ABSTRACT

A SEARCH FOR BIOLOGICAL EFFECTS OF MAGNETIC FIELDS

By Adolph Eric Smith

The potential importance of influencing biological processes by magnetic fields, in conjunction with the long and controversial history of biomagnetism led us to undertake a careful study of some well defined and sensitive phenomenon of biological significance. We have selected the immune reaction, specifically the antigen-antibody reaction as evidenced by agglutination of human erythrocytes. In this thesis we first review the physics of magnetic fields and suggest possible ways in which the small energy of magnetic interactions--as contrasted with thermal effects--might have detectable effects in biological systems. Laboratory-scale magnetic fields would appear to be capable of only very slight action on individual atoms or molecules, but should be able to produce significant effects on groups of associated molecules such as occur in liquid-crystaline or other mesomorphic states. Next we review the background of immunology relevant to the present problem with emphasis on the Rh-blood-group system.

To assess the effects of magnetic fields on agglutination, quantitative scoring methods are necessary. Three methods were considered in the present work: visual scoring, in which the agglutinated material is examined visually for clumping under the microscope; sedimentation rate, in which

the increase in rate of sedimentation is observed when agglutination takes place; particle-size distribution, in which the change in size distribution of particles upon agglutination is observed.

With visual scoring, the magnetic field was observed to enhance the agglutination in the Rh system, but not in other groups studied. In the sedimentation studies, only preliminary experiments were carried out to test the feasibility of the principle; no experimental results are yet available. With particle-size distribution, which were made with an electronic counter, the method had to be developed. A study of the counter operation and of erythrocyte distributions was first made. In a suspension of unagglutinated erythrocytes, the volume distribution of normal erythrocytes in saline was found to follow a lognormal distribution. Upon agglutination the distribution changed significantly. Several statistics were investigated in order to characterize the change in distribution upon agglutination; one of the more useful ones was the ratio of doublet particles to the singlets. This statistic was studied as a function of incubation time, antiserum concentration, and temperature. This statistic failed to show the difference between test samples incubated in the magnetic field and control samples incubated outside the magnetic field. We present a tentative explanation of the discordance between the results for the visual method and for the counter method. The explanation is based on the difference in

treatment of the samples in the two methods.

Finally, the significance of magnetic effects for possible elucidation of the agglutination mechanism is discussed and extension of the magnetic-field studies for this purpose is urged.

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I. INTRODUCTION

Background of Biomagnetism

That gravitational fields, electric fields, and concentration gradients affect biological processes is unquestioned. But the action of magnetic fields, a fundamental force in the universe, is not even considered in many physiology texts. Indeed, the existence of magnetic effects has generally been denied by most scientists for nearly a century and a half.

Recently, however, there have been reports that magnetic fields have profound physiological effects. Supporters of the existence claim that since a uniform field can rotate magnetic dipoles, and an inhomogeneous field can pull them, it is natural to expect effects. The opposition claims that the thermal energies will swamp any effects due to the magnetic field. At present, there is no accepted answer to the question of whether biological effects of magnetism exist.

Historical

Standard works on physiology contain either no mention of the effect of magnetic fields on biological processes, or the statement that magnetism cannot be considered a stimulus for biological processes. Bonner (1), in his essay on morphogenesis, states: "There is every evidence that no

living precess is much affected by reasonable magnetic forces." Heilbrunn's text (2) on physiology is cited in support of this view; it states:

> The above list [of stimuli] includes no mention of magnetic forces. According to Verworm (3) (1899) these have no effect on living systems and, therefore, cannot be considered stimuli. In support of this opinion he cites the extensive experiments of Peterson and Kennelly (4), done in the Edison Laboratory in 1892. These investigators used very strong electromagnets, but were unable to detect any noticeable effect on living material.

In the book of Verworm, which includes a similar list of stimuli, is the observation:

> . . . in this enumeration magnetism is wanting. But it is now known with certainty that magnetism exercises no effect whatever upon living substance, and cannot be properly termed as stimulus. . . . Careful experiments upon the influence of magnets upon the living organisms have always yielded negative results. The recent extended researches with very strong electromagnets by Peterson and Kennelly in America demonstrate the utter ineffectiveness of magnetism upon living matter.

Yet, despite these sweeping statements, there are cases where a magnetic field has been reported to have produced observable effects on biological processes. Table I lists some of the divergent findings on the subject. Here is a curious situation wherein different investigators have arrived at different conclusions about the same subject. Regrettably, the field strength, the field uniformity, the duration of exposure, and the species tested were not exactly the same in the cases of dispute.

TABLE 1

DISPUTED MAGNETIC EFFECTS

Effect	Affirmative	Negative
Yeast and bacteria growth	Kimball (5) Barnothy (6)	1. Leusden (7) 2. Jennison (8)
Tissue-culture growth	1. Huzella (9) 2. Lengyel (10) 3. Lenzi (11) 4. Mulay (12)	Payne-Scott (13)
Mouse growth	Barnothy (14)	Eislein (15)

There are in the literature, however, accounts of several affirmative results that are uncontested to date: (1) Magnetic phosphenes: Thompson (16), Magnusson (17), Barlow (18); (2) Magnetic compass effects on snails: Brown (19); (3) Magnetotropism of plants, Audus (20); and (4) Growing speed of plants: Ssawasotin (21).

But also in the literature are some undisputed accounts of negative results with magnetic fields. These are:

1. The perception of steady magnetic fields by humans: Peterson and Kennelly (4), Beischer (22).

2. Attempt to influence the asymmetric chemical synthesis characteristic of living systems: Pasteur (23).

Detailed investigation of such phenomena shows us why it is difficult to obtain conclusive results. First of all, the effects appear to be slight, and hence the experiments must be highly refined, or a vast number of replications must be made. Secondly, theoretical considerations based on simple physical phenomena fail to give much indication as to how magnetic fields might produce measurable effects on

living processes.

Hence we shall expect that experiments of a high order of refinement or very high degree of replication, will be necessary to demonstrate any effect; and we shall expect that the theoretical explanations for any effect will depend upon complex interactions. These ideas will be developed in the body of our thesis.

Physics of the Electromagnetic Field (24)

When a particle interacts with other particles within a system, it is often found that parameters other than mass must be ascribed to each particle. For a certain class of phenomena one of these parameters is the electric charge, which may be of two different kinds, labeled positive and negative. In describing the behavior of the particles, it is convenient to introduce certain mathematical auxiliaries called electromagnetic field vectors. The part of the interaction depending upon the relative positions of the interacting changes is described in terms of the electric field, characterized by the electric field strength $\underline{E}$ and the electric displacement $\underline{D}$. The electric field is set up by the charge according to the relation:

$$d\underline{D} = \rho \, dV \, \underline{r}/r^3$$

where $\underline{r}$ is the position vector from the point under consideration to the charge element consisting of the volume element dV with charge density ρ. The part of the interaction of the velocity $\underline{v}$ of the particles is described in terms of the

magnetic field, characterized by the magnetic induction $\underline{B}$ and the magnetic field strength $\underline{H}$. The magnetic field is set up by the motion of the charge according to the relation:

$$d\underline{H} = \rho dV \frac{v}{c} \frac{\underline{r}}{r^3}$$

where $\underline{r}$ is the position vector from the point under consideration to the current element consisting of the current density element $\rho \frac{v}{c} dV$ and c is the velocity of light. Here we have assumed that the fields are changing sufficiently slowly in time that displacement current negligible is compared with convection current. These two equations, essentially Coulomb's Law in the electric case, and the Biot-Savart Law in the magnetic case, form the basis of the calculation for the setting up of the field by the sources.

To see how the fields act on charged particles, we need the Lorentz equation for the force $d\underline{F}$ upon a test particle of charge dq, a particle whose charge is so small that it does not disturb the previously existing arrangement of charge and current:

$$d\underline{F} = dq[\underline{E} + (\underline{v}/c) \times \underline{B}]$$

As the equations now stand, no connection exists between the pair of vectors $\underline{D}$ and $\underline{H}$ set up by the sources, and the pair of vectors $\underline{E}$ and $\underline{B}$ which exert influence on the particles. The connection is made through postulating the following two relationships:

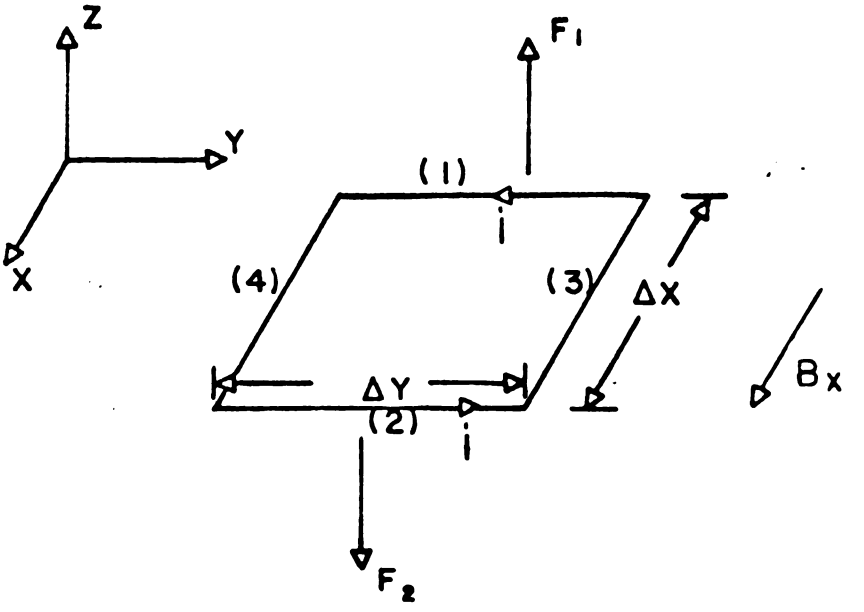
$$\underline{D} = \epsilon \underline{E} \qquad\qquad \underline{H} = \underline{B}/\mu$$

Here, ϵ, the dielectric constant, and μ, the magnetic permeability, are equal to unity in free space in the Gaussian

system of units, and take on values different from unity within material media. In many of the cases of interest ϵ and μ may be taken simply as scalars and, moreover, as constants so far as dependence on $\underline{D}$ or $\underline{H}$ is concerned.

We need not labor the matter of the force on charge elements in the electric case. The direction of force is quite simply given by the direction of the line between two charge elements. In the magnetic case, on the other hand, the forces which depend upon the velocity of the charges--that is, on the currents or on the magnetic field set up by these currents, if one prefers--are more complex. To see in detail how these forces arise, suppose we first consider what happens when a rectangular loop carrying a current i is placed in the homogeneous magnetic field $\underline{B}$. Each side of the loop experiences a certain force, proportional to the current i, the length of the side, and the magnetic field strength B; however, this force is exactly balanced by an equal and opposite one on the part of the loop diametrically opposite, so that the net force is zero. There is, of course, a non-vanishing torque on the loop, given by the product of the force and the lever arm.

The more interesting case is that of an inhomogeneous field, wherein the two forces on the opposite sides of the loop do not exactly balance. To show the main features of the action, we take the special case where the plane of the loop is parallel to the xy-plane, as shown in Figure 1. We see that the force on side 1 is upward, and is given by the



$$F_1 = B_{x1}\, i \Delta y \quad , \quad F_2 = B_{x2}\, i \Delta y$$

$$B_{x2} = B_{x1} + \left[\partial B_x / \partial x \right] \Delta x$$

$$\Delta F_{1-2} = F_1 - F_2 = -\left[\partial B_x / \partial x\right] m_z$$

Similarly

$$\Delta F_{3-4} = -\left[\partial B_y / \partial y\right] m_z$$

$$-\left[\partial B_x / \partial x\right] - \left[\partial B_y / \partial y\right] = \left[\partial B_z / \partial z\right]$$

$$\text{so} \quad F_z = m_z \left[\partial B_z / \partial z\right]$$

$$\text{In general} \quad \underline{F} = (\underline{m} \cdot \nabla)\, \underline{B}.$$

Figure 1 - Illustration of the force exerted by an inhomogeneous magnetic field on current elements.

product of the magnetic induction in the x direction at side 1, multiplied by the current i times the length of the side Δy. Correspondingly, the force on side 2 is proportional to the induction at side 2; that is, B_{x_2} multiplied by i times Δy, but directed downward rather than upward. Now to first-order terms, the induction at side 2 is equal to the induction at side 1 B_{x_1}, plus the rate of change of B_x with respect to x, times Δx. Therefore, the net force on the sides 2 and 1 is equal to the difference between these two quantities, that is, $-(\partial B_x/\partial x)\, i\, \Delta y\, \Delta x$.
The quantity i times the area of the loop, $\Delta y\, \Delta x$, occurs frequently in calculations and is given a separate name, the magnetic moment (this quantity is actually a vector $\underline{m}$, and here we have merely the z component, perpendicular to the area $\Delta y\, \Delta x$). From these considerations we see that the net force from the other two sides is equal to $(-\partial B_y/\partial y)\, m_z$. But now by use of the Maxwell system, $\nabla \cdot \underline{B} = 0$, we have:

$$-\frac{\partial Bx}{\partial x} - \frac{\partial By}{\partial y} = \frac{\partial Bz}{\partial z};$$

hence finally: $F_z = m_z\, (\partial B_z/\partial z)$. The full analysis for the general case leads to the following relationship:

$$\underline{F} = (\underline{m} \cdot \nabla)\, \underline{B}.$$

Because the concept of a current loop is somewhat difficult to visualize, one frequently replaces it by a fictitious source consisting of a pair of magnetic poles (which, of course, cannot exist separately in view of the basic relationship that $\nabla \cdot \underline{B} = 0$). Nevertheless the expansion of the actual field set up by a finite current loop

leads to an expression whose leading term has the same mathematical form as that of a field set up by a pair of monopoles of equal and opposite "magnetic charge" separated by a short distance. It becomes convenient, therefore, to talk about the fields of a magnetic dipole, i.e., the idealization of a pair of equal and opposite poles separated by a certain distance. The limiting form of the dipole is determined when the distance between the charges goes to zero, and the charges become infinite; their product then attains the magnetic moment as its limiting value. In mathematical treatments the magnetic dipole is handled in the same way as the electric dipole. The vast difference in the consequences lies, of course, in the fact that electric monopoles exist, and at large distances become the dominant term, the dipole and higher-order fields becoming negligible. But with magnetic fields there is never a monopole field, and hence the magnetic dipole always becomes the dominant factor. It is for this reason that much of the analysis is carried on in terms of the magnetic dipoles.

The form of the field set up by a magnetic dipole of moment $\underline{m}$ oriented in the direction of a positive z-axis is of the following form:

$$\underline{H} = -(2m/r^3)\cos\theta\,\underline{A}_r + (m/r^3)\sin\theta\,\underline{A}_\theta$$

Here r represents the distance from the origin and θ the angle between the point of observation and the axis of the dipole (zenith angle). $\underline{A}_r$ is the unit vector in the direction of increasing r, with θ and ϕ held constant; and

$\underline{A}_\theta$ is a unit vector in the direction of increasing θ, with r and ϕ held constant. Replacing the magnetic poles by equal and opposite electric charges gives a resulting field which is of the same form as the magnetic field except for changes in the notation.

Electromagnetic Fields in Ponderable Bodies

When an electric field is applied to a body, there is a change in the resulting field from that in a vacuum. Since details of this change are treated in standard texts, we need not review the results here. When the magnetic field is applied to a body there is a similar change in the resulting field. We need to discuss this response in more detail. On the atomic level the change in fields results from the disturbance of the atomic orbitals and the electronic spins. The change in the magnetic field is usually described by introducing the magnetization $\underline{M}$ related to $\underline{H}$ by the following expression:

$$\underline{B} = \underline{H} + 4\pi \underline{M} = \mu \underline{M}.$$

The origin of this relationship is explained in standard texts. The magnetic susceptibility χ is defined by:

$$\chi = \underline{M}/\underline{H}; \text{ whence } \qquad \chi = (\mu-1)/4\pi$$

The atomic basis for the magnetic polarizability is the magnetic moments produced by the motion of the electrons. The magnetic moment of the electron-orbital motion M_z is simply related to the angular momentum L by:

$$M_z = gM_B L \text{ where}$$

$$M_B = \text{Bohr magneton} = 0.927 \times 10^{-20} \text{ erg/gauss}$$

There is also a magnetic moment in the atoms due to the electron spin. Langevin found with classical physics that, for weak fields:

$$\chi = MN^2/kT \quad \text{---------- (1)}$$

where N = number of atoms/cm^3. For stronger fields the expression becomes $\chi = NM_3L(x)/kT$, where $x \equiv MN/kT$ and $L(x) \equiv \coth x - 1/x$.

These expressions give susceptibilities that are always positive. To account for diamagnetism, Langevin considered the induced magnetic moments of the atoms. Each of the electrons moving about the nucleus is similar to a current loop, and by Lenz's law, the magnetic fields induced in this loop will have a direction which opposes the inducing magnetic field. Langevin's formula for diamagnetism is:

$$\chi = -(Ne^2/6mc^2) \sum_i \langle r_i \rangle^2 \quad \text{---------- (2)}$$

where $\langle r_i \rangle^2$ is the average square of distance of the i-th electron from the nucleus. Diamagnetic susceptibilities usually range from 10^{-6} to 10^{-5}. If the molecule has a permanent magnetic moment, the expressions (1) and (2) have to be added.

Energy of Interaction of the Magnetic Field

For charges whose velocity is low compared with that of light--as is the case for all phenomena of chemical and biological interest--the effect of the magnetic field is much smaller than the effect of the electric field, at least in non-neutral systems. To get some idea of the order

of magnitude of magnetic effects, let us consider the electrons in an atom.

At a distance of approximately two Ångstrom units (Å) from a proton or an electron, the electric field e^2/r amounts to about 10^6 volts/cm. The magnetic field, $2m/r^3$, at this distance from an atom with a magnetic moment of one Bohr magnetron, $2\ M_B/r^3$, is about 2500 oersteds. The product $m\partial B/\partial r$ at 2Å from such a dipole is the following:

$$F_B = m\left(\frac{\partial B}{\partial r}\right) \simeq m_B\frac{(-6m_B)}{(r^4)} \cong \frac{6\times(10^{-20})^2}{(2\times10^{-8})^4} \simeq 0.4\times10^{-8}\ dyne.$$

A typical electric dipole moment is about 10^{-18} esu.

$$F_E = p\cdot\frac{\partial E}{\partial r} \cong 10^{-18}\times\frac{2\times4.8\times10^{-10}}{(2\times10^{-8})^3} \cong 10^{-4}\ dyne.$$

Now we see that it is possible to obtain microscopic magnetic <u>fields</u> in the laboratory ($\sim10^4$ gauss) as much as ten times as great as the magnetic fields within the atom ($\sim10^3$ gauss). On the other hand, the <u>gradients</u> obtainable in the laboratory are not likely to be much larger than 10^4 gauss/10^{-1} cm. = 10^5 gauss/cm. This gradient is smaller than microscopic ones by a factor of 10^7. Hence, we see that the <u>forces</u> obtainable macroscopically are negligible compared with those obtainable microscopically; on the other hand, the <u>torques</u> obtainable macroscopically are quite comparable with or even larger than those obtainable microscopically. Moreover, the microscopic magnetic forces are about 10^{-4} times the microscopic electric forces.

To see quantitatively how the externally-applied magnetic fields influence the distribution of the atomic moments, we note that when the field is switched on, the atomic moments

precess about the direction of the applied field, in such fashion that the projection of their moments on an axis parallel to the applied field takes on the value +1/2 or -1/2. In the former case the energy of interaction is decreased, in the latter case increased, by $M_B H$. At equilibrium, the lower-energy state will therefore be slightly more populated than the higher state, by the ratio exp $M_B H/kT$ to exp $-M_B H/kT$, or $1 + 2M_B H/kT$, approximately, since $2M_B H/kT$ is small, amounting to about 2/500 at room temperature for a Bohr magneton in a 10^4 gauss field. We see then at equilibrium for every 250 moments pointing in the direction of the field, there will be 249 pointing against it. In the absence of cooperative effects, it seems unlikely that this slight alignment--which, for a given set of atoms, is continually being disturbed by thermal agitation --could result in any pronounced biochemical effect.

Cooperative and Statistical Phenomena

Although the energies associated with the external magnetic field are small compared with thermal energies, and the field strength is small in comparison with the mascroscopic fields, there are still important responses to the application of the external fields. Ferromagnetism and ferroelectricity are two striking examples. Here an external field brings about a slight alignment, but the result of this new reorientation is to produce alignment of adjacent molecular groups. These groups then cooperate to produce a resulting field which may become very strong and create

an overall effect which produces stability in the presence of disrupting forces. It is unlikely that either ferroelectricity or magnetism is of any interest in biological systems, although there are certainly some special systems that are clearly ferromagnetic, such as radulae of Chitons (Phylum Mollusca) (25). Cooperative phenomena are exhibited by certain large unsymmetrical molecules which group together to present associated effects which may become important on the mascroscopic level. These phenomena occur in the so-called mesomorphic or liquid-crystal state.

Possible Biological Effects of the Magnetic Field

Effect of Orientation

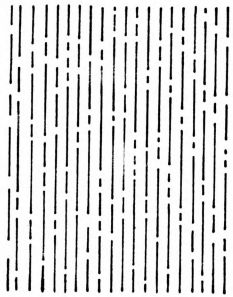
Even though a single atom or group of atoms in a typical molecule could be oriented by the external magnetic field--and we have seen how small is the possibility for this orientation--it is not likely to affect the macroscopic behavior in the absence of a mechanism for coupling this orientation with the rest of the molecule. On the other hand, in certain crystals that do occur in the mesomorphic state, the whole molecule can be oriented by a magnetic field and can transmit this effect to neighboring molecules. Since some protoplasmic material is known to exhibit certain properties of liquid crystals, we examine in detail the mesomorphic state to determine if its occurrence is sufficiently frequent to permit influencing biological processes by magnetic fields.

The mesomorphic state (liquid crystals) (26-28)--For nearly a hundred years it has been known that a few substances in a restricted temperature region have both liquid properties (such as fluidity) and crystal solid properties (such as birefringence). This state of matter is known under various names--"mesomorphic state," "liquid-crystal state," or the "paracrystalline state." Nowadays it is well known that the bonds between atoms in different molecules may be of different kinds and may have different orders of binding energies. Consequently, there are states of matter that are not described simply by the earlier threefold classification of solids, liquids, and gases. In general, we may well expect that if the chemical attractions in different directions are of varying nature, one might be able to destroy the order in one direction, for example, by heat or by a polar solvent, while retaining the order in different directions. Indeed it has been found that perhaps 3,000 substances exhibit the liquid-crystal state.

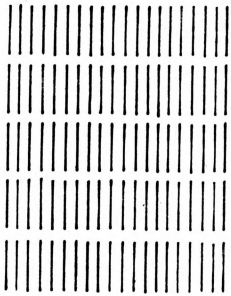
Most of the compounds in the mesomorphic state are classified as either smectic (like soap) or nematic (like a string). But a third state of special interest also exists in certain biological systems, the cholesteric (like cholesterol, the prototype of this class). In the smectic state the molecules are arranged with their long axes approximately normal to the layers in which they are stratified. In the nematic state the molecules are arranged with somewhat less order, the only restriction being that the molecules are

lined up nearly parallel. In the nematic state the molecules can be lined up even by weak magnetic fields, whereas in the smectic state it is difficult to align molecules under the influence of any except the relatively strong magnetic fields. In view of the widespread occurrence of cholesterol and its derivatives in biology, this form has special significance. The cholesterol derivatives as well give cholesterol-type liquid crystals. The cholesterol liquid crystals have the property of being uniaxial and having negative birefringence. These properties are believed to be explainable in terms of a screw structure for this state. There is no sharp transition from the cholesterol form to the nematic form, whereas there is a sharp transition to the smectic form in the cases where the material exists both in the smectic form and nematic form. The optical activity of the cholesterol form is high; there may be as many as 200 revolutions in one mm. Figure 2 is a schematic representation of the three forms of the liquid-crystal state.

Some polypeptides give mesomorphic structures when they are precipitated from solution, and in these there is a layered structure which is sometimes twisted into spirals in spherulites. The polypeptides are of great importance in biology because the protein structure consists of polypeptide chains. In the formation of antibody molecules, the configuration of the polypeptides is of the greatest importance and it is possible that this may have something to do with the ideas of the cholesterol-state.

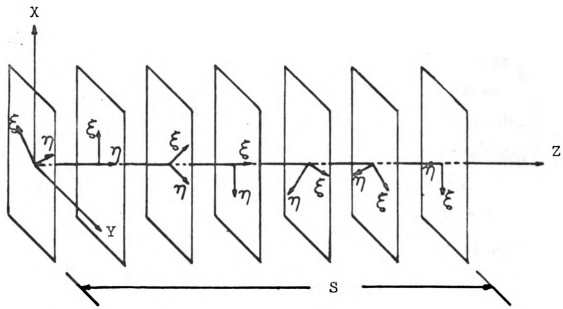


a) nematic state



b) smectic state

Figure 2 - Linear arrangement of molecules in the mesomorphic state.



c) Helical array of the molecules in a cholesterol-like liquid crystal. XYZ is the rectangular coordinate system; ξ and η are the axes of the molecules.

To describe logically the transition between the crystal and the liquid state we may give the following description: (1) True (three-dimensional crystals). The atoms are all fixed at their lattice points and only thermal vibrations about the lattice points are performed. (2) Molecular crystals with rotation of the subunits. The lattice units are fixed at the lattice points, but rotation about one or more axes is allowed for the unit. (3) Smectic structure. The lattice units can move in two directions and rotation about one axis is permitted. (4) Nematic structure. The centers of mass of the molecules are mobile in all three directions and rotation about one axis is permitted. (5) True liquid. The centers of mass of the units are mobile in three directions and rotations about three axes perpendicular to one another are possible.

All the compounds which exist in the mesomorphic state have been found to be elongated molecules of fairly large size. In the crystalline state they are rigidly aligned and as the temperature is raised, a solvent is introduced--the weaker bonds are at first loosened. The molecules then have some freedom in certain directions as the thermal energy or other disrupting forces become great enough to partially destroy the aligning forces, but not so great as to destroy the partial ordering forces.

The nematic structure: In the cases where any progress has been made in understanding the mesomorphic state, it appears that mobile electrons within the conjugated

double-bond systems react against applied magnetic fields so as to produce diamagnetism. The molecule then sets itself up so as to minimize the energy of interaction with the field. In the nematic state the molecules set themselves perpendicular to the lines of magnetic force. The diamagnetic movements for the whole structure and the group of atoms (typically a hundred thousand) have a resultant interaction energy equal to 10^5 times that of a single molecule. This energy is more than enough to overcome the disruptive thermal energies. Hence, it is possible for relatively weak magnetic fields to line up the swarms against thermal agitation. Because of space limitations it is not possible to discuss here the various attempts to produce theories of the liquid state, which are discussed in several review articles or books.

The biological importance of the mesomorphic state is that matter exists in the mesomorphic state in various biological systems. Recently the deoxyribonucleic acid (DNA) in living sperm was found to exhibit certain characteristics of the mesomorphic state (29). The widespread occurrence of optical birefringence in protoplasm (2) suggests the occurrence of mesomorphic phases in this material.

Effect of Magnetic Field on Chemical Reactions

Since the magnetic-interaction energies are so small compared with thermal energies, it is not expected that chemical reactions can be influenced by macroscopic magnetic

fields. Indeed, many experimenters have sought this effect, but none have shown conclusive evidence (Selwood (30)). Certain workers (Bahatnagar, et al.(31)) report positive effects in the reaction of iron with chlorine. The effects are very small and, according to Parker and Ames (32), are made even smaller by stirring the solution during the reaction. To get an idea for the mechanism, these workers calculated the difference in concentration for paramagnetic substances. They took the Boltzmann relationship for particles in a potential field as (33):

$$n = n_o \exp(-W_H/kT)$$

where n is the concentration in the given field,

W_H = magnetic interaction energy,

n_o is the concentration in the absence of the field,

N is Avogadro's number.

If we consider molecular oxygen, whose molar susceptibility χ is about 3400×10^{-6}, in a field where $H = 10^4$ gauss

$$W_H = \frac{1}{2}\frac{\chi H^2}{N} = \frac{1}{2} \times \frac{3400 \times 10^{-6}}{6 \times 10^{23}} \times (10^4)^2 \approx 3 \times 10^{-19}$$

$$n = n_o \exp(-W_H/kT) \cong 1.0002\ n_o .$$

This calculation shows that the magnetic field has a negligible effect in changing the concentration levels in a chemical reaction even where so highly paramagnetic a substance as molecular oxygen is used.

On the other hand, it is to be expected, and indeed it is observed, that chemical reactions can be affected by microscopic magnetic fields. The ortho-para hydrogen

conversion is known to be catalyzed by paramagnetic substances. The catalysis of the cis-trans reactions by paramagnetic substances is attributed by Harman and Eyring (34) to the differing action of the inhomogeneous field on the magnetic dipoles which arises from the spin of the two electrons in double bonds. It is thought likely that in the catalysis of certain reactions, paramagnetic ions have their action through the relatively strong magnetic fields around the highly paramagnetic ions.

Consequences of the Lorentz Force

Membrane transport.--Consider the effect of a magnetic field on the transport of ions across a membrane. The force on the ion is given by $(\underline{v}/c) \times \underline{B}$, where $\underline{B}$ is the magnetic induction parallel to the membrane. The potential difference across a frog skin, for example, is about 100 mv, and the thickness of the skin is about 100 microns. The electric field is then approximately:

$$E = 0.1/0.01 = 10 \text{ volts/cm}.$$

A typical ionic mobility in water is that of sodium:

$$\mu = 0.135 \text{ cm/sec/esu volt} \quad (35)$$

$$= 4.4 \times 10^{-2} \text{ cm/sec/volt/cm}$$

A typical drift velocity is then:

$$v = \mu.E = .44 \text{ cm/sec}.$$

For a field of 10,000 gauss the radius of curvature for a sodium ion is then about:

$$R = \frac{cmv}{eB} \cong 10^{-7} \text{ cm} = 10 \text{ Å}$$

No effect was found by Knoll (36), who used tagged sodium in a homogeneous magnetic field of 10,000 gauss. The result may be due to the sodium-ion mean-free-path being much less than 10 Å.

Background of Blood Grouping (37, 38)

The concept of blood transfusion goes back to ancient times, but no transfusions were actually carried out until the middle 17th century. Death often resulted from a transfusion; consequently, the practice was abandoned for nearly a century and a half. In the 19th century direct transfusions of blood between humans were attempted. There were many disasters and the progress toward a satisfactory technique was slow. In a series of classic papers at the turn of the century, Landsteiner announced the discovery of the existence of blood groups, and laid the foundation for safe blood-transfusion technique.

By 1875 it was known that if red blood cells of an animal were mixed with serum from an animal of another species, or sometimes with serum from even the same species, a clumping (technically, agglutination) occurred. In 1900 Landsteiner (39) found that the cells of some humans were agglutinated by the serum of other humans. He took blood from humans, separated the serum, and prepared saline suspensions of the cells. The cell suspensions were mixed with various sera and sometimes the cells agglutinated. On the basis of his results, Landsteiner was able to classify most human blood into three groups. Within each group the red blood cells of any

individual were not agglutinated by the serum of any other person in that group. In 1902 two of Landsteiner's students discovered a fourth and relatively rare group.

To explain his results, Landsteiner postulated the presence or absence on the blood cells of two substances (antigens), now known as A and B. The classification of people into four groups was based on the following: (1) A only, (2) B only, (3) both A and B, and (4) neither A nor B. The groups are known respectively as A, B, AB, and O. In a given blood sample, the serum did not contain the substances (antibodies) capable of reacting with the substance on the cells (the antigens). In the following the reciprocal relationships are shown.

Blood Group	Antigens on Erythrocytes	Antibodies in Serum	Reaction of Erythrocytes with Anti-A Antibody	Reaction of Erythrocytes with Antiserum with Anti-B Antibody
O	None	Anti-A Anti-B	-	-
A	A	Anti-B	+	-
B	B	Anti-A	-	+
AB	A,B	None	+	+

To determine an individual's blood group, a suspension of the cells is mixed with a serum known to contain only anti-A antibodies and one known to contain only anti-B antibodies; it is then examined for agglutination after a few minutes. The results are compared with the chart above.

From the above considerations the importance of determining the blood groups before performing a blood transfusion is evident. The most important consideration is generally believed to be the effect of the recipient's antibodies on the cells of the donor, since the donor's antibodies are diluted to the point where they fail to exert any harmful influence on the recipient's cells. The transfusion possibilities found feasible are then:

Recipient \ Donor	0	A	B	AB
0	Yes	No	No	No
A	Yes	Yes	No	No
B	Yes	No	Yes	No
AB	Yes	Yes	Yes	Yes

In the period 1900-1940 or so, several important discoveries were made: the inheritance of the ABO groups was determined, some of the subgroups of ABO were discovered, part of the ABO chemistry was unraveled, the blood groups MN and P were discovered, and blood-bank techniques continually improved. A new era in blood group work was opened in 1939 with the discovery of the Rh blood groups (to be described later).

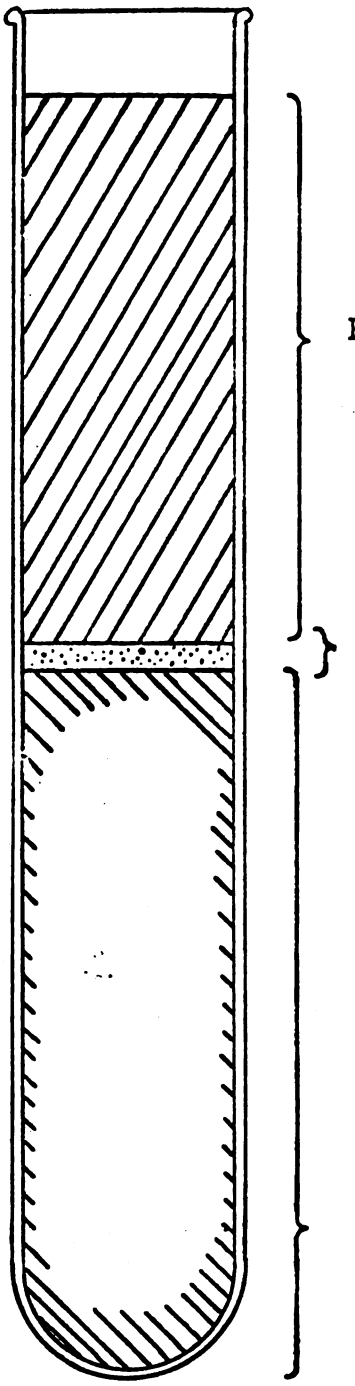
We now summarize the present stage of knowledge. When certain foreign substances (antigens) are injected into an animal, there may appear in the blood serum after a varying amount of time, substances (antibodies) able to react in a

specific manner with the antigen that elicited their production. An animal that produces antibodies in response to injection of antigen is called immunized. The presence of antibody is detected by various reactions characteristic of its interaction with the antigen. These reactions may occur in vitro or in vivo.

A brief description of blood is necessary in order to describe these processes in more detail. Blood is a body constituent transporting food and oxygen as well as substances necessary for the defense of the cells against invaders. The blood also removes waste products, and aids in controlling the temperature and pH of the cells. About 5 to 10 per cent of the body weight, depending on the species, is made up of blood.

When blood to which anticoagulant is added is allowed to settle or is gently centrifuged, it separates into solid material and a supernatant yellowish fluid. The solid material consists primarily of the erythrocytes (red cells), leucocytes (white cells), and platelets. The supernatant is called the plasma. When the plasma is allowed to clot, part of it, called the fibrinogen, comes out as a fibrous mass. The remaining material is called the serum. Figure 3 is a schematic diagram showing the important components of blood. The serum contains a number of simple inorganic and organic compounds, and a large number of proteins.

Mammalian red cells are non-nucleated cells consisting of a complex cell membrane with a "solution" of hemoglobin



Average per cent of total blood volume

PLASMA

Proteins

- Fibrinogen
- Albumins
- Globulins
- Fats
- Sugars
- Amino acids
- Salts, etc.

} SERUM 54

Buffy layer 1
- Platelets
- White cells (leucocytes)

FORMED ELEMENTS

Red cells (erythrocytes) 45

Figure 3 - The essential composition of blood. (After Cushing, reference 40.)

inside, and perhaps an internal structure called stroma. The hemoglobin makes possible the transport of oxygen to a degree approximately 20 times that of mere solution of oxygen in water. The nature of the orienting forces associated with the hemoglobin molecules is unknown, but there is some evidence that the interior of the red cell is in the liquid-crystalline state (63). From evidence based on phase-contrast and electron microscopy, and fluorescent-antibody techniques, the membrane seems to be the site of immunological activity. Yet the antigen-antibody reaction may be very subtle, with the peculiar properties of the interior playing an important role.

The manifestations of the antigen-antibody reactions most important for our purposes are: (a) precipitation, (b) agglutination, (c) combination with hapten, and (4) lysis.

(a) When a soluble antigen is mixed with its appropriate antiserum, a precipitate of antigen and antibody is formed. It is generally believed that the precipitate is the result of a union between the antigen and antibody molecules, the resulting antigen-antibody complex separating from the solution. Quantitative analysis of the reaction may be made by a protein analysis of the precipitate.

(b) Agglutination, the main topic in this work, will be treated in detail later.

(c) The combination of antibody with haptens is described later in the section on antibodies.

(d) Lysis (breaking down of cells) is sometimes the result of the combination of antigen and antibody. These substances alone do not cause lysis. Additional components (viz., complement) in the serum must be present. The conditions for lysis are in general more complicated than for precipitation and agglutination. There are some antigen-antibody reactions, resulting in agglutination *in vitro*, but causing lysis *in vivo*. The complement is normally present in serum, and is not directly connected with the immunization process.

The two general types of *in vivo* response are: (1) hypersensitivity, an increase in the biological response to the antigen; and (2) immunity, an increase in the resistance to the injurious effects of the antigen. Hypersensitivity is manifested as a shock reaction; immunity gives the individual greater resistance to disease. In hemolytic disease the cause is sometimes the presence of an antibody specific for the individual's red-cell antigens. Although a consideration of the physiological response is valuable, it appears somewhat outside the immediate scope of the present work.

In the blood systems with which we shall be mainly concerned, the antigenic material is believed to be on the

surface of the red cell. Antigens may be classified on their biological and chemical behavior as: (1) complete (or functional), and (2) incomplete (or haptenic). The complete antigens are those which react in a specific way with antibodies, and also induce antibody formation. The incomplete antigens are those which do not elicit antibody formation (unless attached to a protein), but will react in a specific way with the antibody once it has been formed.

Antigens are substances of relatively high molecular weight (most are proteins, some are mucopolysaccharides) and their chemical arrangement is known in only a few cases. Landsteiner (41) showed that, by coupling simple chemical substances to protein antigens, antibodies which reacted specifically with the simple substance could be produced. Landsteiner named these substances haptens. The specific activity of the antibody with the hapten may be demonstrated by testing the action of the antibody against the protein and then against the protein with the hapten coupled to it. In this way it becomes possible to observe reactions which are due to the hapten alone. Since the hapten has a known structure, whereas the protein usually does not, it is possible to draw inferences about the nature of the forces involved in the reaction.

At present the physical and chemical characteristics that are necessary to make a substance antigenic are not known in detail. Two generalizations may be made in regard to: (1) molecular weight and complexity, and (2) biological

relationships. In general, good antigens have a molecular weight of at least 10,000. Between molecular weights of 10,000 and 40,000 there is a fair correlation between the ability of a substance to elicit antibodies and its molecular weight. Above 40,000 the relationship breaks down. A certain degree of complexity also seems to be necessary. For example, there are such proteins as gelatin which have molecular weights of about 100,000 and yet are only weakly antigenic, presumably owing to their relatively simple chemical structure. Normally an animal does not produce antibodies against the constituents of its own body or to components which ordinarily enter into the blood. It has been shown, however, that if the proteins of an individual animal are altered chemically and then injected into the animal, they may elicit antibody production that causes the destruction of normal tissue. In vitro measurements of the potency of an antigen has the advantage that the variability inherent in living animals is reduced.

Formation of Antibodies

Until recently, ideas as to the site of antibody formation were only speculative. The antibodies are globulins, and it was expected that those cells which produce normal globulins also produce antibodies. Recently Nossal and Mäkelä (43) demonstrated antibody formation by single lymph-node cells incubated in microdrops. This finding seems to be strong evidence that antibody formation occurs

in lymph cells. There is reason to suppose that antibody formation also takes place in the reticuloendothelial system. Electrophoretic-pattern results have identified most antibodies with the gamma-globulin component of the serum; only a few are identified with the beta-globulin.

The molecular weight of the antibody molecules is approximately that of the normal globulin components. The molecular-weight data are based on ultracentrifuge sedimentation, light scattering, osmotic pressure, diffusion, and viscosity. Boyd (38) cites a value of about 160,000 as typical for man and rabbit, and the anti-pneumococcus antibody in horse, pig, and cow is about 900,000. It is generally believed that the serum proteins approximate prolate spheroids.

The question now arises: how is the body able to produce antibodies? Several theories have been proposed (44). We mention here the template theories and the selection theories. Both types of theory assume that antibodies are developed by a modification of the normal synthesis.

Among the most popular of the template theories is the one proposed by Pauling. He supposes that the presence of an antigen molecule at the site of globulin synthesis modifies the normal sequence in which amino acids are put together to form the polypeptide chains of the globulin. The modified structure of the protein molecule is such that it is complementary to the antigen structure and thus can react specifically with it. The chemical components of the

globulin and the sequence are supposed to be the same, but the antigen influences the folding of the polypeptide chain. The theory is simple and offers a lucid explanation of the specificity of antibodies. The essential distinguishing feature of this class of theories is that the information comes from outside the cell. An objection to the template theories is the persistence of antibody formation long after the antigen has disappeared.

The selection theories, of more recent origin, have been prepared by Jerne, Burnet, and Lederberg. One form of the theory proposed by Jerne supposes that globulin molecules of a very wide variety of configurations (and therefore of specificities) are continually produced by the body. An antigen is presumed to combine with those molecules of the corresponding specificity. The antigen-globulin complex is then phagocytized, transported to the antibody-forming cells, and there dissociated. For some unspecified reason, the body is supposed to discard the antigen, and to continue to make more globulin molecules like those which combined with the antigen. The entry into the circulation of the new specific forms constitutes the rise of antibody level.

In selection theories of antibody formation, the information as to the construction of the antibody is assumed to exist within certain of the protein-forming cells in the animal. The presence of an antigen serves merely to stimulate the proliferation of those cells specific to it. That is, a given antigen selects for multiplication the

clone of cells that can react with it. Each cell of the clone knows how to make the specific antibody even though the complementary antigen may never have entered the body. The primary motivation for acceptance of this theory is that it can explain the differentiation between the self and the non-self. Normally the body does not produce antibodies against its own proteins, although evidently it can produce antibodies against almost any foreign protein or certain other complex molecules. The clonal-selection theory explans this lack of self-selsitivity as follows: during embryonic life the immunological cells mutate frequently, and produce all possible antibody patterns, some of which match antigens native to the body. These antigens will kill cells having the complementary pattern and leave only the cells with antibody patterns corresponding to foreign antigens. Later in life, a foreign antigen will stimulate specific cells to proliferate rapidly and produce the corresponding antibody.

The principal objection to this theory is the hugeness of the information store that must be carried in the body. But if the antigenic determinants--the sites of specific chemical activity--are small enough, their number is not inordinately large. Proponents of the theory estimate that perhaps only 10,000 different patterns are needed.

At present, no experiment has been devised that requires either theory to be rejected. It may turn out that a complete explanation will eventually be provided by a more general theory incorporating the mechanisms of both instruction and selection.

The Rh Blood Groups

In 1939 Levine and Stetson (45) reported the finding of an unusual antibody in the serum of a woman (with a history of several miscarriages) who had given birth to a dead fetus. This antibody did not react with the cells of the mother herself, but did with the cells of the father as well as with those of approximately 80 per cent of individuals in blood group O. Levine and Stetson inferred that the fetus had inherited an antigen from the father, and that the mother had become immunized by fetal red cells.

In 1940 Landsteiner and Wiener (46) immunized rabbits and guinea pigs with blood cells from the rhesus monkey. After removing species--characteristic antibodies from the serum of the rabbit--they found an antibody which agglutinated red cells of the rhesus monkey and also of 85 per cent of white people tested in New York. Landsteiner and Wiener named the new antibody anti-Rh, after the rhesus monkey. Individuals whose cells were agglutinated by the new antibody were called Rh-positive. In that same year Wiener and Peters (47) showed that this anti-Rh antibody was the same as that which caused hemolytic reactions in patients who had received repeated transfusions of ABO-compatible blood.

In 1941 Levine and his associates (48) found evidence indicating the Rh incompatibility to be the cause of the syndrome known as erythroblastosis fetalis. It became evident that this disease occurred in an Rh-negative mother carrying an Rh-positive fetus. The Rh antigens of the fetus,

through placental transfer, caused the mother to produce anti-Rh. More than one pregnancy is necessary to build up a significant level of Rh-negative antibody. This antibody passed back to the fetus and destroyed the fetal cells by hemolysis.

By 1943, several types of Rh antibodies, designated as anti-C, anti-D, anti-E, and anti-c were known. Fisher (37), in a theory presented in 1943, assumed that since the actions of anti-C and anti-c were antithetical, the genes and antigens identified by these two antibodies were alleles. Two other serums, anti-D and anti-E, which were available in English laboratories at that time, were not antithetically related to any known serum. In Fisher's theory each of the six Rh antigens (four known, two proposed) was produced by a single gene, and these occurred in three alleleic pairs: D-d, C-c, and E-e. Another theory, due to Wiener, assumes that a single gene with multiple alleles is involved in the production in all of the six antigens. As the complexities of the Rh system unfolded, Wiener postulated additional genes. Moreover, Wiener, in 1946, challenged Fisher's theory and stated that two additional factors, d and e, predicted by Fisher could not exist. Within a few years, however, the predicted factors were discovered. But Wiener still did not accept the Fisher theory and the controversy is still unsettled. In the words of Race and Sanger (1958):

> The existence of three sites where Mendelian substitution can go on seems to us unassailable, and to argue whether the three sites are to be placed within or without the boundary of gene appears to be particularly unprofitable at the present time when no one seems to know what the boundaries of a gene are.

Each of the two foregoing theories has its own system of notation. The Fisher scheme leads to the Fisher-Race system. The Wiener system is more complicated than the Fisher-Race system, owing largely to its lack of theoretical simplicity. For a thorough discussion of the anti-Rh groups, Race and Sanger (37) should be consulted. Here is an example of the terminologies:

Gene Combinations		Rh Antigens	
Fisher-Race	Wiener	Fisher-Race	Wiener
DCe	R_1	D	Rh_o
DcE	R_2	C	rh'
Dce	R_o	E	rh''
DCE	R_z	d	Hr_o
dce	r	c	hr'
dCe	R'	e	hr''
dcE	R''		
dCE	R_y		

Agglutination

The agglutination reaction is presumably no more than the precipitation reaction occurring at the surface of the particle, the antibody molecule forming a bridge between the antigen molecules on different particles. Because the

particles give large volume relative to the antigen and antibody molecules; the reaction is effectively amplified on a volume basis, as compared with direct precipitation. Hence agglutination will produce a visible reaction at far lower concentrations than will precipitation.

It is sometimes possible to extend the range of agglutination tests by coating red cells or latex particles with an antigen, and then mixing the cells or particles with the appropriate antibody. Fluorescein-labeled antibodies have been used in agglutination tests to locate specific antigens on the surface of red cells, bacteria, and protozoa. Under proper conditions, certain fluorescent aromatic isocyanates will combine with the free amino-groups of protein molecules without interfering with the specificity of the antibody. When the labeled antibody then combines with the antigen, the site of the reaction can be identified by fluorescent microscopy.

Masouredis (49), with I^{131}-labeled anti-D, found that homozygous DD cells bound about 1.6 times as much antibody as did heterozygous Dd cells. He calculated the number of combining sites to be about 6,400 for the heterozygous cells and 10,300 for the homozygous cells. This ratio showed that the D+ blood group antigens were heterogeneous and genetically determined.

Cohen and Zuelzer (50), by use of the fluorescent-antibody technique, identified blood-group antigens in erythrocytes. They were able to demonstrate the factors A,

B, and a variety of Rh antigens.

By hemagglutination-inhibition studies it is possible to gain information about the nature of antigen. The inhibition of the usual agglutination reaction is a result of the combination of a substance with the antibody in question. The substance apparently combines with the antibody because it is structurally similar to the original antigen. It is possible to learn about the nature of the antigen by determining what type of substance will inhibit a particular antigen-antibody reaction. By inference, one also obtains information about the antibody molecule.

In the case of hemagglutination inhibition, a chemical substance is added to a solution containing a known antibody. Red blood cells carrying the corresponding antigen are added. If the usual agglutination does not occur, then it is assumed that the chemical, by virtue of its similarity to the antigen, has combined with the antibody leaving less free antibody to combine with the antigen on the red blood cells.

The inhibition of anti-D, anti-C, anti-E, anti-c, and anti-e by four ribonucleic acid derivatives was reported by Hackel et al. (51, 52). This work suggests that the Rh antigens are at least partly nucleotide in nature. This suggestion was given further support when Hackel and Smolker (53) found that treatment of erythrocytes containing Rh antigens by the enzyme ribonuclease lowered the agglutinability of the cells. Their hypothesis was that if any of the

antigenic specificity of the Rh system was due to ribonucleic acid derivaties, then the enzyme ribonuclease should remove them from the cell membrane. The hypothesis was supported because the Rh antigens were affected and the others were not.

There are antibodies (called incomplete or blocking) which by themselves do not cause agglutination in saline, but which have the same specificity as those antibodies which do agglutinate red cells in saline. These incomplete antibodies will agglutinate erythrocytes suspended in a protein medium. The presence of incomplete antibodies is particularly dangerous because it is not detected by ordinary agglutination or precipitation tests. A special procedure, the Coombs test, has been developed for the detection of these antibodies. It is based on the fact that antibodies are globulins which are almost identical with normal globulins. Now, normal globulin--as well as antibody globulin--in humans is, of course, antigenic in rabbits, for instance. Antiserum from a rabbit immunized by injection with human globulin will then react with human globulin, whether normal globulin or globulin modified into antibody. Normal globulin is not ordinarily attached to the surface of erythrocytes, whereas antibody globulin is. Hence, the presence of an incomplete antibody in a human will cause attachment of globulin molecules on the surface of erythrocytes; and the presence of these globulin molecules will be manifested through agglutination by globulin antiserum. The test then

consists of the following: erythrocytes to be tested are separated from the serum and washed thoroughly in saline. Anti-human globulin is added to the cells. Agglutination occurs if the cells are coated with incomplete antibody; a negative reaction indicates the absence of such an antibody. Anti-human globulin is a specific reagent usually prepared by immunizing rabbits.

Statement of the Problem

General Considerations Concerning the Effect of Magnetic Fields on Biological Processes

As has already been stated, the homogeneous magnetic field can only exert a torque on magnetic dipoles. It has been shown that the action of this torque on individual isolated atoms in material of biological interest is insignificant compared with thermal action. If there exists in some biological process an action which depends on mesomorphic states, in particular, the nematic state, then it is possible that the action could be influenced by magnetic fields. The occurrence of the nematic form of the mesomorphic state, the form which is readily influenced by magnetic fields, is a question which seems to be practically uninvestigated.

We may also infer that the biological effects of magnetic fields are probably subtle; else they would have been discovered a long time ago. Hence, if we hope to detect effects we must be prepared to either investigate very

sensitive biological processes or to develop sensitive instrumentation.

In view of the fact that little is known about the molecular structure, let alone function of the biologically important molecules, we can only make hopeful guesses about what processes should be studied. We should choose an experiment which ideally has the following characteristics: (1) it should be capable of yielding reproducible and unambiguous results, (2) the experiment should be sensitive, and (3) the process should be one which has been fairly well studied so that normal behavior is easily recognizable. With these ideas in mind, we chose the agglutination in the Rh system as the object of study.

Specific Effects of Magnetic Fields in Agglutination, in Particular on Human-Erythrocyte Agglutination

The agglutination reaction in human-blood systems is one of the most intensively studied subjects in biological science. With respect to the items (1), (2), and (3):

(1) Agglutination has the advantage that it is carried out *in vitro*, and thereby relatively independent of variations in a particular animal.

(2) Agglutination, in particular, the Rh system, is one of the most sensitive phenomena occurring in serological reactions.

(3) The antigen-antibody shows high specificity of the type shown throughout the whole biological world.

II. EVALUATION OF THE EFFECT OF MAGNETIC FIELDS ON AGGLUTINATION: VISUAL SCORING

In the first experiments, the visual scoring technique, as developed by Race and Sanger (37), was adopted because of its simplicity and wide acceptance.

Materials and Procedures

Capillary blood was drawn from the fingers of D+ individuals. The blood was collected in a test tube filled with normal saline solution (0.9%) and washed three times with saline. The cells were then made into a 2 per cent suspension. The antiserum was serially diluted with saline in factors of two, from full strength to a dilution of 1024. Ten λ of the cell suspension was added to 10λ of the antiserum (in various dilutions) in 6 x 50 mm test tubes. The mixture was then incubated for various lengths of time, first in an oven, but later in a water bath maintained at 37.5$\pm$ 0.1 C. The work with the A, B, O system was done at room temperature. Antisera from Knickerbocker Biologicals, Inc. and the Ortho Pharmaceutical Corporation were used.

At the end of the incubation time the mixture of cells and antiserum was withdrawn from the test tube and gently smeared on a microscopic slide. The test specimens incubated in the magnetic field and the control specimens

incubated outside the magnetic field were put on the same slide to facilitate comparison.

Magnetic Fields

Magnets

Permanent magnets.--Alnico horseshoe magnets were available from discarded magnetrons. Two magnets were clamped with opposite poles facing, an iron block being placed between one pair of faces, the samples being placed in the gap between the other pair of faces. For homogeneous fields ferromagnetic material was excluded from the gap. For inhomogeneous fields, suitably-shaped soft-iron pole pieces were fastened to the faces of the gap. These faces were 1-inch by 3-inches rectangles, the gap being typically 1-inch. Under these conditions, the field was about 3,000 gauss, with gradient less than 200 gauss/cm over a region about 3/4 inch by 2-1/2 inches. By putting on a wedge pole piece, the field could be increased to 7,000 gauss at the apex, falling to 3,000 gauss at the other side of the gap, to give gradients of about 3,000 gauss/cm and field strengths of about 4,000 gauss at the location of the samples under study. The permanent magnets, along with the control tube holder, are shown in Figure 4.

Electromagnets.--Two kinds of electromagnets were available for the study. A pair of smaller ones (Model R3), Modern and Classical Instruments Corporation, Livermore,

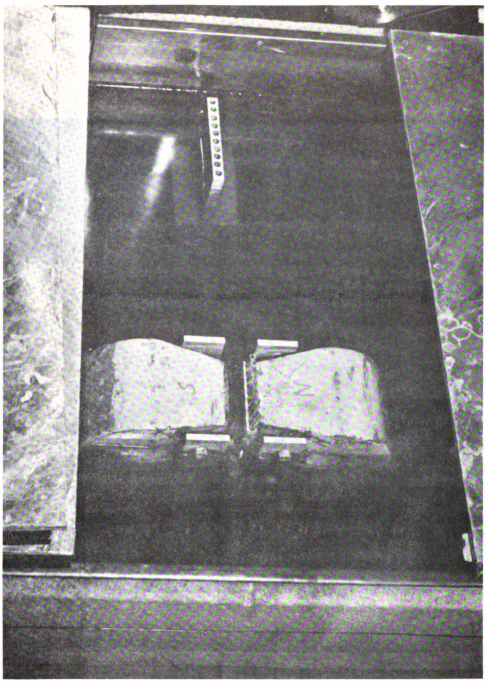


Figure 4. The permanent-magnet fixture in the water bath. The 6 x 50 mm test tubes used in the tests are shown in the magnetic field. The control appears near the magnet.

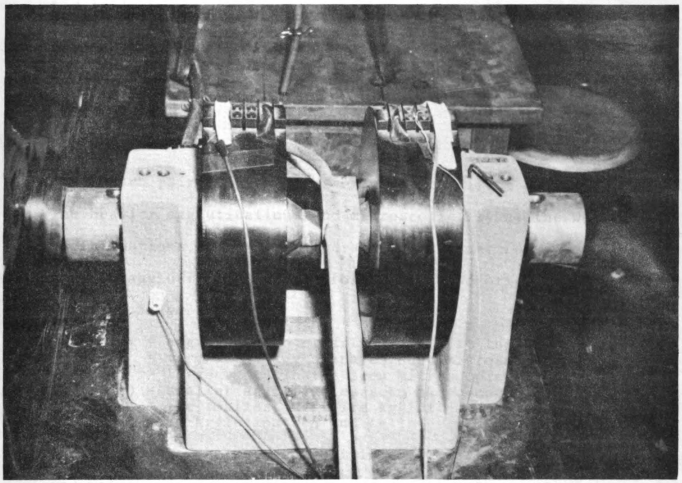


Figure 5. The Model R-3 electromagnet with the temperature fixture in the field.

California, had 1-1/2-inch circular pole faces and a gap adjustable from 0 up to 8 inches. The coils were watercooled, and would set up 15,000 gauss in a 3/4-inch gap when fed with 5 amperes at 100 V d-c. A larger electromagnet (Model L128, Harvey-Wells Corporation, Framington, Massachusetts) had 12-inch circular pole faces and a fixed 2-inch gap. Its water-cooled coils set up a field of 12,000 gauss when fed by 50 amperes at 100 V d-c. The electromagnets are shown in Figures 5 and 6.

Visual Evaluation

Each pair of smears was examined by direct vision at the heavier agglutinations and microscopically at the weaker agglutinations. The observer rated each smear for its degree of agglutination on the following scale originated by Race and Sanger (37).

+++ = agglutination clearly visible to the naked eye
++ = very large agglutinates seen microscopically
+ = large agglutinates seen microscopically
(+) = smaller agglutinates seen microscopically
w = the smallest definite agglutinates
- = no agglutination and cells evenly distributed

These readings are scored by giving the reactions the following values: +++ = 10, ++ = 8, + = 5, (+) = 3, w = 2, and - = 0. To compare the agglutination in a serial dilution of two samples, the scores are totaled and compared.

Field Mapping

Probes.--The field intensity B was measured by Hall-effect instruments (Instrument Systems Corporation, Model A-

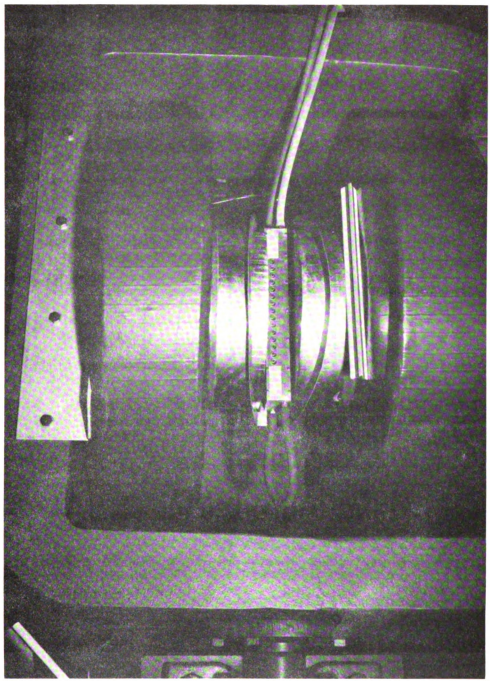


Figure 6. The Model L-12S electromagnet with test-tube holder in position. The temperature-controlled water supply enters and leaves through the rubber tubes shown.

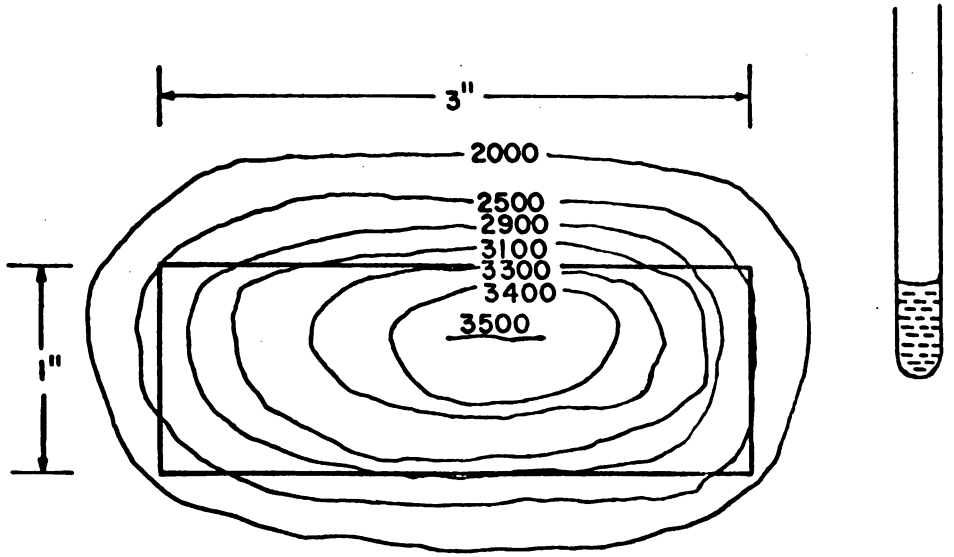


Figure 7 - Map of the magnetic field at the position of test tubes for agglutination studies. The contour lines are loci of constant strength as labeled. The source of the field; the permanent magnet arrangement shown in Figure 4.

102) and by flip-coil methods (Rawson, Model 720). The instruments were checked against a reference magnet (Rawson, Type 721) and against a nuclear-magnetic-resonance magnetometer. The probes were held at the end of a nonmagnetic extension arm clamped to a milling-machine bed. The probes could easily be positioned to 0.01 inch for surveying the field. The gradient, dB/ds, in any given direction was obtained by calculation. The fields were mapped for the various geometries and magnetomotive forces used in the experiments. Figures 7 and 8 show the results of a typical field mapping.

Test-Tube Holder

A fixture to hold the samples in the field of the 12-inch magnet was constructed and is shown in Figure 9. The temperature in the fixture was held at the desired point by circulating water from a controlled bath through it. The temperature gradient between any pair of tubes as determined by thermocouple measurements was less than 0.1 C.-deg.

A similar fixture was constructed to keep the control samples at the same temperature. The water bath fed the fixtures in parallel so that the temperature difference between them could be minimized. The difference was kept to 0.1 deg.-C.

Results

Visual Scoring

Effect of concentration.--With anti-D (anti-Rh) an

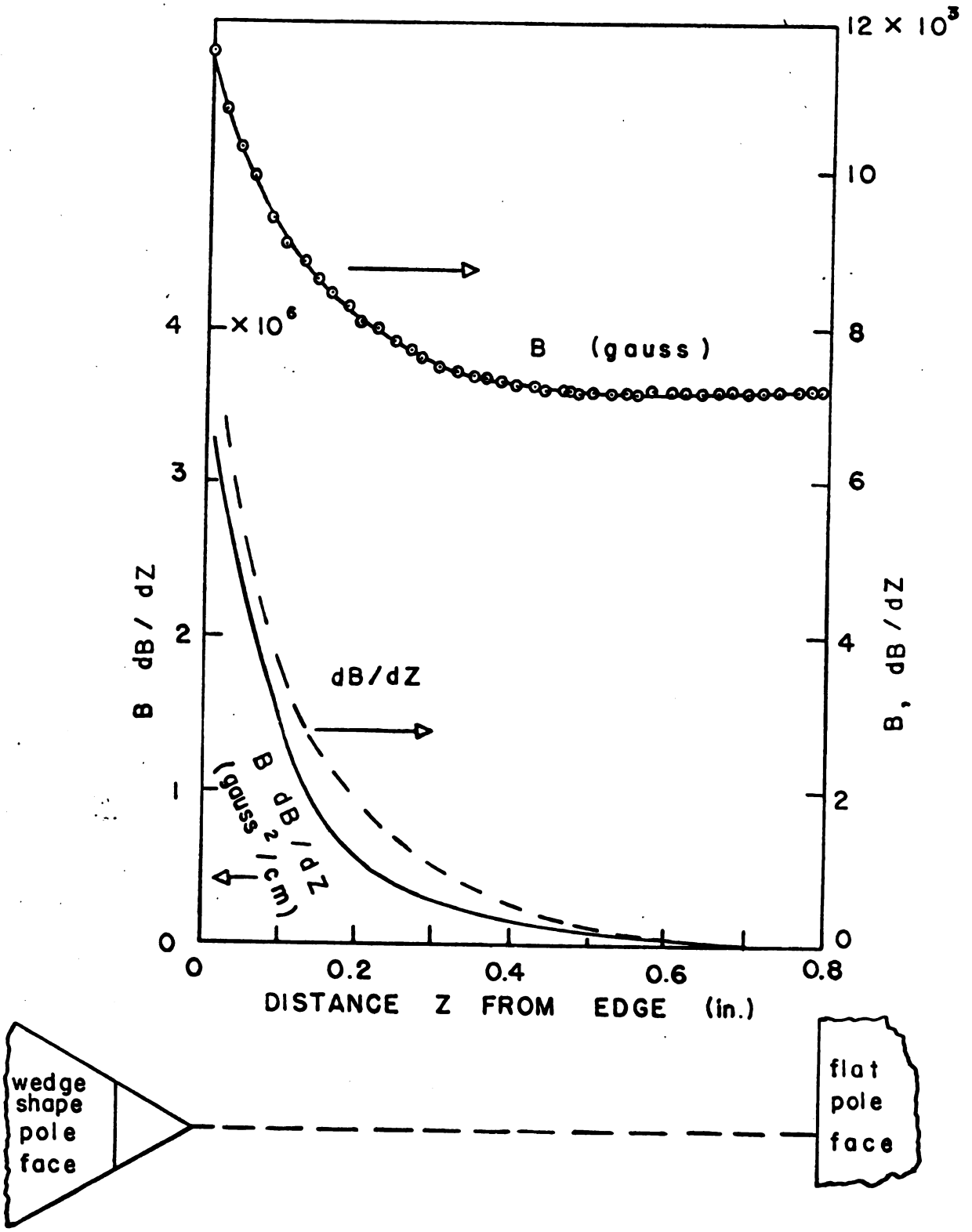


Figure 8 - Plot of magnetic field quantities as a function of distance z from apex of iron wedge placed in gap to produce inhomogeneous field. The magnetic field intensity B determines the force on permanent dipoles in the field; the product B dB/dz determines the force on induced dipoles in the field.

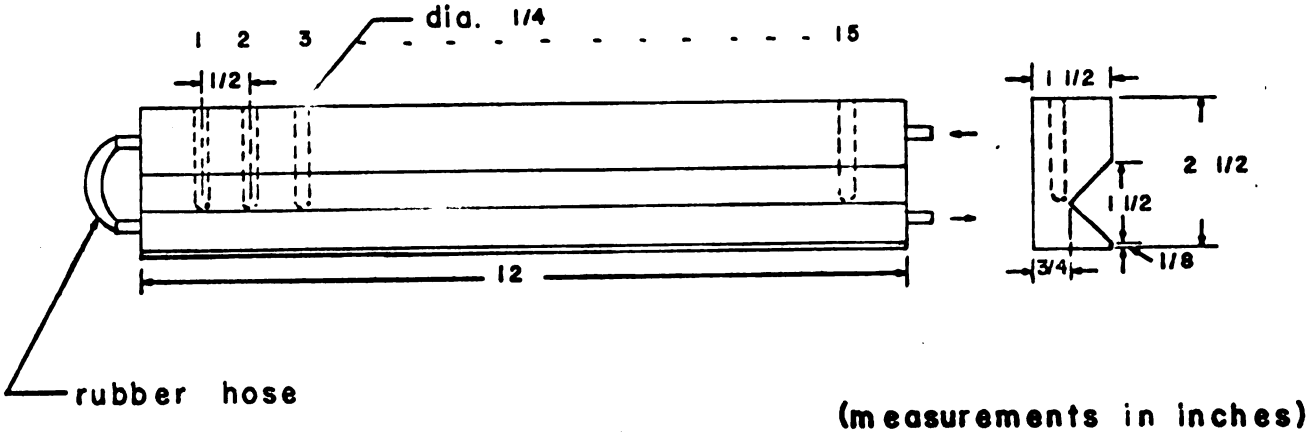


Figure 9 - The test-tube holder used to position the test tubes in the field of the large electromagnet.

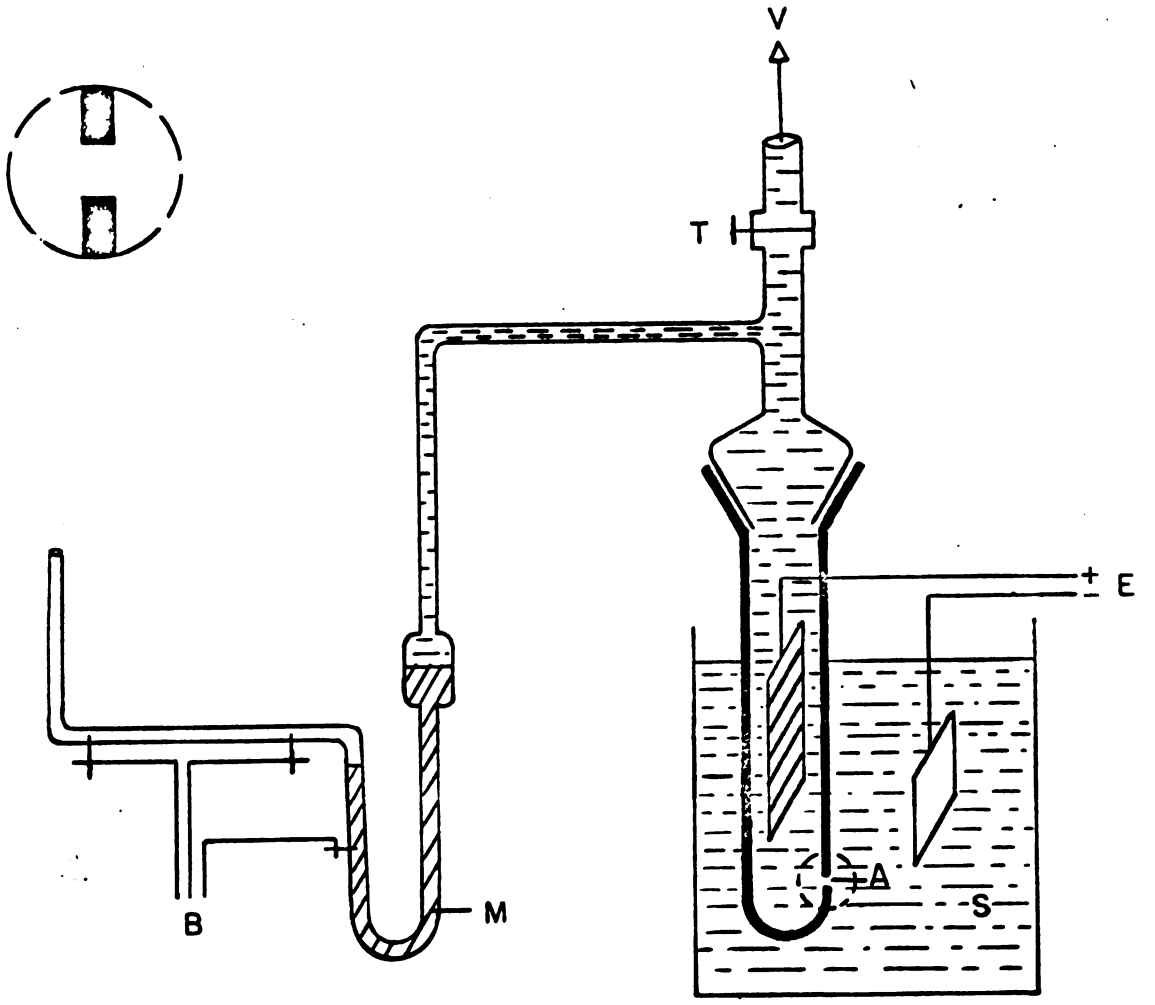


Figure 10 - Schematic drawing of the Coulter counter. The circled insert shows the aperture through which the blood cells are drawn.

A = aperture, S = saline, M = mercury, V = vacuum,
B = breaker contacts, E = electrodes.

enhancement of agglutination in a magnetic field was observed. Table 2 shows the results of a typical experiment with anti-D serum and D-positive cells. At the highest concentrations, no difference was scored, the agglutination being maximal on the Race-Sanger scale even though greater clumping was apparent for the sample incubated in the field. For intermediate and low concentrations, a scoring difference is observed. The overall results on the Race-Sanger scales give a total score of 74 to 56, an enhancement of 32 per cent on this arbitrary scheme.

Effect of field strength.--Similar runs were made in more or less homogeneous fields, the average strengths running from about 20 to 5,000 gauss. The results are shown in Table 3. When D-negative cells were incubated with anti-D serum, no reaction occurred at any of the field strengths used, thereby indicating that the magnetic field does not produce non-specific agglutination. Table 4 shows the effect of a high magnetic field on the anti-D reaction.

Effect of antiserum type.--Similar results were obtained with cells of other D-positive genotypes against anti-D serum. Anti-C (anti-Rh') and anti-E (anti-Rh'') sera against appropriate positive cells yielded essentially the same pattern as anti-D serum. Anti-c (anti-hr') and anti-e (anti-hr'') have not yet been tested because of the increased complexity of the technique without commensurate increase in information likely to be obtained.

TABLE 2

EFFECT OF ANTISERUM CONCENTRATION ON ANTI-D REACTION WITH D-POSITIVE CELLS
(Field Strength = 2,000 Gauss)

Serum Dilution	Field	Control
Neat	+++	+++
2	+++	+++
4	+++	+++
8	+++	++
16	+++	++
32	++	+
64	+	(+)
128	+	w
256	(+)	-
512	(+)	-
Total Scores:	74	56

TABLE 3

ENHANCEMENT OF AGGLUTINATION BY MAGNETIC FIELDS
(Anti-D Serum, D-positive cells)

Field Strength (Gauss)	Titration Scores		Percent Enhancement in Field
	Field	Control	
--	57	57	0
23	56	56	0
29	56	56	0
37	56	56	0
53	68	56	21
85	68	56	21
130	70	57	23
200	70	59	19
400	70	56	25
800	70	56	25
2000	74	56	32
3000	68	52	31
5000	74	57	30

TABLE 4

EFFECT OF MAGNETIC FIELD ON ANTI-D REACTION WITH D+ CELLS
(Field Strength = 16,000 Gauss)

Serum Dilution	Control	Field	Control	Field
Neat	+++	+++	+++	+++
2	+++	+++	+++	+++
4	++	+++	++	+++
8	++	++	++	++
16	++	+++	+	++
32	++	++	++	++
64	++	++	+	++
128	+	++	+	++
256	(+)	++	+	++
512	w	++	(+)	+
1024			-	(+)
2048			-	(+)
4096			-	(+)
	70	88	67	92

When the antibodies of the ABO and MN systems were tested in analogous experiments, no enhancement by magnetic fields was observed under the conditions of our experiment and our techniques of observation. These tests were not exhaustive, however, and we hesitate to claim definitely the absence of effects. Under other conditions, agglutination with these antibodies were, as reported by Foner (65), increased by the magnetic field.

Effect of incubation period.--For antigen-antibody reactions where enhancement is observed, the incubation period at the normal temperature was varied by factors of one-half and twice the normal period. As a rule, increasing the period increased the degree of agglutinations slightly, but definite quantitative relations have not yet been established.

Effect of incubation temperature.--Changes of 1.5 C-deg. above and below the normal temperature showed negligible effects.

Effect of field inhomogeneity.--In the experiments described, the fields were only moderately homogeneous. To see whether adventitious inhomogeneity was possibly the source of this effect, strong inhomogeneity was introduced by placing wedge-shaped iron pole pieces over the magnet faces. When the incubation tubes were placed at the apex of the wedge, the enhancement appeared to be intensified. Experiments to date, however, do not permit meaningful quantitative evaluation of this effect.

III. EVALUATION OF AGGLUTINATION: SEDIMENTATION TECHNIQUES

From Stokes' formula the terminal velocity of a small sphere falling in a viscous fluid is:

$$v = \frac{2}{9} \frac{(D-d)g}{\eta} r^2 = Cr^2$$

where v is the rate of fall of the sphere; D and d are the densities of the sphere and the medium, respectively; g is the gravitational constant; r is the radius of the sphere; η the viscosity of the fuoid; and C a constant. For simplicity, we take erythrocytes, individual or aggregated, as spheres. Aggregates of cells will fall faster than individual cells, and hence the rate of sedimentation may permit an evaluation of the agglutination.

The effect of changed sedimentation rate by agglutinins had long ago been noted. In fact, over twenty years ago, Hirst and Pickels (54) in 1942 developed a photometric instrument to record the course of the settling.

In the present work, where the effect sought may be small, the apparatus must permit simultaneous observation of samples within the field and out of the field. In our laboratory such an apparatus has been designed and built, but results are not yet available.

Sedimentation rate was measured in a simple electrical manner by Schwan in 1948 (55). He measured the change in electrical resistivity between two electrodes in a blood

suspension placed so that the resistivity changed as the cells sank. He found that the resistivity could be simply related to the sedimentation rate. To use this change in resistivity as a measure of the rate of agglutination, Schwan measured sedimentation rate by observing the change in resistance between electrodes placed vertically in the suspension. He was able to measure sedimentation rate with whole blood (containing 40% cells). This method, however, is apparently not feasible for our work with dilute suspensions (2%) because the small change in resistivity is masked by spurious effects from the electrodes and the electronic equipment. Our experiments have shown such small differences in resistance that they cannot be meaningfully compared.

IV. EVALUATION OF THE EFFECT OF MAGNETIC FIELDS ON AGGLUTINATION: PARTICLE SIZING

Introduction

By far the most extensive work on erythrocyte size distribution was that of Price-Jones (56). By photomicrography of highly-diluted blood smears he obtained size distribution curves for samples of very many kinds of blood. He reported a normal distribution with respect to erythrocyte diameter with a mean of 7.202 microns and a standard deviation of 0.172μ. A distribution symmetric in diameter would, of course, give a distribution in volume skewed to the higher volumes. Price-Jones' procedure is time consuming and does not give the distribution of cells as they occur in a liquid medium.

Automatic instrumental methods giving the distribution in liquid medium are thus highly desirable. The Coulter counter, an electronic instrument developed for this purpose, counts and sizes particles suspended in a conducting liquid. The counter was invented by Coulter, U. S. Patent No. 2,656,508, and described by him (57) and Brecher (57). Its use for counting cells was reported in the references just cited and for sizing by Mattern *et al.* (58). Agglutination of platelets and erythrocytes was detected by Halloran *et al.* (59), and by Brecher *et al.* (60), who used the size

distribution. Goodman (61) used the Coulter counter in quantitative hemagglutination.

Description of the Coulter Counter and Particle-Size Distribution Plotter

The instrument operates on the difference in electrical conductivity between particles in a suspended medium. A suspension is pumped through a capillary aperture. The passage of a non-conducting particle changes the conductance to an extent dependent on the system. Accurate solution of the problem of the change in conductance is extremely difficult, but to a first approximation the change is proportional to the volume of the particle. When constant current is maintained through the aperture, the voltage is proportional to the change in conductance. The pulses are then counted and sorted. A schematic representation of the instrument is shown in Figure 10.

In actual use a known volume of sample must be passed through the orifice, preferably at a constant flow rate. The liquid is forced through hydrostatically. In the present experiments, 0.5 ml. is counted. A constant current passes from known electrodes on the high-pressure side of the aperture to a platinum electrode on the low-pressure side. The polarity of the electrodes can be varied at will, and it is advisable to alternate the polarity between successive runs to minimize polarization effects. In the case of fragile particles, such as erythrocytes, it is best to use as low a current density as possible in order to avoid

damaging the cells as they pass through the aperture, the voltage pulses following amplification and shaping are fed into a scaler. In the Model A counter the input threshhold is variable and all pulses greater than a given height are counted. In the Model B counter the upper limit as well can be selected and all pulses with heights between the lower and upper limit are counted. With either model, the size distribution is obtained by successfully varying the limits on the scaler.

Obtaining the distribution can be made largely automatic with the Model B by automatically setting the successive gate widths and recording the number of counts at each setting by means of the Coulter plotter.

The particle size distribution plotter has 25 channels. A stepping switch changes the channels automatically in a sequence from lower to higher ranges. At each step of the sequence a set of pulses is received and the integreated output is fed to a recorder. A typical output curve from the plotter is shown in Figure 11. With this device, using the 100-micron aperture, the 25-channel distribution can be obtained in about 100 seconds.

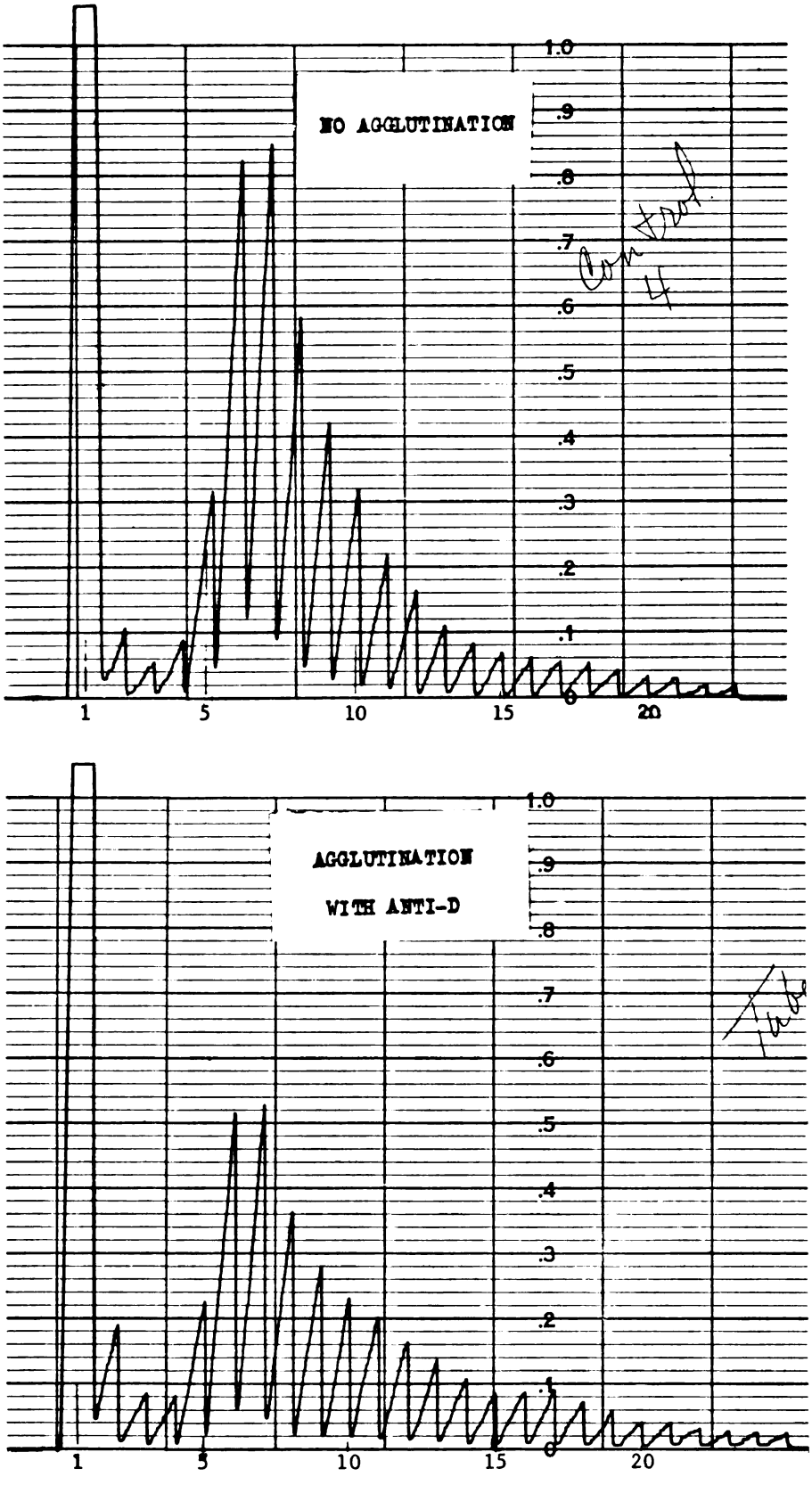


Figure 11. Volume distribution of agglutinated erythrocytes as obtained with Coulter-counter plotter. Note the decrease in spike height in the 7th spike(~singlets), and the rise at about the 17th spike (~doublets).

Choice of Aperture Size

The choice of aperture size is determined by the size and concentration of the particles counted. The aperture size selected should be small enough to give good resolution and small coincidence rate, and large enough so that clogging is negligible. Good resolution is obtained by making the change in conductance relatively large; that is, by making the volume of the particles large with respect to volume of the aperture opening. "Coincidences," that is, the occurrence of more than one particle at the same time to give a pulse simulating the passage of a single large particle can be reduced by using a smaller aperture (or, of course, by decreasing the concentration of particles in the suspension with attendant increase in counting time or statistical error).

The Aperture Current and Amplifier Settings

On the Model B there are controls for the variation of the pulse amplifier and the current through the aperture. Since the output of the electrodes is clamped regardless of

the current passing through the aperture, the pulse height of a given particle will be proportional to the aperture current. For good resolution it is apparent that higher current densities should be employed. The nominal current through the aperture may be varied from 4 ma. to 0.0625 ma. in factors of 1/2. With a 100-micron aperture, a nominal current of 1 ma. corresponds to about a current density of 12.7 amps/cm^2. In the case of erythrocytes, the current density must be kept sufficiently low to avoid damaging the cells. The amplifier settings can be used to increase the pulse height, and an increase of a factor of two in the amplification is approximately equivalent to halving the aperture current. It is preferable to use the lowest aperture current feasible in combination with the highest amplification.

At high current densities we have found that the blood-cell count is changed and we attribute this to several accompanying factors: the heating of the fluid as it flows through the aperture, and the distortion of the cells. The aperture current should be large enough to give pulses high above noise, but should not be so great as to cause excessive heating or disruption of aggregates.

Choice of amplification selection determines the size range of the particles that can be counted and the pulse-height resolution. An increase in amplification decreases the range of particle sizes that can be counted and increases the resolution available for the distribution.

Volume Distribution of Erythrocytes

Distribution in Normal Blood

Figure 11a shows the record made by the plotter of a 2 per cent solution of normal blood cells in saline. For this record the setting of the counter amplifier was 1/2, the control setting (reciprocal of the aperture current) was 2 , and the aperture diameter was 100 microns. The plot is a histogram in which the spike height is proportional to the number of particles whose volume lies between prescribed limits indicated by the abscissa. Each pair of limits defines a "window" or "gate" on the abscissa scale. The first few peaks come from very small particles existing in the saline or other dilutant alone. This debris is of little interest in the present work. At the fifth or sixth peak a maximum in height occurs corresponding to a volume of about 85 cubic microns, attributable presumably to most probable erythrocyte volume.

Later in the work a more precise determination of the volume distribution was obtained by use of a 50-micron aperture and a modification of the size-distribution plotter. The 50-micron aperture made possible a finer resolution of particle size, since here an individual particle causes a pulse large relative to that obtained with a 100-micron aperture.

To see whether the data are under statistical control, we made eleven replications of the actual count in each window for a give blood sample. A specimen of blood was

drawn, washed in saline, and made into a 2 per cent suspension in 150 ml saline. From this same beaker 11 successive size distributions were obtained at intervals of about 5 minutes, each run requiring approximately 4 minutes. The data are shown in Table 5. The suspension was stirred between runs, but not during a run.

Inspection of the table, as well as formal statistical analysis (chi-square), shows that the distribution in the first two runs differs markedly from that in the last 9. To see whether the last 9 were under statistical control, they were analyzed in the following way. The averages for each of the 17 windows (4 to 20) were computed for the 9 runs. These averages were taken as the theoretical distribution. The difference between the actual count and the average count was then computed, with the results shown in Table 6. These values were then summed to permit chi-square tests to be made. The windows 4 and 5, presumably representing debris or perhaps noise, were discarded in the summation.

By the usual rules, a chi-square test would require a chi-square less than 149 at the 5 per cent level. Our value of more than 200 lies outside this, and by usual statistical procedures we should not say that the data come from a single distribution. At this stage we prefer to state that the process is not under statistical control, and wish to refine our technique and investigate our apparatus before we pursue the statistics further.

The skewness of the size distribution shows then the distribution is not normal. We seek a transformation of the abscissa which may transform the distribution to a familiar

TABLE 5

WINDOW COUNTS FOR SAMPLE OF KK BLOOD

Window	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Run 7	Run 8	Run 9	Run 10	Run 11
4	71	106	151	118	91	136	149	146	120	99	149
5	38	47	71	57	68	54	79	[66]	95	65	83
6	48	94	63	55	67	84	72	[68]	72	56	66
7	534	521	294	319	289	439	369	329	333	342	321
8	1558	1621	991	1016	955	1292	1187	1180	1150	1105	1059
9	1978	1877	1723	1729	1688	1692	1795	1826	1850	1733	1847
10	1203	1218	1553	1572	1537	1447	1545	1443	1501	1469	1508
11	798	791	905	929	1003	817	876	912	930	915	907
12	568	574	669	681	693	636	630	661	640	598	677
13	356	532	536	557	555	504	524	505	569	528	561
14	233	271	385	381	397	320	359	361	392	348	356
15	179	200	213	246	247	230	220	222	290	265	217
16	138	132	141	143	140	132	151	143	164	147	148
17	136	107	85	94	87	110	89	89	106	85	87
18	100	101	61	55	55	84	72	81	84	53	76
19	113	116	50	32	45	69	57	51	37	46	48
20	90	67	32	25	30	33	38	37	31	30	26
Total	8141	8375	7923	8009	7947	8079	8212	8120	8294	7884	8136
Average	8102	8102	8102	8102	8102	8102	8102	8102	8102	8102	8102
Deviation	+39	+272	-179	-93	-155	-23	+110	+18	+192	-218	+34
			-144	-58	-120	+16	+145	+62	+237	-183	+69

TABLE 6

CHI-SQUARE TABLE FOR RUNS 3-11 OF TABLE 5

Window No.	Avg. 3-11	3	4	5	6	7	8	9	10	11
6	67	.24	2.15	0	4.31	.3	.37	.3	1.81	0.02
7	337	5.49	.96	68.4	30.3	3.0	3.04	--	0.07	0.76
8	1104	.15	.13	20.1	32.0	6.0	5.23	--	0	1.83
9	1765	1.00	.73	3.36	3.0	.5	2.10	4	0.58	3.81
10	1508	1.34	2.72	5.58	2.47	1.0	2.80	--	1.01	0
11	910	0.03	.39	9.50	9.50	1.2	0.00	.5	0.03	0.01
12	654	0.30	1.11	2.32	0.50	.9	0.08	.3	4.80	0.81
13	538	0.00	.67	.54	2.15	.3	2.02	1.8	0.19	0.98
14	367	0.88	.54	2.45	6.02	.2	.98	1.8	0.98	0.33
15	231	1.40	.97	1.11	.00	.5	.35	.5	5.00	0.85
16	145	0.11	0.63	.17	0.99	.3	.03	3	0.03	0.06
17	.12	0.53	.04	.27	3.52	.4	.10	2	0.53	0.27
18	69	0.93	2.84	2.84	3.26	.2	2.08	4	4.00	0.71
19	48	.08	5.3	.19	9.18	2	.19	3	0.08	0
20	31	.03	1.16	.03	.13	1.5	1.16	--	0.00	0.81
		12.5	20.3	48.8	40.0	18.3	20.5	21.2	19.1	11.3

one. One obvious transformation is the cube root of the volume (corresponding to a distribution in cell linear dimension); another is the logarithm of the volume (corresponding to a distribution in the logarithm of either the volume or a linear dimension).

It turns out that a better fit is provided by the logarithmic transformation, as may be seen from Figures 12 and 13. Although the fit on log-probability paper appears excellent, a chi-square test does not permit acceptance at the 5 per cent level, largely because of the discordance at two adjacent channels; nevertheless, we accept the lognormal as a working hypothesis while awaiting additional data that will give a definite answer on this question.

Similar studies were made by Lushbaugh (62) who used the output of the Coulter counter as input to a 100-channel pulse analyzer. In this way he was able to obtain very accurate frequency distributions of erythrocyte volume. Lushbaugh was able to distinguish normal human blood from abnormal cases by the fractional width of the volume distribution, defined as the ratio of the distribution width at half the mode, to the mode itself. To relate our findings to those of Lushbaugh we have calculated the fractional width of our lognormal distributions, and compared our values with his (Appendix). A close agreement is obtained. We infer that his findings, as well as ours, are consistent with a lognormal distribution.

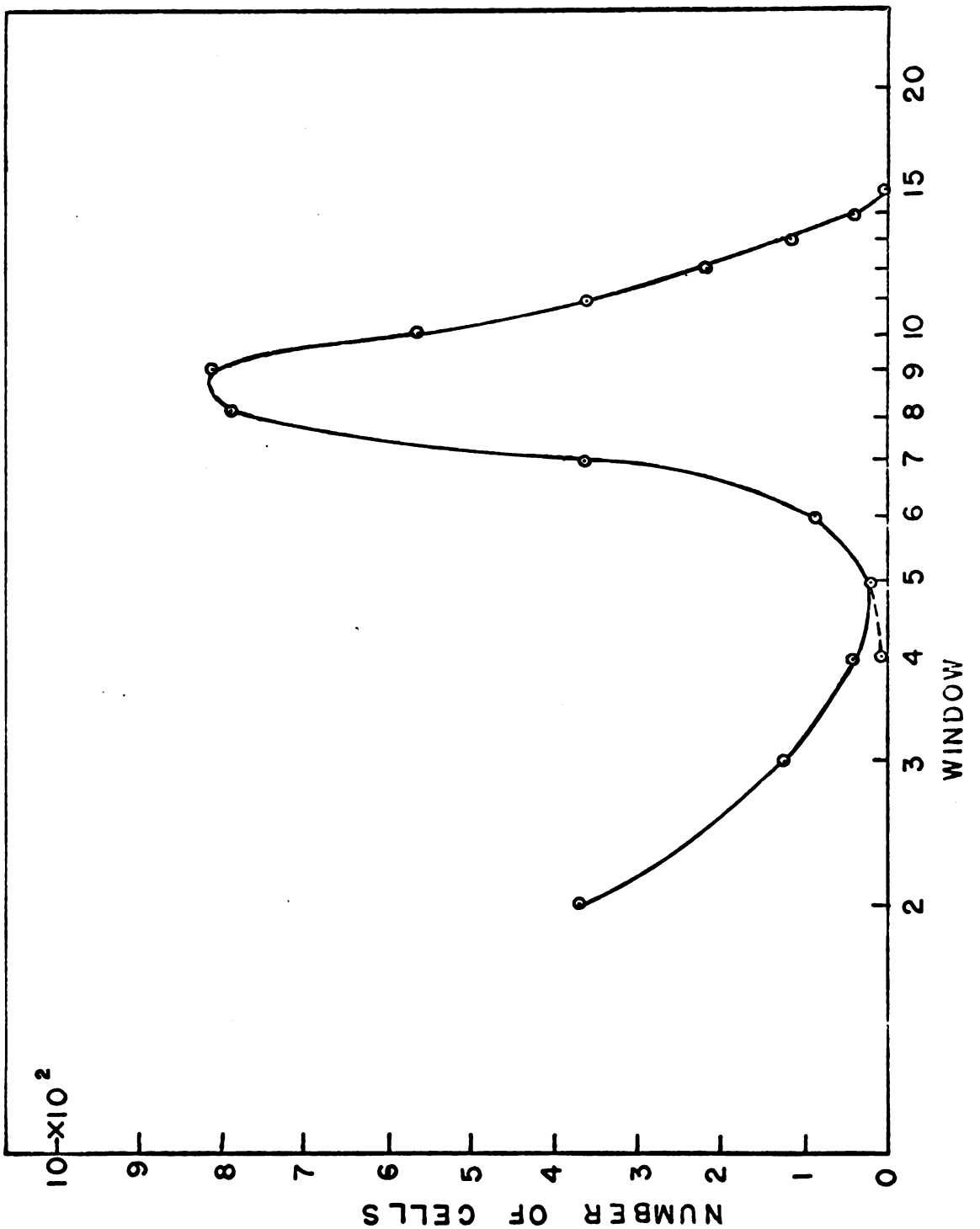


Figure 12 - Volume distribution of KK erythrocytes. Note how use of logarithm of volume as abscissa reduces the skewness.

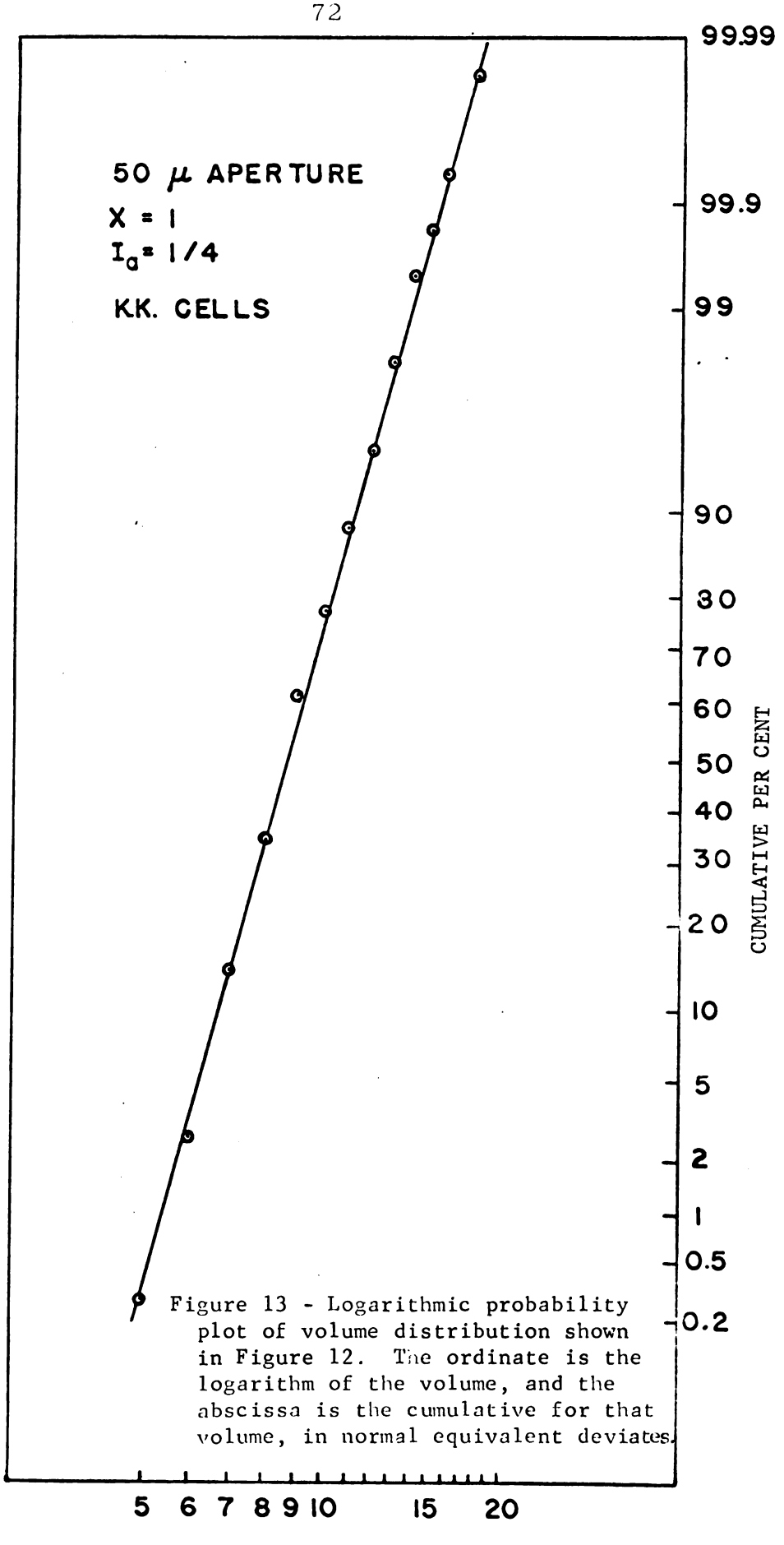


Figure 13 - Logarithmic probability plot of volume distribution shown in Figure 12. The ordinate is the logarithm of the volume, and the abscissa is the cumulative for that volume, in normal equivalent deviates.

Distribution in Agglutinated Blood

Figure 11b shows a typical plot for a 2 per cent suspension of D+ blood incubated with 1:8 dilution anti-D serum for 45 minutes at 37.5°C. The first few peaks again represent dirt and debris that are of little interest in our work. The maximum height occurring again at about 85 cubic microns, presumably the most common erythrocyte volume, but the number is considerably smaller than in the unagglutinated blood. At a volume of about double 85 cubic microns (specifically peaks 13, 14, and 15) another maximum in the height appears. Naturally we attribute this rise to agglutinated pairs of cells. Triplets and quadruplets would be expected to occur, but the resolution of the plotter is not high enough to detect the occurrence.

The new distribution, being bimodal, can no longer be either normal or lognormal. The deviation from either law, of course, may give a measure of the effectiveness of the agglutinin. It is likely that a superposition of two simple distributions can approximate the actual distribution, but we do not consider an exhaustive search on this question worthwhile. Figure 14 shows the deviation from log normality on a cumulative percentage plot.

Figure 15 shows the ratio of corresponding spike heights as a function of peak height for agglutinated blood in comparison with normal blood. The decrease in singlets is shown by the dropping of the curves below unity around the peak 7, and the rise in the doublet peak appears as the rising of the peaks around peak 13.

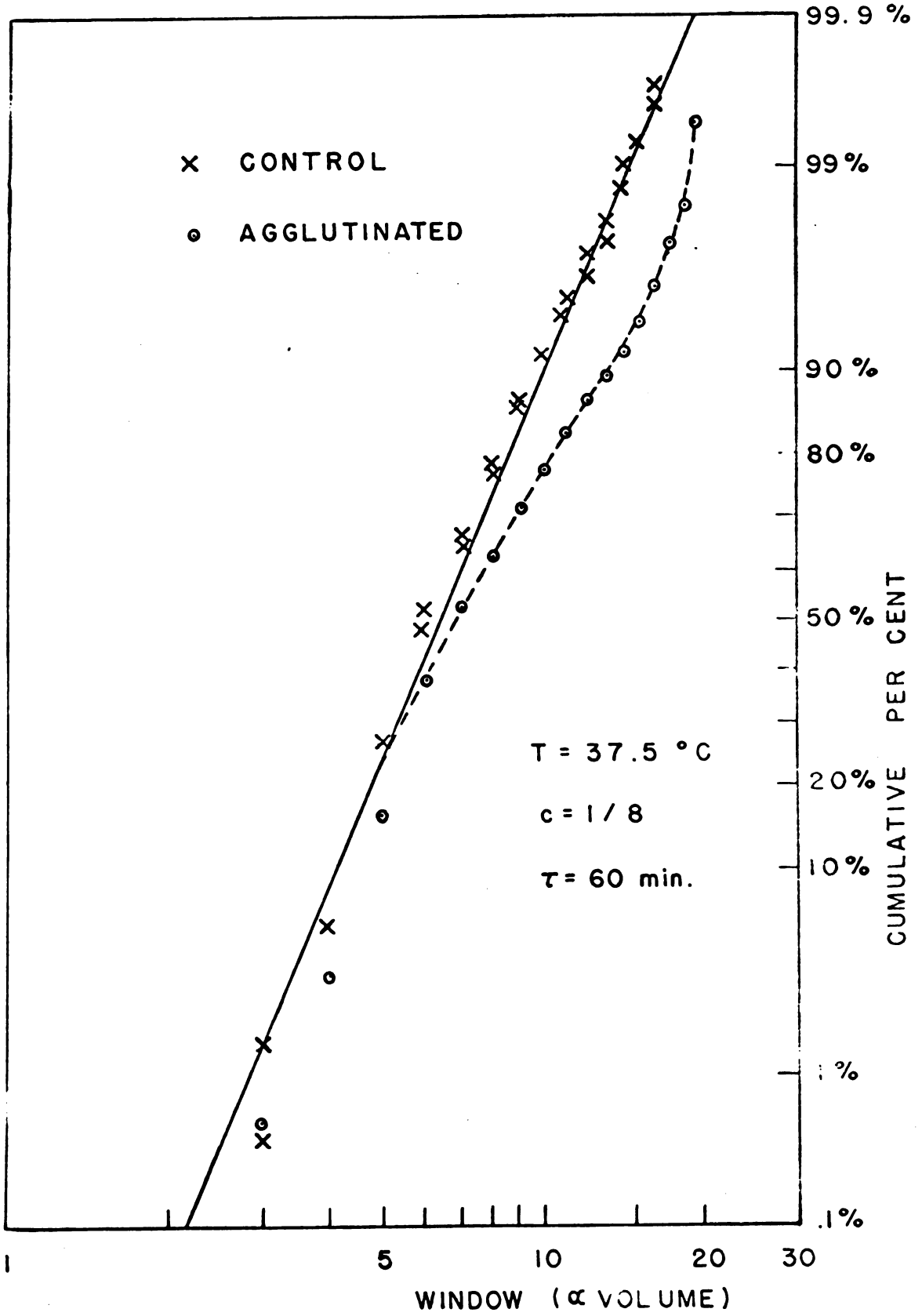


Figure 14 - Plot is similar to that of Figure 13. For the unagglutinated blood, the plot is nearly a straight line, representing a lognormal distribution. For the unagglutinated blood, the

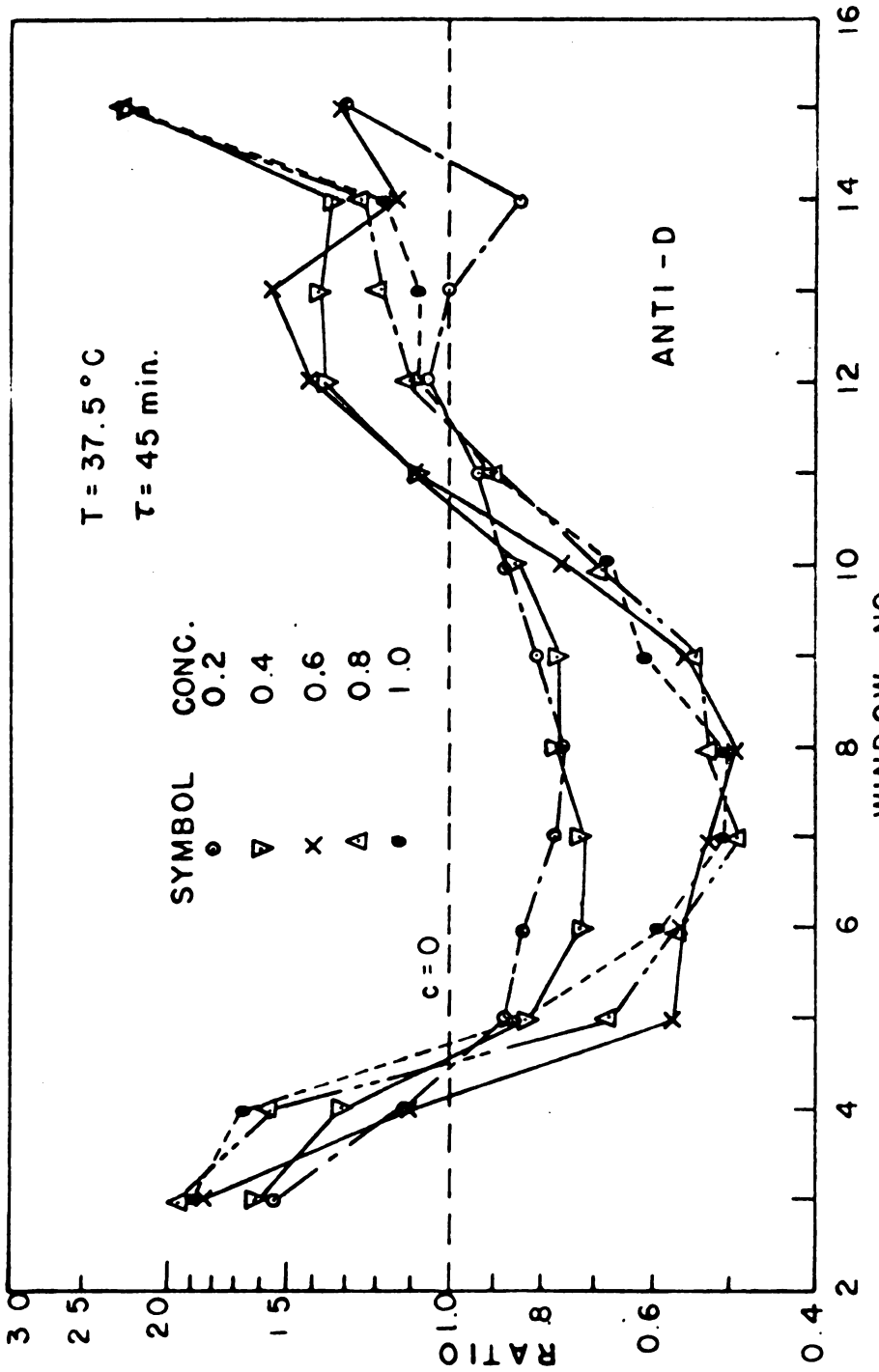


Figure 15 - Semilogarithmic plot of ratio of spike height upon agglutination at various concentrations as shown, to spike height for normal unagglutinated cells, as a function of particle volume (proportional to window number).

Choice of a Statistic to Characterize Agglutination

Since the occurrence of agglutination depresses the total count in causing several particles to appear as a single conglomerate, the total count serves as an approximate indicator of the extent of the agglutination. We might wish, however, to take advantage of the fact that formation of doublets (or even triplets and quadruplets) would independently serve to indicate occurrence of agglutination. We might suspect, therefore, that the increase in height of the "doublet peak" could be used in combination with the decrease in height of the singlet peak to yield a statistic of high sensitivity. Furthermore, the actual concentration of erythrocytes is variable because of difficulties inherent in the sampling procedure; hence, use of a ratio of peak heights, which to first order should be independent of the concentration, is desirable. Accordingly, we define a statistic D/S intended to represent the ratio of doublet to singlets, but spread out over the peaks adjacent to each maximum in order to diminish statistical fluctuations:

$$D/S \equiv (13 + 14 + 15)/(5 + 6 + 7).$$

In the same vein we could define a statistic (T/S), or (Q/S), intended to represent the ratio of triplets to singlets or quadruplets to singlets, etc. Most of our work to date has been concerned with the statistic (D/S). The choice of statistic to characterize agglutination is, of course, arbitrary; only experience will show what are the more useful statistics.

Statistical Behavior of (D/S)

To get some idea of the reliability of the statistic (D/S), we investigate the statistical behavior on replication. When ten replications were made with 2 per cent suspension of freshly drawn unagglutinated blood, the coefficient of variation (CV) of (D/S) value was about 10 per cent. The coefficient of variation for the total count N in the same experiment was 3 per cent. The coefficient CV for agglutinated blood is typically about 6 per cent, and for N about 6 per cent.

TABLE 7

RELIABILITY OF (D/S)

Sample	D/S	
1	3.38	
2	3.19	
3	4.06	Incubation time = 30 min.
4	3.49	Temperature = 37.5°C.
5	4.07	Conc. antiserum = 0.
6	3.98	
7	3.52	Standard Deviation = .346
8	3.35	Coefficient of Variation
9	3.07	(CV) = 9.7%
10	3.70	
	Ave. 3.58	

Dependence of D/S on Concentration C

Figure 16 shows the variation of the D/S value as a function of the concentration for D-positive cells incubated with anti-D for 45 minutes. Each point represents the average of eight samples. The value of D/S at zero concentration

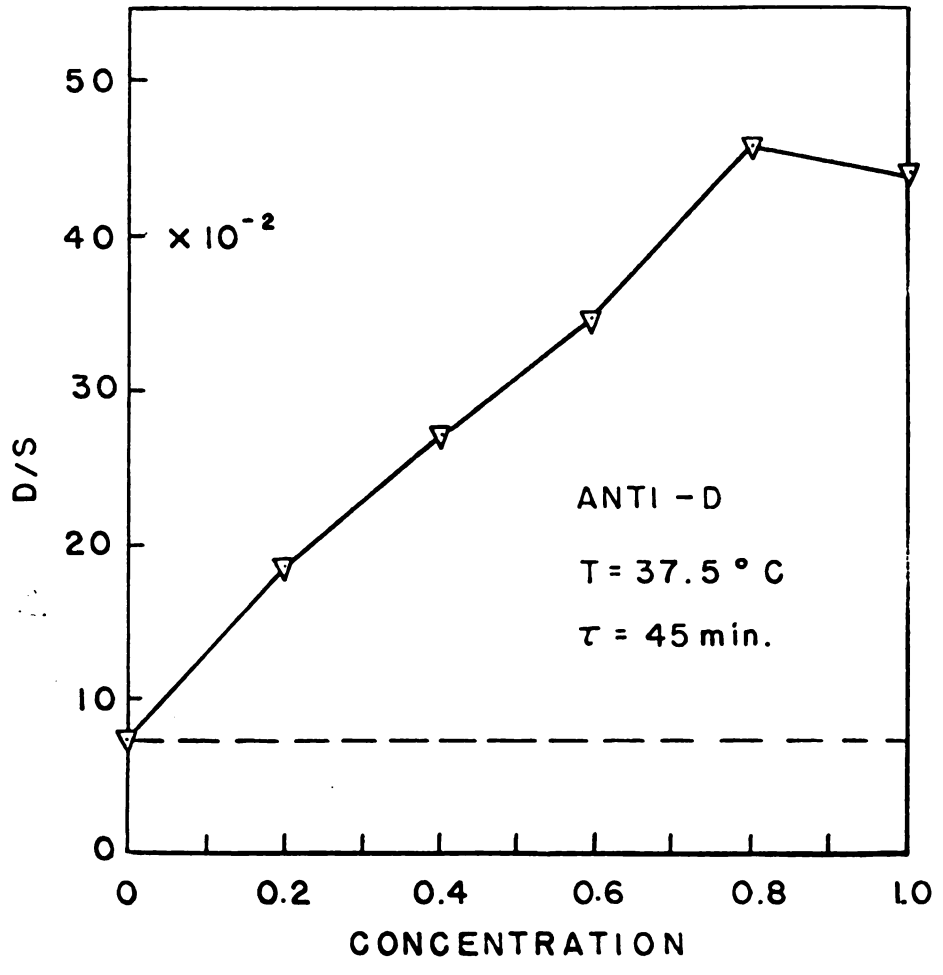


Figure 16 - Agglutination, as measured by D/S, as a function of concentration for D-positive cells incubated with anti-D serum for 45 minutes at 37.5°C.

is appreciably different from zero because the normal distribution of normal blood appears to contain some cells with a diameter double that of the average. There is also the possibility that spurious coincidences due to simultaneous passage of cells through the aperture accounts for this. The existence of rouleaux formation has to also be considered.

The value of D/S increases linearly from its value at c = 0 to about c = 0.8 where it levels off. The slope of the curve in the linear region is about 50 per unit concentration. The ratio of the upper limiting value of D/S to the lower limiting value is about 5 and this is typical of the spread of this statistic over the range of concentration zero to unity.

Dependence of D/S on incubation period.--It is important to establish the sensitivity of D/S to the incubation period--both to determine the timing tolerance for the incubation and to get information bearing on the mechanism of agglutination. Figure 17 shows D/S as a function of τ for D-positive blood incubated at 37-1/2°C. with anti-D serum for periods from 10 minutes to 90 minutes. The linear dependence becomes almost a strict proportionality when the residual value of D/S is subtracted, until a saturation at very high concentrations occurs.

Dependence of D/S on incubation temperature T.--Just as for incubation period τ, it is important to establish the

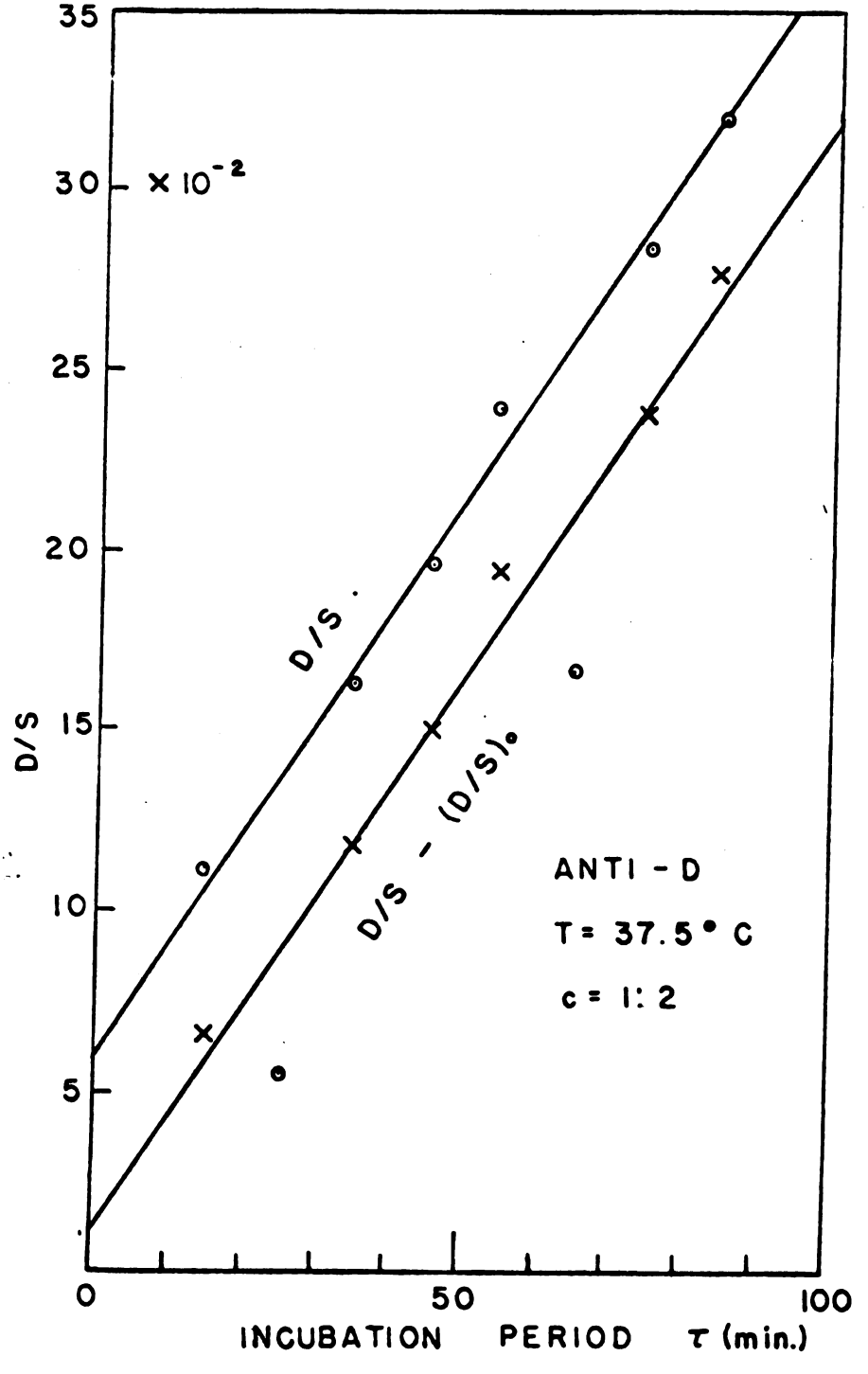


Figure 17 - Agglutination, as measured by D/S, as a function of incubation time for D-positive cells incubated with anti-D serum at 37.5° at two concentrations.

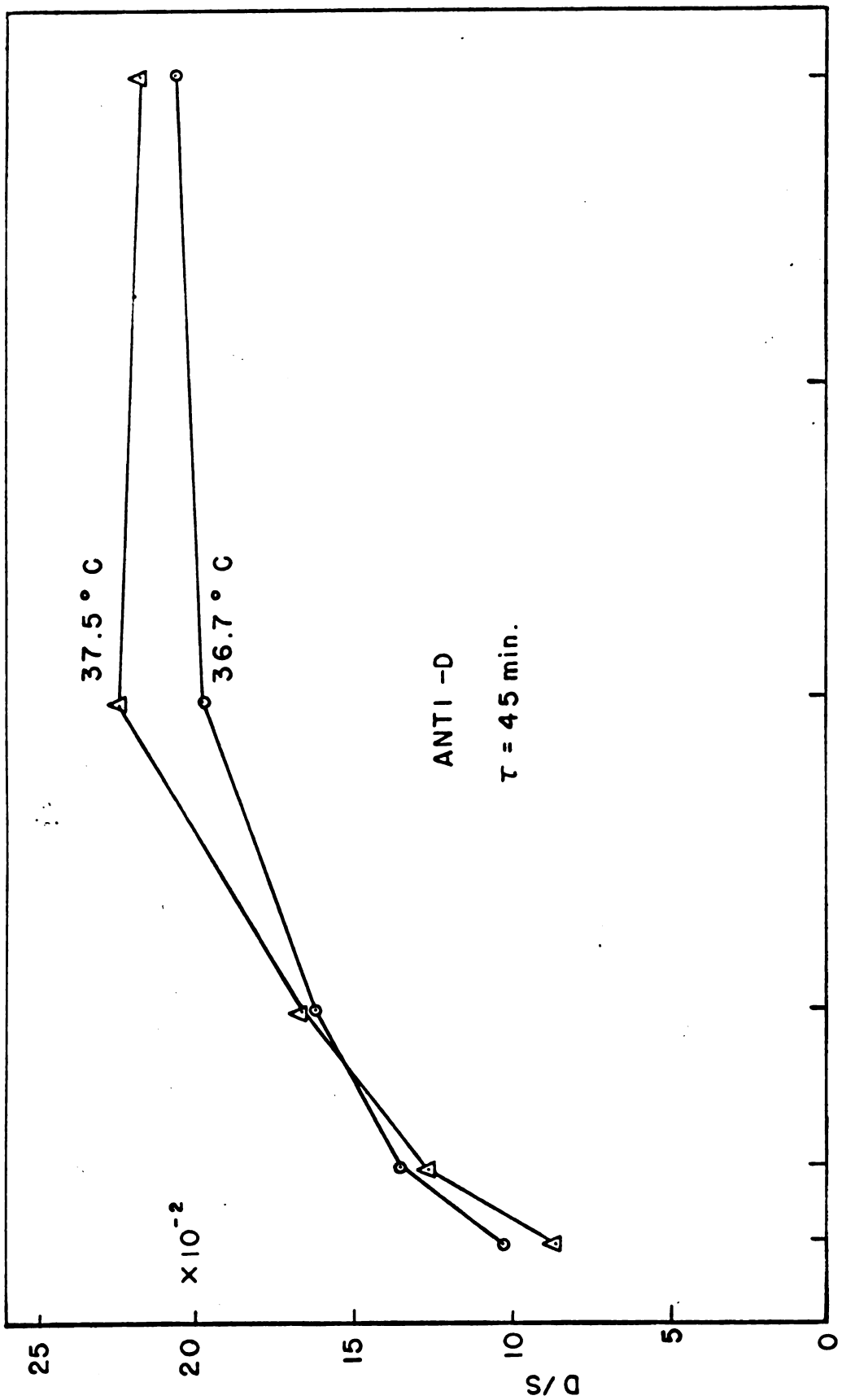


Figure 18 - Agglutination, as measured by D/S, as a function of concentration for D-positive cells incubated with anti-D for 45 minutes, at two incubation temperatures.

dependence of D/S on incubation temperature T. Figure 18 shows D/S as a function of concentration c for D-positive blood incubated with anti-D serum for 45 minutes at 37.5°C. and at 36.7°C. The course of D/S with c is not significantly different between the two curves.

Use of Q/S as a Measure of the Agglutination

The count of various windows representing singlets, doublets, triplets, and quadruplets was taken as a function of anti-D serum used to agglutinate D-positive cells incubated for 45 minutes and shown in Figure 19. It is evident that the ratio of quadruplets to singlets seems to vary more rapidly with concentration than either the ratio of triplets to singlets of the statistic D/S. We call this the ratio of quadruplets to singlets Q/S. Figure 20 shows Q/S plotted as a function of the concentration of anti-D serum for cells incubated for 45 minutes. The variation in Q/S from very dilute solutions of antiserum to full strength is about 100 as compared to a typical variation of 5 in D/S for the same range.

Figure 21 shows the variation of Q/S as a function of time for incubation of various concentrations of anti-D. It is interesting that the variation in time for this statistic is not as great with D/S. The behavior of Q/S is not as linear as D/S and, therefore, for testing of the magnetic field effects, the latter was used. However, further work on the use of Q/S is necessary.

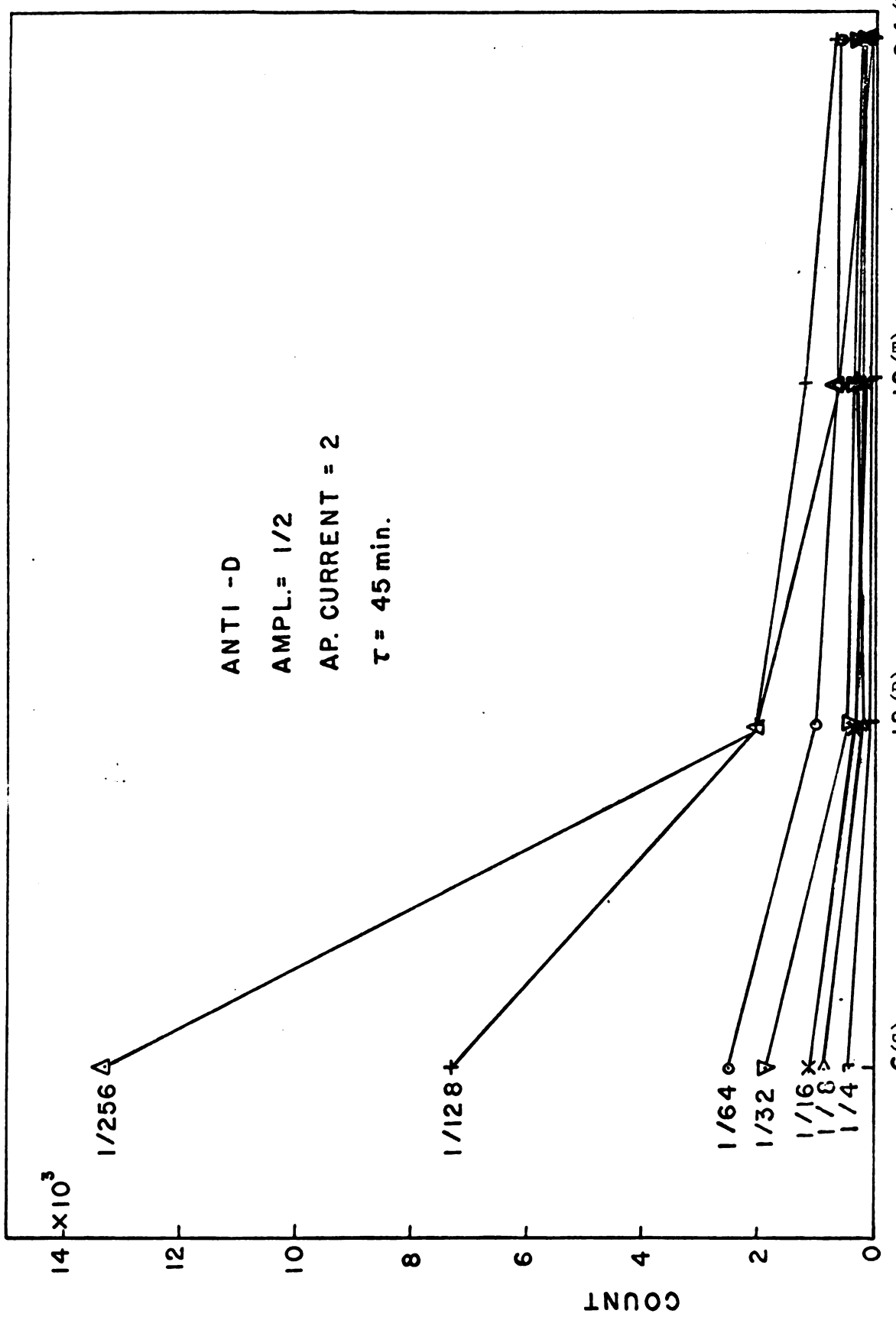


Figure 19 - Counts in various windows representing singlets, doublets, triplets, and quadruplets for various concentrations of anti-D serum.

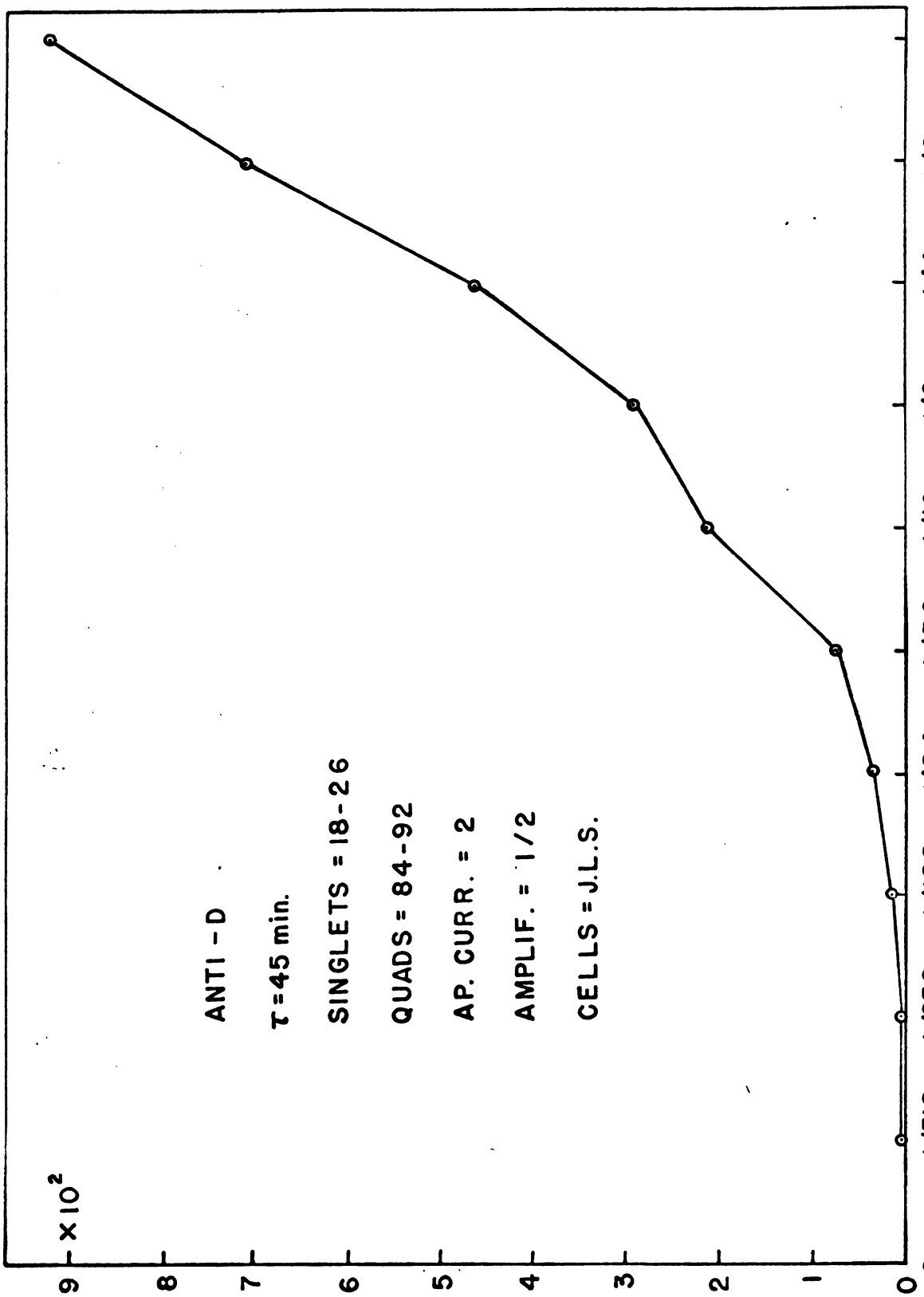


Figure 20 - Agglutination, as measured by Q/S, as a function of concentration for D-positive cells incubated with anti-D for 45 minutes at 37.5°C.

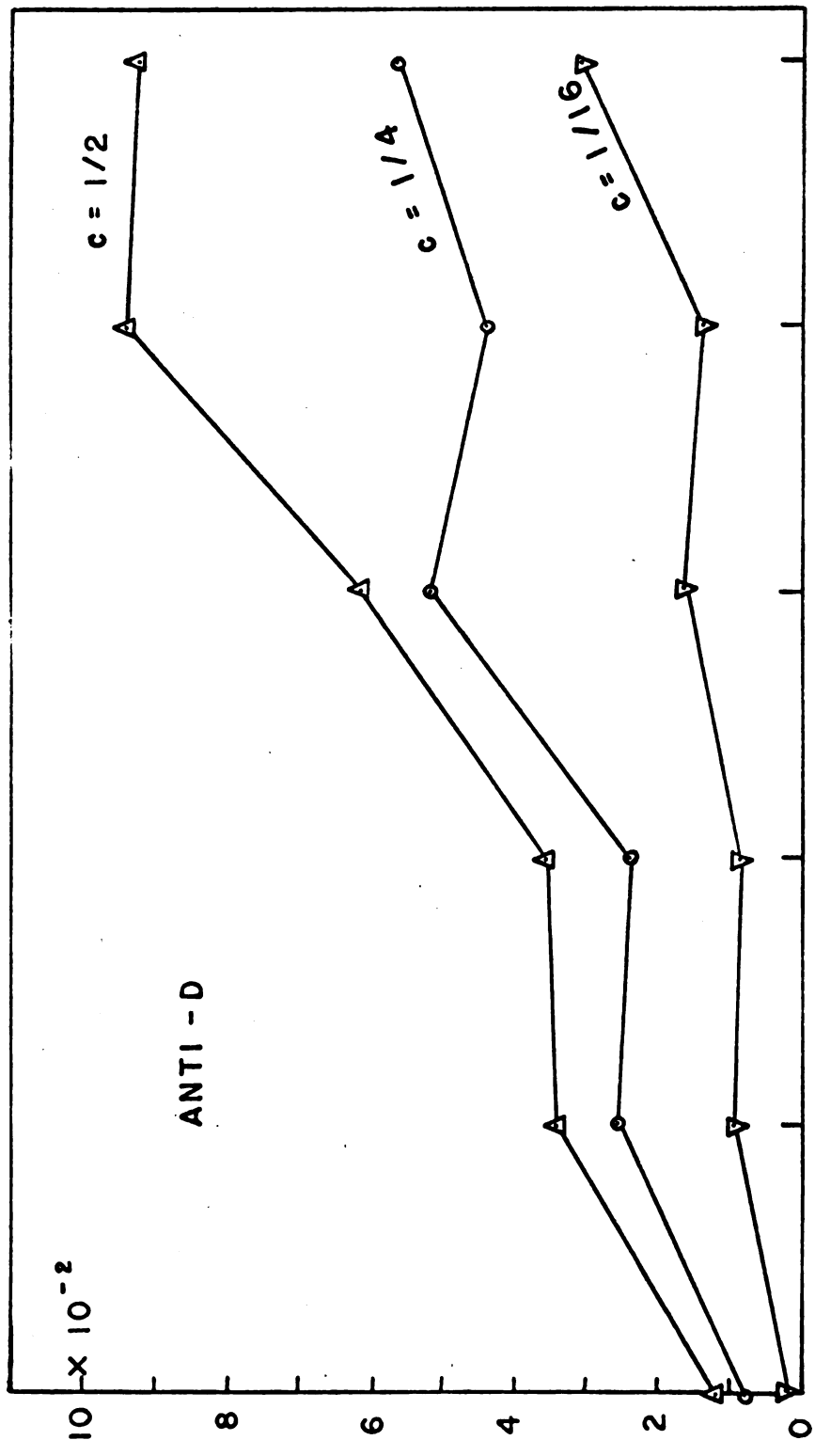


Figure 21 - Agglutination, as measured by Q/S, as a function of incubation time for various concentrations of anti-D.

Correlation between Visual and Particle Sizing Measurements of Agglutination

In two runs with anti-D serum we attempted to find a relationship between the visual scoring of agglutination and the D/S value from the distribution. The samples were prepared in the usual way and incubated for 30 minutes at 37.5°C. Figure 22 shows the visual score plotted against the D/S value. The D/S value over the range studied varies by a factor of about 4, compared to a factor of 10 for the visual score. These values would seem to indicate that the visual scoring is more sensitive. A good correlation between the visual score and the D/S could not be established.

Dependence of D/S on Magnetic Field Strength B

It is plausible that a strong and inhomogeneous magnetic field will be most likely to affect the agglutination reaction. The field strength in the 12-inch electromagnet was brought up to B = 16,000 gauss and wedge-shaped pole pieces were used to produce a gradient dB/dz = 1,500 gauss/cm. Incubation took place at 37-1/2°C. for one hour. To see if there was a change in the distribution at a given concentration, we computed the ratio of the spike height for each window in the distribution obtained for the test sample incubated in the field, to that for the control sample incubated outside the field. A plot of this ratio against window number for various concentrations is shown in Figure 23. No meaningful trend can be detected, and we conclude that the magnetic field has no effect on agglutination as measured by the Coulter counter.

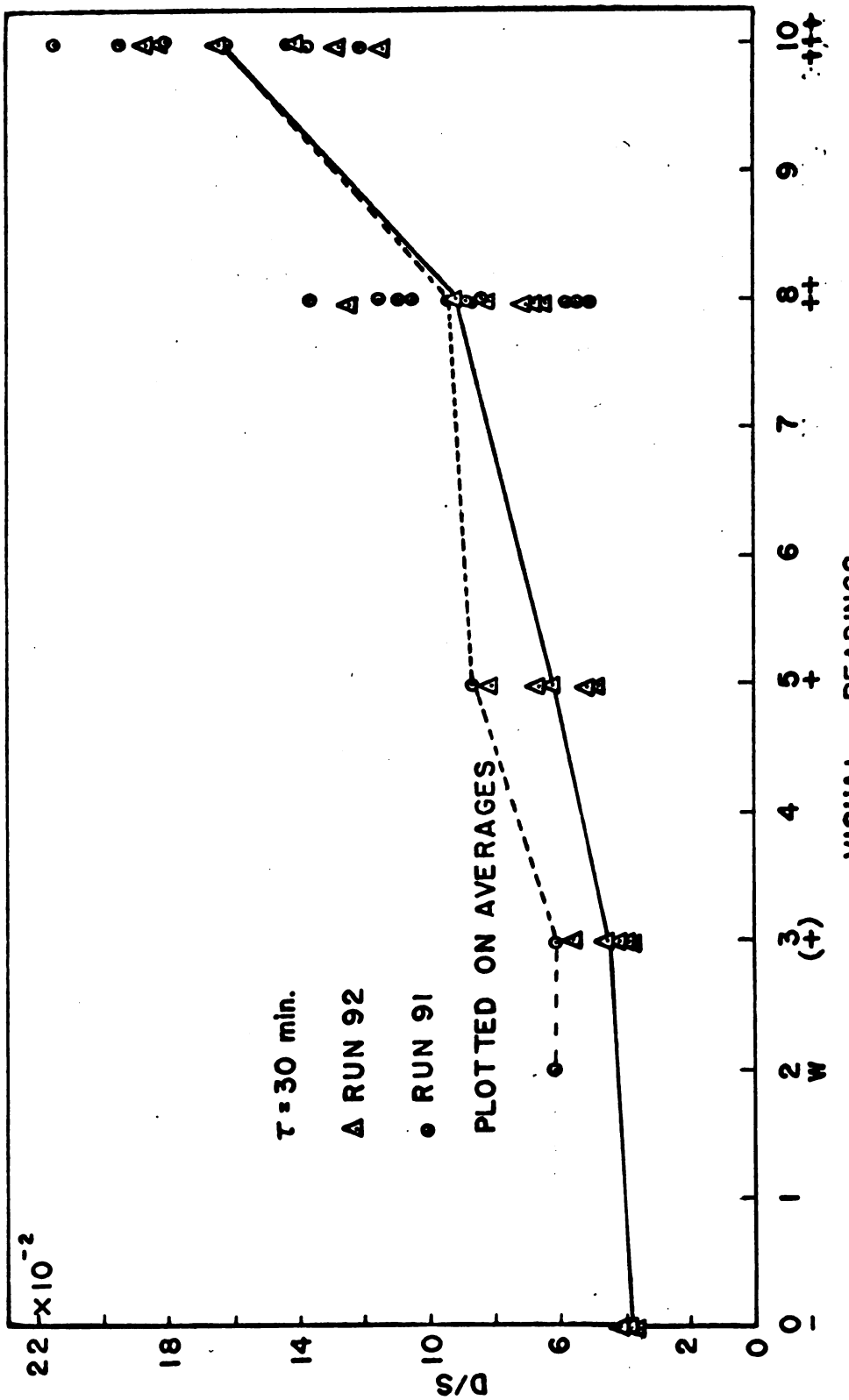


Figure 22 - Correlation between visual data (as scored on the Race scale) and the counter data (as measured by D/S), for the same samples.

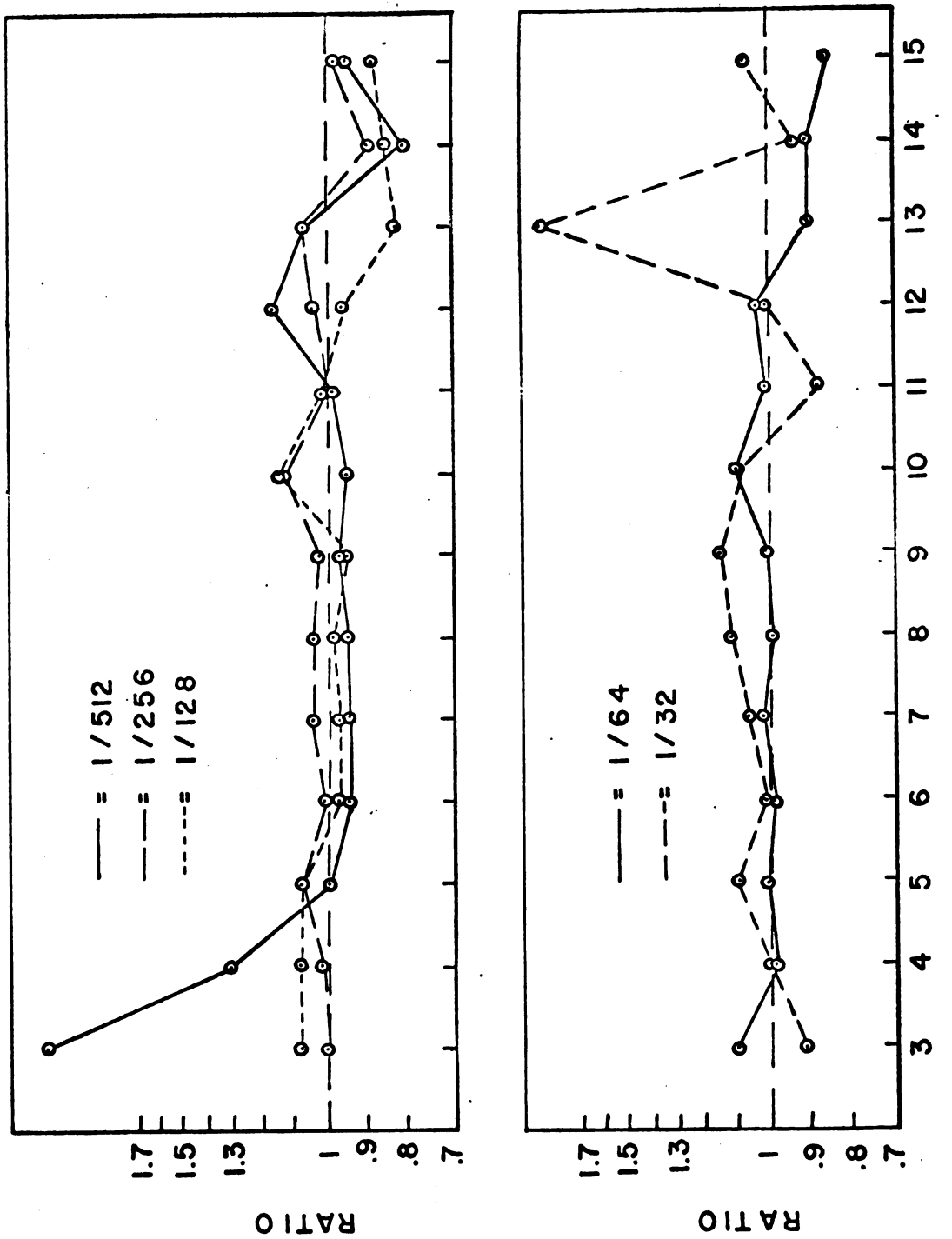

= 1/512
= 1/256
= 1/128
RATIO
1.7
1.5
1.3
1
.9
.8
.7
= 1/64
= 1/32
RATIO
1.7
1.5
1.3
1
.9
.8
.7
3
4
5
6
7
8
9
10
11
12
13
14
15
WINDOW NO.

Figure 23a

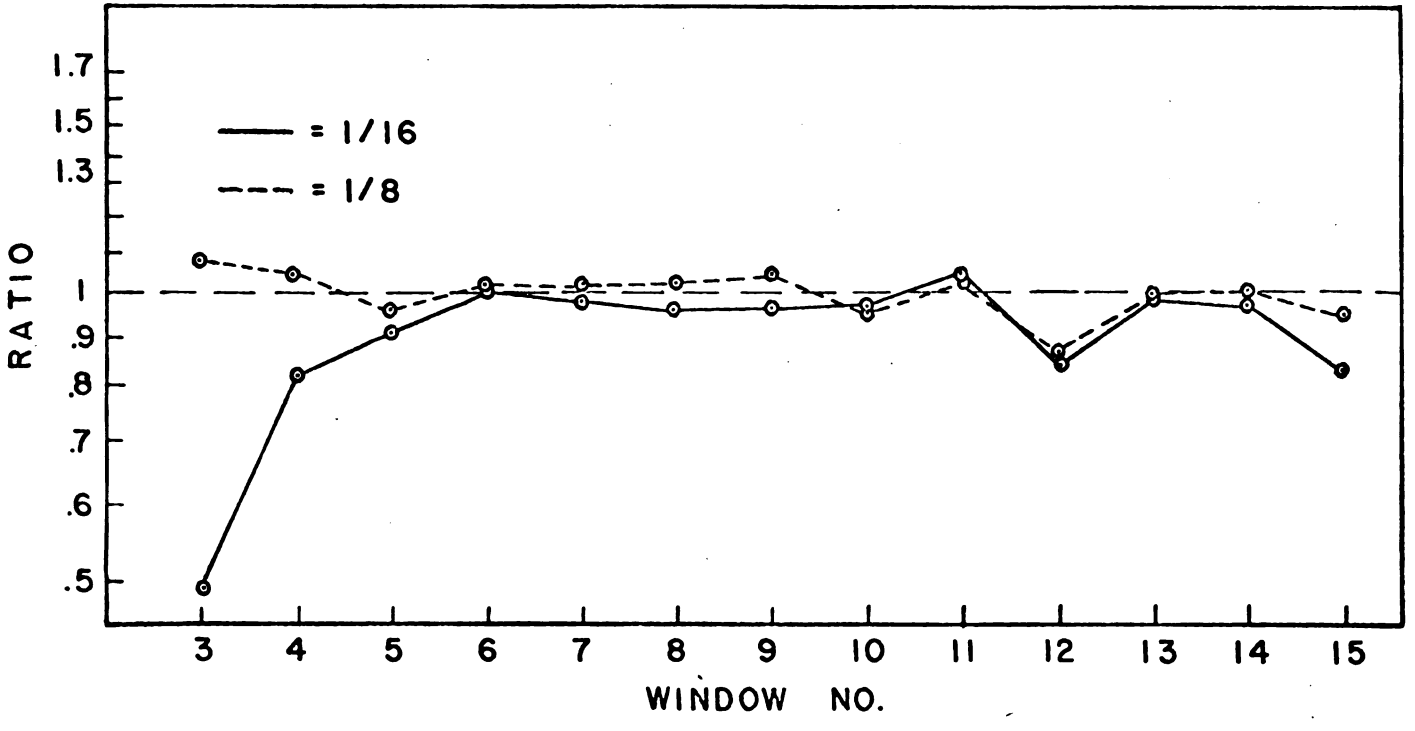


Figure 23b - Semilogarithmic plot of the ratio of spike height for test samples incubated in magnetic field to spike height for control samples incubated outside magnetic field, as a function of particle volume (window number). D-positive cells were incubated with anti-D serum of concentrations shown, for 45 minutes at 37.5°C. Each point represents the average readings for four tubes.

To search for an effect over a wider range of variables, the behavior of the statistic D/S alone was investigated. Run after run failed to disclose any difference between the test samples and the control samples. Figure 24, for a typical run, shows D/S as a function of c for D-positive cells incubated against anti-D serum for one hour at 37-1/2°C. No trend is evident.

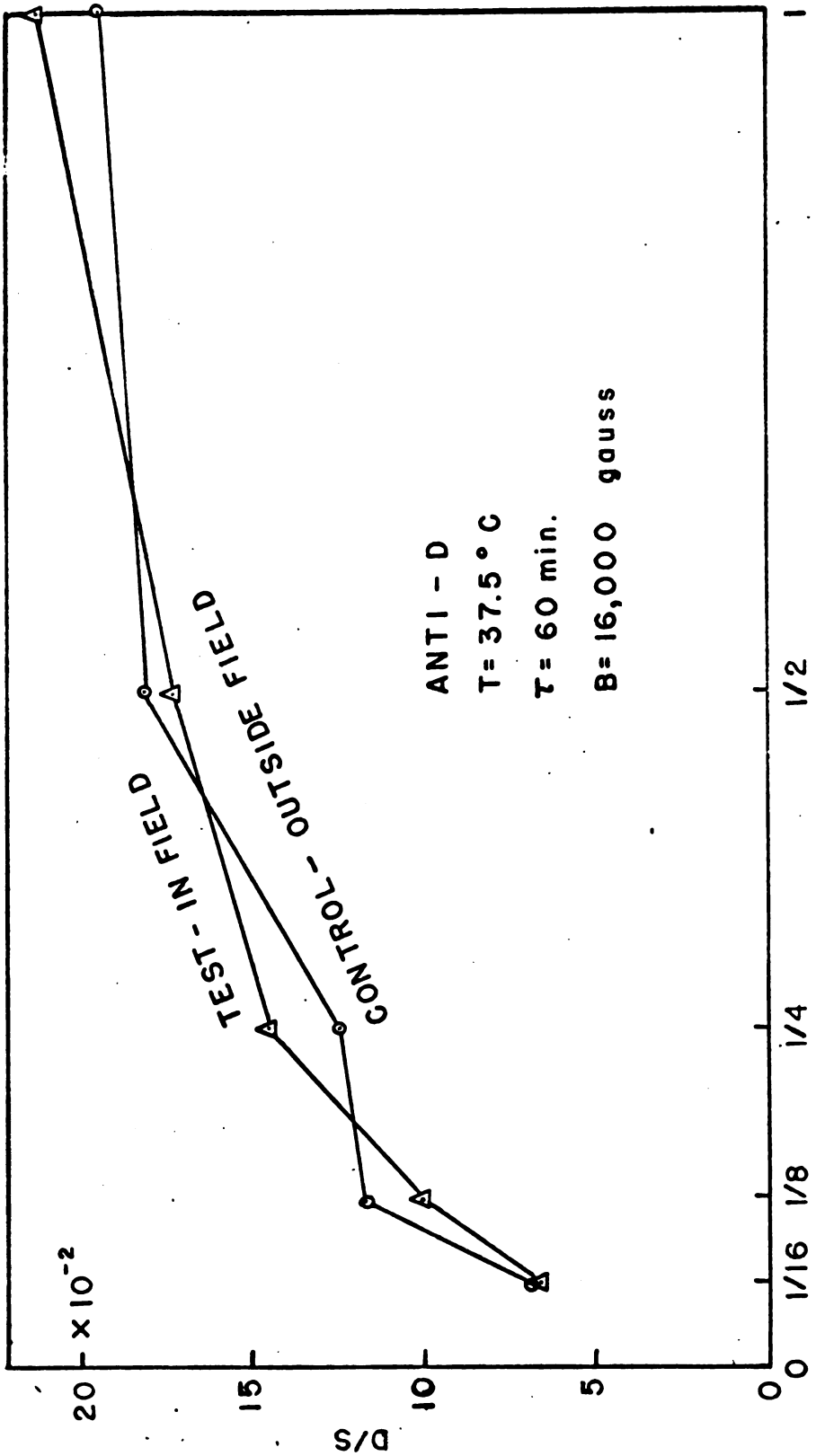


Figure 24 - Agglutination, as measured by D/S, as a function of concentration for D-positive cells incubated with anti-D for 45 minutes at 37.5°C, with magnetic field on and with magnetic field off.

V. DISCUSSION

Visual Method

The experiments in which agglutination was determined visually show definite enhancement of agglutination by magnetic fields of moderate strength. Much more work needs to be done to get quantitative expression of the enhancement, and to see how field strength and field gradient affect the agglutination at varying concentrations of antiserum, and at varying periods and temperature of incubation. Until such information is available, it will be difficult to postulate mechanisms to explain the observed effect.

Counter Method

The experiments in which agglutination was determined instrumentally, on the other hand, show no response to magnetic fields. If the counter completely failed to detect any agglutination, it would, of course, be easy to assume that in the counting procedure the aggregates are destroyed, say by being torn apart when entering the aperture. But, as has been established in the present work as well as that of others, agglutination can be detected by the Coulter counter and, in fact, there is promise that antibody titer can be determined by it.

Reconciliation of Results

Although it may be premature to declare absolutely

that the discordance is not an artifact, we advance the hypothesis that the effect in each case is real, but the differences in methods of observation result in differences in the kinds of aggregates observed. Specifically, we think that in the visual technique the spreading of the suspension on the glass slide favors production of aggregates of two or more erythrocytes lying flat side by side, the cell rims being in contact over only a very small portion. Such aggregates are easily noticable under the microscope. In contrast, aggregates consisting of pairs of erythrocytes with one lying flat above the other would not attract attention in the visual observation, as the upper cell would shield the lower one from view. In the Coulter counter method, on the other hand, aggregates made up of cells lying in the same plane and touching over only a small portion of the periphery would be easily destroyed, and counted as singlets. The aggregates made up of erythrocytes sticking firmly together on their flat sides are probably fairly sturdy and would pass through as multiplets. In sum, the aggregates discerned readily by the microscope are not detected by the Coulter counter; whereas, those detected readily by the Coulter counter are not easily discerned under the microscope. Thus, it is possible that a given method preferentially detects cells agglutinated in a given manner.

If it can be substantiated that the two methods do indeed detect different manners of agglutination, then the

discordance between the two methods in fact gives us a new tool for investigating the mechanism of agglutination. Until then, we must admit that no effect of magnetic fields of weak or moderate strength has yet proved itself discernible by electronic counting methods.

Possible Mechanism for Effect of Magnetic Field on Agglutination

There seems to be little dispute that the magnitude of the magnetic interaction energy $\mu.B$ for a single atom is so small that it will be swamped by thermal energy kT. Therefore, any effect observed at room or body temperature must be based on some sort of cooperative phenomenon (or else some subtle statistical phenomena). Here the magnetic moments are to be coupled in some way so as to have a resultant moment giving an interaction energy large compared with thermal energy. Mathematically speaking, the interaction energy $N\mu.B$ for N coupled atoms would be about N times that for a single atom, while the thermal energy kT would remain the same. Thus the value of about 10^{-3} for the ratio of $\mu B/kT$ for a single magneton at room temperature in a field of 10^4 gauss could be increased to 10 or even 100 for a swarm of 10^4 or 10^5 associated molecules.

The most common cooperative phenomena in biological material are likely those concerned with the existence of the liquid-crystalline or mesomorphic state of matter. It is well known that magnetic fields can produce orientation

of associated groups of molecules in this state (see, e.g., references 26-28). Substances pass into the mesomorphic state from the solid state by decrease of the long-range binding energies relative to thermal disordering energy, either by increase of temperature or by addition of solvents. Substances pass from the mesomorphic state to the true (isotropic) liquid state by further increase of temperature or further dilution with solvent. It is known that some biological materials exist in the mesomorphic state, in particular, certain erythrocytes (63, 64). Hence, it is not fantastic that some component of human erythrocytes might respond to moderately strong magnetic fields.

We suggest tentatively then that erythrocytes are aligned and perhaps even displaced by the action of macroscopic magnetic fields. Such motion causes some active antigen sites on the cell surface to take up positions favorable for reaction with antibody, free or bound on sites on adjacent cells. In the case of the anti-D reaction, this type of reaction produces a weak bond, readily disrupted by any sort of mechanical action, such as stirring, smearing, or rapid passage through a narrow orifice. Indeed, the anti-D reaction has the reputation of needing experience and skill to preserve the aggregates under the microscope. There will, of course, be unstimulated agglutination of the same type, as well as of the type where the flat sides of adjacent discs are stuck together firmly. Hence the Coulter counter would detect agglutination, but only that resulting in formation of sturdy aggregates.

In summary, the salient features of the investigation are the following:

1. The agglutination reaction with human erythrocytes was studied for possible influence by static magnetic fields of moderate strength. Agglutination as detected by visual scoring was enhanced by inhomogeneous steady magnetic fields. Agglutination as measured by the particle sizing methods was not influenced by magnetic fields, homogeneous or inhomogeneous.

2. Particle sizing methods appear capable of furnishing an instrumental method for quantitating agglutination, but only for gross effects.

3. Evidently, differences in methods of observation result in differences in the kind of aggregates observed. Corroboration of such differences might furnish a basis for reconciliation of the discordant results of magnetic tests.

4. Extension of the agglutination studies where enhancement by magnetic fields is found seems worthwhile undertaking for possible elucidation of agglutination mechanism.

5. Extension of the magnetic studies on other immune reactions should be undertaken to find whether the antibody molecules themselves or the erythrocyte membrane are the seat of the response to the magnetic fields.

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APPENDIX

Fractional Width of the Lognormal Distribution

The lognormal distribution is characterized by:

$$\frac{d\Lambda(x)}{dx} = \frac{1}{x\sigma\sqrt{2\pi}} e^{-\frac{(\log x)^2}{2\sigma^2}}$$

where the median is taken as 1, and σ is the standard deviation. The mode is at $e^{-\sigma^2}$ The value of x at which the ordinate is half the mode is given by the equation.

$$\left|\frac{d\Lambda(x)}{dx}\right|_{\Lambda(x)=\frac{1}{2}\text{mode}} = \frac{1}{2}\left|\frac{d\Lambda(x)}{dx}\right|_{mode}$$

$$\frac{1}{x_\pm \sigma\sqrt{2\pi}} e^{-\frac{(\log x)^2}{2\sigma^2}} = \frac{1}{2}\frac{1}{\sigma\sqrt{2\pi}e^{-\sigma^2}} e^{-\frac{(\log e^{-\sigma^2})^2}{2\sigma^2}} = \frac{e^{\sigma^2/2}}{\sigma\sqrt{2\pi}}$$

$$\text{or} \quad e^{-\frac{(\log x_\pm)^2}{2\sigma^2}} = \frac{x_\pm}{2} e^{\sigma^2/2}$$

$$(\log x_\pm)^2 + 2\sigma^2 \log\frac{x}{2} + \sigma^4 = 0$$

Let $\xi_\pm = \log x$ $\qquad \xi = -\sigma^2 \pm \sqrt{\sigma^4 - (\sigma^4 - x^2\ln 2)^2} = e^{-\sigma^2} e^{\pm\sigma\sqrt{2\ln 2}}$

Let Δx = fractional width $= x_+ - x_- = e^{-\sigma^2}\left(\frac{e^{+\sigma\sqrt{2\ln 2}} - e^{-\sigma\sqrt{2\ln 2}}}{2}\right)$

$$\Delta x = 2e^{-\sigma^2}\sinh(\sigma\sqrt{2\ln 2})$$

$$= 2e^{-\sigma^2}\sinh(1.1775\sigma)$$

$$\frac{\Delta x}{\left(\frac{d\Lambda}{dx}\right)_{mode}} = \frac{2e^{-\sigma^2}\sinh(1.1775\sigma)}{e^{-\sigma^2}} = 2\sinh(1.1775\sigma)$$

This is the equivalent of Lushbaugh's $(\frac{W}{M})$ calculated for the lognormal distribution. Various values of $(\frac{W}{M})$ for individuals in our laboratory were computed using a lognormal distribution. The results shown on the following page are in close agreement to those of Lushbaugh.

	Mode (windows)	σ	(W/M)
K.K.	9.0	.20	.48
J.S.	8.2	.26	.62
A.S.	9.2	.21	.60
F.T.	10.2	.18	.43
B.R.	9.4	.25	.59
G.G.	8.6	.25	.59