A STUDY OF THE ULTRA-VIOLET ABSORPTION SPECTRA OF CERTAIN ORGANIC COMPOUNDS

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CONTENTS

INTRODUCTION

The study of the ultra-violet absorption spectra of organic compounds may be considered to begin in 1879 with the publication by Hartley and Huntingdon of the first paper on the connection between the absorption spectra and constitution of various chemical substances. The spark spectrum of an alloy was photographed through a solution of the required chemical by means of a quartz spectrograph. The oscillation frequencies of the edges of the absorption band at a number of different concentrations were plotted against the concentrations of the solution. The resulting curves were used as an aid in determining the structure of unknown molecules by comparison with the curves obtained from substances of known constitution.

Since then a vast amount of work has been done. Studies in absorption spectra have been applied to obtain information on the intimate structure of diatomic and the simpler polyatomic molecules. For the larger polyatomic molecules spectroscopic research has been much more empirical. The selective nature of absorption affords a ready means of detection of small amounts of substances in solution, therefore its value lies in its use as an adjunct to biochemical and organic methods of study. The recent developments in knowledge concerning physiologically active substances, the vitamins in particular, owe much to spectral absorption curves in

the two directions of elucidating photochemical changes and in providing characteristic "labels" for substances, the existence and importance of which rests on biological methods of experimentation. The main service of absorption spectra lies in the possibility of supplementing the physiological description of an unknown substance by means of a physical criterion capable of aiding in identification and analysis.

The application of ultra-violet absorption spectroscopy to the study of blood in an attempt to detect chemical differences between normal and pathological blood as a means of diagnosis of disease was started in 1930 by P. A. Macdonald. In 1931 S. G. T. Bendien published a thesis entitled "Spezifische Veranderungen des Blutserums."(1) In very brief outline Bendien's work is as follows:

Blood serum is treated with a mixture of sodium vanadate and acetic acid. In normal blood with a vanadate acetic acid solution below a certain strength, no precipitate is occasioned beyond a slight milkiness, but with the blood of patients affected with certain diseases, of which carcinoma is one, the same strength of solution yields a precipitate.

The precipitate is filtered with a glass filter, dried, weighed and dissolved in 2% Na_2CO_3.

The solution is then examined with the ultra-violet spectrophotometer. According to Dr. Bendien's statement and thesis, there are definite specific differences in the absorption curves which permit the various diseases to be distinguished, and in particular permit carcinoma to be distinguished with certainty.

The work was immediately investigated in the London Hospital and in the Cancer Hospital, London. The general conclusions of these institutions and of a large number of other investigators were:(2) the chemical test is not specific, the absorption curves are the same in a variety of diseased conditions including carcinoma, and that the curves are unstable, changing with time from the tuberculosis curve of Dr. Bendien to the carcinoma curve.

The problem undertaken by the author, as a continuation of the work begun by P. A. Macdonald in 1930, is the fractionation of the blood into a number of simpler components and the examination of these fractions by ultra-violet absorption studies. A specially developed photoelectric spectrophotometer was used, a great deal more sensitive than any at present on the market.

Blood is composed of four types of form elements, (1) red blood cells or erythrocytes, (2)

white blood cells or leukocytes, (3) blood platelets and (4) blood dust or hemoconein, held in suspension in a fluid, blood plasma. These form elements compose about 60% of the blood by weight.

The blood plasma contains the four protein bodies fibrinogen, nucleoprotein, globulin and albumin. Plasma contains about 8.2% of solids of which the protein constituents named above constitute approximately 84% and the inorganic constituents (mainly chlorides, phosphates, and carbonates) approximately 10%. In common with serum globulin the body known as serum albumin seems to consist of more than one individual substance.

If blood is allowed to coagulate the fibrinogen of the plasma is precipitated as fibrin. The fibrinogen-free plasma is known as serum. Besides the constituents already mentioned other bodies which are found in both the plasma and serum are; glucose, uric acid, urea, fat, amino acids, enzymes, lecithin, creatine, carbamic acid, cholesterol and its esters, acetone bodies, paralactic acid, and ammonia. In addition the blood contains a class of substances called hormones. In many pathological conditions certain normal constituents are present in increased amount.

Bendien's work dealt with the protein constituents of the blood serum. Lewis (3) found the

absorption of blood serum in the ultra-violet region to be due to the proteins. Serum albumin, pseudoglobulin, and euglobulin from human serum gave similar curves. They all begin to absorb at approximately wave number 3200, the absorption increases to a maximum at 3600, falls to a minimum at 4000 and then rises sharply. He also examined the absorption of a number of the constituent amino acids of the serum proteins and found the same absorption maximum and minimum in tryptophane and tyrosine. He therefore attributed the absorption of serum proteins largely to these amino acids. Examination of the non-protein part of the serum revealed no selective absorption.

The present thesis deals with the study of blood fractions to which little attention has up to the present been paid. The description of the experimental work is preceded by a discussion in Chapter I of the underlying general principles. Section A deals with the optical phenomena applied to the construction of the spectroscope for the isolation of different wave lengths of light from a heterogeneous beam; B with the laws and theories underlying the use of absorption spectra as a tool of the chemist. The more common photographic method of obtaining ultra-violet absorption curves is discussed in section C and then, following a brief survey of the principles of thermionic tubes in D and photoelectric cells in E, section F concludes with a description of the photoelectric spectro-

photometer used in these investigations.

The experimental work thus far accomplished by the author consists of the isolation and examination of two of the blood constituents. As described in Chapter II a method to isolate the leukocytes was developed and studies made on extracts, in various solvents, of the white cells of tuberculosis patients and normal people. Chapter III deals with studies on the chemicals of the non-protein part of the serum that were soluble in both absolute alcohol and water. This fraction was studied in the case of normal people and hypothyroid patients.

CHAPTER I

THE PRINCIPLES UNDERLYING THE CONSTRUCTION AND USE OF THE PHOTOELECTRIC SPECTROPHOTOMETER

A. The Principles of the Subject of Spectroscopy.

The amount of deviation suffered by a ray of light in passing from one medium to another will depend upon the relative velocities of light in the two media, and the angle at which the ray falls on the boundary surface. For a given medium the relation of the angle of incidence to the angle of deviation is given by Snell's law.

$$\frac{\sin i}{\sin r} = u \qquad \text{---------- (1)}$$

Where i is the angle between the incident ray and the normal to the surface and r is the angle between the refracted ray and the normal. The incident ray, the normal, and the refracted ray, all lie in the same plane.

u is defined as the index of refraction and is a relative term, solely dependent upon the nature of the two media. The term "absolute index of refraction" refers to the value obtained with the specified substance in a vacuum. Measurements on solids and liquids are made in air due to the great difference in velocity of light. Measurements on gases demand consideration of the effect of the air.

Refraction Through Two Boundary Surfaces:

If the surfaces are parallel the direction of the ray of light will not be altered. If the surfaces are not parallel, as with a prism, the deviation will be increased at the second surface. Fig. (1) represents the refraction, by a prism, of light of a single wave length.

The relation between the angles i and r and between the angles i^1 and r^1 is defined by the index of refraction.

$$\frac{\sin i}{\sin r} = \frac{\sin i^1}{\sin r^1} = u$$

~~θ~~ is defined as the angle of deviation and is a minimum when angle i is equal to angle i^1, i.e. when the ray passes parallel to the base. A is defined as the refracting angle of the prism. By the geometry of figure (1) for a single ray at minimum deviation

$$u = \frac{\sin \frac{A+\theta}{2}}{\sin \frac{A}{2}} \quad \text{---------- (2)}$$

The above theory has considered only a single ray of light; the same would hold for a parallel beam, therefore, in practice a collimating lens is used between the source of light and the prism.

The Total Reflection Prism.

If the light ray is considered to pass through the denser medium to the rarer one, e.g. glass to air,

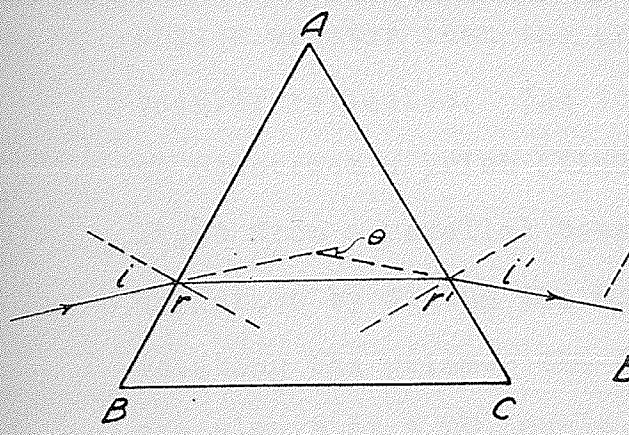


FIG. 1

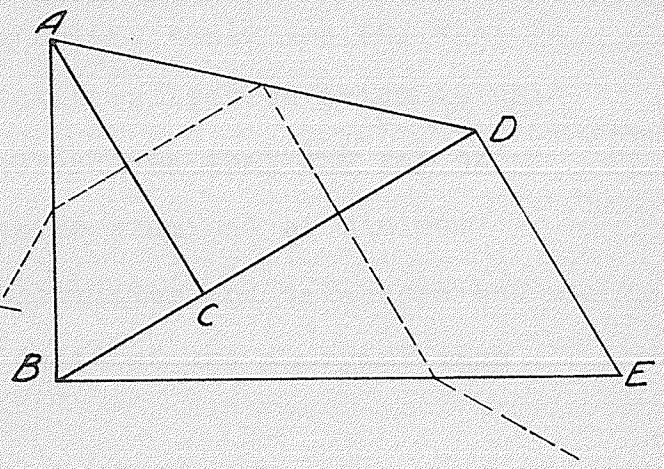


FIG. 2

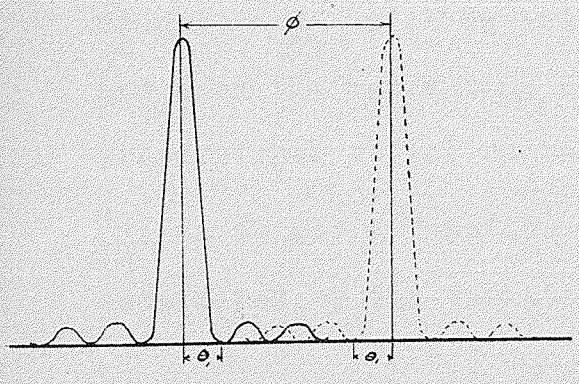


FIG. 3

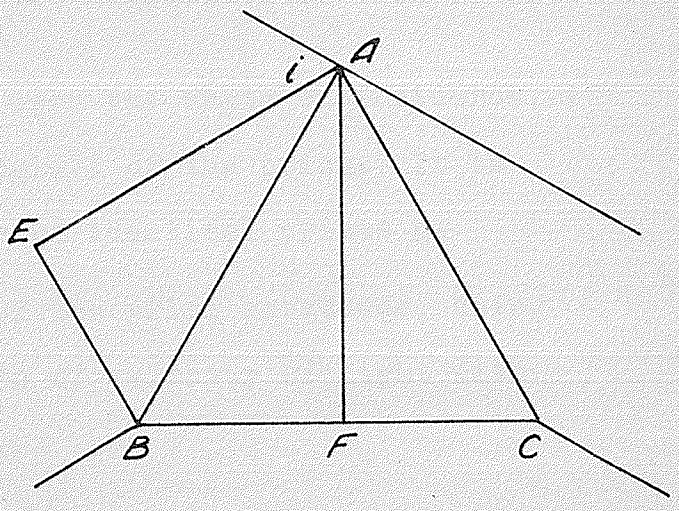


FIG. 4

it follows from Snell's law that for a certain incident angle, called the critical angle, the refracted ray will be at 90° to the normal to the surface. For angles greater than the critical angle the ray will be totally reflected. The total reflection prism is used to construct a prism (figure 2) so that any ray of light from a parallel beam is examined at minimum deviation by fixing the collimator and telescope and rotating the prism. The incident and emergent rays are always at a 90° angle.

Dispersion in a Prism.

In any given substance u varies with the wave-length and the rate of change of u with the wave-length, $\frac{d u}{d \lambda}$ is different for different substances.

Since the angle of deviation θ, is determined by u and the dimensions of the prism we may write.

$$\frac{d\theta}{d\lambda} = \frac{\partial\theta}{\partial\mu} \cdot \frac{\partial\mu}{\partial\lambda} \quad \text{----------} \quad (3)$$

Where $\frac{d\theta}{d\lambda}$ is defined as the dispersion of the substance. $\frac{d\theta}{d\mu}$ will depend upon the angle of incidence, i and upon the refracting angle A of the prism. It is proportional to the number of identical prisms n.

$\frac{\partial\mu}{\partial\lambda}$ is solely a function of the prism substance, being determined by the variation of velocity of light with

wave-length in the substance. The dispersion of a prism may be calculated as follows:

By differentiation of equation (2)

$$\frac{\partial \theta}{\partial \mu} = \frac{2 \sin \frac{A}{2}}{\sqrt{1-u^2 \sin^2 \frac{A}{2}}} \text{ ---------- (4)}$$

This equation will be true only when the indices of refraction of the rays in question vary very little from that of the ray at minimum deviation. In practice the equation is used over wide limits without great error. If u_1, and u_2 be the indices of the extreme rays, then the index of the ray at minimum u_3 should be a mean

$$u_3 = \frac{u_1 + u_2}{2}$$

Whence $$\frac{\Delta \theta}{(u_1-u_2)} = \frac{2 \sin \frac{A}{2}}{\sqrt{1-u_3^2 \sin^2 \frac{A}{2}}}$$

If $\Delta\theta$ is 9° the error is only a few minutes.

A second way of expressing $\frac{\partial \theta}{\partial \mu}$ from equation (2) is

$$\frac{\partial \theta}{\partial \mu} = \frac{2}{u} \tan i \text{ ----------(5)}$$

i.e. as a function of the incident angle.

We may evaluate $\frac{\partial \mu}{\partial \lambda}$ using Hartman's interpolation formulae:

$$u = u_o + \frac{c}{(\lambda - \lambda_o)^a} \text{ ---------- (6)}$$

$$\lambda = \lambda_o + \frac{c}{(\mu - \mu_o)^{\frac{1}{a}}} \text{ ---------- (7)}$$

Where c, μ_o, λ_o and a are constants.

The constants can be calculated for one definite substance if three values of μ with λ are known.

By differentiation of (7)

$$\frac{\partial \mu}{\partial \lambda} = \frac{-c}{(\lambda - \lambda_o)^2} \text{ ---------- (8)}$$

and knowing the constants we may calculate the value of $\frac{\partial \mu}{\partial \lambda}$ for the neighborhood of a known wave length.

A second equation giving u as a function of λ is Cauchy's equation.

$$u = A + \frac{B}{\lambda^2} + \frac{C}{\lambda^3} + \text{ ---------- (9)}$$

Whence $\frac{\partial \mu}{\partial \lambda} = -\frac{2B}{\lambda^3}$ approximately ------ (10)

When the value $\frac{d\theta}{d\lambda}$ has been determined for the medium and angle of a prism, the angular separation between any desired pair of lines may be found. While the ratio $\frac{d\theta}{d\lambda}$ was defined as the dispersion above, the term is often applied to each of the partial ratios, $\frac{\partial \theta}{\partial \mu}$ and $\frac{\partial \mu}{\partial \lambda}$

The Resolving Power of a Prism.

The resolving power of a prism refers to the degree of closeness two spectral lines can have and still be distinguished as two. It is a measure of the narrowness and sharpness of the spectral lines.

In the case of the image of an infinitely narrow slit produced at the focus of a telescope lens,

$$\frac{ae}{m \lambda f} = 1 \text{ ---------- (11)}$$

Where f is the focal length, m a constant, a the linear aperture of the lens, e the linear distance from the principal maximum to the first minimum of the diffraction pattern, and λ the wave-length of the light in question. We may write

$$\frac{e}{f} = \frac{m\lambda}{a} \text{ ---------- (12)}$$

Where $\frac{e}{f}$ is the angular distance from the principal maximum to the first minimum of the diffraction pattern.

In a spectrum each spectral line will be a diffraction pattern of the slit as in Fig. (3) When approached to each other these lines will appear as two until the first minimum of one is in the centre of the principal maximum of the other. i.e. when ~~θ~~ $=$ e Therefore to separate or resolve two rays of mean wave length λ the least possible angle between them:

$$d\,\theta = \frac{m\lambda}{a} \text{ ---------- (13)}$$

With an infinitely narrow slit.

Considering a properly constructed spectroscope in which the beam of light from the collimator just completely fills the prism. Fig. (4)

$$\frac{t}{a} = \frac{2 \sin A/2}{\sqrt{1 - \mu^2 \sin^2 A/2}} \qquad \text{---------- (14)}$$

Whence from equation (3)

$$\frac{t}{a} = \frac{\partial \theta}{\partial \mu} \qquad \text{---------- (15)}$$

and substituting the minimum value of $d\theta = \frac{m\lambda}{a}$ for resolution we have

$$t = \frac{\lambda}{d\,u} \qquad \text{---------- (16)}$$

in words for the minimum condition of resolution of two lines in a prism spectroscope it is necessary that the total thickness of the base of the prism or prisms be equal to the quotient of the mean wave length by the difference in the indices of the two lines. The amount of resolution is independent of A and dependent only on t, the length of the prism base. Approximating m to unity in equations (13) and (15) we have:

$$d\theta = \frac{t(d\,u)}{a} = \frac{\lambda}{a} \qquad \text{---------- (17)}$$

whence $\frac{\lambda}{a}$ is the minimum allowable value of the first two quantities for resolution.

Multiplying equation (17) by $\frac{a}{d\lambda}$ we have:

$$a\,\frac{d\theta}{d\lambda} = t\,\frac{d\,u}{d\lambda} = \frac{\lambda}{\partial\lambda} \qquad \text{--------- (18)}$$

$\frac{\lambda}{\delta\lambda}$ is defined as the resolving power of a spectroscope and is the minimum allowable value of the first two quantities for resolution. Equation (18) defines the resolving power as the product of the linear (effective) aperture and the dispersion.

The equation refers to the condition of an infinitely narrow slit which is unobtainable in practice. Giving a finite width to the slit increases the amount of light available while decreasing the resolving power. The practical resolving power of a spectroscope is a function of the width of the slit as well as the dispersing train, a $\frac{d\theta}{d\lambda}$, and is given numerically by the ratio $\frac{\lambda}{d\lambda}$. For example any instrument that will just resolve the sodium D lines is said to have a resolving power of $\frac{5893}{5896-5890} = 982$.

This characteristic is best determined experimentally.

The Dimensions of a Prism.

The larger a prism, the more light it will pass. In considering the dimensions of a prism account must be taken of dispersion, loss of light by reflection and absorption.

The dispersion has been shown to be

$$\frac{d\theta}{d\lambda} = \frac{\delta\theta}{\delta\mu} \times \frac{\delta\mu}{\delta\lambda} \text{ ---------- (3)}$$

Since $\frac{\partial \mu}{\partial \lambda}$ is constant for one substance, we may consider $\frac{\partial \theta}{\partial \mu}$ the dispersion. With the beam incident at minimum deviation this value will vary directly as the refracting angle A. To compare the relative values of prisms having different values of A, it is preferable to keep the resolving power a constant, i.e. the length of the prism base a constant.

There is no great gain in reduction of dispersion and almost none in reduction of loss by reflection, by decreasing the angle A below 60°. For example, decreasing the angle from 60° to 30° ($u = 1.5$) diminishes reflection loss by only 2.5% and increases the aperture and dimensions of the spectroscope three times. Above 60° any small increase in angle A greatly increases the dispersion without decrease of volume of prism. If a large dispersion does not matter, the refracting angle may be increased with decided advantage until it reaches the limit imposed by diminution of brightness of the image. With plenty of light we may use angles as large as 80° in which case the telescopes and other parts of the spectroscope are only one-third as large as for a 60° angle. 80° prisms are much more expensive since increased distortion of the spectrum is produced by flaws on the prism faces.

By comparing the angles of deviation, the

dispersion, and the amount of light transmitted, the superiority of 60° prisms over 45° prisms is evident.

For a prism to accept all the beam from the collimator, the length of the refracting faces must be greater than their height. From fig (4)

Height of prism = length of face $\times\sqrt{1-u^2 \sin^2 \frac{A}{2}}$

The height of the prism should be equal to the diameter of the collimating lens.

Loss of Light by Reflection.

The light reflected by a surface of a medium is partially polarized. By Fresnel[4], if the light is polarized at right angles to the plane of incidence then the intensity of the reflected light is $\frac{\sin^2(i-r)}{\sin^2(i+r)} = x$

and the intensity of the transmitted ray will be = 1-x where i equals the angle of incidence, r equals the angle of refraction and the intensity of the incident ray is unity. If the light is polarized in the plane of incidence the intensity of the reflected light is $\frac{\tan^2 (i-r)}{\tan^2 (i+r)} = y$ and the intensity of the transmitted ray will be = 1-y.

If we regard ordinary light, intensity equal to unity, as composed of two beams of equal intensity polarized at right angles to one another then,

the amount reflected = $\frac{x}{2} + \frac{y}{2}$

and the amount transmitted $= \frac{1-x}{2} + \frac{1-y}{2}$

at the second surface the amount transmitted is equal to

$$\frac{(1-x)^2}{2} + \frac{(1-y)^2}{2}$$

and at the nth surface the amount transmitted is

$$\frac{(1-x)^n}{2} + \frac{(1-y)^n}{2} \quad \text{---------- (19)}$$

When $i = r = 0^\circ$, for example at the surface of a lens the intensity of the reflected light is equal to $\frac{(u-1)^2}{u+1}$ in both cases.

Loss of Light by Absorption.

If I denote the intensity of the beam of light incident on a prism train, E that of the transmitted beam then:

$$\text{Log } \frac{E}{I} = -\beta x \quad \text{---------- (20)}$$

where β is a constant depending on the medium.

If the intensity of the incident ray be unity then:

$$\text{Log } E = -\beta x$$

In the case of a train of prisms x is equal to the average thickness of the glass multiplied by the number of prisms:

$$x = \frac{a \sin \frac{A}{2}}{\sqrt{1 - u^2 \sin^2 \frac{A}{2}}} \times N \quad \text{---------- (21)}$$

Where a is the diameter of the effective aperture at the position of minimum deviation

From equations (20) and (21)

$$\text{Log } E \propto a \frac{N \sin \frac{A}{2}}{\sqrt{1-u^2 \sin^2 \frac{A}{2}}}$$

$$\text{or Log } E \propto a \frac{\partial \theta}{\partial \mu} \text{ ---------- (22)}$$

By equation (22) the absorption is the same in all prism trains of the same material having the same dispersion. It makes no difference whether we use a large number of prisms of small refracting angle or a few prisms having a large refracting angle.

Materials for Prisms.

Glass is the best prism material for work in the visible region. It is made in a great many varieties, classified by the following constants.

1. u_D is the value of the index of refraction for the sodium D line.

2. $u_F - u_C$ is the dispersion, or difference in the values of the indices, for the two rays C and F. This is defined as the medium dispersion since the brightest rays of the spectrum are between C and F.

3. $\frac{u_F - u_C}{u_D - 1} = V$ is defined as the relative dispersion of a glass.

4. The partial dispersion is the difference between the refractive indices from A to D, from D to F, and from F to G. The relative dispersive powers of the different

glasses are defined by the ratio between the partial dispersion and the medium dispersion, and by the value of V.

For work in the ultra-violet region we may use fluorite, calcite or quartz. Fluorite transmits all the rays as far as the limit but is very difficult to obtain clear. Quartz is transparent as far as $\lambda =$ 1850 and calcite to $\lambda = 2150$. Quartz is used a great deal more than calcite for the following reason.

All natural crystals except those of the cubic system, exhibit the phenomena of double refraction. A ray of light divides into two portions on refraction (1) the ordinary ray which obeys Snell's law (2) the extra-ordinary ray, the relation between the angles of incidence and refraction of which depends upon the angle of incidence. Positive crystals are defined as those in which u for the extraordinary ray is greater than u for the ordinary ray, for example quartz. Negative crystals are those in which u for the extraordinary ray is less than u for the ordinary ray, for example, calcite.

Both quartz and calcite are uni-axial. i.e. there is only one optic axis or direction through the crystal in which double refraction does not occur. Therefore the prism must be cut so that the optic axis is parallel to the base and lies in a principal plane, in

order that the rays at minimum deviation should not undergo double refraction. A principal plane is a plane perpendicular to the plane of the refracting edge.

Calcite has a considerable difference in the values of u between the ordinary and extraordinary rays and so the spectral lines appear sharp only a small angular distance on each side of the position of minimum deviation. This entirely militates against the use of prisms of this material for purposes of photographing anything more than very small limits of the spectrum at one time. In the case of quartz the extraordinary ray is very much weaker than the ordinary ray and this substance finds great use in apparatus for photographing the ultra-violet end of the spectrum.

Quartz however, has a further property which must be taken into account. All doubly refracting substances plane-polarize the light they refract, the plane of polarization of the extraordinary ray being perpendicular to that of the ordinary ray. Quartz rotates the plane of polarization of light passing through it and when light enters the quartz crystal parallel to the optic axis it is resolved into two rays circularly polarized to the left and right respectively.

Due to the different velocities of these two rays they are separated and the image is doubled.

There are two kinds of quartz crystals (1) right handed, in which the dextro polarized ray is less deviated due to its greater velocity, (2) left handed, in which the laevo polarized ray is less deviated. Since the two varieties are absolutely similar in their powers the doubling effect is completely eliminated by making a 60° prism out of a right handed 30° prism and a left handed 30° prism cemented together, the optic axis being parallel to the base in each half. This combination is known as the Cornu prism.

The Lenses:

Two lenses are used in the prism spectroscope. The collimating lens collects the rays which come from the slit and transmits them as a parallel beam on the first face of the prism train. The telescope lens collects the rays leaving the last prism face and brings them to a focus.

The focal length of a single lens may be found from the equation.

$$\frac{1}{f} = (u-1)\left(\frac{1}{r_1} + \frac{1}{r_2}\right) \qquad \text{---------- (23)}$$

Where f is the focal length of the lens, u the index of refraction of the given material, and r_1 and r_2 the radii

of curvature of the first and second surfaces. The focal length differs for each wave-length since u is proportional to the wave length. Thus with white light a single lens will give a series of colored images of different sizes distributed along the axis. This defect is defined as chromatic aberration.

By differentiation of equation (23)

$$d\left(\frac{1}{f}\right) = \frac{(d\ u)}{u-1} \times \frac{1}{f} = \frac{1}{V\ f} \text{ ---------- (24)}$$

The ratio $\frac{(d\ u)}{u-1}$ is defined as the relative dispersion of the medium and is derived from equation (2) for a prism at minimum deviation.

$$u = \frac{\sin \frac{A+\theta}{2}}{\sin \frac{A}{2}}$$

Since the angle A is very small in the case of a lens we may put $\sin A = A$ whence

$$\theta = A(u - 1) \text{ ---------- (25)}$$

differentiating and dividing through by equation (25)

$$\frac{(d\ \theta)}{\theta} = \frac{(d\ u)}{u-1} \text{ ---------- (26)}$$

In words the relative dispersion is the ratio of the difference in deviation for two rays of the spectrum to the deviation of one ray taken as standard.

Chromatic aberration can be corrected since different materials possess different relative dispersions. It is possible to bring two different colored

rays to the same focus using different kinds of glass. A secondary spectrum will remain since the dispersions of two glasses are not geometrically similar.

For two lenses in contact.

$$\frac{1}{F} = \frac{1}{f_1} + \frac{1}{f_2} \qquad \text{---------- (27)}$$

and for achromatism it is necessary that for a given change of wave length

$$d\left(\frac{1}{f_1}\right) + d\left(\frac{1}{f_2}\right) = 0 \qquad \text{---------- (28)}$$

substituting from equation (24)

$$\frac{1}{v_1 f_1} + \frac{1}{v_2 f_2} = 0 \qquad \text{---------- (29)}$$

For visual work the C and F rays are chosen since the brightest part of the spectrum lies between them. Two glasses are chosen that differ considerably in values of v and are cut so that f_1 and f_2 satisfy the equation. In practice it is possible to get glasses differing in v with very similar partial dispersions. Often if the achromatism is perfect from C to F the focal length of the lens is too short for rays beyond F. This is overcome by slightly tilting the photographic plate so that the part intended to receive the blue end of the spectrum is nearer the lens.

<u>The Curvature of Spectrum Lines Produced by Prisms.</u>

The lines seen in a prism spectroscope are curved with the convex sides turned towards the red end

of the spectrum. The explanation is as follows:

In order that the deviation of the rays be a minimum the incident and refracted angles must be equal and the rays must pass through a principal plane. The collimator lens can only make parallel the rays it receives from the centre of the slit and thus only these rays are passed through a principal plane. The rays from the other portions of the slit do not traverse a principal plane and are deviated more, an amount which increases the farther from the centre of the slit they start.

The Construction of the Slit.

The slit is generally formed between two metal jaws, one jaw fixed, the other movable by means of a screw. An improvement on this form of slit is to have both jaws movable and actuated by the same screw. Both sides are made to move equally, and thus the centre of the aperture is not displaced. The dimensions of the slit depend upon the size and quality of the lenses and prisms used. In practice the slit is stopped down with a collar, which in conjunction with the use of large lenses, tends to eliminate the curvature of the lines noted above.

B. The Theory of Selective Absorption and its Use as a Chemical Analyst.

When light passes through any homogeneous

transparent medium it emerges diminished in amount. Part of the light may be scattered at the surface, part scattered in the interior, and part regularly reflected at the surfaces. The rest of the light which is lost is said to be absorbed. It may be transformed into heat, or fluorescent of phosphorescent light of different wave lengths or it may cause ionization or chemical action. Absorption spectroscopy helps to identify substances, estimate strengths of solutions, view chemical changes and check the purity of preparations.

Since only the absorbed part of the lost light is significant to the chemist he must allow for the other lost light in making measurements. The loss by reflection can be calculated from Fresnel's formula or it can be compensated for by arranging that another non-absorbing substance with reflecting surfaces similar to that to be measured is used for comparison. Scattering at the surfaces of the substance must be overcome by careful polishing.

It has been usual to distinguish between two kinds of absorption, general and selective. Where the intensity of absorption varies only slowly from one part of the spectrum to the other, the absorption is said to be general. Where the absorption varies rapidly and in particular where it increases and decreases as one ad-

vances along the spectrum, it is said to be selective, but there is no real fundamental distinction as all absorption is selective.

An emitting atom of a monatomic gas is regarded today as capable of existing in a number of different states according to whether it is the vehicle of a greater or less amount of energy, and it is only when it changes from one of these states to another of less energy that it emits energy in the form of radiation. If we picture the atom as a miniature solar system consisting of a heavy nucleus charged with positive electricity surrounded by a collection of electrons carrying a negative charge, each moving in its own orbit, each electron is characterized by the work which must be done in order to extract it from the atom, and it is the changing from one of the possible orbits to another that constitutes the change from one energy level to another. The radiation is a concomitant of a definite change of energy in the atom, such changes can only take place by definite amounts known as quanta. The relation between the character of the radiation and the change of state of the atom is expressed by:

$$v = \frac{E_2 - E_1}{h} \quad \text{---------- (30)}$$

Where E_2 and E_1 are two different possible energies of the atom, v is the frequency of the radiation, and h is

a constant.

Before emission can occur in this way it is necessary that the energy of the atom be raised above its normal value. This is done by heat or electric discharge. In general, excitation consists of the displacement of an electron from its normal orbit to one in which its energy is greater than normal. When the electron falls back into any one of the possible orbits within the atom, the energy of the electron atom system is diminished and this energy difference manifests itself as a single line of the spectrum.

Just as the energy of the atom may be converted into radiant energy, so may radiant energy be converted into atomic energy. If a collection of atoms is traversed by a beam of light, some atoms under certain circumstances are raised to higher energy levels, and the energy required is taken from the requisite frequency of the beam of light which then shows a line absorption spectrum.

It is natural to suppose that when the molecules of a substance are closer to each other as in a liquid or solution, the energy levels will be no longer always the same for each molecule, owing to disturbance by inter molecular action, and instead of sharp absorption lines we shall get broader regions of absorption.

The chief uses of absorption spectra for the chemist are to be found in their empirical relations. Such measurements must be quantitative, measurement being made of the proportion of energy absorbed at each wave length. The measurements must be particularly precise in the regions of maximum absorption since these regions contain the wave lengths peculiarly characteristic of the molecule.

The Laws of Absorption.

There are two principal laws of absorption. (1) Lambert's law states that the proportion of light absorbed by a substance is independent of the intensity of the incident light. This law is rigidly true. Bunsen and Roscoe assumed only Lambert's law in their definition of extinction coefficient.

Consider a beam of light incident on a medium, then from Lambert's law.

$$I = I_o 10^{-\beta x}$$

Where I_o is the intensity of the incident light entering the medium, I the intensity remaining after its subsequent passage through a path length x, and β a constant of the medium, is defined as the extinction coefficient. It is the reciprocal of the thickness necessary to weaken the light to one-tenth of its incident value.

(2) Beer's Law states that if an absorbing substance is dissolved in a non-absorbing liquid, its absorption of a beam of light is directly proportional to the number of absorbing molecules through which the beam of light passes. This assumes absolutely no change in the frequency of the energy levels of the molecule as it is brought closer to, or removed from other molecules by concentration or dilution. This does not hold in practice. For this reason β is only a characteristic for a substance in one definite state. It may vary in value as the state of the substance varies. Furthermore it is possible for chemically different substances to have identical absorption spectra due to equal energy levels.

C. The Measurement of Selective Absorption in the Ultra-Violet.

Measurements of the amount of light absorbed by a substance are obtained by comparison of the initial intensity with the intensity after passing through the absorbing material. This measurement obviates the necessity for absolute measurement of light intensity and any device that will record accurately changes in light intensity will suffice. The best method of recording results is in the form of a curve. The abscissa may be either wave lengths or frequencies. Frequencies are preferable be-

cause from the theory of the mechanism of absorption, a change in energy level is caused directly by conversion into atomic energy of a characteristic frequency.

As ordinates we may plot the percentage of light absorbed or the extinction coefficient, though it depends on the term most suited to the work in hand. The extinction coefficient is calculated from equation (31) as

$$\beta = \frac{\text{Log } \frac{I_0}{I}}{x} \text{ ---------- (32)}$$

It is in general use because it varies distinctly both with the concentration and chemical nature of substances.

Photographic Methods:

The basic principle of accurate ultra-violet spectro-photometry involves a device by which light from a single source is divided into two beams of which the one is reduced in intensity by known amounts, the other being selectively absorbed by the substance under investigation. This, which is usually known as the photometer, is used in conjunction with a quartz spectrograph to resolve both beams into spectra, a series of spectra taken with successive reductions of the variable beam being inspected for the positions at which the absorbed and reduced spectra are of equal intensity, whence a curve can be drawn relating wave length or frequency with absorption.

There are two types of quartz spectrographs in use. (1) The fixed adjustment quartz spectrograph, for example the Hilger Quartz Spectrograph which gives the whole spectrum from 10,000 Å to 2,000 Å on one 10" plate in whose height a number of spectra can be taken. The lenses are of quartz and the dispersing system consists of one quartz Cornu prism. (2) The Littrow Quartz Spectrograph, for example the Bausch and Lomb Large Littrow Spectrograph requires only half the amount of large optical quartz that would be needed with the other type and gives about three times the linear dispersion. The dispersed spectrum from 2100 A to 8000 A can be covered satisfactorily on three 10" photographic plates.

The photometer device differs in various types of instruments. On some the duration of intermittent exposures is varied, for example the Hilger Twyman Sector Photometer[5] in which one beam of light passes through a rotating section S' of fixed aperture, the other through a rotating section S whose aperture can be varied. A series of photographs is taken with the section S set to different apertures. One of these photographs consists of a pair of spectrum photographs in close juxtaposition, one of which is of reduced intensity throughout its entire length, the other--that which has passed through the material under test--being more

dense than the first in certain parts and less so in others, there being certain wave lengths where the density of the two is equal. The places of equal intensity being spotted, everything necessary is known for the plotting of the absorption curve.

Various methods of varying the aperture of the light path are employed, for example the wire grid and the spekker ultra-violet photometer in which each light path contains an aperture, one being fixed, the other variable, either by a shutter mechanism operated by a fine screw, or by interposing one of a carefully graded series of apertures cut in the circumference of a disc. The variable sectors and shutters are usually graduated in values of Log $\frac{I_o}{I}$.

Of light sources for the ultra-violet we may mention: Jones electrodes, which are carbon rods dipped in solutions of uranium nitrate and ammonium molybdate; the spark between metallic electrodes of selected steel containing tungsten and other metals; the under water aluminum spark; the hydrogen tube which gives a practically continuous ultra-violet spectrum; and the iron arc. If the arc is used there is apt to be some fluctuation, and a quartz disc ground on one side can be put in the position of the arc and illuminated by it. There is no need for this when using the high tension spark.

In some cases a condensing lens is used between the light source and the slit to ensure the proper filling of the collimator and prism with light. The known lines of the source serve as the reference for wave length measurements on the plate though a scale, simultaneously developed with the plate, is often used as an assistance.

D. The Fundamental Principles of Thermionic Tubes.

An electric current may be defined as a flow of discrete elementary charges, either positive ions or electrons. A thermionic tube is a device for controlling the motion of electrons through an evacuated space, and consists essentially of a space evacuated of as much gas as possible and containing an emitter of ions, a collector or plate and a grid system. The emitter consisting of a heated filament of wire, usually tungsten, liberates both metallic positive ions and electrons. The grid of wire is placed between the emitter and plate in such a manner that any electrostatic field established between them will be disturbed by a potential applied to the grid. i.e. the current from the filament to the plate is controlled by the potential of the grid with respect to the filament.

There are two possible paths for the current; from the filament towards the plate and from the filament

to the grid. Also in addition to the electronic current, a gaseous positive ion current is present in the tube due to imperfect vacuum. The ideal tube passes only the plate electronic current. This ideal is approached by keeping all potentials below eight volts which is the critical potential above which gaseous ions form, and by reducing the grid current by reducing the filament temperature. This reduces the light emission from the filament and hence the photoelectric emission from the grid which is the major source of grid current.

The type of tube employed in our spectrophotometer was the F.P. 54, obtained from the General Electric Co. This is a four element tube, highly insulated, especially developed for direct current amplification by Metcalf and Thompson. The fourth element is a space charge grid placed between the filament and control grid with the object of decreasing the positive ion current flowing between these elements.

Special operating characteristics were developed for the present circuit, which along with the rated values are reproduced in Table I.

Table I

	Special	Rated
Filament Potential	2.5 volts	2.5 volts
Plate Potential	8.0 volts	6.0 volts
Space Charge Grid Potential	6.0 volts	4.0 volts
Mutual Conductance	70 $\frac{\text{microamps}}{\text{volt}}$	25 $\frac{\text{microamps}}{\text{volt}}$
Grid Impedance	10^{14} ohms	10^{16} ohms

All vacuum tube circuits are adaptations of the fundamental circuit shown in Figure 5.

It is evident that the tube is a voltage operated instrument and any current to be measured must be transformed to a voltage by forcing it through a high resistance.

In order to quantitatively express tube characteristics and functions the following terms are employed. The amplification factor is the number of volts change of plate potential required to change the plate current by an amount equal to the change caused by one volt change of grid potential. The grid potential is much more effective in controlling the plate current than the plate potential. The effective mutual conductance is the change of plate current with unit change of grid potential, denoted $K_{em} = \frac{di_p}{dE_g}$

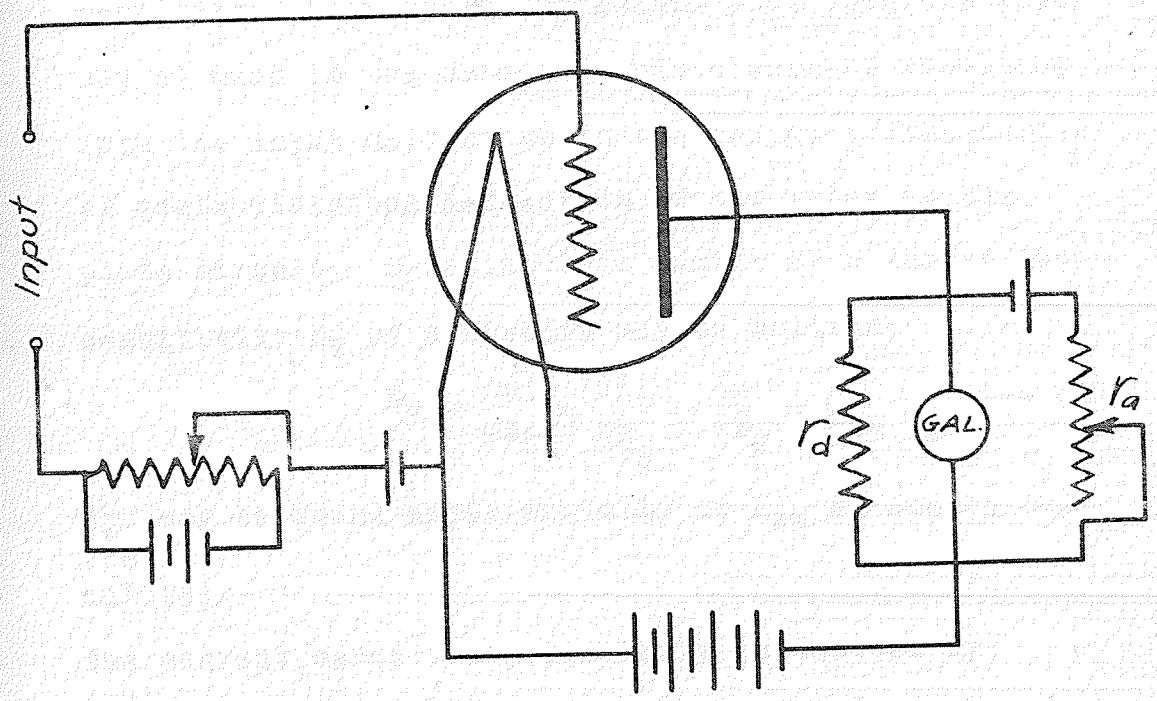


Figure 5.

The plate conductance is expressed as $K_p = \frac{di_p}{dE_p}$ and the plate impedance as $Z_p = \frac{dE_p}{di_p}$

When amplifiers are primarily used for measurement rather than operating relays etc. interest lies not so much in the change in plate current resulting from the input current as in the change of the number of scale divisions deflection of the meter in the plate circuit. Denoting this number by n the voltage sensitivity S_v of a circuit may be written:

$$S_v = \frac{dn}{dE_g} = \frac{di_p}{dE_g} \times \frac{dn}{di_p} = K_{em}\, S_g \quad \text{---- (33)}$$

$\frac{dn}{dE_g}$ may be found experimentally or $K_{em}\, S_g$ can be calculated.

The current sensitivity similarly is denoted by:

$$S_i = \frac{dn}{di} = \frac{dE_g}{di} \times \frac{dn}{dE_g} = S_v \times \frac{1}{k + K_g} \quad \text{----- (34)}$$

Where K_g is the grid conductance and $k = \frac{1}{r}$ is the conductance of the input resistor r. Since in practice K_g is at least one hundred times smaller than k; for practical purposes

$$S_i = r\, S_v \quad \text{----------(35)}$$

There are a number of distinct characteristics of thermionic circuits which place a lower limit upon the value of the current which may be measured.

(1) The time constant deals with the rapidity of response of the change in grid potential resulting from a change in the input current. Assuming that the grid current is much smaller than the input current the rate of growth of the grid potential E is:

$$\frac{dE}{dt} = \frac{i}{c} \qquad \text{---------- (36)}$$

where i is the input current and c is the capacity of the grid system.

The rise in potential E tends to cause a second current to flow through the resistor in the opposite direction to the input current, so after a small interval of time the equation for charging becomes:

$$\frac{dE}{dt} = \frac{i - \frac{E}{r}}{c} \qquad \text{---------- (37)}$$

which has the solution $E_T = E_o (1 - e^{-\frac{T}{rc}})$ ----- (38)

Where E_T is the potential after time T and E_o the total increase in potential at equillibrium. rc is defined as the time constant and its numerical value equals the time taken for the grid to attain to 0.632 of its final potential, in any circuit. After a period given by 10 r c the circuit is for all practical purposes stable.

(2) From equation (35) maximum sensitivity to current will result when the input resistance and the voltage sensitivity are a maximum. In practice the upper limit of input resistance that may be employed seldom exceeds 10^{11} ohms. Since it is hardly possible

to operate with a total capacity much less than 10^{11} farad, this gives a time constant of one second. Furthermore high resistors behave like storage batteries and show a polarization effect, the magnitude of which in resistors greater than 2×10^{11} ohms is sufficient to cause a fluctuation. These factors limit the current sensitivity of this type of circuit to 10^{-16} amp mm.

(3) A third limiting characteristic of thermionic circuits is the random thermal emission of charges with time. The so called steady direct current is only the average value of the fluctuating current measured over a long period of time. The magnitude of this random fluctuation limits the voltage sensitivity of the amplifier. The ultimate practical limit has been shown empirically to be 3×10^{-5} volt mm.

Bridge Circuits.

The principles involved in the common Wheatstone bridge have been made the basis of most effective balanced plate current circuits for neutralizing fluctuations in supply batteries and for eliminating the initial plate current in the galvanometer circuit. In a simple Wheatstone bridge the condition for no current to pass through the galvanometer is $r_1 r_4 = r_2 r_3$; being independent of potential and current. Thus the plate circuit of a tube may be incorporated in a bridge and the galvanometer zero will be independent of

fluctuations in the plate battery when the plate impedance obeys Ohm's law.

The two tube bridge circuit Fig. (6) using matched tubes will be independent of plate battery fluctuations even if not operated on the ohmic section of the tubes. Furthermore, by adjusting the rheostat r, a small change in filament supply will affect both tubes in the same manner.

E. Photoelectric Phenomena.

A photoelectric effect is one in which the absorption of radiation by matter produces an electrical change. Phenomena involving the release of electrons by light fall into three groups.

(1) Surface photoelectric effects. Usually this means the release of electrons by light at the boundary between a solid and a gas or vacuum.

(2) Volume photoelectric effects. This is the separation of electricity throughout a finite volume and is observed as a change in the conductivity of the material when illuminated. The effect is only perceptible with non-metals.

(3) The photovoltaic effect. In the above two groups a battery is necessary in the circuit to produce a current. The third effect involves the generation of an electromotive force in a circuit containing the photo-sensitive material so that a current may flow without

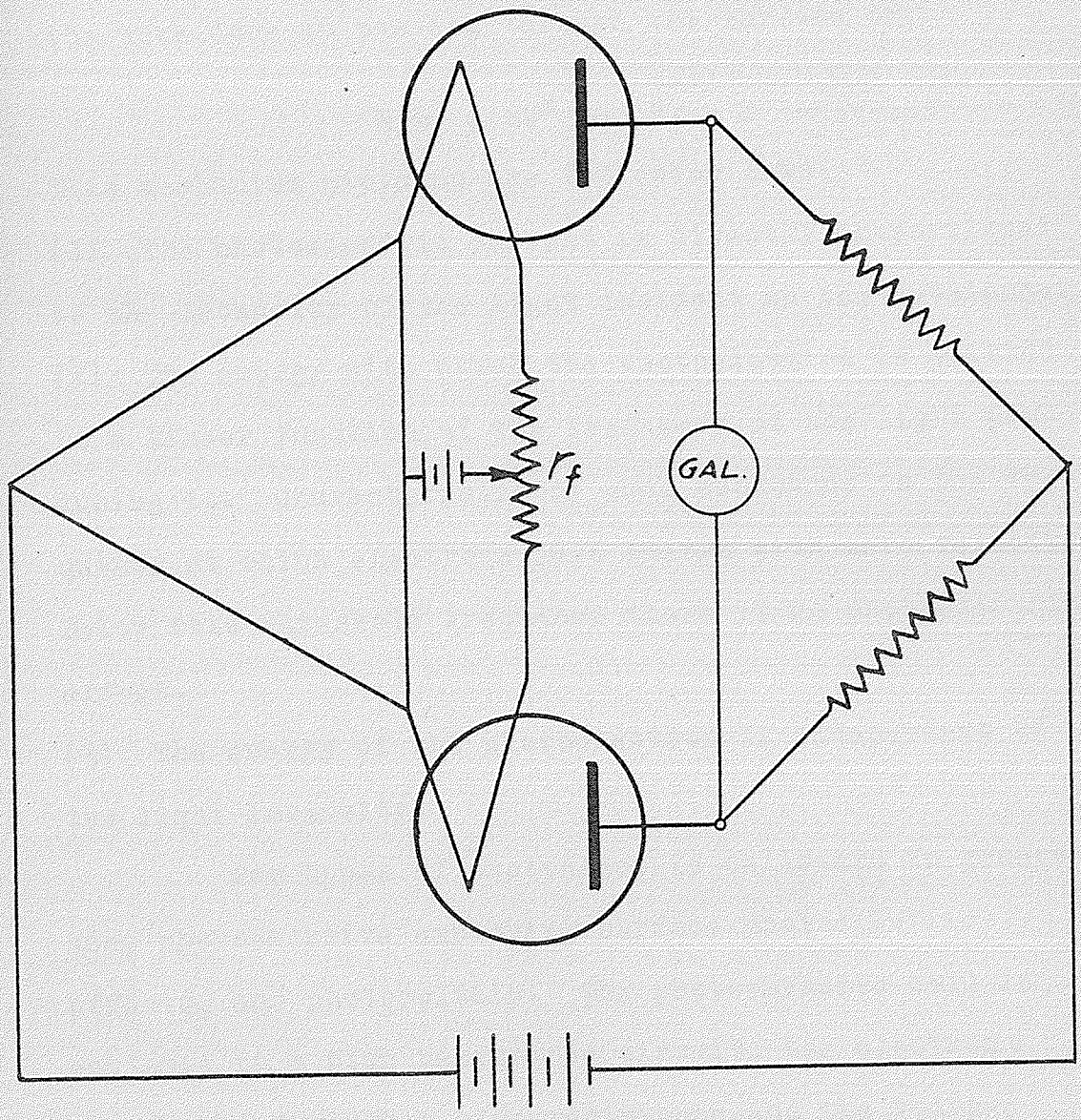


FIGURE 6

the assistance of a battery.

On the basis of light being made up of quanta, each containing energy equal to $h\upsilon$, Einstein stated that the kinetic energy of an escaping electron should equal the energy of the quantum giving rise to it, minus the work done in passing through the surface or,

$$E = \tfrac{1}{2} mv^2 = h\upsilon - P \text{ ---------- } (39)$$

This mechanism explains the following laws:

(1) The photoelectric current is directly proportional to the intensity of the light incident on the substance.

(2) The energy with which the photoelectron is emitted is a linear function of the frequency of the light producing it. Characteristic of each substance is the frequency at which electrons just emerge with zero velocity. Below this threshold frequency there is no photoelectric effect.

(3) The energy of the photoelectron is independent of the light intensity.

Two types of photoelectric cells are in general use, photoemissive employing surface photoelectric effects, and photovoltaic. The photoemissive cell is employed in a high resistance circuit, the electrons are forced through a high resistance and the voltage drop measured. The photovoltaic cell is employed in a low resistance circuit.

Spectral Sensivity
Photoelectric Tubes.

Average of 7 sodium quartz tubes.

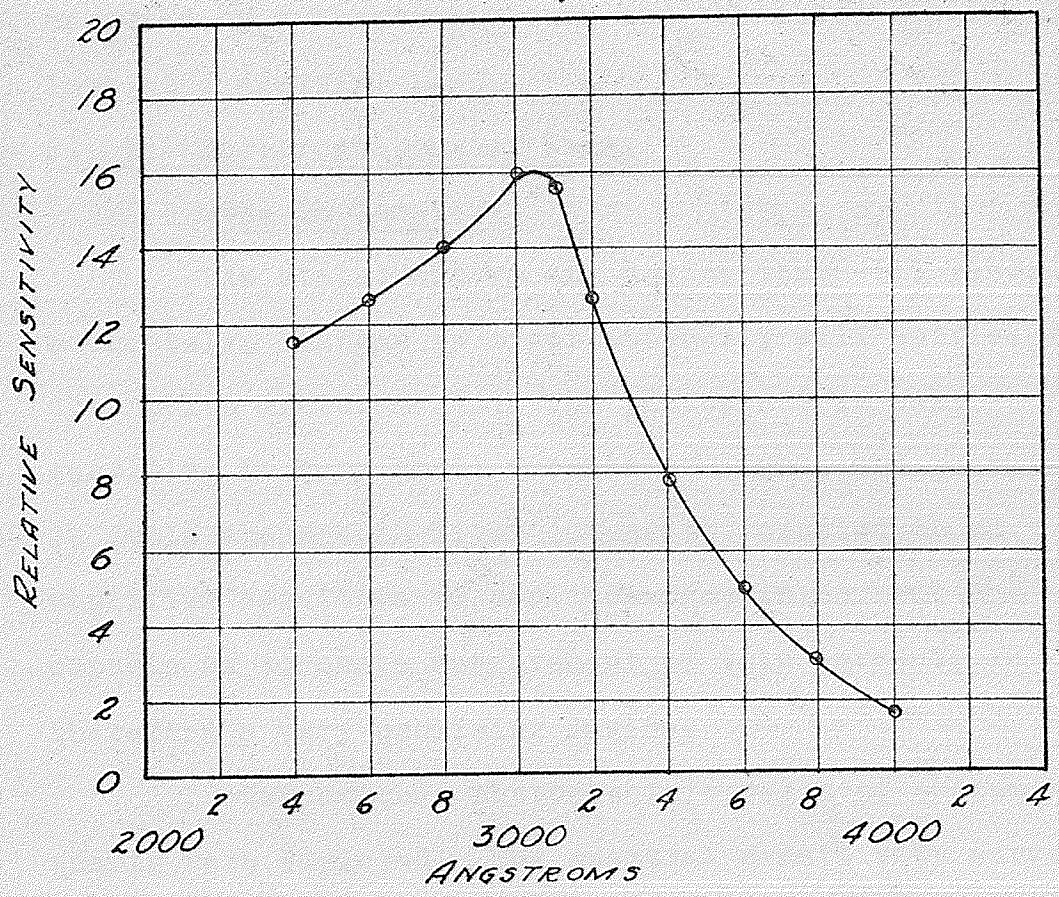


Fig. 7.

There are two types of emissive cells.

(1) Gas filled wherein the current is amplified by ionization of the gas by the emitted electrons. The measured current is not steady and is not proportional to the light intensity.

(2) Vacuum cells. The photoelectric current may be measured by a thermionic tube and the above mentioned laws hold exactly.

F. The Spectrophotometer Used.

The Photometer:

As stated above, any device that will record accurately, changes in light intensity will suffice for absorption measurements. The photoelectric cell, sensitive to very small amounts of light, and therefore to small changes in light intensity, coupled to an amplifier sensitive to small changes in current and capable of accurate measurement of this current, is therefore a very promising device.

Two matched photoelectric cells of sodium in quartz were employed. The spectral sensitivity curve of these cells is shown in Fig. (7). The photoelectric current was measured by vacuum tube amplification, a balanced bridge circuit as shown in Fig. (8) being used. A bridge circuit was necessitated by the use of two photocells and by the fact that these cells were

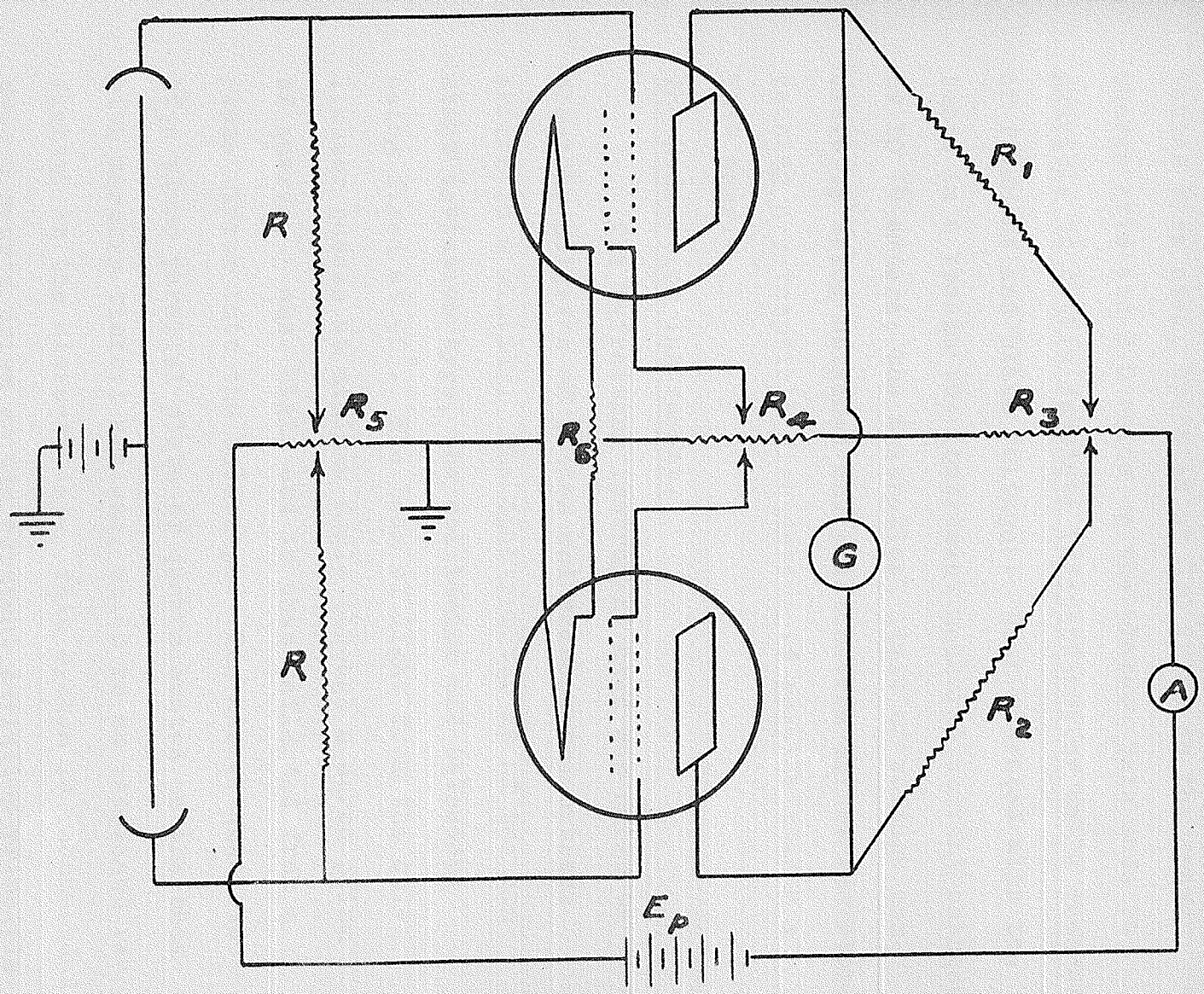


FIGURE 8

illuminated by the 110 A.C. line which was equivalent to introducing a fluctuating voltage on the grids of the tubes. Two F.P. 54 tubes with matched characteristics were used, operated at 6.0 volts on the space charge grid and 2.0 volts on the plate. This made it possible to obtain complete independence of small changes of filament and plate voltages. When operated with the tubes in a vacuum the instrument measured current changes of 10^{-16} ampere; the theoretical limit of voltage sensitivity being obtained and polatization of the resistors causing a perceptible fluctuation if greater than 2 x 10^{11} ohms.

The Refracting Instrument.

The refracting instrument used was built to order by Hilger's of London and reduces the optical errors to a minimum. The spectrometer is of the constant deviation type, the collimator and telescope being fixed at 90°. The beam of light from the collimator passes at minimum angle through a cornu prism of quartz, 5 cms. high, and is then reflected from a plane mirror into the telescope. The mirror consists of a plane parallel plate of quartz coated on the back with mercury tinfoil amalgam, which is an excellent reflector in the ultra-violet. This system is recommended over a quartz prism on the constant deviation principle

due to the greater thickness of quartz traversed with such a prism, and the fact that the absorption of quartz becomes important at wave length 2020 A.

The prism and mirror stand on one table which is rotated by a fine steel screw carrying a drum head graduated in wave frequencies with an accuracy of $\frac{1}{4}$ of 1%. The telescope and collimator are each provided with a scale, engraved in frequencies, for setting the focus of the lenses for the part of the spectrum under observation. The quartz lenses are 5 cm. in diameter.

Both collimator and telescope are provided with symmetrically opening slits which may be varied in width from their smallest value of $\frac{1}{1000}$ inch. The slits may be shut down by means of a collar to utilize only the centre of the lenses and the collimator slit is slightly curved to compensate for the curvature of the spectral lines.

As a source of light a hydrogen tube was employed as giving a practically continuous ultra-violet spectrum.

The Set-up of the Instrument and Method of Making Absorption Measurements.

The actual method of measurement of changes in light intensity due to absorption may be illustrated diagrammatically from figure (9) as follows:

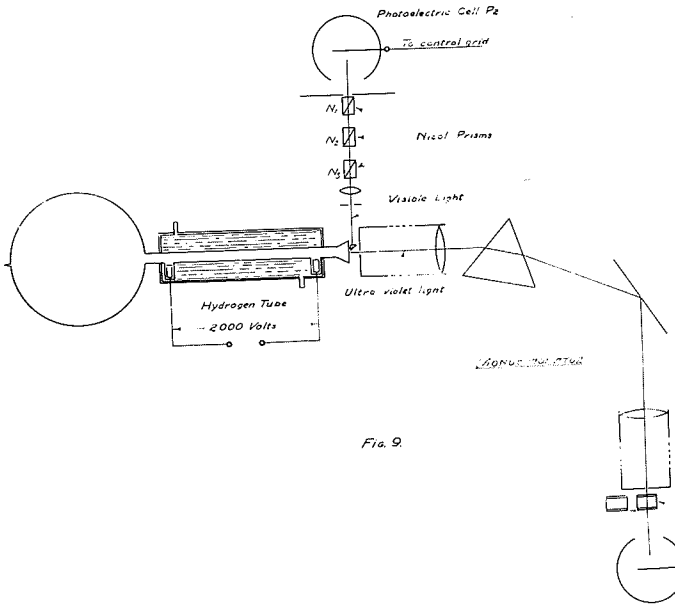

Photoelectric Cell P2
To control grid
N1
N2
N3
Nicol Prisms
Visible Light
Hydrogen Tube
~2000 Volts
Ultra violet light
Absorption Cells
To control grid
Photoelectric Cell P1

Fig. 9.

The light from the hydrogen tube is divided by the right angled prism so that a beam of visible light passes through the three nicol prisms onto photocell P_2, and a beam of ultra-violet of the selected frequency is directed onto photocell P_1. Nicols N_3 and N_2 are movable and their angle of rotation may be read on a vernier. Nicol N_1 is fixed.

Since the absorbing substance is examined in solution, two cells are prepared, one filled with the pure solvent, the other with the solution of the absorbing substance. These cells are interchangeable between photocell P_1 and the spectrometer by means of a sliding tray. The use of a very small telescope opening prevents the light striking the side of the absorption cell.

For work on unknown complex substances liable to contain photosensitive compounds, the mounting of the cell in this position whereby the amount of ultra-violet radiation entering at any one time is exceedingly small, is a decided advantage over the photographic instrument where the full light from the arc enters the cell continuously.

The cell of solvent is first put in front of the telescope and the three nicols are set to give maximum light on photocell P_2. The natural zero of the galvanometer is observed and this instrument is then

shunted into the circuit. By manipulation of R_1 and R_2 the condition of zero current through the galvanometer is obtained.

The hydrogen tube is then switched on and the galvanometer shows a deflection by reason of the photo-electric current set up. By rotating nicol N_3 a condition of no current through the galvanometer is obtained. The absorbing solution is now slipped in between the telescope and photocell and due to the additional absorption of the solute the galvanometer will show a deflection. The galvanometer is brought to zero by rotating nicol N_2.

Since the two photocells are matched and the current is linearly proportional to the light intensity any change in intensity on P_1 is exactly duplicated on P_2 to bring the galvanometer to zero. From the nicol readings this change in intensity due to absorption is calculated as follows:

If I_o be the amount of light that passes through nicol N_3, then I_1 the intensity of light through the solvent is

$$I_1 = I_o \cos^2 P \text{ ---------- (40)}$$

where P is the angle between nicols N_3 and N_2. Similarly when nicol N_2 is rotated to balance the amount of light absorbed by the solution,

$$I_2 = I_0 \cos^2 (P \pm A) \times \cos^2 A \text{ ---------- (41)}$$

where I_2 is the intensity of light through the solution and A is the angle between nicols N_2 and N_1, then the ratio of incident light to transmitted light is

$$\frac{I_1}{I_2} = \frac{\cos^2 P}{\cos^2 (P \pm A) \cos^2 A} \text{ ---------- (42)}$$

Whence from formula (32) the extinction coefficient is

$$\beta = \frac{\log \frac{\text{Cos}^2 P}{\text{Cos}^2 (P \pm A) \text{ Cos}^2 A}}{x}$$

Characteristics of the Instrument.

A measure of the spectral purity obtained with the instrument is given in figure (10) in which the band of wave numbers that pass through the slit width of 10^{-3} inch is plotted against the drum settings. The values were obtained experimentally by moving the spectral line cast on a fluorescent screen across a pointer by rotating the drum. The telescope slit was then made just as wide as a spectral line and the capabilities of the instrument further tested as follows:

Three mercury lines were selected for study

N = 31929

N = 31932

N = 31993

The intensities of the three lines are given

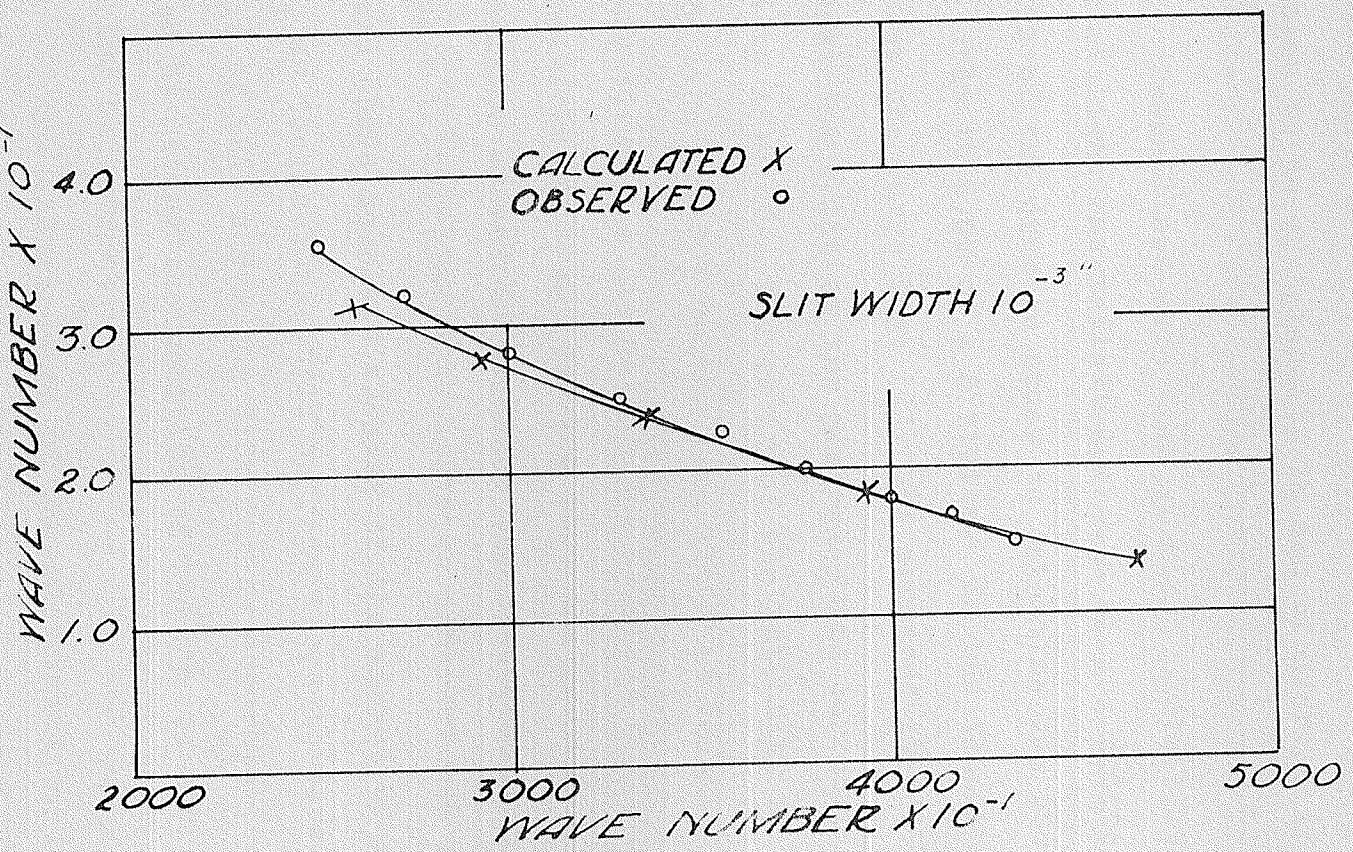

CALCULATED X
OBSERVED o
SLIT WIDTH 10^{-3} "
WAVE NUMBER X 10^{-1}
4.0
3.0
2.0
1.0
2000
3000
4000
5000
WAVE NUMBER X 10^{-1}

FIGURE 10

in standard tables as 8, 8 and 10. Since the lines 31929 and 31932 are not resolved by the instrument they were taken to be of intensity 16 and of average frequency 31930.5.

These lines were then examined photoelectrically. A spot of light was reflected from a mirror mounted on the axis of the drum in order to determine the position of the drum to 0.02 of a division. If the two maximum points on the curve Fig. (11) are compared it will be seen that the ratio of the photoelectric currents involved is 15.9:10. The ratio of the intensity of the average line 31930.5 to the line 31993 is 16:10. These determinations all lie within two adjacent markings on the spectrometer drum, showing that the photoelectric measurements exceed in accuracy the spectrometer settings. The curve illustrates the resolving power of the instrument and shows complete absense of the effect of scattered light, as the photoelectric current drops to zero. With the aid of this curve the drum can be accurately calibrated by moving the position of the quartz mirror with the screw adjustment.

The independence of the instrument of fluctuations in the light source is illustrated by figure (12) wherein the fluctuation was observed with the photocells

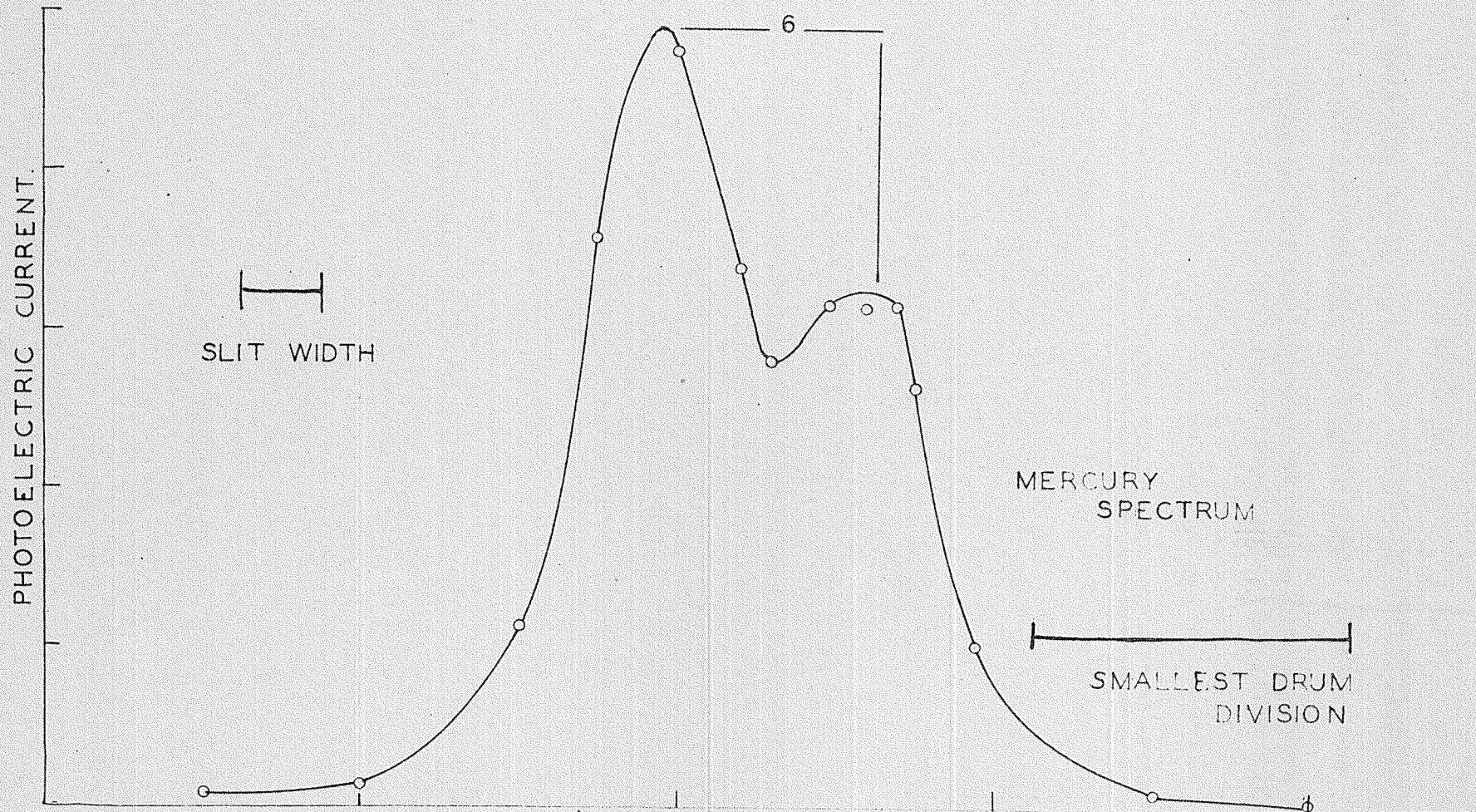

PHOTOELECTRIC CURRENT.
6
SLIT WIDTH
MERCURY
SPECTRUM
SMALLEST DRUM
DIVISION
3210
3220
3230
3240
WAVE NUMBER X 10^{-1}

FIGURE II.

in the dark and again with the two photocells illuminated with equal intensities of light. This graph shows the time response of the instrument to be 120 seconds.

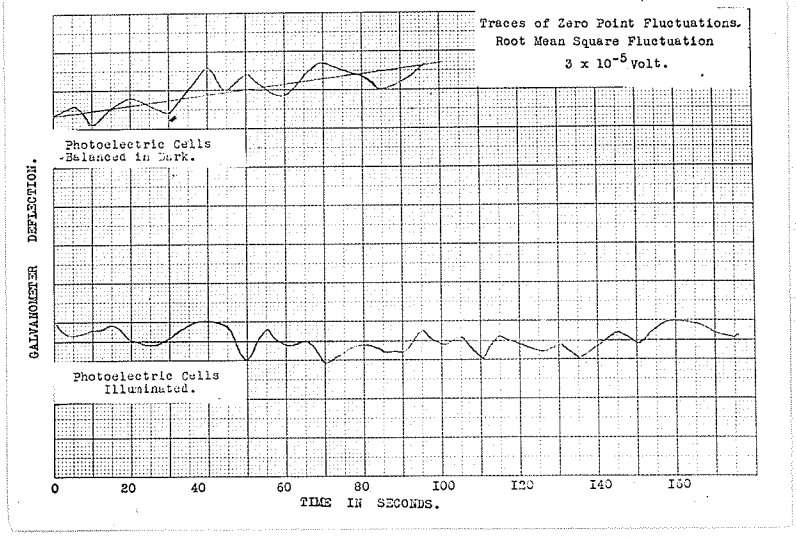

Traces of Zero Point Fluctuations.
Root Mean Square Fluctuation
3 x 10⁻⁵ volt.
Photoelectric Cells
-Balanced in Dark.
Photoelectric Cells
Illuminated.
GALVANOMETER DEFLECTION.
0
20
40
60
80
100
120
140
160
TIME IN SECONDS.

Figure 12.

CHAPTER II

THE PREPARATION AND STUDY OF LEUKOCYTE EXTRACTS.

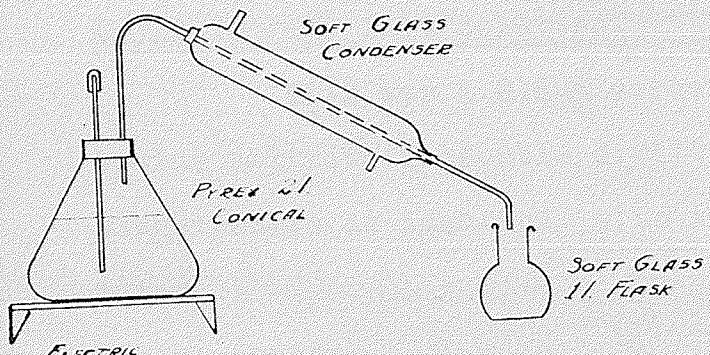
The white cells of the blood were the first constituent selected for examination and a method was evolved to obtain them in a pure state. The description of the technique used will best be prefaced by an account of the preparation of apparatus and reagents.

Water.

To obtain water free from any characteristic absorption was a matter of some difficulty. The technique finally developed was to distill tap water through the apparatus sketched in Fig. (13a) to free it from all non-volatile constituents. The resulting water gave an absorption curve with a peak at wave number 365. This singly distilled water was then redistilled from an alkaline solution of $KMnO_4$ (about 20cc concentrated solution added to 500 cc water) in an all-pyrex still. This second still, figure (13b), was made all in one piece to prevent contamination from stoppers. The double distilled water (figure 14) has stood in pyrex flasks for a period of a month without being spectroscopically contaminated.

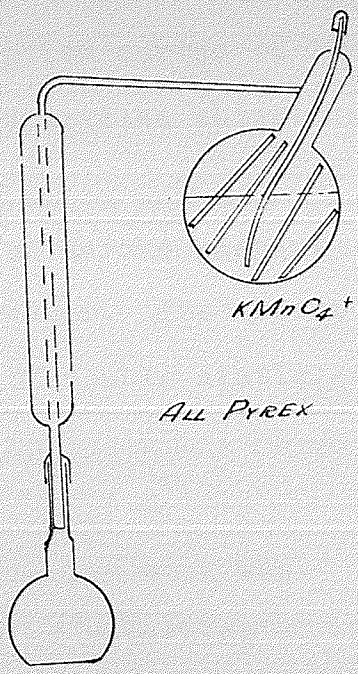
Ethyl Alcohol.

The absolute ethyl alcohol of the Canadian Industrial Alcohol Co. Ltd., was obtained from the Man-



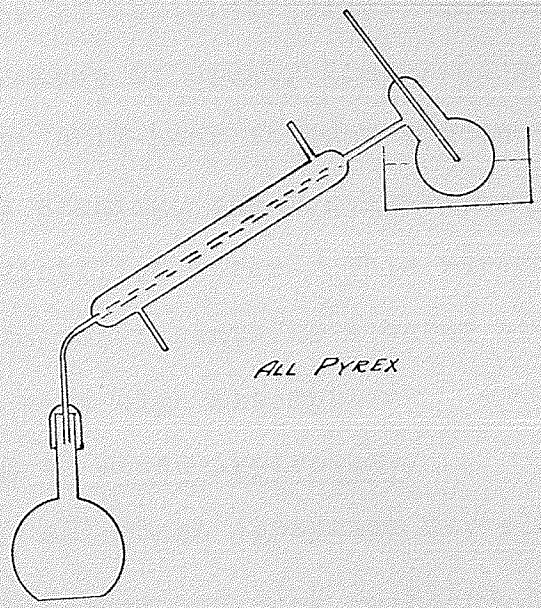
H_2O First Distillation

Fig. 13 a



H_2O Second Distillation

Fig. 13 b.



C_2H_5OH Distillation

Fig 13 c

itoba Government Liquor Control Commission. This was distilled once in an all-pyrex glass still, as in diagram Fig. (13C). The alcohol was used in dilute solution. Fig. (15) shows the absorption curves of 10% and 20% ethyl alcohol in the double distilled water.

Physiological Salt Solution.

This was a 0.85% solution of Mercks reagent sodium chloride in double distilled water. Its absorption spectra is identical with that of double distilled water.

Filter Paper.

The paper used for all filtering work was Whatman No. 50, stated to be capable of retaining particles of colloidal size. The discs were extracted in 10 changes of singly distilled water, remaining about one half-hour in each 200 cc of water, with occasional shaking, the tenth extraction was made with double distilled water. This purification was necessary since the paper contains a water soluble substance which gives a very high absorption.

Cleaning of Glass Apparatus.

All glass apparatus was cleaned just before use in chromic acid cleaning solution and rinsed six times with singly distilled water and once with double distilled water. As a matter of interest a curve on cleaning

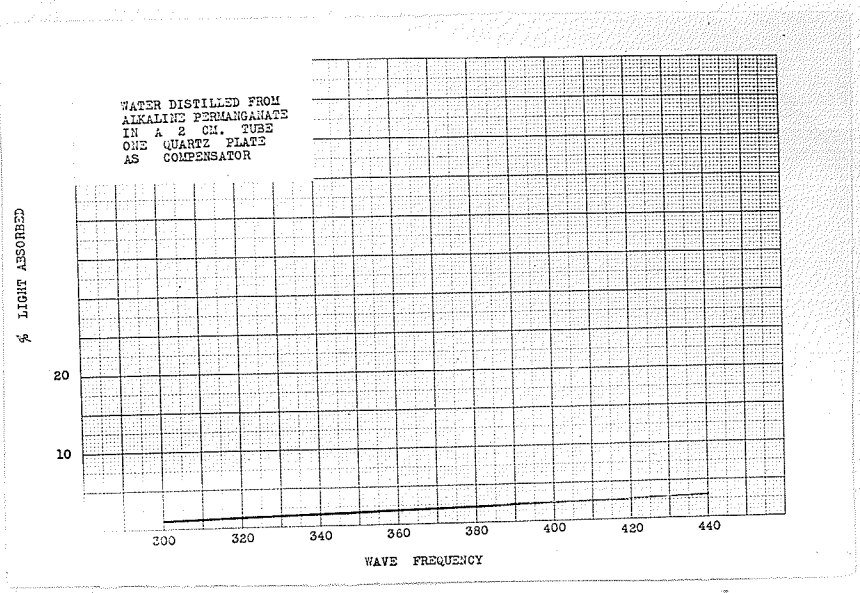


Figure 14.

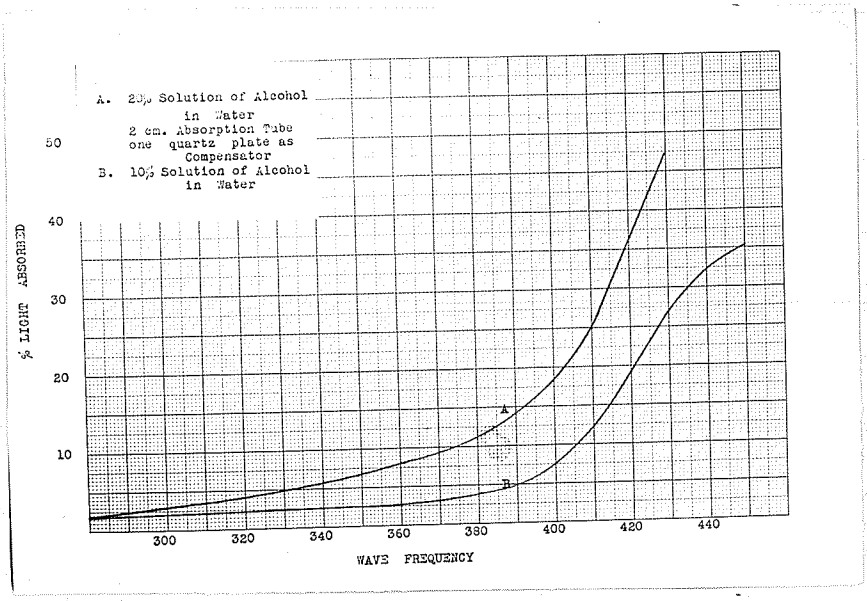


Figure 15.

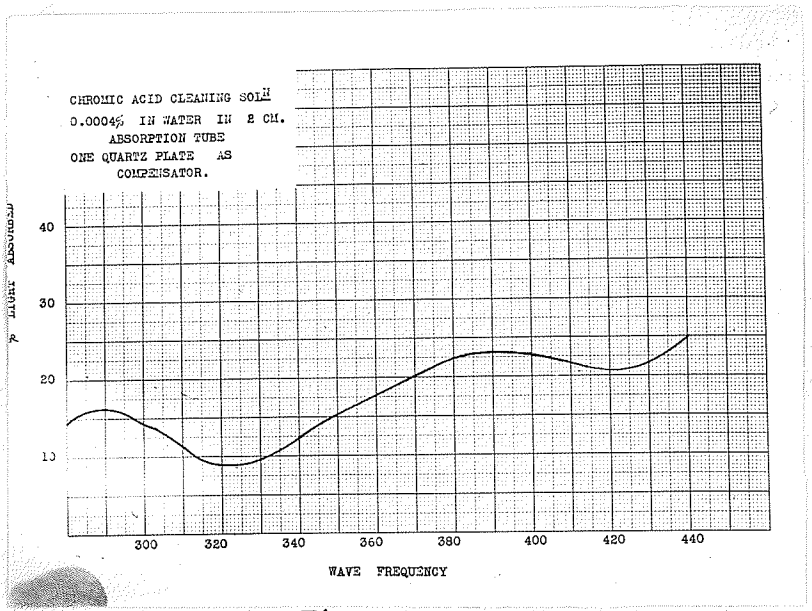


Figure 16.

solution was run, fig. (16). 0.170 grms. $K_2Cr_2O_7$ (commercial) was dissolved in 16.150 grms H_2SO_4 (commercial) and the solution diluted to 0.0004% in singly distilled water.

Isolation of White Blood Cells.

The leukocytes or white cells of the blood are present to the number of between 7,000 and 15,000 per cubic millimeter in normal blood. They are of several forms and constitute a defense mechanism against invading bacteria; consequently there is an increase in their number in disease, particularly with bacterial infection.

The several forms of white cells are differentiated by staining a smear of blood on a slide with one of several available blood stains. Wright's stain is the most common, consisting of a mixture of basic methylene blue and acidic eosin, a red dye. The nucleus of the cell invariably absorbs the blue dye, the surrounding protoplasm is colored differently with the different forms.

In blood at the temperature of the body (37.5 C), the leukocytes are capable of ameboid movement. Use was made of this property of the cells by which they adhere to a foreign body inserted in the blood.

About 10 cc of blood were obtained by venipuncture of the arm using a Luer syringe and treated to prevent clotting or coagulation. Two methods tried were:

(1) Rapid stirring of blood by plunging up and down a coil of plated Cu wire to remove fibrin as fast as it was formed.

(2) Addition of heparin to the freshly drawn blood and gentle stirring; 1.0 mg. heparin will prevent clotting of 5 cc blood. The heparin was used in solution of physiological NaCl of strength 1 mg. heparin in 0.1 cc physiol. NaCl.

The defibrinated blood was then centrifuged in a 3 cc. test tube to produce the characteristic three layers:

(1) red cells at the bottom

(2) "buffy coat" containing the white cells

(3) serum at the top

A large portion of the red cells and serum was then removed using fine nipple pipettes. The test tube was refilled with blood and the centrifuging and removal of red cells and serum repeated until the white cell content of 10 cc blood was concentrated into 3 cc blood. After the final centrifuging a part of the serum layer only was removed, leaving a condition as sketched in figure (17).

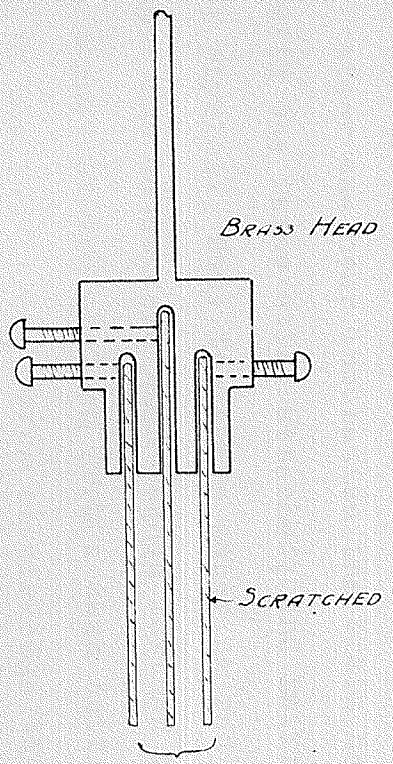


FIG. 18a.

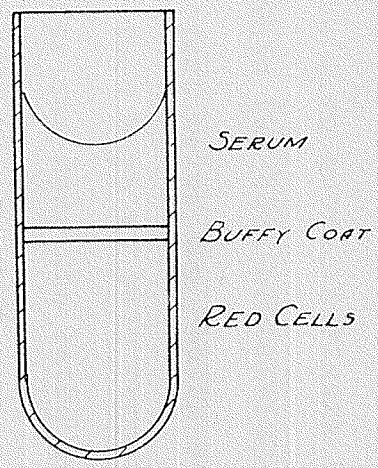
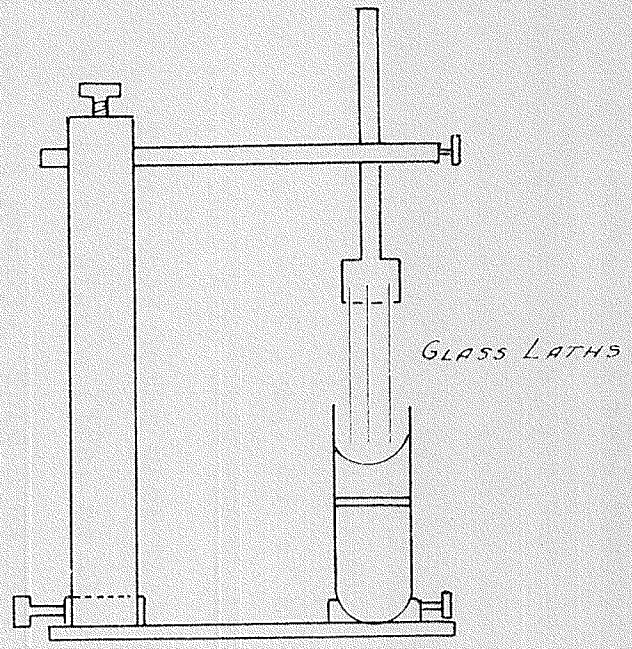


FIG. 17.



COMPLETE BRASS FRAME AND HEAD

FIG. 18b.

The white cells were removed from this tube as follows: Glass laths were made by cutting microscope slides into strips with a diamond cutter. The laths were approximately of size 3.8 cm. x 0.9 cm. These laths were scratched with the diamond about 1 cm. from one end so that about 1 sq. cm. of glass could be easily broken off. They were then thoroughly cleaned.

Three such laths were then screwed into a brass head as in Figure (18a) so that the scratched ends hung free and evenly. This head was then attached to the brass frame also shown in Figure (18b) and the laths were lowered into the tube containing the blood so that the ends were just below the "buffy coat." The whole apparatus was then kept at body temperature (37.5 C) in an incubator for one hour the laths being slightly lowered into the tube every 20 mins. The scratch on the glass must always remain above the "buffy coat."

After one hour incubation the brass head was removed with the laths still attached; and twirled in a beaker of physiol. NaCl to remove adhering red cells and serum. The laths were then washed further by gently dripping normal saline on them from a wash bottle, taking care not to use sufficient force to dislodge the white cells which appeared as milky bands. Moist filter paper

was used if necessary to wipe the edges of the laths. The laths were again twirled around in a beaker of physiol. NaCl when the last traces of serum and red cells were removed.

The end of the lath holding the bands of white cells was then snapped off at the scratch with a pair of clean forceps.

If the blood was defibrinated with the wire coil the bands were broad easily seen, and adhered firmly to the glass.

If the blood contained added heparin the white cells gathered in thick clots on the glass and were invariably lost when the first attempt was made to wash away the red cells and serum, consequently this technique was abandoned.

Physiological NaCl was used as a washing agent rather than pure water for two reasons.

(1) In Physiol. NaCl the red cells are removed as cells and there is no danger of their hemoglobin staining the white cells on the lath. In water the red cells hemolyze and the hemoglobin is freed.

(2) In physiol. NaCl the leukocytes retain their normal appearance, in pure water they cease their ameboid movement, swell and burst due to imbibition of the water.

The white cells were now obtained, free from all impurities, on three squares of glass about 1.0 cm x 1.0 cm. There were approximately 400,000 cells on a lath; or 10% of the number in the original blood sample on the three laths. On staining a lath with Wright's stain for examination, polymorphs; eosinophils, and large monocytes were present, all remarkably clear and well defined. The nucleus stands out a dark blue, sharply differentiated from the lilac cytoplasm of the polymorphs and mono nucleurs and the crimson granules of the eosinophils.

Extraction and Solution of the Leukocytes.

The next step was to suspend the white cells in a solvent and so break them up if necessary that all possible constitutents might go into solution. The effect of the various methods was judged by staining the laths with Wright's stain after the extraction to determine microscopically the condition of the white cells. The following methods were used:

In every case the three squares of glass were put right in the solvent; only enough solvent being used to fill the absorption cell in order to obtain greatest possible concentration.

Solvent	Method	Result
Physiol. NaCl	Laths allowed to stand one hour in cold .85% NaCl.	No change of white cells on lath.
Double distilled H_2O	Laths frozen in the water and thawed out after 1 hr.	Cells appeared crushed though a minority were whole.
Cold 10% C_2H_5OH	Laths allowed to stand in for 1 hour.	Cells appeared whole and well formed.
	Laths allowed to stand in for 10 hours.	Cells distorted and torn.
Hot 10% C_2H_5OH	Laths sealed in tube kept at 60 C.	Only nuclei present on the slides. Protoplasm apparently dissolved.
Hot 20% C_2H_5OH	Laths sealed in tube with 4 cc 20% C_2H_5OH and kept at 100 C for 1 hour.	No resemblance to leukocytes. Just small dark blue spots that must be nuclei. Cytoplasm completely removed by the 20% alcohol.

The solvent after cooling if it was heated, was then filtered into a 2 cm absorption tube through the Whatman 50 filter paper. In every experiment a control of double distilled water was run coincident with the blood sample and this was filtered into the compensating absorption tube.

The purity of the white cell preparations and the exceedingly careful technique required in their isolation from the other blood constituents is demonstrated by the absorption curve Fig (19) of a very dilute solution

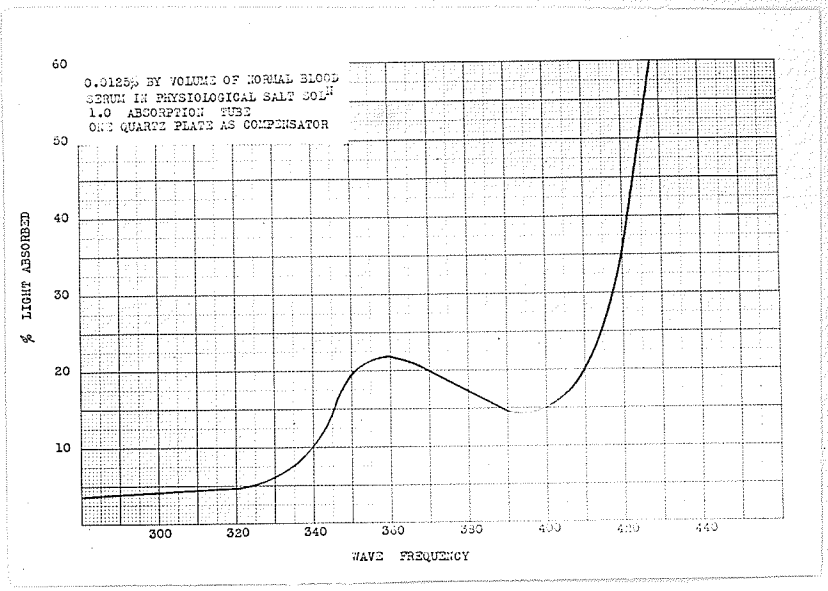


Figure 19.

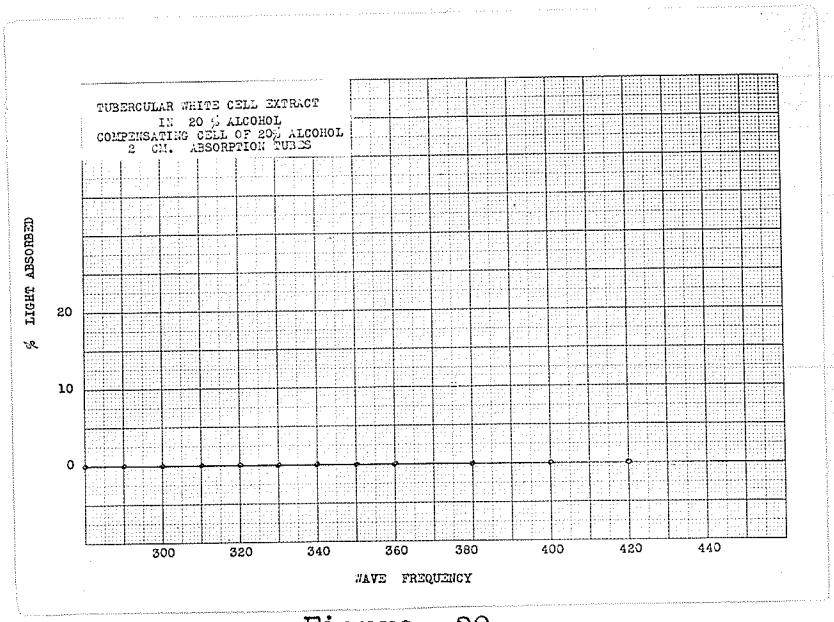


Figure 20.

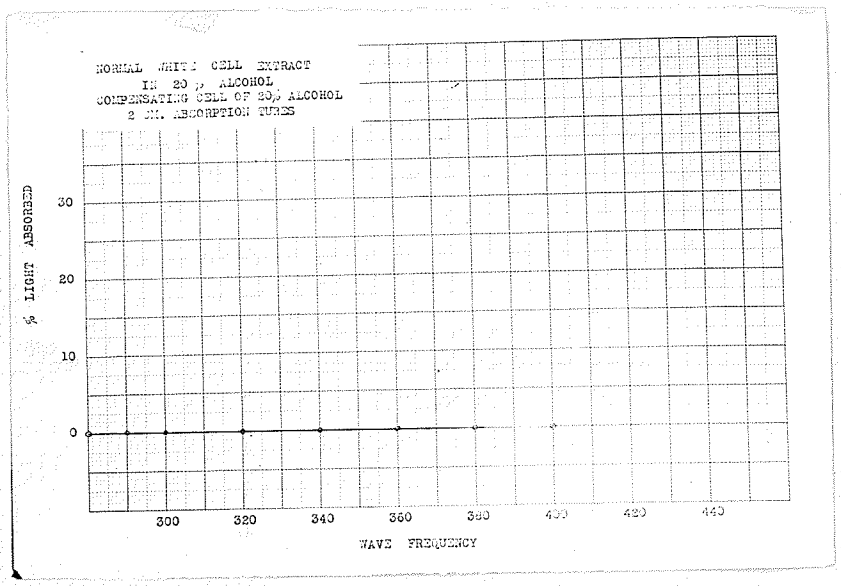


Figure 21.

(0.0125% by volume) of normal blood serum in physiological salt solution.

Extracts were made with the above solvents of white cells obtained from the blood of normal people and tuberculosis patients. The curves run on these solutions showed no selective absorption Figs. (20) and (21). It would appear then that at the concentration of white cells obtainable by the lath method, neither water, physiological salt, nor dilute alcohol solutions extract any substance capable of selectively absorbing ultra-violet light.

CHAPTER III

THE ISOLATION AND STUDY OF SERUM FRACTIONS.

Attention was next turned to the fluid component of the blood known as the serum. This is easily obtained free from the form elements by allowing freshly drawn blood to clot, centrifuging for about ten minutes at 2800 r. p. m., and removing the top layer of straw colored serum with a pipette. As the outcome of a number of investigations it had been stated that the absorption spectra of whole serum was due to the various protein fractions. It was decided to utilize our special instrument in the examination of the non-protein part of the serum.

The first experiments consisted of the removal of the proteins and examination of the total remaining fluid. A number of methods of precipitation of the proteins are known but for our purpose there were two limiting qualifications. In the first place it was necessary to adopt a procedure that was not sufficiently drastic to probably change the composition of some of the blood constituents. It was desirable to examine the blood in a condition resembling as closely as possible its natural state. Secondly no compound should be added whose absorption spectra would mask the absorption spectra of the blood constituents. These considerations were

fairly well met by the process of salting out the proteins with an inorganic salt. Various salts, notably $(NH_4)_2\ SO_4$, $ZnSO_4$, $MgSO_4$, $Na_2\ SO_4$, and NaCl possess the power, when added in solid form to certain definite protein solutions, of rendering the menstruum incapable of holding the protein in solution.

5 c.c. of serum was saturated with $(NH_4)_2\ SO_4$ by adding 3.8 grms of the crystals, sealing in a glass tube, and shaking vigorously at frequent intervals for about 45 minutes. The contents of the tube were then poured into water washed Whatman No. 50 filter paper previously moistened with a saturated solution of $(NH_4)_2\ SO_4$ and allowed to filter into a test tube. The filtrate showed no presence of protein by the nitric acid test. The filtrate was diluted to 36% of its volume concentration with double distilled water and filtered into a 1.0 cm. absorption tube through the Whatman No. 50 filter paper. Absorption curves were run on this blood fraction for normal people and for a number of diseased states, using one quartz plate as the compensating cell. By analysis the concentration of $(NH_4)_2SO_4$ in the final solutions was about 0.6 M. Figure (22) illustrates the absorption spectrum of an $(NH_4)_2SO_4$ solution.

The curves obtained, including curves repeated on the same normal person, varied considerably in shape.

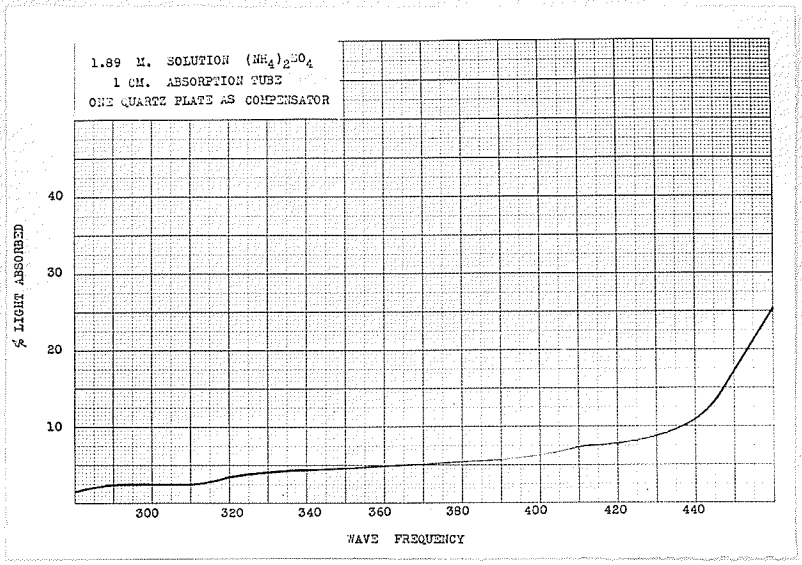


Figure 22.

Figure 23.

Figure 24.

This was traced to the presence of an unstable substance in the solutions, detected by repeating points immediately after running a curve. The unstable factor did not show itself in all cases. Figure (23) shows the change in shape of the absorption curve by allowing the protein-free serum solution to age 24 hours and figure (24) illustrates the change on irradiating the solution for $\frac{1}{2}$ hour with ultra-violet light from the mercury arc.

It was found that the unstable fraction could be removed by extraction with chloroform. The blood solutions prepared for examination were divided into two parts. One part was filtered into the absorption cell and a curve run, the other part was shaken with an equal volume of chloroform three sucessive times in a sealed tube. The difference due to the removal of the chloroform soluble substance is illustrated in figure (25). Curve (b) is constant in shape for all normal cases tested and is stable in cases where curve (a) is unstable. Due to the use of moist filters and the presence of $(NH_4)_2SO_4$ the curves can only be compared qualitatively.

Attention was next directed to obtaining the stable serum fraction in accurate concentration, to be able to quantitatively compare the curves. It was found

Figure 25.

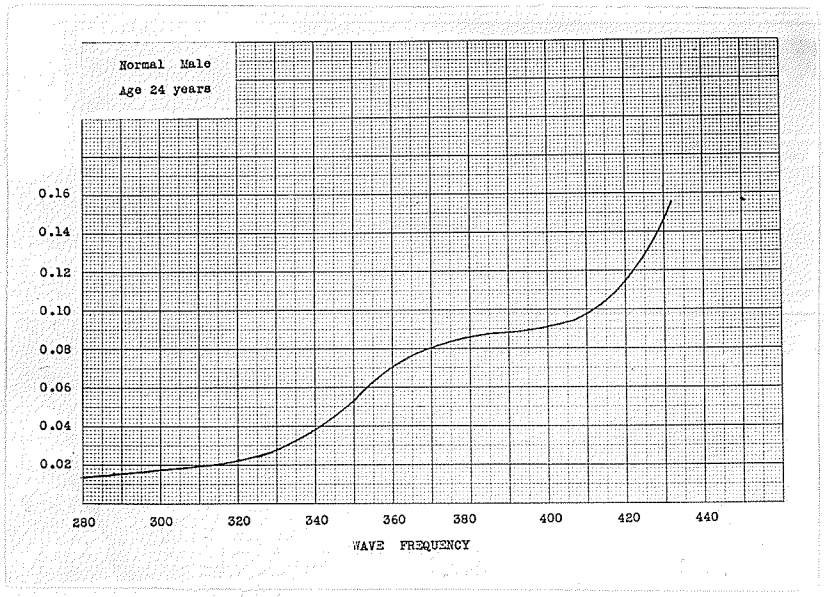


Figure 26.

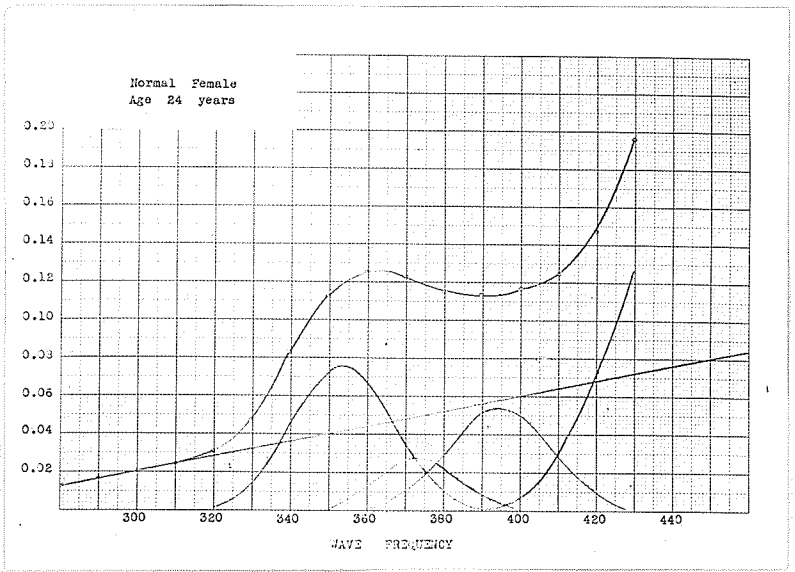


Figure 27.

this particular serum fraction from so-called normal people. These curves are susceptible to mathematical analysis, the methods of which does not concern this thesis; the resulting mathematical constituents of the normal curve are shown in figure (27). The same fraction was further examined with blood obtained from patients diagnosed by the doctor as suffering from hypothyroidism, and the curves found to differ considerably from our results with normal people.

Later we had occasion to examine this serum fraction with a number of cases which could not be diagnosed by the vague clinical symptoms, and in some cases the absorption curves showed the deviation from the normal previously noted in the cases of hypothyroidism. These patients were accordingly treated with thyroid gland preparations, the dose being increased from week to week and the effects followed spectroscopically. The curves changed shape with clinical improvement of the patient, eventually becoming the normal type as the patient approached the limit of tolerance to the gland extract. The absorption curves obtained from three such cases are shown in figures (28), (29) and (30).

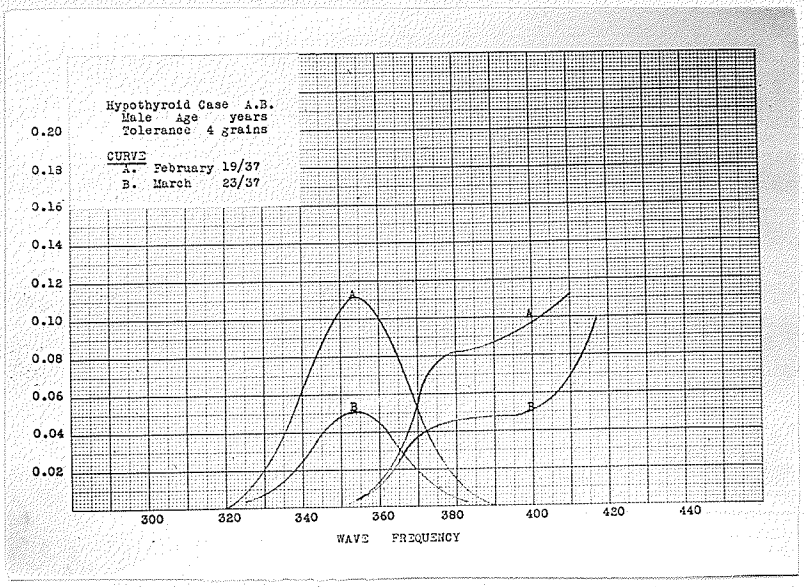


Figure 28.

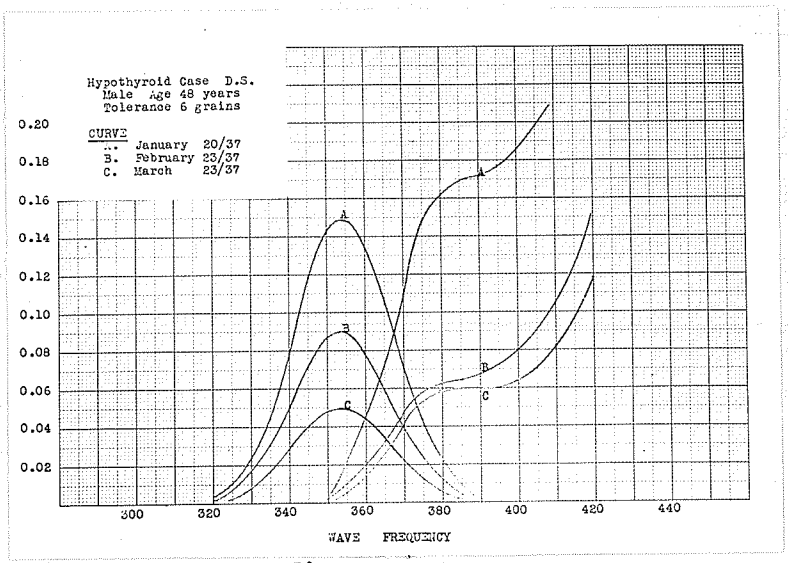


Figure 29.

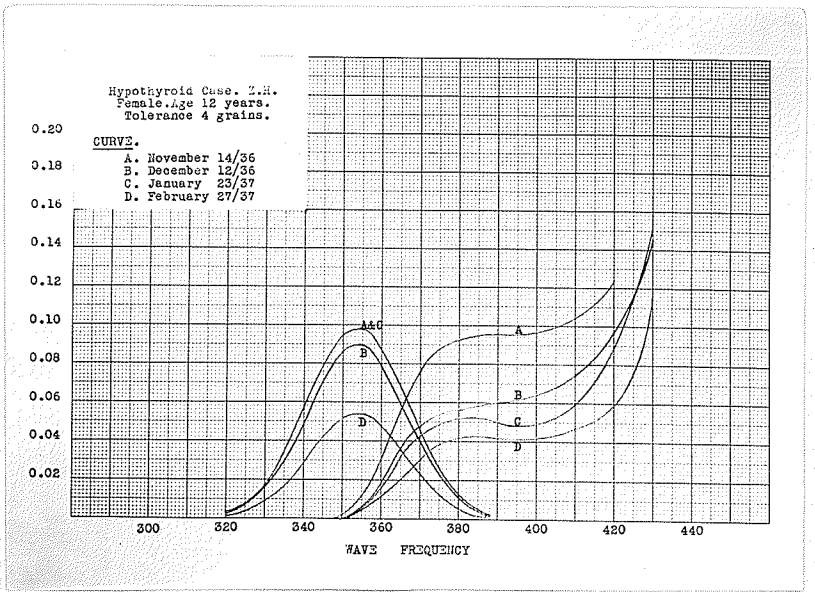


Figure 30.

CONCLUSION

It is possible to fractionate the blood serum and by the removal of the major known substances to examine spectroscopically the residual compounds present in such small amounts as to be undetectable by chemical means. Further it has been shown that variations in one such serum fraction could be correlated in the limited number of cases tested with a specific disease. In this case the value of the work is enhanced, practically by the difficulty of a clinical diagnosis in many such cases, and more fundamentally by the direct relationship it has to the field of the chemistry of the body. The present work is to be taken as indicative of the fact that a large number of serum fractions should be examined and the characteristics noted in relation to health and disease.

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