ANNUAL PROGRESS REPORT

Integrated Research and Training Program
in Molecular Biology
(Ultrastructure and Electron Microscopy)

Humberto Fernández-Morán, M.D., Ph. D.
Professor of Biophysics
Department of Biophysics

THE UNIVERSITY OF CHICAGO

THE RESEARCH INSTITUTES

ANNUAL PROGRESS REPORT

TITLE OF PROJECT: Integrated Research and Training Program in Molecular Biology (Ultrastructure and Electron Microscopy) carried out under:

NASA Grant NsG-441-63

NIH·Grant B-2460, NB-04267

AEC Grant AT (30-1)-2278

INSTITUTION: University of Chicago

PRINCIPAL INVESTIGATOR: Humberto Fernández-Morán, M.D.,Ph.D.
Professor of Biophysics
Department of Biophysics

ANNUAL PROGRESS REPORT

SUMMARY

Following the plan described in the original research proposal, our major effort during the past year has been devoted to:

I. Organization and Initial Operation of the Special Electron Microscope Laboratories for the Proposed Research and Training Program.

With funds provided by NASA (NsG-441-63), NIH (B-2460, NB-04267), AEC (AT (30-1)-2278), and the University of Chicago, a special laboratory facility for high resolution electron microscopy has now been completed and put into operation in the Research Institute. These Laboratories occupy a total of about 4,000 square feet and comprise:

(1) 2,500 square feet of remodeled space in the basement with installation of special floor, wall partitions, ceiling panels, and air conditioning of the type used in "clean rooms" of modern electronic industrial facilities. These laboratories are equipped with two large electron microscopes with attached electron diffraction units. Provisions have been made, and the preparations completed, for installing three additional electron microscopes to be delivered during the next few months. The laboratory facilities include ultra-high vacuum (Varian) evaporation units, four ultramicrotomes, light microscopes, and complete preparation and photographic darkroom facilities. All of the critical equipment has been installed on individual vibration control mountings of special design. Corresponding precautions were taken in the installation of non-magnetic stainless steel ventilation ducts, incandescent lights, and electrical conduit to minimize electrical and magnetic perturbations.

(2) Special highly regulated power supply located in an air-conditioned enclosure on the fifth floor of the Research Institutes. This 50-kilowatt motor generator set, specially designed and manufactured by Westinghouse Company, is equipped with a new solid state regulator, giving better than 0.1% voltage stability and very low harmonic distortion.

(3) Adjoining laboratories with a total area of about 1,200 square feet located on the 2nd floor, are being remodeled for the additional electron microscope and x-ray diffraction facilities.

Most of these laboratories are already fully operational. Preliminary tests clearly demonstrate the exceptional level of sustained performance and high reduction (4 to 6 Angstroms point resolution) which can be consistently achieved by means of this novel combination of optimized operational parameters. These laboratories are generally considered to be the most advanced research facility available at present for high resolution electron microscopy.

The facilities now available embody the following features which are particularly suited for the research and training program:

(a) High resolution electron microscopy can be carried out under ideal conditions, independent of ambient conditions, practically on a 24-hour basis. This stands in contrast to the vast majority of electron microscope installations which are constantly subject to random environmental perturbations (line voltage fluctuations, etc.). Moreover, this unit is designed for a total of at least six electron microscopes operating at maximum efficiency. A broad research and training program, conforming to the highest standards in this field can therefore be implemented.

(b) This is probably the only existing electron microscope facility which is especially equipped to operate under "clean room" conditions. Clean room conditions are, in fact, essential for the proposed research program in order to minimize the danger of contamination of extraterrestrial specimens with ambiaent dust particles.

(c) Favorable location of the Research Unit close to the Low-temperature facilities, the new laboratory for Astrophysics and Space Research, the Institite for Computer Research, and the Enrico Fermi Institute for Nuclear Studies is of key value for the contemplated research and training program.

Under such favorable conditions, this unit could therefore develop as the nucleus of a larger national and international research and training center for molecular biology and electron microscopy.

II. Specific Research Program

The research and training program represents essentially a continuation of the comprehensive, integrated program in the field of molecular biology which is being carried out with support from NASA, NIH, AEC, and the University of Chicago. The following specific projects are being pursued as part of a comprehensive program comprising the following major aspects:

A. Continuation of correlated electron microscope and biochemical studies of mitochondrial membranes which have resulted in the detection and isolation of a fundamental unit of energy transduction (electron transfer particle). A repeating particle associated with the cristae and the inner membrane of the external envelope has been recognized and characterized in beef heart mitochondria by correlated electron microscopic and biochemical studies. Many thousands (ca. 10^4 to 10^5) of these particles, disposed in regular arrays, are present in a single mitochondrion. The repeating particle, called the elementary particle (EP), consists of three parts: (1) a spherical or polyhedral head piece (80 to 100 Å in diameter); (2) a cylindrical stalk (about 50 Å long and 30-40 Å wide); and (3) a base piece (40 x 110 Å). The base pieces of the elementary particles form an integral part of the outer dense layers of the cristae. The elementary particles can be seen in electron micrographs of mitochondria in situ, of isolated mitochondria, and of submitochondrial particles with a complete electron transfer chain. Negative-staining with phosphotungstate is only one of several techniques that can be used for reproducible demonstration of the repeating particles and underlying subunit organization of mitochondrial membranes. A particulate unit containing a complete electron transfer chain can be isolated from beef heart mitochondria. The isolated unit approximates in size that of the elementary particle in situ. The molecular weight of the particle in situ is calculated to be 1.3×10^6. Evidence is presented for identifying the isolated unit with the elementary particle visualized in situ. The elementary particle of the mitochondrion is believed to be a prototype of a class of functional particles or macromolecular assemblies of similar size found in association with membranes generally. Similar integrated studies of related membrane derivatives in photo-receptors, myelin and other cell membrane systems will

be carried out, to gain a better understanding of the fundamental principles underlying cell membrane organization in general (See publication no.92).

B. Electron microscopic and biochemical studies of pyruvate dehydrogenase complex of Escherichia coli carried out in collaboration with Lester J. Reed, Masahiko Koike and Charles R. Willms (See publication no. 93). Examination of the Escherichia coli pyruvate dehydrogenase complex and its component enzymes in the electron microscope indicates that the complex has a polyhedral structure with a diameter of about 300 to 350 Å and a height of 200 to 250 Å. The lipoic reductase-transacetylase aggregate, consisting of about 64 subunits, occupies the central portion of the polyhedron and has the appearance of a tetrad of 130 to 150 Å. Surrounding this tetrad are about 16 molecules of pyruvate decarboxylase and about 8 molecules of dihydrolipoic dehydrogenase arranged into two rings laid one above the other. The sequence of these latter molecules cannot yet be specified. However, many of these molecules are similar in appearance and dimension (70 to 90 Å) to those observed in electron micrographs of the isolated pyruvate decarboxylase component of the complex. The reconstituted complex closely resembles the native complex in appearance.

C. Collateral development work on improvement of preparation techniques and instrumentation for high-resolution electron microscopy will include further application of low-temperature methods (cryofixation), using liquid helium II, and the design of new types of high-resolution "cryoelectron microscopes" immersed in a liquid helium II cryostat, using superconducting electromagnetic lenses.

D. Electron microscope and electron diffraction studies of DNA macromolecules in solution will be continued, using such special techniques as vacuum-tight micro-chambers, low-intensity microbeam illumination, etc. These techniques will also be applied in systematic studies of other nucleic-acid containing systems, including ribosomes, nuclear constituents and virus particles, under conditions approaching the native hydrated state.

E. (1) Correlated electron microscope and electron diffraction studies of certain meteorites (Orgueil carbonaceous chondrite) carried out in collaboration with

Dr. Edward Anders and Dr. Frank W. Fitch of the University of Chicago. Preliminary experiments indicate that the higher resolving power of the electron microscope can be fruitfully applied to further elucidate the composition and structure relationships of its constituents, with particular reference to the "organized elements". A variety of preparation techniques are being applied under carefully controlled conditions, including ultra-thin sectioning with a diamond knife, mechanical and selective chemical dissociation followed by density gradient separation, negative staining, shadow-casting, etc. The ultra-structural data will be correlated with parallel chemical studies of organic constituents and to the results of selected area electron diffraction analysis.

(2) Electron microscopy studies of pre-Cambrian organized systems. Preliminary investigation of nonferruginous cherts of the Gunflint formation of southern Ontario by electron microscopy has been started in collaboration with Dr. Edward Anders and Dr. F. Fitch. S.A.Tyler, of the University of Wisconsin, and Dr. E. Barghoorn, of Harvard University, have reported (SCIENCE, Vol. 119, p.606, 1954) the occurrence of primitive lower plants in these pre-Cambrian rocks, which are the oldest (about 2 billion years) structurally preserved organisms that clearly exhibit cellular differentiation. Electron microscopy reveals the presence of filaments, tubular structures and membranes of apparent organic origin. This work looks most promising and we plan to continue systematic examination of the chert material kindly supplied by Dr. E. Barghoorn, using ultra-thin sectioning with the diamond knife and the Moran-Leitz microtome, mechanical and chemical dissociation techniques and related preparation procedures.

These studies are of great interest in the evolutionary scheme of primitive life, since they may furnish insight into the molecular organization of the oldest known preserved living systems, bearing also on the evolution of membrane ultrastructure.

Electron microscopy of meteorites and of pre-Cambrian fossils should yield uniquely valuable information, and may eventually provide the methodological and constructural basis for future investigation of extraterrestrial (lunar and planetary) matter.

(3) Development of techniques for electron optical examination of extraterrestrial matter. Systematic development work is currently under way for application of electron microscopy, electron diffraction, microprobe analysis, and related electron optical techniques to the examination of samples of extraterrestrial matter, including meteorites, material obtained from lunar and planetary probes, etc. Present procedures for examination of sub-microscopic particles deposited on thin resistant plastic or carbon films can be readily adapted to the special needs of space probes. These suggested techniques, including application of single crystal graphite and microfilms, which are merely extensions of already tested and well-developed electron microscope preparation procedures, would have certain important advantages for sampling of space specimens: (1) Minimum samples of material for analysis, which could be well below the resolving power of light microscopes, can be used; (2) the condition of the specimens, as they existed in the vacuum and low temperature of outer space, can be largely preserved by adequate preparation techniques at a resolution which is accessible only to electron optical methods.

Development work in the field of cryo-electron microscopy and related cryo-electron optical devices immersed in liquid helium cryostat and using superconduction electromagnetic lenses. This type of microscope is uniquely suited for the high-vacuum, low temperature conditions of outer space to permit direct electron-optical studies of lunar and planetary surfaces under special conditions.

Successful application and development of these techniques requires special training and working facilities. It is conceivable that one could work out the optimum parameters for their use with existing types of space vehicles and train the various technicians of electron microscopy laboratories already organized in the different space NASA research centers, so that they may then be carried out routinely. The training could be carried out in seminars or short courses given to technicians at our laboratories at regular intervals.

F. Training. As expected, training has proceeded concomitantly with the various research projects described. In particular, laboratory courses have been conducted for the participants in Biophysics Course 308 and for a number of graduate students from other departments. In addition, our laboratory has already served as a center for consultation and discussion on advanced techniques, cooperating with research laboratories of other Universities, AEC Laboratories at Oak Ridge, Argonne and Livermore, etc. We are now preparing for the wide spectrum of trainees anticipated: graduates, undergraduates, post-doctorals, technicians, teachers. The training program will be coordinated with that of the proposed NASA University of Chicago Center for Science Education.

Acknowledgements

It is a pleasure to thank Dr. H. Stanley Bennett, Dean, Division of Biological Sciences; Dr. Raymond E.Zirkle, Chairman, Department of Biophysics; Dr. William Bloom, Department of Biophysics, University of Chicago; and Dr. William H. Sweet of the Massachusetts General Hospital and Harvard Medical School for their valuable suggestions and whole-hearted support of this project. Dr. H. Stanley Bennett suggested important design features which were successfully incorporated in the construction of the special regulated power supply and electron microscope laboratories.

Sincere thanks are also due to Mr. L. Merrifield, Mr. E. Carlson, Mr. G.E.Rosenfield and associates of Westinghouse Electric Corporation for their valuable assistance in the design, construction and testing of the special regulated power supply for the electron microscopes. We gratefully acknowledge the valuable assistance of Mr. R.H. Samuel, Mr. David C. Norris, and Mr. R. Fredrickson of Unistrut Products Company in the design and construction of the electron microscope facility.

We are greatly indebted to Mr. G. Olson, Asst. Superintendent; and Mr. William M. Connett, Roofing and Tinshop Foreman, and their associates of Building and Grounds; Mr. C.S. Mokstad, Asst. to Dean, Division of Physical Sciences, and his associates of the Research Institutes; to Mr. James R. Turner, Division Budget Office; Miss Irene E. Fagerstrom, Asst. Vice-President of Special Projects; and Mrs. Carol D. Morris, Contract Administrator, Office of Vice-President of Special Projects for their most valuable assistance throughout all phases of this project.

We also wish to thank Mr. Roy Schneider, Services Manager; Mr. George Hudson of Building Service; Mr. George E. Ostrowski, Superintendent-Receiving and Stockroom; and their associates for their valuable cooperation and assistance in this project.

We are particularly obliged to Mrs. P. Ricci, Mrs. S. Schmidt, Mrs. M. Townsend, Miss J. Richardson, Mr. C. Hough, Mr. L. Ouwerkerk, Mr. H. Schilder, Miss G. Hess, Mr. J. Leonard, Miss L. LaDeur, Mr. G. Gibson, Mr. J. Hanacek, and all other members of the staff who have actively participated in carrying out this project.

It is also a pleasure to thank Dr. George J. Jacobs, Chief, Physical Biology and Dr. Orr.E. Reynolds, Director, Bioscience Programs, of NASA for their valuable suggestions and support of this research program.

H. Fernández-Morán

ANNUAL PROGRESS REPORT

Submitted to

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

NATIONAL INSTITUTES OF HEALTH

U. S. ATOMIC ENERGY COMMISSION

ELECTRON MICROSCOPE LABORATORIES

Organization of an Integrated Research Unit for High Resolution Electron Microscopy and Molecular Biology.

This unit has been designed as an integrated series of laboratories representing a basic research facility which is equipped and staffed to effectively carry out the envisaged broad research and training program. As described in the enclosed report, this unit will be equipped with a total of 6 high resolution electron microscopes and accessories for electron diffraction, including low-temperature electron microscopy, cryogenic equipment for liquid helium work, ultra-high vacuum systems, etc. The need for the additional Electron Microscope Laboratories is clearly indicated, since an extensive research and training program and active collaboration with other departments is to be anticipated. Moreover, this would be the only high resolution electron microscopy facility available at the Research Institute, University of Chicago. One of the proposed electron microscope laboratories would be devoted exclusively to continue the described specific research programs. The other facilities will be used mainly to support the special training and research programs in collaboration with other national and international groups. This is of particular importance in connection with the envisaged NASA program, since successful application and development of the new electron-optical techniques will require thorough training of specialized technicians and scientists, as will as adequate bio-instrumentation facilities.

It should be emphasized that under favorable conditions this research unit could develop organically to become the nucleus of a larger research and training center for Molecular Biology, concentrating mainly on correlative biochemical, biophysical, and ultrastructural studies of biological systems. Close association with the Committee on Biophysics and with the Institutes for Basic Research (Fermi Institute for Nuclear Studies and Institute for Study of Metals) affords uniquely favorable opportunities for carrying out a multi-disciplinary program of the type contemplated.

Annual Report 1963/64 -E. M. Labs.- H. Fernández-Morán

Location of the Research Unit in an ideal basement area provided with specia vibration-free foundations, and in the vicinity of the excellent low-temperature and workshop facilities of the Institute is of key value for the contemplated research program.

Location of the Research Unit.

The present program is installed in the basement of the south wing of the Research Institutes Building, (see accompanying campus map). The floor space is about 2,500 sq. ft. and is contiguous. The floors above house the other seven full-time members of the Biophysics faculty. The Low-Temperature Laboratory and the Central Development Shop are about 50 yards down the hall.

Favorable location of the Research Unit close to the low-temperature facilities, the new laboratory for Astrophysics and Space Research, the Institute for Computer Research, and the Enrico Fermi Institute for Nuclear Studies is of utmost value for the contemplated research and training program.

Organization and Initial Operation of the Special Electron Microscope Laboratories for the Proposed Research and Training Program.

With funds provided by NASA, NIH, AEC, and the University of Chicago, a special laboratory facility for high resolution electron microscopy has now been completed and put into operation in the Research Institutes. These laboratories occupy a total of about 4,000 square feet and comprise:

(1) 2,500 square feet of remodeled space in the basement with installation of special floor, wall partitions, ceiling panels, and air conditioning of the type used in "clean rooms" of modern electronic industrial facilities. These laboratories are equipped with two large electron microscopes with attached electron diffraction units (and provision for two additional electron microscopes to be delivered in the coming year), including ultra-high vacuum (Varian) evaporation units, ultramicrotomes, and complete preparation and darkroom facilities. All of the critical equipment has been installed on individual vibration control mountings of special design. Corresponding precautions were taken in the installation of non-magnetic stainless steel ventilation ducts, incandescent lights and electrical conduits to minimize electrical and magnetic perturbations.

(2) Special highly regulated power supply located in an air-conditioned enclosure on the fifth floor of the research Institutes. This 50 kilowatt motor generator set specially designed

and manufactured by Westinghouse Company is equipped with a new solid state regulator giving better than 0.1% voltage stability and very low harmonic distortion. This highly regulated power system consists of the following units mounted and assembled on a common bedplate:

Item 1.

1-S.O. No. 25N4030, Dwg. 474-B-822 M-G set consisting of the following units mounted and assembled on a common bedplate:

1-S.O. No. 25N4026 - 50 KVA synchronous generator single phase, 60 cycles, 220 volts a-c, 1800 rpm, open 40° C. rise continuous, frame 445US, .8 power factor with harmonic content in its output as follows:

0.1% on 2nd harmonic
1.5% on 3rd harmonic
0.1% on 4th and 6th harmonics
0.8% or lower on 5th and 7th harmonics

1-S. No. 450A802G02 -1.0 KW direct connected exiciter generator for 50 KVA synchronous generator, 125 volt d-c for use with regulator covered in Ttem 4.

1- S.O. No. 25N4028 - 60HP synchronous motor, 3/60/220 volt a-c, 100% power factor, 1800 rpm, open - 40° rise continuous.

1-S.O. No. 25N4029 - 1.0 KW direct connected exciter for 60 HP synchronous motor, 125 volt, d-c.

1-S.O. No. N 4030 Bedplate for above.

1-S.O. No. 68x461, Dwg. 842-D-449, M.O. field rheostat for field of exciter for 60 HP synchronous motor.

1-S.O. No. 68x461, Dwg. 842-D-449, set of mounting parts for manual motor field rheostat (the rheostat is to be mounted in Item 3).

1- S.O. No. 68x461, Dwg. 842-D-449, field rheostat for generator exciter.

1- S.O. No. 68x461, Dwg. 842-D-449, set of mounting parts for manual exciter field rheostat (the rheostat to be mounted in Item 5).

Item 2.

Copies of certified test reports showing the actual harmonic content in the output of the 50 KVA synchronous generator at the following generator loads:

3 KVA
6 KVA
9 KVA
12 KVA
15 KVA
18 KVA
31.8 KVA

Item 3.

One - S.O. No. 68x461, Dwg. 441-A-165, Class 14604 Slipsyn synchronous motor control to start the 60 HP, 3/60/220 volt, 100% power factor M-G set driving motor.

Non-reversing, NEMA size4, auto-transformer type, with fusible disconnect.

With space and holes for mounting 60 HP motor direct connected field rheostat being supplied with the M-G set.

With Start-Stop pushbutton mounted on door and with provisions for remote starting and stopping starter rated 60 HP maximum at 100% power factor, 3/60/220 volts a-c.

With 2 extra electrical interlocks (1 - NO and 1 - NC) wired to terminal blocks.

Approximate dimensions of this starter are:

Width: 28"
Height: 90"
Depth: 28"

Item 4.

One - S.O. No. _____ generator control cubicle to control the 50 KVA synchronous generator, including the following components furnished mounted and wired to terminal blocks:

2 - 300 Amp., 2 pole, type GP main line contactors.

1 - Type K241 generator voltage voltmeter; 0-300 volts full scale.

1 - Ambient compensated thermal overload relay.

1 - Current transformer for use with OL relay.

2 - Type AB, 400 Amp., 2 pole, manual air circuit breakers.

1 - Generator ammeter and current transformer.

1 - Control power transformer.

1 - Mounting and wiring of exciter field rheostat.

1 - Set of necessary terminal boards, lugs, etc.

Item 5.

One - S. O. No. _____ special control console containing the devices all mounted on the door and wired to terminal blocks:

1 - S.O. No. 52 Y1949, Dwgs. 434B343
434B342
586C966
507A104
507A105
507A109

Flush mounted Type TRA voltage regulator, ambient compensated as described in attached sheet (accuracy plus or minus 1/10%) and dampening transformer assembly - fixed mounted.

1 - Hewlitt Packard model 302A wave analyzer - rack mounted.

1 - One KVA Precision Loading resistor. (150 parts per million).

1 - Sensitive Research Voltmeter model D (0-300V) - panel mounted. (accuracy 0.25%)

1 - Regulator control switch - type W.

4 - Oil Tite pushbuttons.

1 - Oil Tite selector switch with key - 2 positions.

1 - Spectral precision potentiometer, linear taper, 50 ohms, 7 watts, plus or minus .25 linearity, plus or minus 3% tolerance res., 20 parts per million

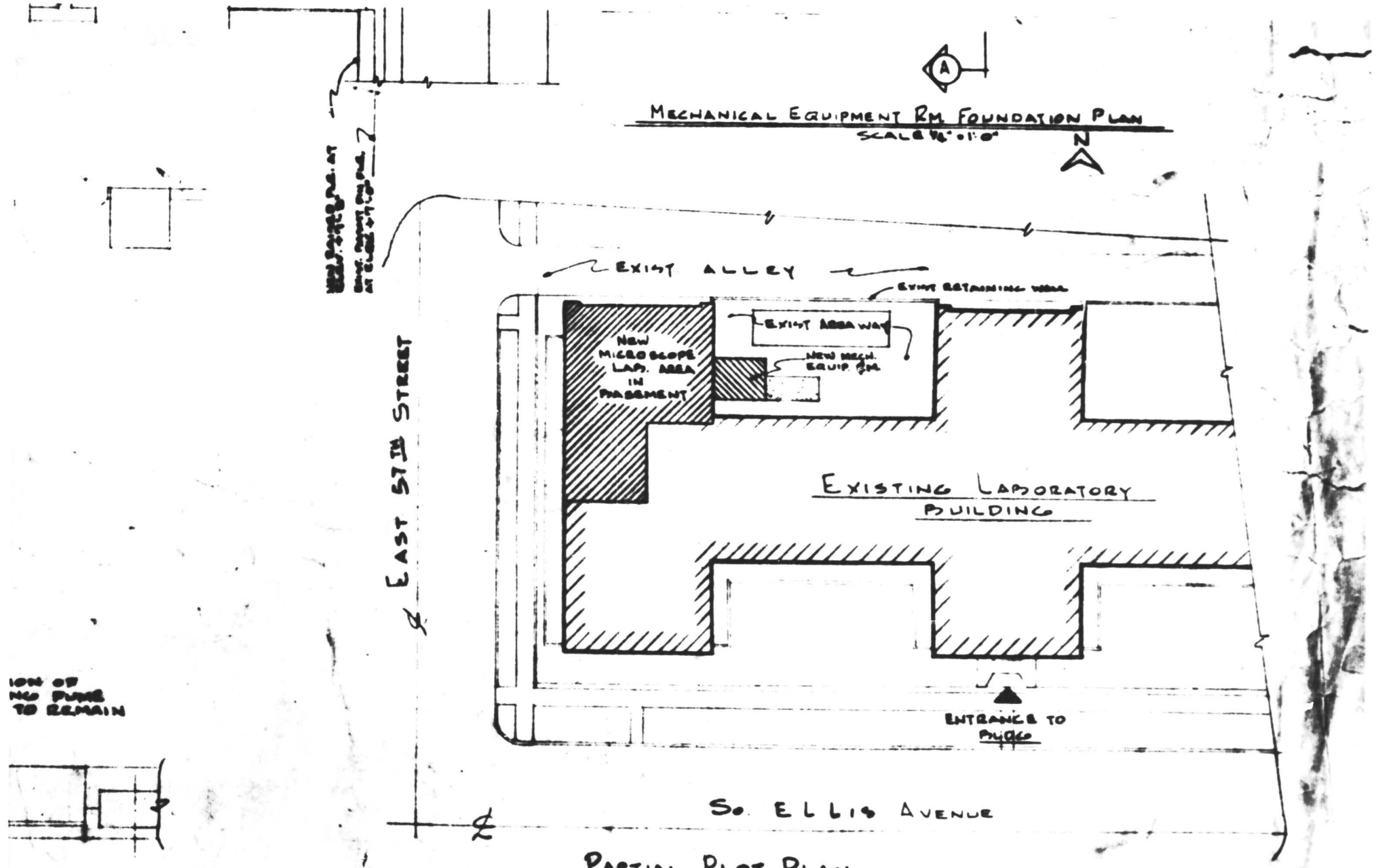
temperature coefficient.

1 - Desk top.

1 - 6 circuit, 2 pole, 30 amp., panelboard (for individual Electron Microscope Feeder protection.)

1 - 110 volt receptacle with cover (for customer's use).

1 - Generator output voltage test point with cover (for customer's use.)



NOTE: ALL MICROSCOPE LABORATORY EQUIPMENT INCLUDING ELECTRON MICROSCOPE COLUMN, POWER SUPPLY EQUIPMENT AND ASSOCIATED PUMPS, LAB TABLES, SINK UNITS, DARKROOM SINKS AND ALL POWER SUPPLY AND CONTROL CABLES AND [illegible] PIPING SHALL BE FURNISHED AND INSTALLED BY OTHERS

FINAL CONNECTIONS TO VACUUM FORE PUMP, SINK UNITS & DARKROOM SINKS SHALL BE MADE BY ELECTRICAL & PLUMBING CONTRACTORS.

BASMT OF SOUTH WING - EQUIPMENT LOCATION & MISC.

ELECTRON MICROSCOPE LAB. FACILITY
FOR
COMMITTEE ON BIOPHYSICS, UNIVERSITY OF CHICAGO
BASIC RESEARCH BUILDING
5640 So ELLIS AVENUE
CHICAGO, ILLINOIS

ROBERT S. TAYLOR
STRUCTURAL ENGINEER

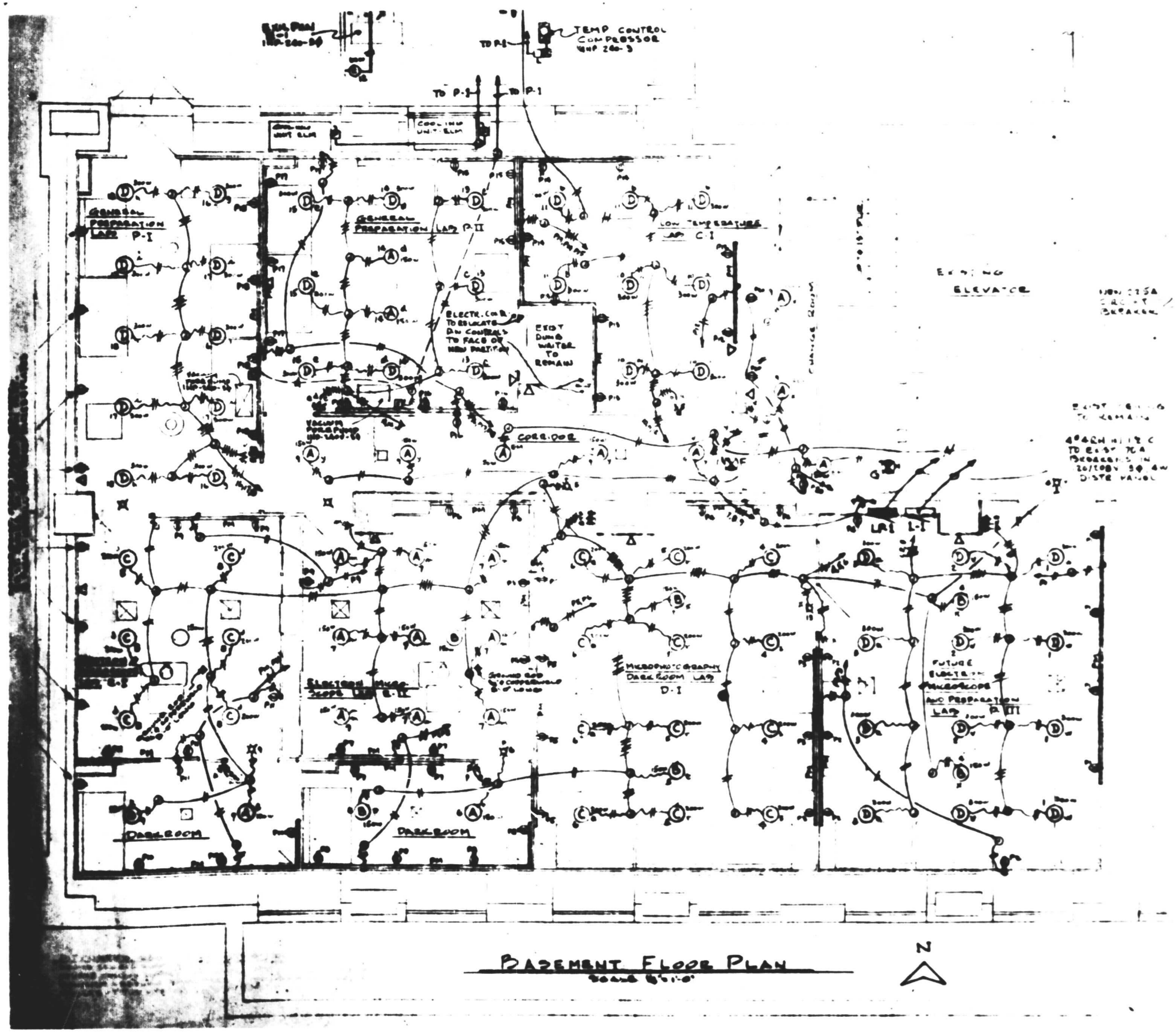
SAMUEL & HICKS CONSULTING ENGINEERS
[illegible]

NO.			
			DATE: 2-19-63

EQUIPMENT CABLE AND PIPING SCHEDULE	
TAG	DESCRIPTION
	POWER SUPPLY M-G REG. CONTROL STATION TO MICROSCOPE POWER SUPPLY
	HIGH TENSION MICROSCOPE CABLE-SUSPENDED BELOW CEILING
	CABLE (A,B,D,E & F) FOR CONTROLLING THE H.T.
	CABLE (G,H,O & L) FOR CONTROLLING LENS CURRENT SUPPLY
	CABLE (C,L,E,M,N & P) FOR A.C. SUPPLY
	GROUND CABLE FROM MICROSCOPE POWER SUPPLY TO MICROSCOPE COLUMN
	FORE PUMP LINES - FROM FORE PUMP TO MICROSCOPE COLUMN
	FOREPUMP RELIEF LINE TO ATMOSPHERE
	DIFFUSION PUMP SUPPLY & RETURN PIPING FROM COOLING UNIT TO MICROSCOPE COLUMN
	LINES FROM MICROSCOPE COLUMN TO GROUNDED END
	ATMOSPHERE FROM HIGH VACUUM EVAPORATING UNIT

FLOOR PLAN - EQUIPMENT LOCATION

N



BASEMENT FLOOR PLAN

...OF DAMPER PAD

...MENT RUBS TO
...BE SET IN PLACE
...WHEN FLOATING MICROSCOPE
...PAD IS POURED

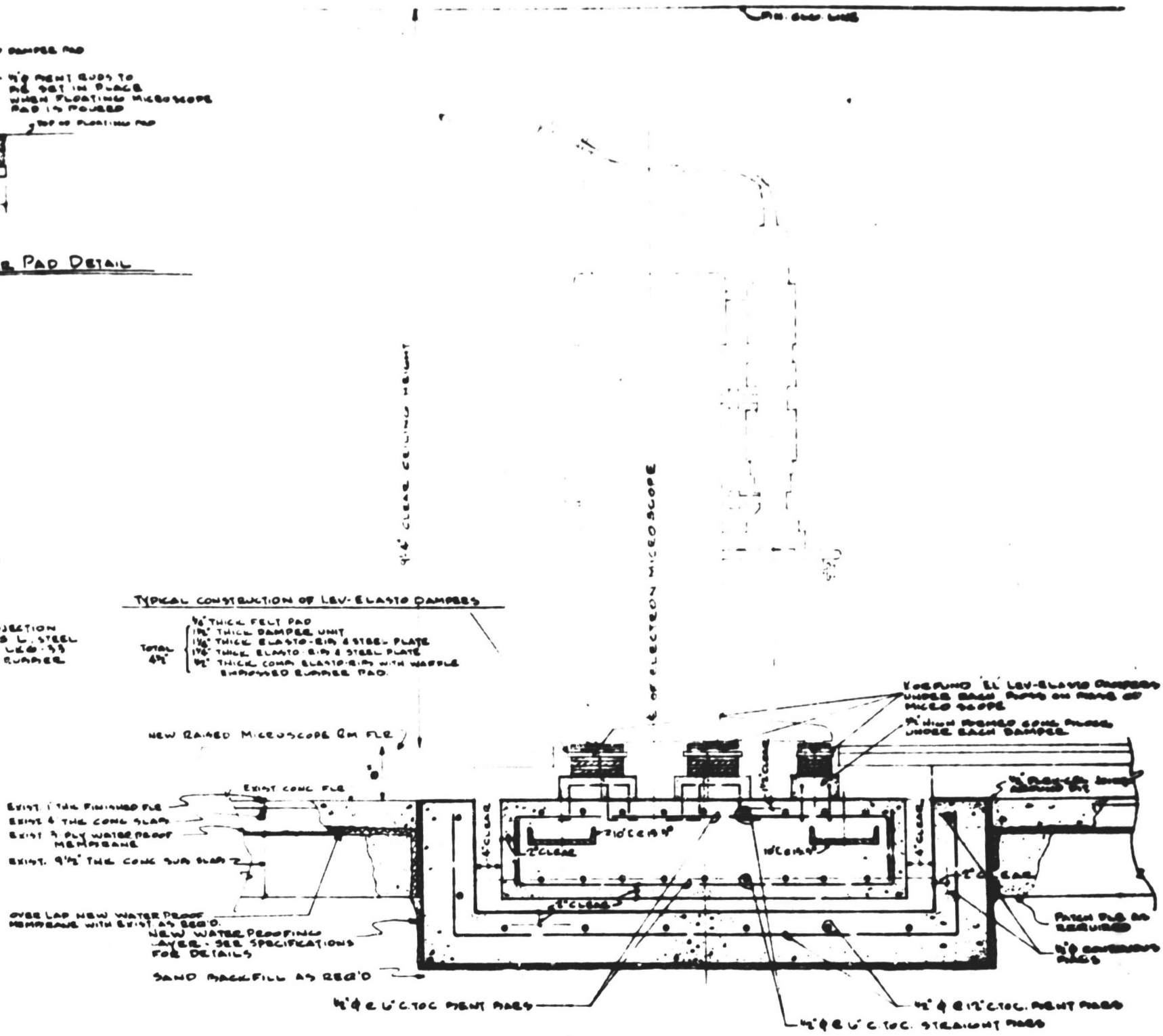
...TOP OF FLOATING PAD

...ER PAD DETAIL

TYPICAL CONSTRUCTION OF LEV-ELASTO DAMPERS

OBJECTION
ES L. STEEL
) LEAD-SS
- RUBBER

TOTAL 4½"
- ½" THICK FELT PAD
- 1½" THICK DAMPER UNIT
  - 1⅛" THICK ELASTO-BIN & STEEL PLATE
  - 1/16" THICK ELASTO-BIN & STEEL PLATE
  - ½" THICK COMP ELASTO-BIN WITH WAFFLE EMBOSSED RUBBER PAD.



SECTION B-B
SCALE ½"=1'-0"

BASMT. OF SOUTH WING —
FLOATING MICROSCOPE PAD DETAILS

ELECTRON MICROSCOPE LAB ADDITION
for
COMMITTEE ON BIOPHYSICS, UNIVERSITY OF CHICAGO
RADIC RESEARCH BUILDINGS
5640 So. ELLIS AVENUE
CHICAGO, ILLINOIS

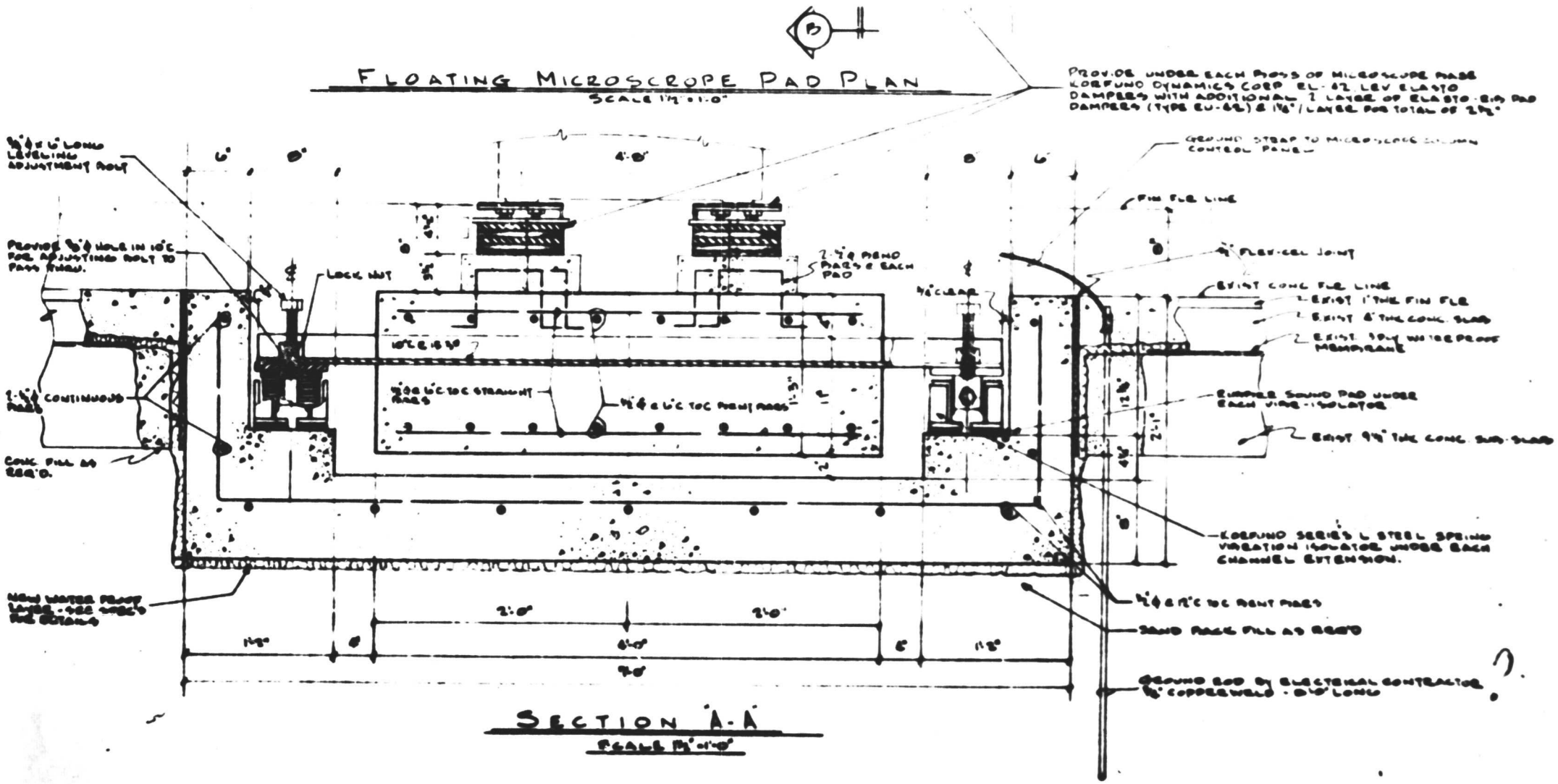
ROBERT S. TAYLOR
STRUCTURAL ENGINEER

SAMUEL A. NICKS CONSULTING ENGINEERS
154 W. LA SALLE STREET CHICAGO 2, ILLINOIS

NO	REVISIONS	DATE

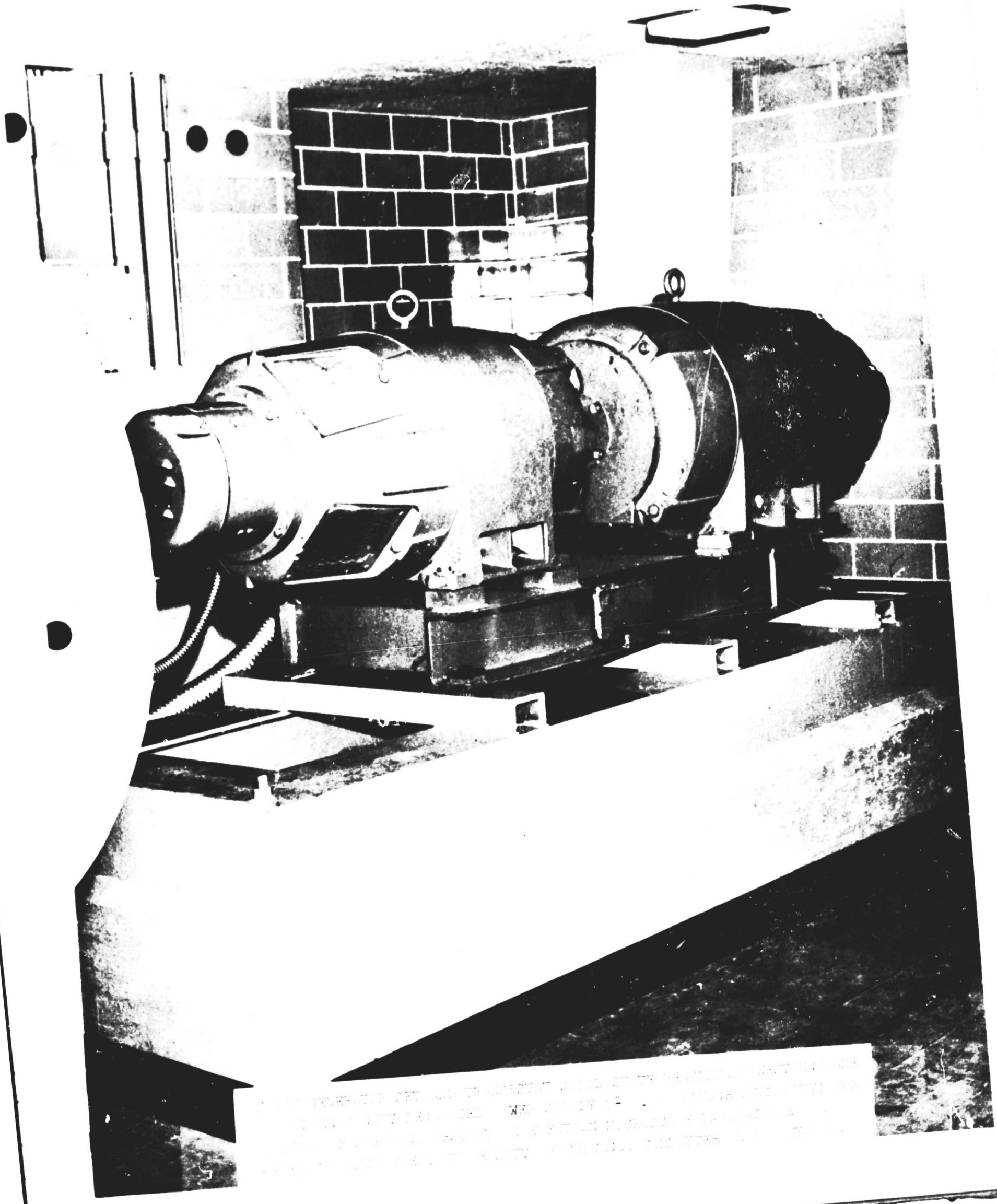
FLOATING MICROSCROPE PAD PLAN

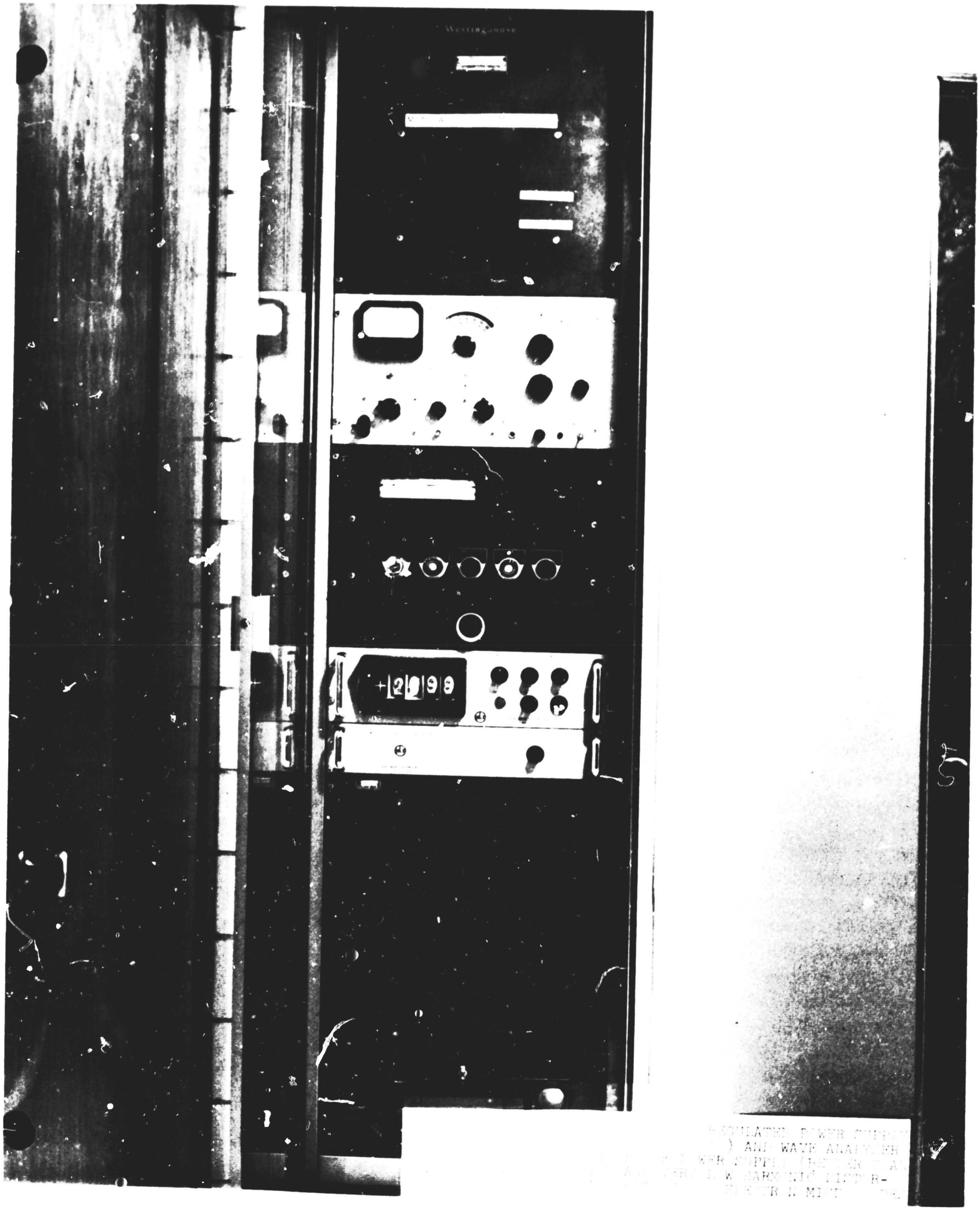
SCALE 1½" = 1'-0"



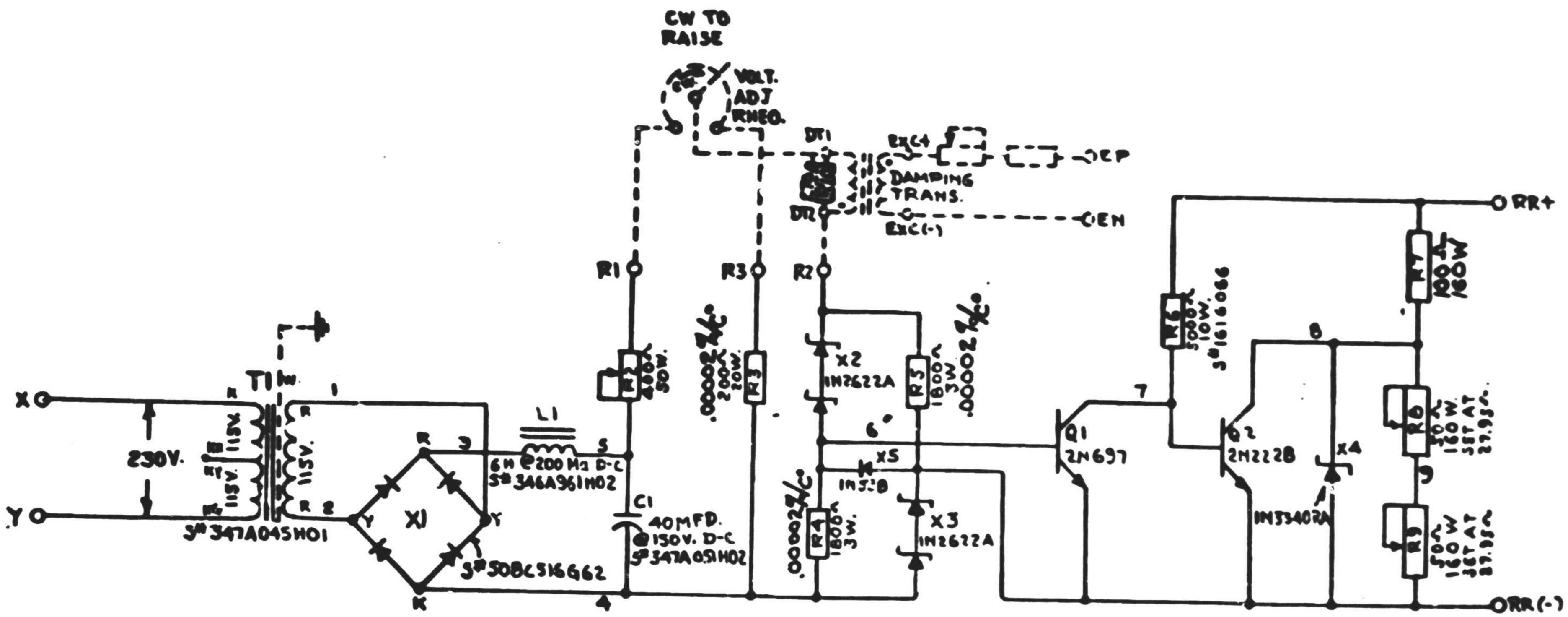
SECTION A-A

SCALE 1½" = 1'-0"




+2198

434B342



1	Z
CHANGE	11-15176 52Y1549 CHANGES MADE PER CUSTOMERS MARKED PRINT R7 WAS 50 Ω R10 WAS ...

EP10032

WESTINGHOUSE ELECTRIC CORPORATION

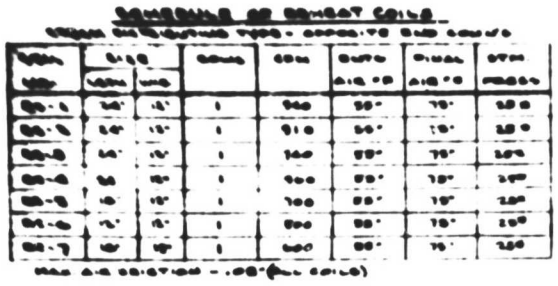
TITLE TYPE "TRA-11" TRANSISTOR VOLTAGE REGULATOR WITH 230V. INPUT, NO CUR. COMP. & EXTERNAL VOLT. ADJ. RHEO

DIMENSIONS IN INCHES—SCALE

SCHEMATIC DIAGRAM

434B342

DIVISION A. S. & D. PLANT LOCATION E. PGH., PA., U.S.A. 6720



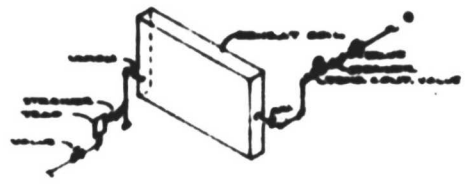
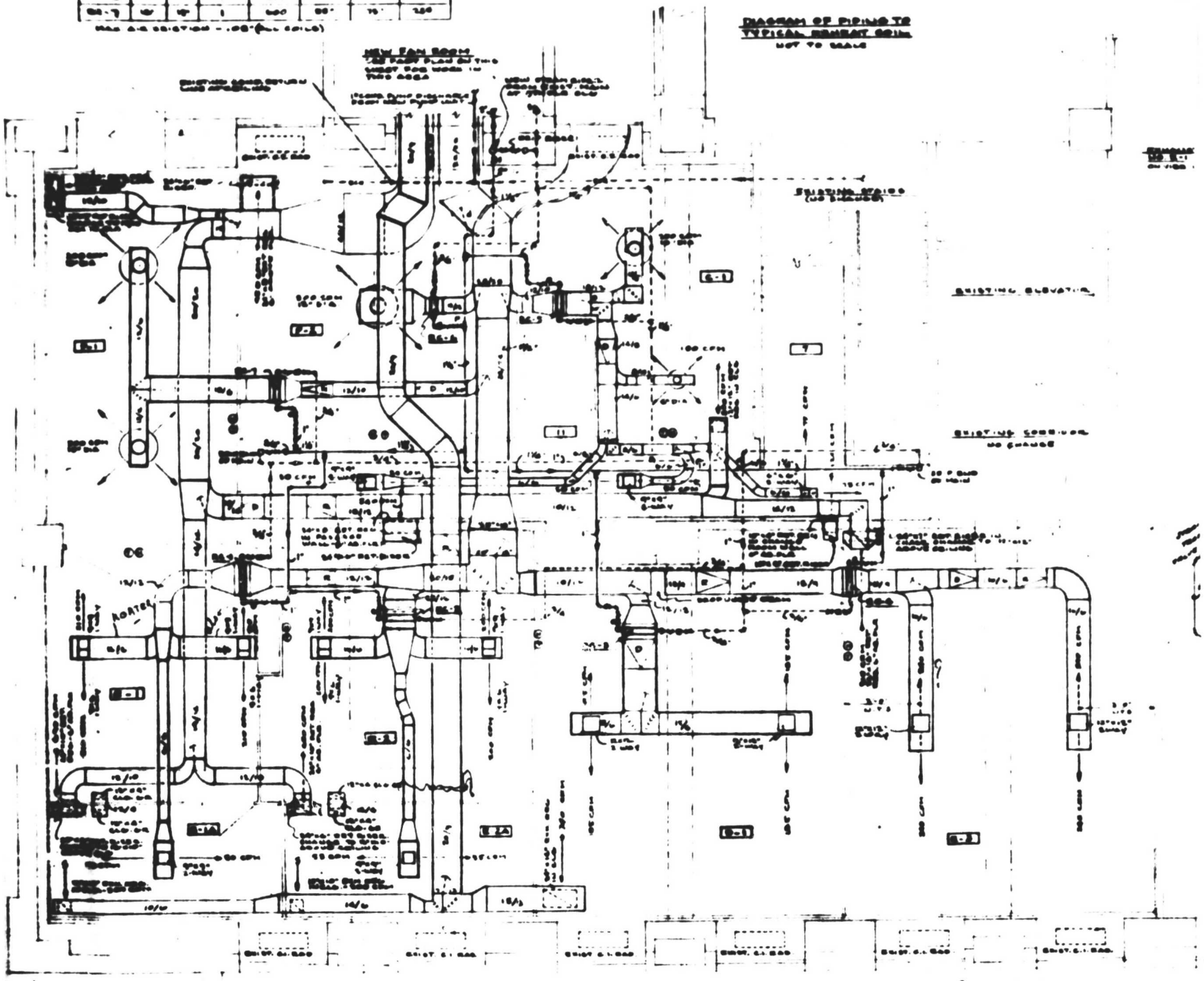
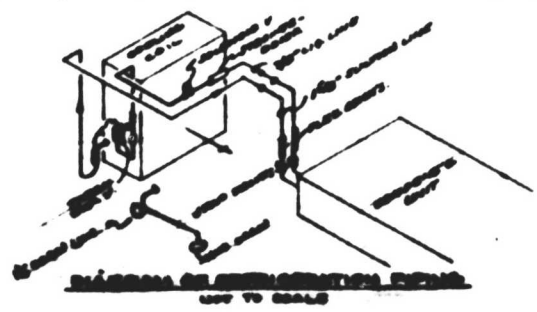
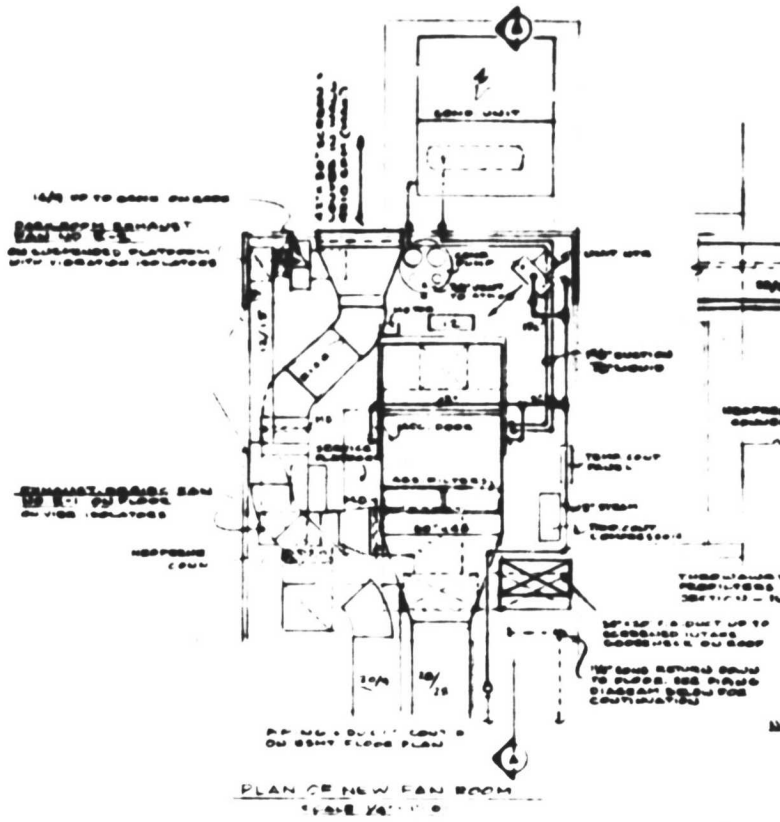


DIAGRAM OF PIPING TO TYPICAL REHEAT COIL
NOT TO SCALE

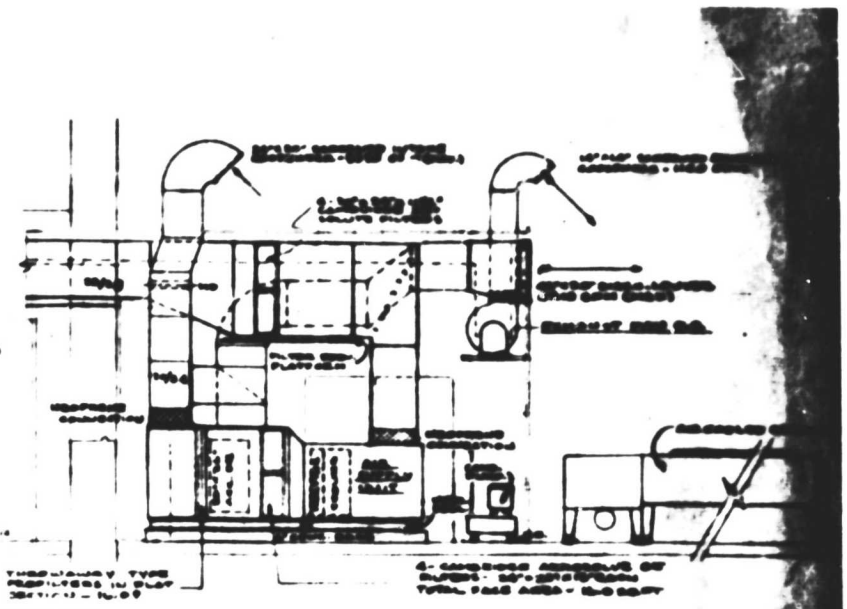


BASEMENT FLOOR PLAN





PLAN OF NEW FAN ROOM



SECTION A-A

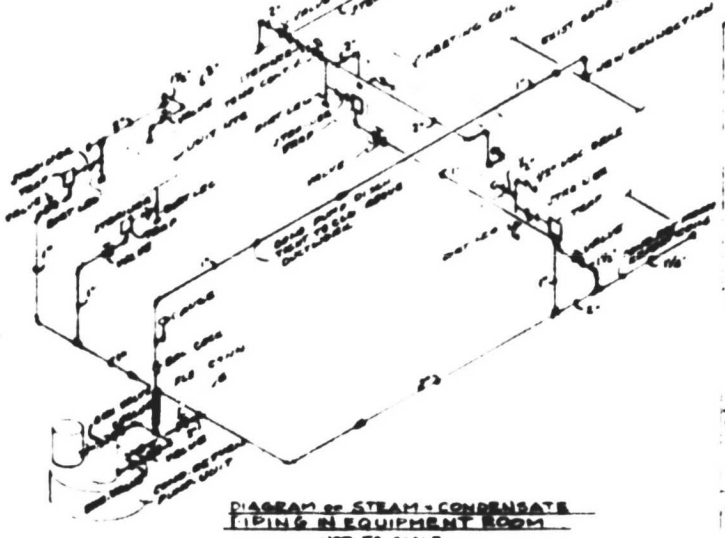
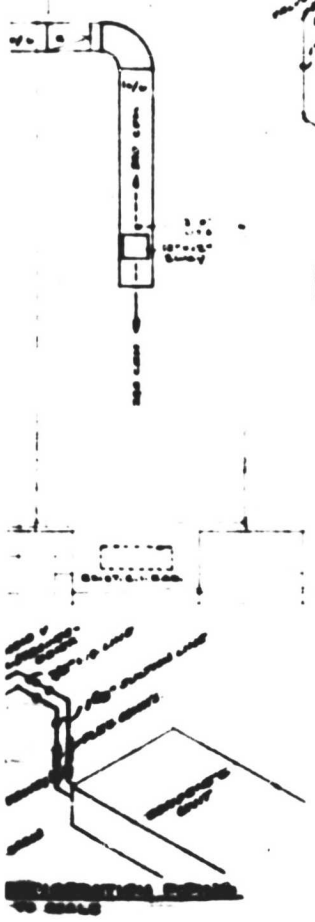


DIAGRAM OF STEAM + CONDENSATE PIPING IN EQUIPMENT ROOM
NOT TO SCALE



NOTE

VIBRATION ISOLATORS FOR AIR SUPPLY UNIT AND ASSOCIATED EQUIPMENT SHALL BE ROBICHAUD TYPE RAA OR EQ. RUBBER-IN-SHEAR ISOLATORS. ISOLATORS FOR UTILITY FAN SET A SHALL BE ROBICHAUD TYPE D OR TYPE D FOR SPRING ISOLATORS WITH ADJUSTABLE MOTOR SLIDE. ISOLATORS FOR CONDENSATE RETURN UNIT SHALL BE THREE (3) ROBICHAUD TYPE RAA RUBBER-IN-SHEAR.

CONCRETE PAD UNDER AIR SUPPLY UNIT & PLATFORM FOR FAN SET SHALL BE PROVIDED BY VENTILATION CONTRACTOR.

EQUIPMENT SCHEDULE

AIR SUPPLY UNIT

FAN SECTION: ONE (1) - 9' DIA. DWDI FAN TO DELIVER 68000 CFM @ 4.98" S.P. @ 690 RPM. FAN OUTLET AREA - 3.68 SQ. FT. MINIMUM.

COOLING COIL: 30" x 52" - 10 R 50 FT FACE AREA - 6 ROW MINIMUM, COPPER TUBE ALUMINUM FIN COIL WITH THERMAL EXPANSION VALVE. (DISTRIBUTING TYPE) COOL 9350 CFM FROM 83.3 DB + 69.2 WB TO 52.6 DB + 51.6 WB WITH 32 # 40# SUCTION. FAN AIR FRICTION - 0.66

HEATING COIL: 30" x 52" - 10 R 50 FT FACE AREA, 2 ROW MINIMUM, COPPER TUBE ALUMINUM FIN STEAM DISTRIBUTING TYPE (DUAL END SUPPLY) TO HEAT 9350 CFM FROM 40° TO 80° WITH STEAM @ 20 PSIG. MAX. AIR FRICTION

FILTERS: NOT FURNISHED WITH UNIT (SEE SECTION AS ABOVE)

MOTOR: 7½ HP, 690 RPM, 60 CY., 220 VOLT / 125 RPM, WITH ADJUSTABLE V-BELT DRIVE AND BELT GUARD

UNIT CONSTRUCTION: NO. 3 HORIZONTAL FLOOR MOUNTED TYPE WITH MEDIUM PRESSURE CASING CONSTRUCTION PROPERLY BRACED AND STIFFENED.

AIR COOLED CONDENSING UNIT

DUNHAM-BUSH TYPE ACU-25M OR EQ. SELF CONTAINED HORIZONTAL TYPE UNIT WITH LEGS, COMPLETE WITH 5 CYL. COMPRESSOR, SCREENED AIR PROPELLOR TYPE CONDENSER FAN WITH 3HP FAN MOTOR, PRESSURE FOR LOW AMBIENT CONTROL, CRANKCASE HEATER, 2 STAGE CAPACITY CONTROL. UNIT CAPACITY - 315,000 BTU/HR @ 20° SUCTION 100° AMBIENT AIR, REFR. 22, MOTORS FOR OPERATION ON 208/230/460 V. CIRCUIT. TOTAL ELEC. INPUT - 24.1 KW

EXHAUST RECIRCULATION FAN E-1

PEERLESS SIZE 20 B 25 OR EQ FORWARD CURVE UTILITY SET WITH SWSI FAN @ 1 HP, 3-60-230 VOLT MOTOR WITH ADJUSTABLE V-BELT DRIVE TO DELIVER 4610 CFM @ 0.8" S.P., 1330 RPM, 1330 RPM OUTLET VELOCITY - UP BLAST DISCHARGE

LABORATORY EXHAUST FAN E-2

PEERLESS SIZE 20 B 25 OR EQ FORWARD CURVE UTILITY SET WITH SWSI FAN @ 1½ HP, 3-60-230 VOLT MOTOR WITH ADJUSTABLE V-BELT DRIVE TO DELIVER 1160 CFM @ 0.8" S.P., 698 RPM, 1070 RPM OUTLET VELOCITY - UP BLAST DISCHARGE.

FAN ROOM UNIT HEATER

McQUAY UD MH 34 OR EQ HORIZONTAL PROPELLOR TYPE WITH 1725 RPM MOTOR. CAPACITY - 10000 BTU/HR @ 20# STEAM + 60° ENTR. AIR + 110 CFM.

CONDENSATE RETURN UNIT

HOLL PUMP CO NO. TVC-25 OR EQ. DUPLEX UNIT. CAPACITY - 5000 GPH PUMP TO DELIVER 3 GPM @ 100 DISCHARGE PRESS. - MOTORS 1 HP - 1725 RPM - RECEIVER - FLOAT SWITCHES - VENT OPENING

VENTILATION SCHEDULE

ROOM NO.	USE OR SPACE	FLOOR AREA SQ FT	NAT'L VENTILATION ORDINANCE CU	NAT'L VENTILATION ORDINANCE V	NAT'L VENTILATION ACTUAL CU	NAT'L VENTILATION ACTUAL V	MECHANICAL VENTILATION ORDINANCE S.	MECHANICAL VENTILATION ORDINANCE E.	MECHANICAL VENTILATION ORDINANCE E.O	MECHANICAL VENTILATION PLANS REQ'MTS S.	MECHANICAL VENTILATION PLANS REQ'MTS E.	MECHANICAL VENTILATION PLANS REQ'MTS E.O
B-1	MICROSCOPE RM	210	210	0.5	—	—	525	735	—	840	630	—
B-2	MICROSCOPE RM	225	112.5	11.25	—	—	270	270	—	800	450	—
B-2A	PHOTO DARKROOM	90	9.0	4.9	—	—	90	90	—	100	100	—
B-2A	PHOTO DARKROOM	100	10.0	5.0	—	—	100	100	—	110	100	—
B-3	MICROSCOPE RM + DARKROOM	435	43.6	21.75	—	—	435	435	—	760	760	—
B-3	MICROSCOPE RM + DARKROOM	445	44.5	21.25	—	—	510	510	—	960	960	—
7	CHANGE RM (SHOWER)	90	—	—	—	—	—	—	—	100	150	—
B-1	CO-TEMP. ROOM	150	15.5	9.0	—	—	230	230	—	300	300	—
B-2	REFRIGERATION RM	250	26.4	13.2	—	—	317	317	—	500	450	—
B-2	REFRIGERATION RM	285	28.0	12.4	—	—	340	340	—	600	550	—
11	PASSAGE	100	—	—	—	—	—	—	—	200	200	—
12	MAIN ENTRY	250	—	—	—	—	—	—	—	—	—	—

BASMT OF SOUTH WING -

HEATING, VENTILATING & AIR CONDITIONING FLOOR PLAN

ELECTRON MICROSCOPE LAB

for

COMMITTEE ON BIOPHYSICS, UNIVERSITY OF

BASIC RESEARCH BUILDING

5640 SO ELLIS AVENUE

CHICAGO, ILLINOIS

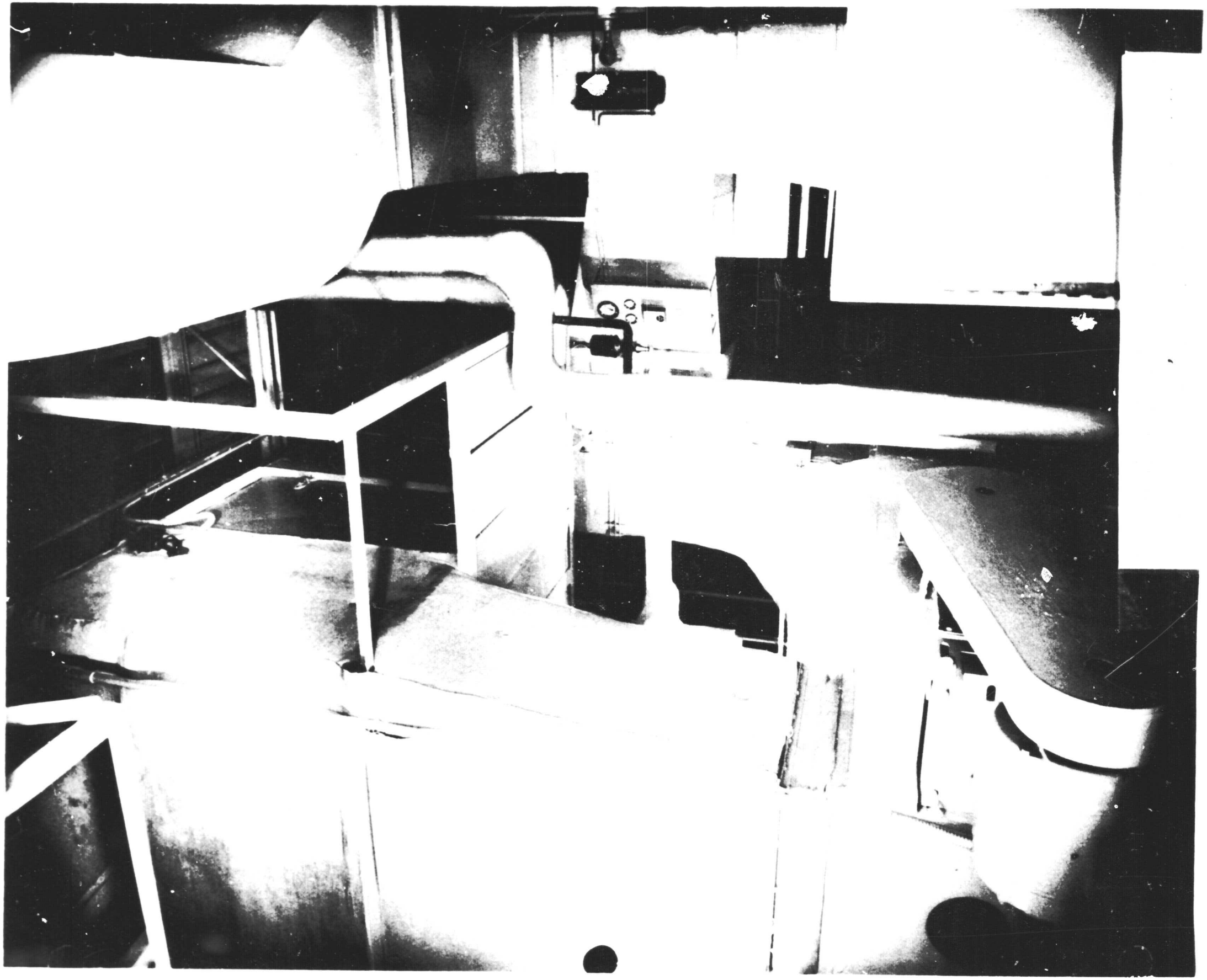
ROBERT S TAYLOR

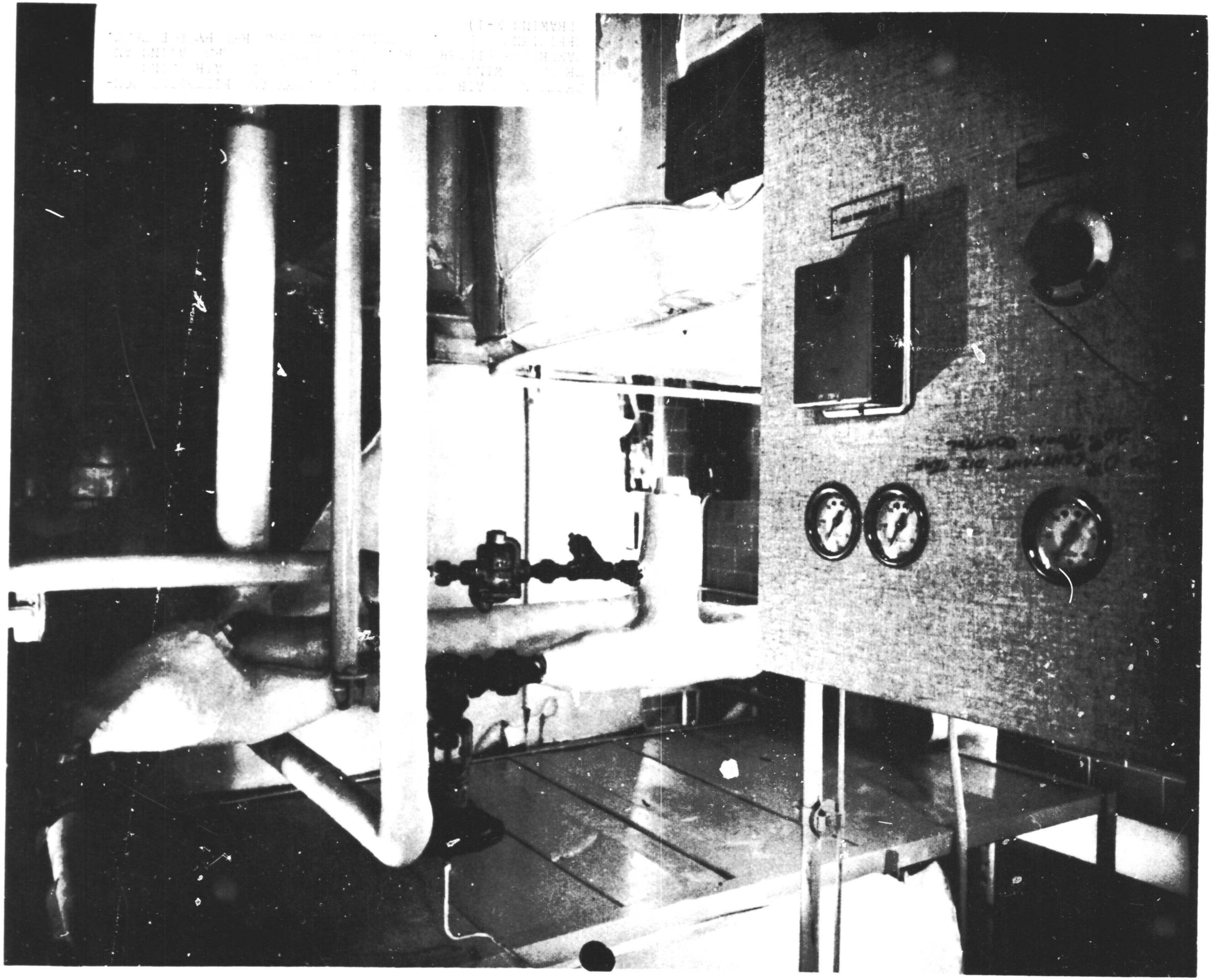
STRUCTURAL ENGINEER

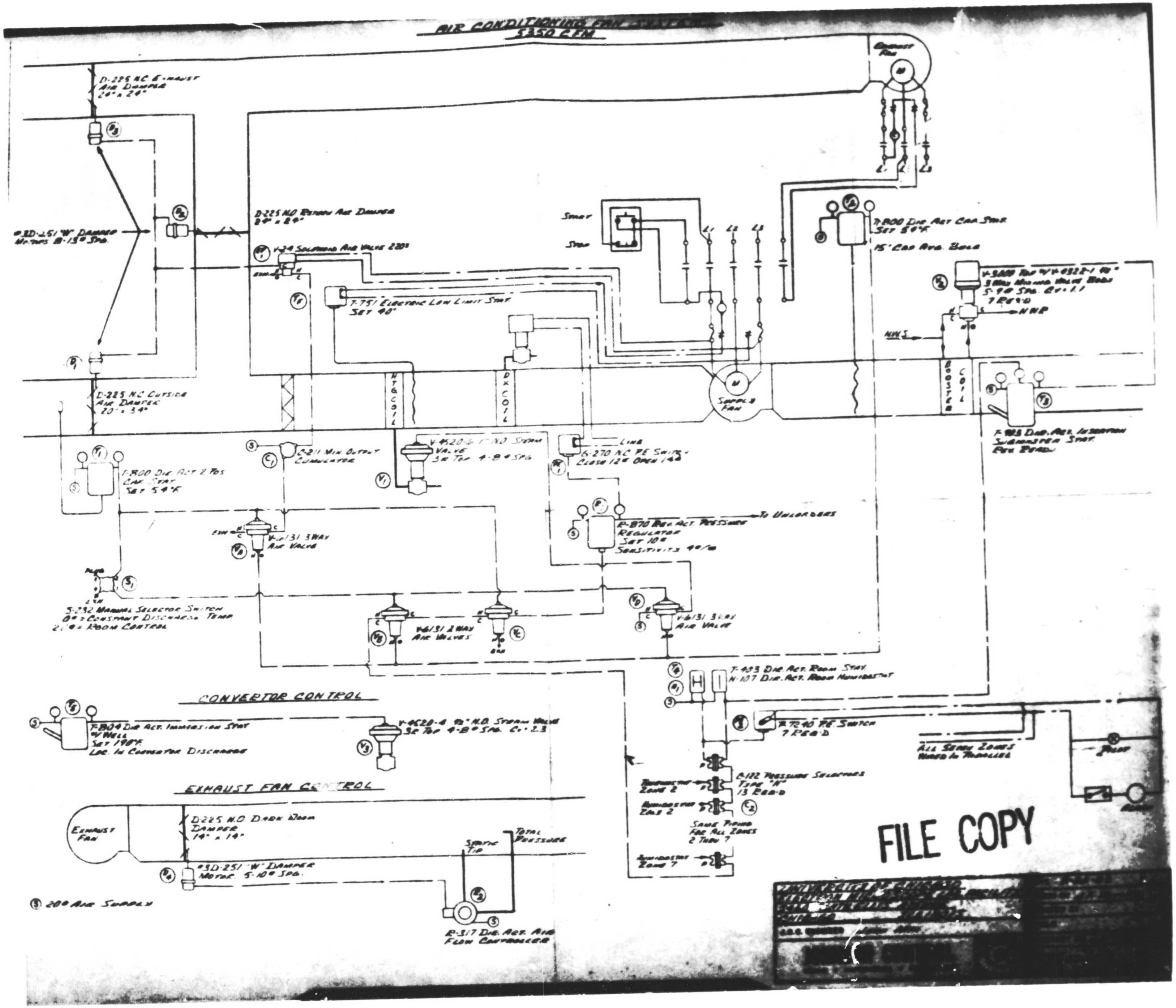
SAMUEL E. HICKS, CONSULTING

1540 LA SALLE ST. CHICAGO

NO	REVISIONS	DATE






5350 CFM
CONVERTOR CONTROL
EXHAUST FAN CONTROL
FILE COPY

General Design Concepts and Requirements for Electron Microscopes:

The area allotted in the basement of South Wing of Research Lab. Buildings comprises approximately 2,500 sq. ft.(main area:48 x 48 ft.;area of ca. 150 sq. ft. in adjacent yard for location of air-conditioning services). This area was formerly occupied by workshops, animal rooms, and had to be modified. (see attached plans).

As indicated in the "Instructions for Installing Siemens Elmiskop I Electron Microscope Labs," the rooms selected must fulfill the following requirements: The room selected for the electron microscope is as large as possible (minimum of 10 x 10 ft., height of 9 ft.) to permit the installation of additional equipment and to provide optimum working conditions. Mechanical vibrations, ambient magnetic and electrical perturbations are kept to a minimum (see enclosed quantitative data). Particularly in the electron microscope and preparation labs, but actually throughout the entire facility, "clean room conditions" should be maintained. In view of the critical nature of some contemplated investigations (e.g., examination of meteorite and extra-terrestrial specimens for NASA research program) the danger of contamination with high ambient dust particle populations should be minimized. After discussing these conditions with Mr. Royce O. Young, Manager of the Clean Room Division of Unistrut Products, and bearing in mind the critical monitoring and design requirements clearly pointed out by him, it was assumed that "Ordinary Clean Room" conditions (as per proposed specifications for Sub-Committee F1-X-A of American Society for Testing Materials) could be met in the proposed area.

In the following pages, a brief description will be given of the proposed layout and equipment (based on attached plans, sketches and specifications) of : 4-5 electron microscope laboratories, 4-5 general preparation laboratories, low-temperature laboratory, microphotography and darkroom laboratories, high-vacuum and preparation laboratory (to be converted later on into Electron Microscope Lab III).

General Description of Electron Microscope Laboratories:

The E,M. Labs for the Siemens Elmiskop I microscopes are located side by side in the (south) end of the basement, as far away from the Air-conditioning installation in the opposite yard as possible, and also providing requisite distance from the Power supplies, cooling units and other sources of magnetic and electric disturbances. All fluorescent lights are eliminated and the electrical

installations (cables, ducts, etc.) must be carefully checked with megnetometer to eliminate possibility of magnetic field disturbances. Rooms are each ca. 18 x 15 ft., with attached small darkrooms for processing of photographic material directly connected with E. M. work.

Rooms have the following characteristics:

Panels: Special Aluminum Double Panels (with acoustic shielding) and light-tight doors, including preferably black anodized finish (for both panels, doors, floors and ceiling). Panels should also have provisions for attaching wall cabinets and shelves, electrical fixtures, etc.

Attached Small Darkrooms: (ca. 6 x 15 ft.) have simple (single) type of panel, with light-tight doors. As indicated in the sketches, the doors are located in each laboratory to permit ready personnel and equipment access to adjacent preparation labs and corridor (through ramp).

Floors: Unistrut floors of special design (Aluminum, or non-magnetic supports) are envisaged for entire area (of Electron Microscope Labs: 29 x 24 ft. = 700 sq. ft.; General preparation Lab I: 22 x 12 = 264 sq. ft.; General Preparation Lab II: 16 x 16 = 256 sq. ft.) comprising total area of approximately 1,220 sq. ft. (Thus, the Low-temperature Lab, Microphotography and Darkroom Lab and High Vacuum Lab plus Corridor do not have Unistrut floors, but instead special continuous vinyl covering of type indicated during discussions). All electrical control and power supply cables, vacuum lines, water cooling lines for each Elmiskop will run below Unistrut floors, (height of 8 inches). Provisions were made for separation of vibration-free mounts for each Elmiskop (Concrete block + Korfund Vibro isolators + beams conforming to base plan of each Elmiskop column and power pack--see attached drawings). Arrangements were made for ventilation ducts to maintain temperature of Elmiskop column (plus diffusion pumps) and of power pack cabinet at desirable constant level (similar to analogous analogous conditions in Computer Labs, etc.).

Floors - Vibration Isolation for Electron Microscopes: Following the general design instructions of Korfund specifications for vibration isolation system proposed by Korfund Dynamics Co.,(att. Jack Harris, Ex. Engineer), an excavation was made in suitable area (between foundation supports, as indicated in plans) and of requisite size to accomodate large concrete block (preferably 6,000 to 9,000 pounds) with provisions for special Korfund spring isolator suspension system. The design strove to place the

embedded steel beams as low as possible (removed from Elmiskop column). Since the two electron microscopes are adjacent (separated by minimum distance of 15-16 ft. from each other), the possibility was considered of making one large common excavation (plus water-proofing) to accommodate two suspended concrete blocks for base of each Elmiskop. Provisions were made to permit lifting and inspection (or change of vibration spring suspension air-cushion,etc.) of each concrete base in order to achieve optimum vibration system later on. Important to consider design of leveling platform and supported beams (see photo-graph of RCA Electron Microscope foundation supplied by Korfund) to permit location of Elmiskop column and cabinet level with respect to the Unistrut floor, while allowing for entry of electrical control cables, cooling (water cooling system) and vacuum fore-pump lines to each microscope (See attached natural-size layout of bottom of Elmiskop column). Since the design of this vibration-isolation foundation is critical, it was suggested to check details with Mr. Harris of Korfund, and with Dr. Fernández-Morán in the pre-design stage.

Floors - Preparation Labs: The Unistrut floors in the corresponding adjacent Preparation Labs made provision for mounting and separation of the Cenco hyvac 28 forepump (mounting base measures 10 x 18 inches--see attached description, contained in sound-proof box with base of 12 x 24 inches located 15-20 feet away from Elmiskop preferably in Preparation Lab, connected through 1.5-2 in. copper vacuum pipe-line). Similar provisions were made for location of the power pack cabin for each Elmiskop (30 x 30 inches base--see attached exact dimensions in sketch provided by Siemens.) This power pack was located 25 to 30 feet away from the Elmiskop column.

Floors - Electron Microscope Power Pack Cabinet: A special high-voltage cable (which runs partly concealed in special vibration-isolated sponge-rubber mount within ceiling) and numerous electrical control cables connect the power pack cabinet with the Elmiskop column. Since the power pack contains the major part of the electronic equipment (ca. 2 KVA power consumption), it is important that it be maintained at suitable constant temperature. Therefore, design of ducts for ventilation (below floor and in ceiling) had to take location of this power pack into consideration. The heavy power pack (weight 605 kg) must be serviced periodically, and provisions had to therefore be made to open both side doors and to remove heavy transformers, Oil Tanks, etc. for maintenance. This in turn required that the adjacent Unistrut floor be designed to: (a) permit separation of power pack and small Korfund base from

adjacent level floor; (b) provisions for heavy marine cable and conduits running to column below suspended floor; and (c) power pack bottom must be level with Unistrut floor panels to permit "wheeling-out" of electronic components and transformer.

Floor Design and Cooling Unit: Although the water cooling unit (contained within refrigerated metal box: ca. 5 ft. long, 3 ft. wide, 3 ft. high) is suspended from ceiling (Foundation beams) on special Korfund vibration mount, the thermally insulated (3) water pipes run down the wall and largely below the Unistrut floor (ca. 30-36 ft.) from the Preparation Lab to the Elmiskop column (as per Siemens diagram). Provisions were therefore made to accomodate these cooling lines without interference from vacuum-forepump lines, and electrical cable conduits from Power Pack. The position of these services lines should be shown clearly in the laboratory drawings. Since all of these service lines require periodic inspection (and in case of leakage of water, oil, or vacuum) the floor design provides for ready access to each service line without undue trouble and dismantling of adjacent Unistrut floor panels.

Floor - Electron Microscope - Electrical Ground Connection: each electron microscope (power pack and column) has a special electrical ground connection consisting of heavy copper cable and copper slab which is embedded deep in the basement floor (in connection with the excavated area for the vibration-isolation bases). Provision were made for exit of these cables and attachment to power pack (in Prep. Lab.) and to Elmiskop column.

Ceiling - General Description: The ceiling has requisite minimum height of 10 feet in the Electron Microscope and Preparation Laboratories. Special tile (smooth, glass fiber, acoustic, etc.) of black color in the Electron Microscope Labs, and of white color in the Preparation Lab area to be selected. Lighting is recessed in ceiling. Incandescent lighting is used throughout instead of fluorescent lights. Particular attention was devoted to selecting new types of incandescent lamps (such as new General Electric 300 watt "cool beam" par lamps which send ca. 70% of radiant heat out the back, and focus intense light beam on laboratory space without overheating or minimum infra-red exposure). In calculating the load and distribution of incandescent lamps it was borne in mind that only the two Preparaton Labs and the Low-temperature Lab (total area of ca. 650 sq. ft.) have a level of illumination comparable to ordinary laboratory standards. The rest of the area (ca. 1,000 sq. ft.) has either low-level ceiling illumination

(electron Microscope Labs) with safety red lights or straight-forward darkroom (red safety lights) illumination. Thus, the total heat load can be kept within reasonable limits.

Services Contained Above Ceiling Panels: High voltage cable for electron microscope, mounted on special channel plus vibration isolated (sponge rubber) base. Mostly concealed except at exit from power pack and electron microscope column; Recess for water cooling unit for each Elmiskop; Auxiliary electrical conduits, regulated power supply cable (from GE motor generator set--see detailed specifications, Westinghouse) and air-conditioning ducts; Panels are readily removable and allowance is made for hooks and suspension mounts from concrete ceiling.

Location of Vacuum Forepump (Cenco Hyvac 45): in sound-proof box, resting on floor with pipe affixed or embedded in concrete base of floor, below one of sinks (maximum distance between forepump and microscope 24 feet, minimum distance 15 feet). Same is true for Preparation Lab II.

Location of Cooling Unit for Elmiskop I and II:In window wells in Preparation Labs I and II. Three pipes to each scope run below floor thermally isolated. Panels for servicing and inspection are readily demountable.

Level of Illumination in Preparation Room Area:Prep. Lab I, Prep. Lab II, C-I plus corridor are kept around 100 ft/candles, about 40-50 ft/candles in E.M. Labs I and II and Darkroom. Present light fixtures can be provided with lamps of required color (i.e. white and red) and wattage.

Provision for first-aid kit, motor shoe-brush, and vacuum cleaner are in change room.

Special lint-free chairs are used in the clean room and slso special table design for labs (6 ft. long x 3 ft., approx.).

Wherever possible, provisions were made for placing special heavy equipment (particularly pumps, high vacuum Varian Unit, etc.) on similar pads resting on floor (basement concrete floor) and separated from Unistrut floor (dust protection by light gasket, etc.) This arrangement is particularly critical in the case of electron microscopes with their peculiar base configuration (see drawings provided by Siemens). The operator emplaced on Unistrut floor must at all times be separated from the electron microscope which rests on vibration-free mount.

General Conditions of Clean Room Facilities:

Clean room facilities differ markedly from ordinary construction. Unique requirements of design and materials must be evaluated against excessive cost, the ultimate aim being the greatest reduction of contamination which is economically feasible. While the completed facility is an attractive result, beauty is not a governing consideration. All efforts and expense are directed to eliminating sources of contamination and places where contamination will collect.

Some of the unique features which have been built into our Clean Room facility are:

Materials: Special material has been chosen because it does not rust, corrode, flake, peel, crack, etc. For this reason, wood, paper, glass fiber, fabrics, plaster and many other common construction materials are eliminated. The basic materials we have to work with are porcelain, plate glass, stainless steel, anodized aluminum, heavy chrome plate, and for certain uses, plexiglas, vinyl and Formica. You will see that these materials have been used in our Clean Room.

Floor: The floor is of heavy vinyl, applied in long, wide sheets to minimize the amount of cracks, which would collect contamination.

Walls: The walls are of porcelain enameled panels.

Ceiling: The ceiling is of 1/4" porcelain enamiled panels, which is non-contributory to contamination and gives maximum fire protection.

All of the ceiling and wall panels have a compressed gasket or non-hardening caulking behind all edges, sealing out any possible source of contamination.

All joints of walls, floor and ceiling are provided with a tight rounded 4" radius cove - again, a special design for Clean Rooms. It eliminates the dead spaces where walls, etc. meet and which in ordinary construction catch so much dust and lint.

Windows: These are flush with the walls, and have no sills; thus, a flat surface is eliminated, which on ordinary construction is a major lodging place for dirt and dust.

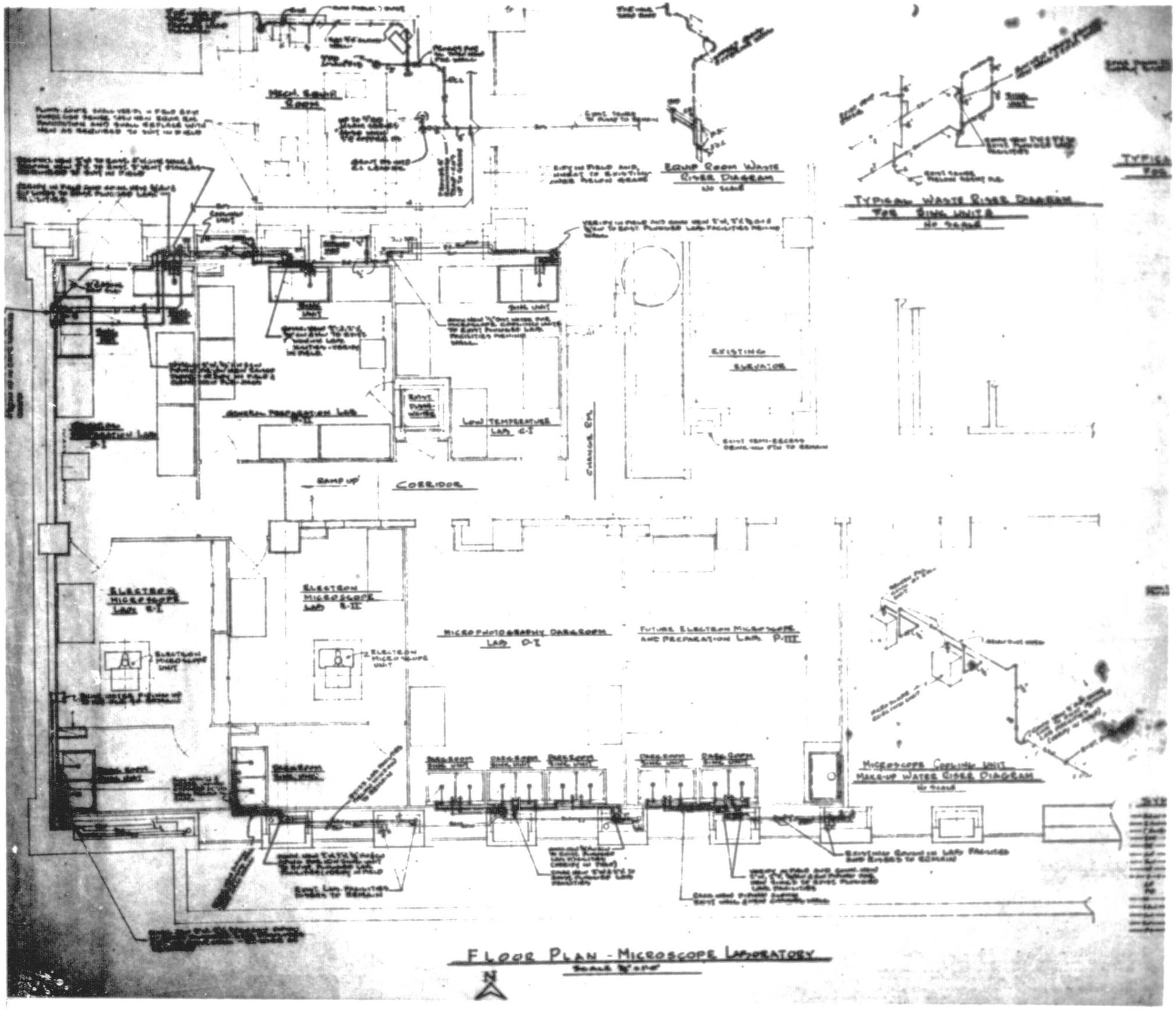
Doors and Frames: These are of anodized aluminum and plate glass

to provide non-contaminating materials (ordinary steel doors are painted and subject to both flaking and rust). Hinges are ball-bearing type to prevent milling of metal hinge parts.

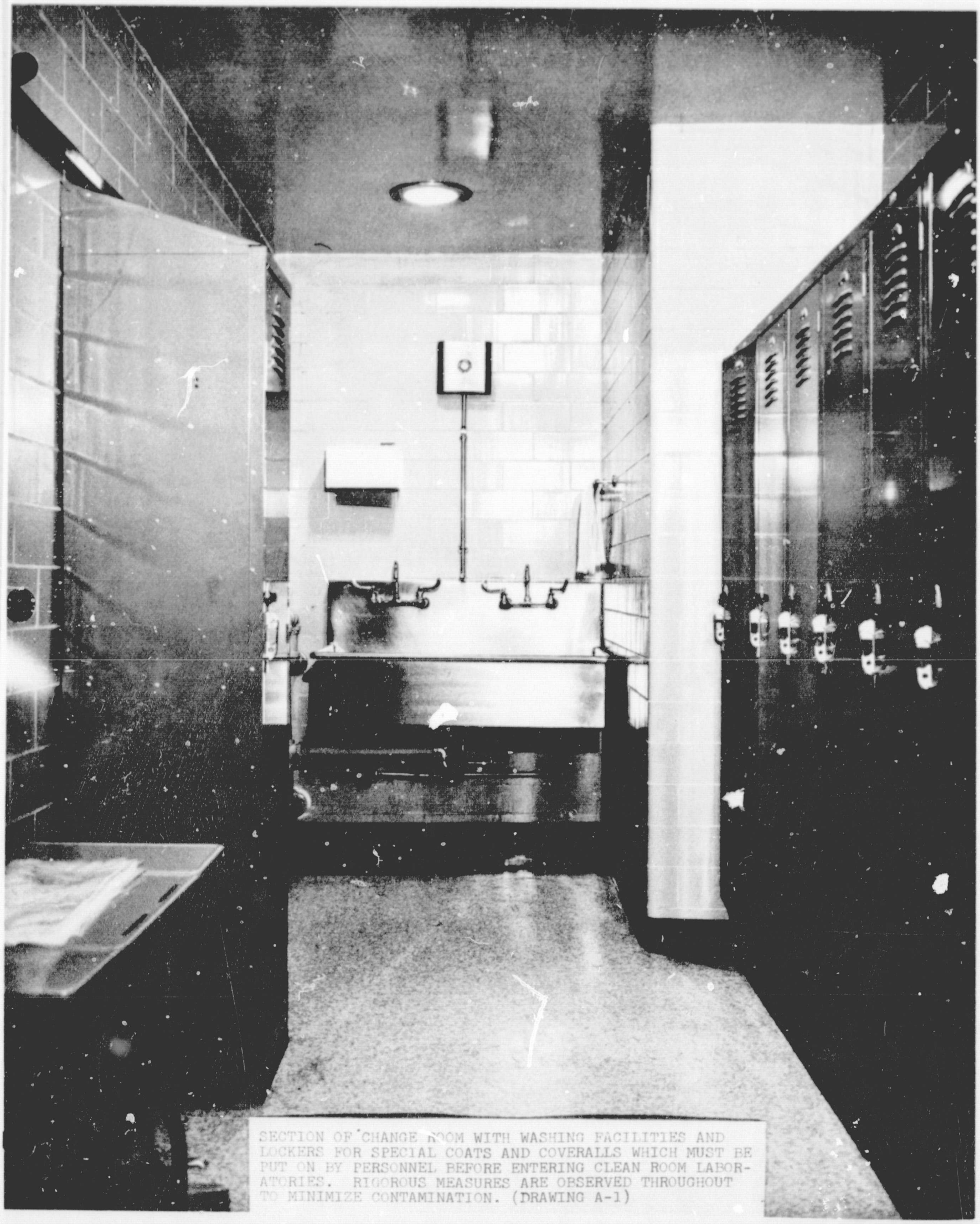
Lights: (Their heat is a collecting source for contaimination) are incandescent for uniform high intensity and are sealed from the room. Lamps are replaced from above to eliminate the contamination which is released from a fixture when lamps are changed from below.

The panel box for all electrical circuits, relays, etc., and all possible other services are located outside the room in a separate service area, reducing to a minimum the need for service-men to enter the Clean Room.

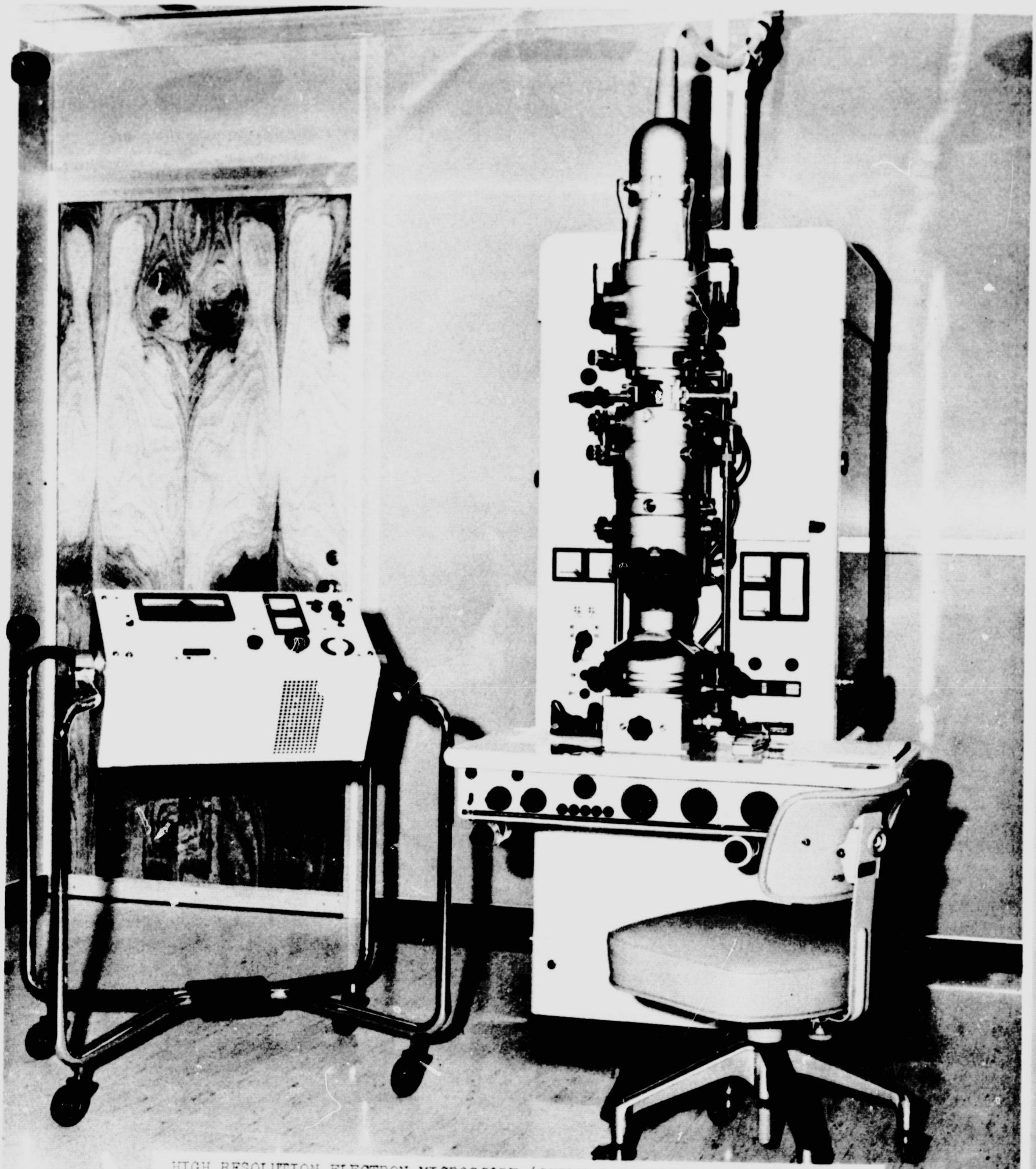
Horizontal surfaces are principal areas for contamination to collect. In our room, all possible are eliminated in conformance with good Clean Room practice, which dictates that only floor, ceiling, and work surfaces remain horizontal.


Equip Room Waste Riser Diagram
No Scale
Typical Waste Riser Diagram For Sink Units
No Scale
Existing Elevator
Corridor
Ramp up
Corridor
Electron Microscope Lab C-I
Electron Microscope Lab C-II
Microphotography Darkroom Lab C-I
Future Electron Microscope and Preparation Lab. P-III
Microscope Cooling Unit Make-up Water Riser Diagram
No Scale
Floor Plan - Microscope Laboratory
Scale 1/4"=1'-0"
N

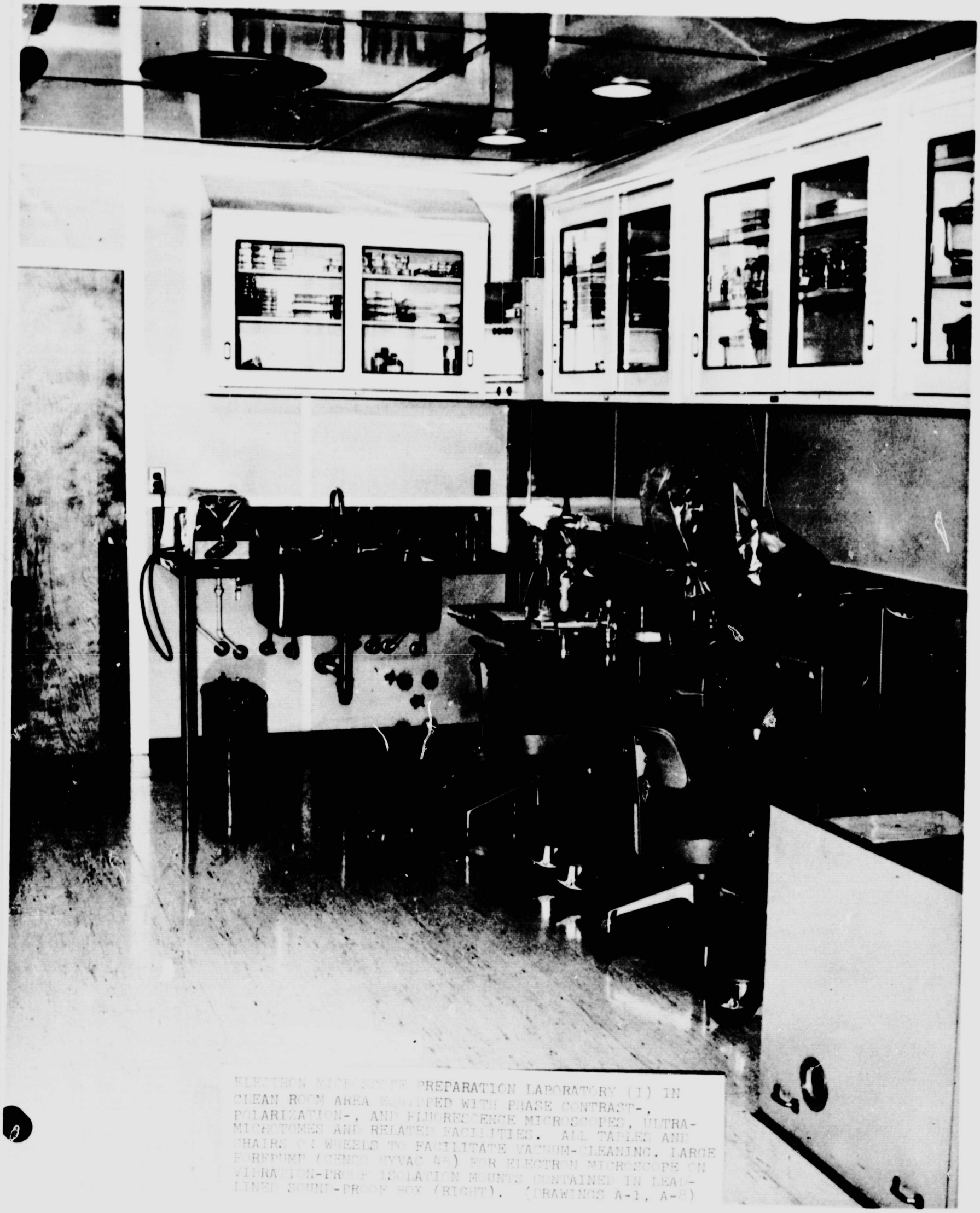
ENTRANCE TO ELECTRON MICROSCOPE LABORATORIES CLEAN ROOM AREA SHOWING SPECIAL WALL PARTITIONS, ENAMELED CEILING PANELS, RECESSED INCANDESCENT LIGHTS AND STAINLESS STEEL VENTILATION DUCT OPENINGS. ALL REASONABLE DESIGN FEATURES ARE INCORPORATED TO CARRY OUT HIGH RESOLUTION ELECTRON MICROSCOPY UNDER CLEAN ROOM CONDITIONS. (DRAWING A-1)



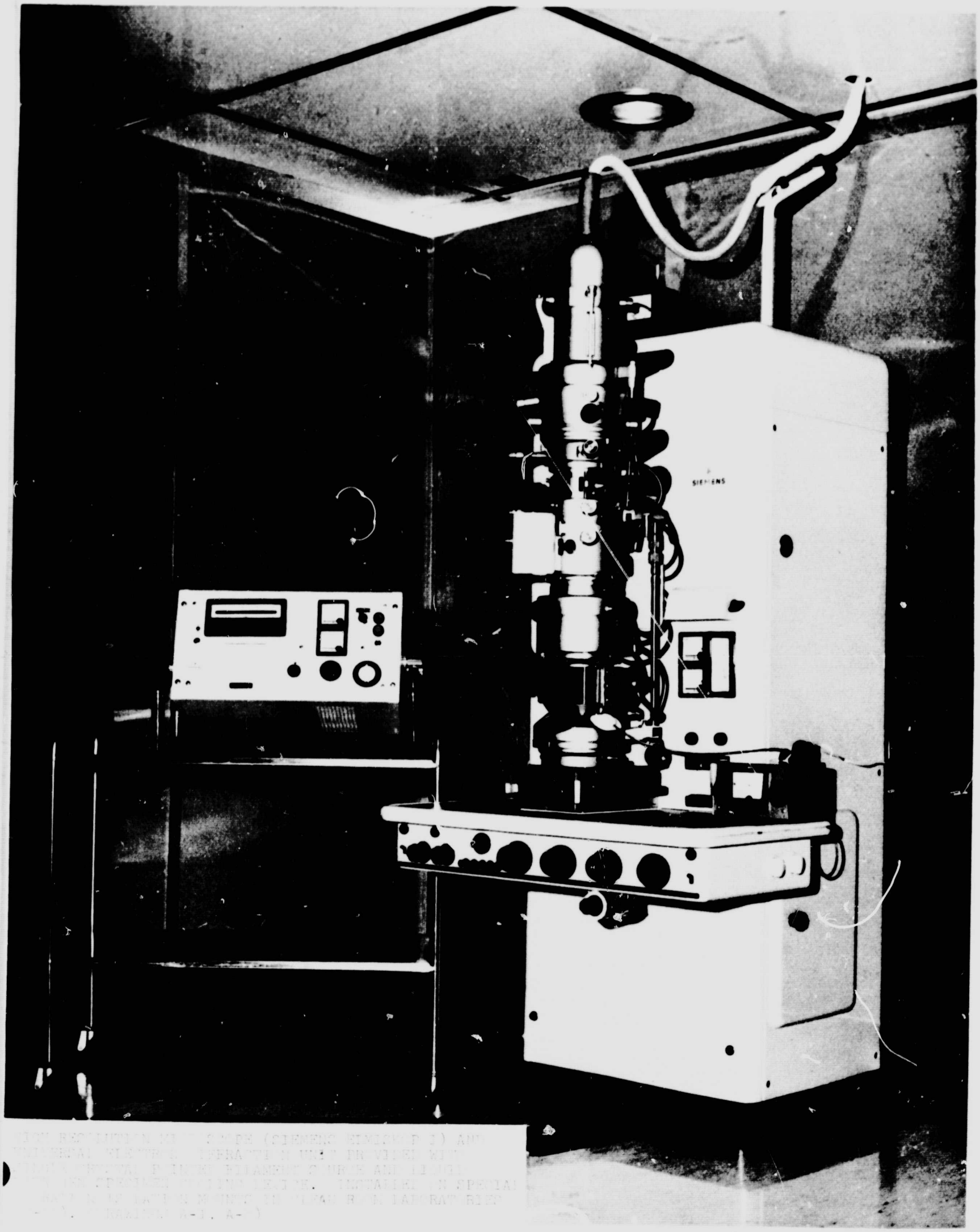
SECTION OF CHANGE ROOM WITH WASHING FACILITIES AND LOCKERS FOR SPECIAL COATS AND COVERALLS WHICH MUST BE PUT ON BY PERSONNEL BEFORE ENTERING CLEAN ROOM LABORATORIES. RIGOROUS MEASURES ARE OBSERVED THROUGHOUT TO MINIMIZE CONTAMINATION. (DRAWING A-1)

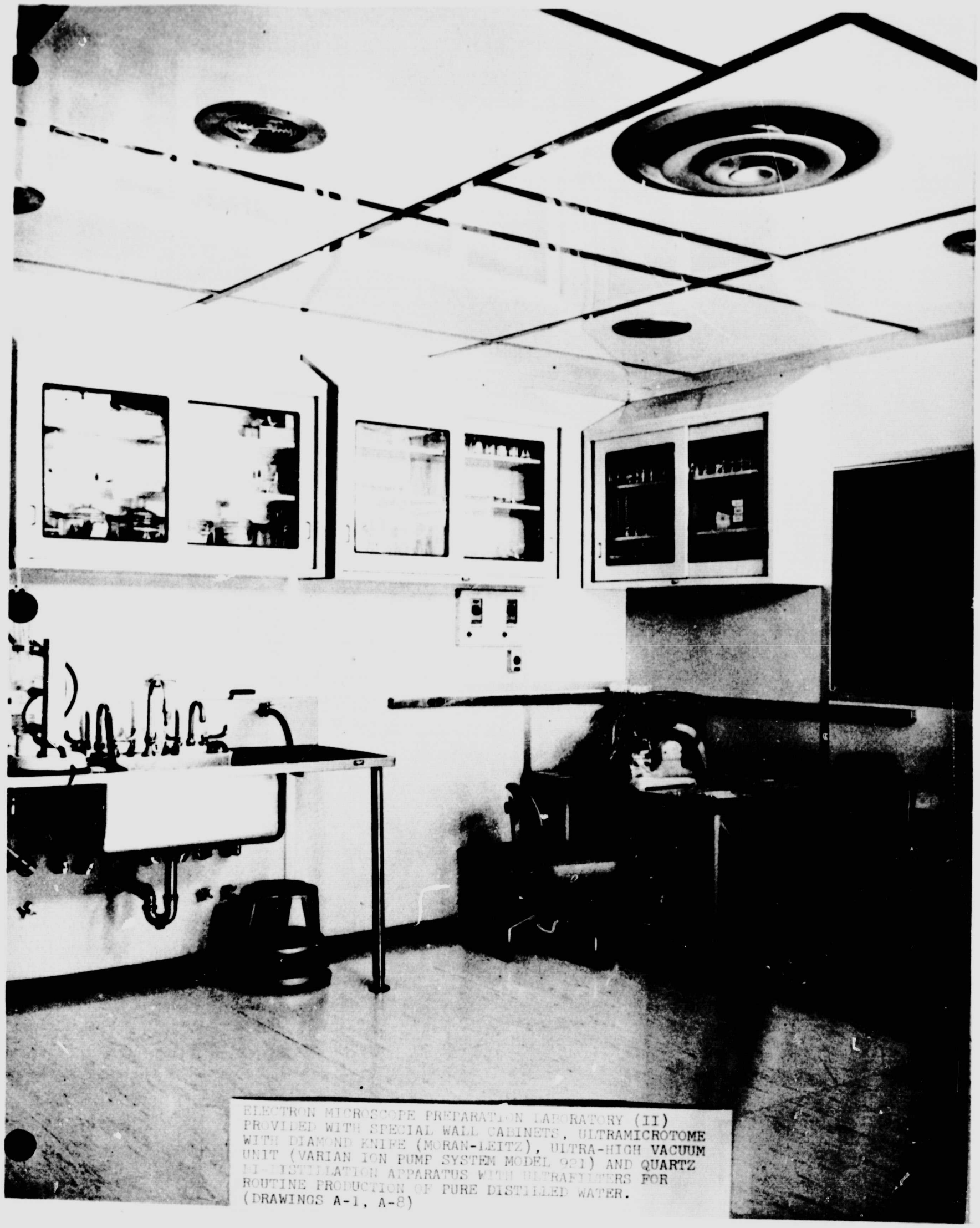


HIGH RESOLUTION ELECTRON MICROSCOPE (SIEMENS ELMISKOP I. 1963) AND UNIVERSAL ELECTRON DIFFRACTION UNIT INSTALLED ON SPECIAL VIBRATION ISOLATION MOUNTS IN NEW CLEAN ROOM LABORATORIES. RESEARCH INSTITUTES. UNIVERSITY OF CHICAGO. COMMITTEE ON BIOPHYSICS.

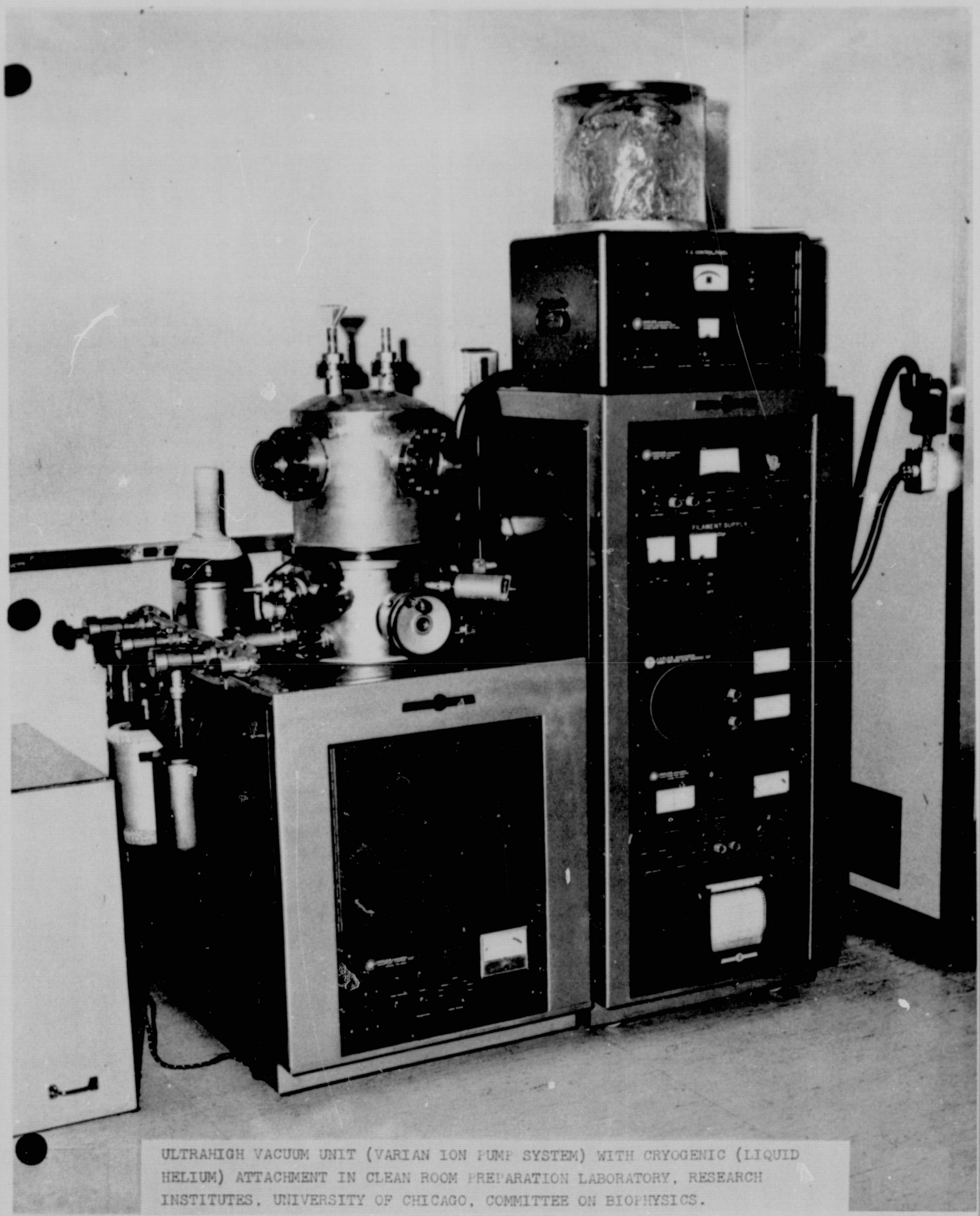


ELECTRON MICROSCOPE PREPARATION LABORATORY (I) IN CLEAN ROOM AREA EQUIPPED WITH PHASE CONTRAST-, POLARIZATION-, AND FLUORESCENCE MICROSCOPES, ULTRA-MICROTOMES AND RELATED FACILITIES. ALL TABLES AND CHAIRS ON WHEELS TO FACILITATE VACUUM-CLEANING. LARGE FOREPUMP (CENCO HYVAC 4F) FOR ELECTRON MICROSCOPE ON VIBRATION-PROOF ISOLATION MOUNTS CONTAINED IN LEAD-LINED SOUND-PROOF BOX (RIGHT). (DRAWINGS A-1, A-8)

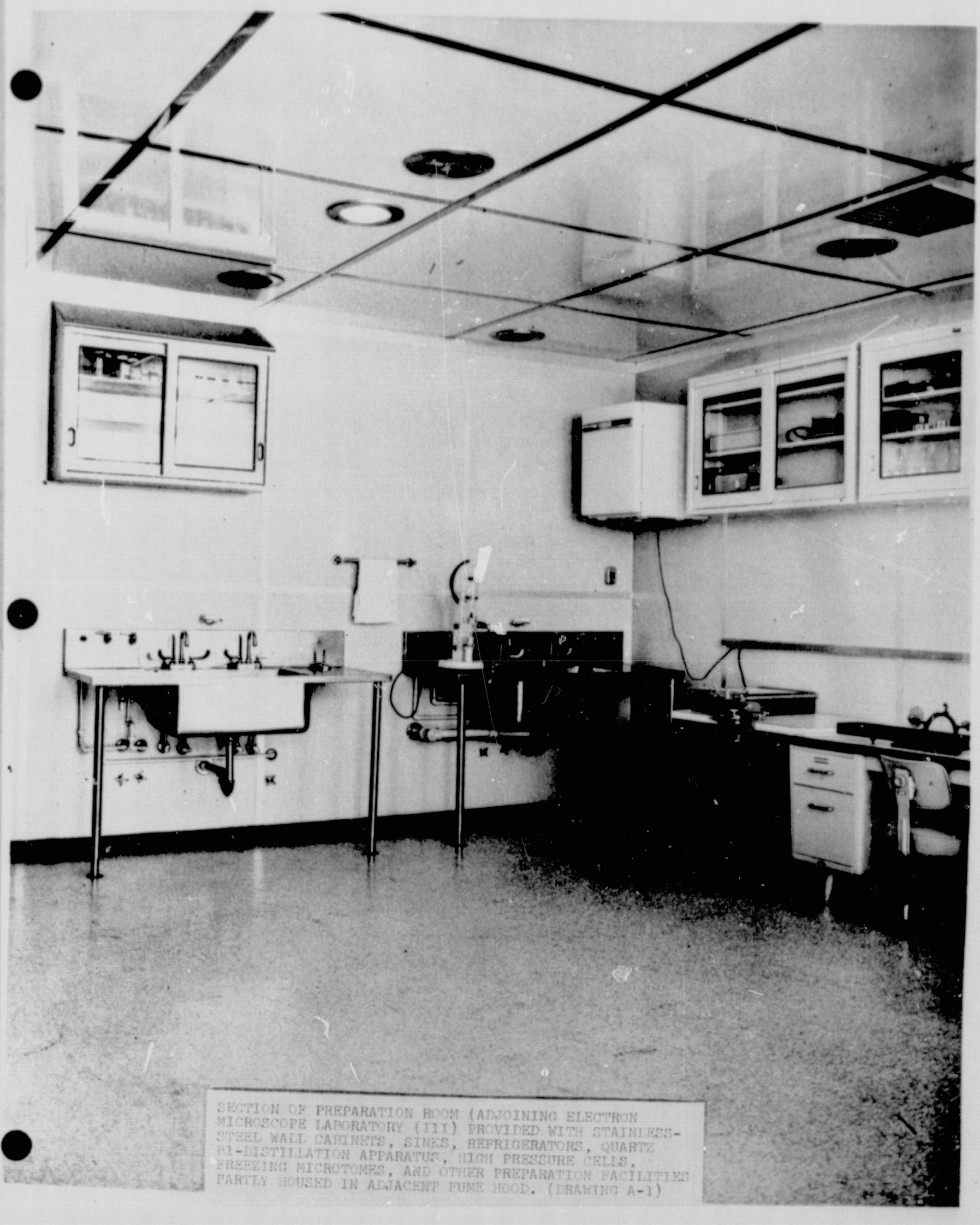

SIEMENS



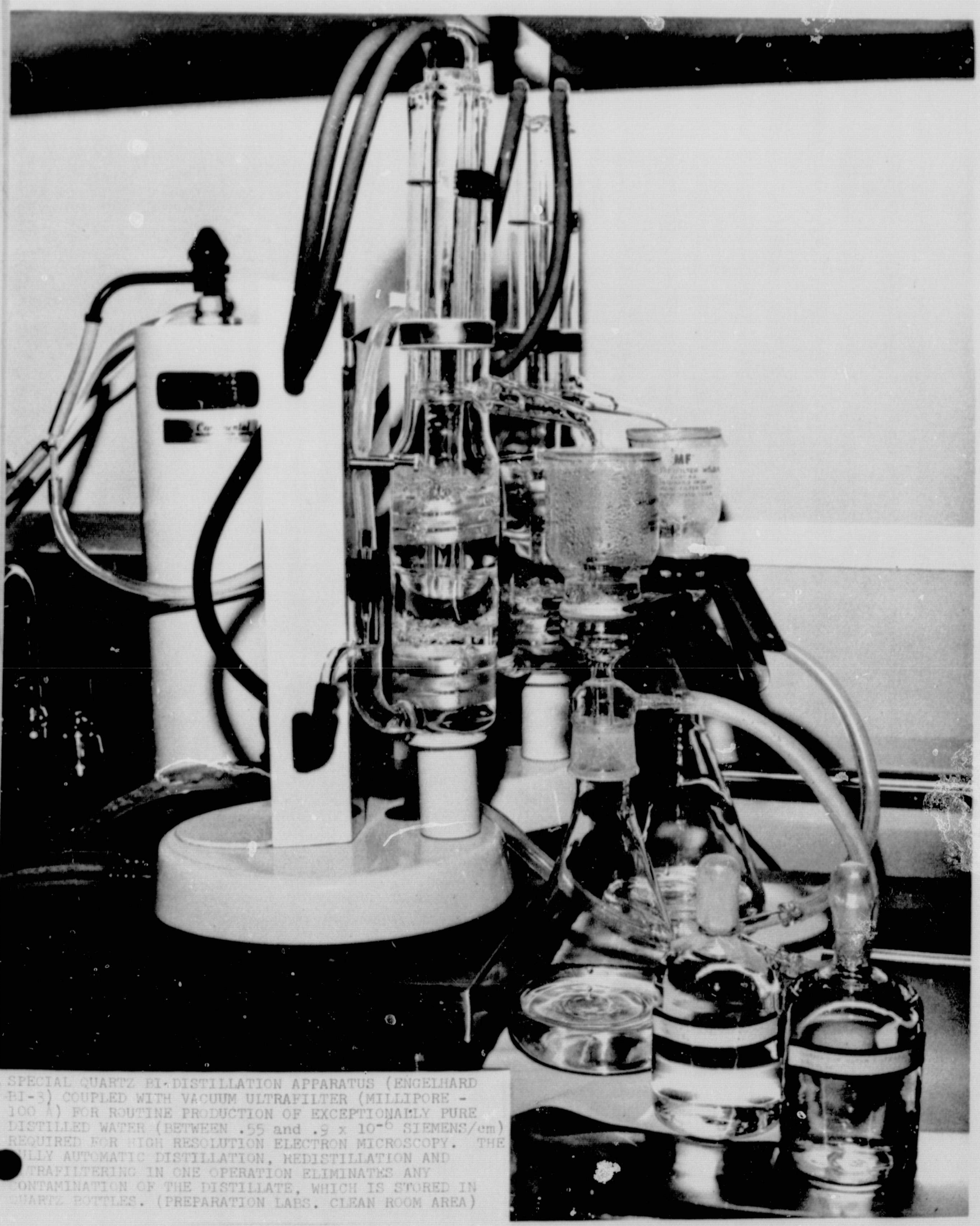
ELECTRON MICROSCOPE PREPARATION LABORATORY (II) PROVIDED WITH SPECIAL WALL CABINETS, ULTRAMICROTOME WITH DIAMOND KNIFE (MORAN-LEITZ), ULTRA-HIGH VACUUM UNIT (VARIAN ION PUMP SYSTEM MODEL 921) AND QUARTZ RE-DISTILLATION APPARATUS WITH ULTRAFILTERS FOR ROUTINE PRODUCTION OF PURE DISTILLED WATER. (DRAWINGS A-1, A-8)



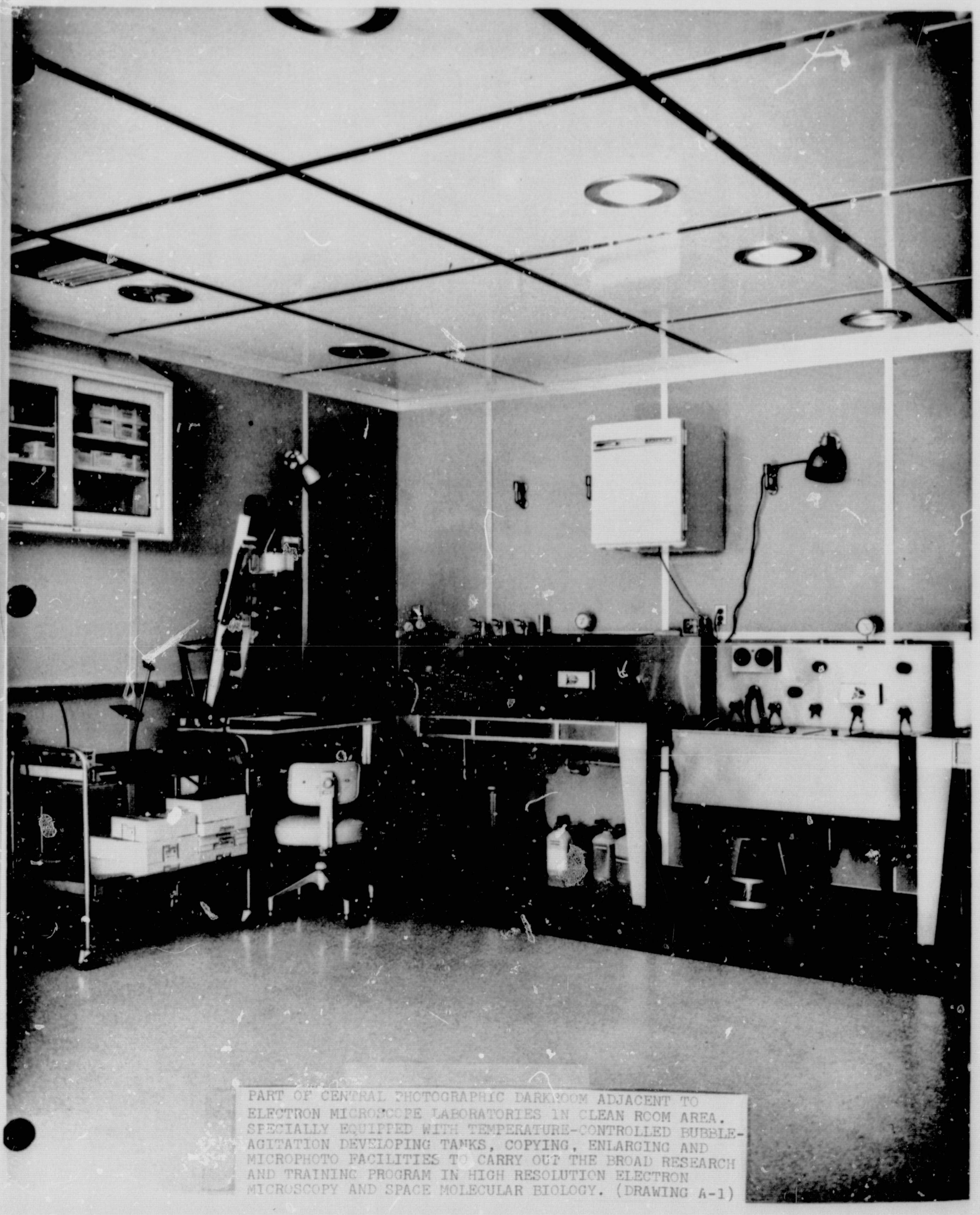
ULTRAHIGH VACUUM UNIT (VARIAN ION PUMP SYSTEM) WITH CRYOGENIC (LIQUID HELIUM) ATTACHMENT IN CLEAN ROOM PREPARATION LABORATORY, RESEARCH INSTITUTES, UNIVERSITY OF CHICAGO, COMMITTEE ON BIOPHYSICS.



SECTION OF PREPARATION ROOM (ADJOINING ELECTRON MICROSCOPE LABORATORY (111) PROVIDED WITH STAINLESS-STEEL WALL CABINETS, SINKS, REFRIGERATORS, QUARTZ RI-DISTILLATION APPARATUS, HIGH PRESSURE CELLS, FREEZING MICROTOMES, AND OTHER PREPARATION FACILITIES PARTLY HOUSED IN ADJACENT FUME HOOD. (DRAWING A-1)



SPECIAL QUARTZ BI-DISTILLATION APPARATUS (ENGELHARD BI-3) COUPLED WITH VACUUM ULTRAFILTER (MILLIPORE - 100 Å) FOR ROUTINE PRODUCTION OF EXCEPTIONALLY PURE DISTILLED WATER (BETWEEN .55 and .9 x 10^{-6} SIEMENS/cm) REQUIRED FOR HIGH RESOLUTION ELECTRON MICROSCOPY. THE FULLY AUTOMATIC DISTILLATION, REDISTILLATION AND ULTRAFILTERING IN ONE OPERATION ELIMINATES ANY CONTAMINATION OF THE DISTILLATE, WHICH IS STORED IN QUARTZ BOTTLES. (PREPARATION LABS. CLEAN ROOM AREA)



PART OF CENTRAL PHOTOGRAPHIC DARKROOM ADJACENT TO ELECTRON MICROSCOPE LABORATORIES IN CLEAN ROOM AREA. SPECIALLY EQUIPPED WITH TEMPERATURE-CONTROLLED BUBBLE-AGITATION DEVELOPING TANKS, COPYING, ENLARGING AND MICROPHOTO FACILITIES TO CARRY OUT THE BROAD RESEARCH AND TRAINING PROGRAM IN HIGH RESOLUTION ELECTRON MICROSCOPY AND SPACE MOLECULAR BIOLOGY. (DRAWING A-1)

Specific Research Program

The research and training program represents essentially a continuation of the comprehensive, integrated program in the field of molecular biology which is being carried out with support from the NIH and AEC at the University of Chicago. Illustrations follow the descriptions of each area of research connected with the Electron Microscope Laboratories.

Continuation of correlated electron microscope and biochemical studies of mitochondrial membranes which have resulted inthe detection and isolation of a fundamental unit of energy transduction (electron transfer particle). A repeating particle associated with the cristae and the inner membrane of the external envelope has been recognized and characterized in beef heart mitochondria by correlated electron microscopic and biochemical studies. Many thousands (ca. 10^4 to 10^5) of these particles, disposed in regular arrays, are present in a single mitochondrion. The repeating particle, called the elementary particle (EP), consists of three parts: (1) a spherical or polyhedral head piece (80 to 100 Å in diameter); (2) a cylindrical stalk (about 50 Å in length and 30-40 Å in width); and (3) a base piece (40 x 110 Å). The base pieces of the elementary particles form an integral part of the outer dense layers of the cristae. The elementary particles can be seen in electron micrographs of mitochondria in situ, of isolated mitochondria, and of submitochondrial particles with a complete electron transfer chain. Negative staining with phosphotungstate is only one of several techniques that can be used for reproducible demonstration of the repeating particles and underlying subunit organization of mitochondrial membranes. A particulate unit containing a complete electron transfer chain can be isolated from beef heart mitochondria. The isolated unit approximates in size that of the elementary particle in situ. The molecular weight of the particle in situ is calculated to be 1.3×10^6. Evidence is presented for identifying the isolated unit with the elementary particle visualized in situ. The elementary particle of the mitochondrion is believed to be a prototype of a class of functional particles or macromolecular assemblies of similar size found in association with membranes generally. Similar integrated studies of related membrane derivatives in photoreceptors, myelin and other cell membrane systems will be carried out, to gain a better understanding of the fundamental principles underlying cell membrane organization in general. (See publication no.92.)

Fig. 1. Pointed filaments with spot-welded and etched, oriented tungsten single crystal tips showing characteristic preservation of critical cathode profile: (a) annealed tip before use; (b) filament after 40 hours, and (c) after 100 hours operation in Siemens Elmiskop I at 40 to 100 kV, followed by "resharpening" of the dulled tip through controlled ion bombardment performed within the electron gun. The tip may also be regenerated by epitaxial growth of tungsten on the single crystal substrate from the vapor phase.

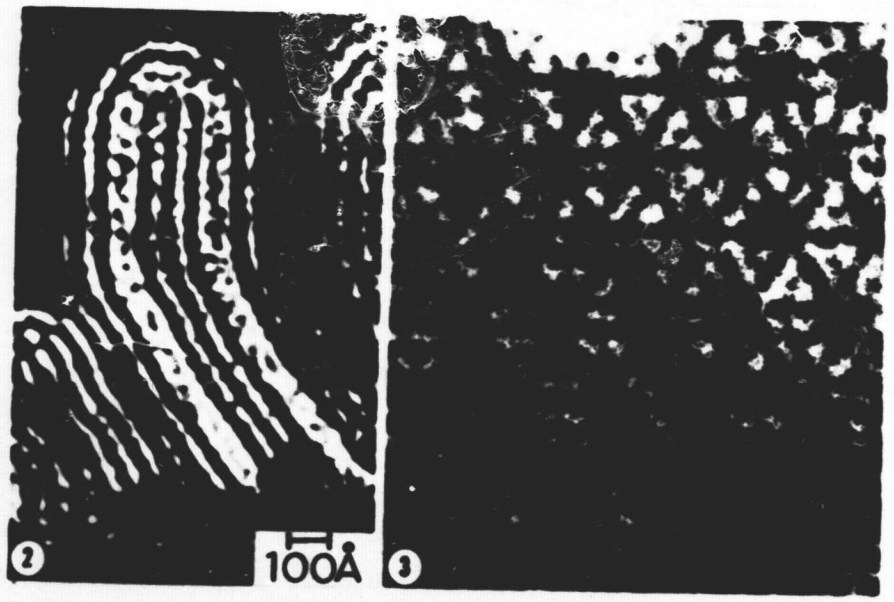
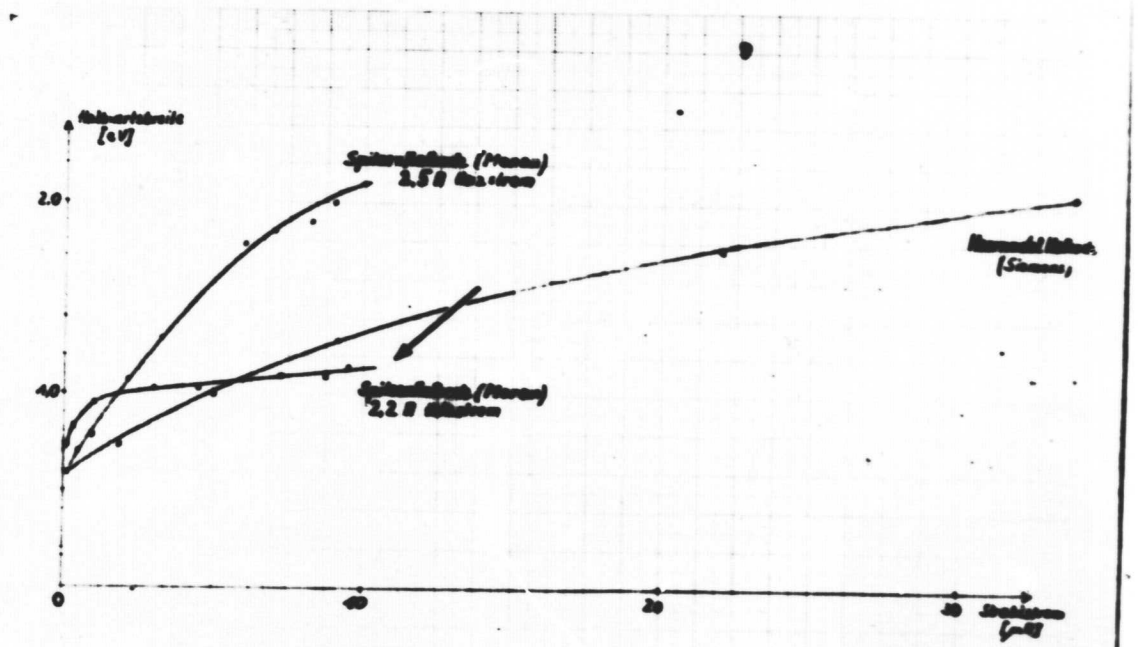


Fig. 2. High resolution electron micrograph of pure Lecithin micelles embedded in thin PTA film showing typical periodic arrangement of the bimolecular lipid layers. Recorded on Ilford high resolution plate with microbeam illumination from single-crystal pointed filament.
Fig. 3. Moiré pattern of overlapping single crystals of basic lead carbonate recorded with microbeam illumination of very low intensity.



IMPROVED SINGLE-CRYSTAL POINTED CATHODES WITH ORIENTED SINGLE-CRYSTAL TUNGSTEN TIP ACCURATELY POSITIONED IN A RE-ENTRANT CATHODE SHIELD HAVE BEEN USED ROUTINELY WITH DOUBLE CONDENSER SYSTEMS TO PROVIDE COHERENT MICROBEAM ILLUMINATION FOR HIGH RESOLUTION ELECTRON MICROSCOPY AND DIFFRACTION AT 40 to 100 kV. AS A RESULT OF THE HIGH DEGREE OF PURITY AND SELECTED CRYSTALLOGRAPHIC ORIENTATION OF THE EMITTER THESE CATHODES HAVE LONGER AVERAGE LIFE AND OPERATIONAL STABILITY THAN CONVENTIONAL SOURCES. THE GREATER INTENSITY OF THIS MICROBEAM ILLUMINATION PERMITS DIRECT SPECIMEN OBSERVATION UNDER OPTIMUM ELECTRON OPTICAL CONDITIONS FOR ATTAINMENT OF ENHANCED RESOLUTION AND CONTRAST. (H. Fernandez-Moran, J. Appl. Phys. 31, 1840 - 1960).

MOREOVER, INCREASED BRIGHTNESS IS OBTAINED WHILE OPERATING THE POINTED FILAMENTS WITH LOW HEATING CURRENT, THUS CONSIDERABLY REDUCING THE ENERGY SPREAD OF EMITTED ELECTRONS (e.g.: 1 eV at 30 kV, as compared with 2 eV for standard filaments, according to velocity distribution measurements by Prof. G. Möllenstedt and K.H. Gaukler at the University of Tübingen, Germany.) MICROBEAM PROBES (100 to 1000 A.U. in diameter) OBTAINED WITH THESE CATHODES HAVE BEEN USED FOR CONTROLLED IRRADIATION OF PRESELECTED MACROMOLECULAR REGIONS IN BIOLOGICAL SYSTEMS. (The valuable assistance of Ernest Shmid and George Leach of the Research Laboratory of Electronics, Massachusetts Institute of Technology, and of Frederick Merk, Mass. General Hospital, in the development of the pointed cathodes is gratefully acknowledged.)

Annual Report 1963/64 -Research Program- H. Fernández-Morán

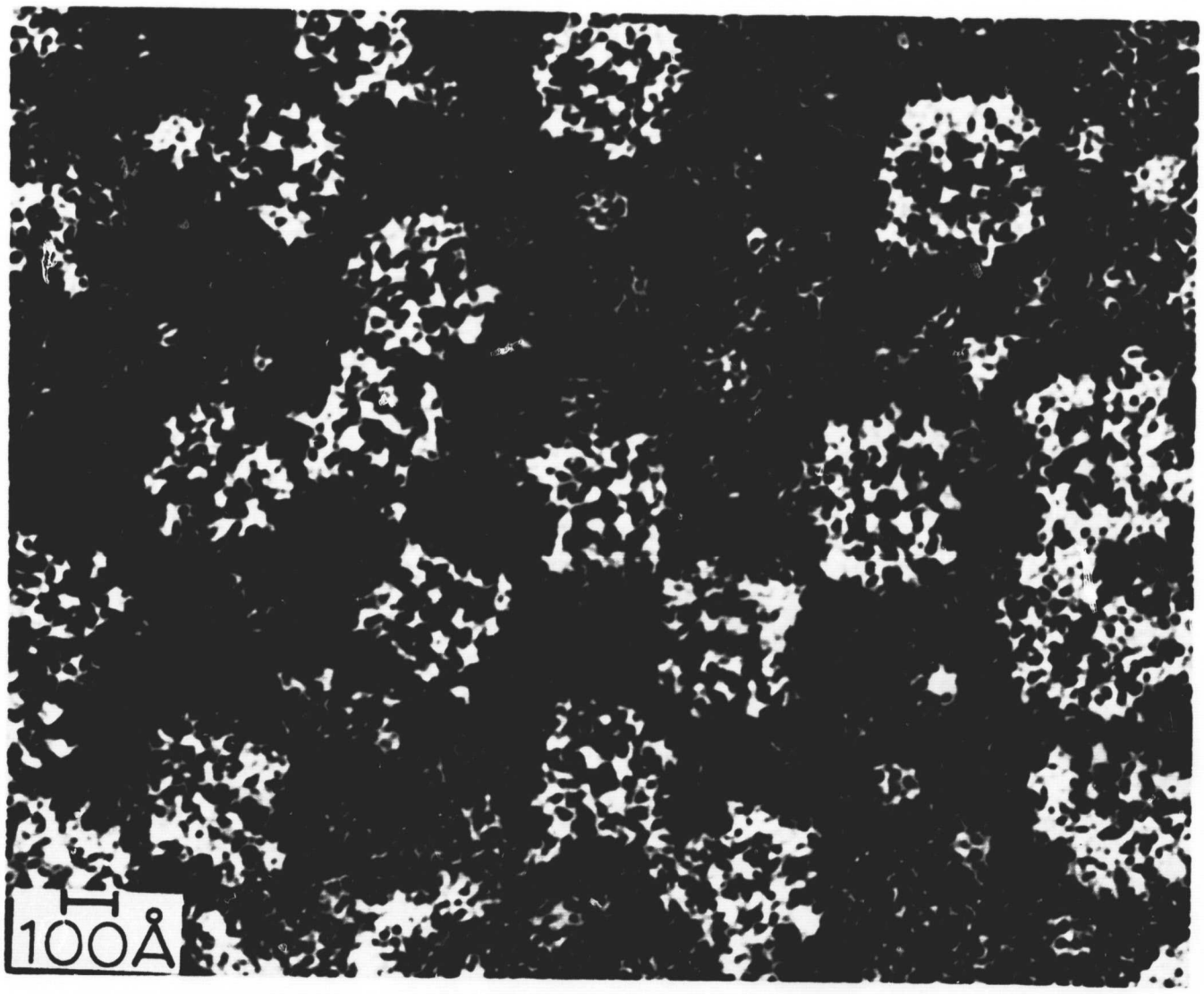
Electron microscopic and biochemical studies of pyruvate dehydrogenase complex of Escherichia coli carried out in collaboration with Lester J. Reed, Masahiko Koike and Charles R. Willms. Examination of the Escherichia coli pyruvate dehydrogenase complex and its component enzymes in the electron microscope indicates that the complex has a polyhedral structure with a diameter of about 300 to 350 Å and a height of 200 to 250 Å. The lipoic reductase-transacetylase aggregate, consisting of about 64 subunits, occupies the central portion of the polyhedron and has the appearance of a tetrad of 130 to 150 Å. Surrounding this tetrad are about 16 molecules of pyruvate decarboxylase and about 8 molecules of dihydrolipoic dehydrogenase arranged into two rings laid one above the other. The sequence of these latter molecules cannot yet be specified. However, many of these latter molecules are similar in appearance and dimensions (70 to 90 Å) to those observed in electron micrographs of the isolated pyruvate decarboxylase component of the complex. The reconstituted complex closely resembles the native complex in appearance. (See publication no. 93).

Texts of Illustrations:

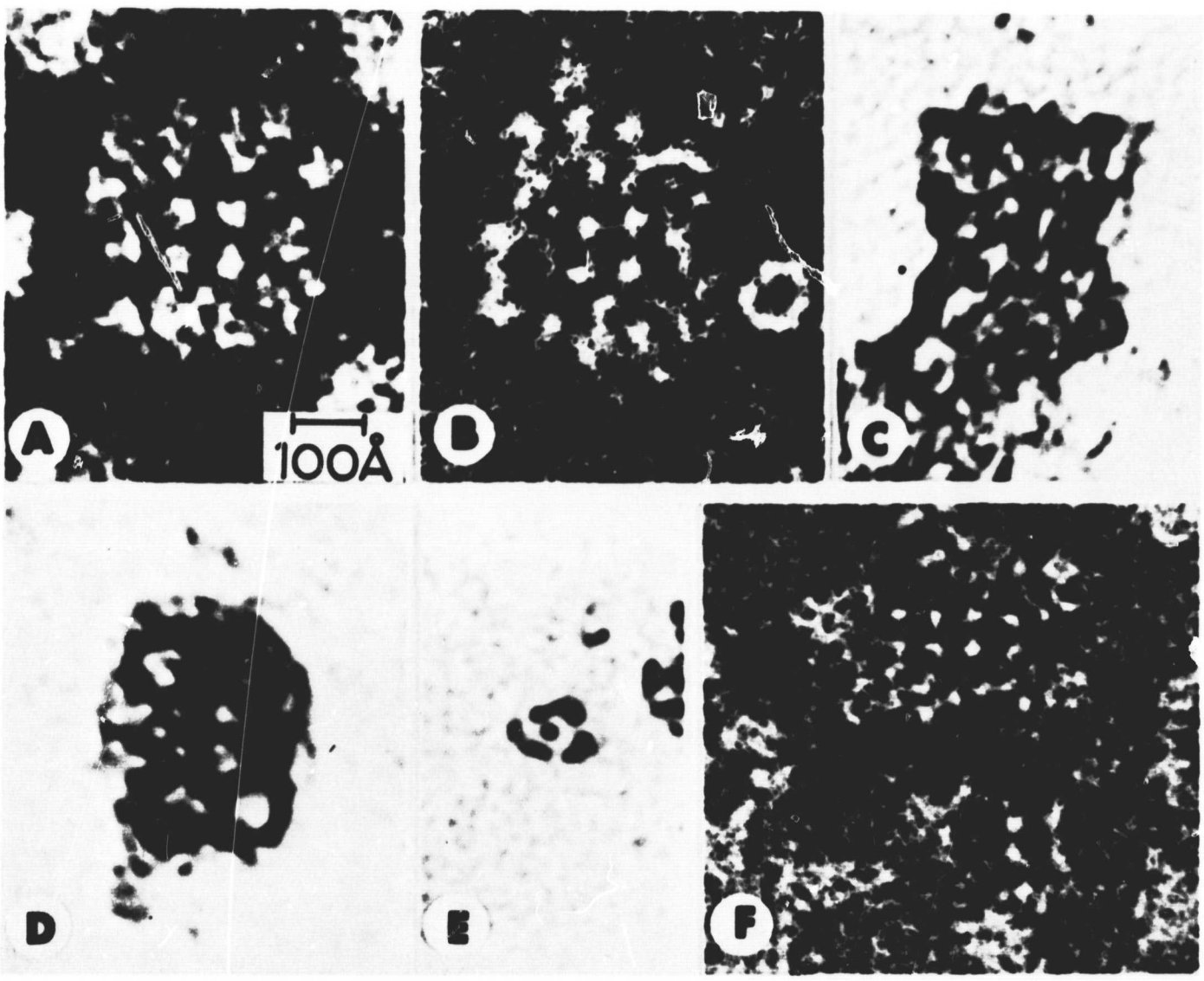
Fig.1: Pyruvate dehydrogenase complex of Escherichia Coli negatively stained with 1 percent phosphotungstate (pH 7.4) using microdroplet cross-spraying technique (X 520,000).

Fig.2: Electron micrographs of E. coli pyruvate dehydrogenase complex (PDC): (A) Native PDC; (B) Reconstituted PDC with Ferritin negatively stained with uranyl acetate; (E) Isolated pyruvate decarboxylase stained with uranyl acetate; (F) Isolated LRT aggregate negatively stained. (X 700,000).

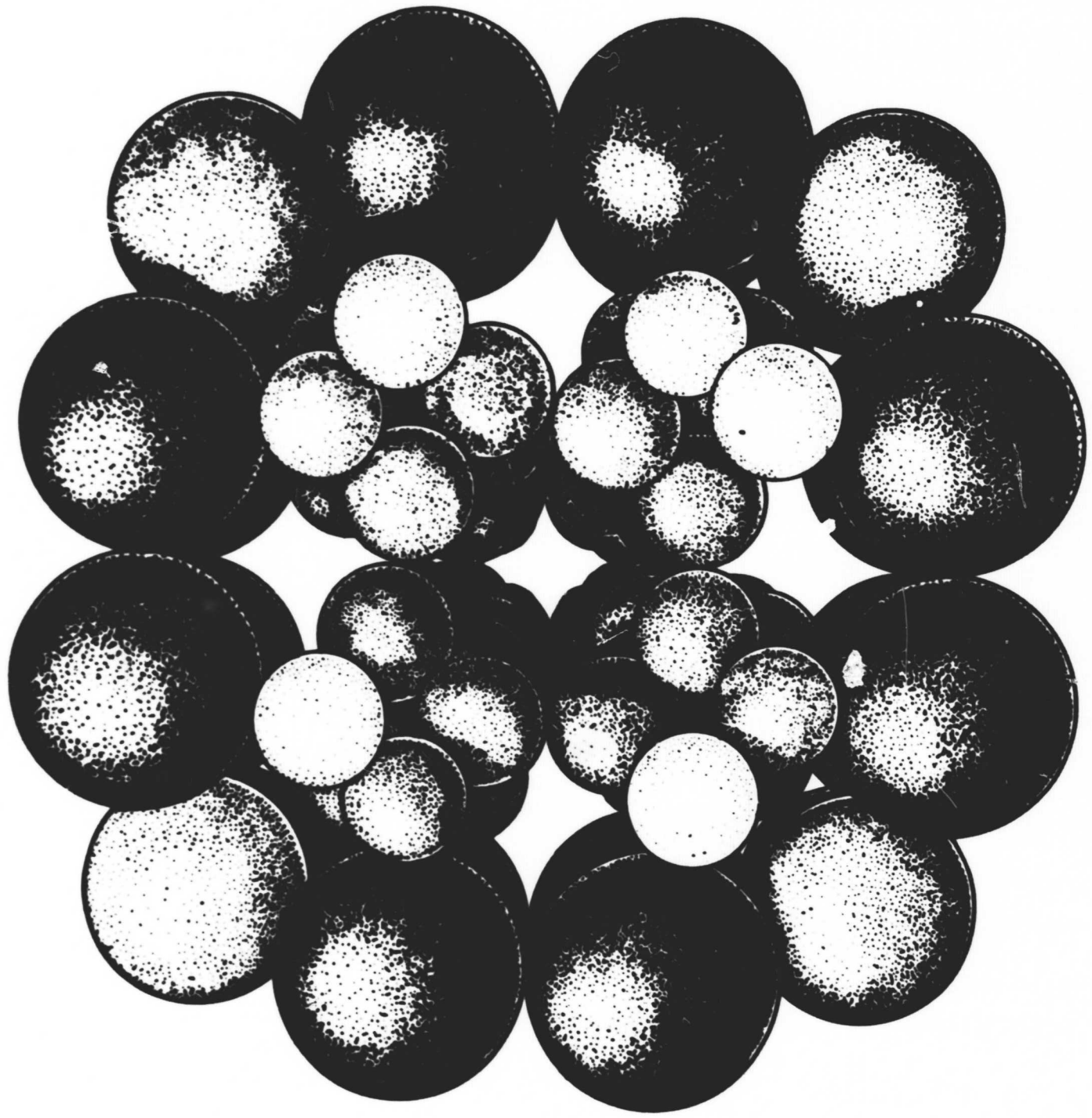
Fig.3: Tentative model of E. coli pyruvate dehydrogenase complex.



① 100Å



(2)



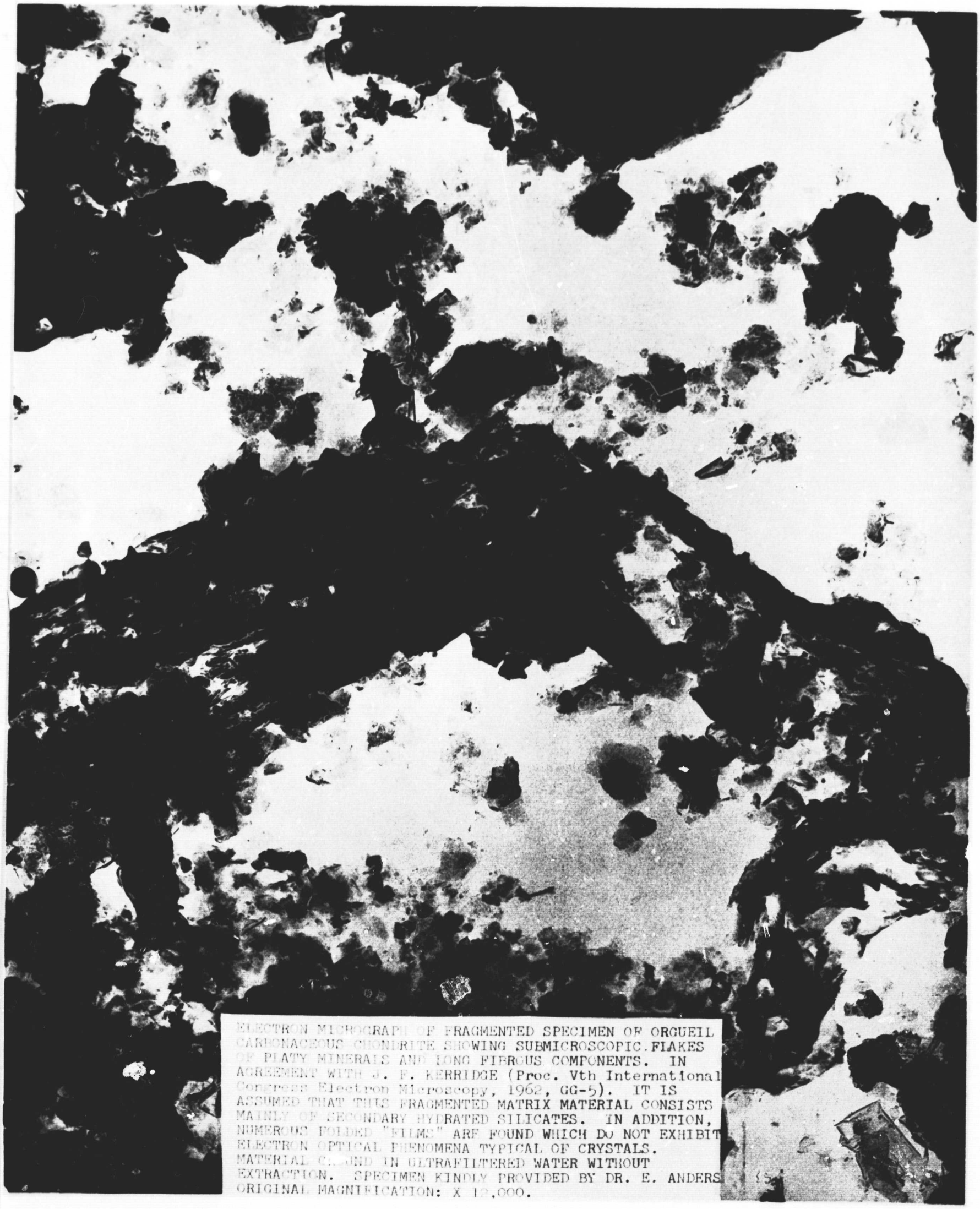
Annual Report -Research Program- H. Fernández-Morán

Correlated electron microscope and electron diffraction studies of certain meteorites (Orgueil) carbonaceous chondrite) carried out in collaboration with Dr. Edward Anders and Dr. Frank W. Fitch of the University of Chicago. Preliminary experiments indicate that the higher resolving power of the electron microscope can be fruitfully applied to further elucidate the compoasition and structure relationships of its constituents, with particular reference to the "organized elements". A variety of preparation techniques are being applied under carefully controlled conditions, including ultra-thin sectioning with a diamond knife, mechanical and selective chemical dissociation followed by density gradient separation, negative staining, shadow-casting, etc. The ultra-structural data will be correlated with parallel chemical studies of organic constituents and to the results of selected area electron diffraction analysis.

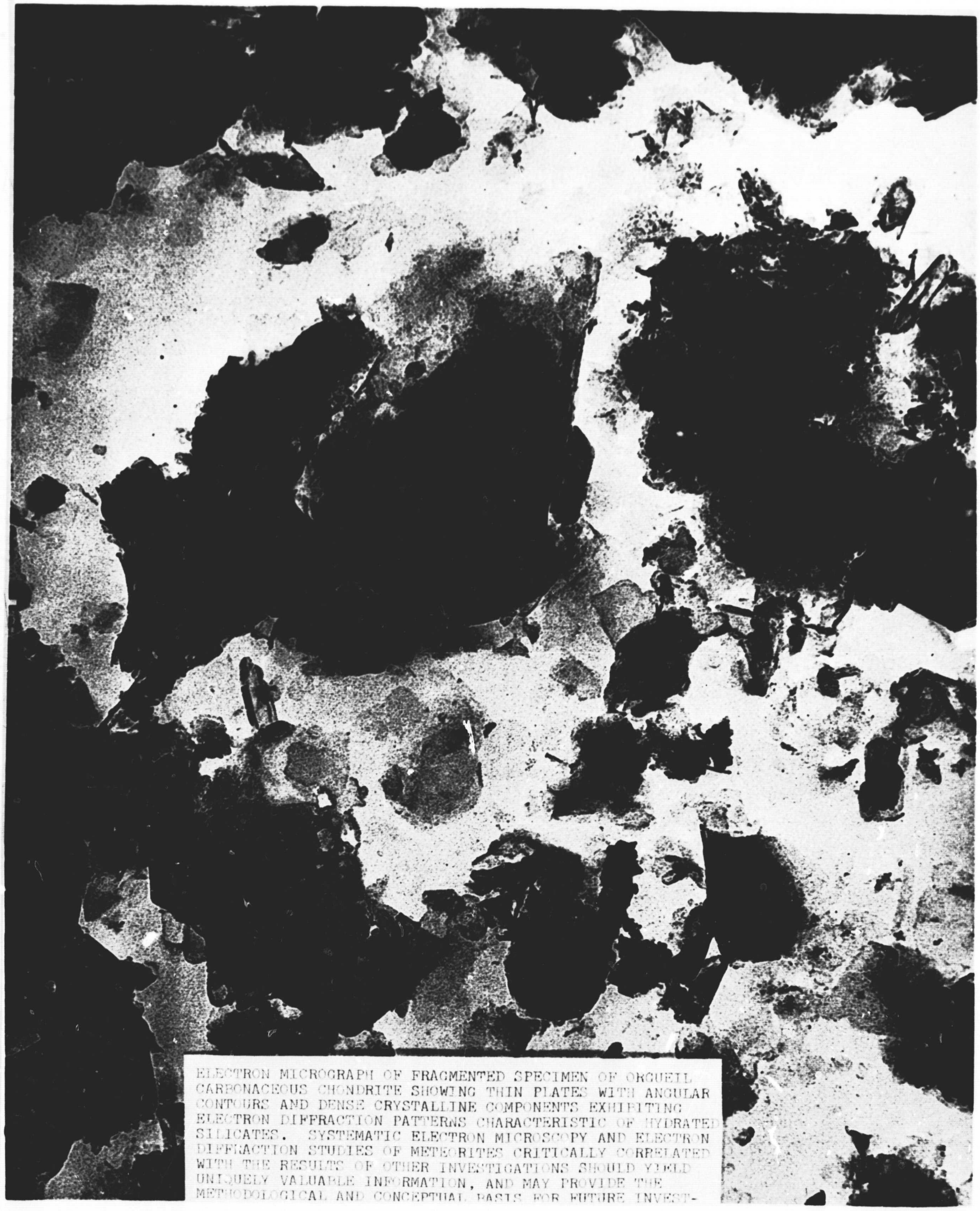
Electron microscopy studies of pre-Cambrian organized systems: Preliminary investigation of nonferruginous cherts of the Gunflint formation of southern Ontario by electron microscopy has been started in collaboration with Dr. Edward Anders and Dr. F. Fitch. S. A. Tyler of the University of Wisconsin, and Dr. E. Barghoorn of Harvard University have reported (SCIENCE, Vol. 119, P. 606, 1954) the occurrence of primitive lower plants in these pre-Cambrian rocks, which are the oldest (about 2 billion years) structurally preserved organisms that clearly exhibit cellular differentiation. Electron microscopy reveals the presence of filaments, tubular structures and membranes of apparent organic origin. This work looks most promising and we plan to continue systematic examination of the chert material kindly supplied by Dr. Barghoorn, using ultra-thin sectioning with the diamond knife and the Moran-Lietz microtome, mechanical and chemical dissociation techniques and related preparation procedures.

These studies are of great interest in the evolutionary scheme of primitive life, since they may furnish insight into the molecular organization of the oldest known preserved living systems, bearing also on the evolution of membrane ultra-structure.

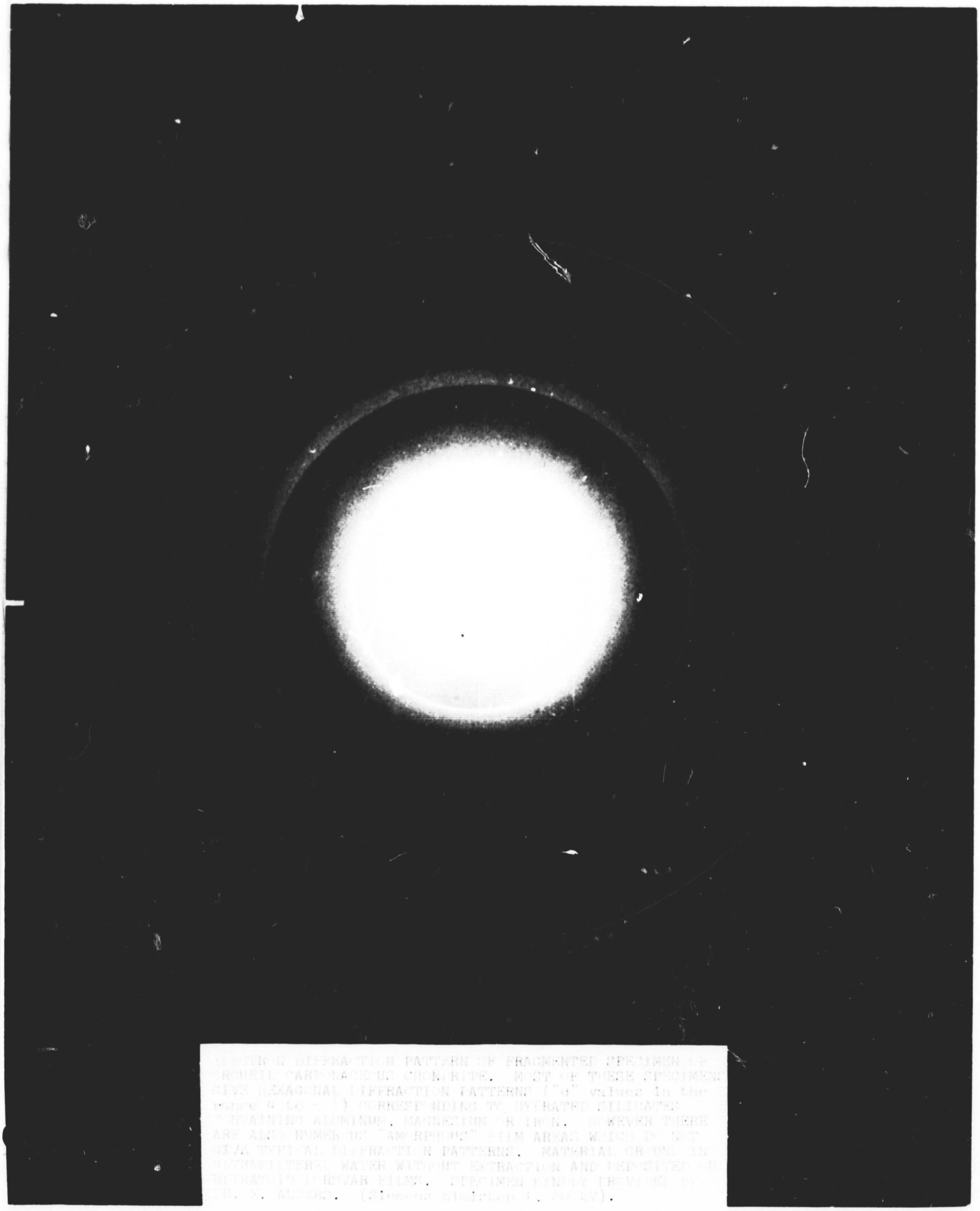
Electron microscopy of meteorites and of pre-Cambrian fossils should yield uniquely valuable information, and may eventually provide the methodological and constructural basis for future investigation of extraterrestrial (lunar and planetary) matter.



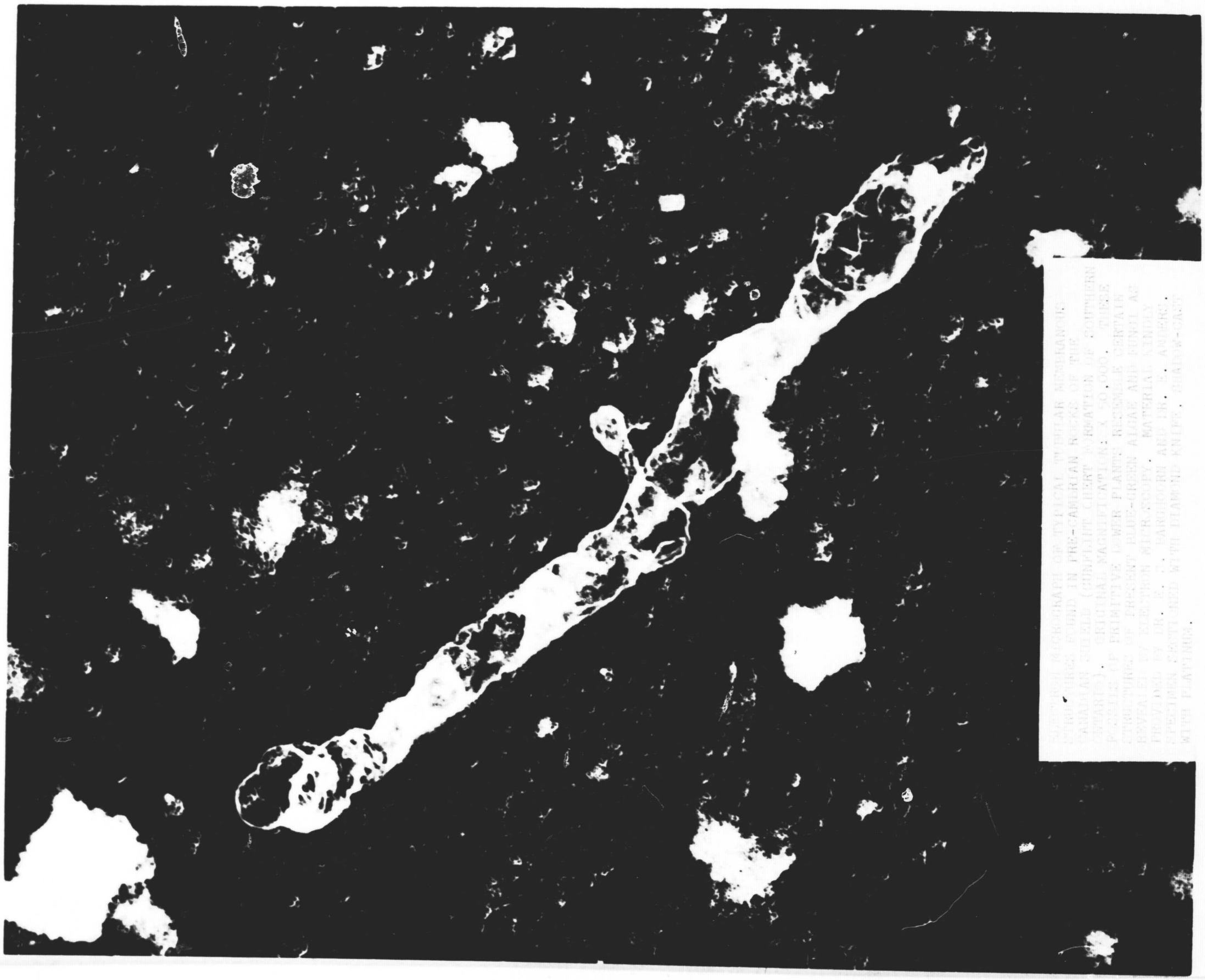
ELECTRON MICROGRAPH OF FRAGMENTED SPECIMEN OF ORGUEIL CARBONACEOUS CHONDRITE SHOWING SUBMICROSCOPIC FLAKES OF PLATY MINERALS AND LONG FIBROUS COMPONENTS. IN AGREEMENT WITH J. F. KERRIDGE (Proc. Vth International Congress Electron Microscopy, 1962, GG-5). IT IS ASSUMED THAT THIS FRAGMENTED MATRIX MATERIAL CONSISTS MAINLY OF SECONDARY HYDRATED SILICATES. IN ADDITION, NUMEROUS FOLDED "FILMS" ARE FOUND WHICH DO NOT EXHIBIT ELECTRON OPTICAL PHENOMENA TYPICAL OF CRYSTALS. MATERIAL C[illegible]UND IN ULTRAFILTERED WATER WITHOUT EXTRACTION. SPECIMEN KINDLY PROVIDED BY DR. E. ANDERS [illegible]
ORIGINAL MAGNIFICATION: X 12,000.

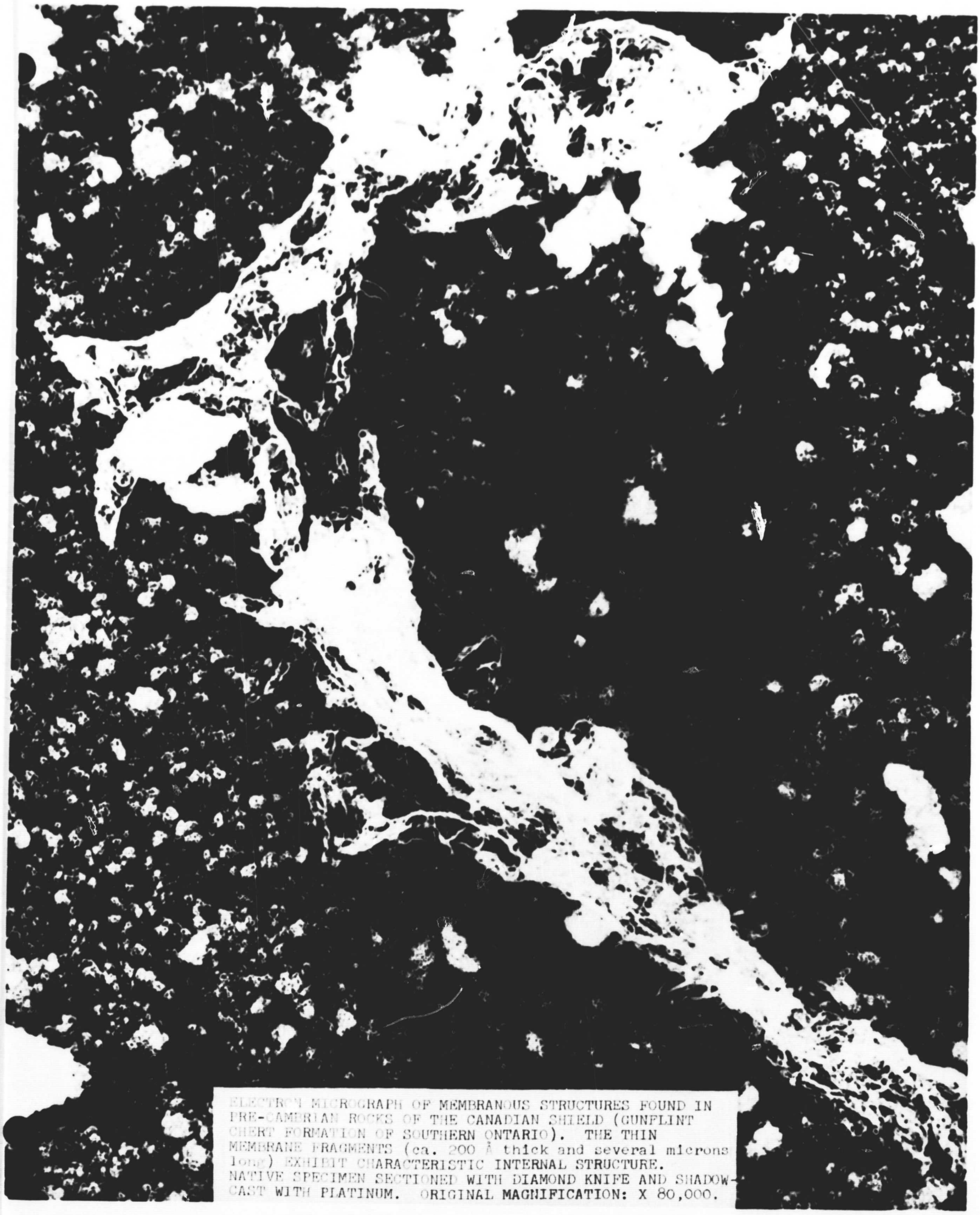


ELECTRON MICROGRAPH OF FRAGMENTED SPECIMEN OF ORGUEIL CARBONACEOUS CHONDRITE SHOWING THIN PLATES WITH ANGULAR CONTOURS AND DENSE CRYSTALLINE COMPONENTS EXHIBITING ELECTRON DIFFRACTION PATTERNS CHARACTERISTIC OF HYDRATED SILICATES. SYSTEMATIC ELECTRON MICROSCOPY AND ELECTRON DIFFRACTION STUDIES OF METEORITES CRITICALLY CORRELATED WITH THE RESULTS OF OTHER INVESTIGATIONS SHOULD YIELD UNIQUELY VALUABLE INFORMATION, AND MAY PROVIDE THE METHODOLOGICAL AND CONCEPTUAL BASIS FOR FUTURE INVEST-

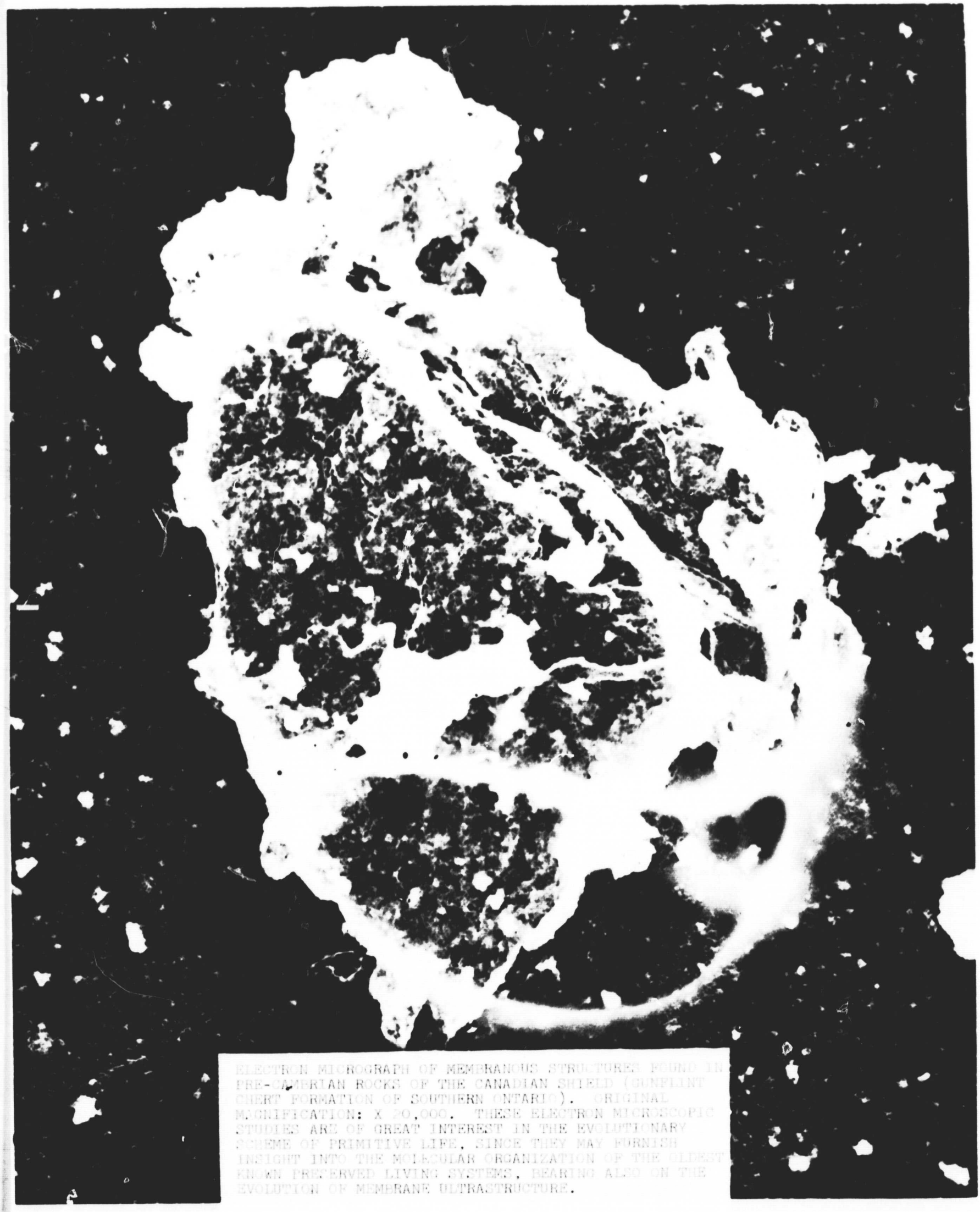


ELECTRON MICROGRAPH OF CHARACTERISTIC TUBULAR MEMBRANOUS STRUCTURES FOUND IN PRE-CAMBRIAN ROCKS OF THE CANADIAN SHIELD (GUNFLINT CHERT FORMATION OF SOUTHERN ONTARIO). ORIGINAL MAGNIFICATION: X 50,000. ELECTRON MICROSCOPE STUDIES OF THESE OLDEST KNOWN (ESTIMATED 1.6 to 2 BILLION YEARS) REMAINS OF LIVING FORMS SUPPLEMENT AND EXTEND THE CLASSIC LIGHT MICROSCOPY INVESTIGATIONS BY S. A. TYLER AND E. S. BARGHOORN (Science, 119, 606, 1954).





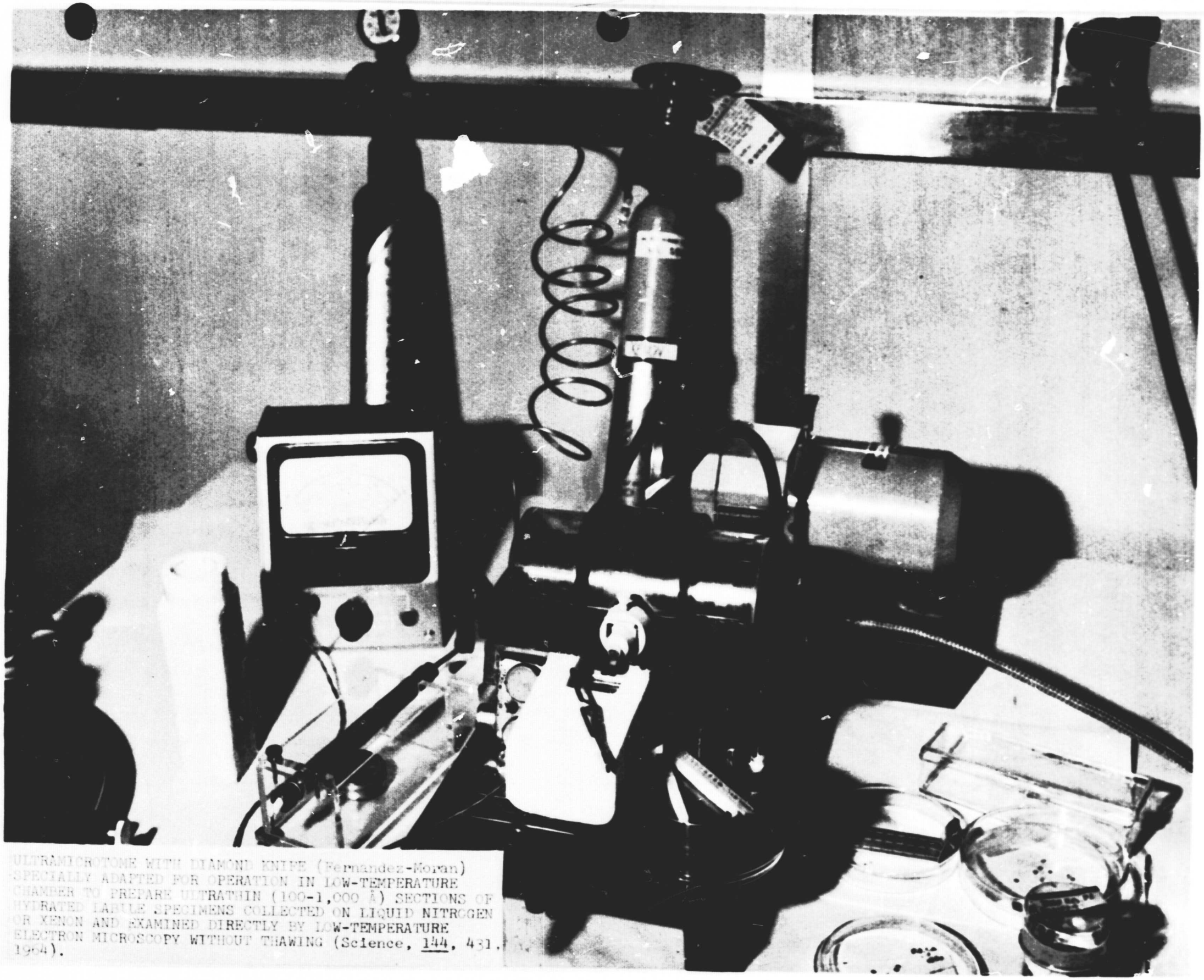
ELECTRON MICROGRAPH OF MEMBRANOUS STRUCTURES FOUND IN PRE-CAMBRIAN ROCKS OF THE CANADIAN SHIELD (GUNFLINT CHERT FORMATION OF SOUTHERN ONTARIO). THE THIN MEMBRANE FRAGMENTS (ca. 200 Å thick and several microns long) EXHIBIT CHARACTERISTIC INTERNAL STRUCTURE. NATIVE SPECIMEN SECTIONED WITH DIAMOND KNIFE AND SHADOW-CAST WITH PLATINUM. ORIGINAL MAGNIFICATION: X 80,000.



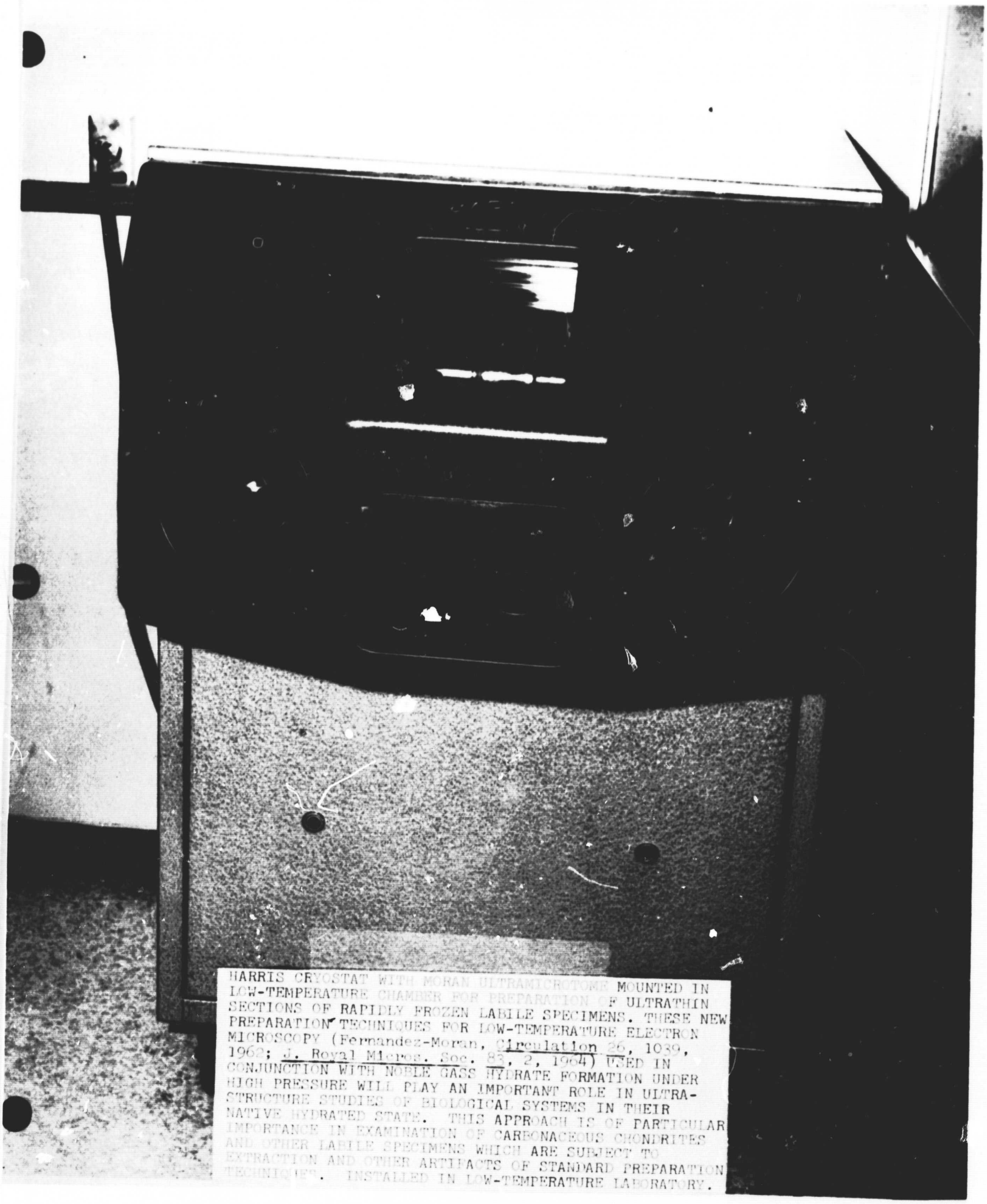
ELECTRON MICROGRAPH OF MEMBRANOUS STRUCTURES FOUND IN PRE-CAMBRIAN ROCKS OF THE CANADIAN SHIELD (GUNFLINT CHERT FORMATION OF SOUTHERN ONTARIO). ORIGINAL MAGNIFICATION: X 20,000. THESE ELECTRON MICROSCOPIC STUDIES ARE OF GREAT INTEREST IN THE EVOLUTIONARY SCHEME OF PRIMITIVE LIFE, SINCE THEY MAY FURNISH INSIGHT INTO THE MOLECULAR ORGANIZATION OF THE OLDEST KNOWN PRESERVED LIVING SYSTEMS, BEARING ALSO ON THE EVOLUTION OF MEMBRANE ULTRASTRUCTURE.

Development of techniques for electron optical examination of extraterrestrial matter. Systematic development work is currently under way for application of electron microscopy, electron diffraction, microprobe analysis, and related electron optical techniques to the examination of samples of extraterrestrial matter, including meteorites, material obtained from lunar and planetary probes, etc. Present procedures, for examination of sub-microscopic particles deposited on thin resistant plastic or carbon films can be readily adapted to the special needs of space probes. These suggested techniques, including application of single crystal graphite and microfilms which are merely extensions of already tested and well-developed electron microscope preparation procedures, would have certain important advantages for sampling of space specimens: (1) minimum samples of material for analysis, which could be well below the resolving power of light microscopes, can be used; (2) the condition of the specimens as they existed in the vacuum and low temperature of outer space, can be largely preserved by adequate preparation techniques at a resolution which is acceptable only to electron optical methods.

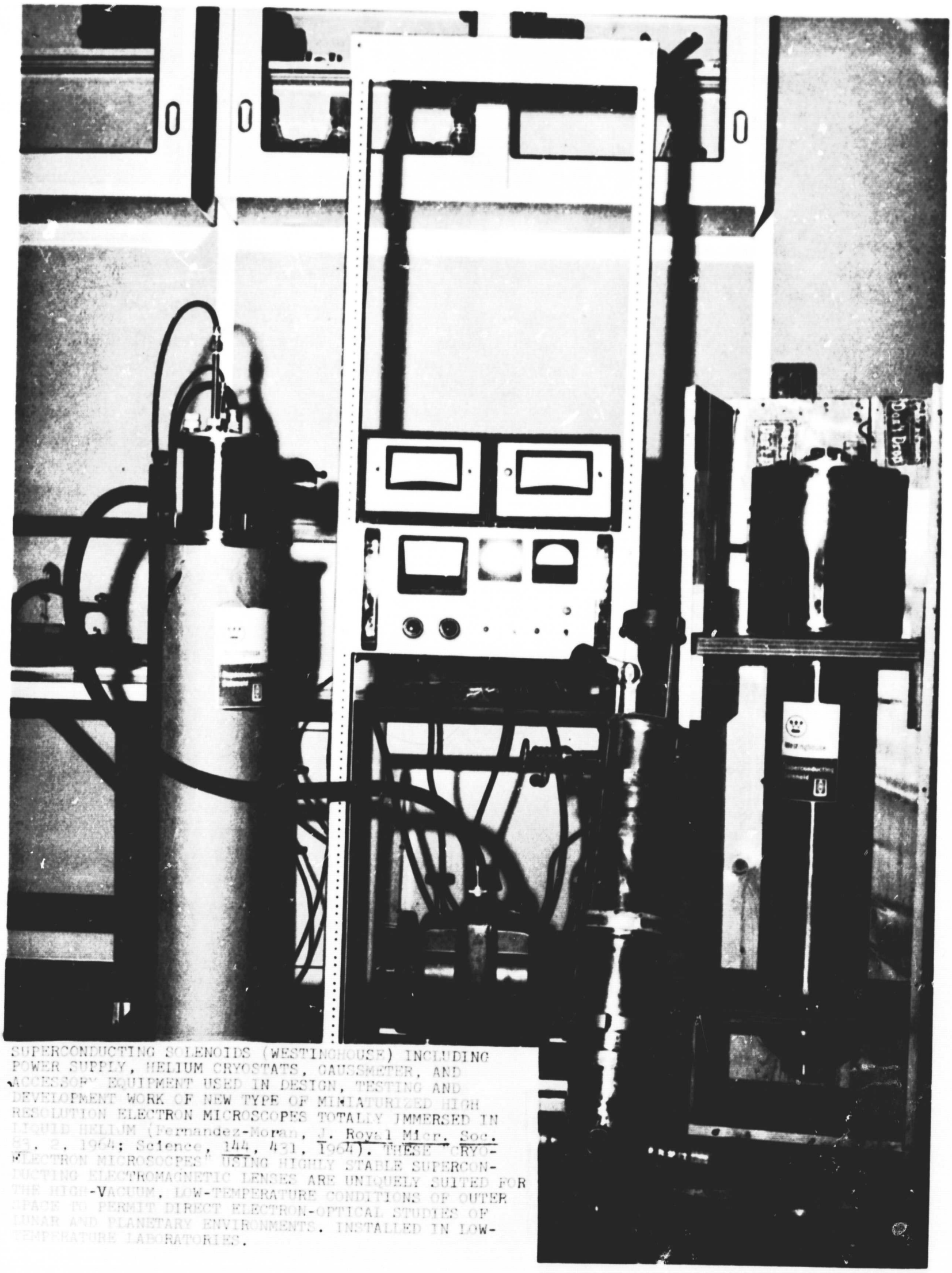
Successful application and development of these techniques requires special training and working facilities. It is conceivable that one could work out the optimum parameters for their use with existing types of space vehicles and train the various technicians of electron microscopy laboratories already organized in the different space NASA research centers, that they may then be carried out routinely. The training could be carried out in seminars or short courses given to technicians at our laboratories at regular intervals.



ULTRAMICROTOME WITH DIAMOND KNIFE (Fernandez-Moran) SPECIALLY ADAPTED FOR OPERATION IN LOW-TEMPERATURE CHAMBER TO PREPARE ULTRATHIN (100-1,000 Å) SECTIONS OF HYDRATED LABILE SPECIMENS COLLECTED ON LIQUID NITROGEN OR XENON AND EXAMINED DIRECTLY BY LOW-TEMPERATURE ELECTRON MICROSCOPY WITHOUT THAWING (Science, 144, 431, 1964).

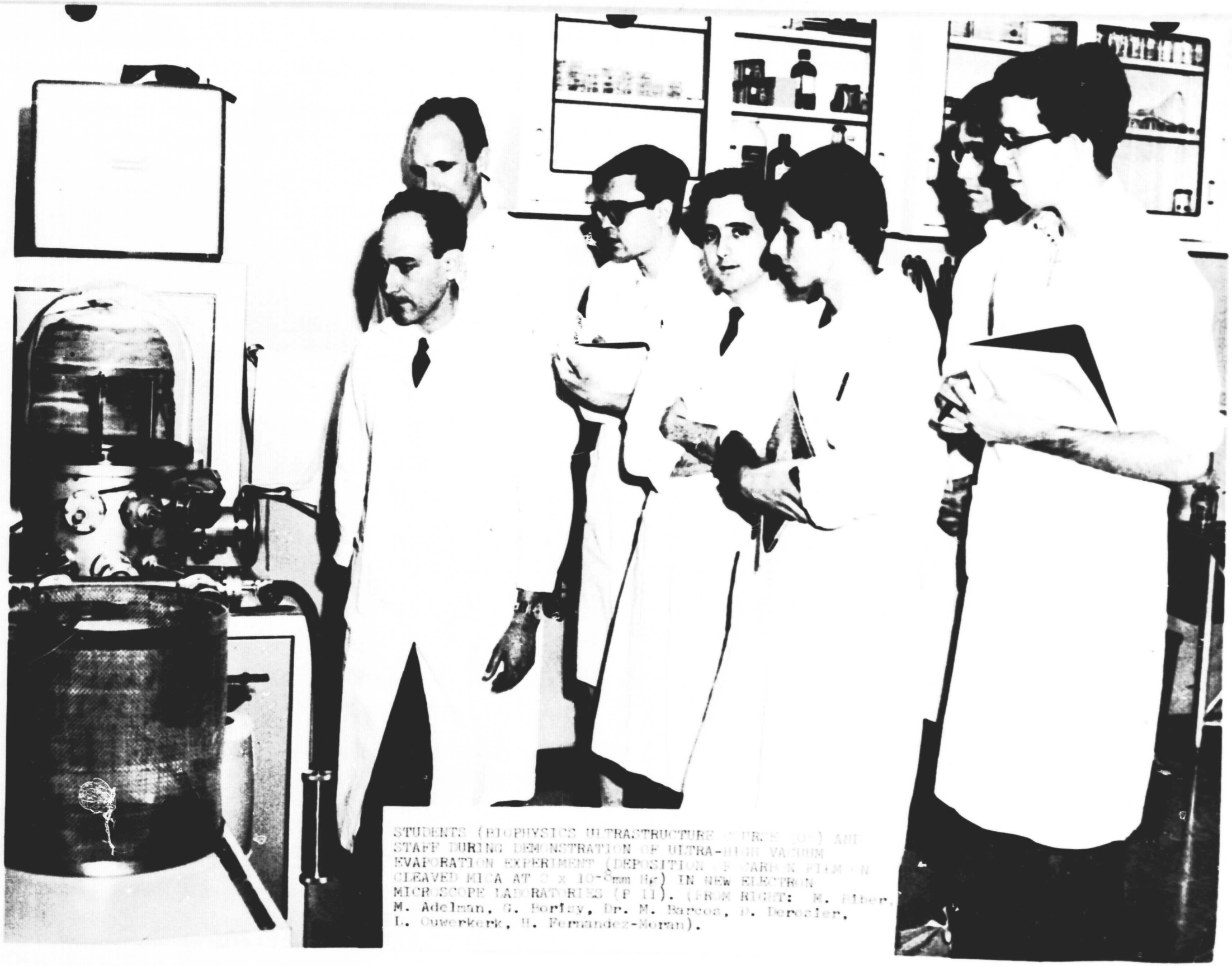


HARRIS CRYOSTAT WITH MORAN ULTRAMICROTOME MOUNTED IN LOW-TEMPERATURE CHAMBER FOR PREPARATION OF ULTRATHIN SECTIONS OF RAPIDLY FROZEN LABILE SPECIMENS. THESE NEW PREPARATION TECHNIQUES FOR LOW-TEMPERATURE ELECTRON MICROSCOPY (Fernandez-Moran, Circulation 26, 1039, 1962; J. Royal Micros. Soc. 83, 2, 1964) USED IN CONJUNCTION WITH NOBLE GASS HYDRATE FORMATION UNDER HIGH PRESSURE WILL PLAY AN IMPORTANT ROLE IN ULTRA-STRUCTURE STUDIES OF BIOLOGICAL SYSTEMS IN THEIR NATIVE HYDRATED STATE. THIS APPROACH IS OF PARTICULAR IMPORTANCE IN EXAMINATION OF CARBONACEOUS CHONDRITES AND OTHER LABILE SPECIMENS WHICH ARE SUBJECT TO EXTRACTION AND OTHER ARTIFACTS OF STANDARD PREPARATION TECHNIQUES. INSTALLED IN LOW-TEMPERATURE LABORATORY.



SUPERCONDUCTING SOLENOIDS (WESTINGHOUSE) INCLUDING POWER SUPPLY, HELIUM CRYOSTATS, GAUSSMETER, AND ACCESSORY EQUIPMENT USED IN DESIGN, TESTING AND DEVELOPMENT WORK OF NEW TYPE OF MINIATURIZED HIGH RESOLUTION ELECTRON MICROSCOPES TOTALLY IMMERSED IN LIQUID HELIUM (Fernandez-Moran, J. Royal Micr. Soc. 83, 2, 1964; Science, 144, 431, 1964). THESE "CRYO-ELECTRON MICROSCOPES" USING HIGHLY STABLE SUPERCONDUCTING ELECTROMAGNETIC LENSES ARE UNIQUELY SUITED FOR THE HIGH-VACUUM, LOW-TEMPERATURE CONDITIONS OF OUTER SPACE TO PERMIT DIRECT ELECTRON-OPTICAL STUDIES OF LUNAR AND PLANETARY ENVIRONMENTS. INSTALLED IN LOW-TEMPERATURE LABORATORIES.

Training: As expected, training has proceeded concomitantly with the various research projects described. In particular, laboratory courses have been conducted for the participants in Biophysics Course 308 and for a number of graduate students from other departments. In addition, our laboratory has already served as a center for consultation and discussion of advanced techniques, cooperating with research laboratories from other universities, AEC laboratories at Oak Ridge, Argonne and Livermore, etc. We are now preparing for the wide spectrum of trainees anticipated: graduates, under-graduates, post-doctorals, technicians, teachers. The training program will be coordinated with that of the proposed NASA University of Chicago Center for Science Education.



STUDENTS (BIOPHYSICS ULTRASTRUCTURE COURSE 305) AND STAFF DURING DEMONSTRATION OF ULTRA-HIGH VACUUM EVAPORATION EXPERIMENT (DEPOSITION OF CARBON FILM ON CLEAVED MICA AT 2×10^{-6}mm Hg) IN NEW ELECTRON MICROSCOPE LABORATORIES (P 11). (FROM RIGHT: M. Elber, M. Adelman, G. Borisy, Dr. M. Barcos, D. Derosier, L. Ouwerkerk, H. Fernandez-Moran).

STUDENTS (BIOPHYSICS ULTRASTRUCTURE COURSE 308), STAFF, AND VISITORS DURING DEMONSTRATION OF NEW ELECTRON MICROSCOPE LABORATORIES (May, 1964). A BROAD TRAINING PROGRAM IS BEING IMPLEMENTED IN THE NEW ELECTRON MICROSCOPE FACILITY WHICH WILL ALSO SERVE AS A CENTER FOR CONSULTATION AND DISCUSSION ON ADVANCED TECHNIQUES, COOPERATING WITH RESEARCH INSTITUTES FROM OTHER UNIVERSITIES, AEC AND NASA LABORATORIES, etc. (FROM RIGHT: Dr. W. Stumpf, M. Adelman, Dr. P. Fitch, M. Biser, G. Borisy, Dr. M. Parcos, L. Ouwerkerk, R. Luftig, Dr. R. Picard, D. Derosier).

REFERENCES

1. Fernández-Morán, H., New Approaches in Correlative Studies of Biological Ultrastructure by High Resolution Electron Microscopy, paper presented at the Celebration of the Tercentenary of the Microscope in Living Biology, the Royal Microscopical Society, Bethesda, Md., April 7-9 (1963). To be published in Journal of the Royal Microscopical Society, Vol. 83, Parts 1 & 2, pp. 183-95, June (1964).

2. Fernández-Morán, H., "High Resolution Low-Temperature Electron Microscopy of Biological Systems," paper presented at the Symposium on "High Resolution Electron Microscopy at the Atomic Level" at the Annual Meeting of the Biophysical Society, Chicago, February 28, 1964; reported in Science, 144, p. 431, April (1964).

3. Fernández-Morán, H., and L.J. Reed, M. Koike, and C. R. Willms, "Correlated Electron Microscopic and Biochemical Studies of a Multienzyme Complex: Pyruvate Dehydrogenase Complex of Escherichia Coli." Submitted for publication in Science, Vol. 145, pp. 930-2, June (1964).

4. Fernández-Morán, H.; Oda, T.; Blair, P. V.; and Green, D.E., "A Macromolecular Repeating Unit of Mitochondrial Structure and Function: Correlated Electron Microscopic and Biochemical Studies of Isolated Mitochondria and Submitochondrial Particles of Beef Heart Muscle," to be published in J. Cell Biology, Vol. 22, pp. 63-100, July (1964).