

Our first measurements in the proof-of-principle experiment with DC applied voltage in air were only measurements of relative energy flux density. This corresponds to measuring an arrival rate of all air ions, most of which (at the estimated electron temperatures) were  $\text{N}_2^+$ . If  $\text{O}^+$ ,  $\text{O}_2^+$ ,  $\text{O}_3^+$  or nitrogen oxide radicals play a disproportionate role in cleaning, this can be determined by comparing the degree of biological cleaning in air and  $\text{O}_2$  discharges, in principle, but not by energy-flux measurements.

## 2. Probe measurements of plasma properties

During January and February of this year we built and tested plasma probes and related electronics for measuring free electron densities and temperatures in the sterilizing ion plasma. The design is shown in Fig. 16 and the first driver circuit is shown in Fig. 17. The high voltage operation of the discharge may make modification of the control circuits necessary and we are currently exploring this. The probes are a fairly simple and standard diagnostic tool that can allow corroboration between theory and experiment.

Although the probe voltage supply was somewhat jury-rigged in our experiments, it was possible to take probe measurements and

partially characterize the ion plasma in both a simple parallel-plate discharge and in the stainless clamshell discharge. Fig. 18 shows a typical probe current vs voltage. By measuring the rate of saturation of the ion current, the plasma ion density can be inferred to be about  $0.9 \times 10^{10}$  ions/cm<sup>3</sup>, which is lower than our initial blind guess but still quite reasonable. The neutral molecule density at 100 mTorr is about  $3.5 \times 10^{15}$  molecules/cm<sup>3</sup>, so that a small fraction of excited neutrals near the cathode can have, in principle, effects as important as the ion bombardment. A total current of 100 mA over about 1000 cm<sup>2</sup> corresponds to a flux of  $6.25 \times 10^{14}$  ions/cm<sup>2</sup>/sec, and this indicates a typical ion speed of  $7 \times 10^4$  cm/s (energy 0.16 eV) in the body of the plasma.

The plasma electron energy is inferred from the 'break' in slope of the electron-current part of the curve in Fig. 18. Inasmuch as one can see a distinct 'break' at all, this occurs about 2V above the "floating" voltage at which the probe draws no current. This implies electron energies in the plasma of order 2eV rather than the 20 - 30 eV that we guessed based on assumed electric field and pressure. In turn, this tells us that the electric field in most of the ion plasma is a good deal less than we had estimated, and the measured potential drop is concentrated more within the "sheath" next to the cathode and implements. Arriving oxygen ions impinging on the implements and cathode must then have higher energy (per ion) than we had estimated earlier, i.e. perhaps 500 eV instead of 100 eV.

The first step in using a plasma probe is to raise it to a voltage such that the arriving electron flux just heats the probe tip to a dull red. This desorbs impurities from the tungsten probe tip. At this voltage the current increases rapidly with any change in voltage. The voltage is then lowered back to the operating range, a few times the electron temperature. The probe cleaning voltage can be seen at the upper right of Fig. 18. The remainder of Fig. 18 is obtained after this probe "cleaning".

Typical separation between neutrals is about  $6.6 \times 10^{-6}$  cm. The thickness of the accelerating layer adjacent to the cathode is probably of order .1 cm (3 Debye lengths), based on the probe measurements. The collision mean-free path of an impinging ion is of order 0.4 cm (or less) among the oxygen neutrals.

## B. STERILIZATION TESTING

### 1. Standard protocol for autoclave sterilization

After meeting with MicroBioTest Inc., a firm involved in sterilization testing, we decided that the use of vesicular stomatitis virus (VSV) used in Phase One research was probably not an adequate or an accepted test for sterilization by our ion plasma. One reason is that VSV is a much smaller organism (~70 X 170 nm) than most bacterial spores (5000 - 10,000 nm), and has a thinner

coating. The ion plasma bombardment basically cleans by surface etching, i.e. material removal. The virus or spore is probably killed when the surface wall is eroded away. Thus a larger, thicker coat/cell wall would represent a more demanding test of sterilizing capability

We also noted that our idea of measuring cleaning, i.e. material removal, on the same samples that we would have evaluated for sterilization, was not practical. To measure the cleaning using radio-isotopes would require dissolving the remaining surface contaminants in toluene for flow through a liquid scintillation counter, and the toluene would almost surely kill the organisms. Upon meeting with MicroBioTest, we also learned their opinion that ordinary residual-protein tests by chemical means were considered quite adequate, and that the additional special handling of (and preparation of) radioisotope-labeled organisms was an unnecessary complication and expense.

Finally, federal agency approval of new autoclave designs is based on sterilization of a certain canonical set of objects soaked in bacterial spores, e.g. those of *B. Subtilis*, as described in the notes on our meeting with MicroBioTest in appendix 1. On MicroBioTest's advice, we decided to try such sets of porcelain hollow-cylinder carriers and silk suture carriers first, even though we were not confident of sterilizing such nonconductors using our technique. Following a first set of runs with these objects, we ran stainless steel spore carriers and implements.

## 2. Sterilization of Nonconductors

From our preliminary testing results with porcelain and suture carriers, we have tentatively verified our conclusion that the objects to be sterilized need to be conducting and need to act as cathodes in the electrical discharges. By the addition of directed ion beams, we could probably engineer the sterilization of the beamward face(s) of nonconducting objects, using (e.g.) a rotating platter. But that refinement is outside the immediate scope of the SBIR contract and the Army's priority need, namely, sterilizing metal implements.

## 3. Experimental device and procedure

The experimental device is shown in Fig. 19. It can be run in either AC or DC modes. In DC mode, the cathode was the lower plate, suspended on a pair of rails that allow easy removal. Spore-impregnated test items are removed from petri dishes in a dessicator and placed on the cathode through the large door on the left end. The door is closed and the device is pumped down to about 50 mTorr or less. The desired gas is bled in through a valve on the right end until the pressure is a bit below the desired operating pressure. The power is turned on and a timer

is set. The pressure quickly rises somewhat due to outgassing, and the bleed rate is adjusted to maintain a steady pressure at the desired level. Voltage is adjusted as necessary to maintain a constant total current as the device cleans. At the end of the timed cycle, the power is turned off, and dry nitrogen is fed into the device from the bleed valve until it reaches atmospheric pressure. The door is opened and a long thin wire, heated in a Bunsen burner to sterilize its last few cm (Fig. 20), is used to extract each spore carrier (Fig. 21), which is dropped into a location- & -run #-labeled test tube of culturing broth, and the test tube is capped (Fig. 22). The extractor is sterilized in the flame before removing each carrier. Several carriers are considered necessary for each run for statistical reliability, because growth of bacteria in the culture broth is a somewhat probabilistic process.

The labeled test tubes were delivered to MicroBioTest at the end of each day. A preliminary report on which samples may have been sterile was taken the following day by phone, with a written follow-up report at the end of the reporting period.

#### 4. Summary of Sterilization-test Runs

First, 14 runs with AC (60Hz) voltage were made, at currents of 100 mA and 300 mA, in nitrogen, air, and oxygen-enhanced air (about 50% O<sub>2</sub>). None of the AC runs reliably sterilized porcelain cylinders or sutures, even though in two of the 30-minute runs the cylinders and sutures were turned over in mid-run to ensure changed points of contact with the electrode. The two AC runs with stainless steel carrier cylinders instead of porcelain, however, sterilized 8/10 and 9/10 of the cylinders, whether or not they were turned over in mid-run.

A batch of 11 runs with DC voltage was then made, at currents of 50 to 175 mA, for durations of 15 and 30 minutes, in air and oxygen-enhanced air (about 50% O<sub>2</sub>). Of these runs, 8 were with the flat electrodes used earlier, with thermocouples near the samples, and 3 were with a pan-shaped stainless steel cathode (Fig. 23). In one run (with flat cathode) 5 of the spore-carrying porcelain cylinders were soaked for 2 min. in alcohol prior to sterile insertion into the experiment. This pre-treatment, however, did not provide sterile control samples, because the spores are alcohol-resistant. Stainless steel cylinder carriers (2 runs) were rather well sterilized, while porcelain cylinder carriers (9 runs) were not, except for one of the two runs using the pan-shaped cathode. Sutures were never reliably sterilized.

In the first run of 11/1, the thermocouples indicated temperatures of 127° C at the cathode after the 30 min. run (at 175 mA DC, in air); nonetheless sterilization was not reported. (Likewise, in the 4th run of 10/26, the thermocouples indicated 101-107° C after 30 min. in "oxygen" at 300 mA AC, but no steriliza-

tion of porcelains or sutures was reported). Heating per se thus does not seem to sterilize such objects. A matrix of the test runs and results is given in Table 1.

Our test runs continued with stainless steel carriers, in order to better map the sterilization requirements in the variables, e.g. current, exposure time, and gas type.

Our sterilization runs in the next period were all done with open mesh cathodes. At first we were concerned that the removal of sterilized cylinders from the wire form described above on Page 9 involved sliding probably-sterilized cylinders over possibly still contaminated (or re-contaminated) regions of the wire form, and thus could conceivably cause a false bias toward "unsterilized" readings. This concern led us to engineer individual mounting wires for the cylinders. These are attached to the mesh cathode. The cylinders are easily slipped on or off their individual wire supports. We made one of the supports stand so that the cylinder would rest about an inch above the main cathode surface, just so we could see what effect this non-proximity might have. (In a hospital environment, we imagine that some implements might have portions or tips an inch or so above the cathode 'tray'). We found no effect from the separation. All cylinders behaved as if they were part of the cathode. The 5 sterilization runs and 1 control run for this period are summarized here in Table 2. All were done with oxygen-enhanced air.

Results reported in the last column signify number of units sterilized/units processed, e.g. "10/10".

TABLE 2

12/13	#1	250 mA	DC	100 mTorr	30 min	9/10
12/13	#2	400 mA	AC	100 mTorr	30 min	10/10
12/14	#1	300 mA	DC	100 mTorr	15 min	10/10
12/20 (double sided, one pan anode)	#1	300 mA	DC	100 mTorr	30 min	10/10
12/20 (double cathode)	#2	300 mA	DC	110 mTorr	10 min	10/10
12/20	#3	~800° C		1 atm	15 min	8/10

The last 'run', 12/30 #3, was a control. All its ten stainless cylinders were heated to about 800 C in a kiln and removed with the usual clean procedure to test tubes of growth medium and analyzed with the other runs by MicroBiotest, which had no knowledge of the control.

We suspect that either the procedure of removing sterilized samples or the statistics of growth evaluation in the culture medium may need improving. We are instituting some improvements in "clean room" technique for the former and providing control tests of the latter.

#### 5. Sterilization tests in the stainless steel clamshell configuration

Three runs were made so far in this device using oxygen, with statistically successful sterilization of spore-coated stainless cylinders in all runs:

Run	Time	Current	Voltage	Pressure	sterilized
1	10 min	200 mA	950-1050	100 mTorr	5/5
2	20 min	200 mA	1050-950	100 mTorr	5/5
3	12 min	200 mA	950-1240	80 mTorr	8/10

The cylinders were supported on small wire clips as before. One cylinder in each run was positioned very near the outer edge of the cathode tray; it was sterilized in every case. The 2 "unsterilized" cylinders of run 3 were found to have been contaminated after sterilization (possibly in transport to MicroBio-Test), with another strain of bacteria, not *B. Subtilis*.

Aside from any questions about statistical reliability of the assay procedure, it was felt that a quicker and simpler (though less official) less costly in-house diagnostic for probable sterilization of stainless steel carriers and implements was desirable at this research stage.

We therefore acquired standard *B. Subtilis* spore suspension (for coating implements and stainless cylinders), nutrient broth, test tubes, petri dishes, dessicator, and an incubator (warmer) etc., to allow in-house testing.

We expect to be carrying out a large number of sterilization tests with several slightly different configurations, rather than an immediate final testing for official approval of a fixed product. Having learned some of the basics of the procedure from MicroBioTest, we feel that in-house sterilization testing is feasible, and that we can return to their service as an independent third-party testing laboratory at a later stage in the program. Our preliminary indications are that in-house informal testing will result in considerable savings to the contract over the 6-month to one-year period following. In addition to the savings, we expect to benefit by the simplicity of having the test gear and results available "on-line" without coordinating schedules with the testing laboratory or shipping gear back and forth.

### C. SURFACE CLEANING EXPERIMENTS

First, one blade of a pair of tweezers was coated with WD-40 lubricant and placed on the cathode. After 20 min. in the DC discharge (at 100 mTorr, 380 V) the WD-40 had not been completely removed, but rather the remaining film had become sticky, indicating probable polymerization of the oil.

An additional 10 min. run with AC voltage still did not remove all of the waxy film. The implication of this is that certain petroleum products (or lipids?) may be difficult to remove, at least at the low power levels of the initial experiments.

In the next cleaning runs, we tested 7 samples, surgical implements, supplied by the contract monitor, for removal of biomaterial. A thin layer of soya oil with phosphatidylcholine was spread on a marked portion of each implement. The implements were run in various single-sided configurations for times up to one hour, at AC currents up to 250 mA and DC currents up to 150 mA. A rhodamine dye solution (0.25% in ethanol) was used as a (standard) lipid indicator, based on our observation of its color change in the presence of a control sample of the lecithin oil. Even without the dye, however, one could see noticeable greyish or brownish film remaining on all the implements, even with the highest currents and longest exposures. The film was sticky, unlike the original oil, and appears to have been "polymerized" or "cross-linked" chemically in the oxygen plasma bombardment. We ran similar tests using the double-sided configuration but this did not make much difference in the material removal.

Changing the location of gas feed to induce more oxygen gas flow across the implements had no effect.

A run with a small amount of acetone in the discharge gas provided no visible assistance to the lipid/phospholipid removal, but caused some clouding of the plastic vacuum vessel.

In order to improve the "ashing" efficiency of the discharge for removing hydrocarbons and the other biological material, we tried sodium hydroxide, potassium hydroxide, and water as catalytic cleaning agents, based on reported results elsewhere [Sjovall et al, J. Vac. Sci. Technol. 1987; Ferreiro, Ernie & Evans, *ibid*, 1987].

Our preliminary finding was that NaOH (placed in pellet form on the cathode about 1" from a soya-oil-and-lecithin band on a pair of tweezers) provided somewhat better removal of the thin film band than a similar run without NaOH. A trace streak of brown discoloration was still visible. A thicker oil and lecithin stain farther away from the NaOH on the tweezers was browned and not well removed. The cathode was largely cleaned of its previous discoloration (surface-deposited material) in the process,

and one anode was discolored by processes not yet understood. It is possible that this anode had acquired a thin film of teflon from the previous experiment and that this film was decomposed in the presence of the NaOH + O<sub>2</sub> plasma.

We then tested potassium hydroxide as catalyst. Several pellets of reagent KOH (potassium hydroxide) were placed on a stainless steel holder on the cathode a few inches from a surplused forceps-like instrument which had marked bands of phosphatidyl choline and lipid (soya oil) rubbed on it. During the 20 minutes of the run, the KOH pellets melted (and some ruptured, probably from evaporation of entrained water of crystallization). The thin film of lecithin oil on the implement was no longer visible, and there was no brown or filmy polymerized band, but the implement and cathode mesh were both plated/discolored with a yellowish-brown metallic-looking surface coating. Using a rhodamine dye there was, we think, some sign of residual lipid in the bands, but our observation technique was not sensitive enough to be sure. In summary, it removed most of the oil but discolored the implement. We suspect the potassium successfully catalyzes the oxidation of the lipid but ends up being deposited on the implements. Also, we were not able to keep the pressure down to 100 mTorr in the face of sublimation of KOH from the melting pellets.

Material removal of oils and lipids is problematical. We are still trying to find new ways to do this. The chemistry here is that the hydrocarbon (etc.) fragments must find it energetically more favorable to leave the surface than to recombine with other molecules on the surface.

## V. SUMMARY OF FIRST YEAR WORK AND PROJECTIONS FOR SECOND YEAR

### A. EXECUTIVE SUMMARY

During the first year of its Phase II work, Anatech has demonstrated that large-area DC or AC ion plasma discharges can sterilize stainless steel implements placed on the cathode, and has designed both experimental test systems and preliminary prototype systems to do this. We have demonstrated that cleaning of organic matter from implements by this process is limited to removal of thin surface films, and that for some oils even that is questionable. Catalyzed removal of material is possible but so far has also discolored the implements.

During the coming second year of Phase II, additional research and development will be done to advance the cleaning function, modifying the design and operation if necessary; we will also test sterilization in the modified devices and will build a prototype fieldable device.

## B. WORK CALENDAR - GANTT CHARTS

A Gantt chart of an administrative or programmatic nature is shown in Fig. 24 and describes our 1990-91 work. A more scientifically-oriented Gantt chart, Fig. 25, describes the same work broken down into its engineering and scientific task areas. Fig. 26 projects this work plan into the second year of development.

## C. ADMINISTRATIVE

Although technician turnover and competing demands on technician time have been potential problem areas, these have so far been dealt with very successfully and the flow of work on the R&D effort has been quite respectable. Funds expended to date total \$97,172. This is less than the planned year two expenditure, which includes considerable additional fabrication and testing expense. Projected year two expenditure is \$315,000.

## D. SECOND YEAR EFFORT PLAN

1. Further work on cleaning organic material from surfaces: we plan to try high-velocity gas or water jets, which could be incorporated into a cleaning cycle prior to sterilization. While we would prefer to avoid using water, its solvation abilities may provide the only effective way to remove macroscopic amounts of biomaterial. We also plan to try pulse and higher-frequency AC voltages to improve the rate of microscopic surface layer cleaning. We would prefer to avoid RF and microwave, both because of military complications of these and because of the implement heating and implement cavity loading.

2. Improvements in sterile handling of sterilized samples: the clean-room techniques, including particulate filters over air ducts, air downflow, and disinfectant/precipitation spray before the runs, may still not be adequate to insure noncontamination of samples on removal from the device for testing. We are considering acquiring a plastic enclosure for this purpose.

3. Integration of alternating-support cathode with vacuum drawer sterilizer design: refinements and system integration will require some engineering time. Interleaving cathodes must also be made to closer tolerances.

4. Design and fabrication of prototype fieldable device: this is the principal effort for the second half of year two.

5. Testing of prototype for quality assurance and effectiveness.

## APPENDIX 1 - MICROBIOTEST SERVICES

### A. MEETING WITH DONNA SUCHMAN & MARY BRUCH OF MICROBIOTEST INC. 10/12/90

Attending for Anatech Ltd. were Robert Barr, George Barr, John Guillory and Mark Wabalas.

Mary Bruch has worked for many years in the area of sterilization diagnostics for Fort Detrick, FDA and other concerns. Donna Suchman is president of MicrobioTest, a 4 year old company in Chantilly, Virginia, involved in sterilization testing, spore culturing, etc. Both are familiar with ion plasma sterilization as proposed by other firms, and are under confidentiality/noncompetition agreements with Anatech.

They toured the Anatech facility and were shown the anode-cathode array, vacuum systems (present and under construction) and some implements. The system was described in general terms. Motion (or actually, changing of contact points) of implements was mentioned - as arising from a proprietary process.

EPA regulates sterilizers and has a standard protocol involving *B. Subtilis* (which seems to be the strain most resistant to plasma sterilization) on standard carriers: unglazed porcelain and silk sutures. George showed them plastic bags full of these standard carrier items. Mary referred to AOAC methods, a set of protocols using *B. Subtilis* on these carriers, as standards for EPA. They recommended using this as opposed to VSV (Vesicular stomatitis virus) because 1) *B. Subtilis* is harder to sterilize/kill than VSV, and 2) the recognition of its use as a sterilization standard would aid in eventual EPA test approval before a product is marketed.

Cleaning is more difficult than sterilization. But, rather than the sophisticated method of radioassay of labeled virus removal, they feel that conventional chemical methods for detection of residual protein are adequate and simpler. They imply that they can do this.

Sterilization evaluation requires careful and artful following of a few principles in handling the samples. On removal from the device, each carrier or implement is handled by a separate sterile hemostat, and placed with minimal travel in a sterile vial or sterile mason jar of culturing broth, and sealed. The air flow in the room should be minimal, and spraying the air 2+ minutes beforehand with cetalcide glycol spray helps precipitate particulates from the room air.

They recommend, initially at least, providing their technician Aline to do the procedure at Anatech and show Anatech's designated person how to do it. They will also initially provide Mary's assistance, and she is in charge of their part of the activity. They are to prepare and fax a proposal including protocol and

cost by Tuesday 10/16, and do testing 10/16-10/29 to satisfy Anatech's timeline requirements.

## B. STERILIZATION TEST PROTOCOL (PREPARED BY MICROBIOTEST)

### 1. Preparation of Contaminated Carriers:

A suspension of spores will be prepared and counted to yield a high count inoculum (approximately  $10^6$ /ml).

Penicylinders will be washed once with Triton X-100, rinsed with water, placed into Petri dishes matted with filter paper and steam-sterilized for 20 minutes. Sutures will be placed into a tube containing petroleum ether and incubated at  $35 \pm 2^\circ \text{C}$  overnight. Sutures will be drained and the ether evaporated, then placed into Petri dishes matted with filter paper and steam-sterilized for twenty minutes. After sterilization, all carriers will be cooled and held at room temperature until use.

The carriers will be brought into contact with the spore suspension. The carriers will remain in contact with the cultures for 15 minutes at room temperature.

After the fifteen-minute contact period, the carriers will be removed from the spore suspension, placed in a vertical position in sterile Petri dishes matted with two layers of filter paper and placed into a desiccator containing  $\text{CaCl}_2$ . A count level will be determined after preparation of the carriers. A vacuum of 27" Hg will be drawn for 20 minutes. Carriers will be held under vacuum for 24 hours. Carriers prepared in this manner can be used for seven days.

### 2. Test:

Carriers in the desiccators will be transported and delivered to Mr. Robert Barr, Anatech ltd., 5510 Vine Street, Alexandria, VA.

Individual carriers are to be placed in the test instrument in a grid fashion by Anatech, Ltd. employees prior to initiation of the experiment. MicroBiotest personnel will instruct Anatech personnel in aseptic technique prior to the actual test.

After exposure to the test plasma process, the carriers are to be cultured directly from the test instrument into recovery media, supplied by MBT. Ten carriers with B. Subtilis spores will be exposed for each time interval.

### 3. Incubation:

Carriers are to be transported directly to MBT where they will be incubated at  $35 \pm 2^\circ \text{C}$  for 21 days. Visible growth in each tube will be recorded. Then cultures will be heat

shocked, incubated at  $35\pm 2^{\circ}\text{C}$  for an additional three days and visible growth recorded as + (growth) or - (no growth).

#### 4. Acid Controls (Resistance of spores to HCl):

Ten-ml aliquots of 2.5N HCl will be prepared in sterile tubes. Each of ten contaminated, dried cylinders and ten contaminated, dried sutures will be added to one control tube each. The temperature of each tube will be maintained at  $20\pm 2^{\circ}\text{C}$ . At 2, 5, 10, and 20 minutes, a carrier will be removed from its tube and inoculated into a tube of MFT and swirled. The carriers will be transferred again to fresh tubes containing MFT and again swirled. All MFT tubes will be incubated at  $35\pm 2^{\circ}\text{C}$  for 21 days and then scored for growth.

In addition, one suture and one penicylinder (per organism) will be placed in tubes of MFT and incubated as the HD1 controls. These will serve as viability controls.

Uninoculated carriers will be exposed to the test agent and used as controls to indicate carryover of any sterilant or inhibition of growth in the viability testing.

#### Data Presentation:

The final report will include the following information in tabular form for both the test and control plates: 1) the number of positive carriers per organism for each time. 2) The results of the HCl spore resistance (controls).

#### Report Format:

MBT employs a standard report format for each assay design. Reports are issued individually by type of test and by test material. Each final report will provide the following information: sponsor identification, test material identification, type of assay, dates of study initiation and completion, interpretation of results and conclusions, signatures of the study director and other responsible individuals, test results presented in tabular form.

#### Records to be maintained:

All raw data, protocol, protocol modifications, test material, dispensation records, and correspondence between MBT and the sponsor will be stored in the archives at MBT.

#### Personnel:

The study director will be Mary K. Bruch. Resumes are maintained and are available on request.

TABLE 1

First Set of Sterilization Tests, 10/24-11/2/90. Sterilization go/no go evaluation by MicroBioTest Inc.

<u>DATE</u>	<u>ID#</u>	<u>GAS</u>	<u>CURRENT</u>	<u>TIME</u>	<u>PRESSURE</u>	<u>CYLINDERS</u>	<u>STERILIZED</u>
10/24	0	N <sub>2</sub>	100 AC	10	110	PORCELAIN	NO
	1	N <sub>2</sub>	100 AC	20	100	PORCELAIN	NO
	2	N <sub>2</sub>	100 AC	30	100	PORCELAIN	NO
10/25	0	AIR	100 AC	10	100	PORCELAIN	NO
	1	AIR	100 AC	30	100	PORCELAIN	NO
	2	O <sub>2</sub>	100 AC	10	100	PORCELAIN	NO
	3	O <sub>2</sub>	100 AC	30	100	PORCELAIN	NO
10/26	1	AIR	300 AC	10	140	PORCELAIN	NO
	2	AIR	300 AC	30	140	PORCELAIN	NO
	3	O <sub>2</sub>	300 AC	10	140	PORCELAIN	NO
	4	O <sub>2</sub>	300 AC	30	140	PORCELAIN	NO
10/30	0	O <sub>2</sub>	300 AC	15 + 15	140	PORCELAIN	T/O NO
10/31	0	O <sub>2</sub>	150 DC	15 + 15	140	PORCELAIN	T/O NO
	1	O <sub>2</sub>	50 DC	10	110	PORCELAIN	NO
	2	O <sub>2</sub>	50 DC	30	110	PORCELAIN	NO
	3	AIR	50 DC	30	110	PORCELAIN	2/10
11/1	0	AIR	175 DC	30	140	PORCELAIN	NO
	1	O <sub>2</sub>	175 DC	30	160	PORCELAIN	1/10
	2	AIR	150 DC	30	145	PORCELAIN	P/C NO
	3	O <sub>2</sub>	150 DC	30	150	PORCELAIN	P/C 5/5
	4	O <sub>2</sub>	150 DC	15 + 15	140	STAINLESS	T/O 8/10
11/2	0	O <sub>2</sub>	300 AC	15 + 15	150	STAINLESS	T/O 8/10
	1	O <sub>2</sub>	150 DC	30	150	STAINLESS	T/O 9/10
	2	O <sub>2</sub>	300 AC	30	150	STIANLESS	T/O 9/10
	3	O <sub>2</sub>	150 DC	15	140	PORCELAIN	P/C NO

T/O = TURNED OVER

P/C = PAN CATHODE

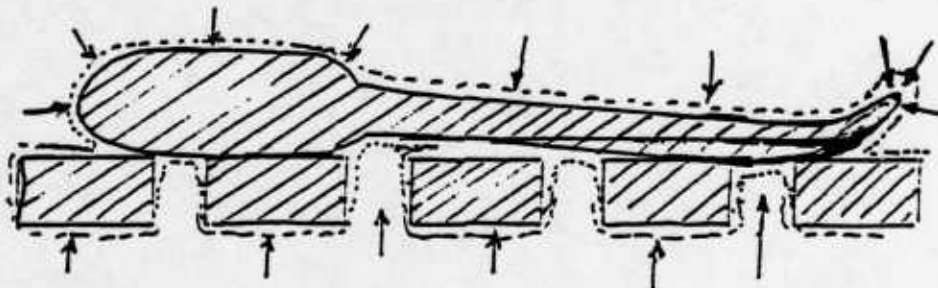
PRESSURE MEASURED IN mTORR

TIME MEASURED IN MINUTES

CURRENT MEASURED IN mA

Fig. 1 Implement and tray as cathode of glow discharge, showing anticipated sheath structure and normally-impinging ions.

DISCHARGE ION PLASMA



# HEAT STERILIZATION

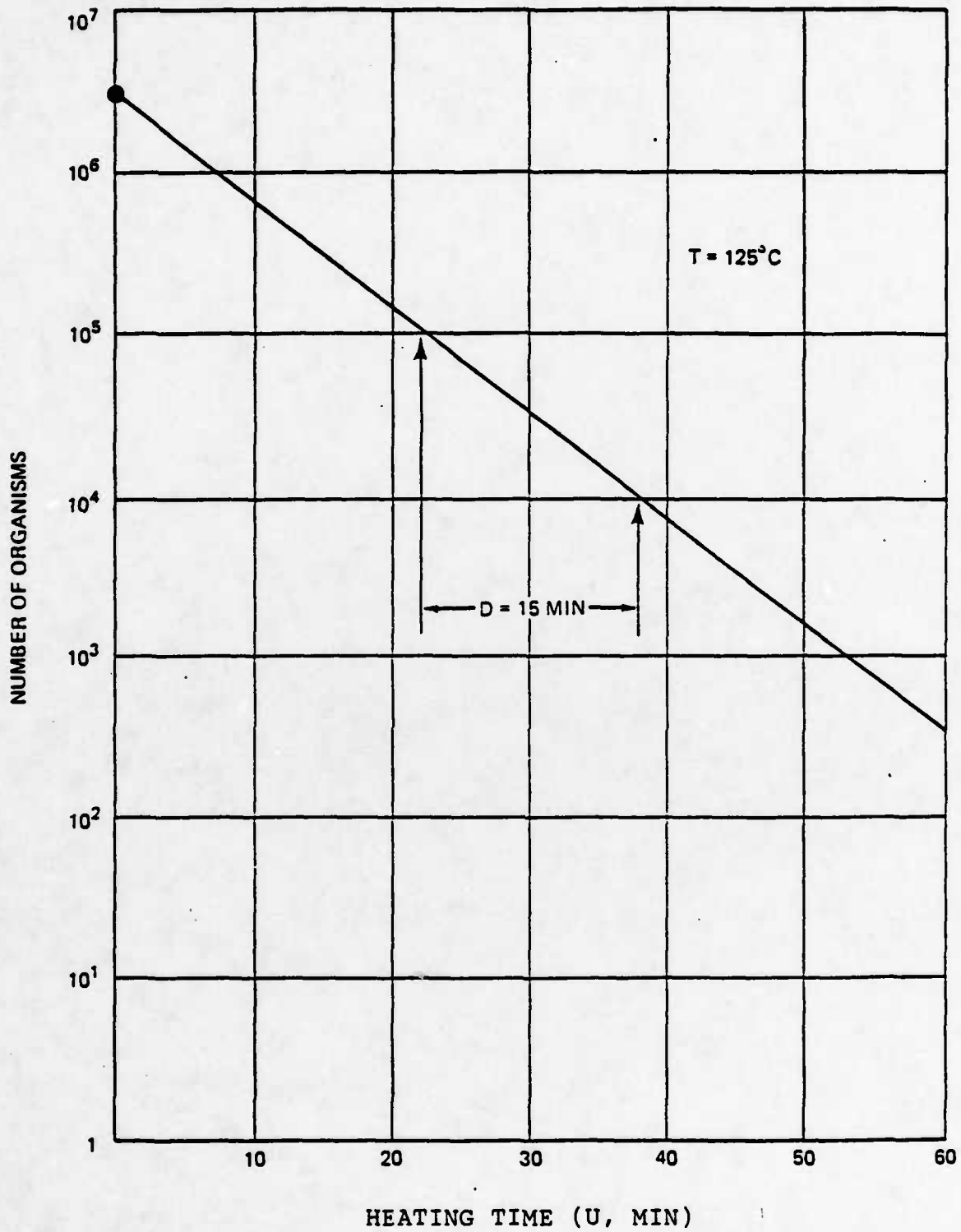


Fig. 2 - Typical straight line semilogarithmic survivor curve for *Bacillus subtilis* var. *niger* spores on a stainless steel surface.

RADIATION STERILIZATION

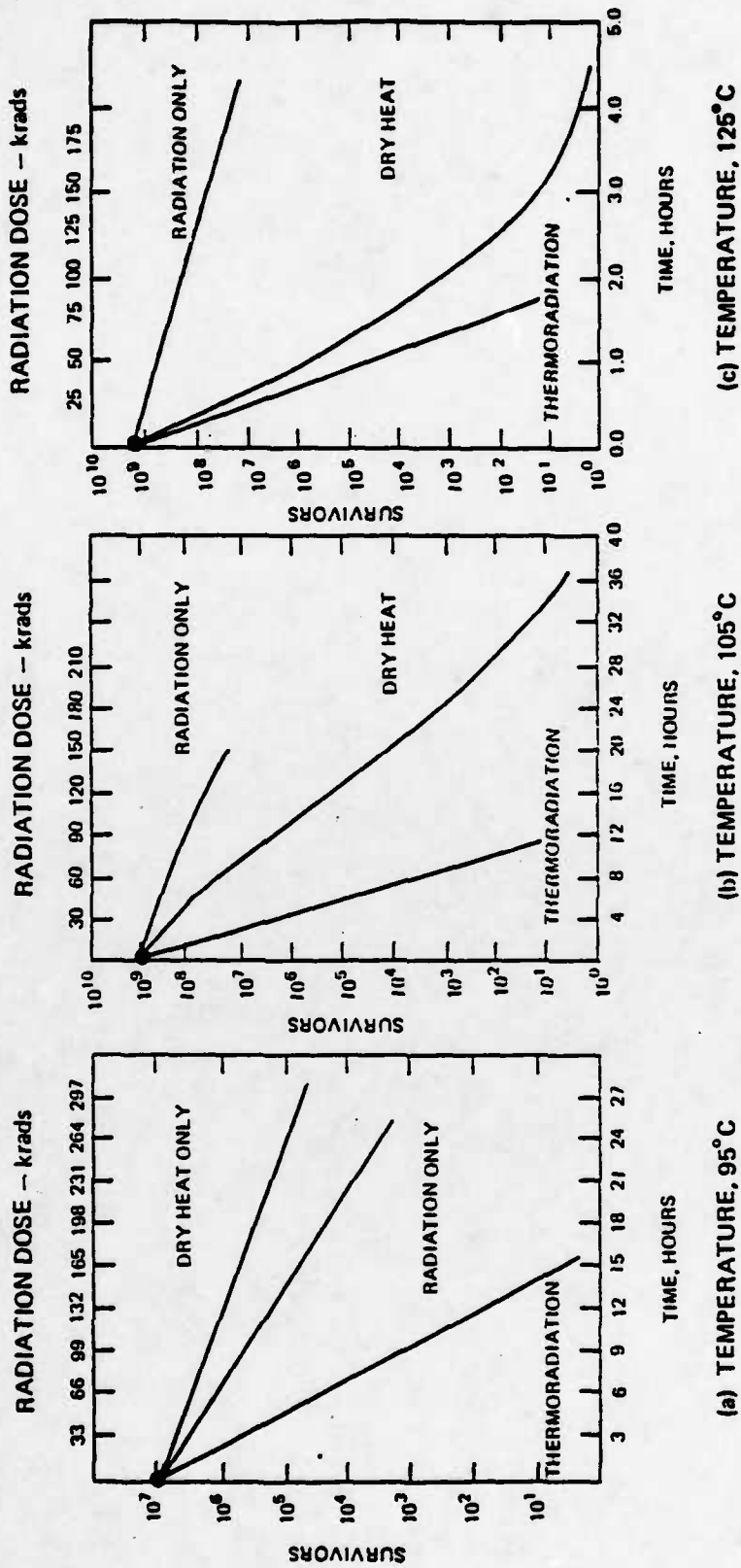


Fig. 3 - Comparison of radiation, dry-heat and thermoradiation inactivation of *Bacillus subtilis* at 95°C, 105°C, 125°C.

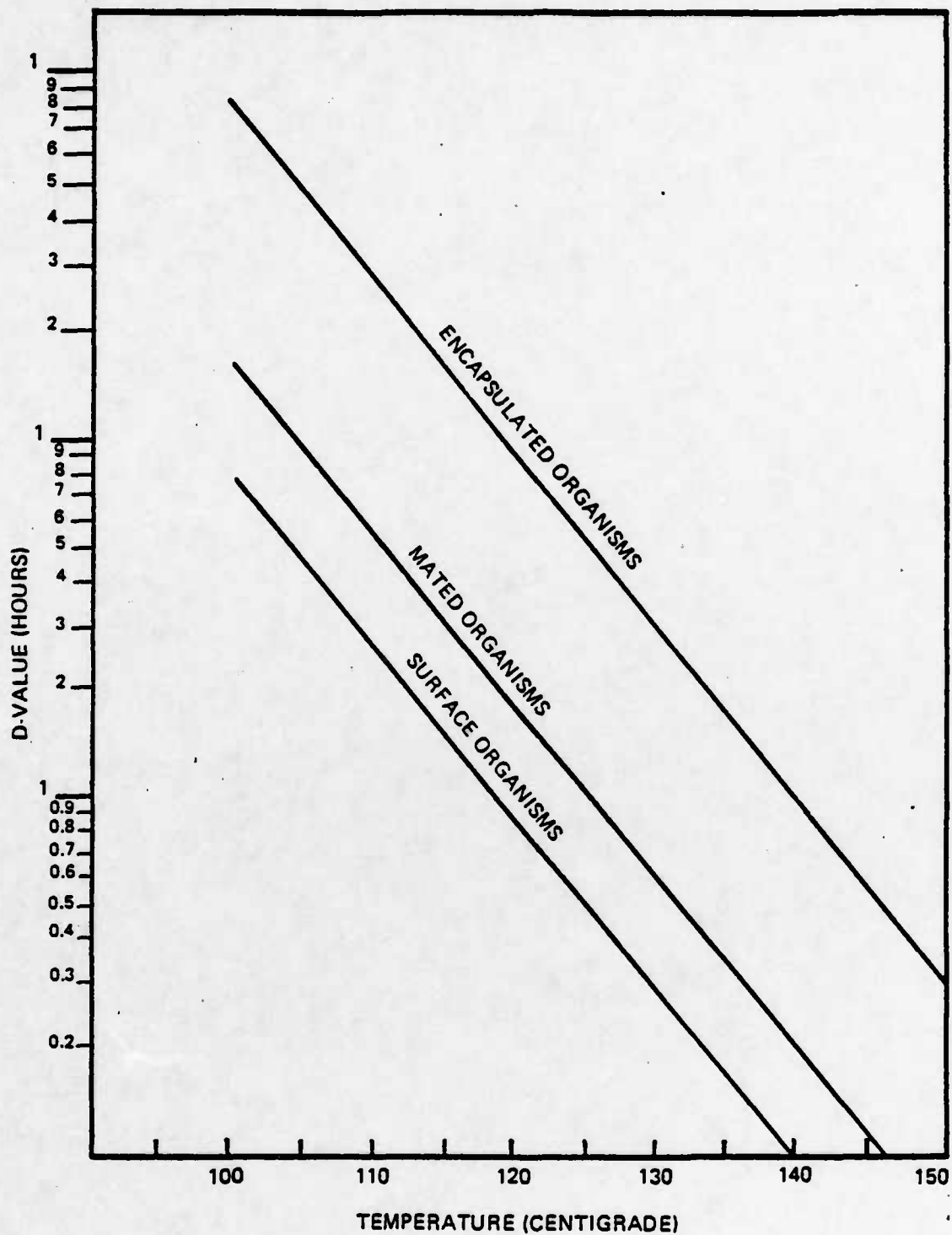


Fig. 4 - D-value as a function of temperature for *Bacillus subtilis* spores.

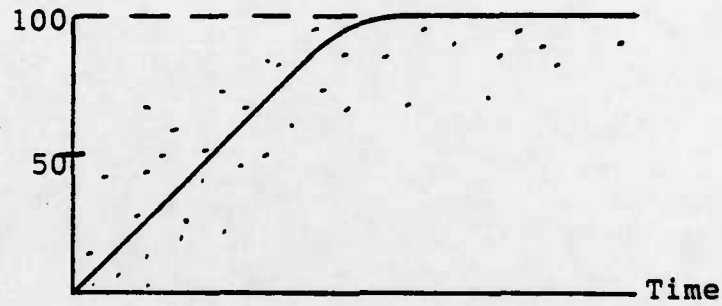
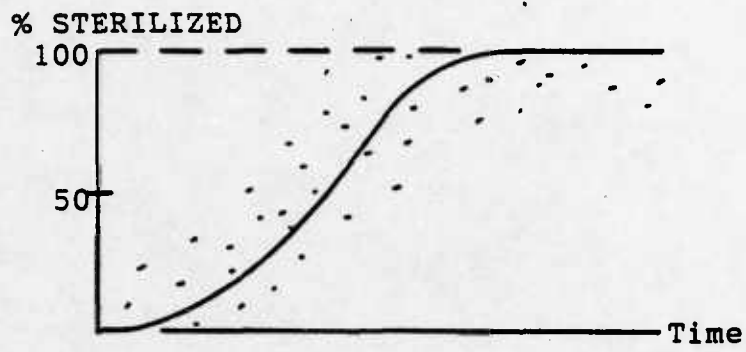


Fig. 5. Two experimentally indistinguishable dependences of sterilization on run time.

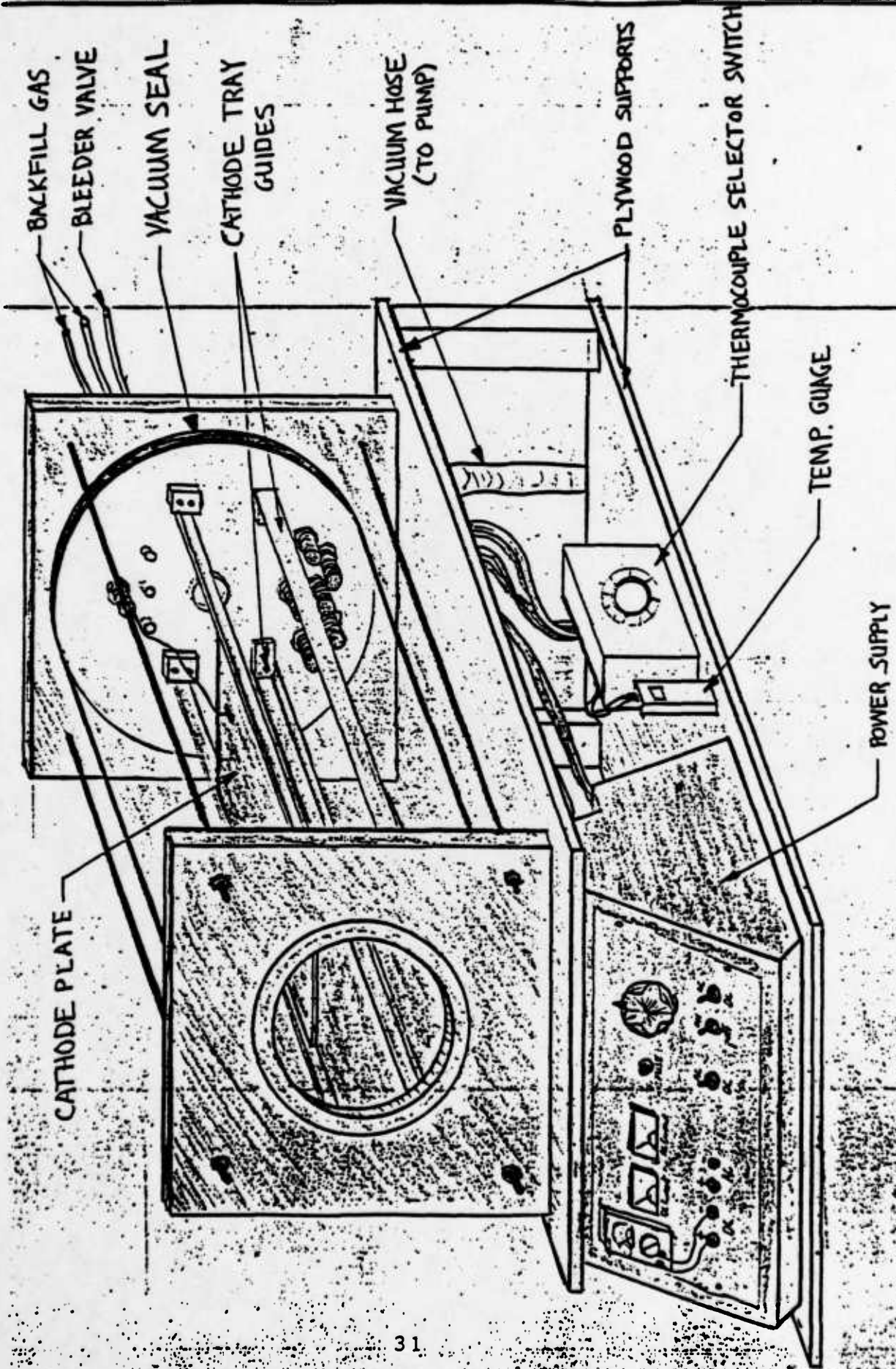


Fig. 6

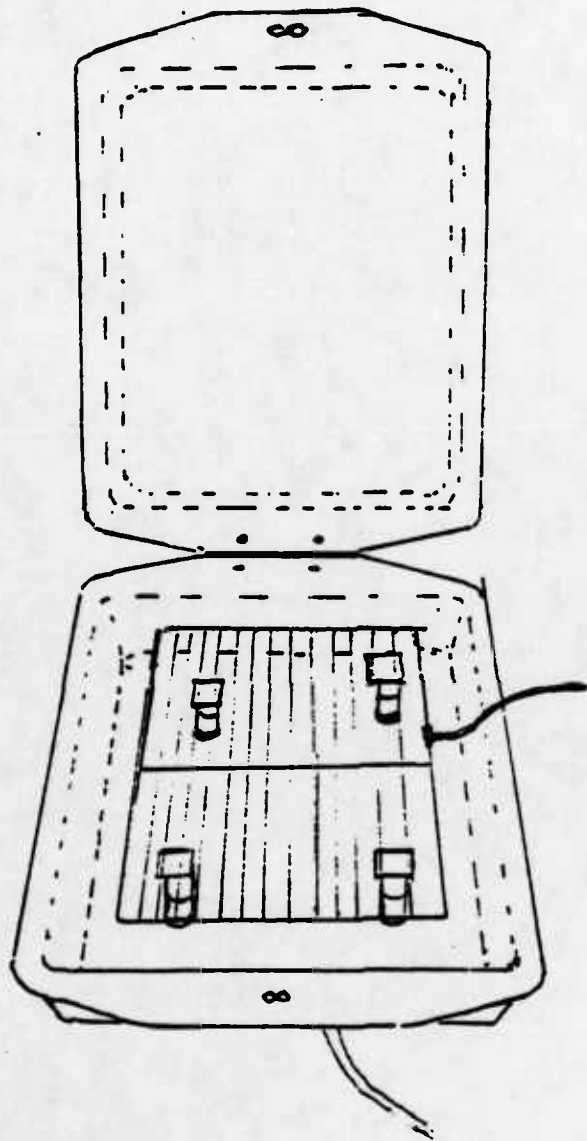


Fig. 7

Stainless steel "clamshell" drawer (conceptual-test model) with cathode rack .

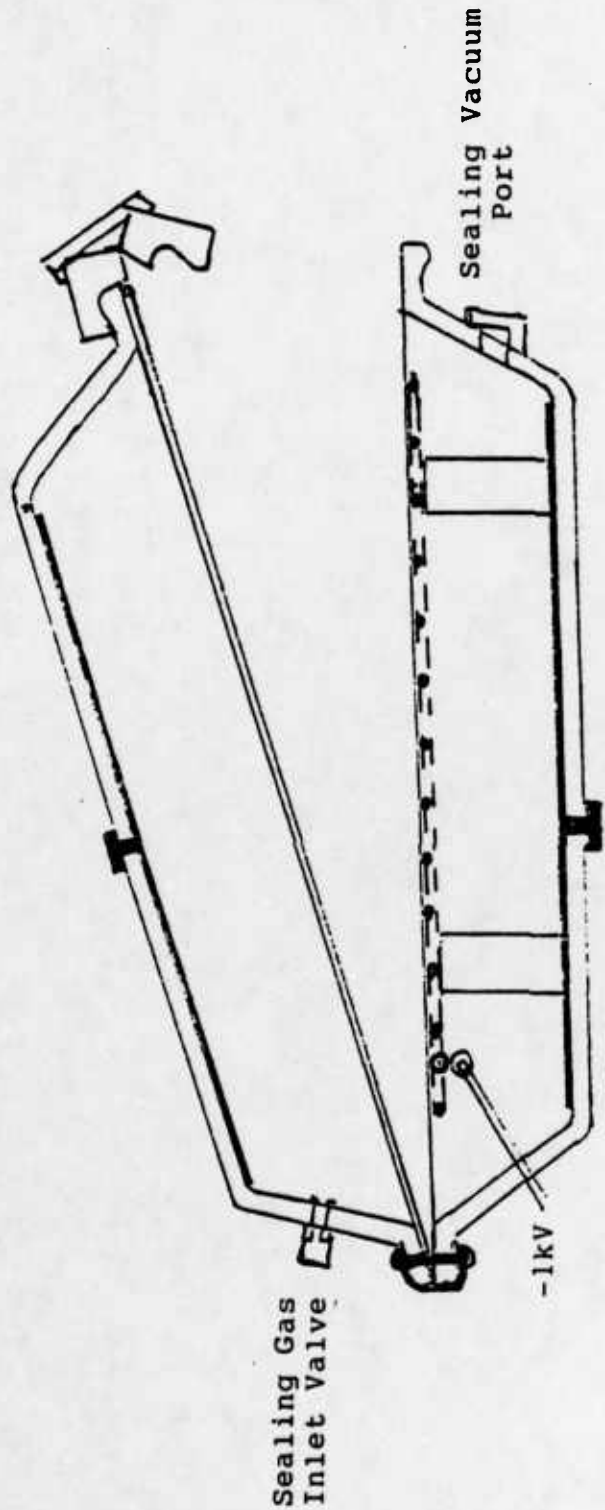


Fig. 8

Prototype design of pyrex clamshed drawer containing high-voltage cathode tray and stainless steel anode plates on top and bottom (grounded).

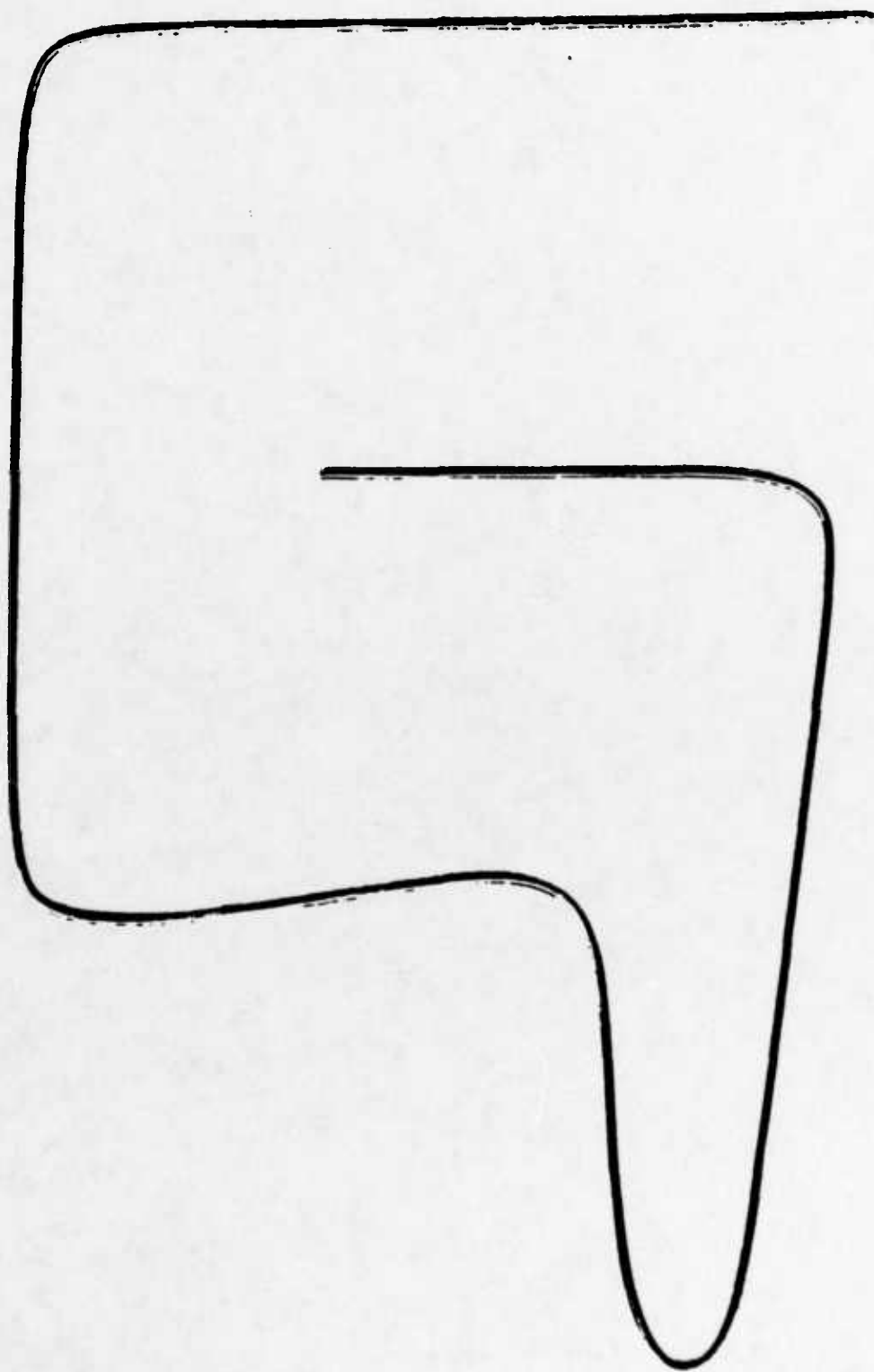


Fig. 9 Wire form for holding hollow cylinder spore-carriers  
(65% of actual size)



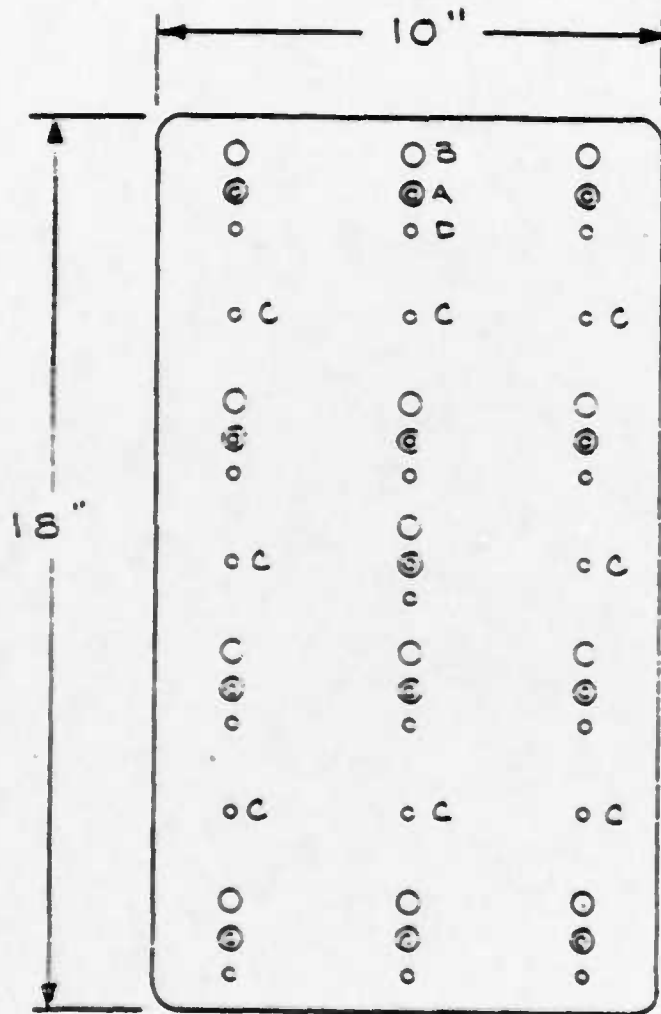
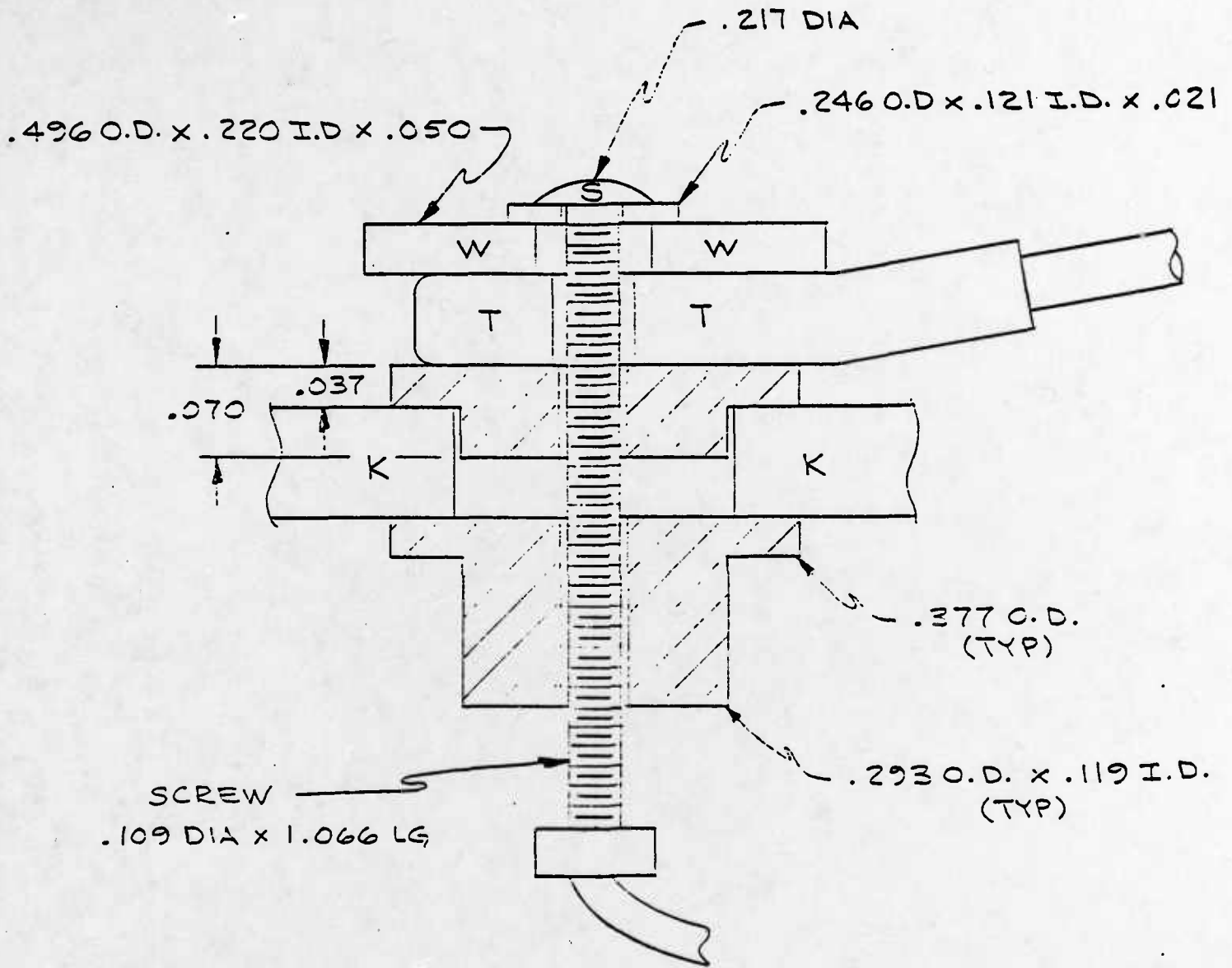


Fig. 11



DIMENSIONS ARE IN INCHES

Fig. 12

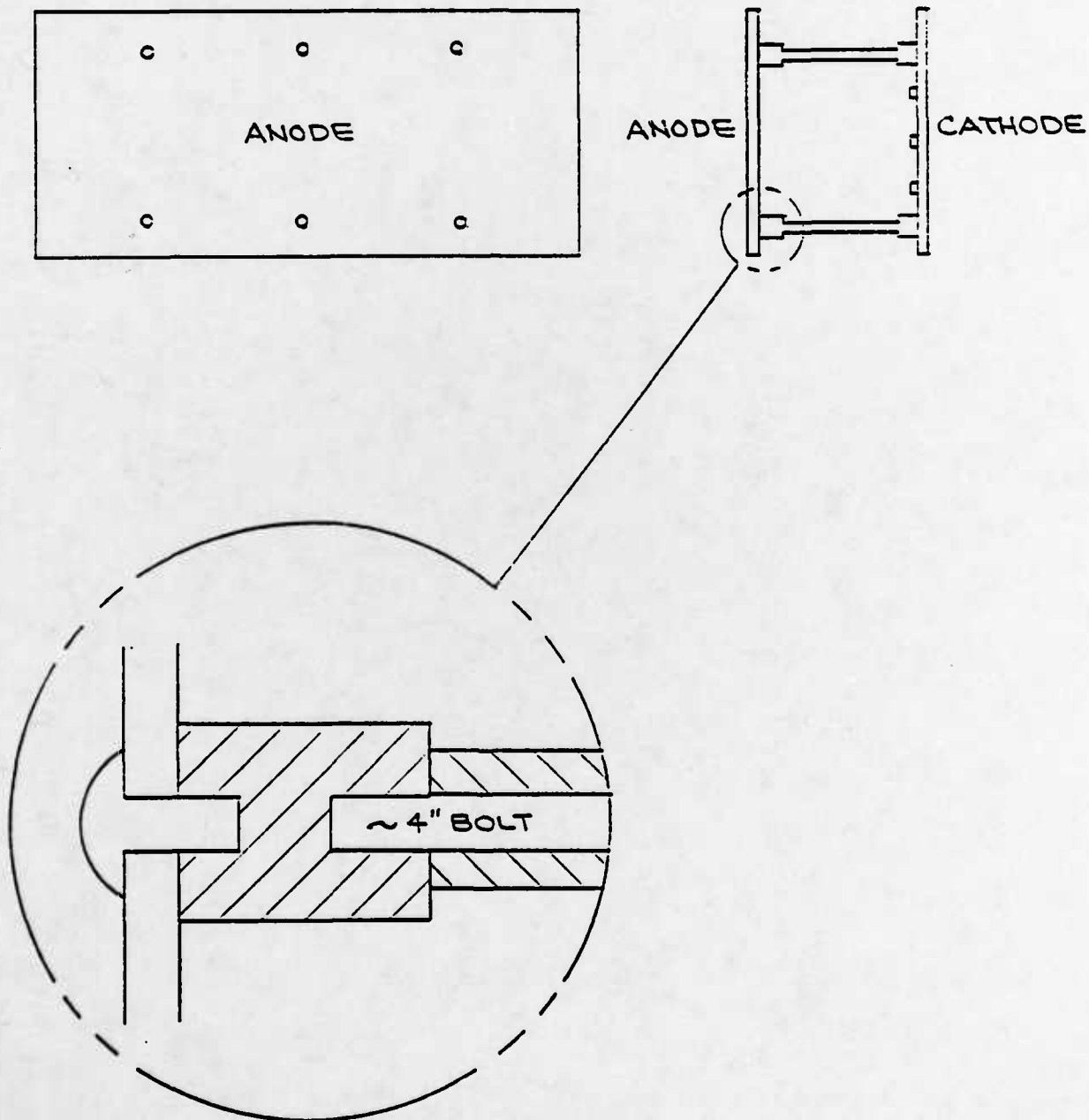


Fig. 13

DC discharge fluence profiles in air and argon at 100 mTorr, 50mA.

Thermocouple temperature changes after 10 minutes.

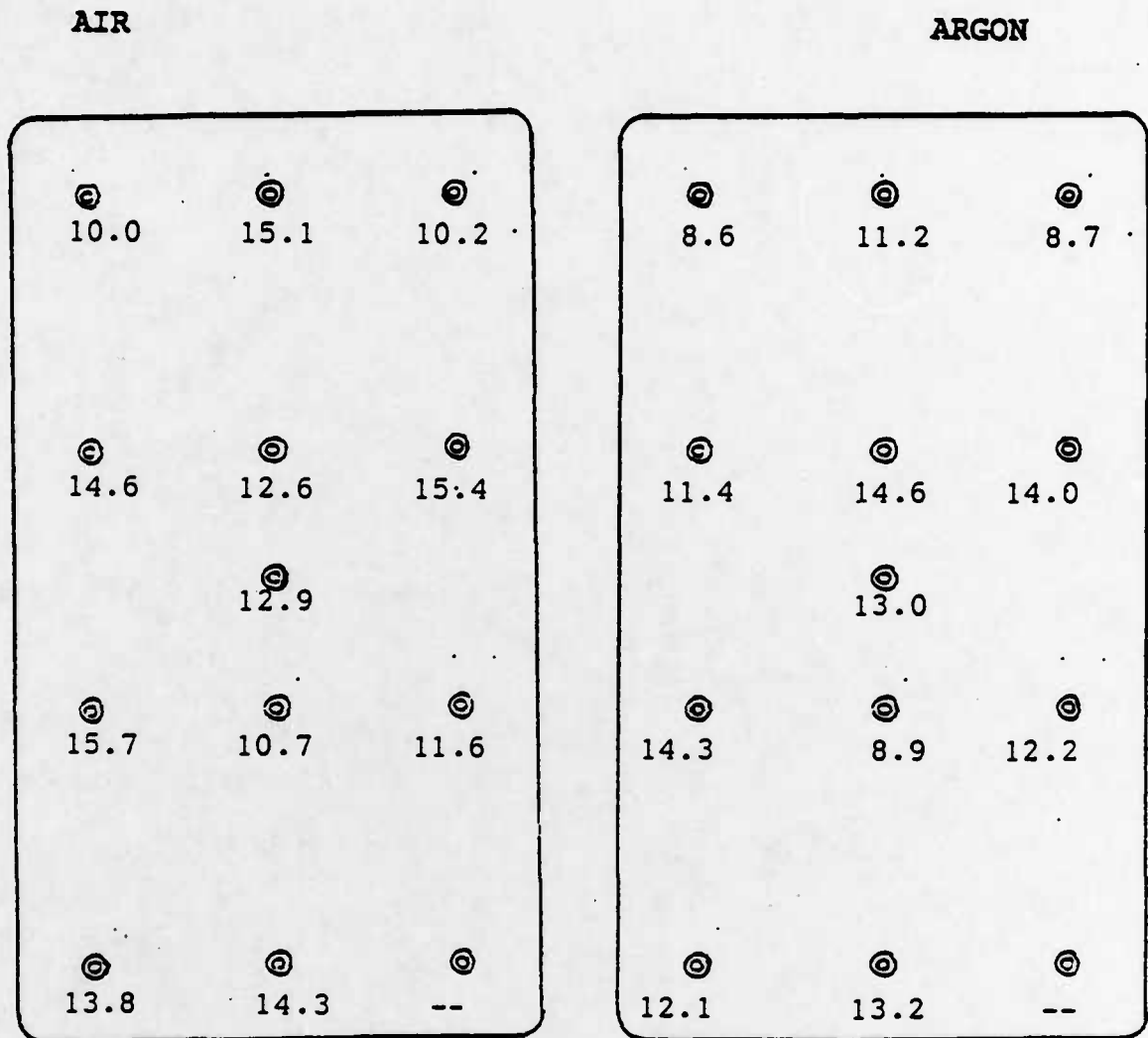


Fig. 14

Relative temperature rise of thermocouples in DC air discharge  
(100 mTorr, 50 mA) after 10 minutes, with hemostat on cathode.

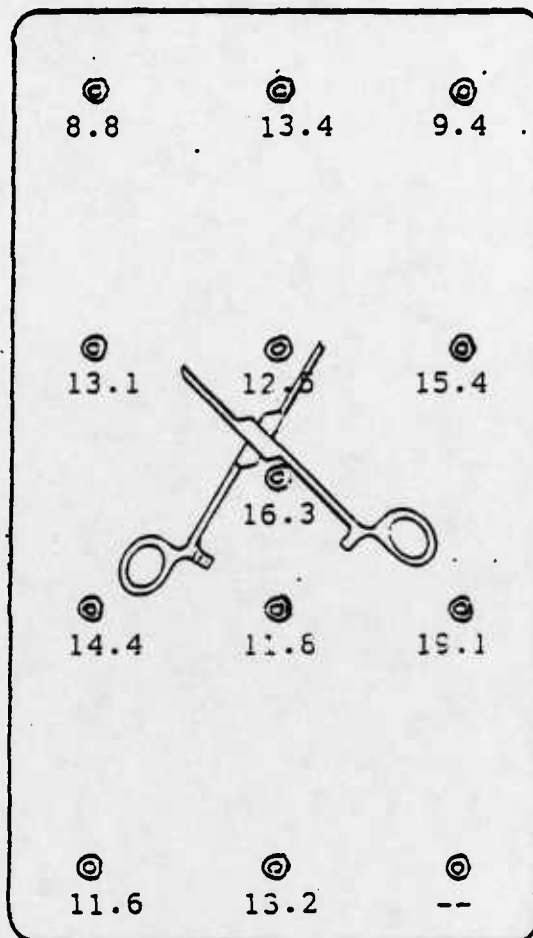


Fig. 15

Plasma probe. Probe element is 007" Tungsten wire  
inside teflon and ceramic insulator tube inside  
ceramic-coated stainless support tube.

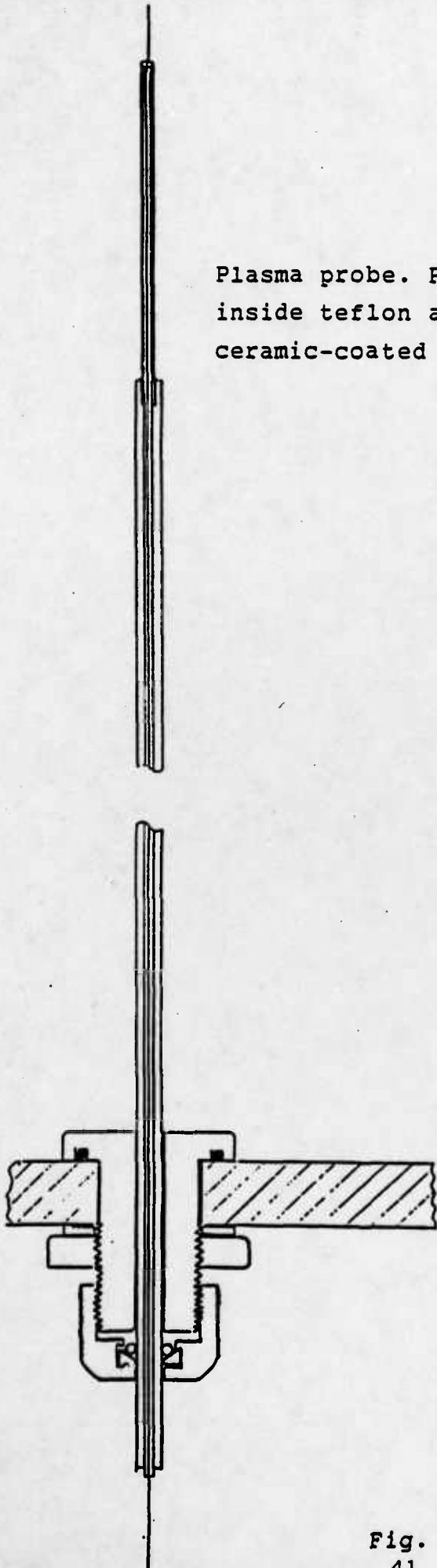


Fig. 16  
41

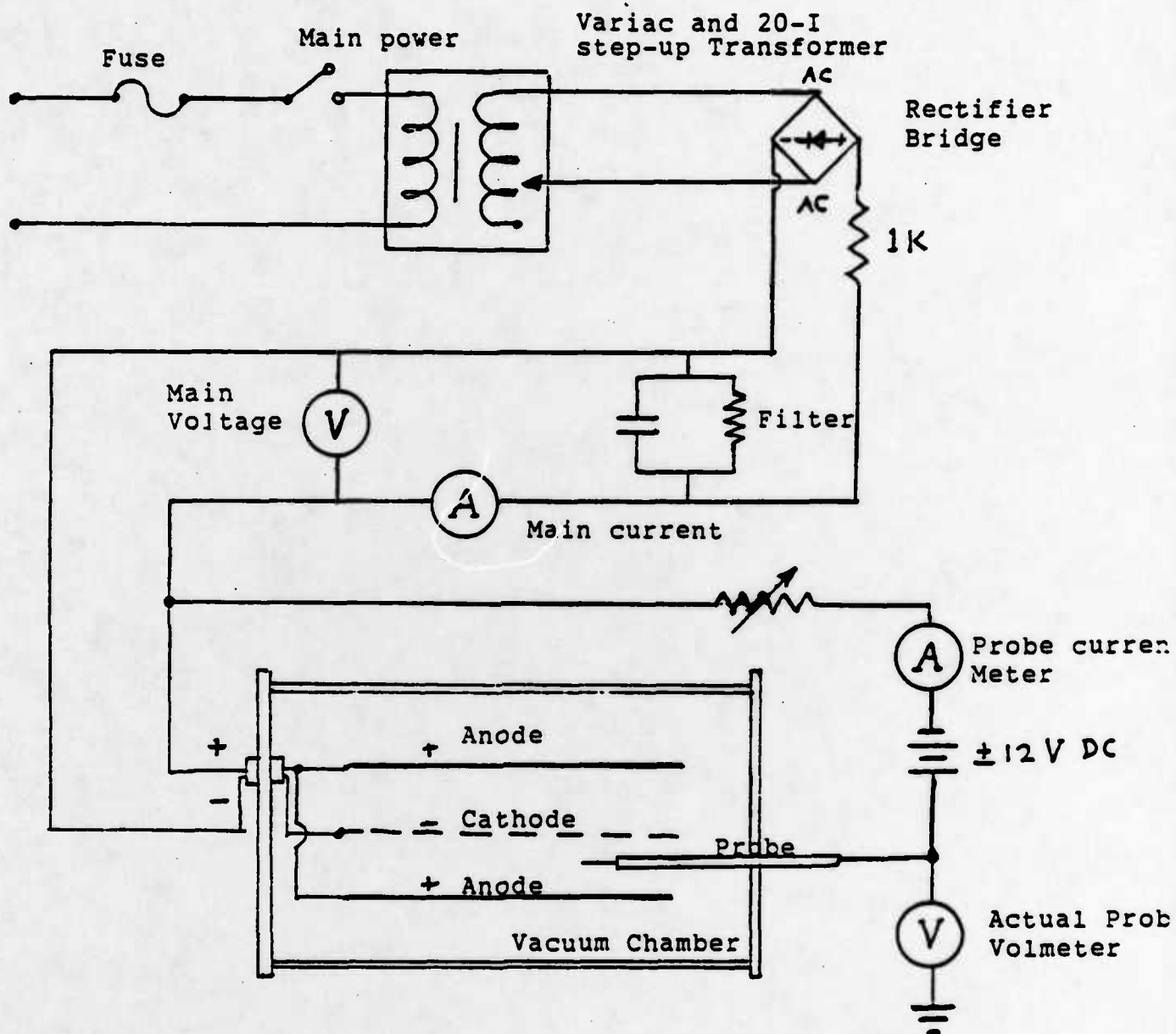


Fig. 17  
 First probe circuit shown with DC power supply to device .

Stainless clamshell anode  
100mTorr  $O_2$  + air  
100mA  
 $V_A = +2.6V$ ;  $V_k = -410V$  DC

AK distance: 1.7" below (4.32 cm)  
2.6" above (6.6 cm)

'Mesh' cathode between anodes  
Probe: .018 cm dia  
1.52 cm long  
.086  $cm^2$  surface area  
2.24 cm above cathode

Note change of vertical scale by X10  
between + and -  $I_p$  values

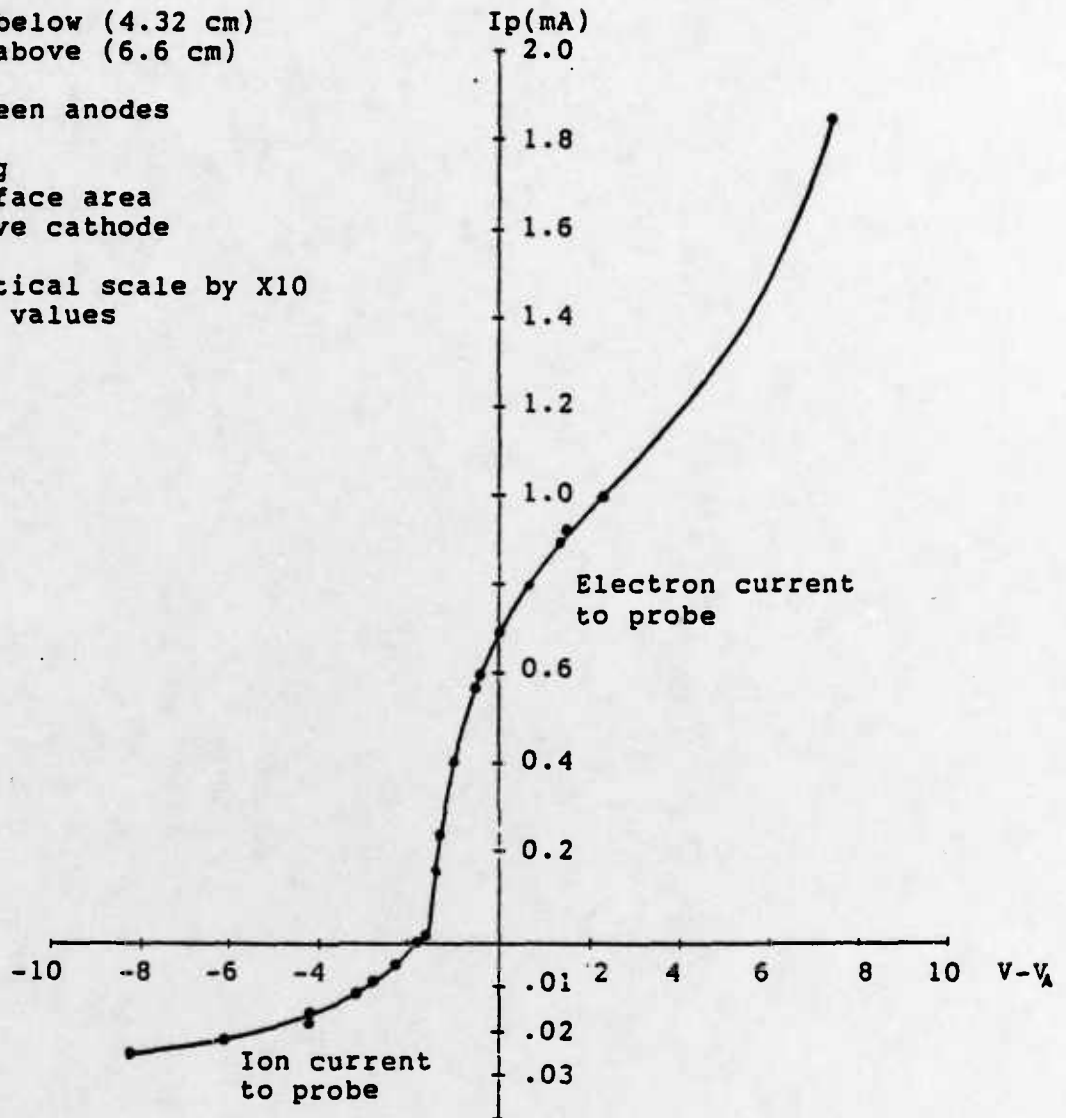


Fig. 18. Current-Voltage Relation for a Cylindrical Langmuir Probe

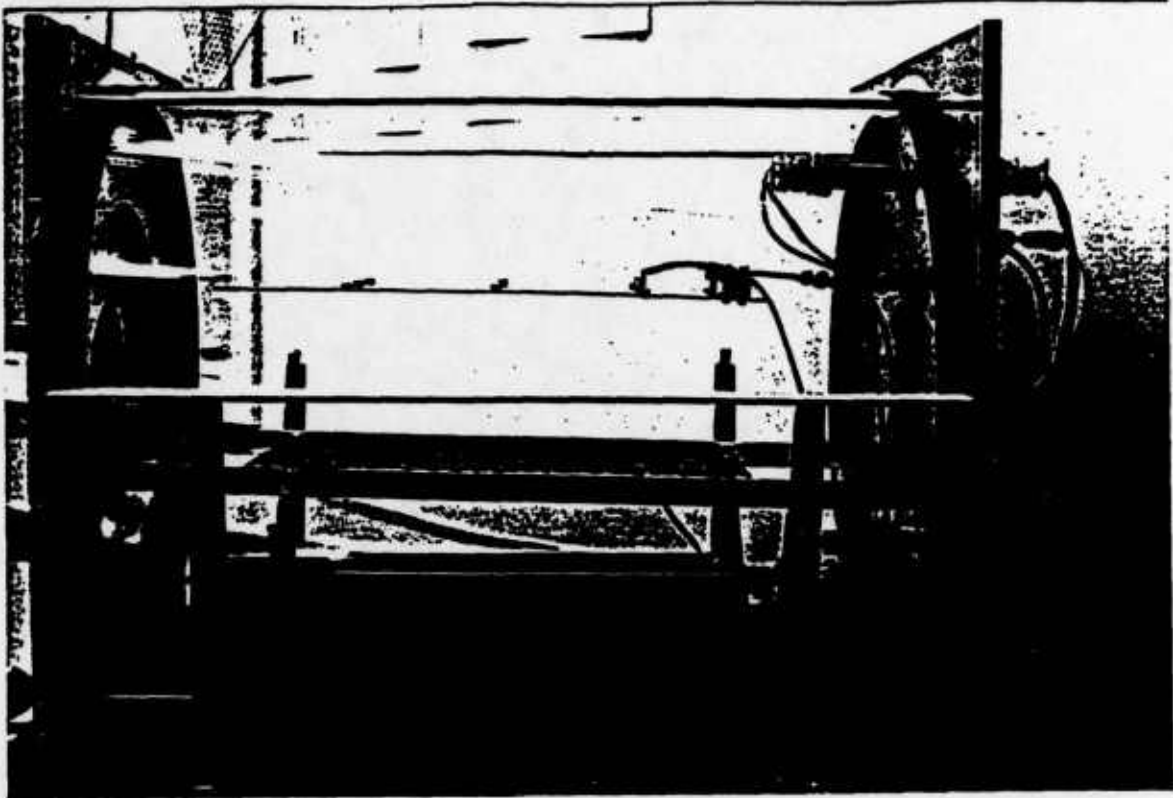


Fig. 19 Experimental device for sterilization by ion plasma

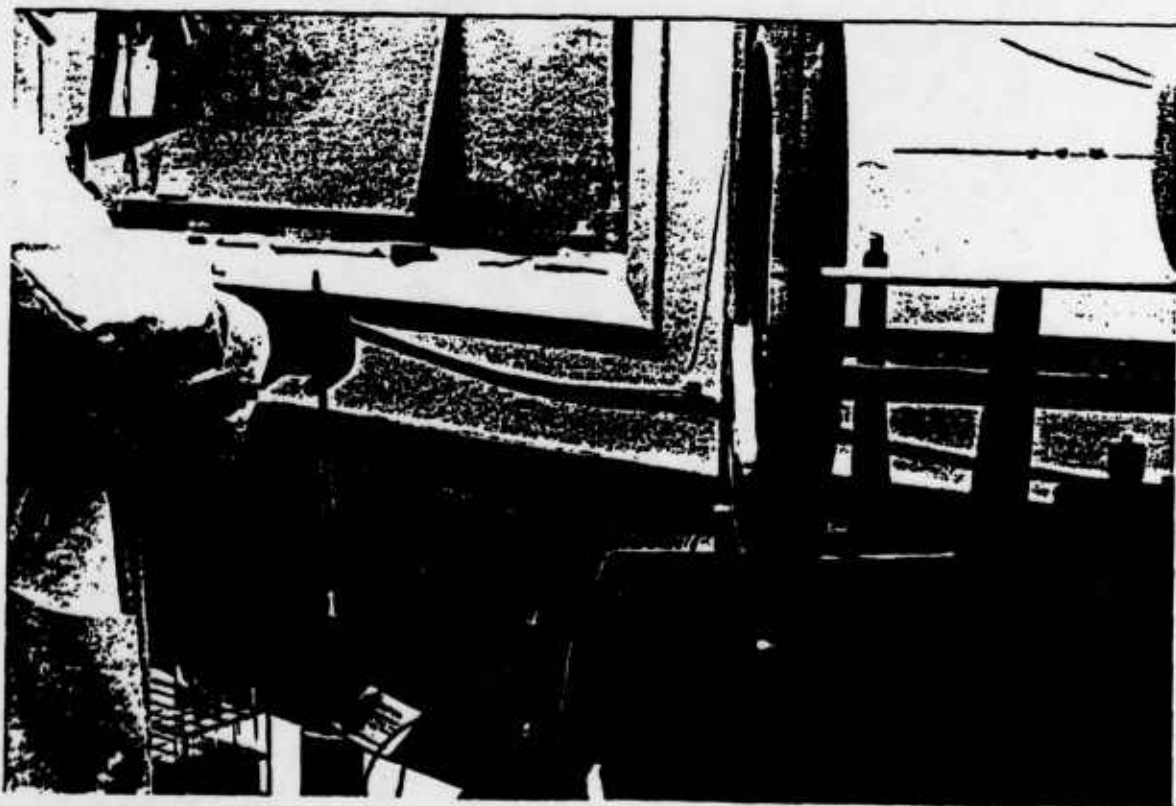


Fig. 20 Pre-sterilization of carrier-removal hook

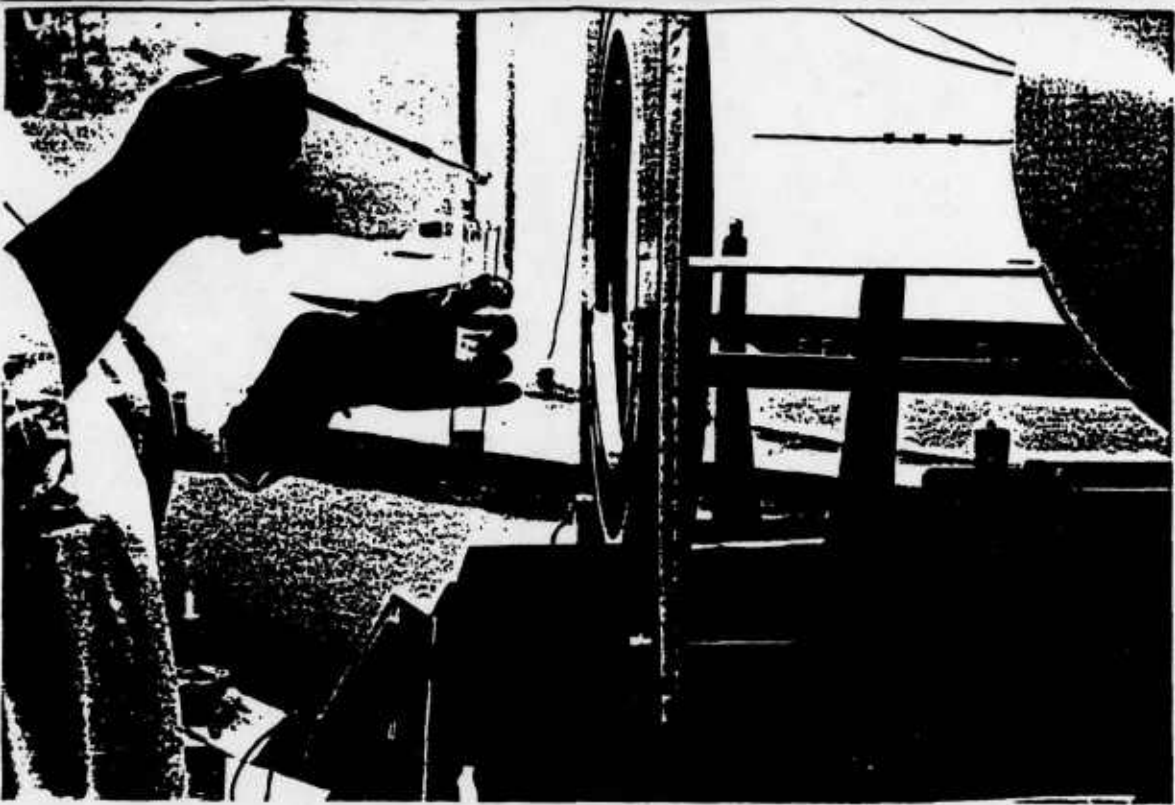


Fig. 21 Removing spore-carrier cylinders to incubation broth after sterilization run

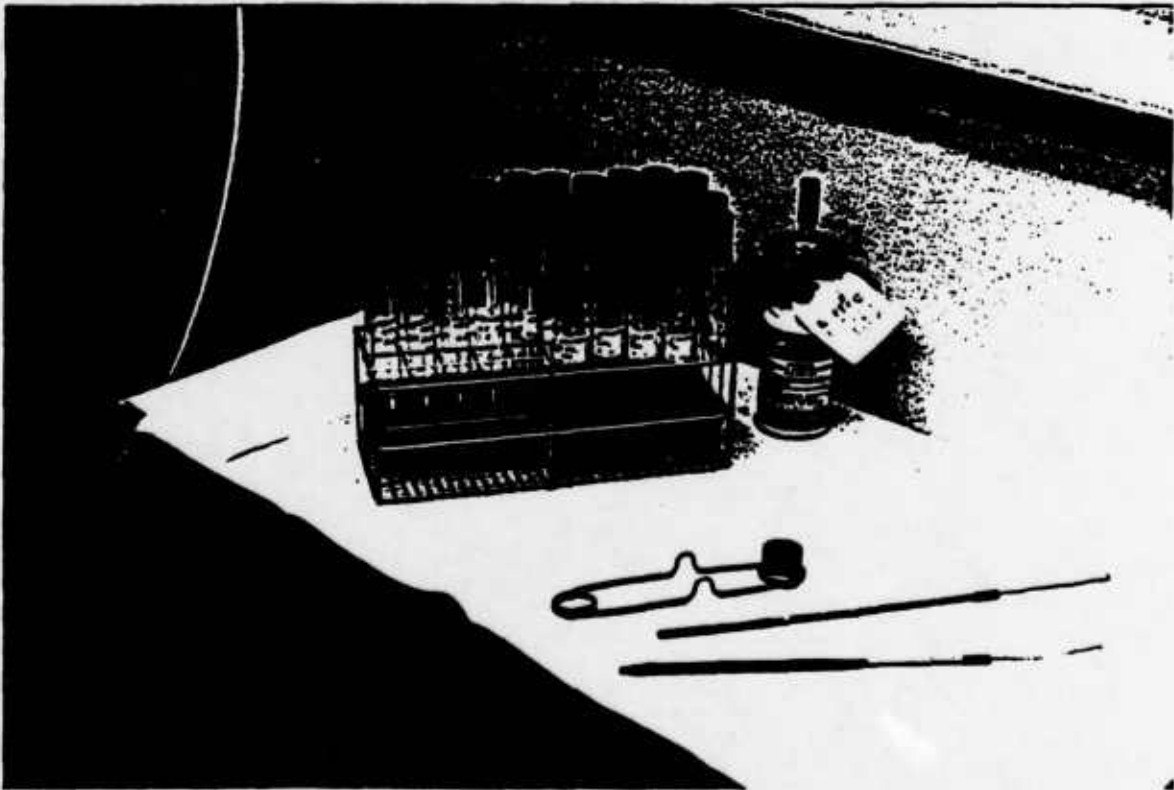


Fig. 22 Carriers in broth test tubes labeled with date, run #, position in run

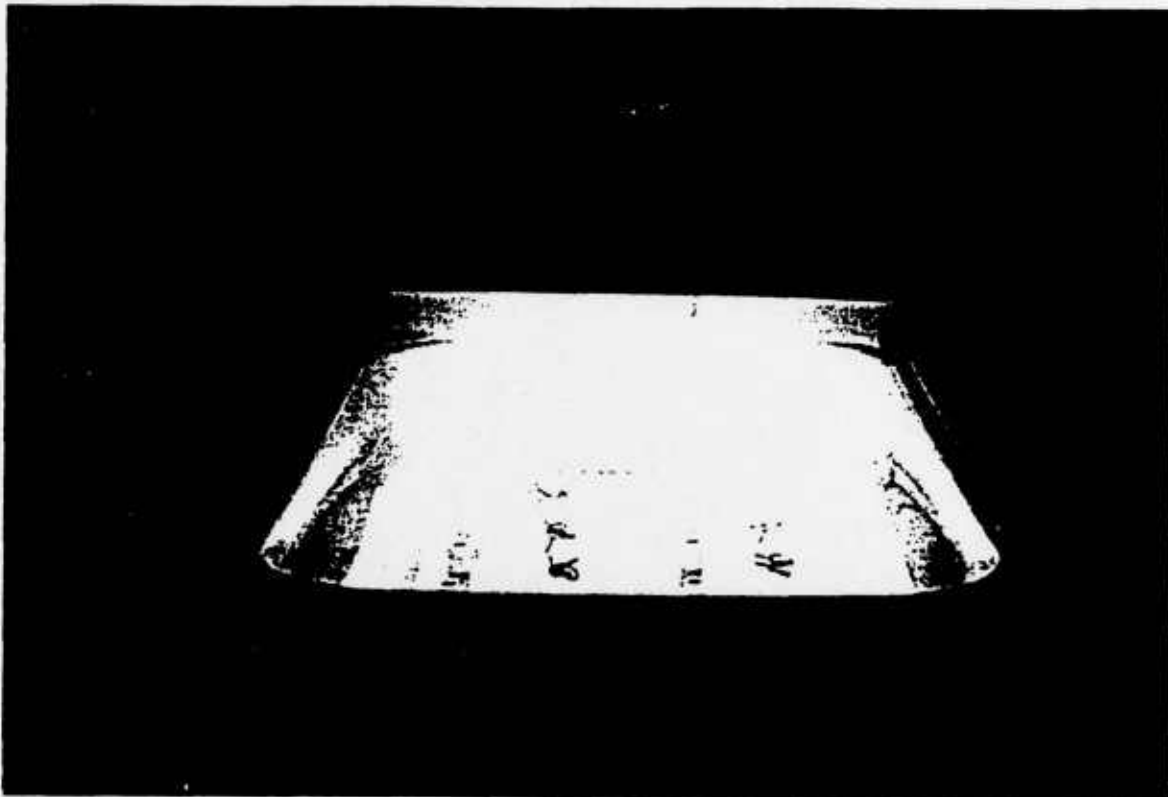


Fig. 23 Pan cathode with carriers, during run

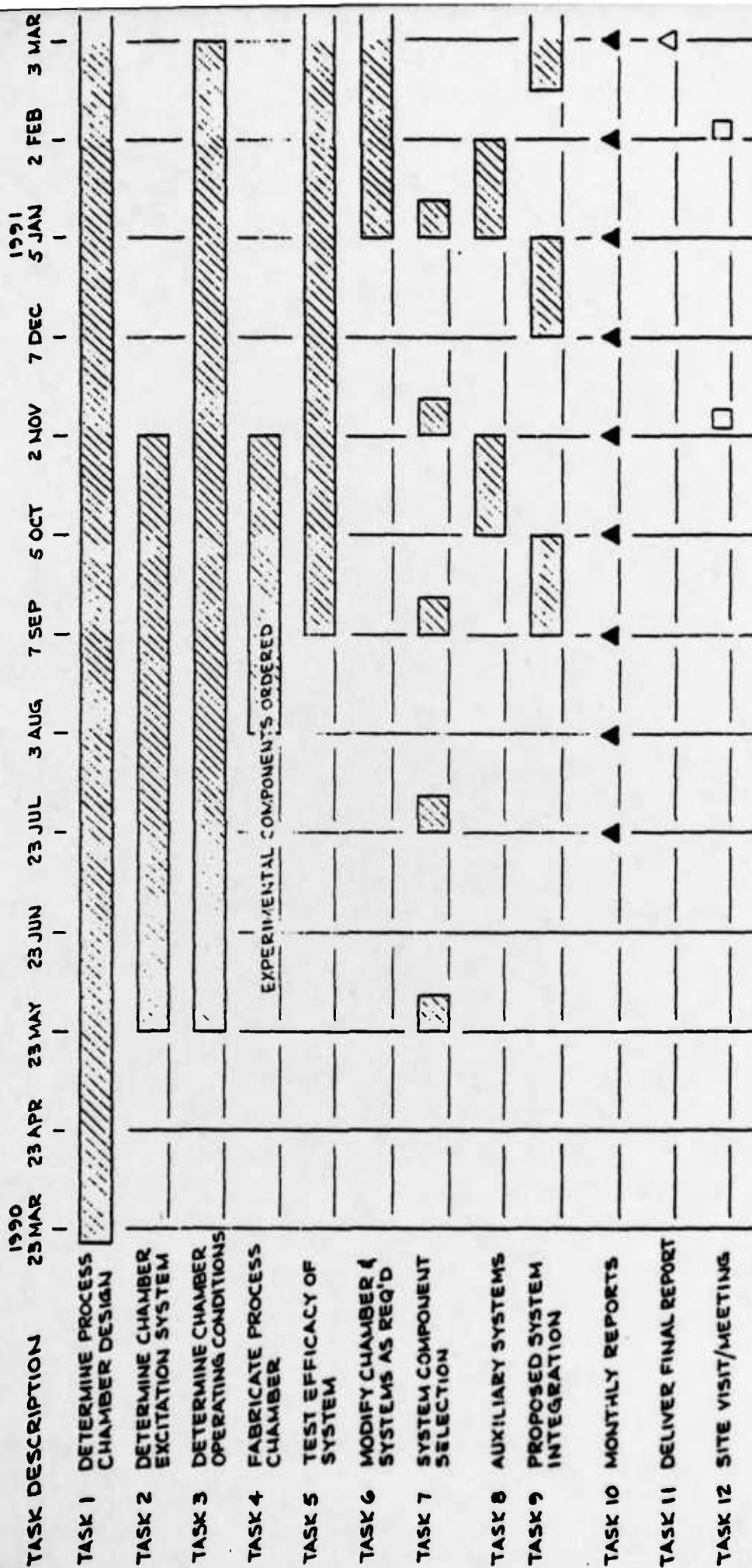
PROJECT TITLE: ENGINEER, TEST, AND BUILD ETCHER TO CLEAN AND STERILIZE SURGICAL INSTRUMENTS

PERFORMING ORGANIZATION:  
ANATECH LTD

PRINCIPAL INVESTIGATOR:  
ROBERT W. BARR

DATE: JULY 23, 1990

CONTRACT NO.: DAMD17-88-C-8190  
MODIFICATION NO. P00005



FIRST OF TWO YEAR FUNDING INCLUDED

Fig. 24

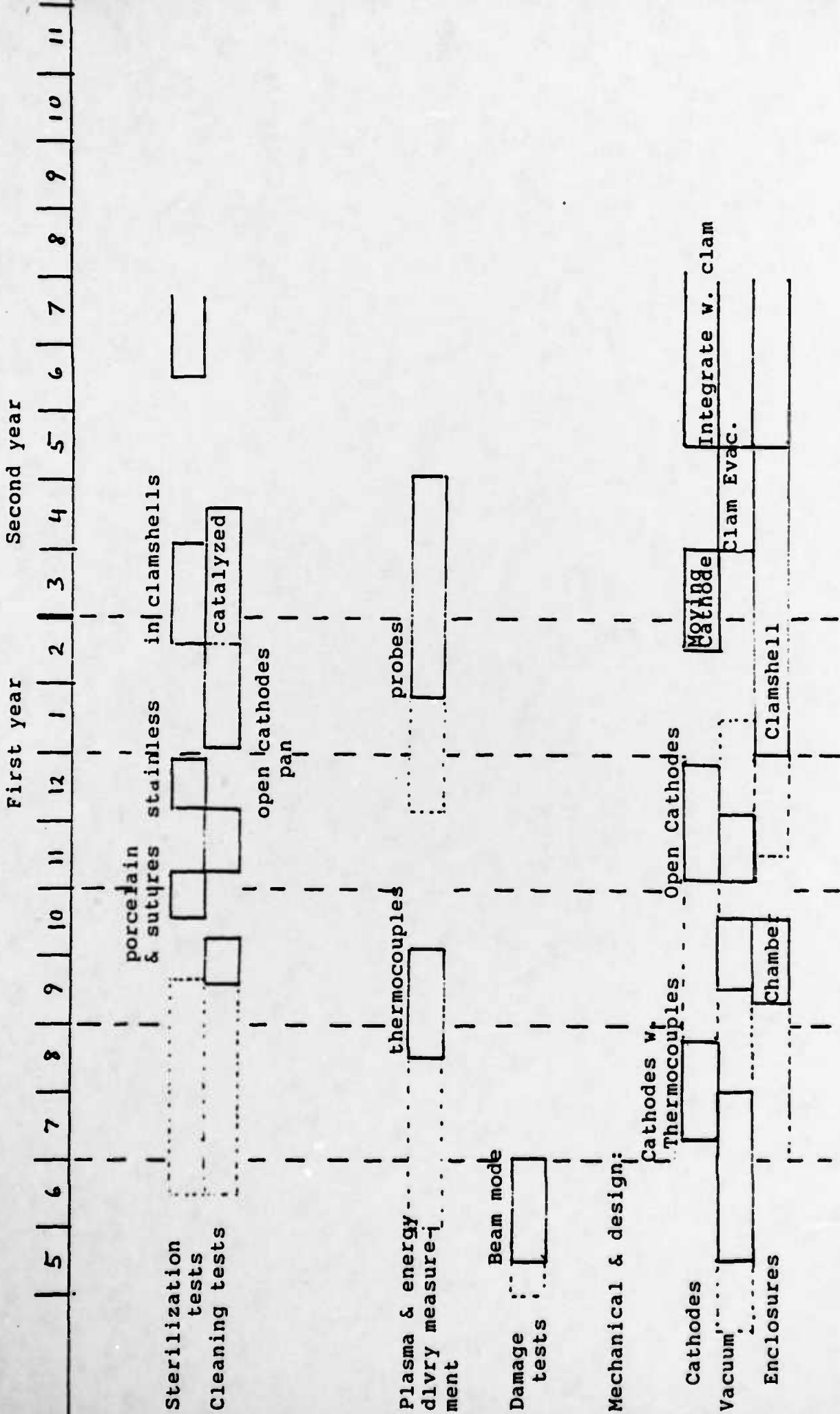


Fig. 25

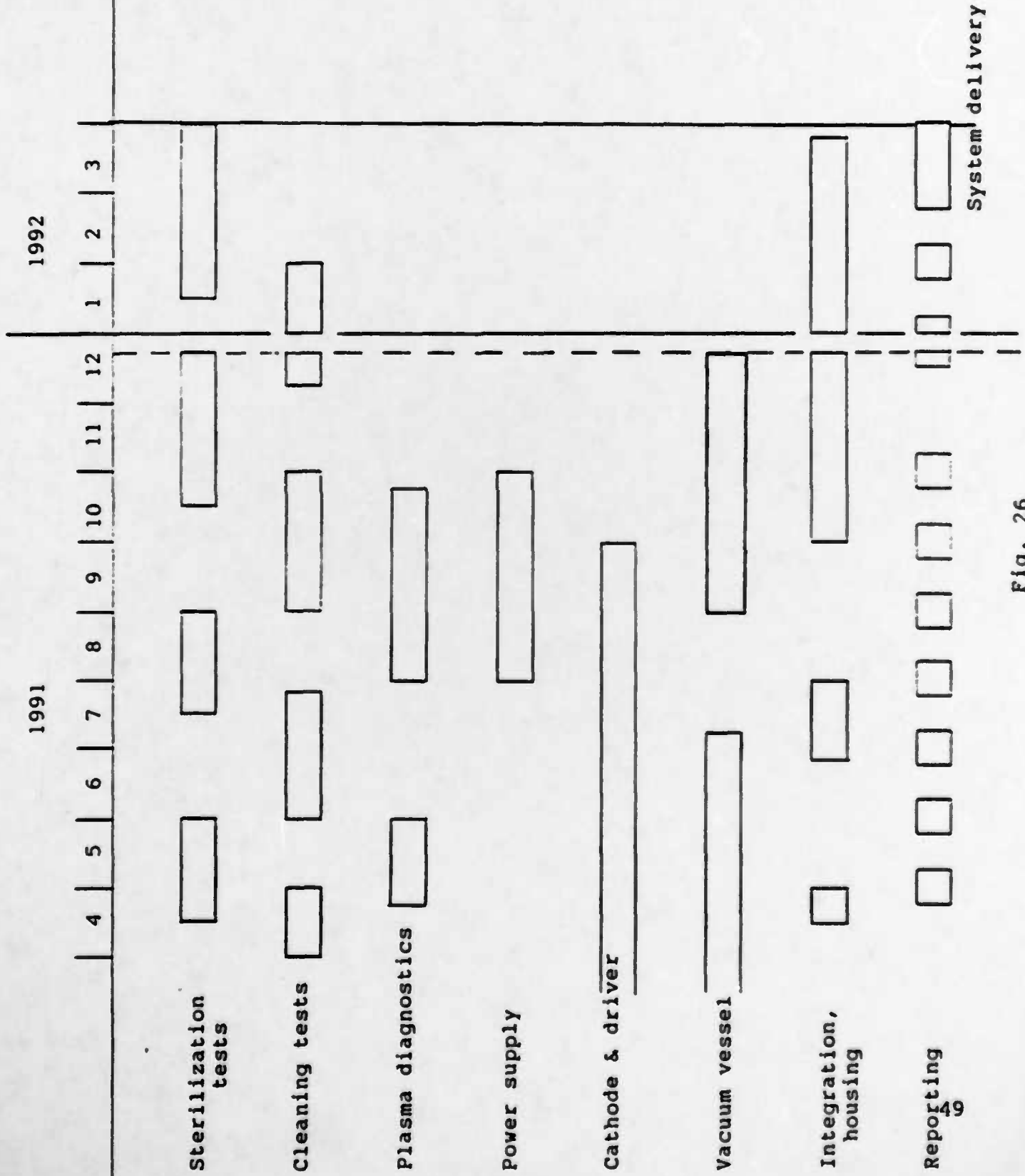


Fig. 26