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I hereby recommend that the thesis prepared under my supervision by John F. Schuler

entitled CONSTRUCTION, CALIBRATION AND OPERATION OF A MASS SPECTROMETER FOR GASEOUS SAMPLES IN BIOLOGICAL RESEARCH PROBLEMS

be accepted as fulfilling this part of the requirements for the degree of DOCTOR OF PHILOSOPHY

Approved by:

Harold J. Forster

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CONSTRUCTION, CALIBRATION AND OPERATION OF A MASS SPECTROMETER FOR GASEOUS SAMPLES IN BIOLOGICAL RESEARCH PROBLEMS.

A dissertation submitted to the

Graduate School of Arts and Sciences
of the University of Cincinnati

in partial fulfillment of the
requirements for the degree of

DOCTOR OF PHILOSOPHY

1949

by

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A.B. University of Dayton (1930)

M.S. University of Cincinnati (1946)

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LIST OF PLATES.

CHAPTER I.

INTRODUCTION: OBJECT OF RESEARCH.

Construction of a Mercury-Free Nier-Type Mass Spectrometer.

The mass spectrometer constructed is commonly called the Nier-type after A.O.Nier, who introduced (Rf.#1) it in November, 1940. Marked improvements were made and published (Rf.#2) by him in June, 1947. The following August, the building of our instrument was undertaken. We profess it to have all of the latest and basic features of this type of mass analyzer besides the unique characteristics of being 100% all rugged metal and of being free from all cold traps due to the elimination of the use of mercury.

This latter feature is a radical departure from the various existing mass spectrographs and mass spectrometers. The good fortune that graced our efforts in the end leads one to forget the numerous uncertainties and inherent perplexities that presented themselves at the outset. Authorities in the field are of one mind that mercury is a hazardous tool with which to work; but no common accord is reached among them as to an acceptable substitute.

If mercury is not to be used, then the characteristics of the liquid replacement must include a vapor pressure that is workable at room temperature. Recent advances with pump oils have been made by Distillation Products Inc., so that S-Octoil satisfies this requirement.

Problems then multiplied as to the necessary alterations in

design needed to incorporate this new method with the basic principles of the instrument. After much experimentation these adaptations proved operational in their performance as individual parts of the system. It was not until the final results of a specific investigation were obtained that such innovations proved themselves in the instrument as a functional whole. This suspense was due to the possibility that the long chained hydrocarbons would crack in quantity such that their efficiency as a high vacuum fluid would be impaired and at the same time introduce into the mass spectrometer cracked products having masses identical with those being studied. This would give rise to spurious readings and vitiate the experiment. The singular advantages of this mercury-free operation together with other fundamental improvements of a mechanical and electronic nature will be stressed as this thesis developes.

Provision of an Analytical Tool for Tracer Work in Biological Research.

Those, who are engaged in research in the fields of Biochemistry and Biology and who are fortunate to have access to a Nier-type mass spectrometer, find it difficult to understand why more work is not being initiated with stable isotopes. The answer lies in the proper appreciation of the instrument. Commercial mass spectrometers are financially beyond the means of the majority of laboratories. Considered as a scientific tool, it is a most delicate instrument.

Development Sketch of the Mass Spectrometer.

J.J.Thomson (1897) was first to measure the value of e/m

for the electron by evaluating the deflection of a beam of electrons in combined electrostatic and magnetic fields which were at right angles to each other. (Rf.#3-p.115) Similarly in 1907, he made the first determination of e/m value for positive ions by observing the deflection produced on a beam of positively charged ions under the combined action of parallel electrostatic and magnetic fields. The positive ions moved in the plane of the electrostatic field and at right angles to the plane of the magnetic field. The net effect was a series of parabolas. Each curve represented a single value of e/m. Ions of different velocities of the same e/m value fell on different parts of the same curve. The parabolas were completed by reversing the magnetic field. Knowledge of the charge left the mass to be calculated.

In 1912, Thomson discovered the stable isotopes of Neon (masses 20 and 22) by this method of parabolas. (Rf.#4-p.559) In 1919, F.W.Aston designed an instrument as an improvement in the method of positive ray analysis and called it a MASS SPECTROGRAPH. This mass analyzer achieved greater dispersion and effected a point focus by passing positive rays successively through electrostatic and magnetic fields which were at right angles to each other. The electrostatic field acted in the capacity of the velocity component of a focal system, in which the slower ions were bent more upward. By this method, Aston confirmed Thomson's measurement of the isotopes of Neon and added the finding of Chlorine masses of 35 and 37.

A.J.Dempster (1918) devised a method of allowing positive ions (Rf.#5-375) to fall through a potential difference and gain a velocity before entering the magnetic field. Different masses with

equal velocity possessed different momenta and would experience a different radius of curvature in a magnetic field over an arc of 180^{o}. Later he added a velocity selector consisting of a radial electrostatic field of cylindrical condenser design, which deflects the accelerated ions through a right angle and brings the divergent bundle of ions of one velocity to a focus at the entrance to the magnetic field. With a proper ratio of the electrostatic and magnetic fields all of the positive ions of varying energies will reunite at the photographic plate. Accurate results in determining mass differences were obtained over a wide range of masses which included the discovery of U-235 isotope. In 1933, K.T.Bainbridge designed a velocity selector similar to Dempster's but with the electrostatic and magnetic fields at right angles to each other. (Rf.#6-p.176; #7-p.237)

CHAPTER II.

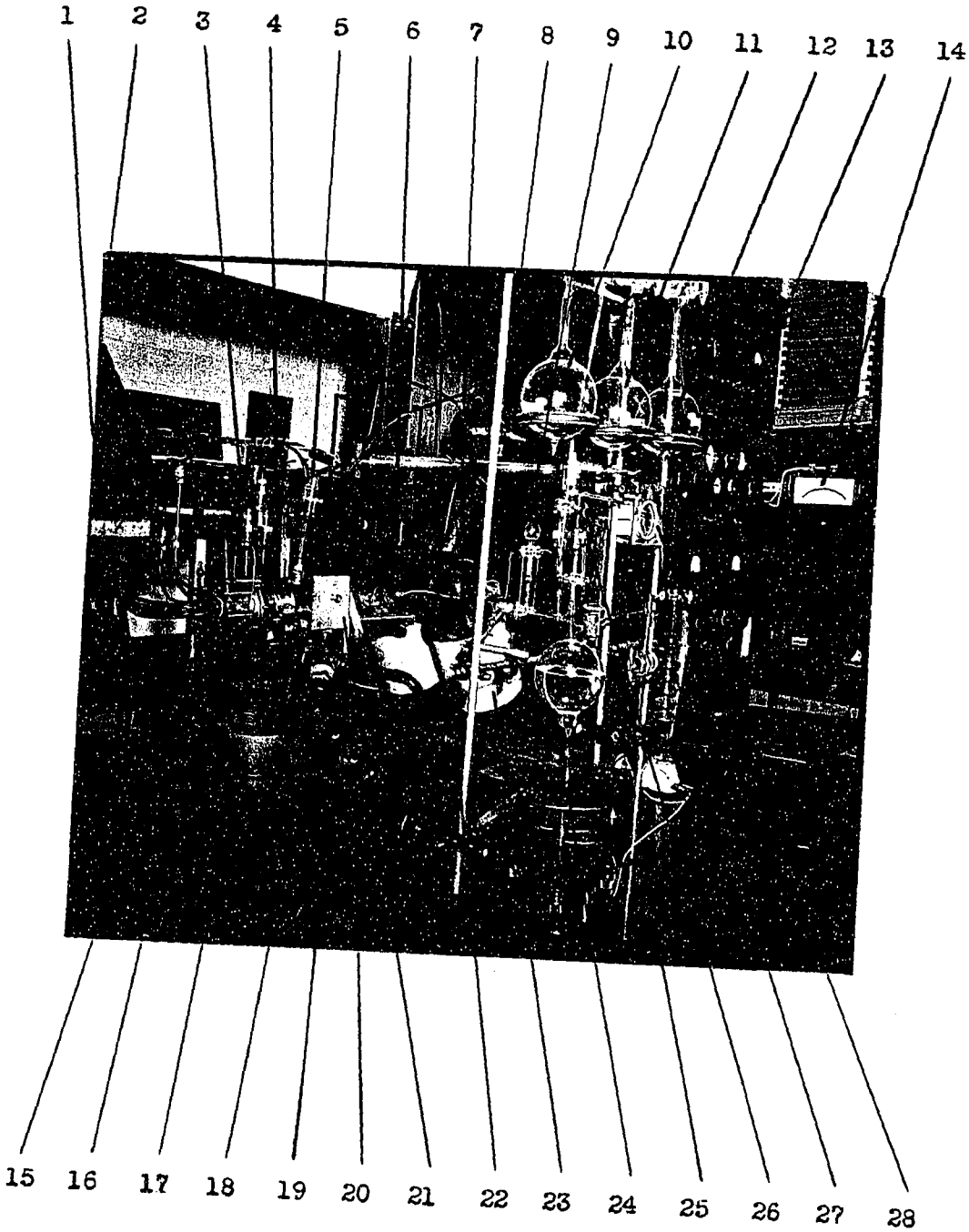
CONSTRUCTION OF THE APPARATUS.

Design and Function of the Analyzer Tube.

The double focusing mass spectrograph for both velocity and direction developed an accuracy and resolving power of one part in one hundred thousand. (Rf.#6-p.178) Without this twofold focusing, the selection of a much narrower bundle of rays, having a very narrow range of velocity, was required to obtain sufficient resolving power. This led to a decrease in beam intensity. Nier supplied the remedy for this defect both by substituting a sensitive electrometer for the photographic plate and by lengthening the defining slit. (Plate #3)

PLATE #1. COMPLETE MASS SPECTROMETER

1 - Control Circuit of Ionization Gauge.
2 - Control Circuit of Pirani Gauge.
3 - Pirani Gauge.
4 - Source; Ionization Chamber.
5 - Ionization Gauge.
6 - 60° Wedge-Shape Pole Pieces.
7 - Copper Analyzer.
8 - Collector; Pre-Amplifier.
9 - Gas Sample Bulb.
10 - Helium Calibrating Gas.
11 - Neon Calibrating Gas.
12 - Argon Calibrating Gas.
13 - Electronic Control Cabinet.
14 - High Voltage Meter.
15 - Adjustable Support of Analyzer.
16 - High Voltage Cable Receptacle.
17 - Metal Oil Diffusion Pump.
18 - Sylphon Vacuum Valve.
19 - Rotary Fore Pump.
20 - Metal Clamp.
21 - Capillary Gas Leak.
22 - Sliding Carriage of Magnet.
23 - Coils of Magnet.
24 - Sylphon Pressure Adjustment.
25 - Rotary Fore Pump.
26 - Pirani Gauge.
27 - Glass Oil Diffusion Pump.
28 - Bourdon Vacuum Gauge.

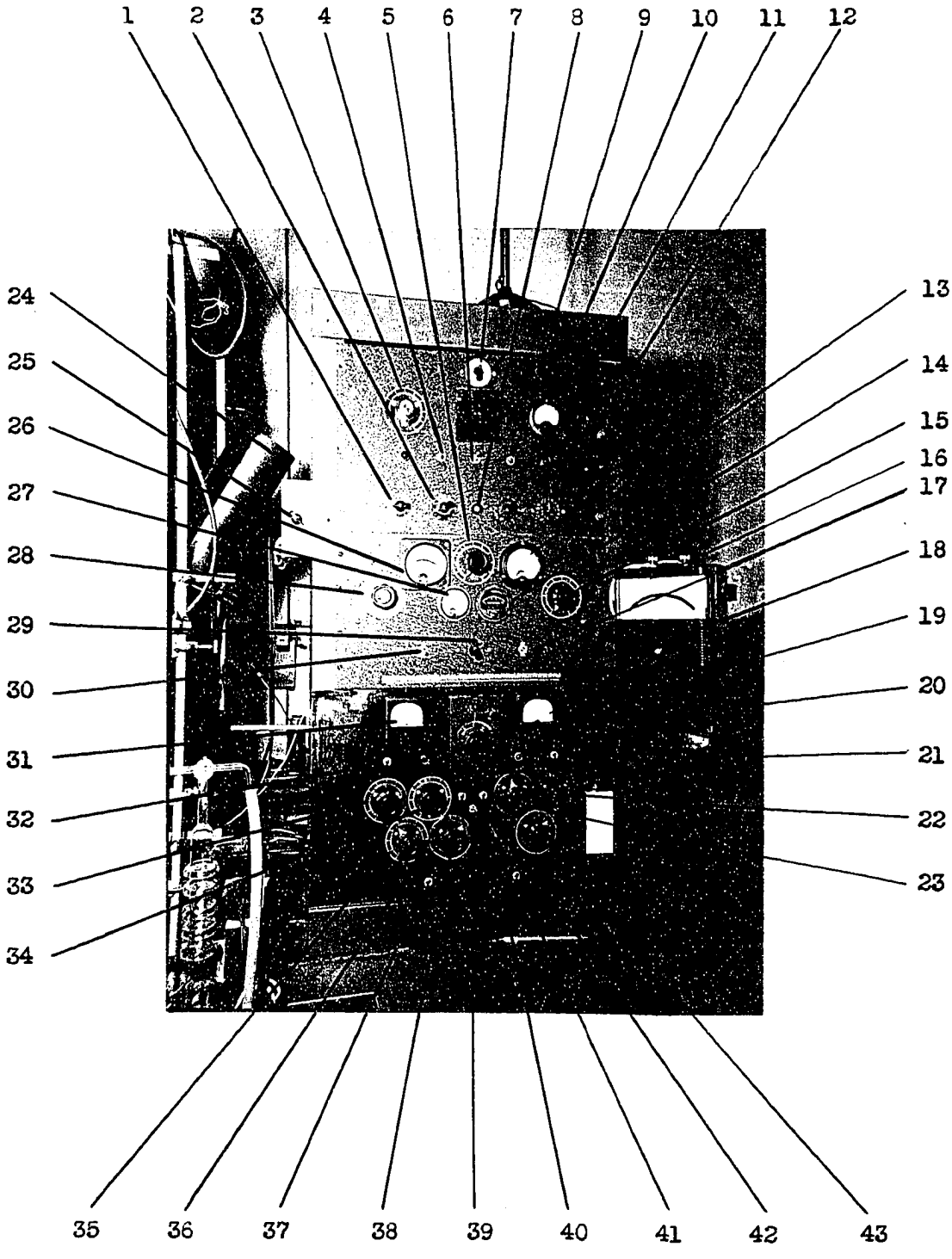


COMPLETE MASS SPECTROMETER

(Plate # 1)

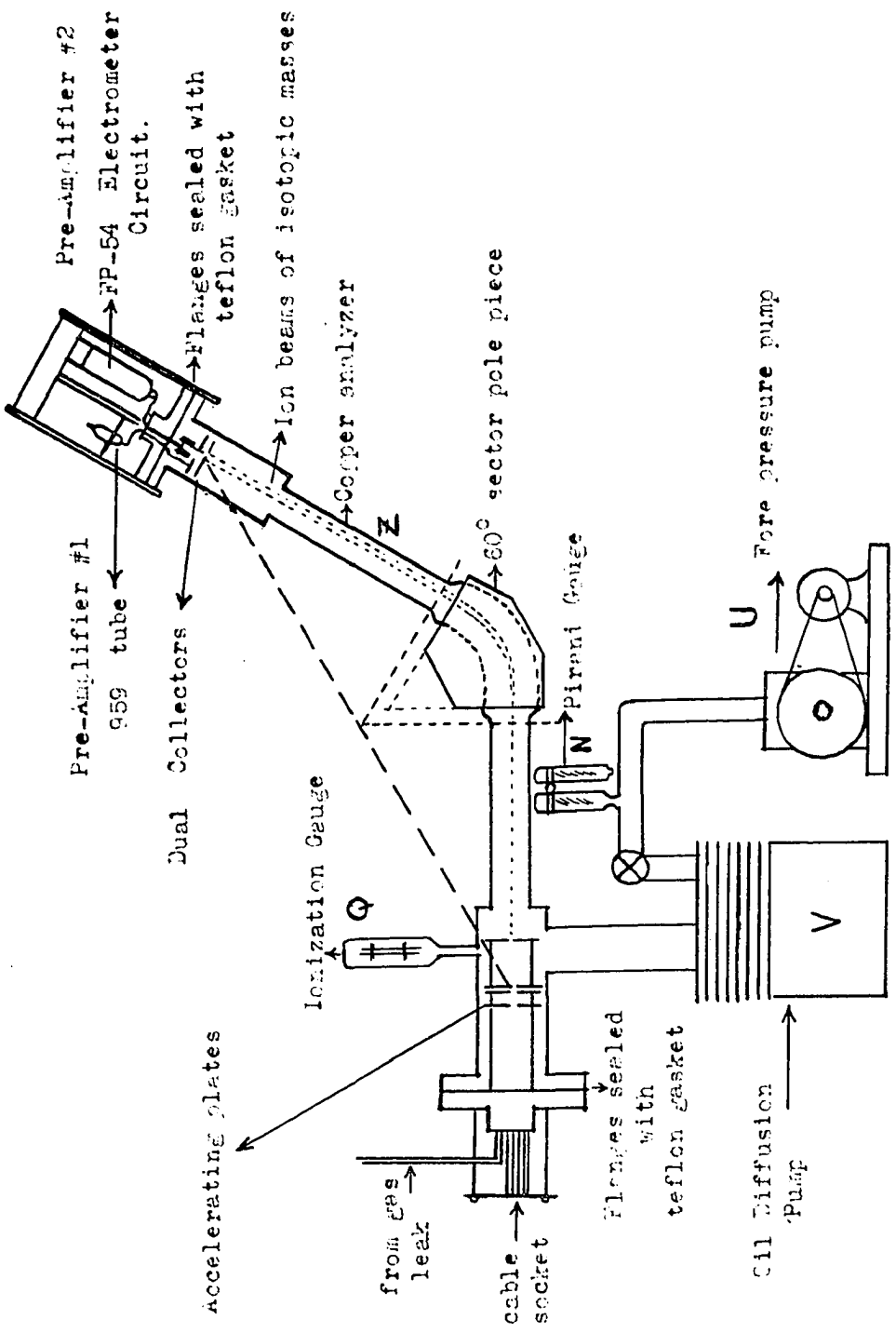
PLATE #2. ELECTRONIC CONTROL CABINET.

1 - Selector Switch: 3 - 500 volt steps.
2 - Selector Switch: 9 - 50 volt steps.
3 - Potentiometer: 0-50 volts.
4 - Control Switch: Filaments of power supply.
5 - Potentiometer: Vernier control of ionization filament current.
6 - Control Switch: Beam cut-off.
7 - Selector Switch: Voltmeter.
8 - Fuse Receptacle.
9 - Control Switch: High voltage of power supply.
10 - Microammeter: Ion beam focusing potential circuit.
11 - Selector Switch: Drawing-out potential.
12 - Potentiometer: Ion beam focus.
13 - Ammeter: Ionization Filament Current.
14 - Microammeter: Electron Beam Emission.
15 - Potentiometer: Ionization filament current.
16 - Ammeter: Calibrated in volts (0-2000).
17 - Control Switch: Ionization filament current.
18 - Ground Glass Scale of Galvanometer.
19 - Ammeter: Plate circuit (Inverse Feedback Amplifier).
20 - Ion Beam Ratio Scale.
21 - Control Switch: Filament current (Inverse Feedback Amplifier).
22 - Potentiometer: Grid bias (Inverse Feedback Amplifier).
23 - Potentiometer: Plate load (Inverse Feedback Amplifier).
24 - Magnetic Shield for Pre-Amplifiers at Collectors.
25.- Potentiometer: Grid bias (Regulated Power Supply).
26 - Ammeter: Electromagnet circuit.
27 - Milliammeter: Total electron emission.
28 - Variable Resistor: Electromagnet circuit.
29 - Selector Switch: Electron Accelerating Potentials.
30 - Control Switch: Electromagnet current.
31 - Milliammeter: Filament current (FP-54).
32 - Control Switch: Filament current (FP-54).
33 - Variable Resistor: Filament current (FP-54).
34 - Variable Resistor: Plate load (FP-54 Electrometer Circuit).
35 - Same as #34.
36 - SPDT Switch: Increasing (Rt.) resistance of #43
37 - Control Switch: 12 volts D.C. (Storage Batteries).
38 - Vernier (Same as #34).
39 - Control Switch: Null point procedure.
40 - Selector Switch: Galvanometer sensitivity.
41 - Control Switch: 115 volts A.C.
42 - Same as #36.
43 - Potentiometer: Vernier of Plate Load (Inverse Feedback Circuit).



ELECTRONIC CONTROL CABINET

(Plate #2)



MASS SPECTROMETER LAYOUT. (Plate #3)

The limitation in the latter case was dependent on the maximum permissible diameter of the analyzer tube, which would pass between the pole pieces and yet not destroy the homogeneity of the magnetic field by a demand for a wider pole gap. This interdependent relation of dimensions had its solution in the flattening of the analyzer tube in the area of the pole pieces, so as to permit the use of copper tubing $1\frac{1}{4}$ inch O.D. with a 1/16 inch wall and an air gap of 9/16 inch.

The use of copper tubing by Nier for the analyzer was a marked improvement over the glass design and provided a definite ruggedness to the instrument as well as the elimination of an internal shield which was required to protect the ion beam from the influence of stray charges on the glass walls.

There were also preventive measures to be taken to insure full utilization of the entire beam as defined at the source slit. This was accomplished by proper alignment so that the defining slit and the collector slit were positioned as elements of a right angle cylinder. Had this precaution not been taken, the effective slit area might have been reduced to one approaching a pinhole in size at the center. The assembly rigged for this adjustment consisted of a lathe bed with the source and collector stainless steel housings clamped in tracks making an angle of 120°. These slit housings were then rotated until a slit image beam of light (perpendicular to the plane of the analyzer) from each end was superimposed on an improvised screen. Then the copper analyzer tube was cut to proper length and silver soldered into place.

Having increased the beam intensity, it remained to improve

the resolving power without making the instrument too unwieldy. This was accomplished with a wedge shape magnetic pole piece with ample allowance for an increased radius of curvature.

The mathematical principle underlying the resolution of different masses and the focusing of equal masses is the basis for all conclusions affecting the size of the apparatus, the economy of construction and the accuracy of determinations.

Positive ions are accelerated in a strong uniform electric field so that those emerging from a very narrow slit have the same kinetic energy.

$$\frac{n\,q\,E}{300} = \frac{m\,v^2}{2\,N_0} \qquad \text{(Eq.\#1)}$$

n — one for singly ionized particles

q — 4.80×10^{-10} e.s.u.

E — accelerating potential in volts

m — grams (O^{16} = 16.000 grams)

v — velocity of ions in cm per sec.

N_0 - 6.02×10^{23} particles per mole.

Solving for v^2, we have

$$v^2 = \frac{2\,n\,q\,E\,N_0}{300\,m} \qquad \text{(Eq.\#1a)}$$

This ribbon of accelerated ions of various masses enters the magnetic field and is sorted out into ionic beams according to their respective masses and having corresponding radii of curvature.

$$F = \frac{B\,v\,q}{c} = \frac{m\,v^2}{N_o\,R} \qquad \text{(Eq.\#2)}$$

F -- force on particle in dynes

B -- density of magnetic field in gauss (lines/cm^2)

v -- velocity of ions in cm/sec.

q -- 4.80×10^{-10} coulombs

c -- 3×10^{10} (conversion factor of esu to emu units)

m -- grams (O^{16} = 16.000 grams)

N_o - 6.02×10^{23} particles per mole

R -- radius of curvature in cms.

Solving for m

$$m = \frac{B\,R\,q\,N_o}{c\,v} \qquad \text{(Eq.\#2a)}$$

Substituting (1a) in (2a) we have

$$m = k\,\frac{B^2\,R^2}{E} \qquad \text{(Eq.\#3)}$$

m -- grams (O^{16} = 16.000 grams)

k -- numerical constant (4.82×10^{-5})

B -- density of magnetic field in gauss (lines/cm^2)

R -- radius of curvature in cms.

E -- accelerating potential in volts.

The resolving power or spread of the various masses (dE/dm) is a function of the radius of curvature (R). Differentiating (Eq.#3) with respect to m and R we have

$$dm = k\,\frac{B^2}{E}\,(2\,R\,dR) \qquad \text{(Eq.\#4)}$$

Dividing (#4) by (#3) and rearranging we obtain

$$dR/dm = R/2m \qquad \text{(Eq.\#5)}$$

Therefore the resolving power is increased by lengthening the radius of curvature (R) or by reducing the mass (m) to be considered. Since the radius of curvature (R) is limited by the size and shape of the magnetic pole pieces, we conclude that the design of the pole face determines in part the resolution as well as the focus.

A second consideration of equation (#3) for a definite mass is

$$R = k' \frac{E^{\frac{1}{2}}}{B} \qquad \text{(Eq.\#6)}$$

Therefore, by affecting the radius of curvature (R), the resolving power of the instrument for the same pole pieces can be increased by reducing the magnetic field (B) or by increasing the accelerating potential (E). This means that the optimum operating condition is for a magnetic setting so that the desired mass will be brought to the collector for the highest working potential available. Since this variation of the strength of the magnetic field is not possible with a permanent magnet, an electromagnet was used.

A third consideration of equation (#3) makes it possible for one to predict the location of any mass within a range of working potentials and for a given magnetic field by merely setting the slide-rule indicator for a known mass-potential relation. Since all masses are collected at the same geometric location by keeping the magnetic field (B) fixed and varying the accelerating potential (E), the radius of curvature (R) is also fixed. Hence with both B and R constant,

equation (#3) becomes

$$m E = K \qquad \text{(Eq.\#7)}$$

and therefore

$$m_1 E_1 = m_2 E_2 = \text{constant} \qquad \text{(Eq.\#7a)}$$

The Electromagnet.

In 1933, Barber (Rf.#8) showed that if a slightly divergent beam of ions with equal momenta enters a homogeneous wedge shaped magnetic field (Plate #4) so that the ion path is perpendicular to one edge, the ion beam will leave perpendicular to the other edge and will be refocused. The nature of this focusing is such that the defining slit of the ion source, the apex of the acute angle of the sector pole pieces and the collector at the point of refocusing are collinear. This principle was used with the double focusing mass spectrograph of Bainbridge and Jordan (Rf.#9) in 1936.

It is evident from the previous consideration of the resolving power that the radius of curvature is the critical dimension and not the wedge-angle whether it is 180° or 30°. It would seem therefore that economy would dictate the use of the smallest angle giving a homogeneous field. However, Barber's principle stated above reveals an optimum angle both for economy and for a well proportioned instrument.

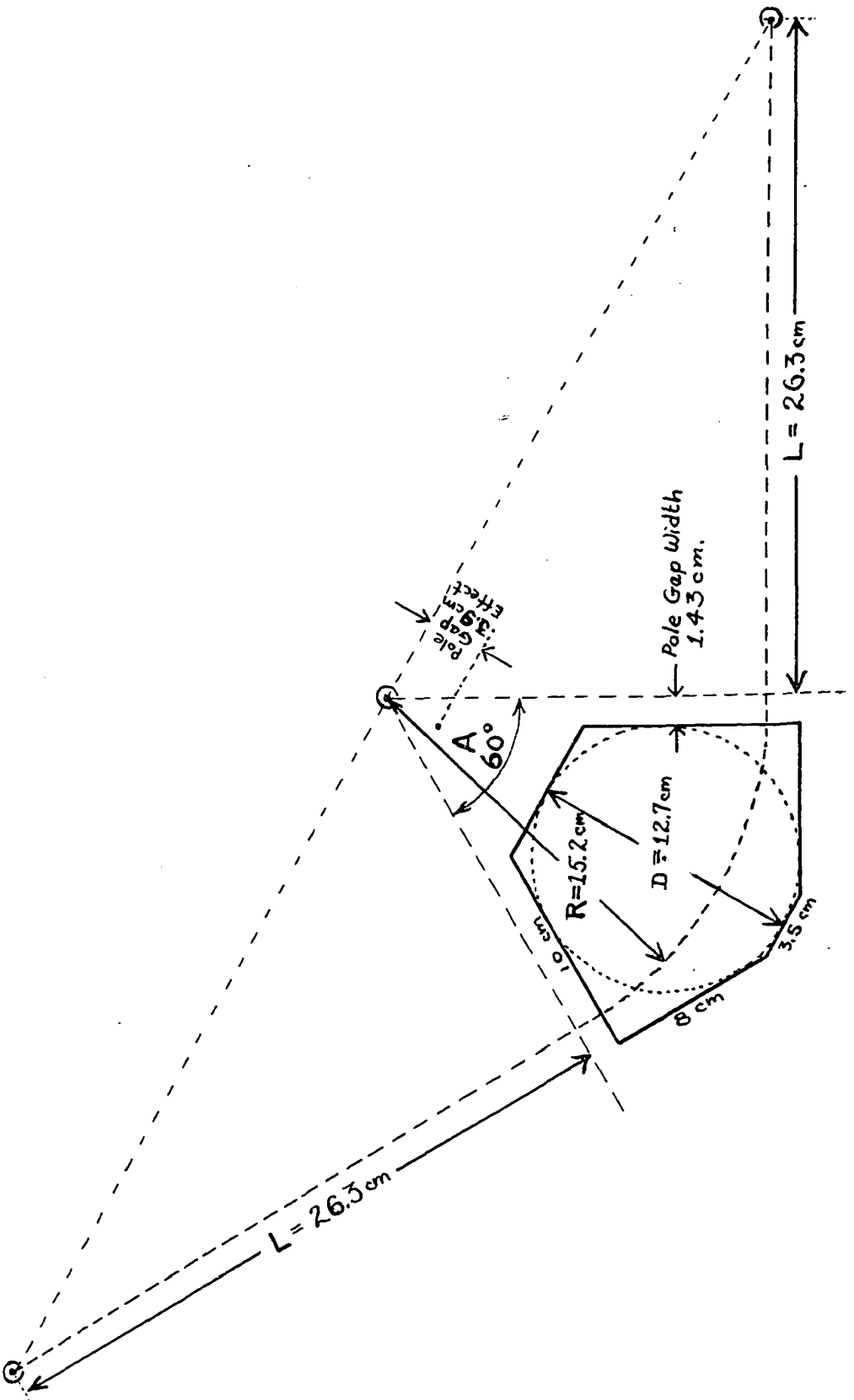
$$L = R \cot A/2 \qquad \text{(Eq.\#8)}$$

L -- distance from defining slits to effective pole boundary (cms)

R -- radius of curvature (cms)

A -- wedge angle (degrees)

Increasing the radius of curvature (R) does not then necessarily demand



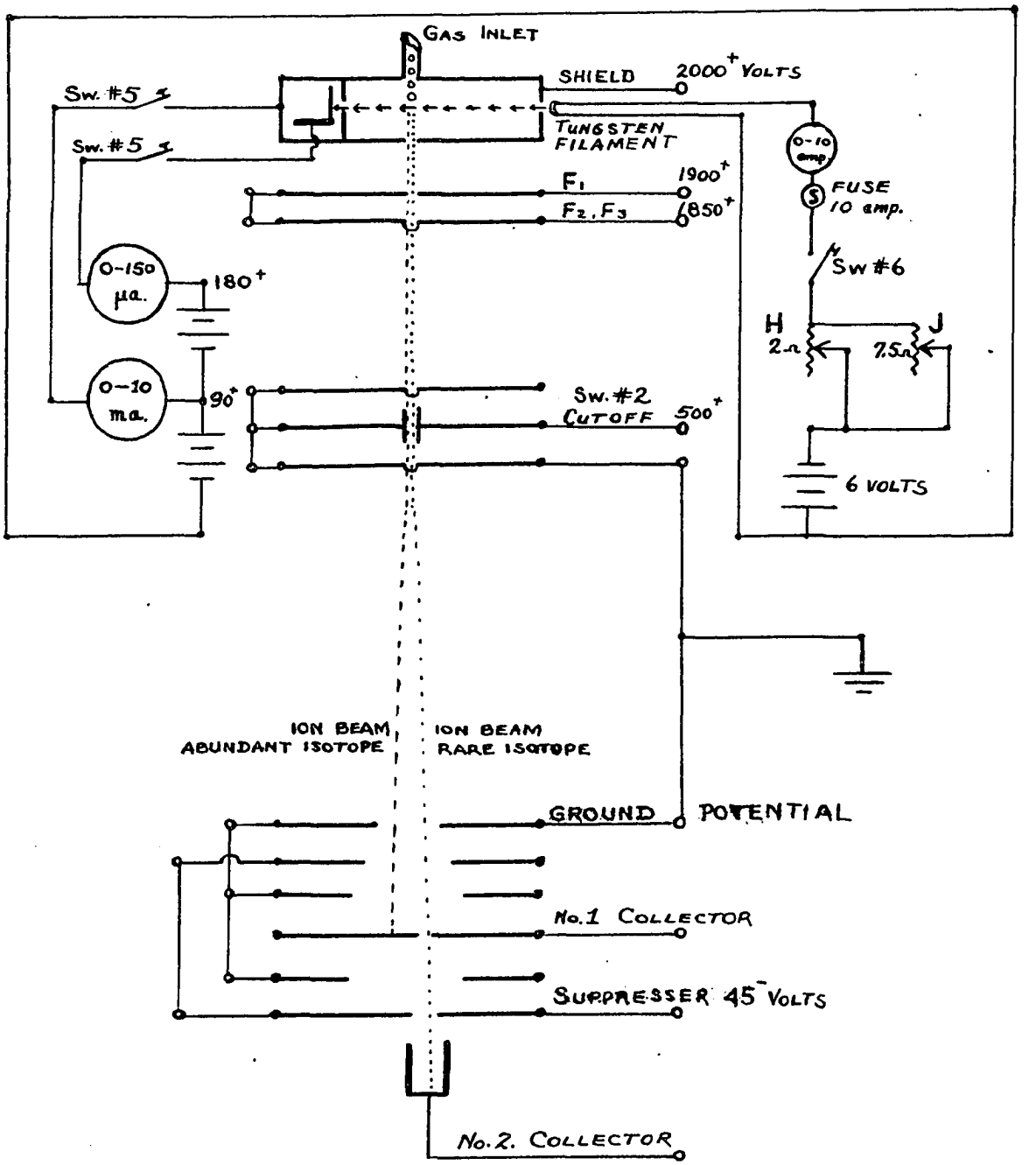
FOCUSING PROPERTY OF A 60° SECTOR MAGNETIC POLE PIECE. (Plate # 4)

a larger area for the pole face, which can be cut away at the apex. However, the length of the arms of the copper analyzer tube becomes greater and unwieldy as R or A is increased.

First having selected R to satisfy requirements of masses to be measured, one has a choice between L and A. For L equal to zero, the angle A would be 180°, which would necessitate an expensive and massive pole face. For an angle A equal to 30°, the length (L) becomes 3.73 R, which adds considerably to the volume to be evacuated and is mechanically unstable and disproportionate. The selection of a 60° angle for A is a satisfactory compromise between economy and a mechanically balanced instrument with a minimum volume.

Only ions moving in a magnetic field are subjected to a force perpendicular both to their path and to the magnetic field.(Rf.#6) This force is radial, because the ions are discrete particles, and it becomes effective when the ions reach the virtual boundary of the magnetic field. In computing this effective boundary, allowance must be made for fringing flux. Coggeshall (Rf.#10) treats at length the trajectory of charged particles affected by fringing flux as a region of uniform magnetic field is approached. The integrated effect is equivalent to extending the physical pole boundary by the approximate distance of the pole gap. In computing the length (L) of the copper analyzer arm previously considered, as well as the magnetic focus alignment described above by Barber, the extended or virtual pole boundary is used.

The physical dimensions of the magnet proper were taken from Nier's (Rf.#1) recommendation for a battery operated unit, while



IONIZATION CHAMBER with electron emission measurements.

ACCELERATION PLATES with relative accelerating potentials.

COLLECTOR ASSEMBLY with simultaneous ratio device.

(Plate # 5)

the design of the pole pieces were patterned after Nier's (Rf.#2) later article describing an electronically operated magnet.

The control circuit of the magnetic field is operated from the left side of the middle panel (Plate #12) of the electronic rack. It is extremely simple in design and consists of two six volt heavy duty storage batteries connected in parallel, a 150 watt potentiometer of 7.5 ohms controling the current as measured by a dual scale (0 - 1) and (0 - 5) ammeter with an accuracy of one percent. Each coil of the magnet has a resistance of 6.5 ohms. For a parallel connection, the total resistance of the two coils is 3.25 ohms. This approximates an available range of current in both coils from one half to two amperes. Due to the low voltage used, good electrical contacts and connections are of extreme importance.

The Ion Source.

A collimated electron beam is used in the ion source (Plate #5) to create positive ions from the gaseous molecules that are to be analyzed. The source of this stream of electrons is a matter of choice between a filament and a heated cathode. In our instrument, the selection of a filament was made because it is more stable, has a longer life and is independent of gas poisoning. The current drain (3 to five amperes) is more severe for the filament type. This demand however was satisfied by two six volt storage batteries connected in parallel. The real advantage of a heated cathode type is the uniform energy of its electrons, which is difficult to obtain with a filament because of the potential difference changes over its length. This difficulty was

partially remedied by making the defining slit only two millimeters long and adjusting the tungsten ribbon (0.025 mm thick, 0.75 mm wide and one cm long) so that its center portion was one millimeter distant from and directly over the slit (0.5 mm by 2 mm). Using the center portion of the filament is desirable because the temperature at that point is higher than at the ends where some of the heat is conducted away by the filament leads. A length of tungsten ribbon less than one centimeter was found to lack this requirement. The small slit area is not a limitation with a tungsten filament because of the abundance of electrons emitted.

The geometry of the construction of the ionization chamber is a critical one. The electron beam shaped by the 2 x 0.5 mm slit must not strike any of the Nichrome walls until it reaches the electron trap which is designed to prevent secondary emission. The beam should be shielded from all insulating material that might collect stray charges. Neither should it be affected by the magnetic field of the analyzer or the ion accelerating potentials. Preferably this beam of electrons should cross the stream of sample gas molecules immediately above the first slit of the ion accelerating system. The collimation of the electron beam can be assisted by an external arrangement of two small permanent magnets forming a field parallel to the electron path.

The choice of electric fields used to accelerate the electrons to the slit barrier and then to the electron trap is a decision not only affecting the energy of the beam but also its focus. The principle of electron optics are applicable both for magnetic and

electrostatic focusing. Hughes (Rf.#11) treated the subject of magnetic lenses, while Copeland (Rf.#12) made a theoretical approximation of potentials for a set of slit barriers to achieve electrostatic focusing. In ordinary optics, lenses are mostly spherical refracting surfaces and as such, together with their image forming properties, they are useful for their electrical optical analogues. Most common electron lenses are thick in the optical sense, but nevertheless can be treated as thin lenses. In dealing with a set of five slits, Copeland placed a tungsten filament in the plane of the first. This design is now being used by A.J.Dempster at the Argonne National Laboratory (Chicago) to obtain electrons of uniform energy. The second aperture was made rather small by Copeland so that the image will be formed by electrons having paraxial trajectories. The third diaphragm contained an opening intended for use as a lens with a diameter of 3.85 cms. The fourth aperture was much larger and was inserted between the lens and the screen to aid in establishing a uniform field in the image space. The final slit was very narrow and placed immediately in front of the screen, which is at the focal distance of 4.9 cms. The geometry of our ionization housing would not permit such an elaborate arrangement; instead, a two slit design was used and various potentials tried for best experimental values. The criterion for judging these values was based on the most stable and intense beam for a minimum filament current without interference with the ion beam. The electron accelerating potentials varied for filament replacements with slightly different configuration. In general, the potential difference between the filament and the slit was 90 volts and between the filament and the trap 180 volts.

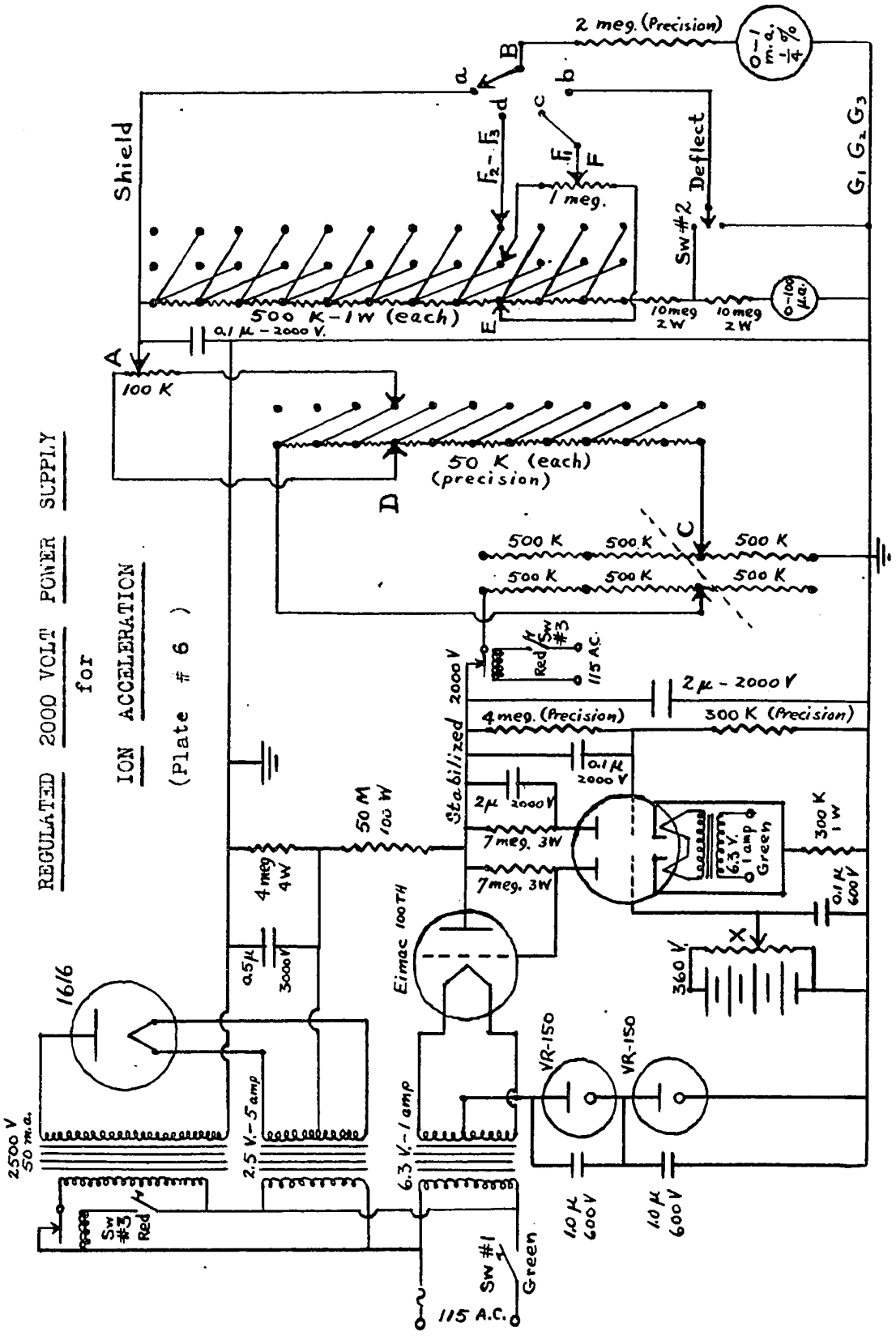
The gas inlet as referred to above should be directed in the path of the electron beam without interfering with it. The gas pressure rise in the ionization chamber should be adequate for a good beam intensity without raising the pressure in the analyzer tube above 2×10^{-5} mm mercury.

The Ion Accelerating System.

The acceleration of the positive ions formed near the exit slit of the ionization chamber (Plate #5) must be drawn out quickly before recombination occurs. The electric field that accomplishes this is essentially divided into a weak and strong relation. The weak electric field set up by plates F_1, F_2 and F_3 immediately next to the exit slit of the ionization chamber is necessary to prevent any influence exercised by this difference of potential over the electron beam. Such an effect, if permitted, would shift the ionization region and cause a spread of ion energies. This weak field (a few volts per centimeter) is sufficient to draw out the ions since the electron beam bombardment of the gas molecules takes place immediately before this exit slit of the ionization chamber; besides, the gas pressure in the ionization chamber is uniformly slightly higher than in the immediate vicinity.

F_1 and F_2 are half-plates in the same geometric plane (Plate #5) and can have their potentials altered separately. This difference of potential creates a delicate electric field change sufficient to deflect the ion beam so as to give it the best clearance of the remaining defining slits.

These weak electric fields set up by plates F_1, F_2 and F_3 are determined experimentally for the maximum intensity of the ion



beam. The strong electric field is positioned about one centimeter distance away and is created by an assembly of plates (Plate #5) at ground potential. A deflecting plate (F_g) arrangement described by Nier (Rf.#2) provides a facility for canceling the beam of ions by changing the potential on the half-plate (F_c) from ground potential to that of a few hundred volts merely by the click of the toggle panel switch (Sw #2). This convenience permits a "relative zero" galvanometer reading while all operating values remain unchanged.

The dimensions of the accelerating Nichrome V (0.025 in.) plates were adopted from Nier's recommendation (Rf.#2). The whole assembly of the accelerating system is mounted (Plate #3) on a flange that can be sealed to the instrument by means of six hexagonal head bolts and a Teflon gasket. (Plate #6a) The final slit (0.25 x 14 mm) is the defining slit which determines one of the three points in the magnetic focusing geometry as described above (Plate #4).

The electrical circuit for the electron beam is outlined in Plate #5 and is controled at the right side of the middle panel (Plate #12) of the electronic rack. The stabalized high voltage (2000 D.C.Volts) is essentially the same as that described by Nier (Rf.#2) and reproduced with modifications as used in Plate #6. Many improvements in this regulated power supply are still to be desired.

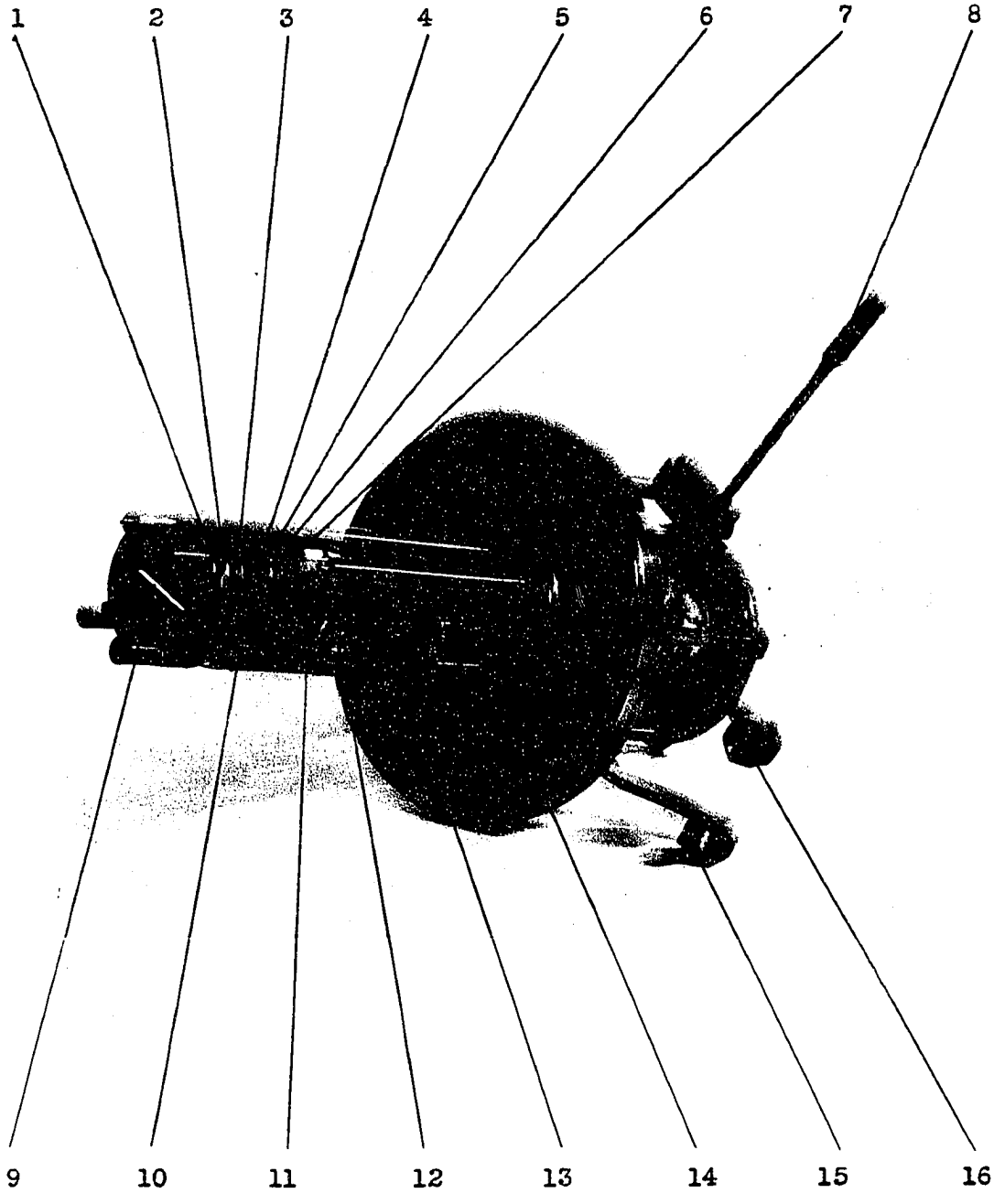
The useful range of this ion accelerating device is within the interval of 800 and 2000 volts.

$$m = k \frac{B^2 R^2}{E} \qquad \text{(Eq.\#3)}$$

tells us that different masses can be brought to the collector for inten-

PLATE #6a. COMPLETE SOURCE ASSEMBLY.

1. Nichrome Plate (Ground Potential) Slit 0.5 x 14 mm
2. Nichrome Half Plate (Beam Cutoff Potential) 3mm apart
3. Nichrome Plate (Ground Potential) Slit 0.25 x 14 mm
4. Nichrome Plate (Drawing Out Potential) Slit 1 x 14 mm
5. Nichrome Half Plate (Focusing Potential) 1 mm apart
6. Exit Slit of Ionization Chamber 1 x 14 mm
7. Ionization Chamber with Electron Trap
8. Connection for Gas Sample Leak
9. Defining Slit for Three-Point Magnetic Focus
10. Pyrex Spacer.
11. Tungsten Filament 0.75 x 0.025 mm Shield
12. Glass-seal for Filament Leads
13. Nichrome Clamp for Glass-seal
14. Teflon Gasket Seated in Stainless Steel Flange
15. Water Cooling Connection
16. High Voltage Cable Receptacle.



COMPLETE SOURCE ASSEMBLY

Ion Accelerating Plates Ionization Chamber

(Plate #6a)

sity measurement by changing the strength of the field of the magnetic analyzer (B) or by altering the electric accelerating field (E). Varying (B) to scan a section of masses is too coarse a procedure since mass (m) varies as the square of (B). In practice, (E) is varied for a definite setting of (B).

However, a study of Equation #6

$$R = k' E^{\frac{1}{2}} / B$$

tells us that the resolution is dependent on our choice of (E) and (B). In the final analysis, we select a setting for (B) so that a workable range of potentials are within the scope of the power supply. The extent of that range of (E) depends on one's ability to calibrate the instrument; too narrow a range would make it difficult to recognize a known calibrating mass. It might, therefore, be necessary to use (E) as low as 800 volts. The resolution is so jeopardized at potentials less than 800 volts that one defeats his purpose to resolve masses properly. Any adjustments provided for increments in potential from zero to 800 volts is then without purpose.

As was pointed out above, the potential difference between the ionization chamber and the first group of accelerating plates F_1, F_2 and F_3 (Plate #6a) is very small. This is a necessity as previously stated. Hence, the provision for a complex voltage divider mechanism with a range, 95% of which is never used, is too without purpose.

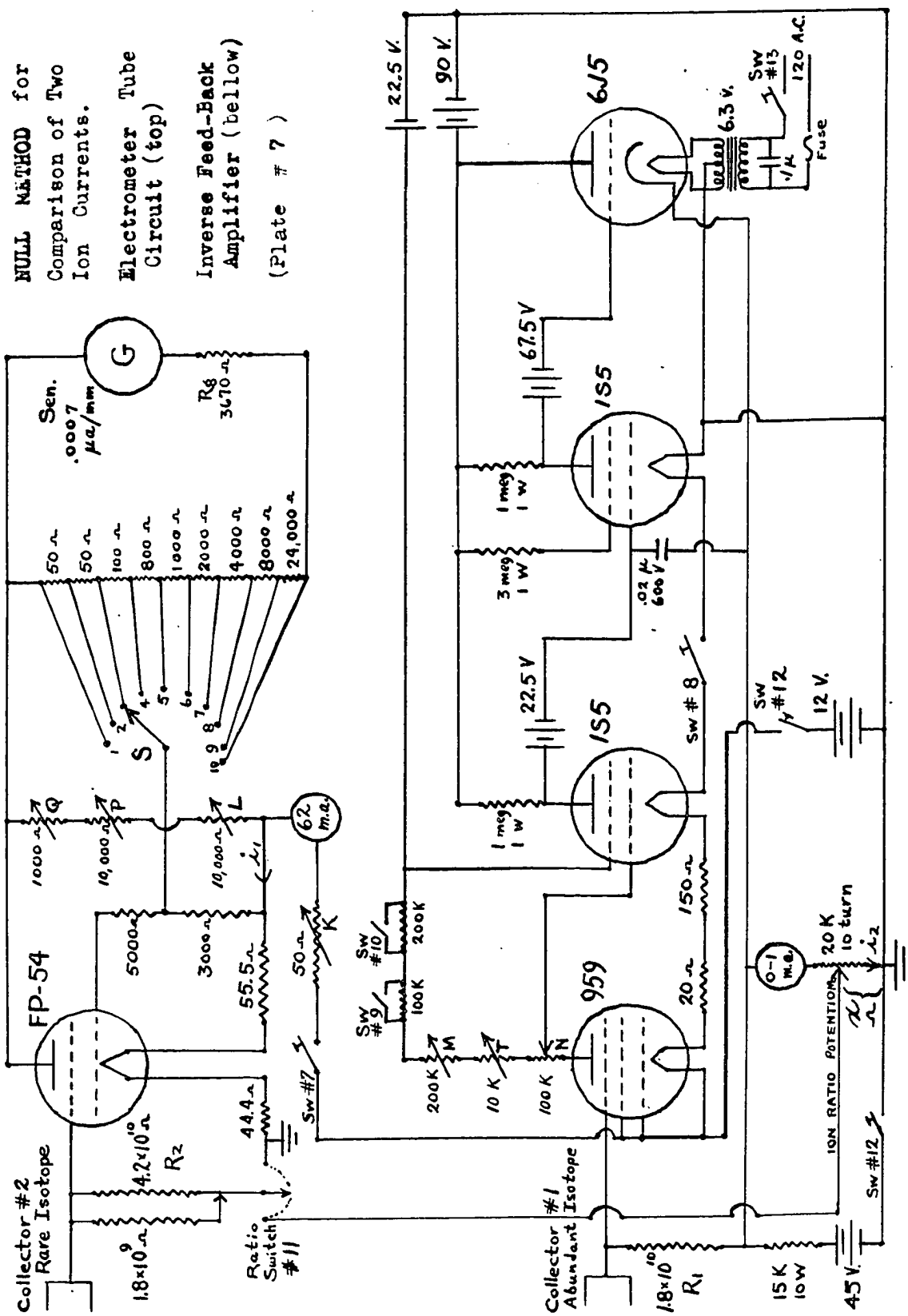
The most serious difficulty with the power supply centers about the selection of precision resistors for the put-and-take

arrangement in the load circuit. If one switches in a slightly larger resistor than that which is automatically switched out, there is a narrow range of potential that cannot be commanded for the ion accelerating potential. The switch in resistors of unequal value effects a reduced load current since the stabilizer maintains an over all constant voltage. However, the reduced current changes the value of individual increments of potential. Thus it was necessary to provide a panel extension adjustment potentiometer (X) (Plate #2) so that the grid bias of the stabilizing circuit could be changed to assure a continuous range of potentials.

The Collector System.

The ion collector assembly (Plate #5) is a duplication of Nier's (Rf.#2) except that the whole unit is detachable similar to that pictured for the ion source assembly (Plate #6a). The slit in the plate marked #1 collector is the defining slit which determines one of the three points in the magnetic focusing geometry as described in Plate #4. The means of suppressing any secondary electrons that are ejected from the two collectors by the impinging ion beams consists in the introduction of a negative plate (45 volts below ground potential) before each of the collectors. These negative plates are designed so that no portion of the positive ion beams will strike them. The #1 collector is protected by a grounded guard ring placed immediately on either side so that any ground leakage currents from the negative plate will be prevented from reaching it.

The dual system of collecting these positive ion beams is a means of eliminating many problems in instability. For a single collector



the energy of the electron beam and the balance of the electrometer circuit must remain so regulated that the intensity of the ion beam being measured will be a proper relation to its isotope being measured an interval of time later. If however the intensity of the ion beam of both isotopic masses could be measured simultaneously, each would respond in the same degree to any change in the electronic circuits just mentioned. Then a true isotopic relative abundance will be had even under those changing conditions.

The geometry of the plates limits the application of this dual collecting system. When the more abundant isotope is made to fall on the #1 collector, the rare isotope should clear the defining slit in #1 and fall on the #2 collector. This arrangement embodies a twofold assumption. First, that the heavier isotope is always the rare one; although this is true in most instances of interest, it is not the case for example with Argon. The second supposition is that when the rare isotope falls on the #2 collector, the abundant isotope will fall within the limits of the width of the #1 collector plate. This will depend on the number of mass units by which the two isotopes differ. The design as used in construction will permit this number to be as great as one twelfth of the average mass considered. Thus if mass 45 falls on the #2 collector, masses 42 to 44 will fall on the #1 collector plate. This dual arrangement is optional, so that either method may be used by mere panel manipulation of control switch (Sw #11).

The individual ion beam measurement is made by a FP-54 electrometer tube circuit (Plate #7) with a sensitive galvanometer (0.0007 microamp/mm) having a wide range of sensitivity factors. Es-

TABLE 1. GALVANOMETER SENSITIVITY FACTORS.

Selector Switch (S on Plate #12) -dial positions-	Resistance Ratio (R/43,390 ohms) -values for R-	Relative Factor of Sensitivity
# 1	zero	shorted
# 2	49.00	612.00
# 3	98.93	306.00
# 4	199.9	153.00
# 5	977.0	33.65
# 6	1,954.	18.00
# 7	3,965.	10.00
# 8	7,909.	6.00
# 9	15,770.	3.605
#10	39,720.	1.000

sentially this electrometer circuit (Rf.#13) is a current amplifying instrument which has very stable operating characteristics for currents ranging between 10^{-11} and 10^{-13} amperes. The spotlight galvanometer used is a rugged taut-suspension type made by Rubicon. It functions equally well for the null point method or for the deflection measurement.

When both the rare and abundant beams are read separately by the galvanometer, the galvanometer must have sufficient range of sensitivity available to detect beam intensities that differ by a factor of approximately 500. Such a maximum range would be required for two isotopes -- one being 99.8% and the other 0.2% in relative abundance. After experimenting with several arrangements, a satisfactory Ayrton shunt was devised to give a range of relative sensitivity factors from 1.000 to 612.0 in ten steps including a direct short (Table 1).

Since the intensity of the ion beam is directly proportional to the grid resistance (4.2×10^{10} ohms), the latter must be of such a value calculated so as to have the galvanometer deflection for the rare beam be significant and yet remain on scale for one of the more sensitive factor positions (#9 or #10); then the abundant beam can be measured by merely increasing the sensitivity factor.

The galvanometer scale is non-linear, hence the smaller the angle of deflection, the greater the accuracy in measurement. This effect is very slight since the radius of curvature is long compared to

the length of the arc read on a flat scale. In the null point method of balancing the dual ion beams, the question of linearity of the galvanometer scale is eliminated.

The inverse feed-back amplifier (Rf.#13) which measures the positive ion beam falling on the #1 collector, uses a high grid resistor (1.8×10^{10} ohms) to control a 959 acorn amplifying tube (Plate #7). The potential (V_i) that appears across the input terminals of this inverse feed-back amplifier has a voltage gain (G) so that

$$V_i = i_1 R_1 / (1 + G) \qquad \text{(Eq.\#9)}$$

and the output voltage is

$$V_o = G V_i \qquad \text{(Eq.\#10)}$$

Substituting equation #9 in equation #10 we have

$$V_o = i_1 R_1 \frac{G}{G+1}$$

Since the voltage gain (G) is approximately greater than 500

$$\text{(output)} \quad V_o = i_1 R_1 \quad \text{(input)} \qquad \text{(Eq.\#10a)}$$

This inverse feed-back circuit is useful only for the null point method of balancing out the two ion beams. The output voltage (V_o) is impressed across a special ten-turn precision potentiometer with a linearity tolerance within 0.05%. A fraction of this potential ($x V_o$) can be picked off and adjusted to buck the potential appearing across the FP-54 grid resistor. This adjustment can be read accurately to four places by

a panel geared mechanism (ION RATIO - Plate #12) when the galvanometer deflection has been returned to zero-reading (i.e. the position for ion beam cut off). The balance condition of the galvanometer is expressed

$$x V_o = i_2 R_2 \qquad \text{(Eq.\#11)}$$

Substituting equation #10a in equation #11 we have

$$i_2 R_2 = x i_1 R_1 \frac{G}{G+1} \qquad \text{(Eq.\#11a)}$$

Rearranging equation #11a we have the current ratio

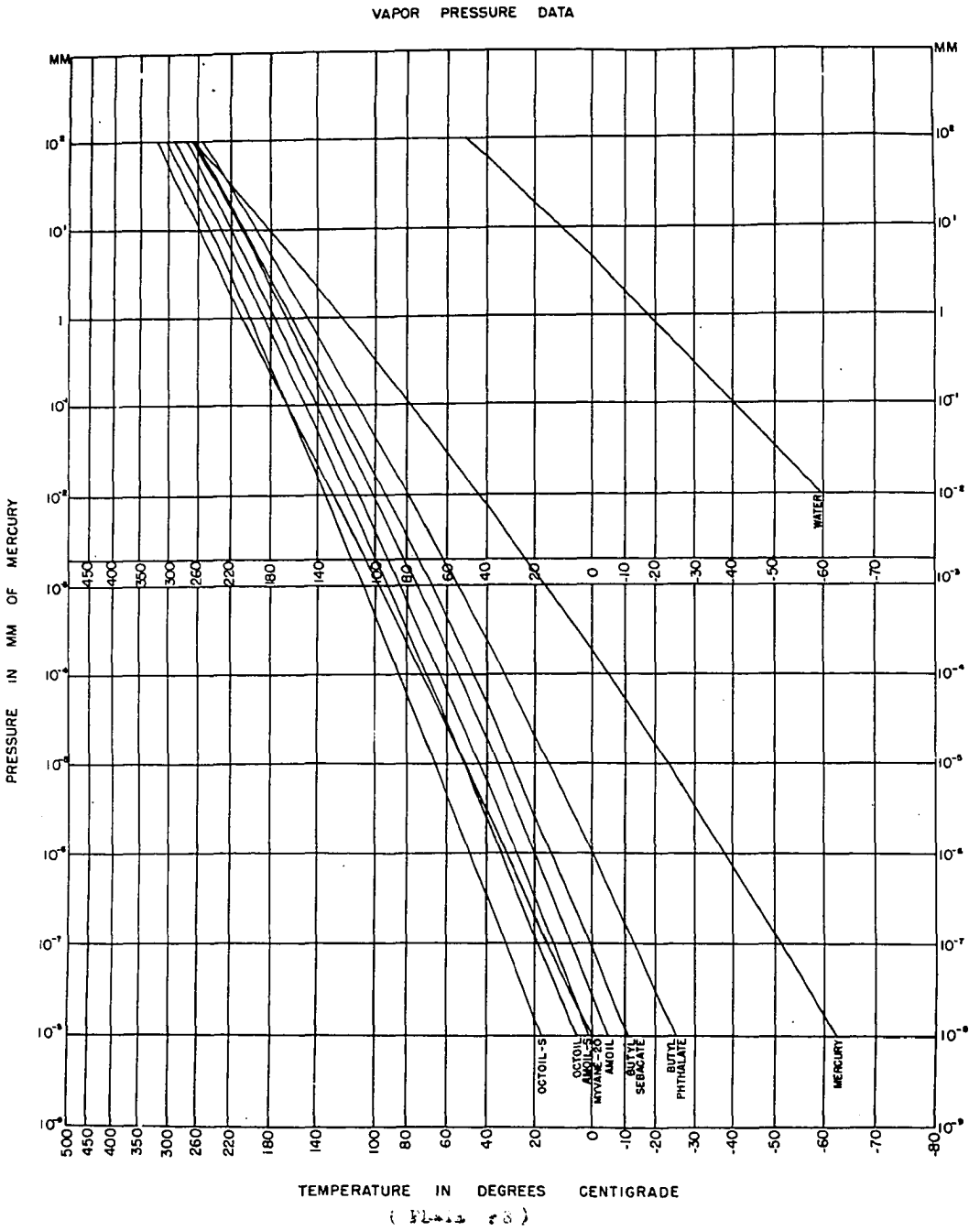
$$\frac{i_2}{i_1} = x \frac{R_1}{R_2} \frac{G}{G+1} \qquad \text{(Eq.\#11b)}$$

Thus the feed-back amplifier output provides the e.m.f. for the ten-turn potentiometer just as a dry cell would on a conventional potentiometer. This relation i_2/i_1 is the ratio measurement of the abundance of the two isotopic masses. This relative abundance is equal to the product of the fraction (x) and the constant known from the R_1/R_2 relation.

Aston (Rf.#14) was the first to propose the use of dual collectors, but he never used the method himself. Straus (Rf.#15) used two ion collectors for balancing out accumulated charges over a time interval. The method adopted as used by Nier gives continuous readings.

Mercury-Free Vacuum System.

The vacuum system (Plate #3) maintains the instrument at a pressure of 5×10^{-6} mm Hg. after a four hour conditioning, which includes torching the metal analyzer (Z) about twenty minutes after the pumps have been operating for about two hours. At this pressure colli-



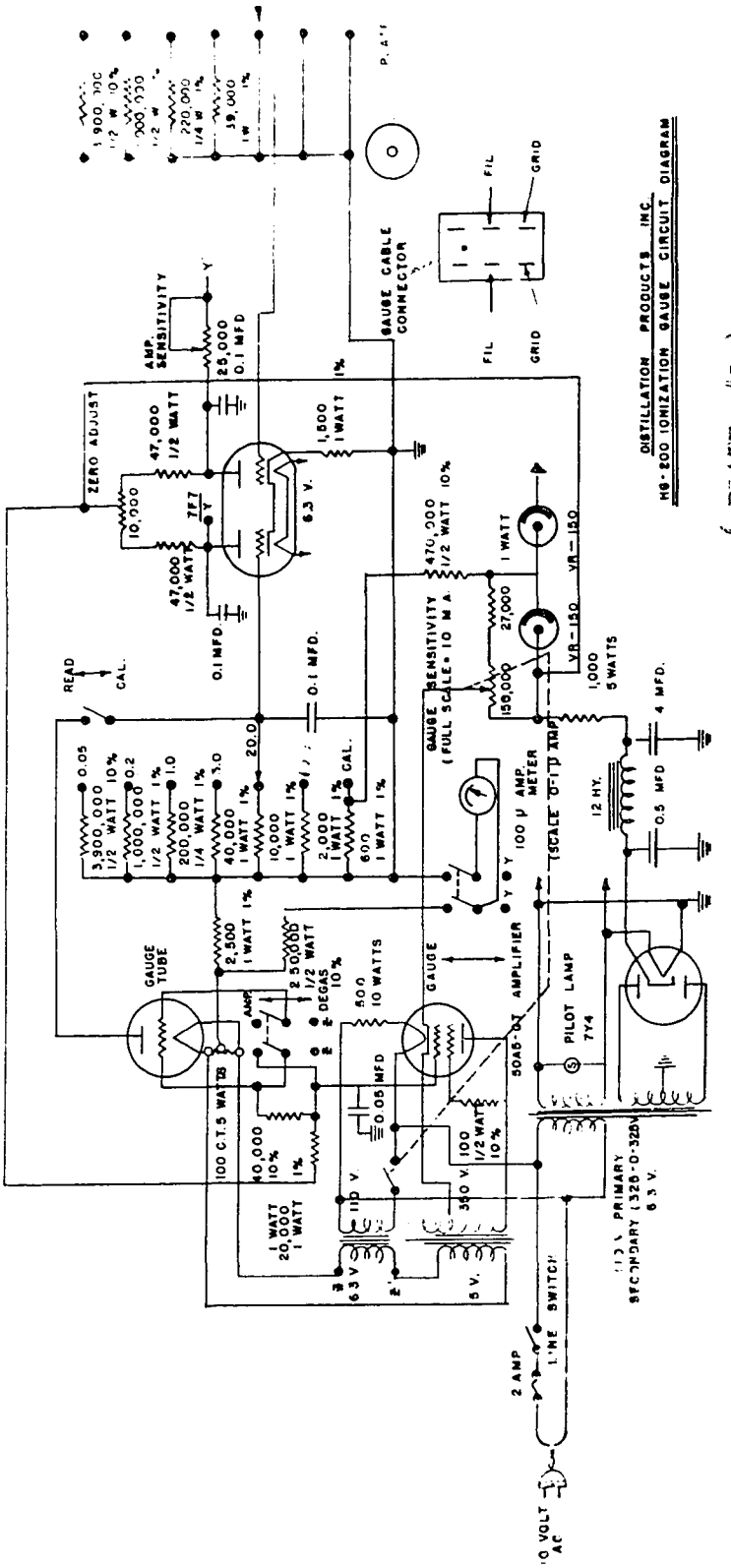
(Plate 28)

sion between accelerated ions and residual gas particles is not likely to occur. The mean free path between intermolecular collisions for Nitrogen at a pressure of 5×10^{-6} mm Hg. and zero degree centigrade is twenty-five meters.

No mercury is used in the instrument at any time. The rotary oil fore pump (U) is capable of pulling a vacuum of approximately 10 microns. The oil diffusion (V) pump (Distillation Products - Type VMF-20) was designed to attain a maximum vacuum of 1×10^{-6} mm Hg. when S-Octoil is used.

The principal disadvantage of mercury is its high vapor pressure at room temperature. A system using a mercury pump demands the use of a cold trap to freeze out these mercury vapors. Each trap increases the volume of the system and offers additional gas-flow resistance. At very low pressures mercury vapor acts as a choke between the system and the diffusion pump. Mercury vapor is toxic and an accident with hot mercury is a health hazard and presents a difficult job to clean up that which is spilled. The elimination of cold traps also means the elimination of their care, which under working conditions calls for around-the-clock vigils. At room temperature S-Octoil has the same vapor pressure (Plate #8) that mercury has when cooled to a temperature of minus 63° C. Thus S-Octoil has advantages comparable to the use of mercury with a trap cooled with a mixture of dry-ice and acetone but without its hazards and functional disadvantages.

The use of oil diffusion pumps makes it possible to attain pumping speeds from ten to one hundred liters per second (VMF-20 rates at 20 liters/sec) while mercury diffusion pumps range from a fraction of



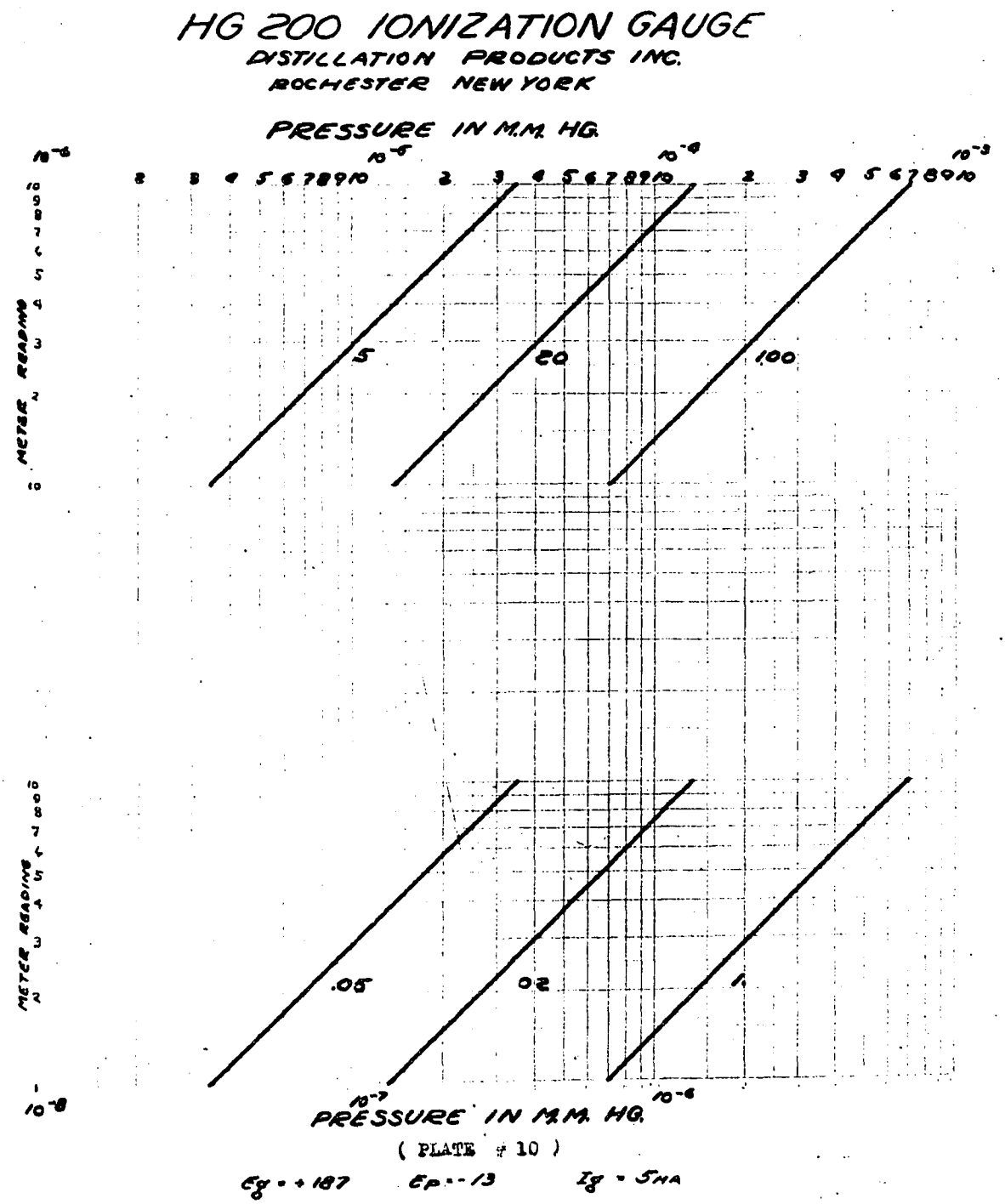


TABLE 2. CALIBRATION READINGS OF IONIZATION GAUGE. (HG-200)

Meter Reading	Pressure in mm. Mercury		
(panel dial)	--A--	--B--	--C--
0.10	0.7	0.14	0.34
0.15	1.07	0.21	0.52
0.20	1.4	0.275	0.68
0.25	1.75	0.34	0.86
0.30	2.1	0.41	1.05
0.35	2.5	0.48	1.2
0.40	2.8	0.55	1.35
0.45	3.2	0.62	1.55
0.50	3.5	0.68	1.7
0.55	3.9	0.75	1.85
0.60	4.2	0.82	2.05
0.65	4.6	0.88	2.25
0.70	4.9	0.95	2.4
0.75	5.3	1.02	2.6
0.80	5.6	1.09	2.75
0.85	6.0	1.15	2.9
0.90	6.3	1.2	3.1
0.95	6.7	1.3	3.3

Sensitivity $\frac{100}{20} = A \times 10^{-4}$ $= B \times 10^{-4}$

$\frac{5}{} = C \times 10^{-5}$

Sensitivity $\frac{1}{0.2} = A \times 10^{-6}$ $= B \times 10^{-6}$

$\frac{0.05}{} = C \times 10^{-7}$

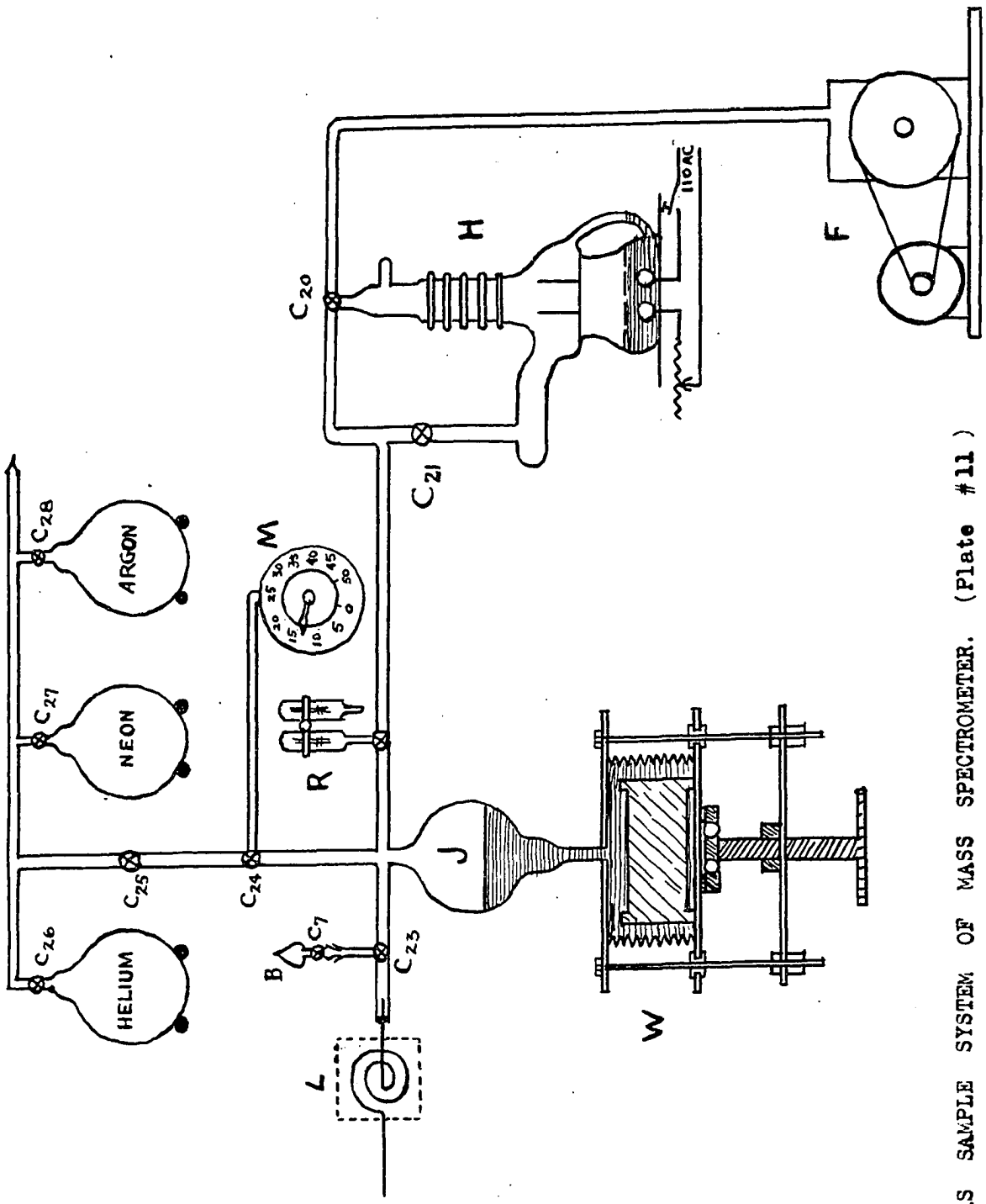
a liter per second to a few liters per second. To exploit fully the use of the oil diffusion pump would be to line at least two of them in series so that the first would keep the oil in the second purified. This procedure also reduces the limiting pressure by a factor of ten.

The great advantage in the construction of an instrument afforded by the oil diffusion pump is the possibility of an all-metal system. The mercury type of diffusion pump requires glass on account of the cold trap needed, which in turn demands a glass-metal ball joint sealed with wax.

The vacuum gauges are selected because of their scale range and their mission. A McLeod gauge was ruled undesirable both because it requires mercury and because it is not a continuous reading device. A Pirani gauge (N) was placed between the fore pump and the diffusion pump. With a scale range from 0.75 mm to one micron, it served as an indicator of the performance of the fore pump as well as an index of the speed of the oil diffusion pump. The ionization gauge (Q) (Distillation Products-Type HG-200) (Plate #9) with a scale range from 10^{-4} to 10^{-8} mm Hg. (Plate #10 and Table 2) was placed near the ion source assembly.

Mercury-Free Gas Sample Assembly.

The gas sample assembly is evacuated by a single stage oil diffusion pump (Distillation Products-Type G-4) backed by a rotary oil pump. Pressures below one micron are sufficient and easily attained if all stopcocks are properly greased with Silicon (Dow Corning). A Pirani gauge reads the low pressures while the high pressures (0.5 to 50 mm Hg.) are indicated by a special Bourdon gauge (Wallace and Tiernan Inc.) which replaces the less desirable mercury manometer.



GAS SAMPLE SYSTEM OF MASS SPECTROMETER. (Plate #11)

When the gas sample is introduced in the mass spectrometer (Plate #11) it may have a variety of combinations of pressure-volume values. This requires some mechanism (W) whereby the pressure of the gas can be controled by altering the volume. Usually this is accomplished by changing the level of a reservoir of mercury. First, the gas is drawn in by lowering the mercury level and then it is compressed at a desired pressure by raising the mercury level. This necessitates dealing with mercury in the system. Whereas it was a simple matter to select an "oil" diffusion pump (H) instead of a "mercury" one, and quite as simple procedure to find a suitable Bourdon gauge (M) to replace a mercury manometer, it proved a difficult task to successfully eliminate the mercury device for changing the sample gas pressure.

The great difference in density between oil and mercury, excluded the use of oil as a replacement of mercury and still keep the same principle of operation as referred to above. To select another liquid of comparable density to mercury would not eliminate the use of cold traps. The only alternative was to devise a mechanism in which S-Octoil could be used. This meant a change in principle in which the gravitational force exerted on the dense mercury would be replaced by some mechanical leverage. Several methods were tried unsuccessfully, which included a plunger type cylinder by which the volume could be controled. The failure centered about the inability to make the device vacuum tight. The final solution was a sylphon bellows (W) containing a reservoir of S-Octoil with a possible differential in volume of one liter. This volume change is controled by turning a wheel operating a screw thread which was designed to give smooth action in small increments.

This assembly for the gas sample is joined to the mass spectrometer by means of several (approximately 10) inches of copper tubing (I.D. 0.006 inch). This gas leak (L) has a delicate opening that is easily obstructed by any foreign particle. The advantages of such a length of capillary tubing are multiple. The mass spectrometer itself requires an insignificant amount of gas for its operation; at pressures of 10^{-6} mm Hg. every cubic centimeter of gas contains about 3×10^{10} particles. With a narrow restriction, a higher working pressure (5 to 20 mm Hg.) can be maintained in the gas assembly. This eliminates much stopcock leakage. Such working pressures would not be possible for gas leaks with relatively large apertures, since the pressure in the mass spectrometer would build up beyond operating conditions (2×10^{-5} mm Hg.).

Another advantage of the capillary tubing is the prevention of back diffusion of gas fractionated at the constriction, where a combination of viscous and molecular flow takes place. The choice of a proper capillary diameter and length guarantees that the mass flow velocity will be greater than the back diffusion velocity; hence the gas entering the mass spectrometer will be representative of that in the gas manifold. R.E.Honig (Rf.#16) made a detailed study of the gas flow for mass spectrometric analysis. He points out three requirements, which are essentially fulfilled, if molecular flow is established by correct choice of pressures, namely:

1) The ion beam intensity is to be directly proportional to the pressure of the sample gas but independent of its molecular weight;

2) In a gas mixture, all mixture peaks are to be formed by linear superposition of individual intensities; and,

3) The gas flow should be constant.

The basic assumption made is that the mean free path of the molecule is large compared to the diameter of the capillary. Knudsen, who was first to derive laws of molecular flow, found experimentally that his calculations for rate of flow of gas through capillary tubing is applicable in the low pressure range provided the mean free path corresponding to the average pressure of the system is greater than about thirty times the diameter of the capillary.

$$Q_m = \frac{d(\bar{p}V)}{dt} = 3800 \frac{D^3}{L} \frac{T^{\frac{1}{2}}}{M^{\frac{1}{2}}} (p_1 - p_2) \qquad \text{(Eq.\#12)}$$

Q_m -- time rate of change of pressure-volume product

$\bar{p}$ -- mean pressure in bars

V -- volume of gas discharged per second

D -- diameter of capillary cylindrical tube

L -- length of tube (cms)

T -- temperature (degrees absolute)

M -- molecular weight (O^{16} = 16.000 grams)

p_1 -- pressure in bars on high side of aperture

p_2 -- pressure in bars on low side of aperture.

But at high pressures where the mean free path is much smaller than the capillary diameter, the gas flow is viscous and follows Poiseuille's law

$$Q_v = \frac{D^4 \pi}{128 \eta L} \bar{p} (p_1 - p_2) \qquad \text{(Eq.\#13)}$$

where (η) is the coefficient of viscosity in poises.

This consideration becomes critical if a chemical analysis is undertaken where there are several different gases present. The sample gas pressure must be reduced from several centimeters (Hg.) to a fraction of a millimeter according to the following reasoning:

$$(30)(\text{diameters}) = (30)(0.006 \text{ in.})(25.4 \text{ mm/in.}) = 4.57 \text{ mm}$$

This dimension (4.57 mm) must be smaller than the mean free path. A mean free path of 4.57 mm represents a pressure between 0.1 and 0.01 mm Hg. Thus to insure molecular flow for the analysis of a complex mixture of gases, the pressure must be reduced by a factor of more than one hundred.

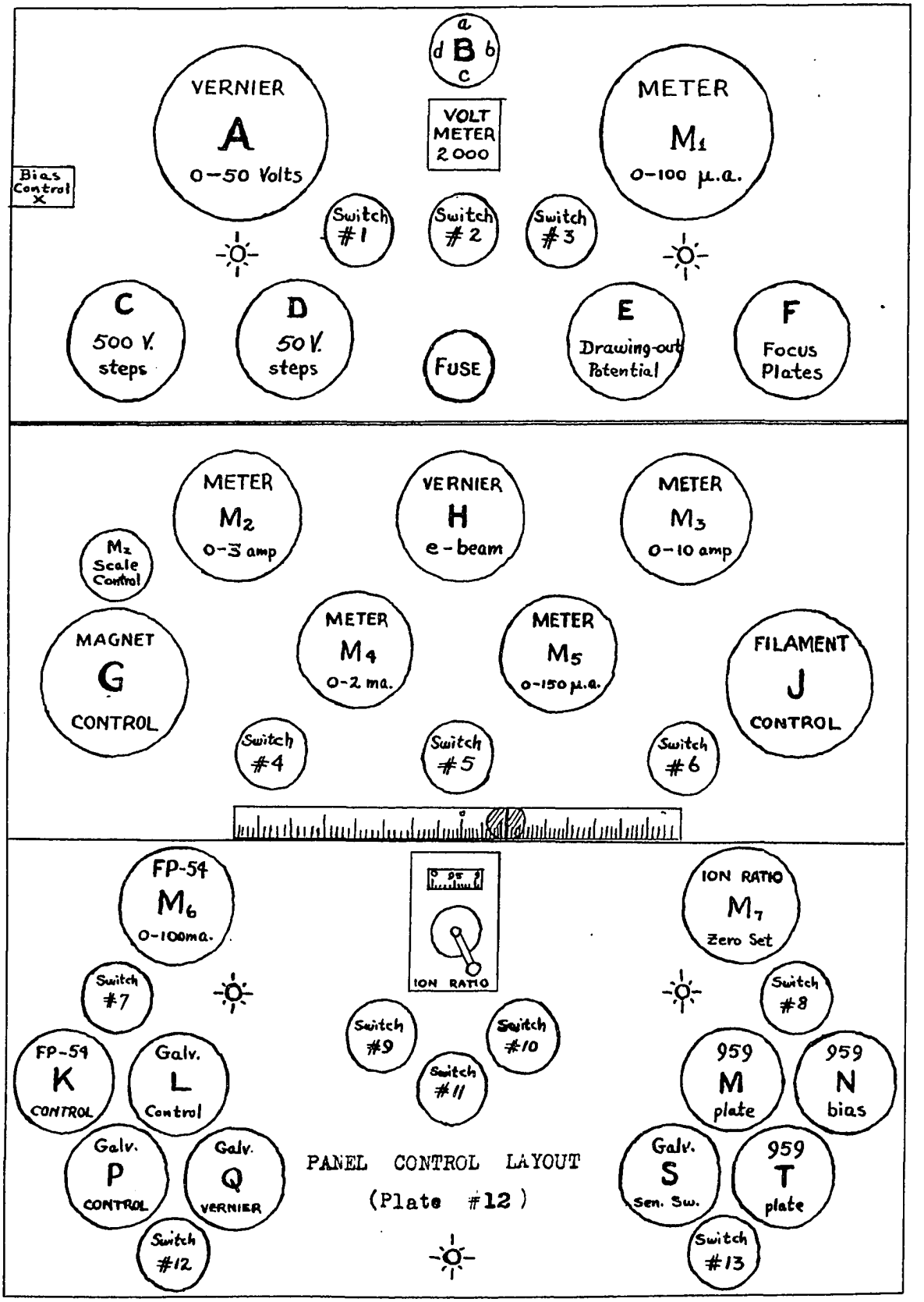
The gas sample system must also include reservoirs of gases (Plate #11) to be used for calibration. The choice of these gases depends on the masses to be analyzed; thus for Nitrogen (mass 28), Neon (mass 20) will serve well; for carbon dioxide (mass 44), Argon (mass 40) is selected; for Oxygen (mass 16), water vapor (mass 18) is always present; and for Hydrogen (mass 2), Helium (mass 4) is chosen. All of these masses are important in isotopic tracer work in biochemistry, so a facility for the three calibration gases was provided including Helium, Neon and Argon.

CHAPTER III.

CALIBRATION AND OPERATION OF MASS SPECTROMETER.

Regulated Power Supply.

The experimental procedure begins after the vacuum ionization gauge reads a pressure in the vicinity of 5×10^{-6} mm Hg. The



electronic circuits are turned on so that a temperature equilibrium can be established. (NOTE: All references for operation refer to the panel layout as illustrated in Plate #12; all toggle switches are in the "ON position" when turned to the right.)

The high voltage supply (Plate #6) is conditioned by throwing the panel switch #1 (green light signal) which controls the filament circuits. After a minute delay, #3 switch (red light signal) connects the high voltage to the plate circuit and the grid bias of the stabilized control circuit is adjusted by means of the panel extension potentiometer (X). For the full output (2000 volts D.C.) of dials A, C and D, this potentiometer (X) is properly adjusted so that one milliampere d.c. flows through the panel extension meter (Position B-a) and through a precision two megohm resistor. This same meter arrangement can be used to measure the potential at the other accelerating plates by selecting the proper setting of the selector switch B. Position B-d indicates the potential at plates F_2 and F_3 (panel control E); position B-c, at plate F_1 (panel control F); and position B-b at the beam cutoff (panel control switch Sw #2). The operating potential of the A-C-and-D combination determines the mass which will be focused on the collector. E and F are then varied to effect the optimum ion beam intensity.

The Electron Beam Adjustments.

The ionization filament is energized by the panel control Sw #6, and its temperature adjusted by potentiometer J and H (Plate #12). The filament current is read on meter M_3. The electron accelerating potentials are connected by panel control Sw #5. The total electron emission is read on meter M_4 and the electron beam intensity in

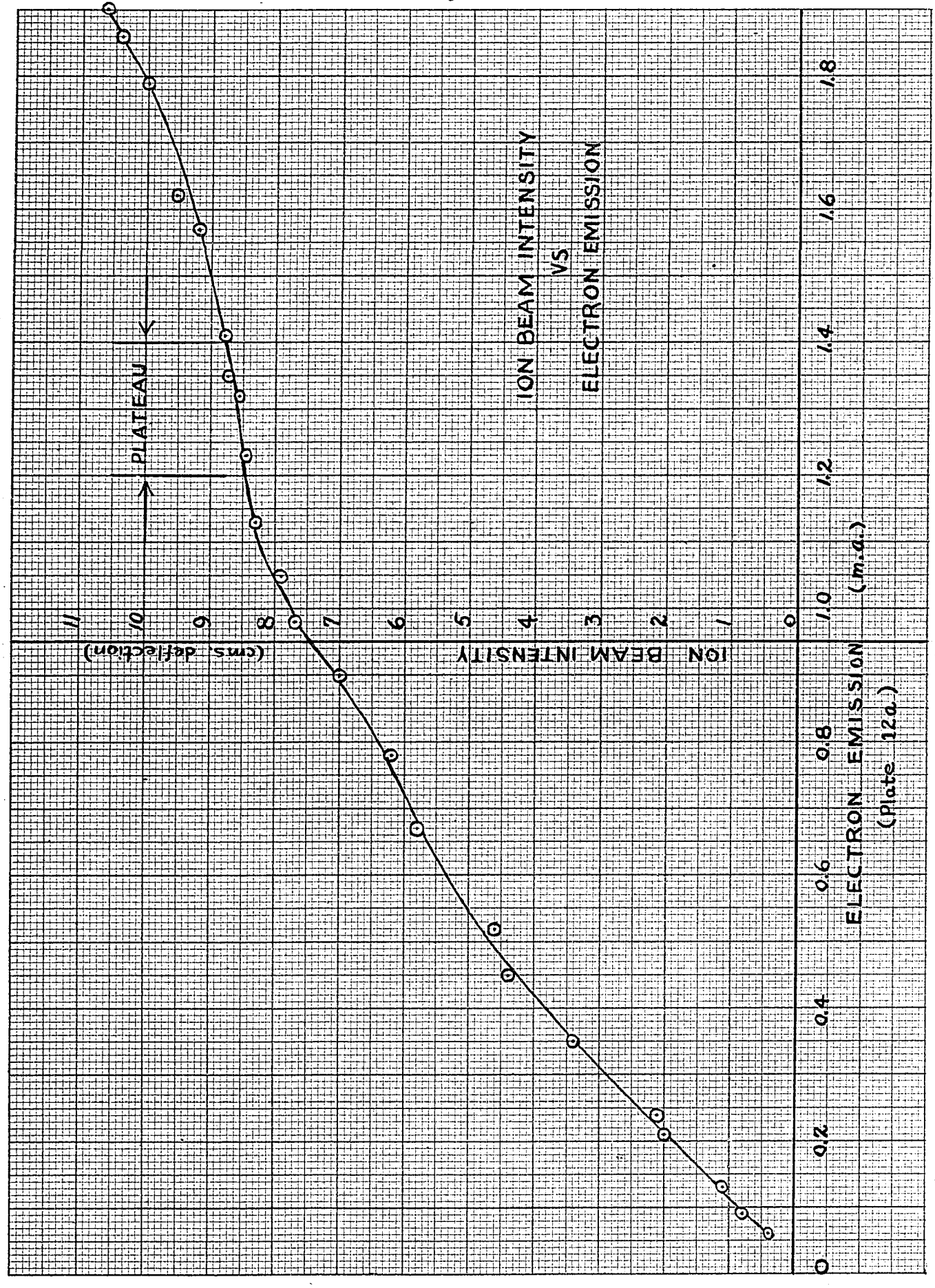
microamperes on meter M_5. After fifteen minutes warm-up, the electron emission becomes constant enough for operating purposes.

The electron beam admits of two considerations. First, its intensity as controled by the temperature of the filament can be viewed as a function of the number of electrons which make up this ionizing beam. Richardson's theory (Rf.#17) of the emission of electrons from hot bodies is in many respects analogous to the kinetic theory of vaporization. Heat possessed by a metal is believed to be stored in the kinetic energy both of random motion of atoms and molecules and of free electrons. The number of electrons that reach the surface in unit time is proportional to the fraction of all the free electrons in the metal. As the temperature of the emitter is increased, the average velocity of the free electrons increases. Hence the rate at which electrons escape from the metal increases with temperature. If the emitted electrons are not drawn away by an external field, they will form a space charge which returns the electrons to the emitter. If a second electrode is placed near the emitting surface and is made positive with respect to the emitter, the current is increased. This increase in electron current is directly proportional to the intensity of the ion beam.

Table III contains experimental data for the intensity of the ion beam as a function of the total emission of electrons from the tungsten filament. The accelerating potentials of the electrons remained constant but high enough so that all emitted electrons would be drawn to the positive electrode. As the filament current was increased the total emission of electrons was read on the milliammeter (M_4). For each value recorded, a corresponding deflection of the galvanometer was

TABLE 3. ION INTENSITY BEAM AS A FUNCTION OF ELECTRON EMISSION CURRENT.

Reading (order)	Electron Emission (milliamperes)	Ion Intensity Beam (cms deflection)
#1	0.06	0.4
#2	0.09	0.8
#3	0.13	1.1
#4	0.21	2.0
#5	0.24	2.1
#6	0.35	3.4
#7	0.45	4.4
#8	0.52	4.6
#9	0.67	5.8
#10	0.78	6.2
#11	0.90	7.0
#12	0.98	7.7
#13	1.05	7.9
#14	1.13	8.3
#15	1.23	8.45
#16	1.32	8.55
#17	1.35	8.75
#18	1.41	8.8
#19	1.57	9.2
#20	1.62	9.56
#21	1.79	10.0
#22	1.86	10.4
#23	1.91	10.7



observed as a measure of the ion beam intensity. These relations were plotted (Plate #12a) and a plateau discovered in the ion beam intensity for an interval of electron emission between 1.2 and 1.4 milliamperes. This was helpful in the elimination of variations in ion beam intensities resulting from fluctuations in electron emission current; as a result, a working value of 1.3 m.a. emission was adopted.

Secondly, the electron beam intensity can be looked upon from the standpoint, not of the number of electrons but of the energy in electron volts that each electron possesses. This is controled by the difference of potential through which these electrons fall. It too is a direct function of the measured ion beam intensity at the collector.

The measure of the intensity of the electron beam as measured in microamperes on meter (M_5) may be constant and yet equal to a variety of product combinations of filament temperatures and electron accelerating potentials. The effect of these various combinations has a distinct operational difference.

The magnetic analyzer of the mass spectrometer cannot distinguish between two different substances of equal mass (e.g. -- mass 18 for $H^1H^1O^{16}$ and $N^{15}H^1H^1H^1$); nor can it differentiate between one mass singly ionized and another mass half as great but doubly ionized (e.g. -- Neon gas molecule with a single charge and Argon gas molecule with a double charge). However, unique selection is possible in both cases by the proper selection of the accelerating potential for the electron beam.

With care and practice, the difficulty of doubly ionized particles can be reduced to a negligible quantity. The other problem

calls for more detailed observation. It concerns appearance potentials. Two molecules of the same mass (as cited above for mass 18) but of different chemical substance (water vapor and ammonia gas) require different energies to ionize them. In other words, the work necessary to remove an electron from a molecule of a particular substance is characterized by its nuclear structure and depends on its quantum numbers. (Rf.#4-p.317) The minimum energy needed to ionize the gas molecule is referred to as its appearance potential. This ionizing work or energy is supplied by the bombarding electron beam, which in turn receives its energy from the electron accelerating potential.

Thus two substances chemically different but with equal masses can be separated by gradually increasing the energy of the electron beam through small increments of electron accelerating potential until the ionizing range is reached for the first of the two. Then the magnetic analyzer, which resolves only moving ions, will complete the separation in regards to the presence of the other ions of different masses.

This electron beam is collimated and focused by a weak magnetic field which is established by two opposite permanent magnetic poles fixed to a brass collar so as to be adjustable around the metal manifold in the vicinity of the ionization chamber. The optimum position is for the maximum ion beam intensity and not for the maximum electron beam intensity as read on meter (M_5). The reason lies in the fact that this weak magnetic field effects also the ion beam by deflecting its path and assorting its masses. This type of interference defeats the fundamental purpose of the instrument and annuls any

advantage of greater intensity in the ionizing electron beam.

Too great an intensity in the electron beam will broaden out the ion peaks so that difficulty will be experienced in tuning in on the actual peaks as well as in resolving congested peaks. This is true for most adjustments intended to increase the ion beam intensity. A notable exception to this generalization is the method of intensifying the ion beam by creating a greater sample gas pressure. The limitation of this procedure has already been discussed.

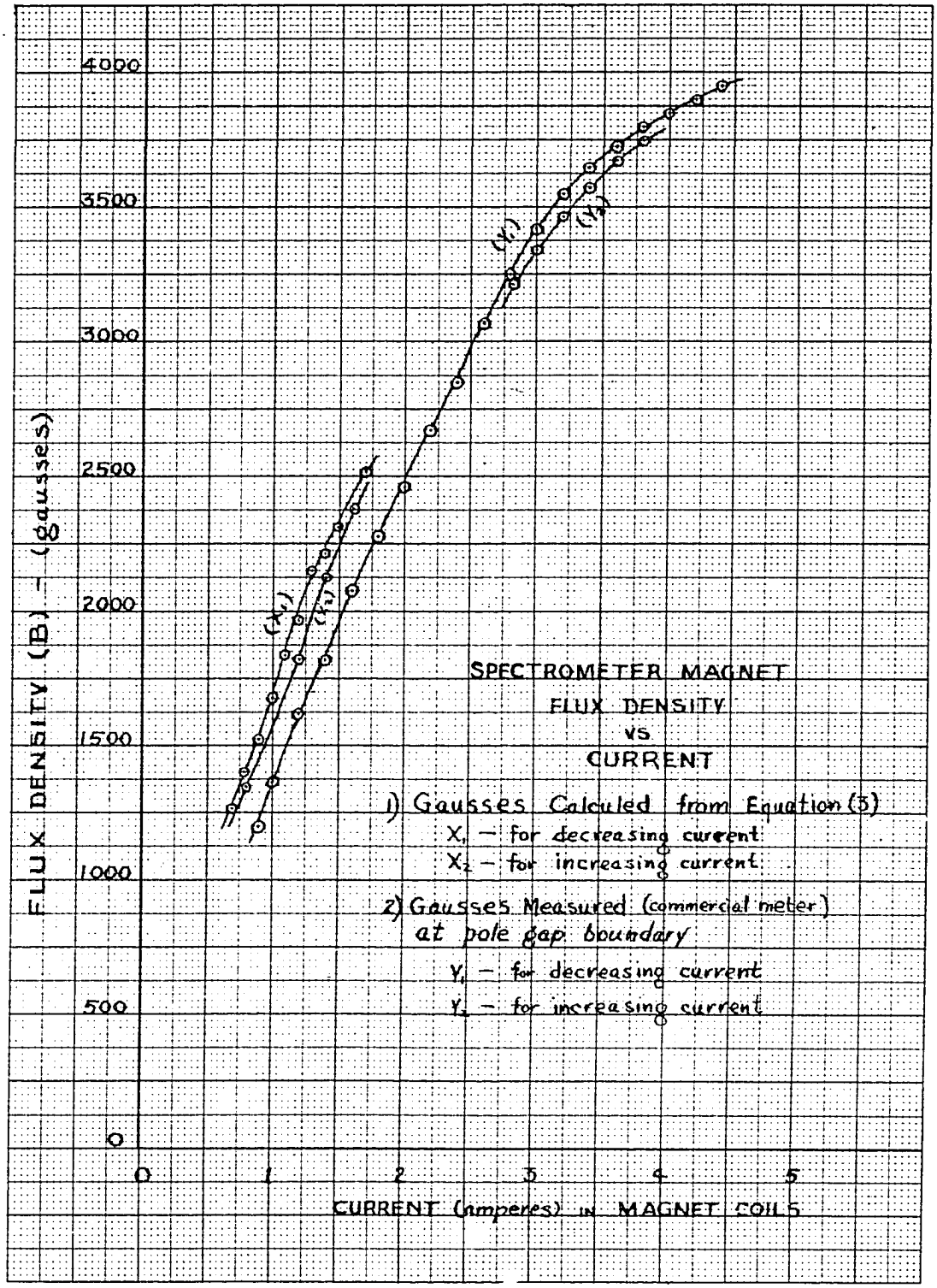
The ion accelerating potentials are increased by manipulating the following three panel controls (Plate #12). Selector switch C has four positions whereby increments of 500 volts can be added. Selector switch D has ten positions whereby steps of 50 volts can be supplied. And potentiometer A is a vernier adjustment for a fractional increase within a range of zero to fifty volts.

The drawing-out potential is controled by the selector switch E which regulates a total potential that increases automatically with an increasing accelerating potential. The focusing property of the potentiometer F is limited within the range of fifty volts.

The path of the ion beam through the strong magnetic field is determined by the position of the copper analyzer tube. The geometric position of the source and collector slits with respect to the theoretical apex of the magnetic field sector aligned roughly. Precise adjustment is made during operating conditions according to the maximum intensity for some particular ion peak. This definite position establishes the actual radius of curvature (R) according to which the length of the analyzer arms were made, the depth of the magnet pole pieces designed,

TABLE 4. DATA FOR B & H CURVE OF ELECTROMAGNET.

---- Calculated Values ----			---- Measured Values ----		
Current (amperes)	X_1 (down) (gauss)	X_2 (up) (gauss)	Current (amperes)	Y_1 (down) (gauss)	Y_2 (up) (gauss)
1.7	2520	----	4.4	3950	----
1.6	2380	----	4.2	3900	----
1.5	2315	----	4.0	3850	----
1.4	2218	2125	3.8	3800	3750
1.3	2150	----	3.6	3730	3680
1.2	1970	1830	3.4	3650	3570
1.1	1843	1740	3.2	3550	3480
1.0	1694	----	3.0	3420	3350
0.9	1528	----	2.8	3260	3240
0.8	1408	1350	2.6	3070	----
0.7	1272	----	2.4	2860	----
			2.2	2670	----
			2.0	2470	----
			1.8	2280	----
			1.6	2080	----
			1.4	1820	----
			1.2	1620	----
			1.0	1370	----
			0.9	1200	----
			0.8	----	----



and now the radial path that every mass must follow to reach the collector slit.

The Magnetic Field Adjustments.

The magnetic field itself is controled by the panel potentiometer G and the current in the coils measured by the panel meter M_2 which has two scale ranges (0 to 1 ampere) and (0 to 5 amperes) accordingly as selected by the panel M_2 scale control switch.

The strength of the magnetic field as a function of the current in the coils was measured by a commercial gauss-meter and plotted as shown in the B & H curve "Y" (Plate #13) for the values listed in Table 4. These readings were limited by the measuring device to one half inch within the pole-gap.

The strength of the magnetic field in the effective area within the pole-gap where the ion beam is bent, as a function of the current in the coils, can best be calculated from equation #3 where all values are known except B. Plotting these calculated values (Table 4.) as a function of the current readings in amperes on panel meter M_3 resulted in the B & H curve "X" (Plate #13). In this way the ammeter M_3 was calibrated to read in gauss.

The selection of this magnetic flux density (B) was referred to earlier in the consideration of equation #6

$$R = k' \frac{E^{\frac{1}{2}}}{B}$$

Since the geometry of the analyzer is fixed for this particular instrument, there is only one radius of curvature (R) that will bring a de-

Table 5. Data for Relative Abundance of Neon.

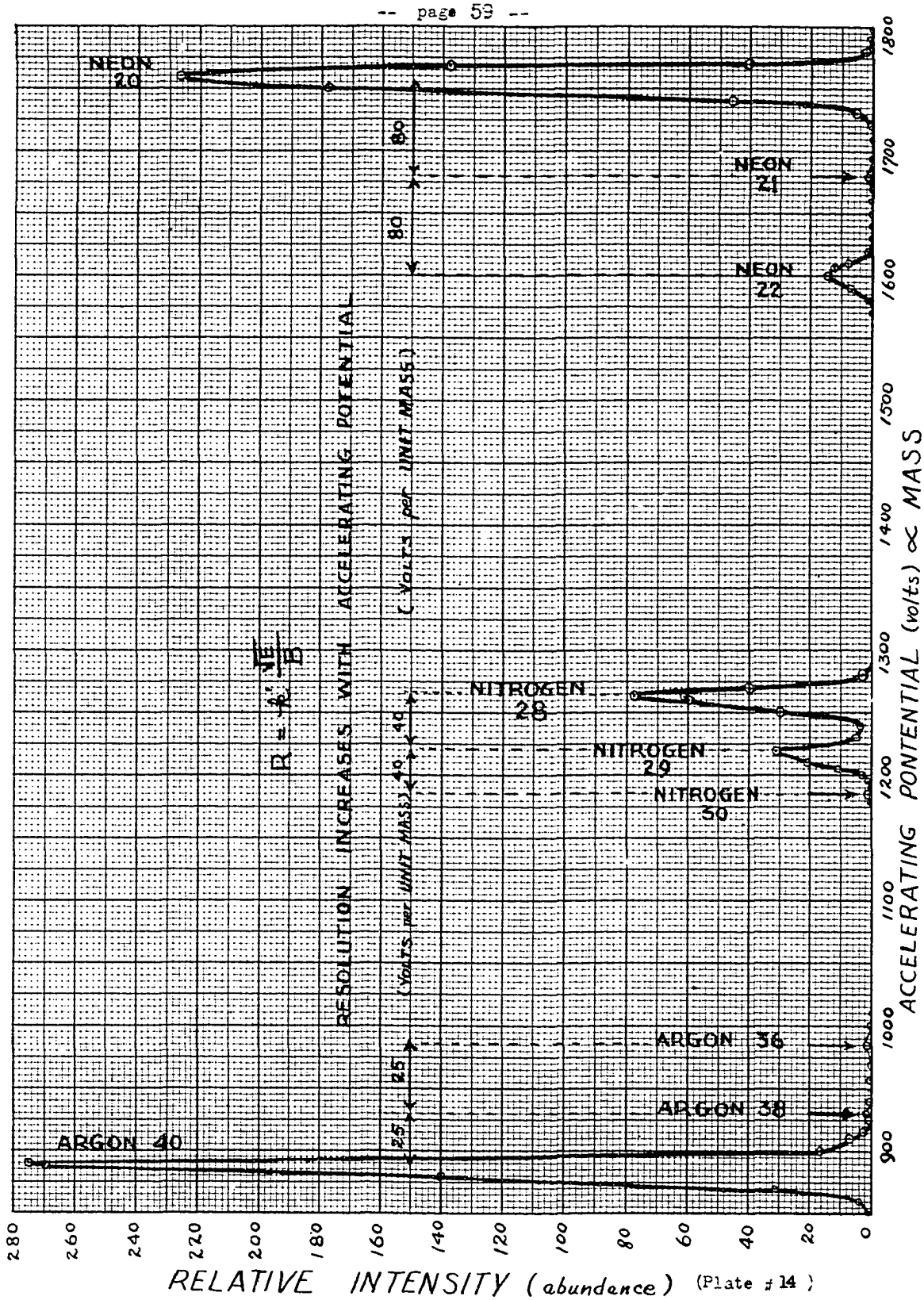
NEON (mass) (volts)	Sensitivity Factors	Deflection (cms)	Relative Abundance -calculated- sen.factor used	Relative Abundance -experimental- sen.factor used	Percent (ratio)
1570	6	0.2	0.2	0.36	
1580	6	1.2	1.2	21.6	
1590	6	7.3	7.3	131.5	
(22)1600	5	7.5	14.2	252.0	7.03
1608	5	6.2	12.4	208.5	
1610	5	4.0	8.0	134.5	
1620	6	1.1	1.1	19.8	
1630	7	0.4	0.2	4.0	
1650	7	0.4	0.2	4.0	
1660	7	0.6	0.3	6.0	
1670	8	1.1	0.5	6.6	
(21)1675	8	1.7	0.8	10.2	0.28
1680	8	1.3	0.6	7.8	
1690	7	0.7	0.3	7.0	
1710	7	0.7	0.3	7.0	
1720	7	1.1	0.5	11.0	
1730	7	10.8	5.4	108.0	
1740	4	4.6	46.0	704.0	
1750	3	8.9	178.0	2725.0	
(20)1760	2	5.6	227.0	3428.0	92.69
1766	2	3.4	138	2080.0	
1770	3	2.1	40.4	642.0	
1780	7	4.9	2.4	49.0	
1810	9	1.9	0.2	6.8	

Table 6. Data for Relative Abundance of Nitrogen.

NITROGEN (mass) (volts)	Sensitivity Factors	Deflection (cms)	Relative Abundance -calculated- sen.factor used	Relative Abundance -experimental- sen.factor used	Percent (ratio)
1180	8	0.15	0.76	o.76	
(30)1185	10	0.8	0.8	0.8	
1187	8	0.15	0.76	0.76	
1190	7	0.2	2.0	2.0	
1200	7	0.6	6.0	6.0	
1205	7	1.2	12.0	12.0	
1210	7	2.1	21.0	21.0	
(29)1218	7	3.1	31.0	31.0	
1230	7	0.5	5.0	5.0	
1238	7	0.4	4.0	4.0	
1250	7	3.0	30.0	30.0	
1260	7	6.0	60.0	60.0	
1262	7	6.5	65.0	65.0	
(28)1263	7	7.8	78.0	78.0	
1270	7	4.0	40.0	40.0	
1280	7	0.3	3.0	3.0	

Table 7. Data for Relative Abundance of Argon.

ARGON (mass) (volts)	Sensitivity Factors	Deflection (cms)	Relative Abundance -calculated- sen.factor used	Relative Abundance -experimental- sen.factor used	Percent (ratio)
850	9	2.3	1.4	8.3	
860	9	6.5	4.0	23.4	
870	5	3.1	31.5	104.5	
880	4	2.8	140.0	428.0	
888	4	5.4	270.0	826.0	
890	4	5.5	275.0	841.5	
(40) 893	3	3.2	322.0	980.0	99.40
900	5	1.6	16.2	53.8	
910	5	0.7	7.0	23.6	
920	6	0.5	2.6	9.0	
930	7	0.5	1.2	5.0	
(38) 935	7	0.55	1.3	5.5	0.05
940	8	0.5	0.6	3.0	
948	9	0.6	0.4	2.2	
970	9	0.6	0.4	2.2	
(36) 985	9	1.5	0.9	5.4	0.55
1000	9	0.3	0.2	1.1	
1010	9	0.25	0.15	0.9	



sired mass to the collector slit. This can be accomplished either by reducing the accelerating potential (E) and the flux density (B) proportionately, or by increasing them proportionately. The limitation is usually with the range of potentials available. It is a point to be demonstrated that optimum resolution is to be had if (B) is so chosen that the smallest mass desired is given a radius of curvature (R) so as to bring it to the collector by the highest accelerating potential (E) available.

Differentiating equation #3 with respect to m and E, we have

$$dm = k'' \, dE / E \qquad \text{(Eq.\#14)}$$

Dividing this equation by equation #3 and rearranging, we have

$$\frac{dE}{dm} = \frac{E}{m} \qquad \text{(Eq.\#15)}$$

Resolution as shown by the experimental data for Neon (Table 5), for Nitrogen (Table 6) and for Argon (Table 7) which are plotted as individual graphs (Plate #14) is actually dE / dm. Mass is interpreted in terms of volts; hence the number of volts per unit mass gives the spread to the peaks for the various isotopes of neon, nitrogen and argon. The lighter masses (about mass 20) as indicated by the graph (Plate #14) have peak separations of approximately 80 volts per unit mass; while the heavier masses (about mass 40) have peak separations of approximately only 25 volts.

Theoretically, equation #15 above substantiates this fact.

Neon (mass 20) ----- $dE/dm = E/m = 1760/20$ or 88.0 volts/unit mass.

Nitrogen (mass 28) - $dE/dm = E/m = 1263/28$ or 45.0 volts/unit mass.

Argon (mass 40) ---- $dE/dm = E/m = 893/40$ or 22.3 volts/unit mass.

In computing the limitation of the instrument with regards to maximum mass that can be analyzed, several assumptions must be made. Included in them is one that refers to the saturation limit of the magnet, which of course can be increased by selecting a better quality of magnetic metal for the pole pieces and yoke. This saturation limit for the present arrangement is approximately 4500 gauss for a coil current of five amperes. Another limiting factor is the range of accelerating potentials available, which presently is 2000 volts.

The most important assumption to be made in discussing the limitation of the instrument centers about the desired resolution required to give acceptable accuracy in measurements. With the quality of peak definitions as obtained, measurements can be made accurately with a resolution of ten volts per unit mass. Hence the range of accelerating potentials only remains to be considered, and the limiting mass to be analyzed is given by equation #15

$$dE/dm \simeq 10 = 2000/m$$

where the maximum mass is approximately 200 ($O^{16} \cong 16.000$).

However, if the range of flux densities available also are to be considered in the limitation of the instrument, equation #3 can be solved for the maximum mass (m) by substituting 10m for E and 4500 gauss for B. Under these restrictions: resolution of ten volts per unit mass, acceleration potential limit of 2000 volts and a flux density capacity of 4500 gauss, the maximum mass for analysis is 150 ($O^{16} \simeq 16.000$).

Methods of Measuring Ion Beam Intensities.

If all ion beams are to be measured by the FP-54 electrometer circuit, panel control switch #11 (Plate #12) is in the left position. This isolates the inverse feedback amplifier and establishes the proper conditions for the measurement of those ions only that fall on the #2 collector.

When the ion beam is cutoff, the panel control switch #2 is in the north position. Effectively, a potential of several hundred volts is applied to one side of a halved plate (Plate #5) in order to deflect the beam of ions from their true course through the slit apertures in the accelerating system of plates.

The spot-light of the galvanometer is turned on by clicking upwards a toggle switch which is in the main lead of the 115 volt A.C. line and found on the right side of the electronic cabinet. Panel switches #12 and #13 control the D.C. for the filaments and the A.C. for the filament transformer respectively. Panel switch #7 controls the D.C. for the FP-54 only and is regulated by the potentiometer K.

The relative position of the spot light of the galvanometer is controled by the three potentiometers L, P and Q which are in the plate circuit; the latter one is a vernier adjustment. The choice of sensitivity of the galvanometer depends on the expected intensity of the ion peak in question. In the last analysis, the ten positions of the sensitivity selector switch S is to keep the deflection of the galvanometer within the range of the frosted glass scale. By adjusting Q this deflection interval can be kept near the center of the flat scale where the length of the chord approaches the length of the arc swept out by the galvanometer.

When all is in readiness, the panel switch #2 (ion beam cut-off) is clicked downwards and any deflection of the galvanometer is a measure of the intensity of that beam falling on #2 collector. This measurement could be converted into an absolute value by calibrating the galvanometer deflection in terms of a known potential impressed across the high grid resistor of the FP-54. However, a relative value of this deflection is sufficient for a comparison with the deflection effected by another mass peak brought to the #2 collector by its proper ion accelerating potential. The abundance ratio indicated by the relative intensities of the two ion peaks is usually about 100 to one; these correspond to ion currents of the order of 10^{-11} and 10^{-13} amperes.

This procedure is not limited to isotopic analyses. It can be followed for the measurement of the relative abundance of any gas having a mass within the range of the instrument. Moreover, this type of analysis can be placed on an absolute basis without calibrating the galvanometer as suggested above. A known gas with a known volume and pressure can be introduced in a mixture of gases which is to be analyzed. The relation established with the known gas gives results with absolute values.

If the null method of balancing the two isotopic beams is to be used, the inverse feedback circuit is conditioned by the panel filament switch #8. After thermal equilibrium has been established, the circuit is adjusted so that the current in the ion ratio potentiometer will read zero on meter M_7. This adjustment is effected by the panel control switches #9 and #10 and by the panel potentiometers M, N and T, the latter being a vernier control. The test for actual

zero current through M_7 with the ion beam cut off is to vary the ion ratio setting while #11 switch is in the right position. If the galvanometer remains motionless, the zero is established.

While control #11 isolates the inverse feedback amplifier, panel switch #2 permits the rare isotopic beam to fall on the #2 collector and create a deflection in the galvanometer. At the same time the abundant isotopic beam falls on the #1 collector and meter M_7 indicates a flow of current through the ion ratio potentiometer. The tap of this ten-turn precision potentiometer can be varied so that when control #11 connects this output to buck the input of the FP-54, the galvanometer will return to its original position for no deflection. The relative abundance of the isotopic masses can be calculated immediately by application of the factor constant in equation #11b.

The use of this null method is limited to the measurement of isotopic masses of the same chemical substance. This limitation is inherent in the design of the dual collector system as previously explained. There may be a further restriction due to some impurities that might be present and have a mass that would fall within the range of the #1 collector plate. This range includes all masses that are within one twelfth of that mass. For example, when CO_2 (45) is measured by #2 collector, the masses impinging on #1 range from 42 to 44; hence the dual method will not correctly measure a ratio between mass 45 and mass 44, if there is an impurity present of mass 42 or 43. This difficulty can be eliminated by bringing masses 44 and 45 successively to the single collector #2.

CHAPTER IV.

DISCUSSION OF EXPERIMENTAL RESULTS.

The Background Count.

In isotopic tracer work, one is more interested in the differences of isotopic abundance than in absolute values. And since all analyses are performed under identical conditions, the correction for background count is not a distinct problem. However, a correct understanding of the background has a decided advantage in dealing with isotopic tracer measurements.

Sometimes it is stated that the systematic background associated with mass spectrometers is somewhat analogous to that encountered in radioactivity detectors (Rf.#18-p.11). This is not true. The question of background in these two cases is strikingly different. As previously mentioned, a manifold of 10^{-6} mm Hg pressure and at room temperature has a density of 3.24×10^{10} molecules per cubic centimeter. These molecules form the residual gases in the instrument and are all potential ions. Some molecules like water vapor are adsorbed on the walls, while others like nitrogen, oxygen and carbon monoxide are admitted through leaks; but the most important source of residual gas is still present when all leaks have been closed and all adsorption overcome by repeated baking or torching. That source is the gas pressure itself which furnishes an enormous number of potential ions at very low pressures.

Therefore the basic source of background in a mass spectrometer is something within the instrument, while in radioactivity detectors the source of background is entirely from without. Vacuum pumps

work on a percentage basis and represent an infinite series in their efforts to create a perfect vacuum. Hence the background question of a mass spectrometer seems to defy remedy, just like in radioactivity detectors (e.g. Geiger Counters), where cosmic rays and some short gammas penetrate the thickest lead shields. But just when the analogy seems to apply, it fails.

In a mass spectrometer the difficulty is not so much the residual gas pressure, but rather with the unwanted kinds of molecules present that create the pressure. Pressure causes interference only when intermolecular collisions occur; types of molecules of mass equal to that being measured introduces spurious readings. Therefore, if the sample gas is permitted to enter the manifold for a period of fifteen minutes before measurements are made, the residual molecules are flushed out of the system and eliminated by the pumps. With the elimination of these molecules, the so-called background count has given way to the actual count to be measured. It is quite different in the operation of Geiger counters, where the background count is superimposed on that due to the radioactive material being analyzed.

This comparison was experimentally observed in tests made with Neon, while calibrating the instrument for mass-voltage relation. A background spectrum was taken which included the following data:

Mass	Relative Intensity	
	Background	Neon Introduced
29	3.3 x 100 = 330	7.7 x 5 = 38.5
18	5.1 x 200 = 1020	6.2 x 20 = 12.4

With the operating conditions of the instrument unchanged, Neon gas

(commercial quality) was introduced at a 40 mm Hg pressure through the capillary gas leak. Here a change in the operating conditions was effected, as the pressure in the manifold rose from 7×10^{-6} to 1×10^{-5} mm Hg. This change, however, favors the argument, since an increase in pressure would warrant an increase in intensity. On the contrary, the peak intensities dropped off.

No effort was made to obtain this reduction of background. Rather an effort was made while plotting the results to explain the phenomenon as to why the background should have been reduced. The only acceptable reason was as explained above: the gas (Neon) introduced flushed out the residual gases which made up the gas pressure. The water vapor measured was either a dilution of that previously present, or was an impurity in the Neon sample. The explanation for mass 29 peak reduction is partly that as given for water vapor, except that mass 29 stems from two substances: $N^{14}N^{15}$ and $C_2H_5^+$. This latter, ethane$^+$, contradicts the assumption made above since its source is from within the system and comes as a product of the cracking of pump oil. Hence its build-up partial pressure would likely be reduced but not its source.

Isotopic Analysis of Neon and Argon.

The analyst is primarily interested in the relative abundance of isotopic masses. In biological tracer work, the emphasis is placed on changes in the abundance ratio between two isotopes. In order to determine this ratio change, measurement is first made of the normal abundance; then some of the same material enriched in one of its rarer masses is synthesized chemically and studied. The excess tracer

isotope present over the normal quantity is then the object of the investigation.

Since Neon and Argon gases were used to calibrate the instrument, the data recorded (Tables 5 and 7) was also used to check the measurement of the relative abundance of the isotopic masses of each of these gases with accepted values. The measure of abundance of each mass is read on the galvanometer as an electrical current of the order of 10^{-12} ampere. This deflection is then converted to a numerical value on an arbitrary scale by the sensitivity factor (Table 1.). The resultant values are compared for the calculation of relative abundance.

With commercial Neon gas introduced in the mass spectrometer, the peaks measured at mass 22 was 252.0, at mass 21 was 10.2 and at mass 20 was 3428.0. In addition to these peak intensities, there are masses of the same value that appear in the instrument at the 11, $10\frac{1}{2}$ and 10 mass positions due to some molecules of these isotopes of Neon becoming doubly ionized. These values are very small and can be ignored. There is, however, an added difficulty encountered if an attempt is made to measure them. The resolution is worse due to the additional kinetic energy acquired when a molecule explodes upon being dissociated; this broadens the base of the peak. Besides, the intensity for very small masses falls off by approximately one tenth in this region; no acceptable reason has been advanced for the explanation of this phenomenon.

Accepting these three masses (20, 21 and 22) as the principal constituents of Neon, the following per-centages were calculated:

NEON	MASS NUMBER (O^{16} = 16.00)	RELATIVE ABUNDANCE (with total mass)	ATOM PER CENT -experimental-	-published-
	20	3428.0/3690.2	92.69	90.00
	21	10.2/3690.2	0.28	0.27
	22	252.0/3690.2	7.03	9.73

Similar treatment of Argon was made and the following data recorded:

36	5.4/985.5	0.55	0.307
38	(0.5)/985.5	(0.05)	0.061
40	980/985.5	99.40	99.632

These values are in general agreement with the normal per cent abundance as found in the literature.

An absolute determination of the relative abundance of isotopes is subject to several slight errors that have their source either in the difference in molecular weights or in the ion currents which differ by a factor of 100 or more. The discussion of molecular flow previously considered when constructing the capillary gas leak, was based on this question of difference in molecular weight. There is also a difference in rate of flow out of the manifold for different gases due to fractionation in the pumping system. These difficulties make it impossible to maintain representative gas samples for absolute measurement.

The source of error due to the ion currents centers mainly within the amplifier circuit. The amplifier grid resistor is very high (4.2×10^{10} ohms) and depends in a small degree for its resistance value upon the current passing through it. The ratio of voltages,

TABLE 8. ISOTOPES OF ORGANIC ELEMENTS.

ELEMENT	PER CENT	HALF-LIFE	ELEMENT	PER-CENT	HALF-LIFE
CARBON			NITROGEN		
10	-----	8.8 sec.	13	-----	9.93-min.
11*	-----	20.5 min.	14	99.62	-----
12	98.9	-----	15**	0.38	-----
13**	1.1	-----	16	-----	8 sec.
14*	-----	5100 yrs.			
SULFUR			OXYGEN		
31	-----	3.2 sec.	15	-----	126 sec.
32	95.1	-----	16	99.76	-----
33	0.74	-----	17	0.041	-----
34**	4.2	-----	18**	0.20	-----
35*	-----	87.1 days	19	-----	31 sec.
36	0.016	-----			
HYDROGEN					
1	99.98	-----			
2**	0.02	-----			
3*	-----	31 yrs.			

(*) Radioactive Tracer

(**) Stable Isotopic Tracer.

therefore, impressed on the amplifier may be in error by a few per cent, if the two ion currents differ by a factor of 100. Another consideration in regard to the amplifier's influence on the accuracy of the instrument is its stability. This is critically important during the measurement of the abundance of a relatively rare isotope. The lack of good resolution and absence of peak definition usually accompany ion currents that differ widely. This is evident in the data listed (Table 7) for mass 38 of Argon. Its peak is feebly perched on the slope of the intense mass 40, which differs by a factor of approximately 2000. This could be partially remedied by selecting a magnetic setting so that the Argon masses would be brought to the collector at a higher potential (from 893 to 1900 volts) where the resolution is twice as good.

CHAPTER V.

PREPARATION OF NITROGEN SAMPLES.

Selection of Nitrogen 15.

"In biological research it is often preferable to use a stable isotope rather than a radioactive one, to avoid the danger of damaging the organism by radiation effects." (Rf.#19) This statement of Dr.Kamen expresses a preference where both types of isotopes --- radioactive and stable -- exist and are both practical and available. There are instances where only stable isotopes meet this demand. The bulk of organic compounds centers around four or five elements. A glance at their radioactive and stable isotopes (Table 8) is revealing.

PLATE #15. AUXILIARY SYSTEM FOR PREPARATION OF GAS SAMPLES.

1 - Tapered Joint (permitting bulb #10 to rotate).

2 - Ball Joint (dismantling of chemical reaction vessels).

3 - Stopcock (flushing system with air).

4 - Gas Sample Bulb.

5 - Electric Blower (Air cooled diffusion pump).

6 - Glass Oil Diffusion Pump.

7 - Lead of Fore Pump.

8 - Control Switch (fore pump).

9 - Control Switch (diffusion pump).

10 - Chemical Reaction Vessel.

11 - Companion Chemical Reaction Vessel.

12 - Dewar Flask and Cold Trap.

13 - Barometric Gauge.

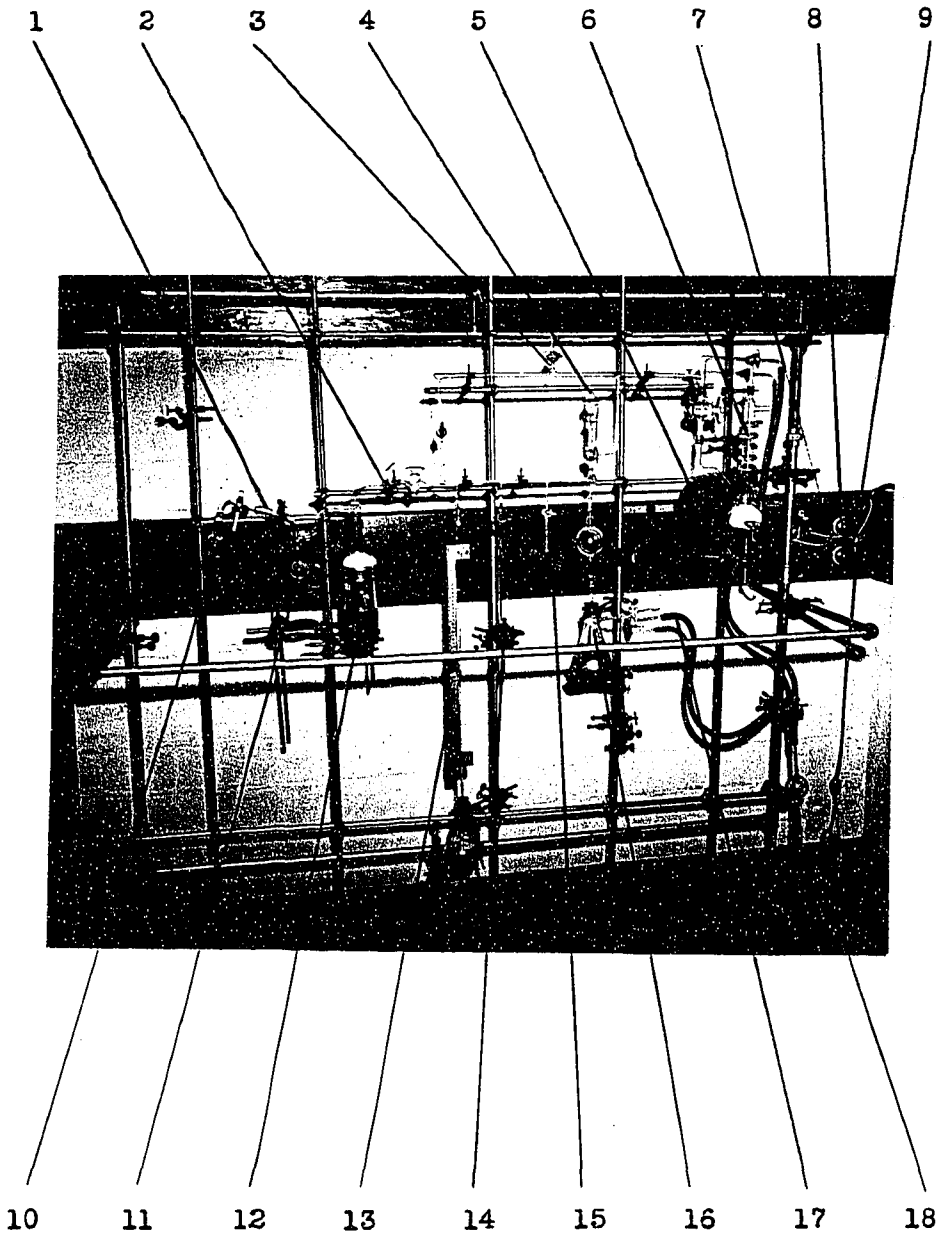
14 - Auxiliary Cold Trap.

15 - Tapered Joint (additional gas bulb).

16 - Toepler Pump.

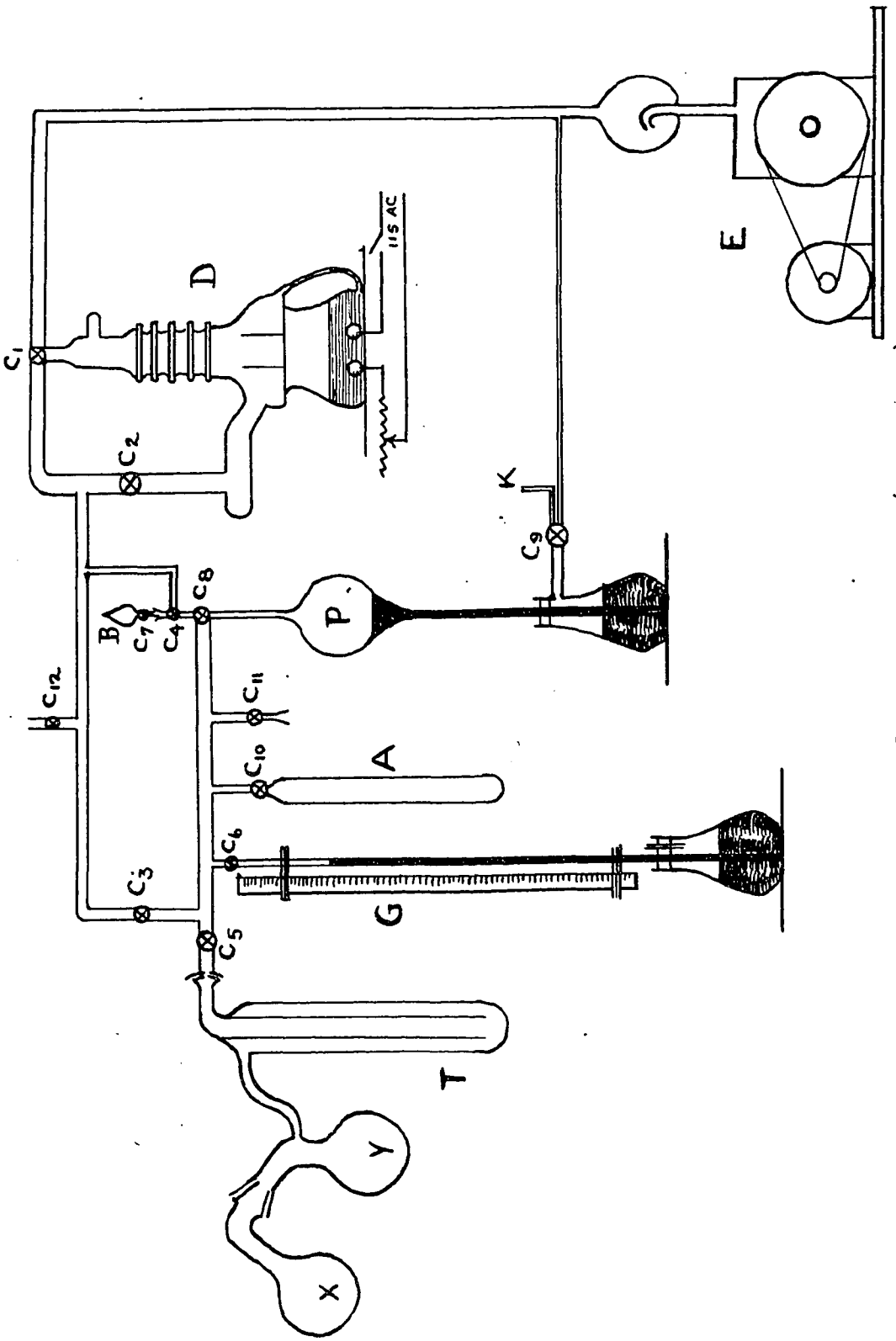
17 - Lead of Fore Pump.

18 - Rotary Fore Pump.



AUXILIARY SYSTEM FOR PREPARATION OF GAS SAMPLES

(Plate #15)



AUXILIARY SYSTEM FOR PREPARATION OF GAS · SAMPLES. (Plate # 16)

There are no suitable radioactive isotopes for Hydrogen, Nitrogen and Oxygen. The half-life pattern for the radioactive isotopes listed is either too long or too short, so that it is difficult to conduct experiments with them. Besides these intrinsic limitations of radioactive isotopes, there is the general health hazard associated with their use. "All the radiation emitted from radioactive isotopes, either as light quanta or charged particles, have energies many times higher than necessary to rupture individual chemical bonds or to initiate chemical reactions." (Rf.#20) Dr.Bale emphasizes that the syndrome of radiation injury has two characteristics that combine to make protection against it difficult. Very small amounts of energy that escape our physical senses is sufficient to injure or kill biological tissue; while these damaging effects do not appear until the injury is irreparable. Thus research work with radioactive isotopes should be conducted in closed or even vacuum tight systems (Rf.#18) because the hazard extends as well to gases, vapors and escaping dusts.

The principal tools of the biologist in tracer work with stable isotopes are H^2, C^{13} and N^{15}. For our particular application, N^{15} was selected. Nitrogen gas (N_2) has been found to be the most satisfactory form to use for analyzing this element. This determination of the concentration of N^{15} of the nitrogen of an organic substance involves two steps. The conversion of the organic nitrogen to ammonia and then the oxidation of the ammonia to elementary nitrogen molecule. This twofold operation requires separate equipment and distinct from the mass spectrometer.

Function of the Auxiliary Gas Sample Assembly.

A mass spectrometer system is divided into two manifolds, one the analyzer (Plate #3) and the other (Plate #11) to introduce the sample. Unless the gas to be analyzed can be easily generated, a separate manifold (Plates #15 and #16) is constructed.

This auxiliary system is evacuated by a single stage (Distillation Products- Type G-4) oil diffusion pump (D) using S-Octoil and backed by a small rotary pump (E). The forepump is started with stopcocks C_2, C_3 and C_{12} closed, C_1 closed to the diffusion pump, and C_4 closed to the pump system. After the barometric gauge (G) indicates a few millimeters Hg pressure, the diffusion pump (D) is started and stopcocks C_1 is closed to the system and C_2 is opened.

After the nitrogen of the organic material has been converted (Rf.#21) to ammonia by the Kjeldahl process, it is absorbed in dilute hydrochloric acid. It is this ammonium chloride concentrated that is introduced in flask (Y). A hypobromite solution is prepared for the companion flask (X), so that upon revolving (X), the chemical reaction will be initiated and the ammonia oxidized to molecular nitrogen.

The system is completed by connecting the small (15 cc) flask (B) in which the nitrogen sample will be transported to the mass spectrometer. The trap (T) is cooled with dry ice and acetone mixture. With parts (X), (Y) and (B) assembled, stopcocks C_3, C_4 and C_5 are opened. As the mercury rises in the Toepler pump (P), stopcock C_9 is opened to the forepump, so as to equalize the pressure on both of the mercury levels.

The test for a suitable vacuum is made by closing stopcocks C_4 and C_8 so as to isolate the Toepler pump. Stopcock C_9 is then so oriented as to admit atmospheric pressure slowly through the capillary tube (K) to the lower mercury level. If the mercury rises to the top of the flask (B), the vacuum is suitable for the chemical release of nitrogen gas. The mercury level is lowered in the Toepler pump by manipulating stopcock C_9 so as to reduce the pressure on the lower level of mercury. (NOTE: While this is being done, stopcock C_1 must be closed to the rotary pump.)

The evacuating system is isolated from the rest of the apparatus by closing stopcock C_3. Then the Toepler pump is connected to the rest of the system by opening stopcock C_8. The hypobromite solution in (X) is now introduced in flask (Y) containing the ammonia. Molecular nitrogen is liberated by the oxidation reaction

$$2\ NH_3 + 3\ NaOBr \longrightarrow N_2 + 3\ H_2O + 3\ NaBr$$

The water vapor is trapped in (T) while nitrogen gas fills the Toepler bulb. When the chemical reaction is completed, stopcock C_5 is closed, and the Toepler pump again isolated by turning stopcock C_8. By raising the mercury level in (P), the nitrogen gas can be forced into bulb (B). When the mercury level reaches the height of stopcock C_7, the gas in (B) has a volume of 15 cc and a pressure of approximately 10 cm Hg. (This approximation is based on a barometric pressure of 740 mm Hg.) Bulb (B) is sealed off by stopcock C_7. By lowering the mercury level and opening stopcock C_8, the procedure can be repeated by additional strokes of the Toepler pump until practically all the nitrogen has been forced into (B).

The chamber (A) makes the system practical for carbon dioxide gas samples. By means of liquid air, all vapors are first trapped in (T); then the liquid air is transferred to (A), while (T) is cooled with dry ice. Under these conditions, the carbon dioxide gas first trapped in (T) will sublime over to (A), where it can be isolated by stopcock C_{10} while the rest of the system is evacuated of all remaining gases and vapors. Stopcock C_{12} serves both as a means of quickly breaking the vacuum and as a means of piping tank oxygen or nitrogen into the system. Stopcock C_{11} and its 7/25 standard tapered joint is a facility for another flask. If enough gas is produced for more than one sample, a new bulb (B) can be connected and the volume above stopcock C_8 evacuated by manipulating stopcock C_4. However, if a different sample is to be prepared, the system is contaminated and must be flushed out with air. This wipes out the instrument's memory of the previous sample.

The gas sample bulb (B) now becomes part of the gas assembly (Plate #11) of the mass spectrometer. The forepump (F) is operated until the Bourdon gauge (M), having a scale range of 0-50 mm Hg, reads about one mm Hg pressure, when the single stage oil diffusion pump (H) is started. This pump has a limit of 10^{-5} mm Hg; however, when the Pirani gauge (R) reads a few microns, the gas system is isolated by C_{22}.

If the instrument is to be checked with one of the calibrating gases, a volume of He, Ne or A is trapped between C_{24} and C_{25} and then admitted to the special Toepler pump (J). The gas pressure is read on gauge (M) and introduced into the mass spectrometer through the capillary leak (L) by manipulating stopcock C_{23}. After calibration, the system is pumped out and the nitrogen sample in bulb (B) is admitted to (J) by opening stopcock C_7. The pressure is adjusted by expanding or con-

tracting the sylphon bellows (W) which changes the oil (S-Octoil) level in (J). The gas to be analyzed is then introduced into the mass spectrometer as before.

CHAPTER VI.

INVESTIGATION OF NITROGEN IN CHLORELLA VULGARIS BEIJERINCK

Preparation of the Algae.

In order to demonstrate biological tracer technique with the stable isotope of N^{15}, an organism CHLORELLA VULGARIS Beijerinck was selected. These algal cells were obtained by Dr. V. M. Diller from a pond in Loveland (Ohio), isolated in a pure culture and identified at the Natural History Museum in Chicago.

The medium used to grow the algae was Moore's modification of Beijerinck's salt solution (Rf.#22) with 1% glucose added:

$K H_2 P O_4$ (40 mg)

$Mg S O_4 \cdot 7 H_2 O$ (40 mg)

$Ca Cl_2 \cdot 2 H_2 O$ (20 mg)

$Fe S O_4 \cdot 7 H_2 O$ (0.06 mg)

$N H_4 N O_3$ (0.1 gm)

Anhydrous glucose (10.0 gm/ liter)

pH adjusted to 7.0

Three liters of this mediumwereput in each of two four-liter serum bottles; the one contained medium with the labeled N^{15} in $N^{*}H_4 NO_3$ (Eastman Kodak Co. #C 917241) and the other contained medium with the normal N^{15} in $NH_4 NO_3$.

Table 9.

NOT LABELED						LABELED					
BACKGROUND			GAS SAMPLE			BACKGROUND			GAS SAMPLE		
Medium Before						Medium Before					
28	4.8 x 33.65 --	161.4	28	3.7 x 153 ---	566.0	28	9.4 x 33.65 -	316.0	28	11.2 x 33.65 -	376.5
29	2.5 x 33.65 --	84.0	29	2.4 x 33.65 -	80.7	29	2.9 x 33.65 -	97.5	29	6.8 x 33.65 --	228.7
30	0.1 x 1.0 ----	0.1	30	0.1 x 1.0 ---	0.1	30	0.1 x 1.0 ---	0.1	30	5.7 x 10 -----	57.0
Algal Cells						Algal Cells					
28	9.2 x 33.65 --	309.0	28	8.5 x 306 ---	2600.0	28	10.3 x 33,65	346.5	28	430.5 & 428 -	429.0
29	2.9 x 33.65 --	97.5	29	2.8 x 33.65 -	94.2	29	9.3 x 10 ----	93.0	29	9.5 x 33.65 --	319.3
30	0.1 x 1.0 ----	0.1	30	0.1 x 1.0 ---	0.1	30	0.1 x 1.0 ---	0.1	30	11.4 x 18 ----	205.5
Medium After						Medium After					
28	9.2 x 33.65 --	309.0	28	5.5 x 153 ---	841.0	28	4.1 x 153 ---	627.0	28	4.0 x 153 ----	612.0
29	2.9 x 33.65 --	97.5	29	3.5 x 33.65 -	117.8	29	2.9 x 33.65 -	97.5	29	8.4 x 33.65 --	282.5
30	0.1 x 1.0 ----	0.1	30	0.1 x 1.0 ---	o.1	30	0.1 x 1.0 ---	0.1	30	50.7,49.8,49	49.8
Total Nitrogen Check by Kjeldahl Method						$NH_4^*NO_3$ Salt					
Medium Before - 5.4(mg)%			Medium* Before 5.6(mg)%			28	4.8 x 33.65 -	161.4	28	3.9 x 153 ----	597.0
Algal Cells --- 7.5(mg)%			Algal Cells* - 7.5(mg)%			29	2.5 x 33.65 -	84.0	29	3.3 x 153 ----	504.0
Medium After -- 3.3(mg)%			Medium After* - 3.3(mg)%			30	0.1 x 1.0 ---	0.1	30	7.6 x 18 -----	136.8

The atom per cent of Normal Nitrogen is N^{14} (99.62) and N^{15} (0.38) while the atom per cent of Labeled Nitrogen is N^{14}(38.5) and N^{15} (61.5).

The liquid inoculum culture was grown in the same medium as above with normal NH_4NO_3. The cell count of a five day culture used for inoculation was 8.6×10^6 per ml. Two ml inoculum was added to each of the above two four-liter serum bottles, and incubated at 21° C for twelve days with continuous radiation from four 40 watt white fluorescent lamps. At the end of this time interval, the cell count of both bottles was 0.55×10^6 per ml, with a pH of 3.1. The algal cells were removed from the medium of each of the two four-liter bottles by centrifuging, washed with distilled water and lyophilized.

The nitrogen of 25 mg samples of each of these cells was then converted to ammonia by the Kjeldahl process using mercuric sulphate as recommended by Dr. Rittenburg (Rf.#21).

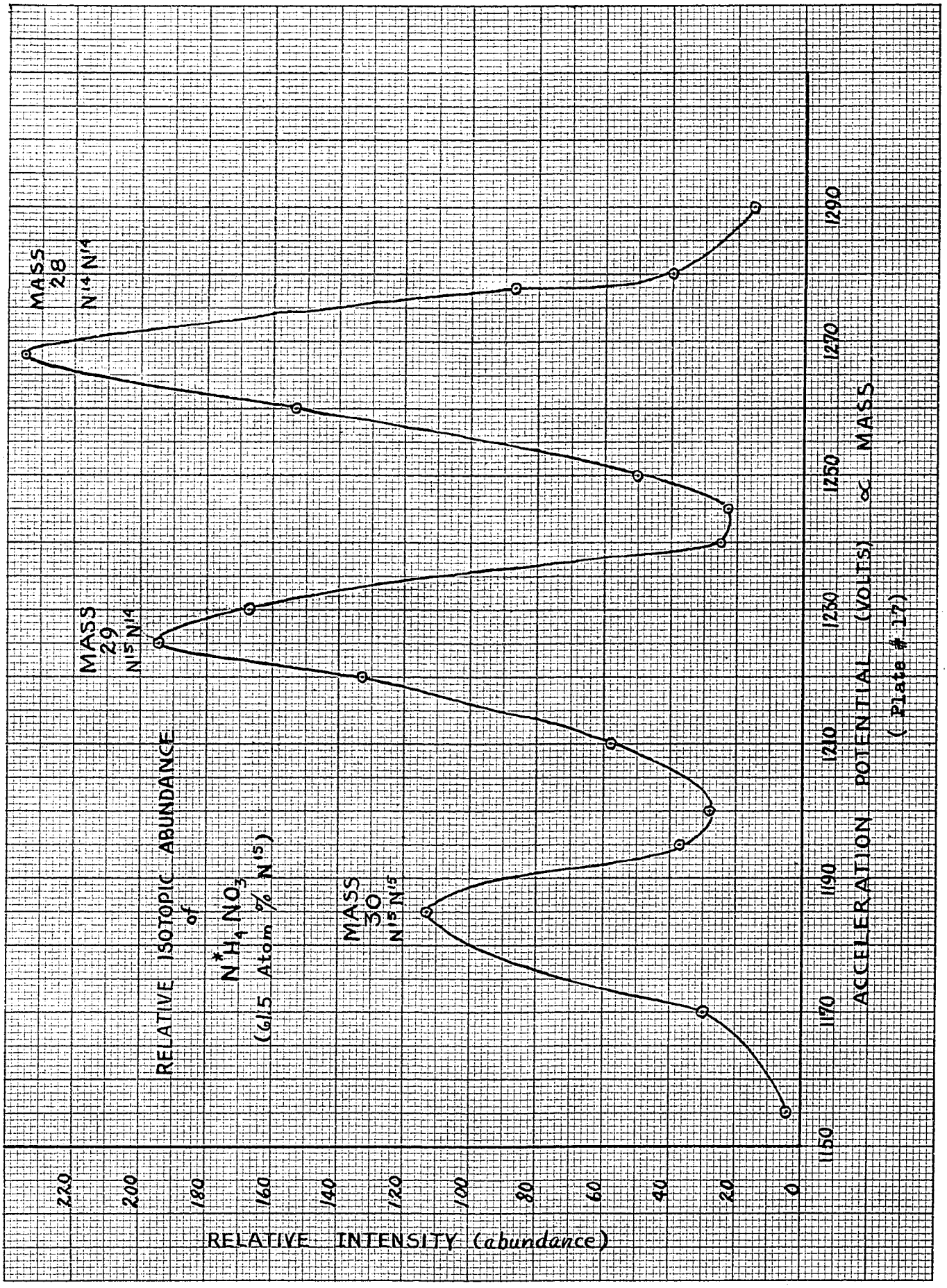
The ammonia obtained from the two samples was oxidized by a hypobromite solution (Rf.#21) to obtain molecular nitrogen as described above (page 76).

The relative abundance ratios for $N^{15}N^{15}/N^{14}N^{14}$ were then made with the mass spectrometer and the results obtained as recorded in Table 9.

Since the relative abundance of both normal and enriched nitrogen is given in terms of atom per cent, it was necessary to find the relative abundance of the labeled salt ($N^*H_4NO_3$) in the same terms as determined for the algal cells. This data deals with molecular nitrogen (N_2) and as such is subject to three different mass combinations, namely: 28 ($N^{14}N^{14}$), 29($N^{14}N^{15}$) and 30 ($N^{15}N^{15}$). The cal-

Table 10. Isotopic Spectrum of Nitrogen in $N^*H_4 NO_3$

NITROGEN (mass)	(volts)	Sensitivity Factor	Deflection (cms)	Relative Abundance (Arbitrary Scale)
	1155	7	0.3	3
	1170	7	3.0	30
30	1185	7	11.3	113
	1195	7	3.7	37
	1200	7	2.8	28
	1210	7	5.8	58
	1220	6	7.4	133
29	1225	6	10.8	194.5
	1230	6	9.3	167.5
	1240	6	1.4	25.2
	1245	6	1.3	23.4
	1250	6	2.8	50.5
	1260	6	8.5	153
28	1268	5	7.0	235.5
	1278	5	2.6	87.5
	1280	6	2.2	39.6
	1290	6	0.8	14.4
32	1110	6	0.5	9.0
18	1965	6	11.8	212.5



culated ratios from experimental data taken were made on the basis of the ratio of $N^{15}N^{15}/N^{14}N^{14}$ because other substances present in the mass spectrometer have significant intensity for mass 29.

To obtain this control ratio of masses 30/28 from both the enriched and normal salt (NH_4NO_3) used in the growth of the algae, the ammonia was first distilled off and then oxidized by the hypobromite solution as before. Titration checks were made on the distillation procedure to insure against dilution by nitrogen from any unknown source; satisfactory agreement was obtained within one tenth of a mg.

The isotopic spectra of these values were:

Normal NH_4NO_3 -------	28 - 4.8 x 153	735	
	29 - 8 x 6	48	
	30 - (0.1)	(0.1)	
Normal Cells ---------	28 - 8.5 x 306	2600.0	
	29 - 2.8 x 33.65	94.2	
	30 - (0.1)	(0.1)	
			RATIO $\frac{N^{15}N^{15}}{N^{14}N^{14}}$
Enriched $N^*H_4NO_3$ in N^{15} ----	28 - 7.0 x 33.65	235.5	
	29 - 10.8 x 18	194.5	0.480
	30 - 11.3 x 10	113.0	
Cells Grown with Enriched $N^*H_4NO_3$ -----	28 - (430.5) & (428)	.429.	
	29 - 9.5 x 33.65	319.3	0.478
	30 - 11.4 x 18	205.5	

The peaks of the isotopic spectra of the enriched ammonium nitrate salt were scanned with the mass spectrometer and the data recorded in Table 10 and plotted on Plate #17 to demonstrate both resolution and definition.

CONCLUSIONS

The nature of the nitrogen supply is a chief concern in the preparation of the medium for cell growth. Nitrites are eliminated; nitrates and ammonium salts are of interest. In 1926 Dr. Pringsheim stated (Rf.#23-p.36) that no chlorophyll-containing organism had so far been found which could not utilize either type of inorganic nitrogen. In 1946 he adds that such a conclusion is still not in conflict with any more recent experience. "When both ammonia and nitrate are present in the form of NH_4NO_3, the ammonia only is usually absorbed, or it is taken up preferentially" (Rf.#23-p.36).

The presumptive evidence obtained by the mass spectrometric analysis that the nitrogen in the organism comes primarily from the ammonium radical (NH_4) seems to confirm this statement. Since the isotopic ratio of $N^{15}N^{15}/N^{14}N^{14}$ in the salt (NH_4NO_3) enriched in ammonium N^{15} was 0.480 and remained identical (within experimental error) 0.478 in the algal cells grown in the enriched salt, any nitrogen taken from a different source would infer that the algae exercised a preference for nitrogen in the exact same ratio as contained in the enriched salt.

In the future it is planned to do a complete nitrogen balance experiment which will require a host of investigations with a series of mass spectrometric analyses predicated of each. The object of interest is not merely to trace nitrogen to the cell but also to trace its course within the organism.

FINIS

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