AN OPTICAL INSTRUMENT TO RECORD SEDIMENTATION RATE OF AGGLUTINATING ERYTHROCYTES

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Henry Puryear Cole Jr.

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ABSTRACT

AN OPTICAL INSTRUMENT TO RECORD SEDIMENTATION RATE
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by Henry Puryear Cole Jr.

This thesis describes a quantitative method intended to determine the strength of the antigen-antibody reaction by measuring the sedimentation rate of red blood cells. A beam of polychromatic, unpolarized light sweeps a row of sample tubes; the transmission through each tube depends on the degree of settling. The settling, in turn, varies with the size of the settling aggregates formed in the immune reaction. By mounting the light source and photocell on a moving boom, samples placed in the path of the light beam can be studied simultaneously. The transmittance of each cell is recorded and a settling curve for each blood sample is obtained. The bulk of the thesis deals with the construction, operation and theory of the machine.

The apparatus performs well. It records the optical density accurately, and provides direct comparisons between samples agglutinating under different experimental conditions. The settling rates of four types of suspensions were examined: blood mixed with anti-serum; blood with anti-B serum, blood with anti-D serum, both in and out of a magnetic field, and blood with saline.

There is a difference in the curves for agglutinating and

non-agglutinating blood, but more data are required before the instrument forms the basis of a precise method. A few runs were made on samples placed in a magnetic field, but the data were insufficient to permit conclusions to be drawn.

The process of sedimentation was found to be more complex than had been supposed. The experimental difficulties--convection currents, hindered settling, multiple scattering of the light--could be overcome, but there remain serious theoretical problems to be resolved: development of the hydrodynamical laws for settling of non-spherical particles and estimation of the Mie scattering coefficient for the non spherical particles. These problems are discussed, and an approach to a solution is suggested.

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By

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CHAPTER I

INTRODUCTION

The experimental problem of this thesis is to measure the strength of the immune reaction by simultaneously recording the transmittance of light through several tubes of red cells (erythrocytes) settling in saline (physiologically-equivalent) or serum. The associated analytical problem is to relate the time changes in transmittance to the size-distribution of the settling particles.

This thesis deals with the solution of the first problem, and outlines the theoretical basis for the second. The introduction presents a sketch of the requirements for the machine, a survey of its construction, and a description of the agglutination process. The second part discusses the biochemistry of agglutination, and gives a qualitative discussion of settling. The third deals with the scattering, absorption, Stokes Law, and particle size-distribution. The fourth gives experimental results and conclusions. The appendix is reserved for a survey of previous methods of measurement, and details of the construction of the apparatus and circuitry.

The measurement of the strength of the agglutination reaction could be made in a number of ways. Sedimentation is a visible accompaniment of agglutination and the rate of sedimentation depends in some fashion on the strength of the immunological reaction. Thus sedimentation may serve as the basis of an instrumental method which

for several reasons may be preferable to a subjective method. As factors in the choice of a specific method, we note that the binding between the agglutinated cells is very weak, so the settling suspension should not be disturbed, and a number of samples must be studied simultaneously and as a function of time. Finally, enough room must be allowed for the sample to be placed in a water jacket or magnetic field to suit specified experimental conditions. These specifications are effectively met by use of optical transmittance to measure sedimentation rate. Since devices have been described previously which measure transmittance, what is original in this work is the ability to study samples simultaneously and automatically. For a discussion of the features of other existing methods, one may refer to Appendix 1.

The antigen-antibody reaction has been chosen for study for several reasons. First, the sensitivity of the reaction permits the unequivocal detection of substances in amounts of hundreths of a microgram. And since a future aim of this project is to assay the effect of magnetic fields on biological reactions, this high sensitivity makes it likely that no magnetic effect would fail to be detected. Second, the antigen-antibody reaction may be easily observed in vitro with only small amounts of blood and serum, and the test sample can easily be maintained in the desired experimental conditions. Third, since this reaction is one which is not entirely understood, the invention of a quantitative method to measure the rate and extent of agglutination (to supplement or to supersede the existing visual methods) will be a useful tool in determining the nature of the reaction.

In the apparatus, a boom holding the light source, two condensing lenses, and a photo-cell, swings above the samples which are placed on a semicircular table. (See Figure 1) As the boom swings, the light beam, which is blocked off between samples, shines through each sample tube and stimulates a voltage from the photo-cell on the other side. As the beam reaches a particular sample tube and light begins to enter the photocell, the ensuing mechanical motion of the recorder pen energizes an eddy-current brake which decreases the sweep speed. This slowing gives time for the recorder to respond fully to the signal from the photocell. The total arc swept may vary from 10 degrees to 180 degrees, and may contain a maximum of thirty samples along the path. The boom is allowed to sweep forward slowly in one direction while collecting data, and then to return quickly. If the machine is sweeping 8 samples at a typical rate, the forward sweep would take two minutes and the return one-half minute. The runs last from half an hour to twelve hours, and the sweep rate just mentioned gives a sufficient number of points to determine a good curve.

The sample is a suspension of human erythrocytes in saline or serum contained in a square glass cell from a Beckman - DK 2 Spectrophotometer. A window in a brass plate at a specific height on the cell governs the position and flux of the light beam traversing the cell. At the start of the run, the erythrocytes are evenly distributed throughout the saline, and the transmittance is at its lowest value. As the erythrocytes settle, the transmittance increases, and soon a division between the clear medium and the erythrocytes appears near the top of the tube. Settling continues for the next several hours, the

maximum transmission occurring when all the cells have settled into a dense mass at the bottom and the upper portion of the liquid is clear.

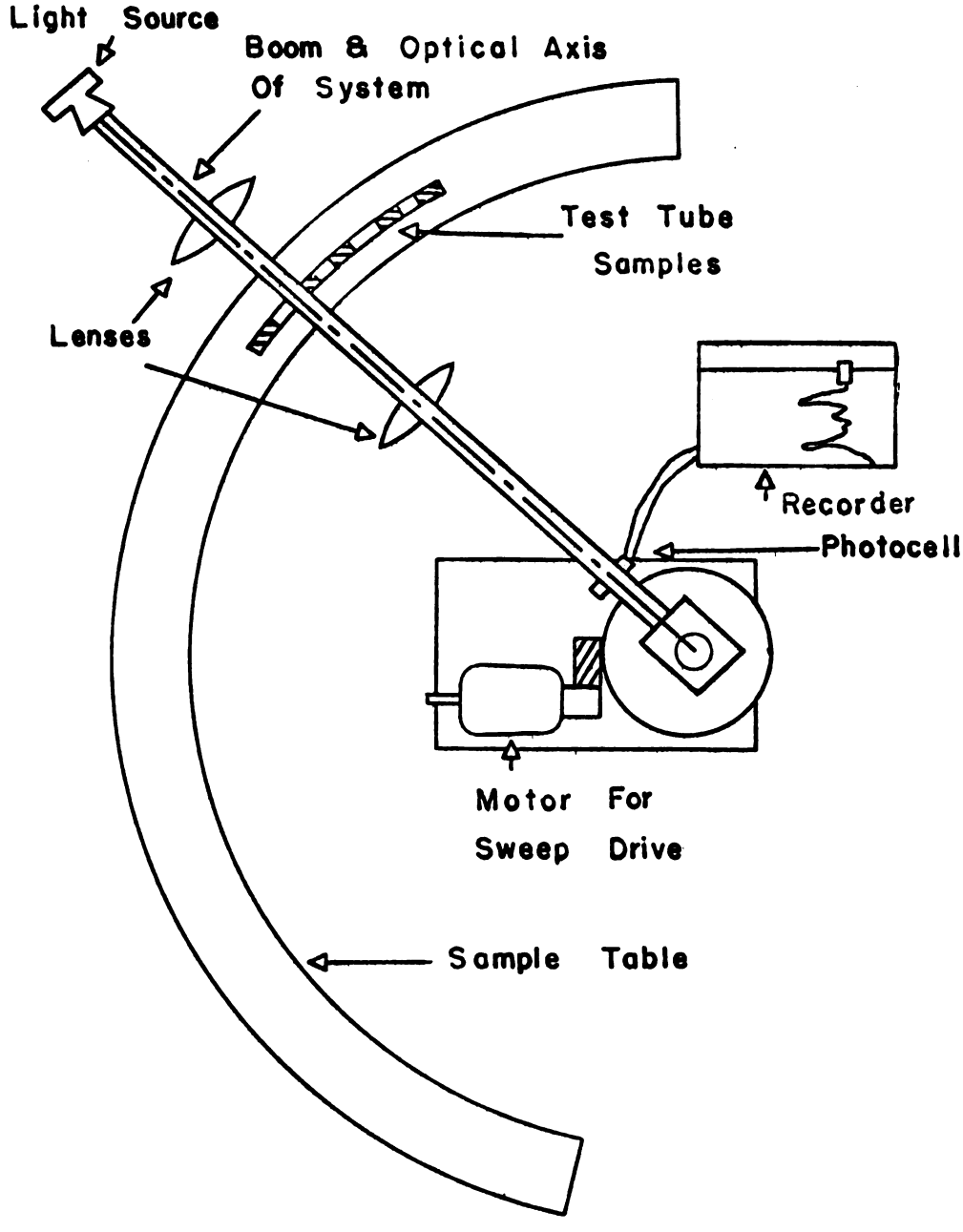

Fig. 1.--Diagram of the Main Components of the Apparatus.

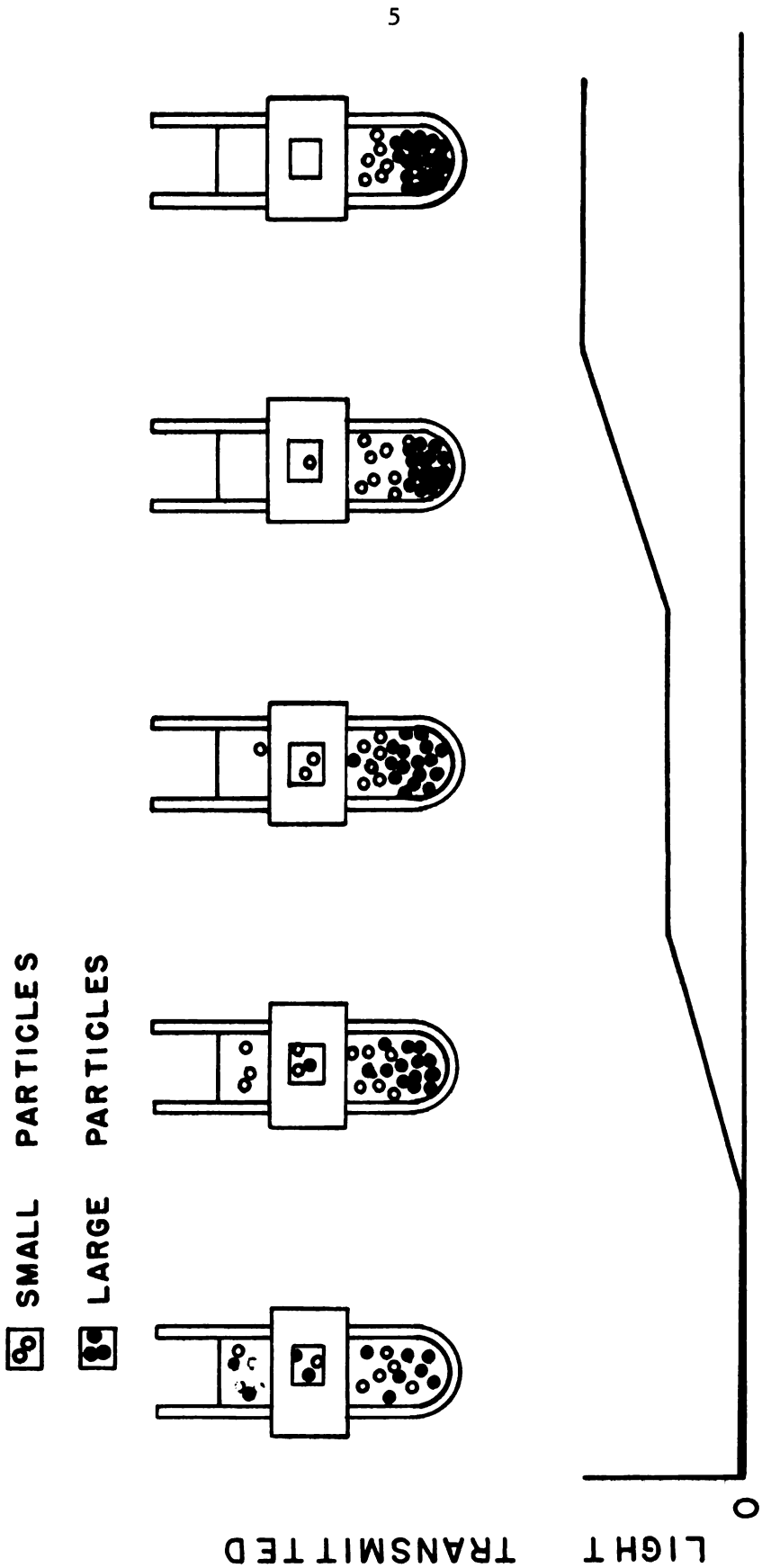

Fig. 2.--Four phases of sedimentation process and corresponding light transmission curve.

CHAPTER II

THEORY OF AGGLUTINATION

An antigen is a foreign substance, usually a protein of a very wide class, which has the ability to induce the formation of antibodies when it is introduced into the blood stream of an animal. Antibodies are proteins of the class of serum globulins, with molecular weights on the order of 160,000 or 900,000,[1] which have the ability to join with the specific antigen and render it inactive. This specificity is attributed to the presence of specialized combining sites, small areas of the antibody molecule which have a configuration complementary to the specific antigen. These sites of chemical activity which are responsible for bringing the antigen and antibody together in combination may extend over only one percent of the antibody molecule. The active area consists of a series of amino acids arranged in a specific sequence and stereoconfiguration, but the rest of the molecule is inert in this reaction. It is this stereoconfiguration joining a spot of complementary configuration on the antigen which is responsible for the very high degree of specificity of the immunological reaction.

Certain substances, called haptenes, have the property of uniting with an antibody once it is formed. This union takes place because the haptenes possess the complementary configuration which matches the

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[1] Advances in Biological and Medical Physics Vol III. p. 102. (Academic Press, 1953).

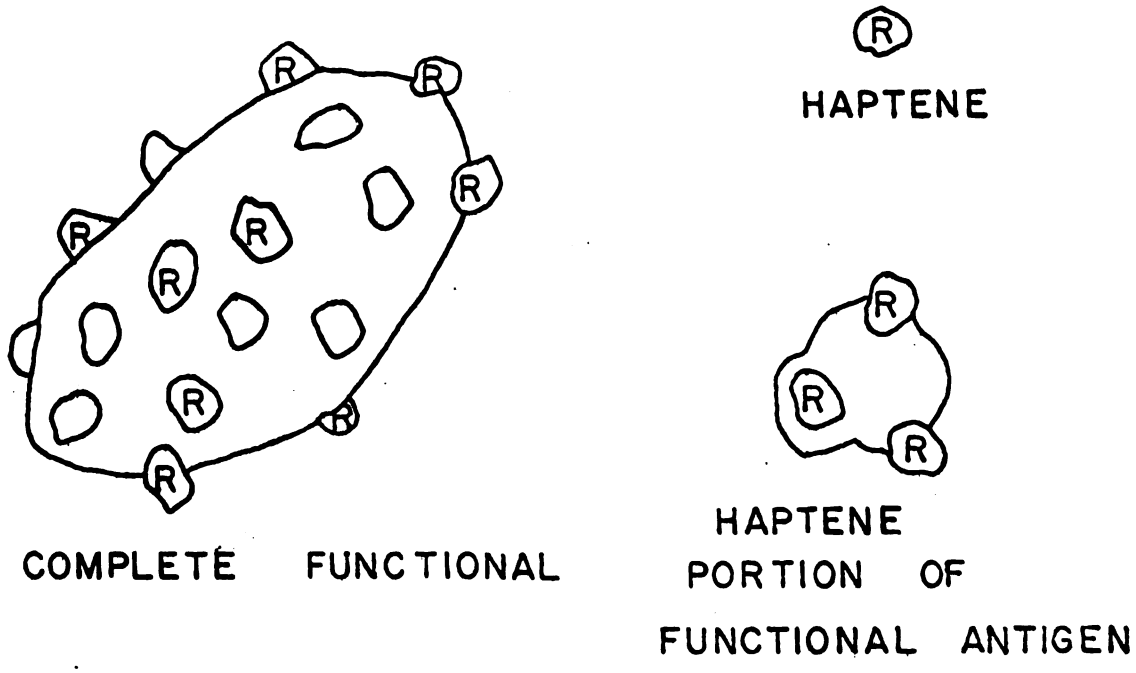

Fig. 3.--Conceptual representation of functional and haptenic antigens. The R group represents a single determinant of antibody specificity and is the actual group that combines with the antibody molecule.[2]

active site of the antibody. Alone, however, the haptene is unable to stimulate the production of antibodies. But when combined with a protein molecule, it can not only stimulate the production of antibodies but join with them. In such a case, the haptene governs the specificity of the reaction. For example, the pneumococcus owes its pathogenic properties to an attached carbohydrate group which alone cannot produce antibodies. Combined with the base protein, however, the carbohydrate group elicits the formation of an antibody which neutralizes its own pathogenic properties.[3] The ability to add functional groups to the base protein enables testing and altering the specificity

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[2]Cushing, J. and D. Campbell, Principles of Immunology. p. 37. (McGraw Hill Book Co., Inc. New York. 1957).

[3]Houssay, Bernado A., Human Physiology. p. 79. (McGraw Hill Book Co., Inc. New York. 1953).

and extent of a particular immunological reaction, and serves as an important tool with which to study the mechanism.

Two theories have been proposed to explain the formation of antibodies: one, called the template theory, suggests that polypeptide chains fold and wrap themselves around the active sites of the antigen molecule and form a shape complementary to it, and thus create the antibody specific to this antigen. The other theory, called the clonal-selection theory, asserts that the body has previous information on the structure of the particular antigen, and that it uses this information to manufacture the antibody to fit the specific antigen when stimulated by the antigen. References to the formation of antibodies may be found in Advances in Biological and Medical Physics, Cushing and Campbell, Kabat and Mayer.[4]

Human blood can be classified into four blood types according to the presence or absence of two antigens A and B on the wall (stroma) of the red cell. There are four combinations of the antigens resulting in the following blood types: Type AB, with A and B antigens; Type A, with antigen A alone; Type B, with antigen B alone; and Type O with neither antigen A or B. Existing in the serum of the blood and reacting specifically with the foreign antigens A or B on the cell, are the antibodies. In blood typing, these antibodies are called alpha and beta agglutinins, and they react specifically with the A and B antigens, respectively. The type AB blood contains neither alpha nor

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[4] Advances in Biological and Medical Physics, op. cit.
Kabat, Elvin A., and Manfred Mayer, Experimental Immunochemistry (Charles C. Thomas Pub., Springfield, Ill. 1948).
Cushing and Campbell, op. cit.

beta agglutinin in its serum, for if it did it would react internally to agglutinate its own cells. Type A contains only the beta agglutinin, type B contains only alpha, and type O contains both alpha and beta agglutinins in its serum. These facts are tabulated below for each blood type.[5]

Blood Group	Antigens on Red Cells	Antibodies in Serum	Agglutinins	Reaction with Erythrocytes: Anti A Antibody	Reaction with Erythrocytes: Anti B Antibody
O	None	Anti A Anti B	α,β	-	-
A	A	Anti B	β	+	-
B	B	Anti A	α	-	+
AB	A,B	None	0	+	+

For a large number of erythrocytes to agglutinate in a single aggregate the antibody molecule must be polyvalent. The rate of combination depends on the amount of antiserum available to form bonds between erythrocytes, and on the extent and frequency of contact between erythrocytes.

Brownian movement and gravitational descent are the two motions experienced by the erythrocytes which would cause them to come in contact. We can calculate the extent to which the cell wanders in a given time owing to Brownian movement, and we can calculate the distance between cells.

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[5]Houssay, op. cit., p. 60.

1) The motion due to Brownian Bombardment may be expressed by:

$$\frac{X^2}{t} = \frac{RT}{3\pi\eta r}$$ where X = distance moved in time t

$$R = 1.38 \times 10^{-16} \frac{\text{erg}}{\text{deg}}$$

$$T = 3 \times 10^{2} \text{ deg}$$

$$= .9 \times 10^{-2} \frac{\text{dyne - sec}}{\text{cm}^2}$$

t = 30 min. = 1800 sec.

η = .9 centipoises (viscosity)

$r = 10\mu$

$$X^2 = \frac{1.38 \times 10^{-16} \times 300 \times 1800}{3 \times 3.14 \times 9 \times 10^{-2} \times 10^{-4}} \quad \frac{\text{dyne x gm x deg x sec}}{\text{deg x } \frac{\text{dyne x sec}}{\text{cm}^2} \text{ x cm}}$$

$$X^2 = \frac{1.38 \times 1.8}{\times .9} \times 10^{-5} \text{ cm}^2$$

$$X^2 = .88 \times 10^{-5} = 8.8 \times 10^{-6} \text{ cm}^2$$

Particle Moves $X = 2.96 \times 10^{-3}$ cm = 29.6 μ in 30 min.

Particle Moves $X = 1.7 \times 10^{-3}$ cm = 17 μ in 10 min.

2) A cell count of 27×10^{6} $\frac{\text{erythrocytes}}{\text{ml.}}$ = 27,000 $\frac{\text{e}}{}$

$$(27 \times 10^{6})^{1/3} = 3 \times 10^{2}$$

$$\frac{10^{4}\ \mu}{3 \times 10^{2}} = 33\mu \text{ between centers of each cell.}$$

We see that the effect of Brownian motion will be such as to make the particle wander in a area of radius 17μ in a period of 10

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[6]Moelwyn-Hughes, E. A., Physical Chemistry. p. 64, equation 145. (Pergamon Press, N.Y. 1957).

minutes. Thus after 10 min. there will be ample contact between erythrocytes for combination if contact alone insures it. Although the fraction of active sites is small, there are probably enough that a single contact will result in a union.

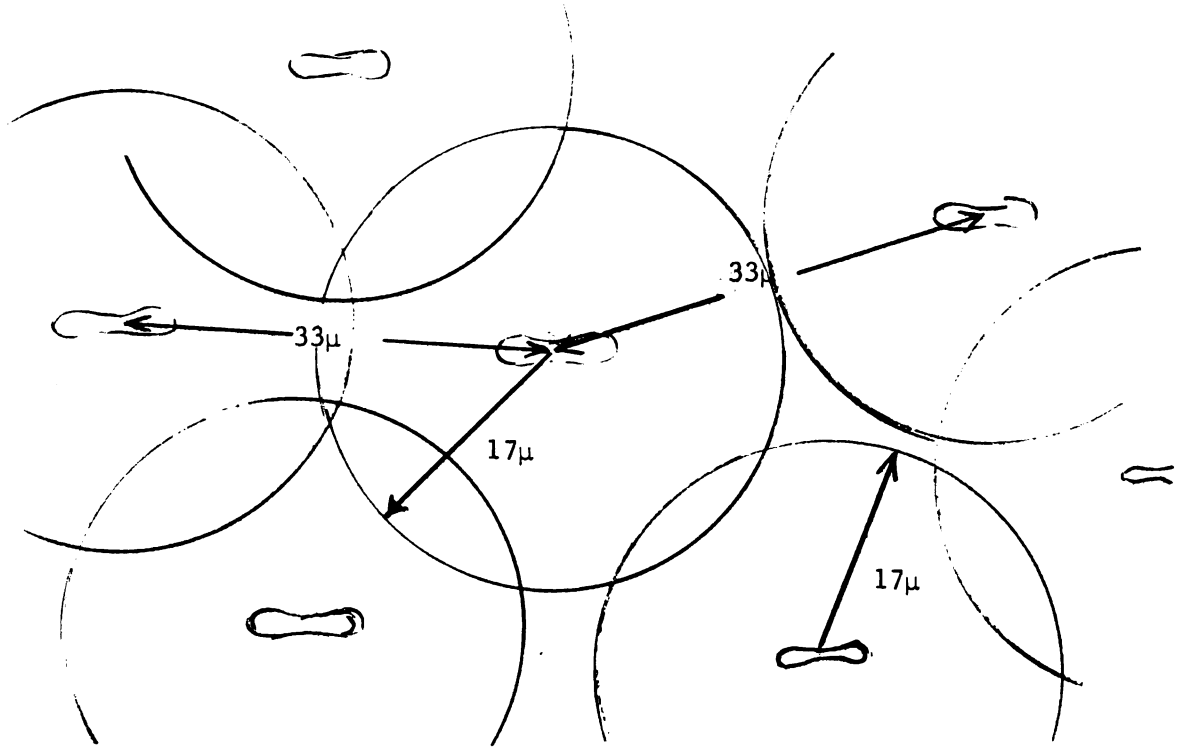

Fig. 4.--Radial distance the erythrocyte wanders under Brownian Bombardment in scale with the actual distance between cells for a concentration of .27 x 10^8 erythrocytes/ml.

We may visualize the agglutination then in the following manner: In a sample mixed with the appropriate antiserum, certain erythrocytes become potentially reactive. Convection and Brownian motion bring them near enough to form dimers (a coupling of two erythrocytes); these descend through the solution faster than the single cells; they have a weight equal to that of two cells but a perimeter less than that of two

cells.[7] As they fall, they will overtake and combine with other single erythrocytes and form larger aggregates. They will fall faster and an avalanche will occur if enough antiserum is present to make all cells potentially reactive. (See curves A and B Fig. 5 on the following page.)

If there is only enough antiserum to supply antibodies for the union of half of the erythrocytes, then dimers mostly would be formed, and avalanching would not be important.[8] (See curve C Fig. 5 on the following page.)

Fig. 6 curve B shows the increase in rate of settling due to the formation of dimers. As the dimers fall out of the path of the beam, the transmittance curve due to the settling of the residual single cells has the same slope as curve A with the virus absent.

The effect of the concentration of the erythrocytes is simply to control the sensitivity of the transmission measurements. With high concentration, the opacity of the suspension makes it difficult to detect changes attributable to the formation of the relatively small number of dimers. A more important factor is whether there is antiserum enough to agglutinate the erythrocytes present regardless of their concentration. Increased experimental time merely allows more collisions between erythrocytes, and thus formation of larger and more numerous aggregates, and attainment of a more advanced stage of settling.

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[7] Kunkel, W. D., J. Appl. Phys. 19, 1056-8. (1948).

[8] Kabat and Mayer, op. cit., p. 117.
Hirst, G. K., and E. G. Pickels, J. Immunol. 45, 273 (1942).

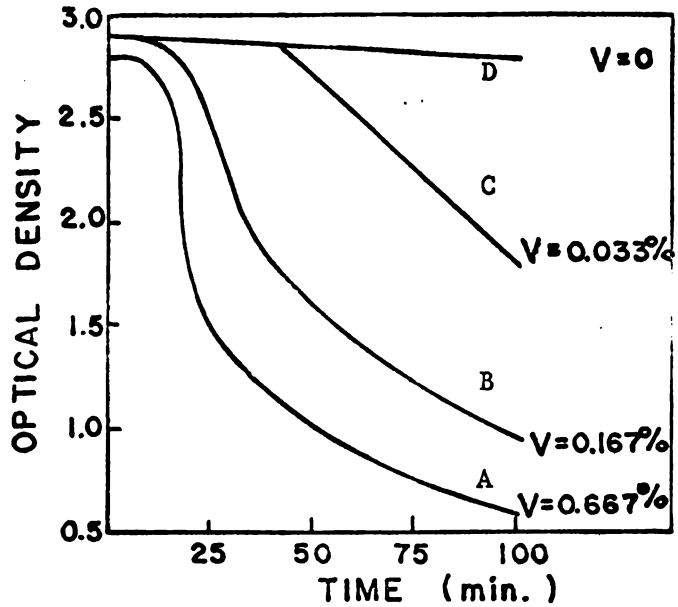

Fig. 5.--The time course of the optical density through a region slightly below the center of tubes containing a constant amount of red cells and varying quantities of influenza virus suspension. As the amount of virus decreases, the form of the curve changes from one with steep inflection (A and B) to a linearly falling optical density C. D is control with no virus.[9]

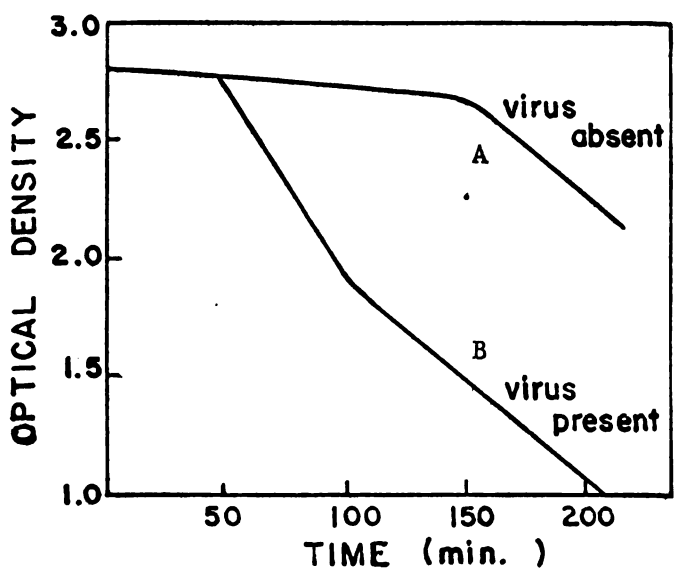

Fig. 6.--The lower curve illustrates that appregates larger than dimers have not been formed in appreciable numbers. The break at 100 min. marks the time when the boundary of the dimers has passed out of the field scanned by the light beam. Thereafter the optical density of the tube containing the virus is parallel to the control. The difference in cell density in the two tubes when the curves fall at parallel rates is a measure of the number of cells dimerized.[9]

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[9]Levine, Puck, and Sagik, J. Exper. Med. 98, 521 (1953).

CHAPTER III

THEORY OF SCATTERING AND STOKES LAW

Most of this thesis is concerned with the experimental problem of designing a machine capable of differentiating between agglutinating and non-agglutinating suspensions of erythrocytes. This chapter, on the other hand, introduces a related and much more complicated problem, analytical in nature: What is the dependence of the transmittance on the size-distribution of the erythrocytes? We shall try to outline a direction of solution by discussing first, what happens to the light beam as it penetrates the sample, and second, what are the factors affecting sedimentation.

Consider an electromagnetic wave at any point in empty space. For its complete description we must know its wavelength, direction, intensity and state of polarization at each moment.

Let I be the intensity, $\underline{k}$ wavevector, $\underline{r}$ position vector and t time.

Then $I = I(\underline{k},\underline{r},\underline{t})$ for horizontal polarization

$I = I(\underline{k},\underline{r},\underline{t})$ for vertical polarization

Since the intensities of the horizontally and vertically polarized components will be equal, and we are considering a steady state condition,

$$I = I(\underline{k},\underline{r})$$

When a beam of light impinges on a molecule, the energy may be absorbed in several ways: in rotational states if the molecule

possesses a dipole moment; in vibrational states, if the charged atoms could vibrate about a fixed position; and in vibrational states of the electrons. Some of the incident energy is reemitted and termed scattered radiation. If the atoms are sufficiently close as in a lattice, the reemission of the energy by the electrons takes place as a cooperative effect, and may result in reflection. The remaining energy is lost as kinetic energy of vibration in the molecule, and is termed absorbed radiation. These are the basic phenomena affecting the beam.

The expression for the differential change in light intensity as the beam traverses a slab of thickness dx, containing n scatterers/ unit vol. with cross-sectional area A is

$$dI = I(K_{sca} + K_{abs})\ nAdx + jdx$$

The first term represents the energy removed from the beam; K_{sca} and K_{abs} are the scattering and absorption coefficients respectively. The second term jdx represents processes which could add energy to the beam:flourescence and secondary scattering back into the beam of rays previously scattered out of the beam. Flourescence may be disregarded and if we neglect multiple scattering also then the jdx term does not appear. The integrated extinction formula for single scattering and absorption through a thickness l is

$$I = I_o \exp-(K_{sca} + K_{abs})\ Anl$$

The coefficients K_{sca} and K_{abs} are both very complicated functions of the size and shape of the particle, wave number and the refractive index of the particle relative to the medium.

To test the applicability of this expression, the following experiment was performed: Starting at concentrations of 5.7×10^8

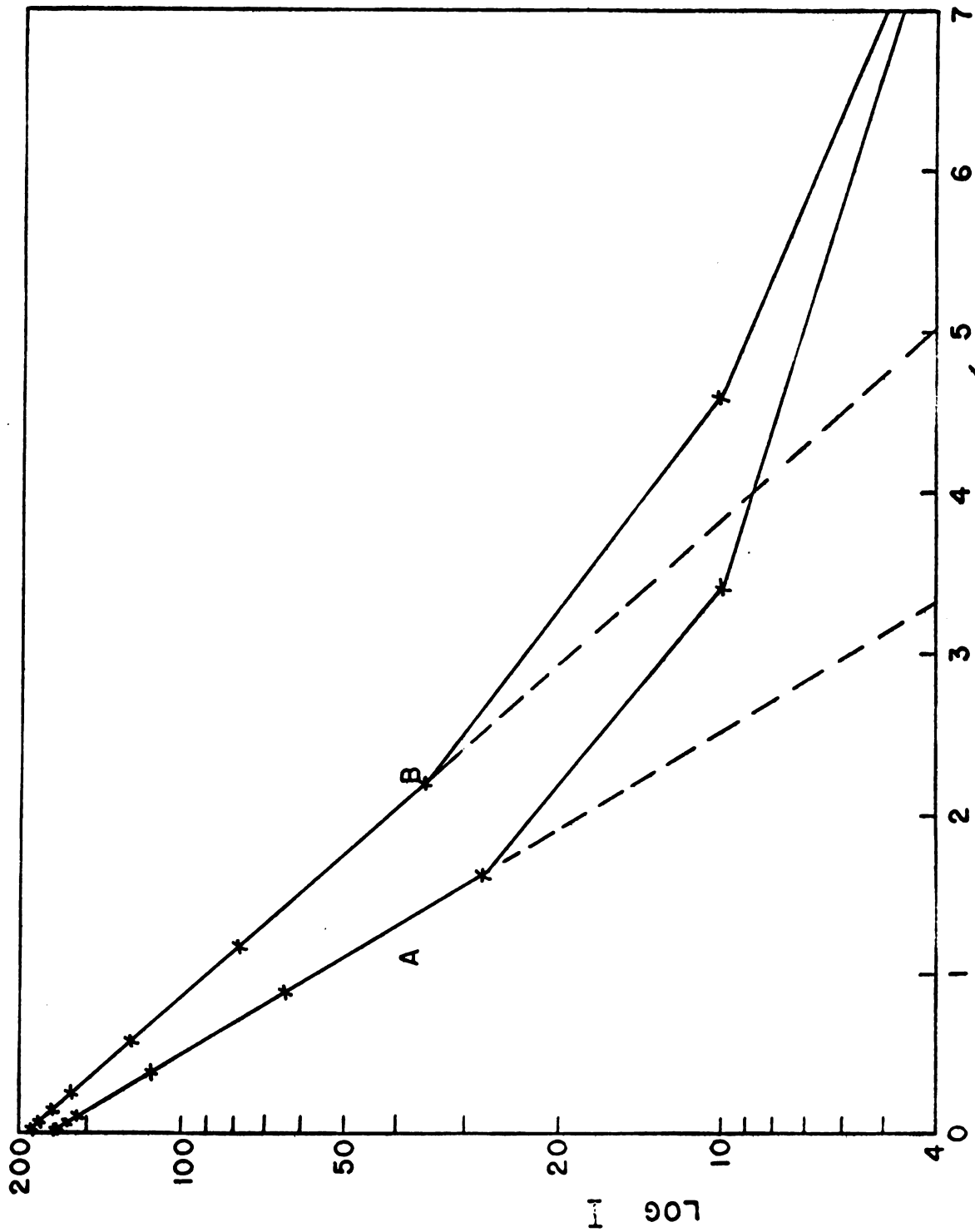

Fig. 7.--Plot of Concentration against Light Transmitted in two serial dilutions A and B.

erythrocytes/ml and again at concentrations of 1.8×10^8 erythrocytes/ml, serial dilutions of the suspension were made and the transmittance of each dilution was recorded. The log of the transmitted intensity as ordinate was plotted against the concentration as abscissa (See Fig. 7). At low concentrations the plot is linear in conformity with the above law representing single scattering and absorption. The contribution by multiple scattering is small except at higher concentrations where the curve deviates markedly from the straight line. Here other factors such as the opacity of the cells may affect the curve. The problem of fully describing the transmitted intensity in terms of the parameters of the problem is extremely complicated and beyond the scope of this thesis. It does seem to be possible, however, to do experiments in the range of low concentration where the exponential extinction law seems to apply.

There are many excellent references which deal with various facets of this problem. A lucid text which surveys the whole field of scattering is Light Scattering by Small Particles[10] by VanderHulst. This is highly recommended as a general introduction to the problem. Mie's original papers develop the general expressions for the absorption and scattering coefficients from Maxwell's Equations.[11] Max Born and Emil Wolf in their Optics[12] and Stratton in Electromagnetic

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[10] VanderHulst, H. C., Light Scattering by Small Particles. (John Wiley and Sons, Inc. N.Y. 1957).

[11] Mie, Gustav, Annalen der Physik (4) 25 (1908).

[12] Born, Max and Emil Wolf, Principles of Optics. (Pergamon Press, N.Y. 1959).

Theory[13] also use this approach. Multiple scattering receives full theoretical treatment by Chandrasekar in Radiative Transfer[14] and by V. V. Sobolev in A Treatise on Radiative Transfer.[15] Gumprecht and Sliepcevich[16] develop specific application of these theories for a light-transmission experiment where loss by scattering alone is considered. Their approach is nonetheless instructive since they introduce Stokes law and develop an expression relating transmission to particle-size distribution.

Let us consider losses due to reflection.

The Beckman Spectrophotometer Cell is made of glass with 1 cm x 1 cm inner cross-section and wall thickness of 1/32". The filament image comes to focus at a spot 1/2" in front of the cell and the light beam diverges only 4 degrees in passing through the cell. Essentially then, the rays traverse the cell normally to the interfaces.

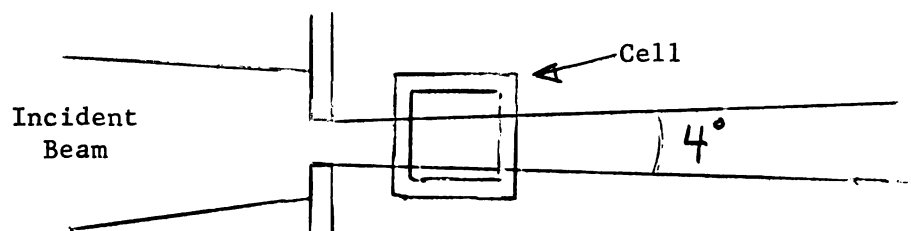

Fig. 8.--Alignment of Beckman cell with respect to incident beam.

Estimating losses due to reflection shows that they are constant with respect to time and concentration and reduce the amount of light by 8%.

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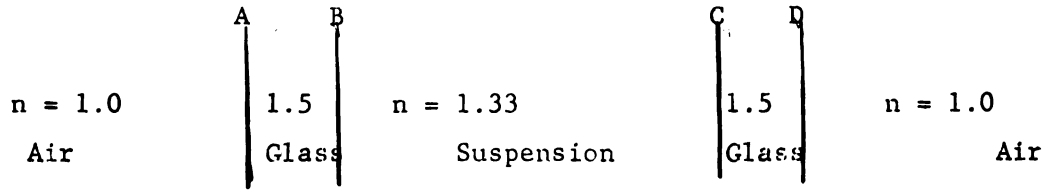
[13]Stratton, Julius, Electromagnetic Theory. (McGraw Hill Book Co., Inc. N.Y. 1941).

[14]Chandrasekar, S., Radiative Transfer. (Dover Pub. Inc. N.Y. 1960).

[15]Sobolev, V. V., A Treatise on Radiative Transfer. (D. VanNostrand, Inc. N.Y. 1959).

[16]Gumprecht, R. O., and C. M. Sliepcevich, J. Chem. Phys. 57, 95-97 (1953b).



$$r_A = \frac{(n_1 - n_2)^2}{(n_1 + n_2)^2} = \frac{(1.0 - 1.5)^2}{(1.0 + 1.5)^2} = .04$$

$$r_B = \frac{(1.5 - 1.33)^2}{(1.5 + 1.32)^2} = .0004$$

$$r_C = .0004$$

$$r_D = .04$$

Total Losses $\cong .08 = 8\%$

The limiting speed of spherical particles falling through a liquid is described by Stokes Law:

$$d_e = \sqrt{\frac{18\eta}{(p - p_o)g}} \cdot \frac{X}{t}$$

where d_e = effective diameter of particle
η = viscosity
p_o = density of saline
p = density of erythrocyte
g = acceleration due to gravity
x = height fallen in time t which is the distance the light beam is below the interface.

In our application, the Reynold's Number is low; .0001 to 2.0, and Stokes law may be used in the form given above which neglects inertial forces.[17] For non-spherical particles, an effective diameter is used in the equation. Kunkel has pointed out that objects fall so

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[17]Perry, J. H., Chemical Engineering Handbook. (McGraw Hill Book Co., Inc., N.Y. 1950). 3rd Ed.

as to offer the greatest possible viscous resistance, that is, with the major axis of inertia parallel to the velocity for typical particles.[18] The deviation of the erythrocyte from sphericity thus results in its falling slower than a sphere of the same mass. The size estimates of maximum radius by Stokes Law therefore, would be erroneously small, perhaps by 50 percent.[19]

For fall times of the order of hours, the effect of Brownian bombardment on the rate of fall is negligible. For a time t = 60 min, the size of a critical radius for which the extent of motion under Brownian bombardment is equal to fall under gravity, is equal to Thus Brownian bombardment will not keep the erythrocyte of diameter 7μ from falling. But as we noticed earlier, Brownian motion is a significant factor in the formation of dimers.

The ionic strength of the saline solution is 0.14 N, which is strong enough to eliminate any electroviscosity effects of the descent of the erythrocytes.[21] This problem is discussed in detail by Booth[22] and Elton and Hirschler.[23]

Hindered settling refers to the phenomenon in settling concentrations where a very sharp interface is formed between the settling particles and the supernatant liquid. In such a case, the rate of fall

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[18] Kunkel, op. cit.

[19] Ibid.

[20] Alexander, Jerome, Colloidal Chemistry. (Chemical Cat. Co., N.Y., 1928).

[21] Orr, Clyde and J. M. Dallavalle, Fine Particle Measurement: Size, Surface and Pore Volume. (MacMillan Co. N.Y. 1960).

[22] Booth, F., J. Chem. Phys. 22, 1956-58.

[23] Elton, G. A. H. and F. G. Hirschler, Brit. J. Appl. Phys. Supp. 3 s60-s62. 1954.

of a sphere is less than that given by Stokes Law. If V_s is Stokes Velocity, Q is the velocity in a vertical plane, and ϵ = the fraction of the total suspension occupied by the supporting liquid, the rate of fall would vary from that given by Stokes Law according to:[24]

$$Q = V_s \epsilon^2 \, 10^{-1.82(1 - \epsilon)}$$

For a $.27 \times 10^8$ cell count, the fraction by volume of cells is:

$$.27 \times 10^8 \times 70 \times (10^{-4})^3 = .00189 \text{ cc.}$$

Therefore $\epsilon = .9981$

And $Q = V_s(.9981)^2 \, 10^{-1.82(.00189)}$

Or $Q \approx V_s$

Thus there are very small deviations from Stokes Law due to hindering at $.27 \times 10^8$ cell count. The largest and at this time unknown discrepancy is due to the departure of the shape of the erythrocyte from that of a sphere.

The conclusions which may be drawn from the above are as follows.

1) A simple exponential relationship between transmittance and concentration does exist at low concentrations (below $.54 \times 10^8$ erythrocytes/ml.). At higher concentrations, the problem becomes much more complicated requiring accurate expressions for the scattering and absorption coefficients for non-spherical particles.

2) Reflection losses are not difficult to calculate and constant in time. Electroviscosity, brownian movement and hindered settling affect the sedimentation process by a negligible amount below $.27 \times 10^8$ erythrocytes/ml.

3) Stokes Law is applicable but corrections still must be made to account for the non-spherical shape of the erythrocyte.

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[24]Steinour, Harold, Industrial Engineering Chemistry. 36 840-7. 1947a.

When the above problems are solved, it will be possible to express particle-size distribution in terms of light transmission measurements.

CHAPTER IV

PREPARATION FOR RUNS

Ten ml of whole blood is removed from the arm of a volunteer, and preserved in 1 ml of sodium citrate under refrigeration. Before the run, 1 ml of whole blood is placed in a saline solution and washed by centrifuging three times for 3 min. at 3000 rpm. The various concentrations of erythrocytes are prepared by using a certain amount of packed cells to saline.

In a normal human adult there are 5,400,000 erythrocytes/ml of blood. Since 30% of the volume of whole blood is occupied by erythrocytes, then the count is:

$$\frac{5.4 \times 10^9}{.3} = 18 \times 10^9 \frac{\text{erythrocytes}}{\text{ml of packed erythrocytes}}$$

$$\frac{3 \text{ ml of packed ery.}}{100 \text{ ml of Saline}} \times 18 \times 10^9 = 5.4 \times 10^8 \frac{\text{erythrocytes}}{\text{ml}}$$

TABLE 1

AMOUNT OF PACKED ERYTHROCYTES IN ML. TO BE MIXED WITH SALINE FOR A DESIRED COUNT

Volume of Saline	Desired Counts: ($\times 10^8$)						
	5.4×10^8	2.7	1.35	1.07	.54	.27	
4	.12 ml	.06	.03	.024	.012	.006	
20	.60	.30	.15	.12	.06	.03	Amount
25	.75	.375	.186	.15	.075	.037	of
30	.90	.45	.225	.18	.09	.045	packed
35	1.05	.525	.26	.21	.105	.052	cells
50	1.5	.75	.375	.30	.150	.075	(ml)
100	3.0	1.5	.75	.60	.3	.15	

Since anti-A and anti-B sera will cause agglutination at room temperatures, the desired blood suspension and the serum dilutions must be prepared separately just before the start of the run: 2 ml of double the desired cell concentration is added to 2 ml of serum of half the desired dilution. The 2 ml of blood will dilute the serum concentration and the serum will halve the cell count, to make 4 ml of the desired preparation. After the suspensions with anti-A and anti-B have been prepared, they may be placed directly in the machine, whereas the suspensions mixed with anti-B serum must be placed in the 37° water bath for the serum to react. Two methods were used to mix the erythrocytes and serum. The tubes for one group of anti-D suspensions were inverted every 10 minutes during an hour to mix the reactants, and the tubes for the other were centrifuged for 45 seconds at a half hour and returned to the bath for the rest of the hour before being pipetted into the sample tubes.

Before the suspensions are pipetted into the cells, a certain number of adjustments must be made on the machine:

1) The cells must be washed with distilled water and dried, to prevent hemolysis of the erythrocytes.

2) The slit heights should be adjusted for each sample to give a uniform amount ($\pm$ 10%) of light through each.

3) The pen slide must be cleaned and oiled for quick response, and the friction clutch must be tightened enough to maintain contact during pen rise, and to break contact during pen fall.

4) The light source should be turned on at least a half hour before the start of a run to stabilize the intensity. Slight differences in flux through each cell offer no great difficulties since these

variations are constant during the course of a run, the resulting curves can each be normalized by a suitable factor and replotted.

5) The reversing arms on the shaft should be adjusted to reverse the sweep 2" beyond the end samples.

6) The Variac setting should be set to give a sweep speed slow enough for full record response as the light traverses the sample, but fast enough to collect an adequate amount of data.

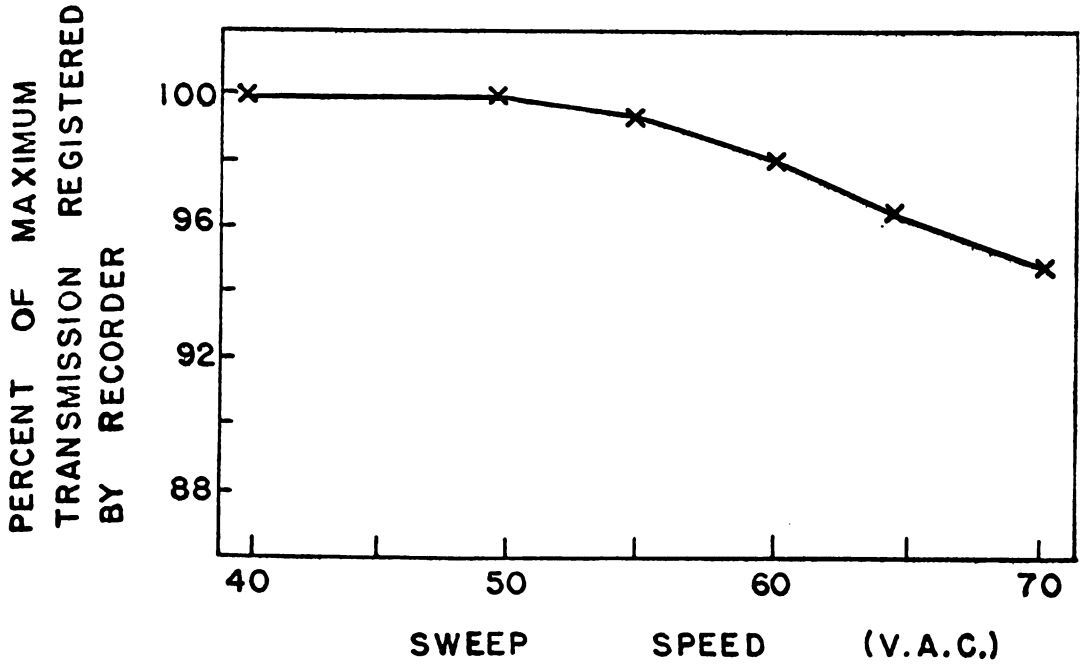

Fig. 9.--Plot of Percent of Maximum possible transmission registered by recorder against Sweep Speed in Volts A.C.

7) Typical Running Values are:

Recorder Scale:	12.5 mv., 25 mv.
Recorder Speed Chart:	0.2"/min.
Variac Setting:	55 vac., 60 vac.
Steady Brake Current:	1.7 amp.
Pulse Brake Current:	3.0 amp.
Capacitance:	2000 μf.
Recorder response with clear saline in Beckman	3/4 of full scale

Height of Light Beam	1/8" below meniscus
	3/8" below meniscus
Number of Samples Run	8
Number of Spaces for Samples	8
Number of Spaces for Magnetic Samples	3

CHAPTER V

EXPERIMENTAL RESULTS

Because of the gradual development of the machine, it is convenient to describe the results and innovations on the machine in chronological order. A run consists of preparing a blood suspension of the desired count, diluting it in serum or saline, and placing it in the machine. The on-off timers are set so the machine will stop sweeping when all particles have definitely settled and the transmittance is maximum.

In tabulating the runs, the numbers in the columns of Saline, Anti D, Anti-A, Anti-B, denote the number of samples in the corresponding soluent. Slit refers to the height of the rectangular window in front of the Beckman Cell. The available sizes are .03 x 3/16", .06 x 3/16", .02 x 3/16". Level means the distance between the top of the light beam and the meniscus of the suspension.

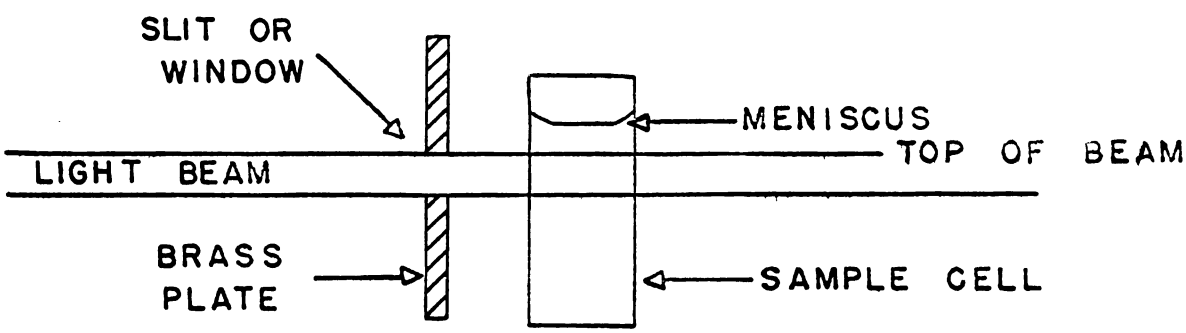

Fig. 10.--Diagram showing arrangement of cell and slit.

a) C - 1 to C - 16

Each run consists of two samples of erythrocytes with saline

and two samples with Anti-D serum, counts of 5.4 x 10^8 erythrocytes/ml in a 4 mm. round test tube. Because it was difficult to position the cell so the light beam would be undeviated in passing through and the sweep was not retarded, these runs were invalid for collecting data.

b) T - 1 to T - 40

A series of T (test) runs were devised to test the machine operation but not to collect data. The effects tested and corrected were, for example: source constancy (of the arc lamp in use at this time) electrical noise in the photocell circuit, transformer noise, recorder response with control specimens in the tubes, effect of sweep speed on recorder response.

c) C - 17 to C - 42

Run	Count (10^{8e}/ml)	Number of Samples in Medium of		Remarks
		Saline	Anti-D	
C -17	5.4	3		Used round 4-mm. test tubes
C -19	5.4	10		6 test tubes, 4 Beckman
C -20	5.4	4		4 Beckman
C -21	5.4	4		4 Beckman
C -22	4.6	4		4 Beckman
C -23	4.6	single		4 Beckman
C -24	4.6	single		4 Beckman
C -25	2.7	5		Round Tubes and Beckman
C -26	2.7	4		Beckman
C -27	2.7	4		Beckman
C -28	2.7	single		Beckman
C -29	2.2	2	2	Beckman
C -30	1.2	2	2	Beckman
C -31	2.2	2	2	Beckman
C -32	1.3	2	2	Beckman
C -33	2.7	2	2	Beckman
C -34	3.2	4		Latex Suspension: No settling,
C -35	3.2	4		colloidal.
C -36	3.2	4		
C -41	3.2	4		Brake on
C -42	5.4	4		Brake on

This series was run with high erythrocyte count suspensions contained in the Square Beckman Cells at sweep speeds of 60 vac (sweep cycle time = 2 min.). The light beam was located 1/8" below the meniscus, and was powered by a 6v. storage battery; and the eddy-current brake was not utilized until the last runs.

No meaningful data were collected; the curves were not smooth, and the individual ordinates fluctuated owing to the lack of an eddy current brake.

d) C - 43 to C - 55

For these runs the machine was improved considerably. A pen motor with the faster response time (1/2 sec. full scale) was installed; the eddy-current brake, with 3-a. pulse for 1.3 sec. and a 1.5-a. steady current, was devised to retard the sweep at the time of measurement; the light beam was collimated by a 0.03" x 3/16" aperture in front of the cell.

Run	Count (10^8e/ml.)	Number of Samples in Medium of Saline	Remarks
C -43	5.4	4	1.5 amp. steady brake current
C -44	5.4	4	1.5 amp. steady brake current
C -45	5.4	4	1.5 amp. steady brake current
C -46	5.4	4	1.5 amp. steady brake current
C -47	2.7	7	1.5 amp. steady brake current
C -49	2.7		
C -50	2.7	4	1.5 amp. steady brake current
C -51	2.7		.03" slits
C -52	2.7	4	Introduction of Pulse
C -53	2.7	4	brake
C -54	2.7	4	.04" slits
C -55	2.7	4	.04" slits

The results were considerably better. The machine worked well, and the improvements enabled us to obtain a number of reliable curves

of the settling interface. The curves were normalized and replotted on smaller linear graph paper to facilitate comparison. The extent of agglutination could not be determined from the comparison of slopes, since with counts of 5.4×10^8 erythrocytes/ml and 2.7×10^8 erythrocytes/ml the interfaces are composed mainly of unagglutinated single cells. Run C -53 demonstrated the first evidence of the formation of convection currents: The transmission increased as the interface fell, then decreased as convection in the Beckman Cell lifted the erythrocytes back into the path of the light beam.

e) To eliminate convection which could be due to heat from the source, an infra-red filter and a 2"-thick water cell was used to filter out the infra-red in a series of runs with 1 or 2 samples each: A - 1 to A - 13.

The resulting settling curves were smooth and valid for 60 to 90 minutes, before convection disturbed the curve. If the beam is 1/8" below the meniscus, the convection currents take longer to circulate the erythrocytes into the path of the light beam than if the beam were farther below the meniscus.

f) A - 14 to A - 49

These runs were similar to C - 14 to C - 55 (d) in experimental set up. The purpose was to check a filling procedure standardized so all samples would settle at the same time. The curves were normalized and replotted. A t run A - 42, the source storage battery was replaced by a 6.3-v. filament filament transformer.

Runs	Count (10^8e/ml)	Number of Samples in Medium of Saline	Anti-D	Anti-A	Slit	Level	Remarks
A -14	2.7	4			.059"	1/8"	Good curves
A -15	2.7	4			.059"	1/8"	Good curves
A -16	2.7	4			.059"	.05"	Good curves
A -17	2.7	4			.059"	.05"	Good curves
A -18	2.7	4			.059"	.05"	Good curves
A -19	5.4	4			.059"	.05"	Good curves
A -20	stirred 19	6			.059"	.05"	Good curves
A -21	5.4	4			.030"	.18"	
A -22	5.4	4			.030"	.12"	
A -23	5.4	8			.040"		
A -24	5.4	8			.060"	.10"	
A -25	5.4				.060"	.10"	
A -26	5.4				.060"	.10"	Good run
A -27	5.4				.060"	.10"	Bad run
A -28	5.4				.030"	.10"	Bad run
A -29	5.4				.030"	.10"	
A -30	5.4				.030"	.10"	
A -31	5.4				.030"	.10"	
A -32	5.4				.030"	.10"	
A -33	5.4				.030"	.10"	Bad pulse
A -34	5.4	2		2	.030"	.10"	Bad pulse
A -35	5.4	2		2	.030"	.10"	Bad pulse
A -36	5.4	2		2	.030"	.10"	Bad pulse
A -37	5.4	8			.030"	.10"	Bad pulse
A -38	5.4	8			.030"	.18"	Bad pulse
A -39	5.4	4	4		.030"	.12"	Bad pulse
A -40	5.4	4	4		.030"	.12"	Good curves
A -41	2.7		4		.030"	.12"	Good curves
A -42	2.7		4		.030"	.12"	Good curves
A -34	2.7		4		.030"	.12"	Good curves
A -44	5.4		4		.030"	.12"	Good curves
A -45	2.7		4		.030"	.12"	Good curves
A -46	5.4		4		.030"	.12"	Good curves
A -47	5.4		4		.030"	.12"	Good curves
A -48	5.4		4		.030"	.15"	Good curves
A -49	5.4	4	4		.030"	.15"	Good curves

The performance is now quite reliable. No striking differences in the curves between the agglutinating and unagglutinating blood samples appear as yet.

g) The beam was allowed to set on 7 single-sample runs to see if the fluctuations in curves were due to machine operation. The runs were

1.35 x 10^8 erythrocytes/ml. count, level was 1/8" below meniscus, with saline and Anti-D.

The curves from these stationary runs were similar in shape and slope to those where the beam was sweeping several samples of suspension made with saline and Anti-D. This similarity, plus the ability of the machine to register the same value of light intensity for a sample which had completed settling, together with the reliability of the individual operations of the machine (switching, braking, recording, constancy of the light intensity) demonstrates that this machine provides an accurate method of measuring the optical transmittance through any number of samples over an indefinite period. (See Fig. 11.)

h) Runs A - 53 to A - 62 were made under the same experimental conditions as the (e) series, but with serial dilution of serum: 1:8, 1:16, 1:32, 1:64.

Runs	Counts (10^{8e}/ml)	Number of Samples in Medium of			Level	Slit	Results
		Saline	Anti-D	Anti-A			
A -53	1.35	1	3 8:1		.18"	.03"	Good run
A -54	1.35	2	2 8:1		.18"	.03"	
A -55	1.35,.9,.6	1	3		.18"	.03"	Fair run
A -56	1.35,1.07		5		.15"	.03"	Good run
A -57	1.35	1	6serial		.15"	.03"	Good run
A -58	.27	1	6s		.15"	.06"	Good run
A -59	1.07		6s		.15"		Good run
A -60	.54,.27	2	4		.15"		Good run
A -61	.54, .27			6s	.15"		Fair run
A -62	.54, .27				.15"		Incomplete
S101	5.4, .27	6			.15"		Inc.

Owing to the blocking power of the erythrocytes at these high concentrations and the convection, no difference between agglutinating and unagglutinating samples was ascertainable.

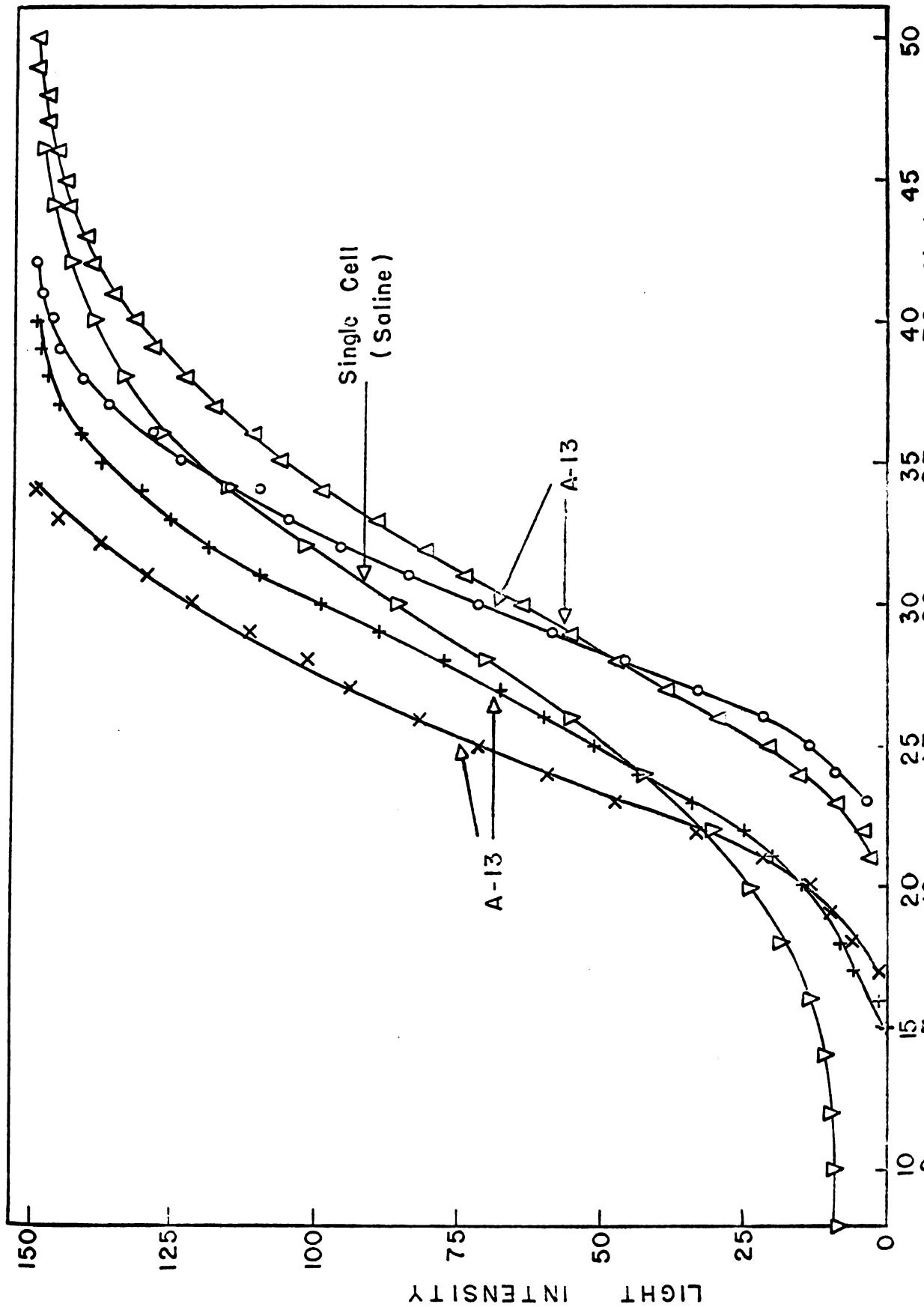

Fig. 11.--Plot of four curves from Run A - 13 compared to a single-cell run to illustrate similarity of shape and slope.

i) A - 64 to A - 73

These runs were made at 0.54×10^8 to 0.27×10^8 erythrocytes/ml counts. The beam was now 3/8" below the top of the meniscus, in order to observe the change in transmittance as the dimers dropped from the center of the tube. Anti-A and Anti-B sera were used, since they would react at room temperatures and would not need to be incubated.

Runs	Counts (10^{8e}/ml)	Number of Samples in Medium of Saline	Anti-D	Anti-A	Level	Slit	Remarks
S101	5.4, .27	6			.18"	.06"	Inc. run
	5.4				.3"		
A -63	5.4	1	5serial		.3"		Inc. run
	5.4				.3"		
A -64	1.07,.54	1	5s		.3"		Fair run
	1.07,.54,.27						
A -65	.54,.27	2	4s	4s	.3"		Good run
	.54,.27				.3"		
A -66	1.07,.54	2	4s	4s	.3"		Good run
A -67	1.07,.54	2	4s	4s	.3"		Good run
A -68	.54,.27	2	6s	6s	.3"		Good run
A -69	.54,.27	2	6s		.3"		Good run
A -70	.54,.27	8			.3"		Good run
A -71	.54,.27	3	5s	5s	.3"		Good run
A -72	.54,.27	3	5s	5s	.3"		Good run
A -73	.54,.27	2	6s	6s	.3"		Good run

Graphs of A -67, A -72, A -73 are included as figures 12, 13, 14.

The curves are now generally excellent, and some difference in slope is evident between the antiserum samples and the saline samples. Convection still causes a decrease in intensity of the suspensions after the initial rise in intensity. The agglutinating samples settle at a steadier rate, and their curves do not show the initial sharp rise which is characteristic of the saline. These differences, however, have not been observed frequently enough that they can be used to differentiate between samples. These results show, however, that the machine is

capable of measuring the transmittance through a sample, although it is premature to use it in differentiating between agglutinating and non-agglutinating samples.

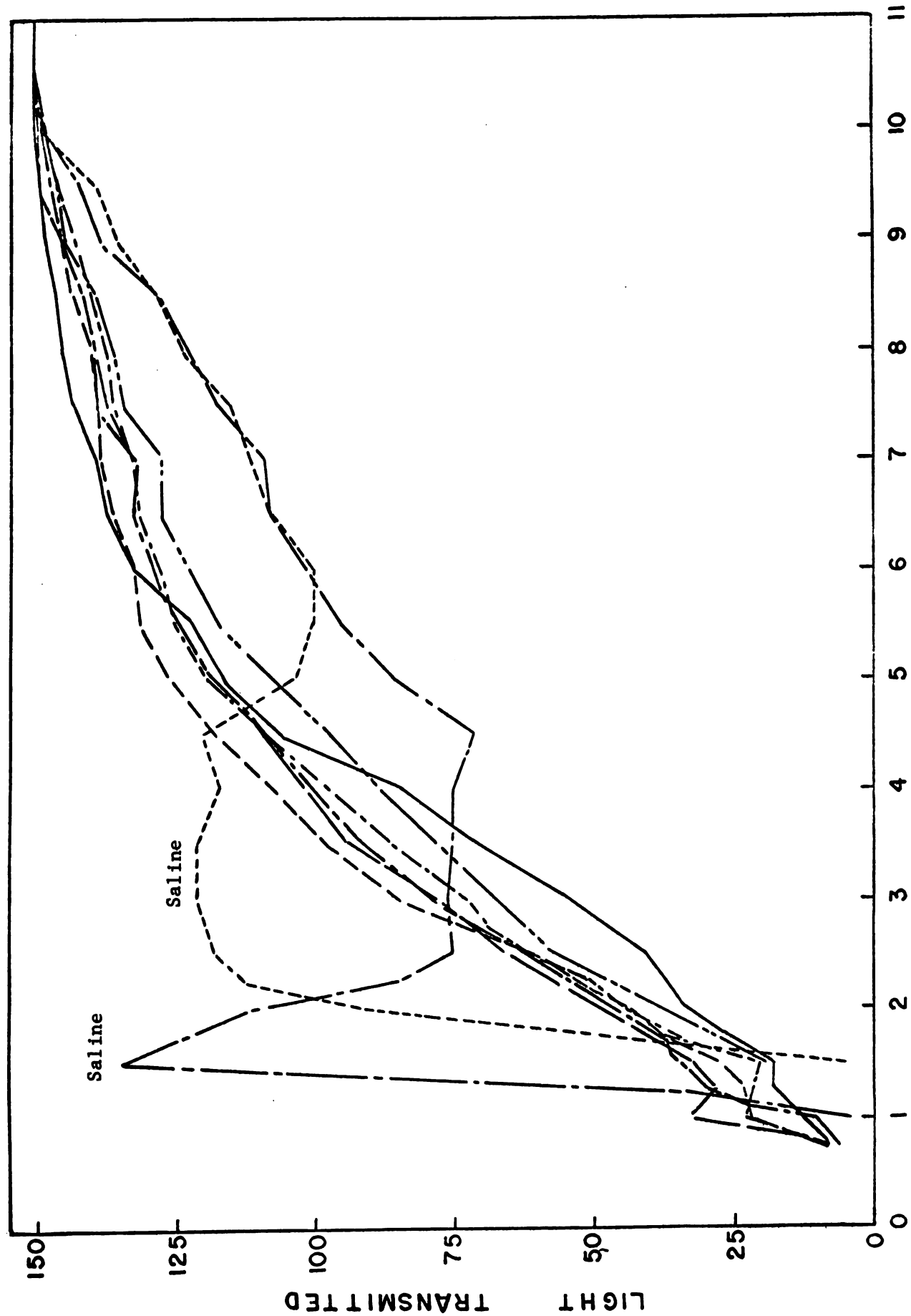

Fig. 12.--Plot of Run A - 67 showing the abrupt rise of the saline samples due to convection compared to the steady rise of samples mixed with serum.

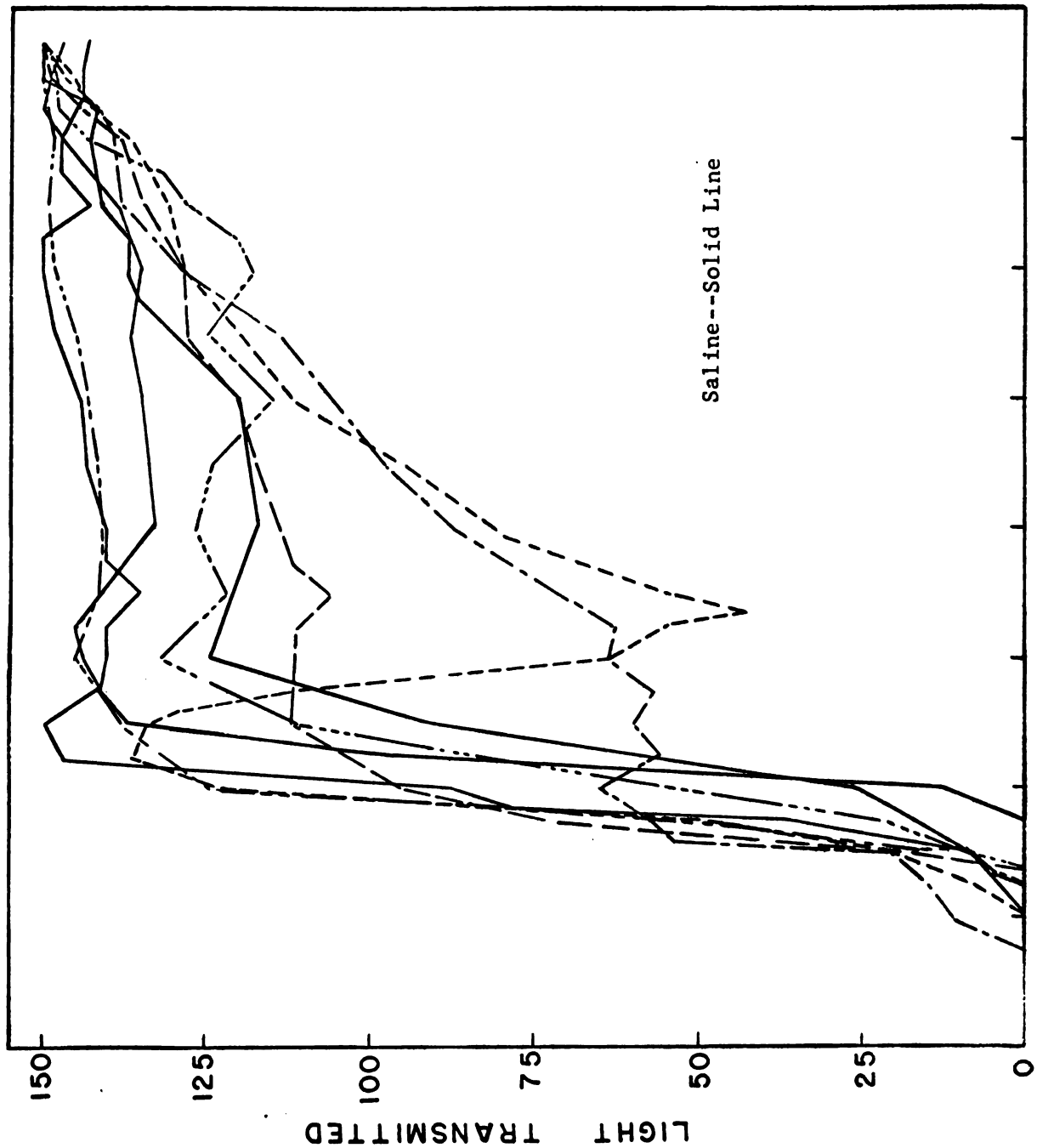

Fig. 13.--Plot of Run A - 72 showing comparative curves of saline and serum.

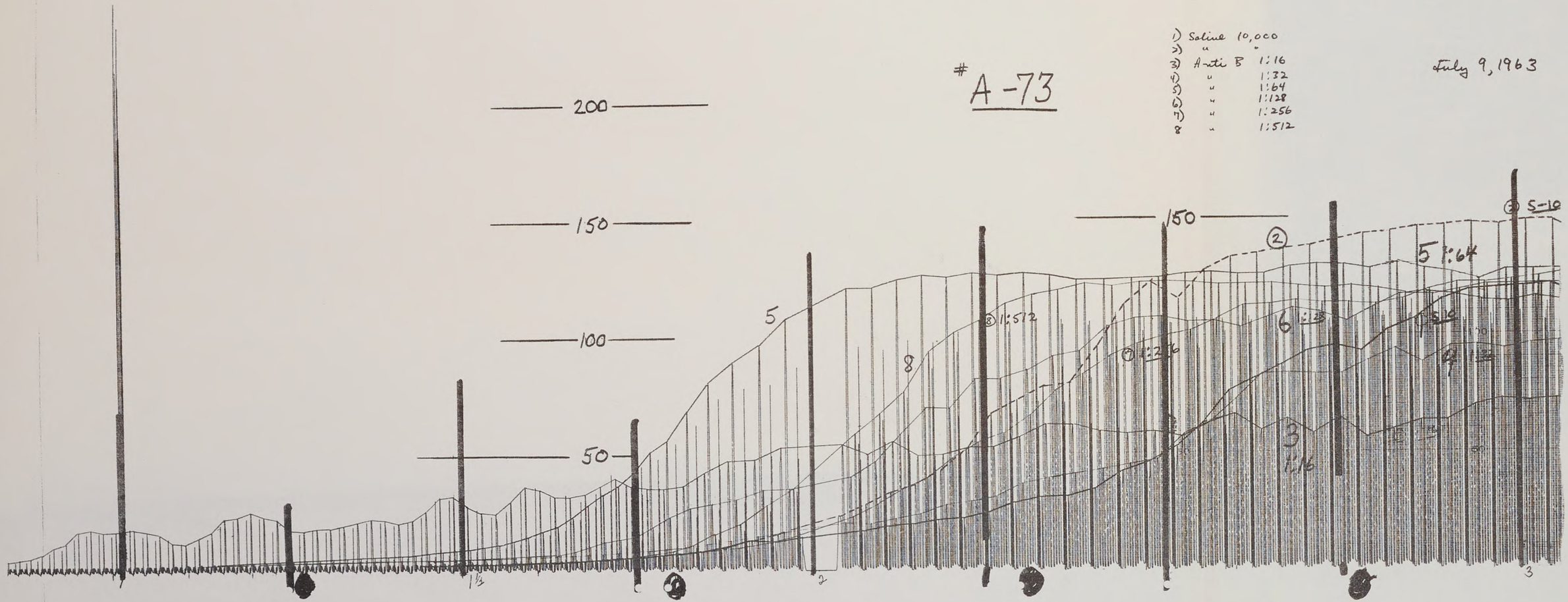

Fig. 14.--Photograph of original recorder chart for run A - 73.

CHAPTER VI

CONCLUSIONS

1) The apparatus which this thesis describes is able to accurately and simultaneously measure the transmittance of light through a number of samples and permit direct comparisons of transmittance of samples under differing experimental conditions.

2) There seems to be some difference in the curves of samples of erythrocytes mixed with saline and erythrocytes mixed with serum, but many more data must be collected to use the machine as the basis of a precise method of differentiating between samples and estimating their degree of agglutination.

3) Settling is a more complex phenomenon than was originally thought. The analytical problem of expressing the particle-size distribution in terms of the light-transmittance measurements will require knowledge of scattering and absorption, and the effect of the non-sphericity of the erythrocytes on Stokes Law.

APPENDIX 1

SURVEY OF METHODS OF MEASURING SIZE-DISTRIBUTION

For solving the analytical problem of this thesis, that is, measuring the particle-size distribution of the erythrocytes, the experimental method chosen should have certain features: ability to handle multiple samples; enough room to place the sample in controlled environment, e.g., water bath, magnetic field. The suspension should not be disturbed and the amount of sample required should be small. Further, the tube holding the sample should be large enough such that wall effects are negligible,[25] and the concentration of the particles in the suspension should be low so that the tendency to hindered settling is reduced.

For the reader to get perspective on the problem, this section describes critically some of the methods of measuring particle size distribution.

There are two basic methods of measurement: incremental, where the changes in the concentration of particles in a certain portion of suspension are measured, i.e., pipette, diver, hydrometer, photo-extinction, and light scattering; and cumulative methods, which measure the overall changes in concentration over the whole testing period:

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[25] Donoghue, J. K., Brit. J. Appl. Phys. 7, 333-6 (1956).

e.g. manometer.

a) The pipette method consists in weighing a small volume of the sample from a specific height in the suspension at a specific time. If C(0) represents the concentration at the start of settling, and C(t) at the time of sampling, from a plot of C(t)/C(0) against a particle-size distribution curve could be drawn.[26]

Suitable Particle-Size Range	0-45μ
Initial Dust Concentration	1% by weight
Accuracy of Concentration Determination	± 0.5%

Remarks: Simple, though much weighing is involved if many points on the size-concentration curve are required. It is the most accurate method tested.[27]

b) In the hydrometer method, the density (the volume doncentration) of the suspension is measured at a specific depth.

Particle Size Range	0-25μ (may be extended to 35)
Initial Dust Concentration	.01 suspension 1.6% by wt.
Accuracy of Determination	0-25μ ± 1.5% 25-35μ ± 2.5%

Remarks: A very simple method. Hydrometer bulb affects the density reading, especially where comparatively large particles are present. The upper size to which the method is applicable is limited by this affect.

c) The diver method consists of placing a number of small glass divers of known density in the sample. They settle at the levels in the suspension whose densities are equal to that of the divers themselves. The divers are located electronically or visually, and thus give the density and rate of fall of a series of levels.

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[26] Orr, Dallavalle, op. cit., p. 52.

[27] Jarrett, B. A., and H. Heywood, Brit. J. Appl. Phys. Supp. 3, 21 (1954).

Particle Size Range	0-30μ
Initial Dust Concentration	.01 suspension
Accuracy of Determination	± 1%

Remarks: Very simple. On larger particle sizes, rapid readings must be made, before deposition of dust on the diver affects the accuracy. Use of a large diver facilitiate readings for larger particle sizes.

d) In the manometer method, the difference in density at two points in a suspension is measured by two indicator tubes immersed at different levels in the settling suspension.

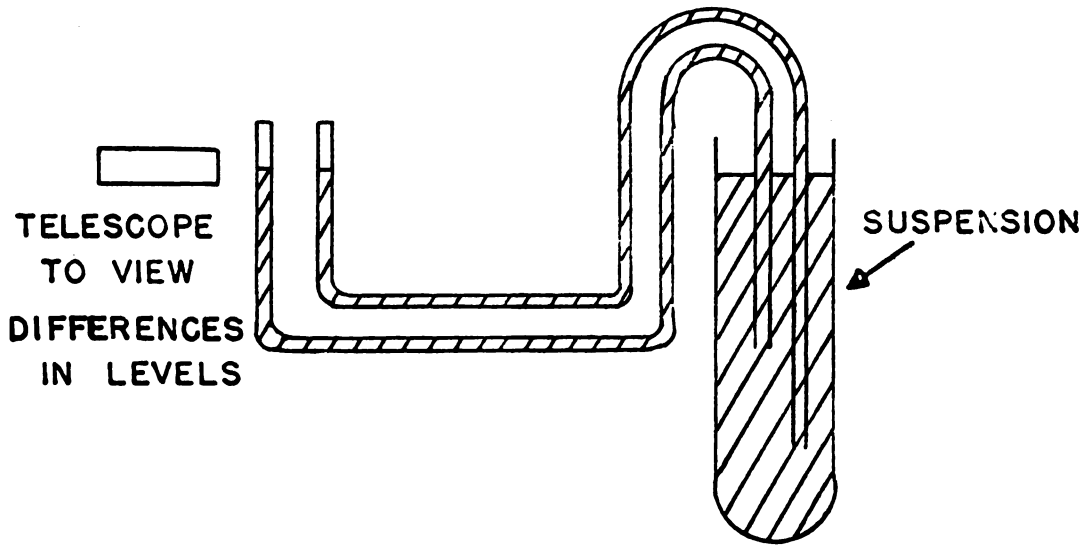

Fig. 15.--Diagram of Manometer.

Suitable Size Range	Tested over range 12-53μ, but unsatisfactory
Initial Dust Concentration	.01 suspension
Accuracy of Determination	± 15%

Remarks: Equipment is difficult to use, and very inaccurate; unsuitable to analysis.

It is of interest to include the following method which is not

based on a sedimentation process but has proved to be very successful in measuring particle size-distribution.

e) Electrical Resistance method or Coulter Counter determines particle size by measuring the resistance of particles as .5 ml. of the suspension flows between two electrodes. The resistance measured is proportional to the volume of the erythrocyte.

Suitable Size Range	2-15μ
Initial Concentration	.01
Accuracy of Determination	± 2%

Remarks: This is an extremely easy and fast method.

All the above methods have a common shortcoming: the instruments are in the suspension, and distort the property which they are trying to measure.

f) Photoextinction, an incremental technique, avoids this problem. In this method, the optical transmission of a number of samples is measured simultaneously at any height and as a function of time. This transmittance may then be related to particle size.

Suitable Size Range	0-25μ
Initial Concentration	.01 by volume
Accuracy of Determination	0-25μ ±3%
	0-53μ ±6%

Remarks: Equipment is very simple to operate. It is the only method which can be used if only a very small sample is available. The method is suitable for comparative tests, such as are required for industrial control, but the theory is complex when absolute measurements are required. Coarse particles have relatively small surfaces, and are difficult to detect; slight errors in reading the photocell output can cause large divergencies in the calculated size-particle distribution curves in this region.

Excellent references on the present methods of measurements are Orr and DallaValle,[28] who discuss the practice and theory of a variety

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[28]Orr, Dallavalle, op. cit., p. 38-133.

of methods in detail; Donaghue,[29] who discusses primarily the cumulative methods; The Physics of Particle Size Analysis Symposium,[30] where a variety of industrial methods and their theory are discussed in detail.

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[29]Donoghue, op. cit., p. 333.

[30]"The Physics of Particle Size Analysis Symposium." Brit. J. Appl. Phys. Supp. 3, 1954.

APPENDIX 2

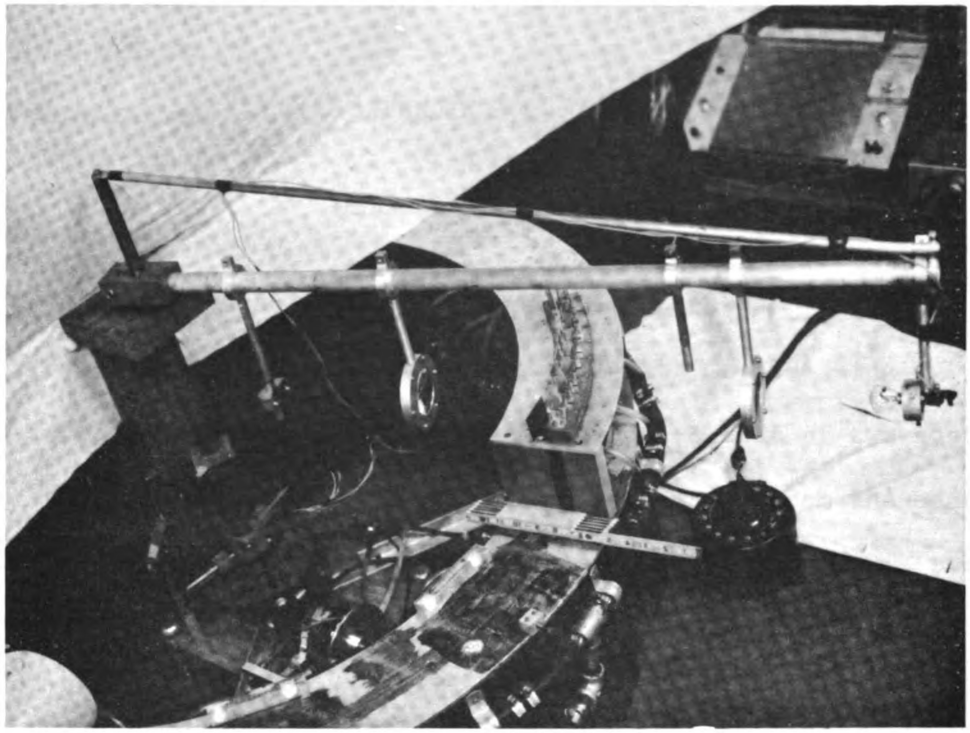
BENCH DESIGN

The boom is fastened to the top of a steel shaft mounted vertically in ball bearings in a yoke of 1-1/2" thick magnesium stock standing 24-1/2" high. An 8" drive gear is mounted near the bottom end of the shaft and is driven through a worm gear by a 1/80-th hp. series-wound Bodine Motor, Frame type NSE- 11R, 4.0 inch lbs. torque and 324:1 downgearing ratio.

The sample bench is in the shape of a semicircular annulus: 6" wide with a center-line radius of 22-1/2". One quadrant of the annulus stands 17-1/8" high and the other quadrant stands 12-1/8" high. The light from the boom hits 1-1/2" above the upper level and 6-1/2" above the lower. This split level is necessary so that the control specimens and the test specimens (resting in magnets) will be at a common height for the sweep of the light beam. (See Fig. 1.)

The top surface of the bench is made of 1/4" aluminum plate bolted to four wooden platforms 11-7/8" high, each made of four 2" x 4" boards which are further stabilized by 30-lb. lead bricks resting on the cross supports. The annular bench is centered with respect to the center yoke by two 1-1/2" x 1-1/2" 1/4" thick aluminum L-angles bolted to the bench and yoke. The bench and the yoke rest on a leveled 1/4" masonite slab, 1/2" - thick blackboard slate, and a table built from 3/4" plywood with legs and cross-braces of 4" x 4" beams.

To maintain a temperature of 37° for the anti-D studies, a water bath has been built to circulate water through the cells holding the sample tubes. The pump, a 1/4-hp. Westinghouse Motor Type FZ, circulates the water through a Sargent Thermal bath whose temperature is regulated by a resistance sensor made by the Yellow Springs Instrument Co. Although the equipment has been built and tested, it has not been yet possible to use it during a run.



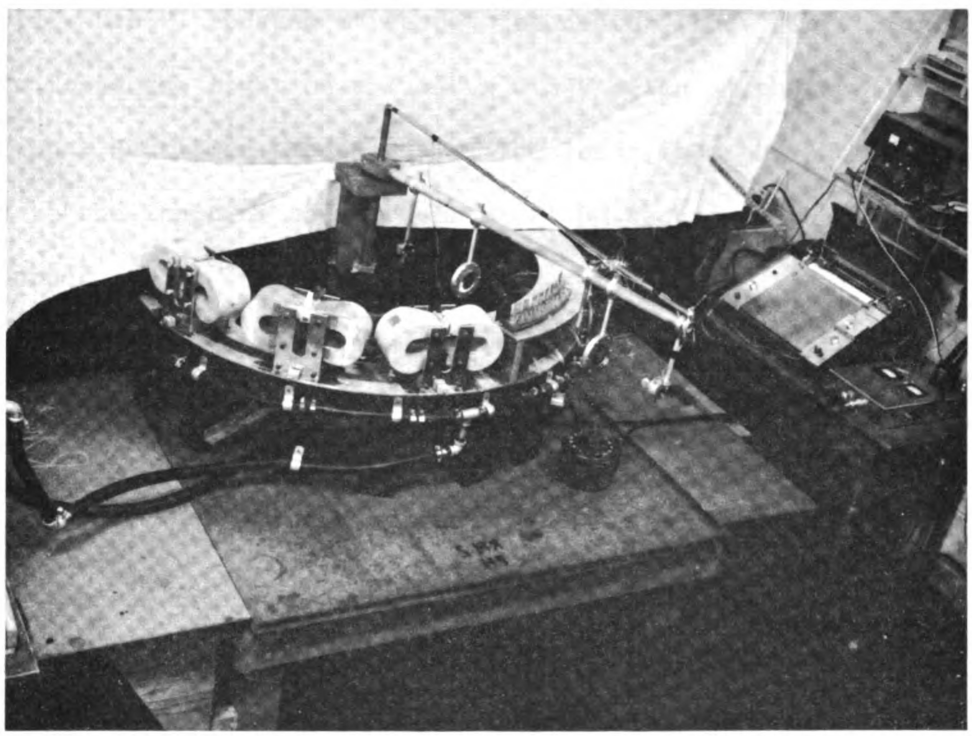


Fig. 16.--Photographs of apparatus.

APPENDIX 3

OPTICAL DESIGN

The optical system is the heart of the apparatus. Suspended 6-1/2" below the free end of the boom is a 32-watt GE 1184 single-filament incandescent lamp. The image projected is that of two spots of light one 1/8" above the other. The filament is 6" from the center of the first 3" condensing lens (f = 3.64 cm.) whose aparture is stopped to 1-1/4" diameter to reduce spherical aberration. The image comes to a focus 9-1/4" beyond the center of the first lens at a spot 1/2" in front of the center line of the bench, at the point where the samples are placed. After passing through a sample cell, the light hits another 3" lens (f = 3.64") located 8" beyond the center line. This final lens serves to focus the light on a 1.44" x 0.64" selenium photocell from International Rectifier Model B5p1. The leads from the photocell go directly to the input of the Sargent Model SR Recorder, the negative lead grounded.

The Sargent Recorder is a self-balancing potentiometer with a full-scale balancing speed of 1/2 sec. ranges from 5 mv. to 125 mv., and chart speeds from 5"/min to 0.2"/min.

The sample tubes are 1/2" x 1/2" x 1-3/4" square Beckman-DK2 Spectrophotometer Cells mounted on a 1/2"- thick brass plate. This plate holds eight cells, and rests on three rubber phonograph shock mounts that eliminate some of the vibration from the motor.

Small windows (cut from brass sheet) of dimensions .03" x 3/16", .06 x 3/16", and .02 x 3/16", may be fastened in place at any height in front of the samples to control the amount of incident light.

The 32-watt source is powered from the line through a Sola Isolation Transformer and a 6.3-volt. filament transformer.

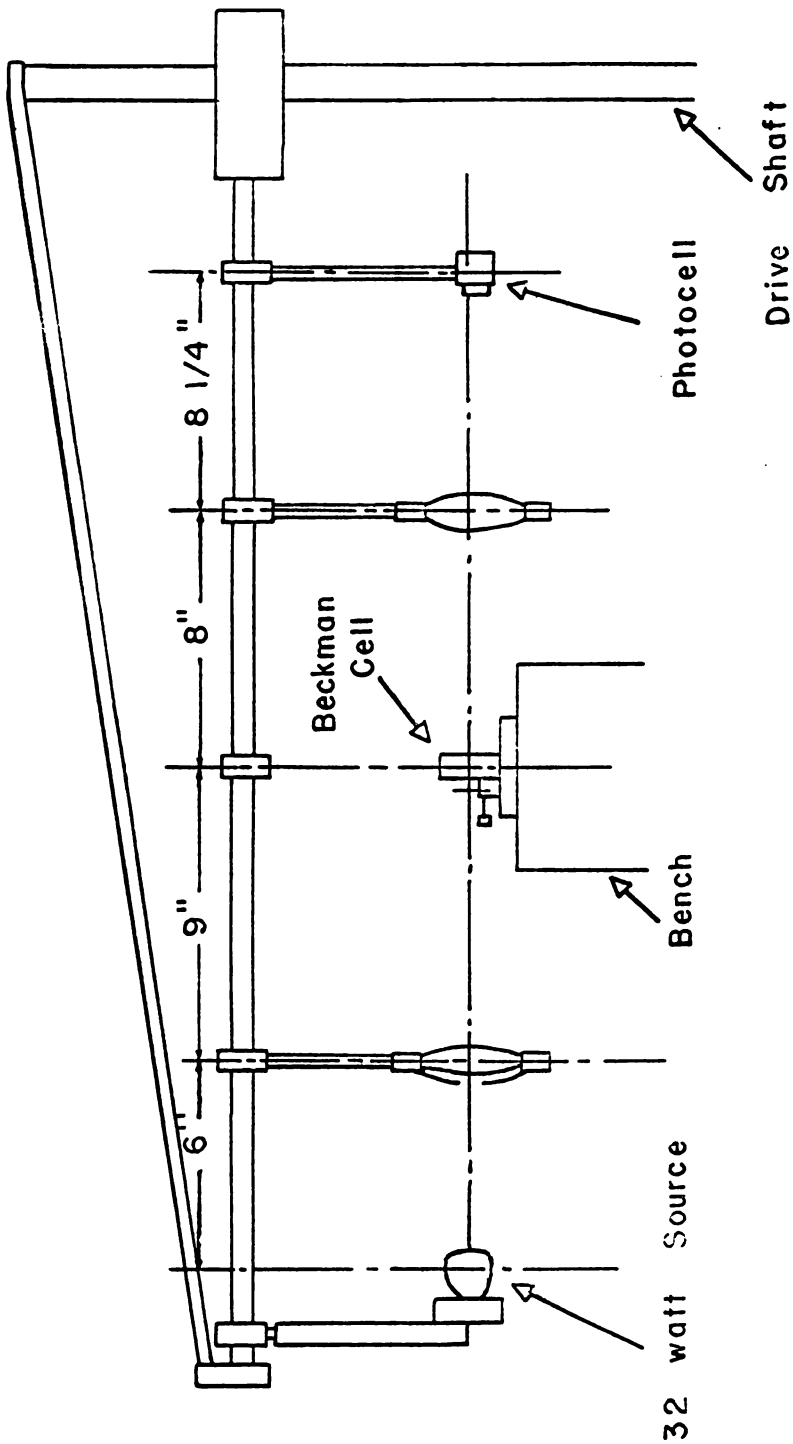


Fig. 17.--Diagram of boom and measurement of distances.

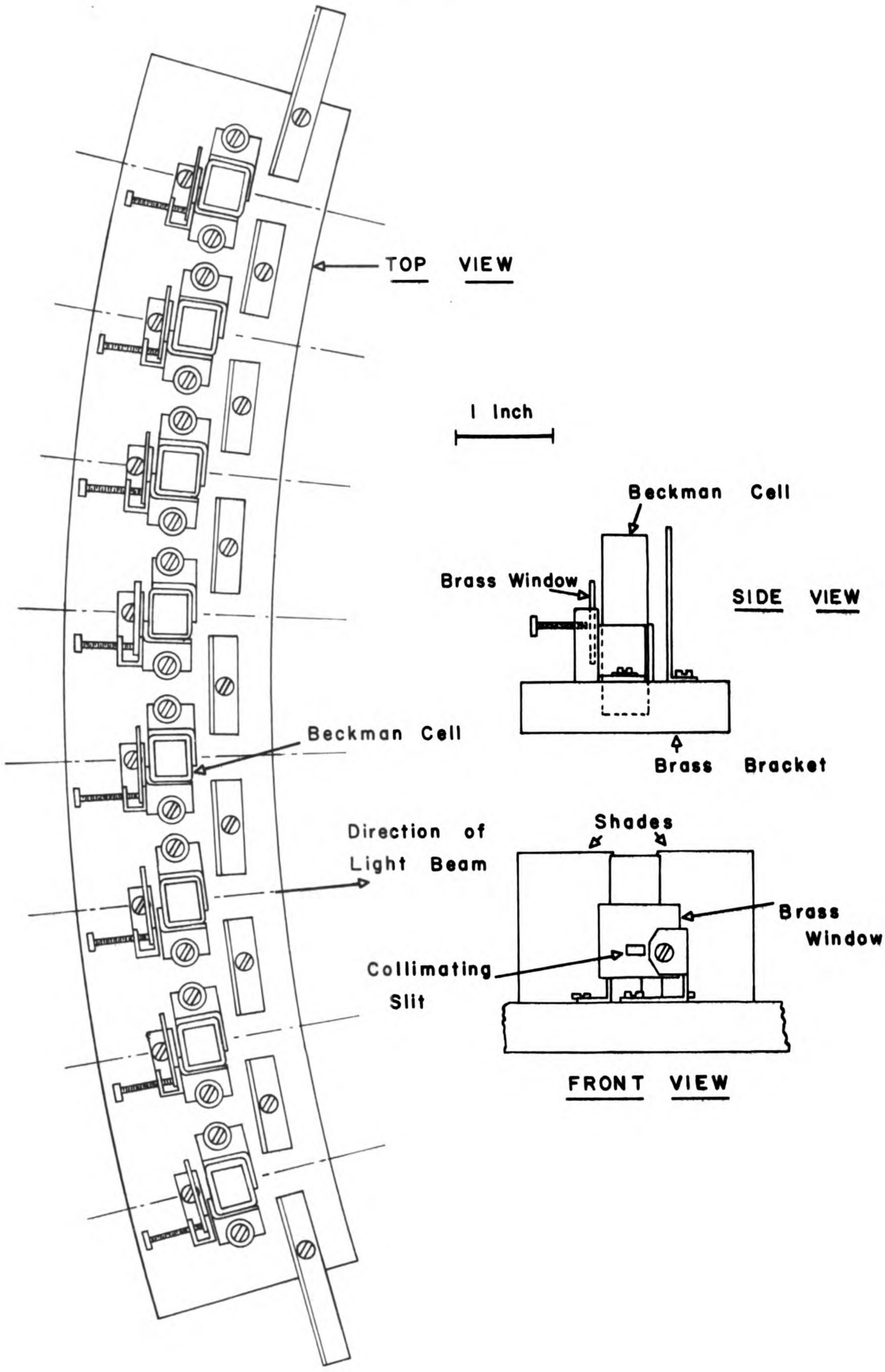


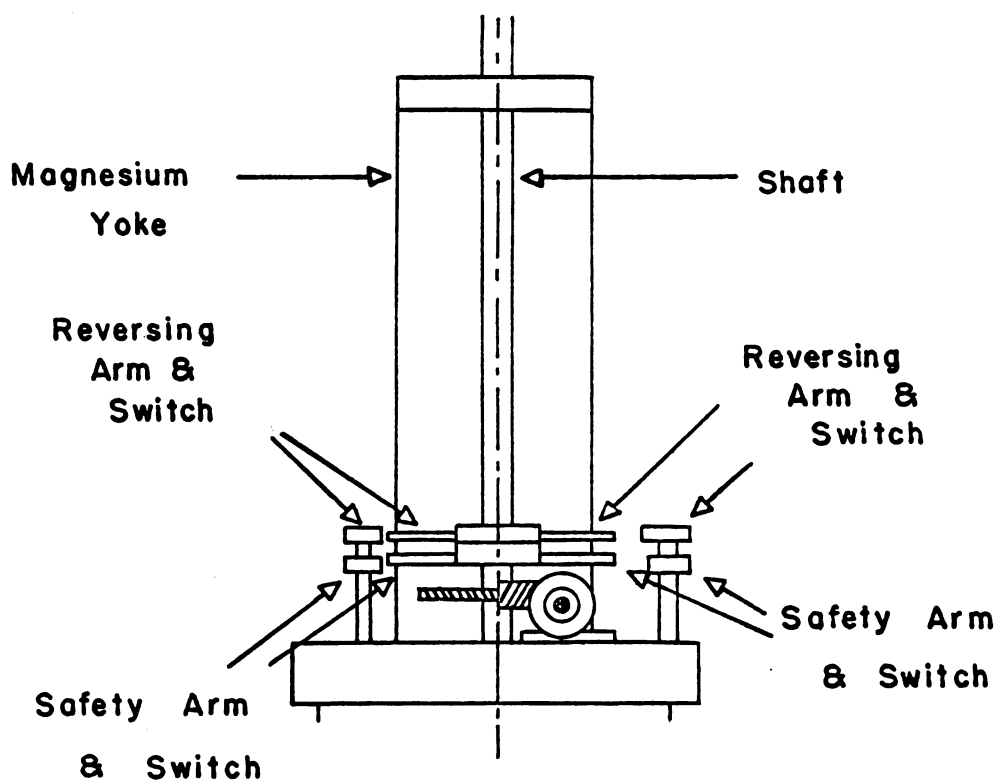
Fig. 18.--Design of bracket for eight Beckman cells.

APPENDIX 4

SWITCHING

To reverse the direction of sweep of the boom, two 4" brass rods are mounted on the main shaft just above the drive gear. They stick radially away from the shaft, and the angle between them is adjustable. When the boom reaches one end of its sweep, one arm trips a microswitch, ISM1 Model L4, which changes the direction of the motor drive for the return. These microswitches are mounted on the magnesium yoke, one to the right and one to the left of the shaft.

Fig. 19.--Illustration of supporting yoke and microswitches.



If these two reversing microswitches fail to reverse the motor direction, limit switches mounted just below them are tripped by other arms. When failure occurs at the end of a sweep, the safety microswitch will be tripped after the boom has been driven an additional 5 degrees in the same direction. This action will break the drive circuit for the motor and prevent the boom from being driven into the supporting post. Before operation can be resumed, however, the relay in the safety microswitch circuit must be reset by hand to complete the drive circuit.

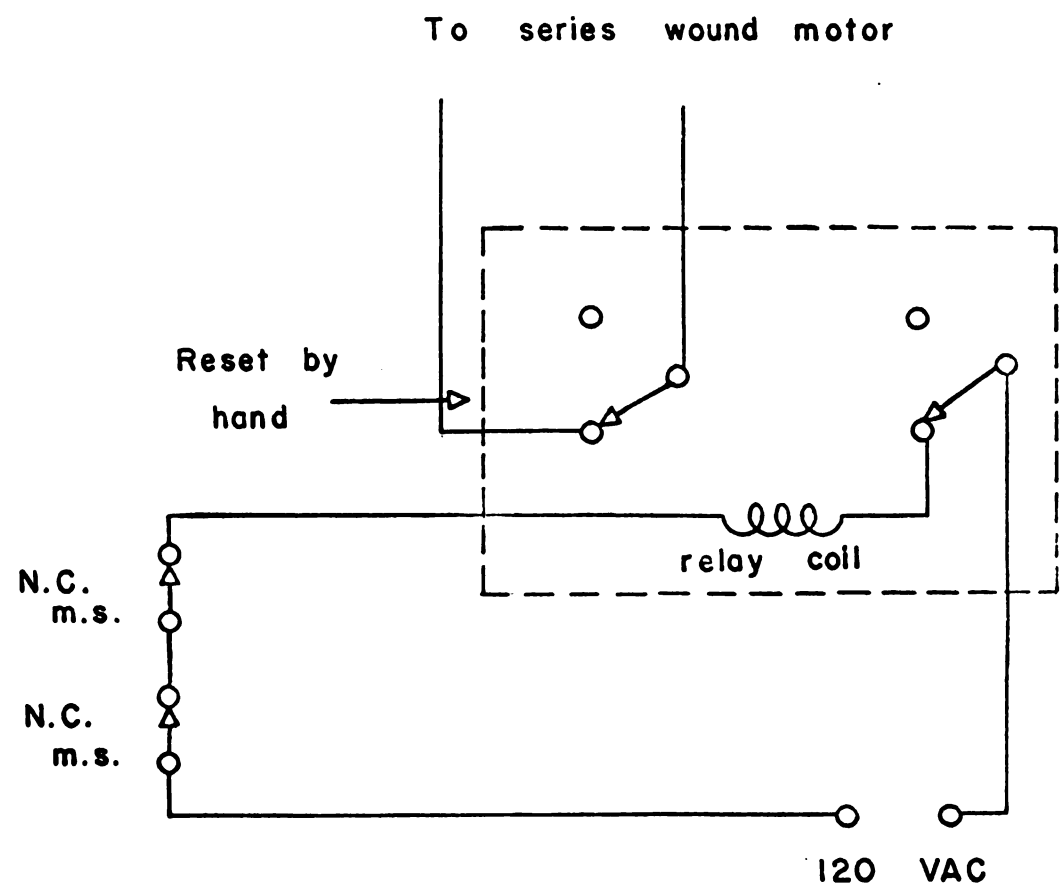


Fig. 20.--Safety circuit diagram.

Another set of switches actuates the eddy-current brake to reduce the sweep speed as the light hits a sample. The brake is a 0.150"-thick aluminum disc of diameter 4-7/8" mounted on the motor shaft and rotating between the poles of a small electromagnet. When the electromagnet is energized, the eddy currents induced in the disc set up fields to oppose the original field; this repulsion causes the disc to slow down. The electromagnet is energized by a switch mounted on the recorder frame.

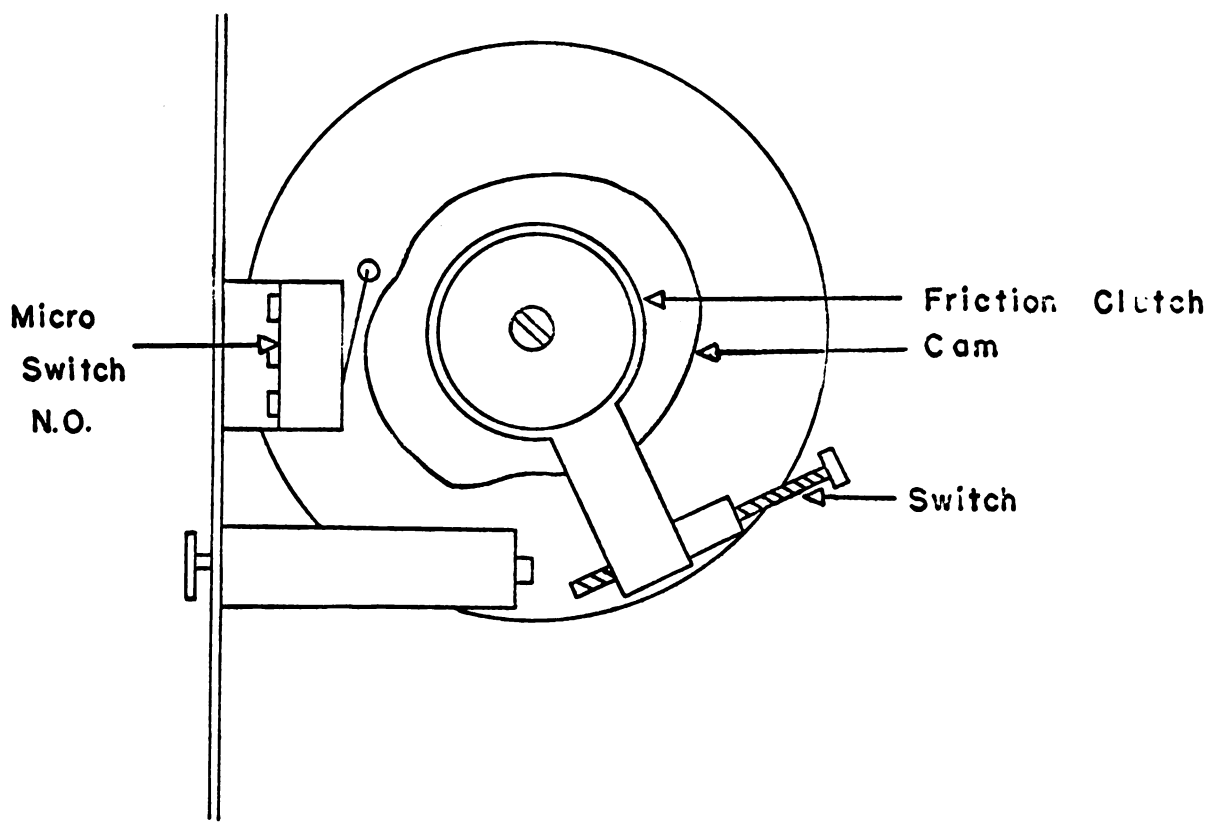


Fig. 21.--Illustration of cam and friction clutch for breaking circuit.

As the pen responds to the voltage rise from the photocell, this switch energizes the magnet which remains closed until the pen has reached a maximum value. This action allows the brake to operate during the rise of the pen. A friction clutch keeps the contacts of the switch together while the pen rises to a maximum and breaks the contact when the pen starts to return to zero.

The steady current through the electromagnet is 1.7 a. at 9 v., from a Heathkit Battery Eliminator. For effective braking at sweep speeds of 60 VAC., 1.7a was found to be insufficient, and a higher current was needed at the start of the braking. Therefore, a 3 a. pulse for .8 sec. in now produced the following fashion: A 2000-μf. capacitor with a 6 v. relay across it is charged to 15 v; this charge holds the relay shut, shorting out a 5-ohm resistor in the electromagnet brake circuit. When the recorded pen starts to respond the brake current is

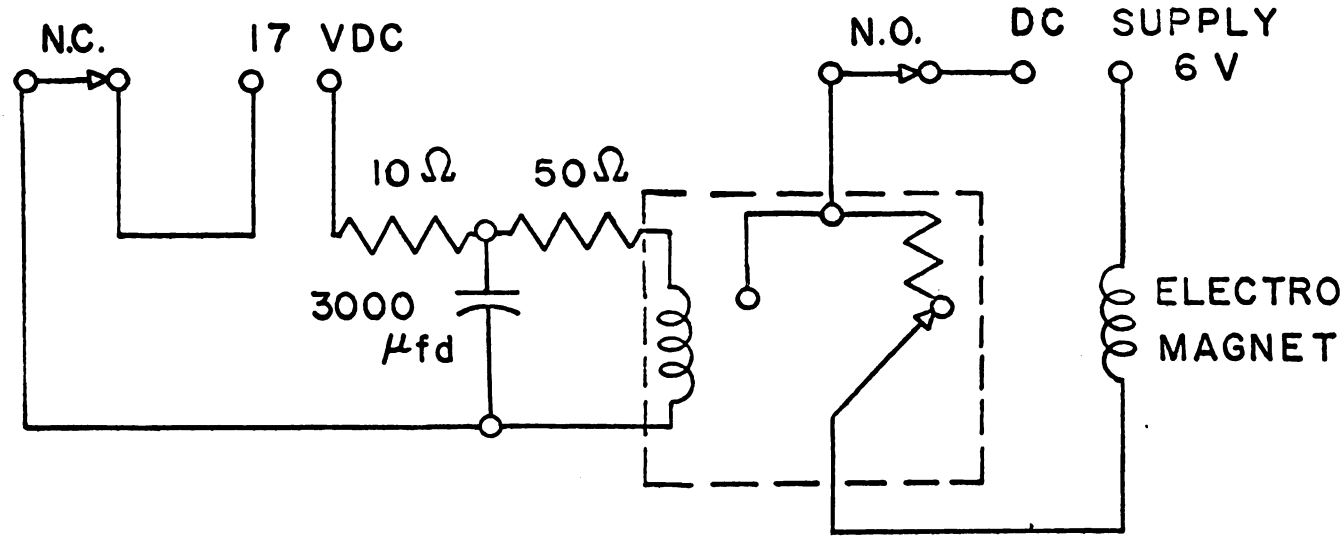


Fig. 22.--Braking circuit.

3a. and the capacitor begins to discharge with a time constant of 1 sec. When the voltage across the capacitor drops to less than 6 v., the relay opens and the 5-ohm resistance is put into the braking circuit to reduce the current from 3 a to 1.7a.

The Bodine Motor is series-wound, with external controls arranged to provide 120 v. for one direction of rotation, and variable voltage in the other.

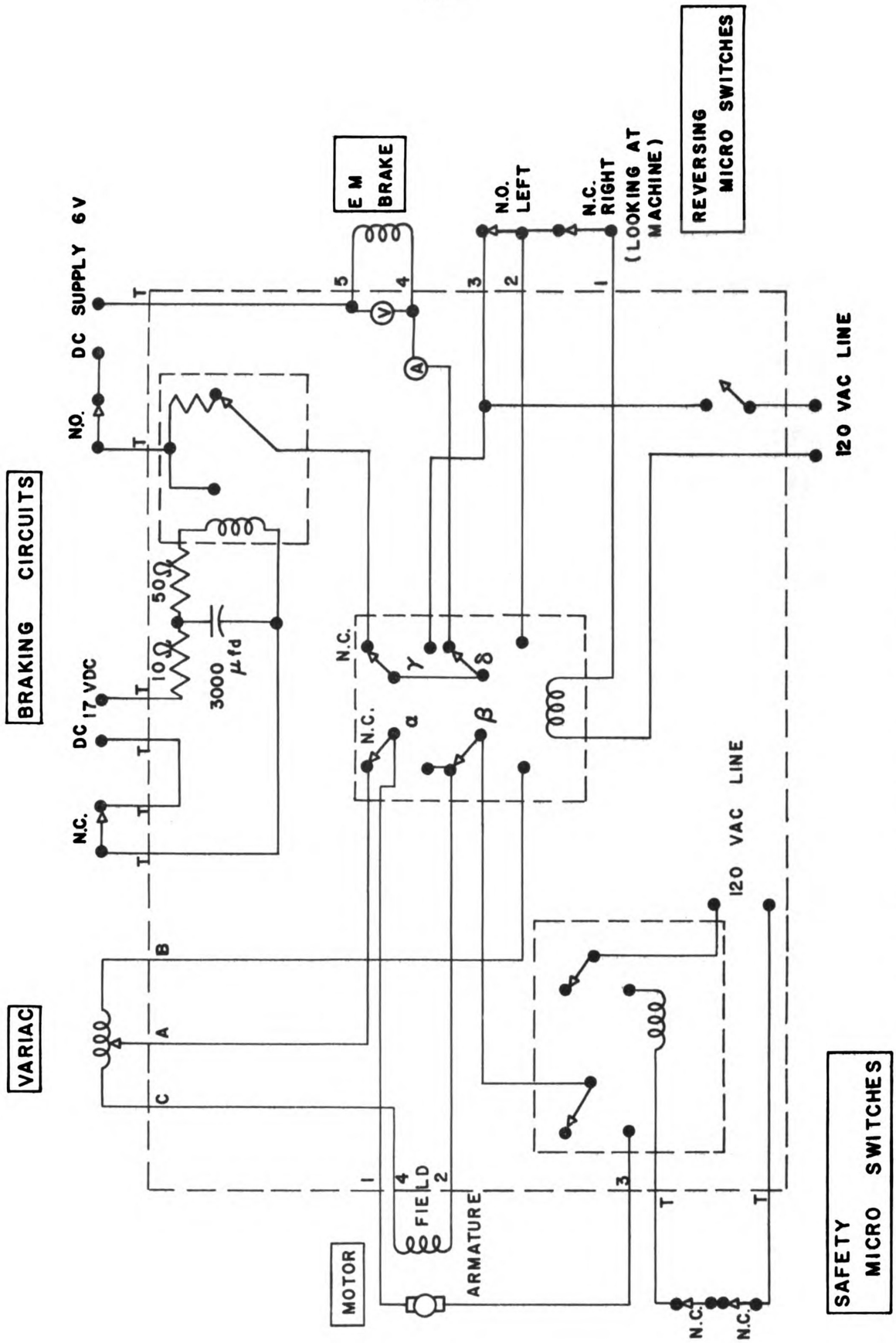


Fig. 23.--Complete circuit diagram.

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