

# BIOLOGICAL EFFECTS OF MAGNETIC FIELDS

Edited by

**Madeleine F. Barnothy**

*Professor of Physics,  
University of Illinois, College of Pharmacy*



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## Preface

Study of the biological effect of magnetic fields is both a very old and a very recent area of investigation. A connection between health and the mysterious force of the lodestone has been suspected since the dawn of human culture. Nevertheless, only during the last decades has reliable evidence of biological effects of the magnetic field been discovered.

The purpose of this book is to bring together in one volume the present-day knowledge in all the active fields of biomagnetic research and at the same time to provide a theoretical and practical background to all scientists who wish to engage in investigations in this new discipline. The need for such a comprehensive survey of current information became evident to the editor from the interest manifested in the biomagnetic symposia and from the extended correspondence maintained by the Biomagnetic Research Foundation. It is hoped that the book will aid in attracting the interest of specialists and may thus serve as a catalyst for interdisciplinary exchange of ideas.

Biomagnetism is a borderline discipline, the successful investigation of which requires proficiency in biology and physics alike. In setting up experiments, there are certain practices which are obvious to those learned in one of the arts, but are not evident to those versed in the other alone. It is not uncommon that such seemingly unimportant features play a decisive role in experimental results. All contributors were asked, therefore, to stress comprehensiveness and to furnish detailed descriptions. Thus the reader will find the parts dealing with questions in his own discipline perhaps somewhat elementary. Each contributor has attempted to be critical in the evaluation of previous information in the field of his specialty, so that the book is not merely a catalog of findings, but a sound analysis of progress. Some chapters contain hypotheses regarding the mechanism by which the magnetic field may exert its influence. Even if these speculations should fail to be supported by future observations, their critical reading or refutation may lead someone to an acceptable explanation.

The book has been divided into four parts: theoretical considerations, experiments *in vivo*, experiments *in vitro*, and effects of very weak fields, followed by an up-to-date bibliography. Effects of time-variable magnetic fields are not included in this volume, because such fields induce electric currents in the biological systems and the effect of these currents in most instances overrides the biological effect of the magnetic field itself.

Looking over the content of this volume, I feel justified in expressing my hope that the magnetic field will in due time develop into a powerful new analytic and therapeutic tool of medicine.

It is a pleasure to express my thanks to the contributors of this volume for the magnificent cooperation and support they have offered, my gratitude to those scientists who graciously undertook the unrewarding task of acting as referees for the papers, and my appreciation to Mr. E. M. Coleman and Mr. J. Matzka of Plenum Press, for their helpful assistance in publishing this first book on biomagnetism.

M. F. B.

June, 1964  
Chicago, Illinois

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*Part I*

## Theoretical Considerations

## *Chapter 1*

# Introduction

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Einstein has shown in his general theory of relativity that the mysterious gravitational force acting between masses can be interpreted as a peculiar state of space, characterized in the neighborhood of masses by a deviation of its geometry from the Euclidean geometry of the flat space. Masses cause the flat Euclidean space to become curved, to bulge out. A test particle possessing a mass "senses" the varying curvature of this bulge as a real physical force which causes the particle to deviate from its "straight-line" path. We could even say that mass is nothing else than a local curvature of generally flat space, the bulge representing all the properties of the mass.

Physicists still have a long way to go before a unified gravitational-electromagnetic field theory is developed. Nevertheless, I believe that we are not very far from the truth when we surmise that a magnetic field could be similarly interpreted as a peculiar state of space, but with the difference that the deviation from the Euclidean geometry is now sensed by test particles endowed with an electric charge or magnetic moment.

## THE MAGNETIC FIELD

Faraday succeeded in presenting a vividly visualizable description of the magnetic field and its properties by introducing the concept of lines of force. In the case of a bar magnet, with the north pole on one end and the south pole on the other, the lines of force start from the north pole and, following a smaller or wider curved path, return to the magnet at the south pole. In the case of a moving charge, or a conductor in which an electric current flows, the lines of force are circles, with the charge or conductor as center.

According to Faraday, the lines of force are endowed with the property of elastic fibers, which tend to contract, but neighboring lines repel each other. This explains why iron filings in a magnetic field will cling to each other in continuous rows, but at the same time the rows remain separated in individual threads. If a current-carrying

conductor is placed in a magnetic field, the circular pattern of lines of force produced by the current will distort the pattern of lines of force due to the external field. The lines of force, in trying to straighten themselves, push the conductor in a direction perpendicular to the direction of the external field.

The strength or intensity ( $H$ ) of a magnetic field can be represented by the density of the lines of force. It was agreed that a magnetic field of unit intensity, that is, of 1 oersted (Oe) strength, should be represented by one line of force penetrating every  $1 \text{ cm}^2$  of area and, furthermore, that  $4\pi$  lines of force should emerge from a magnetic pole of unit strength. A field of 1 Oe strength exerts upon a unit pole a force of 1 dyne.

Contrary to electric charges, where positive or negative charges can exist independently, it is impossible to separate the north pole from the south pole; they always appear in pairs. Faraday has, therefore, introduced the concept of the magnetic dipole. He called the product of pole strength and distance between north and south pole the magnetic moment ( $m$ ) of the dipole. It is measured in Oe-cm<sup>3</sup>. Whereas the electric field around an electric charge decreases with the square of the distance from the charge, the magnetic field around a magnetic dipole decreases with the cube of the distance from the dipole.

It is, however, not enough to know the intensity or strength of the field; we also have to know its direction. Plotting the strength and direction of the magnetic field everywhere within a given region, we obtain a vector field which completely describes the magnetic field in this area.

The field strength, or magnitude of the field vector, may change from point to point in space. This variation can be described by a second vector, called the gradient of the field. Its magnitude is measured in oersteds per centimeter, and the vector points in the direction in which the greatest increase in the field strength occurs.

The strength of the magnetic field is measured with gaussmeters operating on different principles. For biomagnetic research, instruments with an absolute accuracy of 5% and a relative accuracy of 1% will usually suffice. Gaussmeters operating on the Hall-effect principle have the advantage that the probe is very thin and can be used in a narrow gap; furthermore, the sensitive portion of the probe has a very small area, hence allowing the field strength to be mapped over the volume of a small sample and its gradient determined.

Biomagnetic experiments require magnetic fields of various strengths, homogeneity, and extension. Basically, it does not make any difference how the required field is produced, whether with permanent bar or horseshoe-shaped magnets, electric coils or solenoids with air or iron core, or superconductive coils. But, under given conditions, one particular method might be more favorable.

A magnetic field is always a corollary to an electric current.\* The simplest way to produce a field is to take a circular conductor of radius  $R$  (cm) with  $N$  turns and let a current  $i$  (amperes) flow through it. The field strength  $H$  (oersteds) in the center of the loop will be  $H = 2\pi Ni/10R$ . In a coil, the length of which is long compared to its diameter—a solenoid—the field is  $4\pi ni/10$ , where  $n$  is the number of turns per centimeter of length of the solenoid. Inside the solenoid the lines of force proceed parallel to the axis of the solenoid and the field is quite homogeneous, that is, the gradient is very small. Outside the solenoid, the lines of force spread out and converge again at the other end of the solenoid. In a distance large compared to the length of the solenoid, the field is the same as that produced by a bar magnet of magnetic moment  $m = NiR^2\pi/10$ . This similarity in behavior is a consequence of the circumstance that a permanent bar magnet produces the external field with the help of a large number of aligned ring currents. The lines of force continue inside the bar magnet and inside the molecular ring currents, just as they continue inside the loops of the solenoid.

As we can see, the concept of lines of force and of magnetic poles is extremely useful for describing the properties of the magnetic field, but neither of them actually exists. It would be a great mistake to imagine the lines of force as some kind of radiation which emanates from the north pole and is absorbed by the south pole.

There is a great formal similarity between electric circuits and magnetic circuits:

a. The electric current in an electric circuit is proportional to the total electromotive force acting in the circuit and inversely proportional to the resistance of the circuit; the flux, or total number of lines of force in a magnetic circuit, is proportional to the total magnetomotive force and inversely proportional to the reluctance of the circuit.

b. The resistance of an electric circuit is proportional to the length of the conductor and inversely proportional to the cross section and electrical conductivity of the material; the reluctance of a magnetic circuit is proportional to the length ( $L$ ) of the lines of force and inversely proportional to the cross section ( $A$ ) and the magnetic conductivity, called permeability ( $\mu$ ), of the material through which the lines of force proceed.

c. If the magnetic circuit contains sections of different materials, (e.g., iron and air) the reluctances of the sections have to be added (in the same way as electric resistances connected in series have to be added) to obtain the total reluctance of the magnetic circuit:

$$\text{Flux} = M/(L_1/\mu_1 A_1 + L_2/\mu_2 A_2 + \dots) \quad (1)$$

\*The author has shown that this is true even for elementary particles.<sup>1</sup>

where  $M$  is the total magnetomotive force, measured in gilberts. (A ring current of 1 A produces a magnetomotive force of  $4\pi/10$  gilberts.)

Vacuum has a permeability  $\mu = 1$ . Diamagnetic substances have permeabilities slightly smaller, paramagnetic substances slightly greater, than one. Ferromagnetic substances, such as iron, cobalt, nickel, and some alloys, are exceptional in the sense that their permeability can reach values up to 25,000.

Ferromagnetic materials contain microscopic domains inside which the molecular magnetic dipoles are naturally aligned. However, in the virgin state of a ferromagnetic material the magnetic dipoles of these microscopic domains are not aligned; a soft-iron bar will not show any magnetism. By applying an external field, one can more or less align these microscopic domains and hence magnetize the material. The measure of the magnetization of a material is the magnetic moment produced per unit volume, called the intensity of magnetization ( $I$ ). The magnetomotive force produced by the magnetic moments of the aligned domains is superimposed on the magnetomotive force producing the external field. Inside the material the total field strength, called magnetic induction ( $B$ ), will be

$$B = \mu H = H + 4\pi I \quad (2)$$

$B$ ,  $H$ , and  $I$  have the same dimensions. But the unit of magnetic induction is the gauss and the unit of magnetic field strength the oersted. In vacuum (and for all practical purposes in air), where  $\mu = 1$ , the magnetic induction measured in gauss is numerically equal to the field strength measured in oersteds. This is the reason why in the literature we often find the field strength given (somewhat incorrectly) in gauss, instead of in oersteds. For historical reasons, the strength of the geomagnetic field is always given in gauss.

At high field strength, most of the microscopic domains become aligned and the intensity of magnetization asymptotically approaches a limiting value. Any further increase in the induction is due solely to an increase in the external field strength: the ferromagnetic material becomes saturated, and permeability decreases asymptotically to the value 1. Some iron alloys retain a permeability  $\mu > 10$  up to  $B = 30,000$  gauss.

In air-core electromagnets, the reluctance of the magnetic circuit is large, because  $\mu = 1$ . If the current intensity  $i$  is increased, the magnetomotive force will increase with the first power, the energy loss in the form of heat generated in the coils with the second power, of  $i$ . The property of high permeability of iron alloys can help us to decrease the reluctance of the magnetic circuit and to reach the same flux values as with air-core electromagnets at a lower magnetomotive force and consequently at lower operating cost. In iron-core electromagnets, with the exception of an air gap to accommodate the specimen under investi-

gation, the magnetic circuit is made of iron; the lines of force proceed through the iron core of the coils and a yoke, made of high-permeability magnet iron.

Certain iron-cobalt alloys (Hyperco) which saturate only at very high field strength can be used in the form of conical pole caps to concentrate the total flux of the magnetic circuit to a small cross section; in this way flux densities up to 60,000 Oe can be obtained in the gap containing the specimen.

Another way to overcome high electric power requirements is to use superconductors. The electric resistance of most metals, but particularly that of certain niobium alloys, decreases at very low temperatures, in the vicinity of the absolute zero point, to a negligible fraction. A current started in such a superconducting coil will keep flowing for weeks without further energy input. The maximum field strength which can be produced is, however, limited since superconductivity ceases in strong magnetic fields. The Nb-Zr alloy used in present superconducting magnet coils loses its superconductivity at about 80 kOe. The coil is cooled with liquid helium, supplied from a refrigerator which uses a minimum electric power of 5 kW. The biological sample must be kept in a second dewar flask to insulate it from the liquid-helium temperature of the coil. Superconducting magnets, like air-core electromagnets, cannot produce fields with high gradients.

In some ferromagnetic materials not all of the microscopic domains will return to their original random orientation when the magnetizing field is reduced to zero. Some of the magnetic moments of the domains will remain aligned and the magnetomotive force produced by these will create an observable permanent external field. The amount of induction remaining (after previous magnetization up to saturation) is called "retentivity." To reduce this remaining induction to zero, a reversed external field has to be applied. The field strength required to achieve complete demagnetization is called the coercive force of the material. For soft-iron, retentivity and coercive force are negligible. In materials used for permanent magnets, both have high values.

In large ram-horn type magnets the permanent magnet supplies the magnetomotive force, but otherwise the assembly has, like the electromagnet, a yoke to close the magnetic circuit and pole caps to concentrate the lines of force to the desired area. The product of field strength and working volume in the air gap is proportional to the weight of the permanent-magnet portion of the magnet assembly. Permanent-magnet material is commercially available in a great variety of shapes.

To summarize and evaluate the various means of producing magnetic fields for biomagnetic experiments, one could say that solenoids or air-core electromagnets are economical if homogeneous fields below 800 Oe and extending over a large area are required. But solenoids also at present represent our only method of producing fields in the range of 100 to 250 kOe. Here their power consumption is in the

megawatt range and the problems of the mechanical strength and cooling of the coils are so severe that they can only be met by special high-magnetic-field laboratories. Fields of 200 to 500 kOe can be produced with small solenoids in pulsed operation for the duration of a few milliseconds. Even if the short duration of the field is disregarded, the currents induced in the biological sample during the steep increase and decrease of the field strength render such magnets useless for biomagnetic research.

Iron-core electromagnets are to be recommended when large specimens (mice, rats) have to be exposed to homogeneous fields of 10 to 30 kOe, or small samples (tissues) to fields up to 60 kOe, or when an inhomogeneous field with a gradient of several thousand oersteds per centimeter is needed. Iron-core electromagnets have, like solenoids, the advantage that their field can be continuously adjusted to the required value, reversed without changing the position of the specimen, and reduced to zero to facilitate the exchange of pole caps. The disadvantage of large electromagnets is their high current consumption and the necessity of auxiliary equipment such as power supplies and cooling devices, which can be sources of trouble in long-term exposure of specimens. If the magnet is cooled by tap water, the temperature of the pole caps may change by 20°C during 1 day. Methods by which this temperature variation can be reduced are (in order of increasing efficiency): a water-pressure regulator, a large water reservoir with circulating pump, and an electronic pole-cap temperature regulator.

Superconducting magnets are more economical in the range of 30 to 60 kOe than iron-core electromagnets when highly homogeneous fields are required in a large volume.

Permanent magnets have the advantages of zero operating cost and no need for any auxiliary equipment or thermal regulation of the pole caps. They can be recommended for long-term exposure of specimens in medium-large containers (below 20 cu. in.) in homogeneous fields up to 8 kOe, or in inhomogeneous fields with gradients up to 1000 Oe/cm, and, furthermore, when small samples have to be exposed to homogeneous fields up to 20 kOe, or very small samples (hanging-drop cell cultures) to gradients up to 50,000 Oe/cm. Their disadvantages are that the field strength can be changed only by changing the opening of the gap and the field direction cannot be reversed nor reduced to zero. The latter constitutes a major problem when exchanging pole caps in large permanent magnets.

A field of very low gradient (but also of low strength) can be found at large distances from a bar magnet or a solenoid. Helmholtz has shown a way to produce medium-strong fields with very low gradients. Two coils are placed coaxially at a distance apart equal to their radius. The field between the coils at the midpoint of the axis is very homogeneous; around this point, the field will change by less than  $10^{-4}$  up to a distance equal to  $\frac{1}{10}$  the radius of the coils. This method can be also used with superconducting coils to produce very homogeneous fields.

A strong homogeneous field (up to 20 kOe) can be obtained between the parallel flat pole caps of a permanent magnet or electromagnet, if the gap is small compared to the face diameter of the pole caps. Homogeneity can be further improved through correcting shims or correcting coils. However, between flat pole caps, in a gap equal to the diameter of the caps the field measured in the median plane between the poles will decrease by 20% from the center toward the edges.

Highly inhomogeneous fields with large values of  $HdH/dx$  can be obtained with conical or wedge-shaped pole caps opposite a flat or concave pole cap. (Examples of such arrangements can be found in Part II, Ch. 8 and Part III, Ch. 3.) It should be noted, however, that on the point or edge the field strength can reach values at which even pole caps made from special alloys saturate. This saturation has the same effect as if the point or edge had been cut off.

#### CHARACTERIZATION OF THE FIELD

The observation of a new phenomenon cannot be considered a discovery unless the observer describes the circumstances completely enough to enable others to repeat his experiment and reproduce his results. In biomagnetic experiments, some of the most important "circumstances" are the strength, the gradient, and the direction of the field to which the biological sample is exposed.

To characterize the field with the value of the field strength alone, as is often done, would be sufficient in the exceptional case when the field strength is constant in value and direction throughout the entire volume of the sample. Experiments with transplanted tumor, with bacteria, and with leukocyte and erythrocyte counts made in the laboratory of the Biomagnetic Research Foundation indicate that inhomogeneous fields have effects different from, sometimes even opposite to, the biomagnetic effects of homogeneous fields. It would, therefore, be advisable to distinguish clearly between what should be meant by homogeneous field and what by inhomogeneous field from the point of view of biomagnetism.

The inhomogeneity of a magnetic field is usually characterized either by the gradient or by the relative variation of the field strength over the volume in which the specimen is confined. The physical difference between an inhomogeneous and a homogeneous field is that an inhomogeneous field exerts an accelerating force upon particles which are more para- or more diamagnetic than their surroundings, whereas a homogeneous field does not exert such a force. We have to assume that the difference in the observed biomagnetic effects has its source in this physical difference of the two kinds of field.

The force ( $P$ ) exerted upon a para- or diamagnetic particle is proportional to its volume ( $V$ ), the intensity of magnetization ( $I$ ), and the gradient of the field ( $dH/dx$ ). But, since the ratio of intensity of mag-

netization to field strength is called the susceptibility ( $K$ ) of the material, we may write

$$P = VKH dH/dx \quad (3)$$

As we can see, neither the gradient alone, nor the relative change of the field strength over the volume of the specimen, is a proper characteristic to describe the force, that is, the difference in the biomagnetic effectiveness of homogeneous and inhomogeneous fields.

In the past I have advocated that the product of field strength and gradient of a magnetic field should be called the paramagnetic strength of the field; its unit should be the par, defined as the paramagnetic strength of a field which exerts a force of 1 dyne on 1 ml of a substance of susceptibility  $10^{-6}$ . That is, 1 par is equivalent to  $10^6$  Oe<sup>2</sup>/cm. I suggested, furthermore, that fields used in biomagnetic experiments always be described by giving two data: the field strength, as a measure of its effectiveness related to phenomena occurring in homogeneous fields, and its paramagnetic strength, as a measure of its efficiency related to phenomena occurring solely in inhomogeneous fields.

As logical as the above conclusions appear to be, the experiments which reveal a definite connection between effect and inhomogeneity of the field, such as inhibition of bacterial growth during the logarithmic phase<sup>9</sup> and the lethal effect on Drosophila,<sup>10</sup> do not prove that the effect depends on the paramagnetic strength of the field. The Drosophila experiments seem to indicate that the value of the field strength is entirely irrelevant.

If, on the other hand, only the magnitude of the gradient counts, then we have to assume that the magnetic field acts on an entity endowed with a permanent magnetic moment. This is difficult to understand, because such entities (atoms, protons, etc.) are not encountered in a "free state" in living matter. If, however, they form a part of a more complex system, such as a cell or a macromolecule, then space quantization and statistical laws lead us back to paramagnetism, where the magnetic moment of the entity is an induced magnetic moment proportional to the external field, and hence force and displacement are proportional to the paramagnetic strength.

Future experiments in fields of greatly different gradients but of constant paramagnetic strength will help us to elucidate the mechanism by which inhomogeneous fields produce biological effects.

Reviewing the list of well-established biomagnetic effects, we note that none of these effects shows up immediately upon exposure of the specimen to the field, but only after the lapse of a certain length of time. The reason for this feature of biomagnetic effects may be that the cumulative physical effects of the field must surpass a certain threshold before they are capable of triggering biological processes. Since in a magnetic field of greater intensity this threshold will be reached earlier, it is tempting to introduce a "magnetic dose" unit (say, Oersted-hour) in analogy with the roentgen, an X-ray dose unit.

Of course, the total biological effect observed after an exposure to a magnetic field is a complex process. Besides a physical reaction time—which we may define as the time necessary for the cumulative physical effect to reach the biological threshold—a biological reaction time, during which the biological process already initiated is amplified in the system to produce an observable change in some biological functions, certainly plays a role. The biological reaction time can be shorter, but also can be several orders of magnitude longer, than the physical reaction time.

It is conceivable that, as is the case with irradiation with X- or gamma rays, biomagnetic effects will depend on, and change their aspect with, the rate at which the "magnetic dose" is delivered. Since the magnetic dose rate equals the field strength, investigations of the same phenomenon in fields of different strength should give new insight.

### THE GEOMAGNETIC FIELD

William Gilbert of Colchester, the physician of Queen Elizabeth I, was the first to realize that the earth is a large magnet, with the magnetic axis oriented almost parallel to the earth's axis, its magnetic north pole pointing toward the geographic south pole. In the three and a half centuries elapsed since the publication of his work "De Magnete," many theories were proposed to explain the origin of geomagnetism, but none of these theories are entirely satisfactory. Astronomical observations support the suggestion of Schuster (1891) and Wilson (1923) that most celestial bodies have magnetic moments proportional to their angular momenta. The author proposed a solution for this phenomenon which would, however, necessitate a strong deviation from our currently accepted nuclear physical and cosmological ideologies.<sup>1</sup>

But whatever the explanation of the origin of the geomagnetic field may be, we have no reason to assume that, during the millions of years life was evolving on our planet, the earth's magnetic field may have been nonexistent or considerably different from the present field. It is, therefore, not very farfetched to assume that living creatures became not only accustomed to the geomagnetic field as a part of their natural habitat, but that in some of them biological processes have evolved which could be influenced by fields of the order of the geomagnetic field (that is by few tenths of an oersted). This may be true in spite of our finding that fields of several thousand oersted strength are needed to demonstrate the existence of a biological effect of magnetic fields.

Experiments aimed to clarify the biological effect of the geomagnetic field are faced with a severe handicap; namely, that the geomagnetic field cannot be switched off at will like the field of an electromagnet, nor can the specimen be removed from the field.

One way to overcome this difficulty is to increase the intensity of the geomagnetic field by a factor of two to three, or to change or reverse its direction with the help of the field of small bar magnets. Such ex-

periments are described in Part IV, Ch. 1. Another way is to compensate the geomagnetic field with bar magnets (astatization). However, this would not compensate for the variations of the geomagnetic elements and would leave a field of about  $\pm 100$  gammas ( $1 \text{ gamma} = 10^{-5} \text{ Oe}$ ).

A much better way is to shield the control specimens from the geomagnetic field by placing them in closed boxes made of sheets of an alloy which has a very high permeability at low field strength (Superalloy, Mu-metal). It is claimed that three Mu-metal boxes nested in each other, but separated with nonmagnetic spacers, can reduce the field to 1 gamma.

To obtain larger "zero-field" working areas, each of the three components of the geomagnetic field has to be compensated by fields produced in Helmholtz coil-pairs. The currents in the vertical, north-south, and east-west coil-pairs have to be modulated, with the help of servo systems activated by suitable variometers, to follow the earth's magnetic field changes. A small laboratory of this kind, constructed by the Kettering Foundation in Oakland, Michigan, can maintain a field of zero intensity with a deviation of only  $\pm \frac{1}{10}$  gamma.

Since astronauts would encounter very low fields on the moon, the interest in "zero-field" laboratories of larger size, suitable for housing human guinea pigs, has increased. The U.S. Naval Ordnance Laboratory, Maryland, has a room with a working area of about  $10 \times 10 \times 10$  ft in which a zero field with 50-gamma deviation can be maintained. Since the effects one may expect from a difference of only a few tenths of an oersted must be very small, and we do not know where to look for them, only experiments continued over years and with controls living in identical quarters have any hope of success. It is not astonishing that no physiological or psychological changes were observed in the two Navy volunteers who lived for 14 days in this zero-field laboratory.

Neither the strength nor the direction of the geomagnetic field is constant. The magnetic elements vary daily, semidaily, lunarily, annually, etc., and show secular and transient variations and dependence on sunspot activity. The periodic variations in intensity are very small—a few gammas. Exceptions are the secular variations and magnetic storms connected with sunspot activities, when deviations from the normal field strength amounting to several hundred gammas may occur. But since so many other environmental factors such as heating, illumination, air contamination with gases in the laboratory; temperature, humidity, ion and pollen content of the atmosphere; composition and strength of solar radiation, earth-potential, and cosmic radiation have similar variations and periodicities, it would be completely erroneous to hold the geomagnetic field responsible for biological effects solely on the basis that the effect shows a good correlation with the variation of some geomagnetic elements. Such correlations do not have the "cause-and-effect" relationship. It should also not be forgotten that, when computing the correlation between two or more parameters which

are all time-dependent—as are most environmental factors—before the true correlation coefficient between the parameters can be determined, the time parameter has to be eliminated.<sup>2</sup>

### PHYSICAL PHENOMENA IN MAGNETIC FIELDS

We shall here merely attempt a phenomenological description of the various physical phenomena which occur in a living organism or biological material exposed to a static magnetic field. A quantitative discussion of some of these phenomena can be found in the following chapters. The physical phenomena can be classified in four categories:

- a. Generation of electromotive force in moving conductors.
- b. Force exerted upon moving charge carriers.
- c. Torque exerted on permanent magnetic dipoles and nonspherical para- or diamagnetic particles.
- d. Force exerted on permanent magnetic dipoles or para- and diamagnetic particles.

All these physical phenomena are of vector character. The direction of the phenomenon vector relative to the coordinate system of the specimen depends on the direction of the field vector or on the direction of the gradient vector relative to the specimen. Whenever the enumerated phenomena persist for a certain time, they will lead to cumulative physical effects, such as increase or decrease in temperature, displacement of the constituents of a material, etc.

Some of the cumulative physical effects themselves have vector character, that is, the direction or sign of the effect depends on the direction of the phenomenon vector. In such instances we have a reversible physical effect, such as the heat produced in the Ettingshausen effect: if the field direction is reversed, the sign of the temperature gradient will be reversed. But the cumulative physical effect may have scalar nature, such as Joule heat produced through induced currents: the heat produced is independent of the direction of the induced current. This cumulative physical effect is irreversible.

Phenomenon (a) occurs whenever the specimen moves relative to the magnet producing the field, while phenomena (b), (c), and (d) may occur when the specimen is at rest relative to the magnet. Phenomena (a), (b), and (c) will occur in homogeneous as well as in inhomogeneous fields; their magnitude and direction will depend on the magnitude and direction of the field vector alone. Phenomenon (d) can occur only in inhomogeneous fields and its direction depends on the direction of the gradient vector, whereas its magnitude depends on the product of field strength and gradient for para- and diamagnetic particles and on the gradient alone for permanent magnetic dipoles. Phenomena (a) and (b)

can be called nonparamagnetic and phenomena (c) and (d) paramagnetic phenomena, since the latter two occur only when para- or diamagnetic substances or magnetic dipoles are present.

The presence of an unpaired electron in the outer molecular orbital, as in the case for free radicals, endows the molecule not only with an extremely high chemical reactivity, but also with a magnetic moment due to the uncompensated spin motion of the odd electron. This latter property should render chemical reactions in which free radicals play a role vulnerable to magnetic fields.

Phenomenon (a) (generation of an electromotive force in a moving specimen) will lead in homogeneous fields to a rearrangement of the electric charges and therefore to polarization currents. In inhomogeneous fields, where different parts of the specimen are in fields of different strength, it will produce conduction currents. Polarization currents and conduction currents are in general distributed over all the specimen and depend on the conductivity, capacity, speed, and acceleration of the specimen (see also Part I, Ch. 2 and Part IV, Ch. 3). These currents can generate heat and cause electrolytic dissociations, but can also affect the nervous system. The heat effect is always irreversible, the electrolytic dissociation as well as the activation of the nervous system can be reversible or irreversible.

Phenomenon (b) (force exerted upon a moving charge carrier) can change the motion of ions in an electrolyte, leading to an aggregation of chemical substances at variance with their normal distribution. When a current is flowing through an electrolyte, the deflection of the ions by the field is, through friction, transmitted to the liquid and entails a pumping effect (used in atomic reactors). It can change the path of charge carriers in solids, conductors, and semiconductors and initiate the 12 effects called the Hall-effect group.

An electric current can be considered as a stream of electrons. When a magnetic field is applied at right angles to the direction of the current, the electrons traveling in the conductor will experience a deflection. As a consequence, an electric field, or potential difference, is set up in the direction transverse to both the magnetic field and electric current (Hall effect). Faster electrons follow a different path through the conductors than slower electrons when a magnetic field is applied. This means that, at the side toward which the electrons are deflected, the percentage of slow electrons will increase, resulting in a temperature decrease on that side; hence a transverse temperature gradient will be set up (Ettingshausen effect). If instead of an electric current a temperature gradient is applied along the conductor, the electrons will tend to diffuse from the hot to the cold end. The diffusing electrons are deflected by the magnetic field and cause a transverse electric potential difference as in the Hall effect (Nernst effect). For the same reason as explained in the case of the Ettingshausen effect (accumulation of slower electrons on the deflection side), a transverse temperature gradient will also be produced (Righi-Leduc effect). In

semiconductors, not only electrons, that is, negative charge carriers, but also positive charge carriers contribute to the electric conductivity. The sign of the Hall effect and Righi-Leduc effect depends on the sign of the charge carrier, while the sign of the Ettingshausen effect and the Nernst effect is independent of it. All these effects are inversely proportional to the concentration of the charge carriers. The cumulative physical effects caused by the enumerated phenomena are vector effects and reversible.

In a magnetic field the motion of electrons in an atom is to a first approximation the same as in the absence of the field, except for the superposition of a common precession of the orbital axes around the field vector. This precession produces a magnetic field which is opposite in direction to that of the external field, and will decrease the magnetic induction inside the material. It is the explanation for diamagnetism and a general property of all materials.

Phenomenon (c) (torque exerted upon elementary magnetic dipoles and nonspherical para- or diamagnetic particles) can lead to the orientation of nonspherical para- or diamagnetic particles (such as blood cells) and lead to a change in their biological reactions. It can also alter the orientation of molecules having a magnetic moment and thereby alter the probability of bond formation (see Part I, Ch. 6).

According to wave mechanics, an orbital angular momentum vector cannot assume an arbitrary direction in an external magnetic field; rather, it is restricted to those particular orientations for which the component of the momentum vector along the direction of the magnetic field is an integral multiple of  $h/2\pi$ : the orientation of the angular momentum is quantized. Consequently, the possible orientations of the magnetic dipole moment resulting from the orbital motion of the electron are similarly quantized and so too is the magnetic potential energy of the energy levels. The change in the energy of a state with quantum numbers  $l$  and  $m_l$  is, in a magnetic field of flux density  $B$ ,

$$\Delta E_m = m_l (eh/4\pi m)B \quad (3)$$

where  $e$  and  $m$  are the charge and mass of the electron and  $h$  is Planck's constant. This energy difference causes a splitting of spectrum lines in magnetic fields—the normal Zeeman effect.

The electron has, besides its spin, a magnetic moment. In a magnetic field the spin of the electron can have only two orientations with respect to the field direction. These two possible orientations represent two different orientations of the spin magnetic moment and—as in the case of the different orientations of the orbital angular momentum vector—different energy states. The energy difference between these states is

$$\Delta E = 2(eh/4\pi m)B \quad (4)$$

and is the explanation of the fine structure of the spectrum lines—the anomalous Zeeman effect.

In an atom which has a magnetic moment, the electrons will be subject to the magnetic field associated with this moment, and therefore an internal anomalous Zeeman effect, a splitting of the energy levels, will take place even without an external magnetic field. (This interaction between orbital and spin moments is called the spin-orbit coupling.) For example, in sodium, which has a single valence electron, the magnetic field is produced by the orbital motion of the valence electron. (All its other electrons are so arranged in closed shells that their total angular momentum and total magnetic moment are zero.) The orbital magnetic moment causes the splitting of the sodium D-line into two lines separated by 6 Å. Computations show that in sodium the strength of the internal magnetic field is 200 kOe. If an external magnetic field which is considerably stronger than the internal field is now applied (e.g., in lithium just 30 kOe would suffice), the spin-orbit coupling would be destroyed and the effect of the external field would prevail.

By the procedures of quantum mechanics it is possible to compute properties of an atom such as its energy in the absence or presence of an external magnetic field, as well other measurable characteristics. Indeed, in principle it should be possible to predict theoretically from quantum theory the strength of chemical bonding in the presence or absence of magnetic fields. In practice, such a program cannot be easily carried out, inasmuch as formidable mathematical difficulties arise when dealing with systems having many component particles. Nevertheless, it can be inferred that an external magnetic field will change the energy levels of atoms and molecules and thus, under special conditions, may lead (as discussed in Part I, Ch. 7) to a considerable alteration of biological processes.

An atom will have a permanent magnetic moment if it has an odd number of electrons, or if all the electrons are not paired off. The magnetic moment of an atom consists of two parts, the orbital contribution and the electron-spin contribution. Molecules which have one or more unpaired electrons also have a magnetic moment, but in most cases the orbital contribution is negligible. If now an external magnetic field is applied, the molecules will assume orientations in which the component of their angular momentum vectors along the direction of the magnetic field is an odd integral multiple of  $h/4\pi$ . In a substance consisting of such molecules, about one half of the molecules will be oriented so that their magnetic moment is more or less aligned with, the other half so that their magnetic moment is more or less aligned against, the direction of the external field. The energy state of a molecule with its magnetic moment aligned with the field is somewhat lower than its energy when its magnetic moment is aligned against the field. Statistical considerations show then that in thermal equilibrium more molecules will be in the lower energy states. Consequently, we will have an excess of molecules with their magnetic moments aligned with the external field. The magnetic induction inside such materials will be higher than the external field; this is the explanation of para-

magnetism. It is not a general property of all materials as diamagnetism is, but when present it is generally at least 10 times greater than the diamagnetism. Since thermal agitation of the molecules tends to disturb the orientation of the molecular magnets, paramagnetism is temperature-dependent.

It is perhaps of interest to mention that the energy difference between consecutive orientations of a molecule in a magnetic field of 10 kGauss flux density is  $\frac{1}{220}$  of the rotational energy of the molecule at room temperature. This means that the energy obtained from the magnetic field to orient the molecule will become comparable to the randomizing thermal energy at a flux density of 2 MGauss.

Most of the enumerated physical effects of phenomenon (c) are vector effects and therefore reversible. An irreversible cumulative effect is, however, the heat generated in paramagnetic substances as a consequence of the alignment of dipoles against thermal agitation, as well as the heat generated in ferromagnetic substances through hysteresis losses.

Phenomenon (d) (force exerted upon elementary magnetic dipoles or para- and diamagnetic particles) can lead to a displacement of para- and diamagnetic molecules and free radicals, and thus to an accumulation of certain constituents and to an alteration of diffusion processes. Since the force acts in the direction of the gradient vector, the cumulative physical effect of phenomenon (d) is reversible.

Little is known as to which of the enumerated physical phenomena is the prime mover in producing any of the observed biomagnetic effects; yet there is no reason to assume that biomagnetic effects are triggered by other than known physical effects. The extreme smallness of these effects forces us to assume that in most instances some amplification mechanism is involved, producing observable biomagnetic effects. In biological processes which have a positive feedback, such as multiplication and growth processes, even an extremely slight change in the feedback factor may cause, in due time, a large change in the final value. A mathematical analysis of processes of this kind can be found in Part III, Ch. 3. Another mechanism could be the production of a few molecules of different chemical structure with strong catalytic actions, such as growth-promoting enzymes, or a change in the DNA matrix (discussed in Part I, Ch. 7).

### CLASSIFICATION OF BIOMAGNETIC EFFECTS

Since Faraday's time, electrotechnics have provided us with strong magnetic fields. The circumstance that reliable biological effects were observed only during the last decades indicates that biological effects of static magnetic fields are certainly not spectacular, but rather of an elusive nature. During the last 15 years we have subjected several thousand mice as well as some other specimens such as molds, bacteria, tissue cultures, plants, etc., to strong magnetic fields. Surveying the

results of these experiments together with the results of other investigators, we came to the tentative conclusion that the observed biological effects of the magnetic field can be classified into three groups with respect to the mechanism, or factor, which causes them.

In the first group, which we could call the "sensory group," belong those effects which seem to be based on a kind of sensory organ—probably developed during the hundred millions of years of evolution—through which the specimen can sense fields of the order of the geomagnetic field. This organ is certainly not a compass needle, but rather some kind of extremely sensitive current device. In this category belong most probably the navigation of migrating birds,<sup>3</sup> the orientation of planaria, and the experiments on dowser reflexes (see Part IV).

In the second group, which we could call the "stress group," belong those effects which are caused by the many physical phenomena which, according to physical laws, should occur in every biological system subjected to a static magnetic field. The cumulative physical consequences of these phenomena, which were enumerated above, will tend to disturb the normal functioning of the organism, representing a kind of stress, to which the organism has to adapt and will respond with some kinds of countermeasures.

The stress caused by the magnetic field can produce effects similar to other stress factors such as confinement, heat, cold, bleeding, starvation, etc. The stress effect of the magnetic field, like other stress effects, will show up after a few hours, days, or weeks of exposure to the field. Furthermore, the effect seems to be attenuated if the specimen frequently changes its position relative to the direction of the field or gradient vector. This attenuation can be expected, because the overwhelming majority of the cumulative physical effects of the magnetic field are reversible effects.

In the stress-effect group belong, in our opinion, the retardation of the growth of young mice, rejection of transplanted tumors, hematological changes, retardation of wound healing and tissue regeneration, effects on the central nervous system, plant growth responses and magnetotropism (see Part II, Chs. 1-3, 6, 8-10), drop in body temperature, disappearance of the oestrus cycle,<sup>4</sup> resorption of embryos in the uterus,<sup>5</sup> decrease in tissue respiration, inhibition of bacterial cultures during their maximum stationary phase (see Part III, Chs. 1 and 4), and pathological changes in the liver (see Part I, Ch. 7).

In the third group belong those effects which are most probably caused by a stabilizing or labilizing effect of the magnetic field upon the genetic code. We have here in mind the quantum-mechanical tunneling of protons in the hydrogen bonds between the complementary nucleotide bases in the DNA molecule (see Part I, Ch. 7). In this "genetic-code group" belong, in our opinion, the decrease in the incidence of the spontaneous mammary gland carcinoma of the C3H strain, the retardation of aging, the pathological changes in the adrenal, and

perhaps the decrease of spontaneous wing abnormalities of Tribolium confusum.<sup>6</sup>

There are, of course, effects for which it would be difficult to decide whether they are of the stress or genetic type or a combination of both. This may be the case with the inhibition of bacterial cultures during their logarithmic growth phase (see Part III, Ch. 3). A mathematical analysis of the growth curve shows that a new, magnetic-resistant strain appeared. But whether this strain was selected under the stress of the field or the magnetic field produced a mutation cannot yet be decided. Similarly, the lymphoblast, observed in the spleen,<sup>4</sup> and the decrease in the number of megakaryocytes in the bone marrow<sup>7</sup> cannot yet be classified.

#### THE ENVIRONMENT OF THE SPECIMEN IN THE MAGNET

As we have already mentioned, the stress caused by the magnetic field can produce effects which are similar to those of other stress factors. When we expose an animal or other biological sample to a magnetic field, we at the same time, knowingly or unknowingly, change its environment in other respects, too. At the present stage of biomagnetic research, it is of utmost importance to take every possible precaution to ensure that the observed biomagnetic effect is not an artifact caused by the stress of the changed environment of the specimen.

One of the most dangerous stress factors in this respect is the confinement of living animals, since a certain degree of confinement cannot be avoided with larger animals. The author found that young mice confined to a circular cage 3 in. in diameter and 2 in. high remain somewhat stunted and during the first days of confinement their leukocyte count drops by 1000-2000 WBC/mg compared to littermates housed in large cages; these effects are similar to those caused by an exposure to magnetic fields of a few thousand oersteds. The only remedy against the danger that an artifact caused by an unknown stress factor of the confinement is misinterpreted as a biomagnetic effect is to use the same or larger number of controls housed under completely identical conditions as those in the magnet. To state that no identically housed controls were used because no differences were found between confined and normally housed samples is of little value. (It would imply that at least a nine-times larger statistical material was used for this investigation than was used in the main experiment!)

It is well known that temperature, humidity, air currents, light, traces of gases, etc., are capable of causing biological effects in living animals and plants. In a laboratory these factors can differ greatly from one place to the other, between different levels in the room and even between center and edge of a table. Therefore the controls should be kept as close as possible to the magnet, shielded, of course, from the stray field of the magnet.

One factor often forgotten is the influence of the bulk of the magnet and particularly that of the pole caps in close vicinity to the sample. Its proximity will disturb air currents and change humidity conditions, and its shadow or the light reflected from its surfaces change the illumination. It is not enough to house the controls identically; the pole caps, too, have to be simulated with dummy pole caps, made from iron or brass. The shadow of the coils and of other structures should be simulated by baffles. Of course, in experiments with organisms where light is one of the main factors influencing growth or other parameters, even more extreme precautions are needed to ensure equal lighting conditions of magnet and dummy groups.

Some methods to keep the temperature of the pole caps of large electromagnets constant have already been mentioned. Smaller permanent-magnet assemblies can be placed directly in thermostats, or water baths. When working with specimens such as bacteria or tissue cultures, which are highly sensitive to small temperature differences, magnet and dummy samples should be kept in identical thermostats fed by the same thermocirculator. Whenever the temperature of the pole caps differs considerably from that of the thermostat, the water of the thermocirculator should pass through the two thermostats twice, the second time in reversed direction.

In experiments in which the investigated parameter is a genetic change, or a somatic mutation, the role of cosmic radiation as an environmental factor cannot be neglected. The primary cosmic radiation arrives at the top of the atmosphere mainly in the form of protons of extremely high energy. Sea level is reached, with few exceptions, only by secondary particles, 70% consisting of mu-mesons and some neutrons (hard component) and 30% of positive and negative electrons and high-energy gamma rays (soft component). The hard component is only slightly absorbed even by a 100-cm-thick iron layer; the soft component is completely absorbed in 20 cm of iron. In materials of high atomic number, such as iron, the hard component will initiate penetrating showers, composed of a large number of highly ionizing heavy particles. The soft component will produce electron showers, consisting usually of 10 to 100, but sometimes several thousand, electrons. These showers usually have a core where many particles travel closely together. Not very far from their origin, several shower particles may pass together through one cell or one chromosome. Experiments with rabbits and mice placed under lead shields indicate that these showers can cause genetic damages, resulting in a greater mortality of the offspring.<sup>8</sup>

Neither the absorption in the iron core of the magnet nor the negligible deflection of the high-energy charged particles through the magnetic field will appreciably lower the intensity of cosmic radiation; but we can expect an increased number of showers in the space between the pole caps, particularly if the field is vertically oriented. Since the

number of shower particles emerging from iron or brass shows saturation above 1 in. thickness of the layer, it should suffice to simulate the pole caps in the dummy magnet with a 2-in.-thick iron or brass disc to ensure equal cosmic-ray shower intensities.

Mention should be made of experiments in which the specimen is simultaneously exposed to a magnetic field and to some other agent, such as irradiation. The observed difference in the biological effect of the agent in the presence or absence of the magnetic field may not necessarily represent a biological effect of the magnetic field. It could be due to the influence of the magnetic field on the agent. Particularly in the case of X- or gamma-irradiation, the path of the soft secondary electrons will be altered by the magnetic field. This alteration can change, as a purely physical effect, the total number of ions produced per unit volume and thus indirectly alter the biological effect of the irradiation; but it also could alter the sensitivity of the biological system against the biological effect of irradiation. Some considerations along these lines were made by A. Forssberg.<sup>11</sup>

#### MODERATE OR HIGH FIELDS

The first question which one has to ask before starting biomagnetic experiments is: how strong a magnetic field should I use? If either the specimen or the parameter were already investigated by others, or the experiment is based on some theoretical considerations, some clues can be found. But, if the experiment has no theoretical or empirical precursors, it is difficult to find an answer. This may have discouraged many from starting researches in this field; and understandably so, because nobody wants to work for years, merely to learn later than the effect was discovered with the use of more powerful equipment.

Investigators who can perform biological experiments in high-field laboratories are apt to justify the involved high operating costs, space limitation, and short exposure times with the reasoning that the energy density of an electromagnetic field increases, according to Maxwell's equations, with the square of the field strength. Consequently, the likelihood of observing biological effects similarly increases with the square of the field strength. The following examples indicate what we should expect if this reasoning were valid without restriction for biomagnetic effects; the actual results are also given.

The decrease in the growth rate of young mice, which gives a well-measurable weight difference of 2.2 g after 11 days of exposure to a field of 4.2 kOe, should increase by a factor of 6 in a field of 10 kOe. I found a weight difference of 6.2 g in the stronger field. This corresponds to a ratio of 2.8, suggesting a linear increase of the magnitude of the effect with the field strength.

In the experiments made in the Lawrence Radiation Laboratory of the University of California (see Part I, Ch. 4) with a field of 14 kOe,

the effect on growth rate and leukocyte count should have been 12 times larger than that found in the laboratory of the Biomagnetic Research Foundation in a vertical field of 4.2 kOe. The effects actually found in the 14-kOe field were at most  $\frac{1}{3}$  of those found in the weaker field. This result would imply an attenuation by the unlikely high factor of 33, due to the random motion of the mice in the horizontal field!

The decrease in the leukocyte number in the circulating blood of a mouse, an effect which is easily observed after an exposure of 200 hr to a field of 4.2 kOe (see Part II, Ch. 3), should have become observable after an exposure of 15 min to a field of 120 kOe. Yet no changes in the number of leukocytes were noticed after a 1-hr exposure, either immediately or 1 day later (Part II, Ch. 11).

Beischer<sup>10</sup> found that a field with a gradient above 6 kOe/cm has a lethal effect on Drosophila. But no effect was observed below 6 kOe/cm gradient even when the field strength was raised to 100 kOe.

As we can see, the enumerated examples do not support the view that the magnitude of the biomagnetic effect is proportional to the square of the field strength.

If the magnetic field acts upon a particle which has no permanent magnetic moment, the magnetic field has to induce magnetism in it in order to be capable of exerting a force upon the particle as a whole. In this case, the energy transferred to the particle is proportional to the square of the field strength, or to the paramagnetic strength. But, as shown in the next chapter, orientation and displacement of para- or diamagnetic biological entities (macromolecules, cells) can hardly play a role at room temperature in biomagnetic effects, even in fields of 300 kOe intensity, or  $10^9$  Oe<sup>2</sup>/cm paramagnetic strength.

If, on the other hand, the magnetic field acts through a mechanism as proposed in Part I, Chs. 5 and 6 (through an orientation of the molecules endowed with a permanent magnetic moment), or as proposed in Part I, Ch. 7 (through a space quantization of the magnetic moments of lone electron pairs), then the energy transferred will increase with the first power of the field strength.

The yet scanty experimental results and the above theoretical considerations seem to indicate that the magnitude of biomagnetic effects is proportional to the product of field strength and exposure time, or to the product of gradient and exposure time.

Table I compares the doses  $tH$  and  $t(dH/dx)$  which can be delivered with different equipment in a volume of 1 in. in diameter and 2 in. long. The continuous operation time of the Bitter, Kolm, or Montgomery type high-field solenoids is limited to about 2 hr. The operation time of laboratory electromagnets or permanent magnets is practically unlimited. To permit a reasonable comparision, a maximum exposure time of 10 days was arbitrarily assumed in the latter cases.

As can be seen from this table, if the aim is to find a yet unknown biomagnetic effect, the chance of success for experiments made in a field

TABLE I

Maximum Continuously Delivered Dose in a Volume 1 in. in Diameter and 2 in. Long

Magnet type	Exposure, hr	Field, kOe	Gradient, kOe/cm	Dose		Electr. energy, MW-hr
				$tH$	$t(dH/dx)$	
Permanent, 300 lb	250	8	2	2000	500	0
Electromagnet, 4-in. core	250	20	5	5000	1250	0.5
Bitter or Kolm solenoid	2	125	10	250	20	4
Montgomery solenoid	2	250	20	500	40	28

of 4 to 20 kOe strength is about 20 times greater than for experiments made in the 100-kOe range. One should not forget here that, should the gradient of the field be the critical factor, a 12-in. electromagnet can produce much higher gradients than any air-core solenoid.

It would be premature to state threshold values of field strength, gradient, or paramagnetic strength; but as general information Table II lists the values at which biological effects have been observed. For gradient sensitive phenomena, gradient and paramagnetic strength are listed whenever it is not known which of the two factors plays the dominant role.

TABLE II

Field sensitive phenomena	Oe	Gradient sensitive phenomena	Oe/cm	MOe <sup>2</sup> /cm
Orientation of Mud-snails (Magnetophosphenes)	1.5 200	Dowser reflexes	$10^{-6}$	—
Central nervous system (rabbits)	800	Rejection of tumor homo transplants	600	2
Plant growth (barley)	1,000	Inhibition of bacteria	2,300	35
Embryo resorption (mice)	3,000	(logarithmic phase)		
Retardation of development; hematological changes; wound healing; pathological changes; isolation of tumor isotransplants (mice)	4,000	Magnetotropism	5,000	20
Enzyme activity change	5,000	Lethal effect (mice)	5,000	100
Change in oxygen consumption and degeneration of sarcoma cells	8,000	" " (Drosophila)	6,000	—
Inhibition of bacteria (stationary phase)	14,000	Arrest of tumor growth	10,000	200
Oxygen consumption of potato; survival of leukemic mice	18,000			

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## *Chapter 2*

# Simple Theoretical Models for Magnetic Interactions with Biological Units

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We can calculate what happens to physical entities in magnetic fields. If these entities can serve as appropriate models for biological units such as blood cells or neurons, definite predictions become possible and meaningful experiments can be designed.

Two kinds of action of static magnetic fields with and without gradients lend themselves to clear-cut calculations of this sort. One of these is the magnetomechanical force which can produce both translation and rotation of "particles." The other is the production of electric voltages and currents in conductors which move relative to a magnetic field. The equations for the Hall effect will also be discussed, but there are insufficient data to determine their applicability to biological conductors at this time.

### I. MAGNETOMECHANICAL ACTIONS ON SPHERICAL AND ELLIPSOIDAL PARTICLES

I shall consider a model appropriate to red blood cells, but its extension to particles of any size will be apparent. Both the translational redistribution of spherical particles and the alignment of one axis of an ellipsoidal particle will be calculated. The importance of Brownian motion will be demonstrated by calculating the dynamics of the redistribution in addition to the final state of equilibrium.

#### A. Translational Redistribution in a Gradient Field

First, consider the redistribution of spherical particles of volume  $V$  ( $\text{cm}^3$ ), radius  $r$  ( $\text{cm}$ ), and volume susceptibility  $K$  in a fluid of susceptibility  $K_0$  when acted upon by a magnetic field  $H$  (oersteds) which has a gradient  $dH/dx$ . If there is a concentration of  $n$  particles/ $\text{cm}^3$ , which is a function of  $x$ , then adapting the equations derived by Einstein<sup>1</sup>

$$n/n_0 = \exp(FVx/kT) \quad (1)$$

where  $F = H(dH/dx)(K - K_0)$  dynes/cm<sup>3</sup> is the magnetic force of translation per unit volume and  $n_0$  is the concentration at  $x = 0$ , where  $H$  is greatest.

For a red blood cell in isotonic saline, we may take as a model a sphere with  $r = 3 \times 10^{-4}$  cm and  $V = 1.1 \times 10^{-10}$  cm<sup>3</sup>;  $H(dH/dx)$  can, with reasonably priced equipment, be made as high as  $10^9$  Oe<sup>2</sup>/cm over a distance of 0.1 cm for experiments *in vitro* in small-volume containers;  $K - K_0$  is at the very most  $0.2 \times 10^{-6}$ , using Drake's<sup>2</sup> data;  $kT = 4.2 \times 10^{-14}$  at room temperatures and  $x = 0.1$  cm. Then

$$F = 200 \text{ dynes/cm}^3$$

(equivalent of  $\frac{1}{5}g$ , where  $g$  is the acceleration due to gravity) and

$$n/n_0 = e^{52,500} \approx 10^{23,000}$$

i.e., a magnetic field gradient of this size will separate red blood cells from saline very completely, if the process is allowed to go to equilibrium.

This equilibrium calculation tells us nothing about how fast equilibrium is reached. To find this out, we can use Stoke's Law. The velocity  $v$  of a sphere in a fluid of viscosity  $\eta$  is given by

$$v = FV/6\pi\eta r = 2r^2F/9\eta \text{ [cm/sec]} \quad (2)$$

using the same symbols as above. If  $\eta = 0.01$  poise, as in water at 20°C,

$$v = 3.9 \times 10^{-4} \text{ cm/sec} = 1.4 \text{ cm/hr}$$

for the hypothetical red blood cell discussed above. Obviously, the equilibrium separation will not be reached quickly and if the suspending fluid is moving with a velocity much larger than 1.4 cm/hr, it will be impossible to observe.<sup>3</sup>

Most of the reported biomagnetic experiments *in vivo* have been made in fields for which  $H(dH/dx)$  is  $10^7$  or less; for them the viscosity of whole blood of 0.03 poise at 37°C would be appropriate to use; these two factors result in a magnetic drift velocity of less than 0.005 cm/hr. Additionally, the value of  $(K - K_0)$  chosen above is for completely reduced hemoglobin in red cells. For most cells  $(K - K_0)$  would be less than  $0.2 \times 10^{-6}$ . The conclusion that translational forces of gradient fields will not redistribute the red blood cells under these conditions is inescapable. For smaller particles, the velocity will decrease as the square of the radius according to (2), so that small cell components, even if they were more highly magnetic, such as ferritin, are influenced even less; no known mobile, larger and more highly magnetic biological entity than a red blood cell is known.

## B. Rotational Alignment of Ellipsoids in a Uniform Field

We saw above how translational redistribution is produced by a gradient field. In a uniform field, there would be no such forces, but a

torque can be exerted on a magnetic particle, provided the particle is, magnetically speaking, not spherically symmetrical.

As actual red blood cells are not spherical, we shall calculate the rotation of an ellipsoid of a material of uniform and isotropic magnetic susceptibility  $K$ , immersed in a fluid of susceptibility  $K_0$  in a uniform magnetic field  $H$  having a negligible gradient  $dH/dx$ , and use this as the appropriate model in this situation.

The simplest method is to consider the energy  $U$  (in ergs/cm<sup>3</sup>) of such a body in a magnetic field:

$$U = \frac{K_0 - K}{8\pi} \int_V \vec{H}^- \cdot \vec{H}_0 dV \quad (3)$$

where  $\vec{H}^-$  is the field inside the body and  $\vec{H}_0$  the undisturbed field. If our ellipsoid has semiaxes  $a$ ,  $b$ , and  $c$ , then its energy  $U_{\perp}$ , when it is oriented in a field  $H$  with  $a$  and  $b$  perpendicular to  $H$  and  $c$  parallel to  $H$ , is given by

$$U_{\perp} = \frac{H^2}{1 + 4\pi KL_3} \left[ \frac{abc}{6} (K_0 - K) \right] \quad (4)$$

If  $H$  is parallel to  $a$  and perpendicular to  $b$  and  $c$ , then its energy  $U_{\parallel}$  is

$$U_{\parallel} = \frac{H^2}{1 + 4\pi KL_1} \left[ \frac{abc}{6} (K_0 - K) \right] \quad (5)$$

using the equations derived in Stratton,<sup>4</sup> but in Gaussian units as above.  $L_1$  and  $L_3$  are given by Osborn,<sup>5</sup> who designated them by  $L$  and  $N$ , respectively; for  $a = b$  and  $c/a = 0.34$ ,  $L_1 = 0.186$  and  $L_3 = 0.629$ . Subtracting (5) from (4), we get

$$\Delta U = U_{\perp} - U_{\parallel} = (2/3)\pi abc H^2 (K_0 - K) K (L_1 - L_3) \quad (6)$$

Taking the volume  $4/3\pi abc = 1.1 \times 10^{-10}$  cm<sup>3</sup> and  $(K - K_0) = 0.2 \times 10^{-6}$  in conformity with the values used above, with  $K_0 = -0.7 \times 10^{-6}$ ,

$$|\Delta U| = 2.4 \times 10^{-24} H^2 \text{ erg} \quad (6a)$$

In order that any preferred orientation be produced, even for the equilibrium case,  $\Delta U$  must be appreciably larger than  $kT$ . As  $kT$  at room temperature is  $4.2 \times 10^{-14}$  erg,  $H^2$  has to be of the order of  $10^{11}$  before any angular alignment will become observable. This means that extremely high fields only just within range of the latest powerful magnet installations will be required. Let us assume that we have a

field of 300,000 Oe, and let us calculate the rate of preferential alignment. The order of magnitude of the angular velocity at  $H = 300,000$  Oe can be obtained from the energy difference

$$\Delta U = 21.6 \times 10^{-14} \text{ erg}$$

obtained from (6a). Because the energy equals the product of torque and angular displacement, the average torque  $M$  for a  $90^\circ$  rotation will be given by

$$M_{\text{ave}} = \Delta U / (\pi/2)$$

This makes  $M_{\text{ave}} = 13.7 \times 10^{-14}$  dyne-cm. The angular velocity  $\omega$  of a small sphere in a fluid is given<sup>1</sup> by

$$\omega = M / 8\pi\eta r^3 \quad [\text{rad/sec}] \quad (7)$$

Hence  $\omega = 0.02 \text{ rad/sec} = 1.1^\circ/\text{sec}$  or  $68^\circ/\text{min}$ .

This compares with the root mean square of the angular displacement  $\sqrt{\bar{\Theta}^2}$  due to random thermal agitation<sup>1</sup> or "Brownian" motion:

$$\sqrt{\bar{\Theta}^2} = \sqrt{t} \sqrt{kT / 4\pi\eta r^3} \quad (8)$$

which is equal to  $6.3^\circ$  in 1 sec or  $49^\circ$  in 1 min in our case. Note that the random displacement increases only as the square root of time, making this effect observable.

No reports of the observation of this magnetic rotation of red blood cells in vitro exist. However, this method of lining up the short axis of red blood cells normal to a very high magnetic field might possibly find application in the study of agglutination; it could also be used to determine whether in addition to the "shape" anisotropy, the blood cell has some additional magnetic anisotropy, due to nonrandom alignment of its hemoglobin. Under the conditions of nearly all the reported biomagnetic experiments, this rotation could not have been a causative factor.

## II. ELECTROMAGNETIC EFFECTS. THE "GOLDFISH" MODEL

It is a well-known fact that moving conductors have voltages "induced" in them when their motion takes place relative to a magnetic field. The real problem is to know whether the magnitude of the voltages and the duration of the currents which may be associated with these voltages is large enough to cause any known biological effects. I shall show that experimental evidence indicates that certain neurons are sensitive enough to be measurably affected by voltages produced in a model biological system in motion in a magnetic field.

To make our calculations we only have to assume a few properties which are quite generally applicable to any tissue of reasonable con-

ductivity. However, for the sake of providing a picture, the reader may consider the model to be a running mouse or a swimming goldfish. The conducting tissue, which contains neurons, is moving in a magnetic field with a velocity  $v$  (cm/sec). With a suitable choice of coordinates, we can solve some simple cases which will give us the order of magnitude of the effects to be expected.

### A. Gradient Field Case

In the first case, consider that the velocity  $v$  (cm/sec) is along  $x$  in a straight line, and the magnetic field  $H$  (oersteds) is at right angles to it and has a gradient  $dH/dx$ . Because of this velocity  $v = dx/dt$ , an imaginary conducting loop of area  $A$  ( $\text{cm}^2$ ) normal to  $H$  will have passing through it a flux  $\phi$  (maxwells) equal to  $AH$ , which will be a function of time. In fact,

$$d\phi/dt = A(dH/dt) = A(dH/dx) \cdot (dx/dt) = vA(dH/dx) \quad (9)$$

Now the voltage  $V$  induced in any current loop is given by

$$V = -10^{-8} (d\phi/dt) [\text{volts}] \quad (10)$$

and from (9)

$$V = -10^{-8} vA (dH/dx) \quad (11)$$

If in our model  $v = 100$  cm/sec (approximately 2 miles/hr),  $dH/dx$  is 500 Oe/cm, and  $A = 1$   $\text{cm}^2$ , the voltage along a circular loop enclosing this area will be 0.5 mV and the voltage gradient  $E$  will be 0.14 mV/cm.

If the magnetic field is vertical, this represents voltages and accompanying currents in the horizontal plane. The velocity we have considered does not have to be that of the tissue or animal as a whole. The argument applies equally to moving components; if the velocity of the blood stream in an artery is considered, for instance, we get a similar voltage produced. The principle has been used to measure the velocity of blood flow. However, there are only a few points in the circulation where velocities as high as 100 cm/sec are encountered. In the human circulation, this order of magnitude of flow velocity is only reached in the aorta, and there only during the ejection period of the start of the systole.

### B. Uniform Field Case

Basically, a voltage is produced in a conductor moving so as to cut any magnetic field lines. We may call this voltage a polarizing voltage because, as we accelerate a conductor from rest to where it is cutting

magnetic field lines at some given rate, the charges in it will be redistributed so as to balance this polarizing voltage. If at this point we change the velocity in magnitude or direction, a continued redistribution of charges will take place. For the gradient field case we had simply picked out a rather special aspect of this problem, where the velocity was not changing, but the number of field lines cut per unit time was changing because of the gradient of the magnetic field. Now we shall instead calculate the polarizing voltage, in the uniform field case. If the velocity is 100 cm/sec again, and if the uniform field has a magnitude of 5000 Oe at right angles to  $v$ , the voltage induced in the conducting tissue will be  $10^{-8} \cdot 5000 \cdot 100 = 5$  mV/cm. If the conductor reverses direction, so will the voltage; suppose our "goldfish" turns around  $180^\circ$  in 1 sec in a plane at right angles to the field  $H$ . The changing polarizing voltage, producing a current, will still be given by (10), where now  $d\phi/dt$  will simply be  $2 \times 5000$  per second. Therefore,  $V = 0.1$  mV per  $1 \text{ cm}^2$  of enclosed area is the average voltage maintained throughout a  $180^\circ$  turn, taking 1 sec to execute. This is slightly smaller, but of the same order of magnitude as, the voltage calculated for the gradient field case. The case of accelerated motion can be similarly calculated; in fact, the above example of the  $180^\circ$  turn is equivalent to an acceleration of  $200 \text{ cm/sec}^2$  or  $\frac{1}{5} g$ .

Although these voltages have been calculated by very elementary methods for particularly simple geometries of field and motion, they are clearly representative of the voltages occurring in animals moving fairly rapidly in magnetic fields of the order of a few thousand oersteds. It remains to show that such voltages are of the right size to affect neurons. This has clearly been demonstrated only in the case of a number of "electric" fish by Lissman and Machin,<sup>6,7</sup> who have shown that these fish can sense voltage gradients as low as  $0.03 \mu\text{V}/\text{cm}$ . Specialized nerve cells and a specially developed part of the brain of these fish are involved in this, and the fish can use this electric gradient information to orient themselves. However, the main point of interest for us is that a biological mechanism exists to sense such small voltages. It could exist in other organisms without their having to make "intelligent" use of this sensitivity. This speculation is borne out in part by the observations of Terzuolo and Bullock,<sup>8</sup> who were able to modulate neuronal firing in the stretch receptor of crayfish and in the cardiac ganglion of lobster by imposed voltage gradients of only 10 mV/cm applied across the saline bathing solution of these preparations. Although these voltages are greater than the ones effective in the live electric fish, they do indicate that gradients much smaller than the membrane depolarizing voltage gradients are effective in producing measurable changes in neuronal response. The results of Kholodov may also have some bearing on this.<sup>9</sup> (See Part II, Ch. 10.)

The voltages we have calculated for our goldfish model lie in between the levels found to give observable effects in these two papers; there is, therefore, some justification in claiming that our model

shows a possible mode of action of stationary magnetic fields on moving conducting tissue in moderate magnetic fields.

### C. Hall Effect

If a current flows in a conductor, electric charges are, of course, in motion. Therefore, even if the conductor itself is stationary, a magnetic field will interact with the moving charges, deflecting them. The cyclotron is a well-known example of this effect for charges moving in a vacuum. In a conductor, the effect is referred to as the Hall effect.

The force  $\vec{F}$  on a charge  $e$ , with an average drift velocity  $\vec{v}$  in a conductor is given by

$$\vec{F} = (e/c)(\vec{v} \times \vec{B}) \text{ [dynes]} \quad (12a)$$

in Gaussian units, where  $c$  is the velocity of light. In translating this into terms of currents and voltages, it is preferable to use MKS units, where

$$\vec{F}' = e'(\vec{v}' \times \vec{B}') \quad (12b)$$

where now  $e'$  is in coulombs,  $\vec{v}'$  in m/sec,  $\vec{B}'$  in webers/m<sup>2</sup>, and  $\vec{F}'$  in newtons.

The current density in A/m<sup>2</sup> will be given by  $\vec{J}' = N'e'\vec{v}'$ , where  $N'$  is the number of free charges per unit volume (m<sup>3</sup>). The Hall intensity  $\vec{E}'_H$  which will just compensate this force  $\vec{F}'$  is given by the equation

$$\vec{F}' = -e'\vec{E}'_H \quad (13)$$

Therefore

$$\vec{E}'_H = -(\vec{v}' \times \vec{B}') = (1/N'e')(\vec{J}' \times \vec{B}') \text{ [volts]}$$

Actually, to account for the random drifting motion of the charges, a numerical factor must be used to correct this equation and we get

$$\vec{E}'_H = -(3\pi/8N'e')(\vec{J}' \times \vec{B}') \text{ [volts]} \quad (14)$$

Unfortunately, it is necessary to know  $N'$  and  $\vec{J}'$  for all charge carriers, both negative and positive, before the magnitude of this Hall voltage, which appears at right angles to the current flow and the magnetic field, can be calculated.

In some semiconductors, the Hall voltage can be almost as high as the voltage driving the current forward in the conductor, but there is insufficient data for biological conductors from which to make any reasonable calculations. It would appear rather unlikely that conducting tissue such as a nerve axon would have an appreciable Hall effect, at least on any model based on ionic conduction. If any crossed-field voltages are observed,<sup>10</sup> they may be an active response of the organism rather than the "passive" Hall effect.

### III. CONCLUSION

We saw that under favorable circumstances static magnetic fields can produce observable effects on models which are representative of living tissue. Very much larger fields are needed for magnetomechanical than for electromagnetic effects. The basic reason is that mechanical alignment of spins against thermal agitation requires each spin, i.e., each "magnetic unit," to be acted on independently, while in the electromagnetic case it is the integrated electrical effect over a large volume which we calculated. Whether neurons are generally sensitive to small currents much below the threshold values for depolarizing axons, and to what degree, in quantitative terms, would perhaps make a good subject for new experiments.

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## *Chapter 3*

# Basic Concepts Related to Magnetic Fields and Magnetic Susceptibility

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## INTRODUCTION

Magnetism has been known since ancient times; references to lodestone (leading stone) are found in the Vedas, the most ancient religious scriptures of the Hindus, dating back to about 1000 BC, in the Platonic dialogues of Socrates, and in the ancient literature of the Chinese. Scientific studies of magnetism began with William Gilbert of Colchester (1540-1603); he showed that the earth itself behaves like a magnet, that iron ceases to be attracted while red hot, and that substances such as paper and cloth do not affect the force of attraction between a magnet and iron. The early contributions of John Mitchell (1724-1793), of John Robison (1739-1805), and of Coulomb (1736-1806) helped to establish the well-known Coulomb's "inverse square" law. Faraday is regarded as the founder of magnetochemistry; he based his investigations on the early researches of Ampére, Oersted, Arago, and Biot. Faraday showed that all matter is magnetic in one sense or the other; that is, that matter is either attracted or repelled by a magnetic field. Today we know that the former category embraces para- and ferromagnetism and the latter corresponds to diamagnetism. Stoner<sup>1</sup> has given an excellent historical introduction and many references to early work. Faraday may be also regarded as a founder of biomagnetics or magnetobiology. Many writers have noted the historical fact that on November 8, 1845, he investigated the properties of dried blood and noted that he "must try recent fluid blood." If he had done so he might have discovered the difference between arterial and venous blood. Brief historical reviews of other aspects of biomagnetics are given in Part II, Chs. 7-9, and Part III, Ch. 3.

The rigor of the mathematical analysis of magnetochemistry was developed in the past century by Poisson (1820), Weber (1854), and Ewing (1890). The theories of Langevin, Honda, Oxley, and Stoner

established the present-day basis for a quantitative interpretation of magnetic properties of atoms in terms of electronic structure. G. N. Lewis showed the relationship between magnetism, electrons, and valence. This constitutes the basis for chemical interpretations of magnetic susceptibility. The outstanding theoretical contributions of Van Vleck, Purcell, Bloch, and others in the United States are well known to physicists and physical chemists.

Thus far, no reference to the accomplishments of the French School, notably of Pierre Curie, P. Pascal, and Pacault,<sup>2</sup> has been made because they occupy a unique position in the history of magnetochemistry. As a matter of fact, the applications of the Curie-Weiss law to a study of paramagnetism and of Pascal's constants to diamagnetism constitute the practical bases for the analytical applications of magnetic susceptibility measurements.

#### GENERAL LITERATURE

The first treatise in English dealing with physical principles and the applications of magnetochemistry was put forth in 1935 by S. S. Bhatnagar and K. N. Mathur<sup>3</sup> in India. This was followed by W. Klemm's Magnetochemie<sup>4</sup> and two editions of Selwood's Magnetochemistry,<sup>5</sup> which is by far the most comprehensive and up-to-date contribution in this area.

During the past few years, the general literature on magnetism has shown a phenomenal growth. Recent books by Selwood,<sup>6</sup> Goodenough,<sup>7</sup> Rado and Suhl,<sup>8</sup> Dorfman,<sup>9</sup> Belov,<sup>10</sup> ter Haar,<sup>11</sup> and Brown<sup>12,13</sup> have made significant contributions to our understanding of magnetism in its various aspects. Special books have been written on ferrites by Smit and Wijn,<sup>14</sup> Gurevich,<sup>15</sup> and Lax and Button.<sup>16</sup> A number of books under the general title of "Electricity and Magnetism" have been published; however, only a few describe magnetism in relation to matter. Texts dealing with a basic theoretical approach have been written by a number of physicists.<sup>17-21</sup> Information on magnetic properties of materials such as semiconductors and alloys is available in reviews and books<sup>22,23</sup> specially written in these areas and in solid-state physics.<sup>24</sup> These works discuss the general concepts of lattice and charge-carrier susceptibilities that contribute to the overall susceptibility of a semiconductor.<sup>25</sup> Some information on applications of magnetic susceptibility has appeared in Technique of Organic Chemistry<sup>26</sup> and other similar compilations and reviews.<sup>2,27-31</sup> Most extensive tables of magnetic susceptibility constants are given by Foex, Gorter, and Smits<sup>32</sup> and by Landolt and Börnstein;<sup>33</sup> recent trends in magnetic susceptibility are discussed by Palmer.<sup>34</sup> Two reviews by the author of this chapter<sup>35,36</sup> describe instrumentation and some analytical applications of magnetic susceptibility; important susceptibility constants have been reported separately.<sup>37</sup>

The study of electrical superconductivity and superconducting magnets has opened up new areas of research. Kolm and co-workers<sup>38</sup> have edited papers dealing with high magnetic fields. The "Index to the Literature on Magnetism"<sup>39</sup> is expected to provide valuable help to readers; however, judging from the bibliography, some magnetic investigations of significance to chemists and biologists do not seem to have been covered. This index was compiled by members of the Bell Telephone Laboratories, and very likely it may have been compiled to meet their special requirements.

Short reviews,<sup>40,41</sup> compilation of papers<sup>42</sup> on ferro- and antiferromagnetism and related topics, and a chapter by this author<sup>43</sup> which should be of special interest to workers in biomagnetism also appeared recently.

### BASIC CONCEPTS

"What is a magnetic field?" and "Why does a magnet attract iron?" are questions that cannot be answered precisely and in a simple fashion. Hence, we will use the approach of several texts on magnetism and, considering the limitations of this discussion, will accept the phenomena of magnetism as commonly observed; we shall, therefore, define certain concepts within its own domain and in relation to the properties attributed to the fundamental particles of matter.

Magnetic Field. The region surrounding a magnetized body which is capable of inducing magnetism in other bodies is called a magnetic field.

Magnetic Dipole. This is a macroscopic or microscopic magnetic system in which the north and south poles of a magnet, equal and opposite in character, are separated by a short but definite distance. A magnetic dipole will tend to orient itself parallel to an applied magnetic field in the same way an electric dipole does in an electric field.

Unit Pole. Unlike an electric charge of either sign (+ or -), a single magnetic pole (north or south) cannot be isolated. However, the purely fictitious concept of a unit pole helps to develop other useful quantitative aspects of magnetism. Hence, a unit pole may be defined as one that will repel an equal and similar pole placed 1 cm away in vacuum with a force of 1 dyne. The repulsion or attraction is governed by Coulomb's law.

Pole Strength. The strength, that is, the attractive (or repulsive) power of a magnet, is measured by the number of unit poles to which each pole is equivalent.

Intensity or Strength of a Magnetic Field. If a unit pole is placed at a fixed point in vacuum in a magnetic field, it will be acted upon by a force that is taken as a measure of the intensity or strength of the magnetic field. It follows from previous definitions that unit magnetic intensity exists at a point where the force on the unit pole is 1 dyne. The

unit magnetic intensity was formerly called the "gauss," and this term is used even today by many manufacturers and users of magnets. According to the recommendations of the International Conference on Physics at London (1934), the unit is called the oersted. Some authors use the abbreviation "Oe" for the oersted. A smaller unit is the gamma,  $\gamma (= 10^{-5} \text{ Oe})$ .

Magnetic Flux or Flux Density. This is defined in terms of the lines of force of a magnet. The free path that would be traced by a unit pole in a magnetic field due to the magnetic forces acting on it is called a line of force. The number of lines of force per square centimeter is taken to be numerically equal to the strength or intensity of the field at that point. The total number of lines of force emanating from the (north) pole face of a magnet is called the total magnetic flux. The number of lines of force per unit area is termed the flux density. The unit of flux used for theoretical purposes is the maxwell. The number of lines of force emanating from a pole of strength  $m$  is  $4\pi m$  maxwells.

Magnetic Moment. This is a term most widely known to chemists and probably is the least understood with respect to its physical significance. The "magnetic moment," as in the case of "moment of a force," refers to the turning produced under certain conditions. When a magnetic dipole is placed in a magnetic field, it experiences a turning effect, which is proportional to a specific character, called the magnetic moment.

If a field of strength  $H$  acts on a dipole N-S of length  $l$  and strength  $m$ , its N and S poles will each experience a force of  $+mH$  and  $-mH$ , respectively (Figure 1). These two equal and opposite forces constitute a couple, the turning moment  $M$  of which is given by  $M = \text{force} \times \text{distance}$ ; hence  $M = mH \times PN = mH \times l \sin \theta = \mu H \sin \theta$ .

Thus the quantity  $\mu = ml$  defines the magnetic moment and serves as a measure of the turning effect. It is measured in dyne-centimeters per oersted or in ergs per oersted. Although no practical unit for magnetic moment was formulated, experiments with the basic electrical

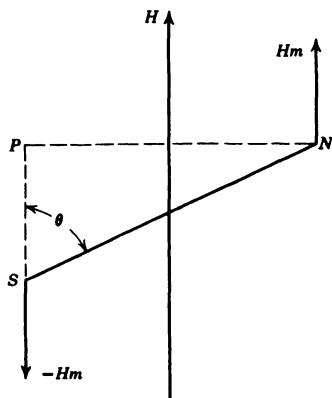


Fig. 1. Forces on a magnetic dipole, showing the "moment" or turning effect.

and magnetic properties of fundamental particles have revealed the existence of a fundamental unit of magnetic moment, the Bohr magneton, which, like the charge of an electron, is just as real a quantity and may be placed among the "universal constants." The Bohr magneton, often abbreviated BM or designated by  $\mu_B$ , is equal to  $eh/4\pi mc$ , where  $e$  is the charge on the electron,  $m$  is the mass of the electron,  $h$  is Planck's constant of action, and  $c$  is the velocity of light.

Substitution of the values of  $e$ ,  $h$ ,  $m$ , and  $c$  gives  $\mu_B = 0.917 \times 10^{-20}$  erg-Oe<sup>-1</sup>.

Coulomb's Law and Magnetic Permeability. Magnetic permeability is best understood in terms of Coulomb's law for magnetic attraction, which is treated in the same manner as electrical attraction.

If two poles of strength  $m_1$  and  $m_2$  are  $r$  centimeters apart, the force between them is given by the inverse square law:

$$\text{force} = \frac{Km_1m_2}{r^2}$$

The north and south poles are denoted by positive and negative signs, respectively. Hence, a positive value for the constant of proportionality  $K$  indicates repulsion, and a negative value indicates attraction.

This law is strictly true for a vacuum and is approximately true for air. However, in many media the force between magnetic poles is quite different than in a vacuum. Hence the concept of permeability is introduced to measure the extent to which a medium would be permeable to the magnetic (lines of) force. Thus,

$$\text{force} = \frac{m_1m_2}{\mu r^2}$$

where  $\mu$  is the magnetic permeability of the medium in which the poles are located. For vacuum,  $\mu$  is taken to be unity, and this equation furnishes the definition for the unit magnetic pole stated earlier. The factor  $\mu$  may be regarded as a constant of proportionality, depending on the nature of the medium and the units used for the measurement of force, distance, etc. It may be noted that, in an analogous situation in electrostatics, one considers a constant  $k$ , called the specific inductive capacity or the dielectric constant, for measuring the force between two electric charges  $q_1$  and  $q_2$  placed  $r$  centimeters apart. In this case

$$\text{force} = \frac{q_1q_2}{kr^2}$$

Intensity of Magnetization. The amount of pole strength induced over unit area represents intensity of magnetization. Thus,

$$I = m/A$$

where  $m$  is the induced pole strength over a total area of  $A$  square centimeters. An alternative definition is obtained by multiplying the

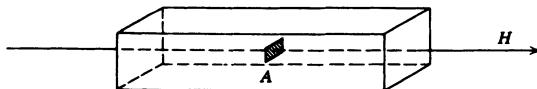


Fig. 2. Magnetization induced in unit area inside a sample in a magnetic field.

numerator and denominator by the distance  $l$  cm. This gives

$$I = \frac{m \cdot l}{A \cdot l} = \frac{\mu}{\text{Volume}}$$

that is, the magnetic moment per unit volume.

Gauss' Law and Magnetic Induction. According to the definition of field intensity, one unit line of force must pass through every square centimeter. If one considers a sphere of 1 cm radius (with surface area of  $4\pi \text{ cm}^2$ ) enclosing at its center a unit pole, it follows from the previous definition that  $4\pi$  unit lines of force emanate from a unit pole. Gauss' law states that the total magnetic induction over a closed surface is  $4\pi$  times the total amount of pole enclosed. Hence, for a pole  $m$ ,  $4\pi m$  maxwells emanate from its surface.

A bar of unmagnetized material when placed in a uniform magnetic field will become magnetized. Now consider a unit surface  $A$  within the material at right angles to the direction of applied field  $H$ , as in Figure 2. If  $I$  is the intensity of magnetization induced, there will be  $4\pi I$  unit lines of force across the unit surface. In addition to this, there will be  $H$  lines of force of the applied magnetic field superimposed on the induced magnetization. Therefore, the magnetic induction  $B$ , representing the total number of lines of force across the unit surface, is given by

$$B = 4\pi I + H$$

If there is a vacuum in place of the magnetic material, one expects that  $B = H$ , because the magnetic permeability for vacuum is taken as 1. It also follows that for a magnetic material of permeability  $\mu$ , the magnetic induction  $B$  will be given by

$$B = \mu H$$

Magnetic Susceptibility. The intensity of magnetization  $I$  induced at any point in a body is proportional to the strength of the applied field  $H$ :

$$\begin{aligned} I &\propto H \\ I &= KH \quad (\text{or } K = I/H) \end{aligned}$$

where  $K$  is a constant of proportionality, depending on the material of the body. It is called the magnetic susceptibility per unit volume and may be verbally defined as the extent to which a material is susceptible to (induced) magnetization. For an isotropic body the susceptibility is

the same in all directions. However, for anisotropic crystals, which are discussed in the next section, the susceptibilities along the three principal magnetic axes are different and measurements on their powdered samples give the average of the three values.

Obviously, magnetic susceptibility is related to the magnetic permeability, and the following relationships may be derived.

As shown in the preceding section,

$$B = \mu H = 4\pi I + H$$

$$\mu = 4\pi I/H + 1$$

$$\mu = 4\pi K + 1$$

or

$$K = (\mu - 1)/4\pi$$

It should be noted that, since  $K$  is a ratio of the intensity  $I$  of induced magnetization to that of the applied field  $H$ , the susceptibility  $K$  should be strictly a dimensionless quantity if  $I$  and  $H$  are measured in the same units. This situation is comparable to that of specific gravity, in which the ratio of the density of a material to that of water, both measured in the same units, is expressed as a number without units. However, magnetic susceptibility is still expressed in terms of cgs units, more as a matter of convention than of scientific thought. [Some writers use the letters emu (electromagnetic units) in place of cgs, whereas others use a combination (emu-cgs) to designate susceptibility.] This is so because there exist, to start with, uncertainties both in the nature of measurement and in the units of magnetic permeability that have led to confusion as to whether  $B$  and  $H$  are quantities of the same kind. In order to simplify matters, the convention of expressing susceptibility in some units will be followed here.

Mass (or Specific), Atomic, and Molar Susceptibilities. If  $\rho$  is the density, then the susceptibility for 1 cc or  $\rho$  g of the material corresponds to the volume susceptibility  $K$ . Hence, the susceptibility per gram of the material, called the mass or specific susceptibility  $\chi$ , is given by

$$\chi = K/\rho$$

The atomic susceptibility  $\chi_A$  and the molar susceptibility  $\chi_M$  are simply defined as the susceptibility per gram-atom and per gram-mole, respectively. Hence,

$$\chi_A = \chi \cdot \text{atomic weight}$$

$$\chi_M = \chi \cdot \text{molecular weight}$$

Some authors have used the term "molal susceptibility" to designate "molar susceptibility." The term "molal" does not seem to have any relationship to "molal concentrations" of solutions.

The magnetic susceptibilities are occasionally expressed in units of the rationalized Georgi system, based on the mks (meter, kilogram, second) system. For volume susceptibility  $K$  the ratio of units

$$\text{Georgi/cgs} = 4\pi$$

which makes the Georgi unit 12.56 times larger than the cgs unit. However, for conversion to mass susceptibility  $\chi$ , the Georgi system employs density in units of  $\text{kg/m}^3$ , making the ratio of units for

$$\text{Georgi/cgs} = 4\pi \times 10^{-3}$$

Magnetic Anisotropy. Many crystals, except those with cubic symmetry, show magnetic anisotropy. The principal susceptibilities, that is, the susceptibilities along three mutually perpendicular axes of principal magnetism, are different. An anisotropic crystal when suspended in a magnetic field is found to rotate in such a manner that the axis of maximum susceptibility in the plane of rotation sets itself parallel to the direction of the applied field.

In the ultimate analysis the anisotropy of the unit cell in the crystal has its origin in (1) the anisotropy of the molecule and (2) the geometry of the molecules in the unit cell. The anisotropy of a crystal containing anisotropic molecules will depend largely on their relative orientation. A preferred orientation of molecules, such as in parallel layers, results in maximum anisotropy, whereas a random orientation will give a small anisotropy or occasionally result in an isotropic crystal if the anisotropies of individual molecules cancel one another. However, if the molecule itself is anisotropic, the crystal will exhibit a feeble anisotropic character.

### PHYSICAL BASIS

The magnetic behavior of bulk matter often is explained in terms of the magnetic properties of the constituent molecules and atoms. In the ultimate analysis, one must consider the magnetic properties of the fundamental particles of matter. A discussion on these lines needs a thorough understanding of the electronic structure of atoms in terms of the language of physics in general and of spectroscopic nomenclature in particular. The reader is urged to clarify for himself the concepts of similar-sounding but often confusing terms, such as momentum and moment.

An attempt will be made here to present the physical basis of magnetic susceptibility by considering simply the magnetic effects arising from the two distinct types of motion of the electron, namely, its orbital rotation and spin around its axis. These effects often are expressed in terms of the turning moments or magnetic moments, although one does not know, in the classical sense, the precise magnitude of the strength of the poles of an electron and the distance separating them. The mag-

netic susceptibility arising from electrons often is called the "electronic susceptibility" to distinguish it from the susceptibility of the nucleus; the latter is of a very small magnitude ( $\sim 10^{-10}$ ). The susceptibility of the nucleus is detectable at very low temperatures and may be ignored relative to the electronic susceptibility.

If  $l$  is the orbital quantum number, that is, the angular momentum of the electron, the magnetic moment  $\mu_l$  for the orbital motion is given by

$$\mu_l = l \cdot e\hbar/4\pi mc$$

$$= l \text{ Bohr magnetons}$$

This orbital magnetic moment can be expressed as a vector opposite to that of  $l$  since the electron charge is negative.

Now, an electron spinning around an axis may be said to behave like a tiny magnet and to give rise to a magnetic moment. A theoretical value for the magnetic moment due to the spin of the electron cannot be derived, as nothing is known about the shape of an electron or its charge distribution. However, in order to obtain agreement with experimental results, and using wave mechanics, a spin magnetic moment  $\mu_s$  given by the following relations has been assigned to the electron:

$$\begin{aligned}\mu_s &= 2\sqrt{s(s+1)} eh/4\pi mc \\ &= 2\sqrt{s(s+1)} \text{ Bohr magnetons} \\ &= \sqrt{3} \text{ Bohr magnetons} = 1.62 \text{ erg-Oe}^{-1}\end{aligned}$$

Here  $s = \frac{1}{2}$  represents the spin angular momentum of the electron. In vectorial representation,  $\mu_s$  has a direction opposite to the mechanical moment  $s$ .

The observed magnetic moment arises from a combination of the orbital and spin moments. The resultant moment may be calculated theoretically by a vectorial addition of the two, taking into account various coupling mechanisms that arise in a system containing many electrons.

The effect of a magnetic field on different systems containing electrons may now be considered. Whether this effect will be one of attraction or of repulsion between the system and the applied field will depend on the presence or absence of unpaired electron(s) in the system. The ferric ion, for instance, is said to contain five unpaired electrons in its  $d$  shell, and a free radical such as 2,2'-diphenyl-1-picryl-hydrazyl is said to contain one unpaired electron somewhere inside the molecule. Hence, such systems have a permanent magnetic moment to start with and will be attracted appreciably toward an applied field. The magnetic moment of most paramagnetic systems may be expressed by the "spin only" formula and correlated with the magnetic susceptibility.

There can exist systems, comprised of practically all inorganic and organic compounds, which do not contain any unpaired electrons. In these, the magnetism due to the spin of one electron is cancelled out in some fashion by that of another spinning in an opposite direction; as such these systems do not have any permanent magnetic moment. One would then ordinarily expect no effect due to an applied field. However, a very feeble yet significant repulsion is observed. This diamagnetic behavior is attributed entirely to the effect of the magnetic field on the orbital motion of the (paired) electrons, and the susceptibility in this case may be correlated with the radii of these orbits.

According to the classical theory, an electron carrying a negative charge and moving in a circular orbit is equivalent to a circular current. If a magnetic field is applied perpendicular to the plane of the orbit, the revolving electron will experience a force along the radius, the direction of which depends upon that of the magnetic field and of the moving electron. Application of the well-known Lenz's law, which predicts the direction of motion of a current-carrying conductor placed in a magnetic field, to this situation shows that the system as a whole will be repelled away from the applied field. An elaborate mathematical picture is presented by the Larmor theorem (cf. Ref. 44), which describes the behavior of a system of particles, all having the same ratio of charge to mass, in the presence of a constant uniform field. According to this theorem, the superimposed field leaves the form of the orbits and their inclination to the magnetic lines of force, as also the motion in the orbit, unaltered and merely leads to the addition of a uniform "precession" of the orbit about the direction of lines of force. At this juncture, it will be most appropriate to clarify the meaning of precession as applied to the orbital and spinning motions of an electron. Of these, the latter is rather easy to visualize by its comparison with the behavior of a spinning top or a gyroscope. As shown in Figure 3, when a top originally spinning erect around an axis is subjected to an external force  $F$ , it does not topple over completely but continues to

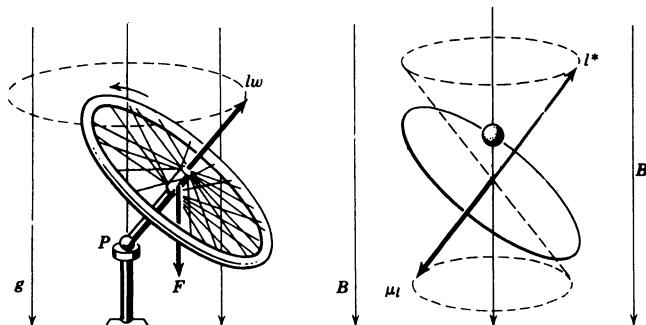


Fig. 3. A mechanical top precessing in a uniform gravitational field  $g$  is analogous to an electron orbit precessing in a uniform magnetic field. Reproduced by permission of Holt, Rinehart and Winston (New York) from Semat and White, Atomic Age Physics (1959).

spin around its own axis  $PW$ , while this axis itself keeps on rotating in an orbit around the axis  $P$ . This characteristic motion of the axis is called precession. This spin precession indeed arises in the case of a free (unpaired) electron subject to a magnetic field and forms the basis of the electron paramagnetic resonance techniques. Similarly, the precession of spins of certain nuclei, such as protons, for example, forms the basis of nuclear magnetic resonance methods. It should now be possible to visualize the precession of the entire orbit of an electron around the direction of the applied field, as shown in Figure 3. It is this type of orbital precession of an electron that gives rise to diamagnetism and forms the core of the mathematical theory of diamagnetism.

It is beyond the scope of this discussion to derive any equations for the atomic or molecular susceptibilities. However, some of the equations derived from classical and quantum mechanical considerations will be given here.

#### TYPES OF MAGNETISM OBSERVED

Tables I and II summarize several aspects of the common and special types of magnetic behavior,<sup>29</sup> some of these are described below.

##### Diamagnetism

This term refers to the phenomenon in which the intensity of magnetization induced in a body by an applied field is less than that produced in a vacuum by the same field. In practice, the net effect manifests itself as one of repulsion between the body and the applied field. Hence, the susceptibilities are shown with a negative sign. Such substances are called diamagnetics. Practically all organic and inorganic compounds with the exception of free radicals and compounds of transition elements are diamagnetic. Diamagnetic susceptibility is independent both of temperature and of the applied field. Any significant change in diamagnetic susceptibility with temperature in most cases may be attributed to a change in the physical or chemical structure of the material.

It will be appropriate to consider atomic and molecular diamagnetism separately and to point out the limitations in extending the classic derivation for the susceptibility of an atom to that of a molecule.

##### *Atomic Diamagnetism*

According to the classical theory of Langevin,<sup>44</sup> the susceptibility per gram-atom is given by

$$\chi_A = -\frac{Ne^2}{6mc^2} \sum r_i^2$$

TABLE I  
Types of Magnetic Behavior Commonly Observed

Type	Effect of external field on substance	Examples	Comments on Origin	Magnitude of specific susceptibility $\chi$ at 20°C	Dependence of susceptibility on temperature	Dependence of susceptibility on field
Diamagnetism	Feeble repulsion $I < H$	Most inorganic compounds, except those containing ions of transition elements. Organic compounds except free radicals. Certain compositions like stainless steel, special Cu-Ni alloys (e.g., 5-cent coin).	Caused by orbital motion of electron(s). Hence, it is a universal property. Most perceptible when all electrons are "paired," that is, when they have no permanent "spin" moment.	Negative and very small ( $\sim 1 \times 10^{-6}$ ).	None theoretically. Small dependence attributable to change in state of aggregation of system with temperature.	None
Paramagnetism	Attraction $I > H$	Salts and certain complexes of transition elements, "odd" electron molecules like $\text{NO}_2$ and oxygen. Free radicals such as triphenyl methyl.	Caused by spin and (usually) orbital momentum of (unpaired) electrons. The system contains permanent magnetic dipoles (moments) without interaction.	Positive and small ( $\sim 100 \times 10^{-6}$ ). It is sufficiently large to mask the underlying diamagnetism.	$\chi \propto 1/T$ (Curie law) or $\chi \propto 1/(T + \Theta)$ (Curie-Weiss law)	None
Ferromagnetism	Intense attraction $I \gg H$	Metals like iron, cobalt, nickel, and their alloys. $\gamma\text{-Fe}_2\text{O}_3$ .	Caused by 'domains' or lattice of particles containing electrons with parallel spins. Positive interaction among dipoles.	Positive and very large ( $\sim 1 \times 10^2$ )	Beyond a certain temperature (Curie point) magnetism drops and shows paramagnetic behavior.	Dependence described by hysteresis curves.

TABLE II  
Special Types of Magnetic Behavior

Type	Effect of external field on substance	Examples	Origin	Magnitude of susceptibility at 20° C (approx.)	Dependence of susceptibility on temperature
Temperature-independent or Van Vleck paramagnetism	Feeble attraction	KMnO <sub>4</sub> , Co (III) ammines	Atom with upper state separated from ground state by energy interval large compared to kT. System has no permanent magnetic moment.	Positive and very small ( $\sim 1 \times 10^{-6}$ ).	None.
Pauli or free electron paramagnetism.	Feeble attraction	Metallic K and Na (vapors)	Paramagnetism of an "electron gas."	Positive and very small ( $\sim 1 \times 10^{-5}$ ).	Very slight, generally for vapors $\chi = 1/T$ (Curie law).
Antiferromagnetism	Feeble attraction	KNiF <sub>3</sub> , MnSe, Ti <sub>2</sub> O <sub>3</sub> , ferrites	Two lattices of particles having electron spins in one lattice antiparallel to those in another lattice. Negative interaction among magnetic dipoles.	Positive and very small ( $\sim 1 \times 10^{-7}$ to $1 \times 10^{-5}$ ).	Complex dependence. Up to a critical temperature * magnetization increases with temperature, then it decreases.
Ferrimagnetism	Feeble attraction	FeCr <sub>2</sub> O <sub>4</sub> , ferrites	Interpenetrating lattices with unequal numbers of electrons with antiparallel spins. Simultaneous unequal interaction among dipoles.	Positive and small ( $1 \times 10^{-3}$ ).	Positive dependence.
Metamagnetism. † It shows field-strength dependence.	Feeble attraction	NiCl <sub>2</sub> or CoCl <sub>2</sub> at liquid H <sub>2</sub> temperature	Parallel or antiparallel alignment of moments in domains	Positive and small ( $1 \times 10^{-3}$ ).	Positive dependence.

\* Antiferromagnetic Curie point or Néel temperature.

† May be regarded as a special case of antiferromagnetism with low Néel temperature.

where  $e$  and  $m$  refer to the charge and mass of the electron, respectively,  $c$  is the velocity of light,  $N$  is Avogadro's number, and  $\bar{r}_i^2$  is the sum of the mean square radii of the orbit of the  $i$ th electron projected perpendicular to the direction of the applied field. Diamagnetism thus depends only on the effective radius of the electronic orbits; the electrons in the outermost orbits contribute most to the atomic susceptibility. Langevin's<sup>44</sup> equation also shows diamagnetism to be independent of temperature, but this is true in practice only to a first approximation. In many cases an increase in temperature causes a change in intramolecular forces, in hydrogen bonding, etc. This is sufficient to cause small changes in the diamagnetism. The negative sign of diamagnetism is a consequence of the Larmor precession; as such, all atoms possess the universal property of diamagnetism. It must be emphasized that the equation does not apply if the atom or ion contains a free unpaired electron or is not in a spherically symmetrical state.

The result of Van Vleck's quantum mechanical treatment<sup>21</sup> for the atomic susceptibility  $\chi_A$  may be mentioned here briefly before proceeding to a discussion of molecular susceptibility:

$$\chi_A = - \frac{Ne^2}{6mc^2} \left( \frac{\hbar^2}{4\pi^2 Ze^2 m} \right)^2 \left[ \frac{5}{2} n^4 l(l+1) - \frac{3}{2} n^2 l(l+1) + \frac{1}{2} n^2 \right]$$

where  $n$  and  $l$  are the principal and subsidiary quantum numbers, respectively,  $Z$  is the atomic number,  $\hbar$  is Planck's constant of action, and other terms have the same meaning as before.

#### *Molecular Diamagnetism*

Langevin's theory<sup>44</sup> is strictly applicable to mononuclear systems; this implies that the electrostatic potential should be symmetrical about an axis parallel to the magnetic field. This stipulation is easily fulfilled by atoms; however, in considering molecules, the classical treatment may be extended only to linear molecules in the  $S$  state, on the following lines. The magnetic field is presumed to act along the molecular axis in the  $z$  direction, so that the cloud of electrons may be said to rotate freely around this axis with an angular velocity of  $eH/2mc$ . This gives

$$\chi_M = - \frac{Ne^2}{4mc^2} \sum \overline{x_i^2 + y_i^2}$$

Langevin's theory cannot be applied to other molecules, as the electric field acting on the electrons ceases to be spherically symmetrical. This is so because, in the classical treatment, if one refers the motion of electrons adequately to a rotating frame of coordinates, one cannot apply the equations of motion of a nonrotating system to a rotating one. Here the fixed nuclei, other than the one taken as the

origin, produce an electric field that changes with rotation around the origin. Any electrical asymmetry about the direction of the magnetic field will hinder free circulation of the electrons and will thus reduce the total diamagnetism.

Van Vleck<sup>21</sup> has treated this particular problem in terms of quantum mechanics and the reduction in diamagnetism is ascribed to a mixing of the wave function for the ground state with that of some of the excited states brought about by the magnetic field.

It may be now pointed out that the calculation of susceptibility even for the simplest element, hydrogen, is formidable, because the wave functions are not known. The first and second terms for hydrogen are  $-4.71 \times 10^{-6}$  and about  $+0.51 \times 10^{-6}$ , respectively, which gives  $\chi_M = -4.20 \times 10^{-6}$ . This is in fairly good agreement with the experimental value of  $-4.005 \times 10^{-6}$ , determined by Havens<sup>45</sup> and generally accepted as the most accurate value.

A major problem in the application of these equations is in computing  $\Sigma r^2$ . Several workers, notably Stoner,<sup>46</sup> Pauling,<sup>47</sup> and Slater,<sup>48</sup> have suggested theoretical and semiempirical derivations for this purpose.

It should be noted that an approximate method<sup>3</sup> for correlating the molar susceptibilities of isoelectronic compounds has been shown by this author<sup>49</sup> to be without any significance and to fail in many cases.

### Paramagnetism

If the intensity of induced magnetization is greater in a substance than the applied field in vacuum, the substance is called paramagnetic and the phenomenon of attraction of the substance towards the magnetic field is observed. It is indicated by a positive sign. Paramagnetism occurs especially among the transition-group elements. Such substances are called paramagnetics. It may be noted that all substances, even though paramagnetic, have an underlying diamagnetism, because such diamagnetism is a universal property; however, the magnitude of paramagnetism that manifests itself as a force of attraction is usually so great that it masks the feeble underlying and opposing diamagnetism. Paramagnetic susceptibility is independent of the applied field, but often is inversely proportional to the temperature. In some special cases paramagnetism independent of temperature arises.

For the sake of clarity, this topic is discussed under two categories, (1) normal, temperature-dependent, and (2) temperature-independent paramagnetism, although a reference to the second must invariably be made in discussing the first category and, as seen before, in discussing molecular diamagnetism.

#### Normal Paramagnetism

According to the classical theory of Langevin,<sup>44</sup> the molar susceptibility is

$$\chi_M = N \mu^2 / 3kT$$

where  $\mu$  is the permanent moment,  $T$  is the absolute temperature,  $N$  is Avogadro's number, and  $k$  is Boltzmann's constant. P. Curie had established experimentally prior to Langevin's work that the paramagnetic susceptibility  $\chi_M$  is inversely proportional to the absolute temperature  $T$ , and the expression

$$\chi_M = C/T$$

is known as Curie's law;  $C$  is called the Curie constant.

Curie's law is generally applicable to a magnetically dilute system, that is, one in which magnetic interactions between neighboring molecules is negligible.

P. Weiss introduced the concept of a molecular field to account for molecular interactions and proposed the following modification:

$$\chi_M = C/(T + \theta)$$

where  $\theta$  is a constant known as the "Weiss constant." The magnetic susceptibility of many compounds is much better expressed by the Curie-Weiss law than by the Curie law alone. The significance of this is discussed by Selwood.<sup>5</sup>

More precise derivations for  $\chi_M$  include a term for the underlying diamagnetism. However, for all practical purposes this term, being small, may be neglected.

In paramagnetism, the magnetic moment  $\mu$  may be regarded as a fundamental quantity relative to susceptibility, which varies inversely with temperature. Hence, in a comparison of experimental and theoretical results, the values of "effective Bohr magneton numbers"  $\mu_{\text{eff}}$  and the theoretical values may be used;  $\mu_{\text{eff}}$  is obtained very simply from Langevin's equation

$$\mu_{\text{eff}} = \sqrt{3k \chi_M T / n \beta^2} = 2.48 \sqrt{C}$$

where  $\beta$  is the Bohr magneton and  $n$  is the number of unpaired electrons.

The magnetic moment  $\mu_{\text{eff}}$  on the basis of classical theory may be calculated from

$$\mu_{\text{eff}} = g \sqrt{J(J + 1)}$$

in Bohr magneton units. Here  $g$  is the Landé splitting factor and  $J$  is the resultant angular momentum, which is a vector sum of  $L$ , the total angular momentum of the orbital motion of the electrons, and  $S$ , the corresponding spin angular momentum. For atoms in the  $S$  state, for which the orbital moment  $L = 0$ , the magnetic moment is entirely due to the electron spins, so that  $J = S$  and  $g = 2$ , giving

$$\begin{aligned} \mu_{\text{eff}} &= 2 \sqrt{S(S + 1)} \\ &= \sqrt{2S(2S + 2)} \\ &= \sqrt{2ns(2ns + 2)} \end{aligned}$$

TABLE III  
Theoretical and Effective Bohr Magneton Numbers  
for Ions of First Transition Group

Ion	3d electrons	Term	$\frac{\mu_{\text{eff}}}{\sqrt{n(n+2)}}$	$\mu_{\text{eff}}$ (obs.)
$\left. \begin{matrix} \text{Sc}^{+3} \\ \text{Ti}^{+4} \\ \text{V}^{+5} \end{matrix} \right\}$	0	$1S_0$	0.0	0.0
$\left. \begin{matrix} \text{Ti}^{+3} \\ \text{V}^{+4} \end{matrix} \right\}$	1	$2D_{3/2}$	1.73	1.77-1.79
$\text{V}^{+3}$	2	$3F_2$	2.83	2.76-2.85
$\left. \begin{matrix} \text{V}^{+2} \\ \text{Cr}^{+3} \\ \text{Mn}^{+4} \end{matrix} \right\}$	3	$4F_{3/2}$	3.87	3.68-4.00
$\left. \begin{matrix} \text{Cr}^{+2} \\ \text{Mn}^{+3} \end{matrix} \right\}$	4	$5D_0$	4.90	4.80-5.06
$\left. \begin{matrix} \text{Mn}^{+2} \\ \text{Fe}^{+3} \end{matrix} \right\}$	5	$6S_{1/2}$	5.92	5.2-6.0
$\text{Fe}^{+2}$	6	$5D_4$	4.90	5.0-5.5
$\text{Co}^{+2}$	7	$4F_{9/2}$	3.87	4.4-5.2
$\text{Ni}^{+2}$	8	$3F_4$	2.83	2.9-3.4
$\text{Cu}^{+2}$	9	$2D_{5/2}$	1.73	1.8-2.2
$\left. \begin{matrix} \text{Cu}^+ \\ \text{Zn}^{+2} \end{matrix} \right\}$	10	$1S_0$	0.0	0.0

Finally, this value equals  $\sqrt{n(n+2)}$ , because  $S$ , the total spin momentum, is equal to the total number  $n$  of the unpaired electrons times  $s$ , the spin momentum for each electron, which is  $\frac{1}{2}$  quantum unit. This formula is applicable to some ions of the transition-group of elements. Moments calculated on the basis of this "spin only" formula are given in Table III for the transition metal ions, some of which play an important role in biological matter.

It may be mentioned briefly now that, in the Langevin-Debye formula,<sup>21</sup>

$$\chi = N\mu^2/3kT + \alpha$$

the term  $\alpha$  was introduced to account for magnetic effects other than those arising from the permanent magnetic moment of a molecule.

#### Temperature-Independent Paramagnetism

It will now be in order to point out the physical significance of the temperature-independent term in Van Vleck's equation. One may im-

agine a resultant magnetic moment  $\mu_R$  precessing regularly around a direction  $J$  in the absence of a field, but when  $J$  itself is made to precess about an applied field  $H$ , the precession of  $\mu_R$  ceases to be symmetrical and gives rise to a small increase in the magnetization parallel to  $H$ . An analysis of this situation shows this increase to be independent of temperature for a given  $L, S, J$  state.

Van Vleck's expression refers only to atoms with Russell-Saunders coupling, that is, in those cases in which the spin angular momenta  $s$  of the electrons can combine to form a resultant  $S$  and the orbital angular momenta  $l$  of electrons combine independently to give a resultant  $L$ . Application of Van Vleck's theory<sup>21</sup> to various paramagnetic systems is rather complex, but it accounts for departures from the Curie-Weiss law. It depends entirely on the magnitude of the spin multiplet intervals as compared to the Boltzmann distribution factor  $kT$ . This gives rise to three specific situations, in which the spin multiplet intervals may be (1) small, (2) great, or (3) nearly equal as compared to  $kT$ . Of these, the first can be described by a relatively simple equation and will be of special significance. This gives

$$\chi_M = \frac{N\beta^2}{3kT} [4S(S+1) + L(L+1)]$$

The expression is used for calculating susceptibilities of ions of most transition-group elements (see Table III). Note that for ions in the  $S$  spectroscopic state,  $L=0$ , this gives the well-known "spin only" formula  $\mu_{\text{eff}} = \sqrt{n(n+2)}$ . (For other cases and significance of  $kT$ , reference should be made to a chapter by the author in Ref. 43.)

Van Vleck's theory and its recent modification<sup>(20)</sup> are quite satisfactory in that they explain a number of experimental facts. According to the theory, Curie's law should be obeyed in the limiting cases in which the multiplet intervals are large or small compared to  $kT$ , neglecting the temperature-independent paramagnetic contribution to susceptibility arising from high-frequency elements. When the multiplet intervals are comparable to  $kT$ , a Boltzmann distribution of the normal states occurs, which results in serious departures from the Curie-Weiss law.

### Ferromagnetism

This falls under the category of attraction between a substance and an applied field; however, the forces of attraction are very great as compared with those of paramagnetism. Ferromagnetism is quite field-dependent, and ferromagnetic substances show typical hysteresis curves. Hence, many ferromagnetic properties are measured at saturation, that is, by using high applied fields that cannot bring about any further increase in the intensity of induced magnetization at a given temperature. Ferromagnetic materials, when heated, start losing their magnetism gradually. Beyond a critical temperature, called the Curie

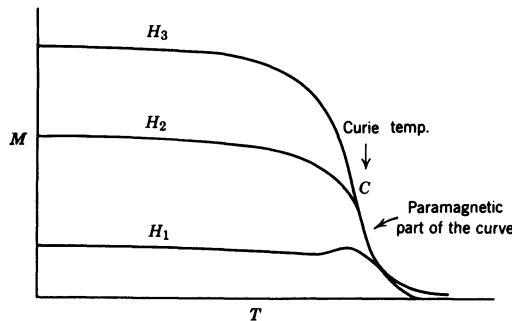


Fig. 4. Intensity of magnetization  $M$  vs. temperature at three different fields for a ferromagnetic substance.

temperature or the Curie point, these start behaving as regular paramagnetics (Figure 4).

In nature, ferromagnetism is restricted to only a few metals such as iron, although for several technological applications a number of alloys and compounds have been prepared. It must be noted that the term ferromagnetism and a new term "ferrimagnetism," suggested by Néel to indicate ferromagnetism arising from atoms in two kinds of sites, have no relation with the valence-state nomenclature such as ferro(cyanide) and ferri(cyanide) employed by chemists.

Ferromagnetism is by far the most complex area of magnetism. It cannot be defined simply, but may be understood by its comparison with paramagnetism and in relation to the dependence of the intensity of magnetization both on the applied field and on the temperature.

The magnetization induced varies directly with the applied field in a ferromagnetic material and may become a thousand times greater than its value at lower field strengths; however, with certain limiting values for the applied field that can be obtained rather easily, a magnetic saturation is produced. Hence, it is necessary to consider specific magnetization or the magnetic susceptibility of such materials at or above the saturation point. The induction as a function of the applied field shows the typical hysteresis curve and the phenomenon of residual magnetism. In this sense ferromagnetics are materials that can be permanently magnetized.

The magnetization produced at different values of magnetic fields may be destroyed by increasing the temperature. However, even here ferromagnetics show the typical behavior shown in Figure 4, in that at the Curie temperature the drooping curve up to the Curie point changes suddenly into the hyperbolic curve, which represents the characteristic behavior of a paramagnetic.

In ferromagnetism, it is necessary to distinguish between the susceptibility moment,  $\mu = n(n+2)$  Bohr magnetons, and the saturation moment, which is the maximum component of the magnetic moment in

the direction of the applied field, given by  $\mu = n$ , where  $n$  is the number of unpaired electrons. The saturation moment is obtained from specific magnetization  $\sigma$ , studied both as a function of temperature and of applied field strength. Extrapolation of the  $\chi$  vs.  $T$  curve to  $T = 0$  gives values for  $\sigma_0$  corresponding to different fields. These, when plotted against reciprocal field and extrapolated to zero (which amounts to finding  $\sigma_0$  at infinite or "saturation" field), give the value for  $\sigma_{0,\infty}$ , that is, for magnetic moment per unit mass. From this, the magnetic moment per gram-atom is computed.

It is important to recognize that, whereas paramagnetism is an atomic or a molecular property, ferromagnetism is a group effect. The ferromagnetic moment arises from electron spins only; their orbital contribution is completely quenched. Elements such as iron, cobalt, and nickel with incomplete low-energy levels, such as the  $3d$  level, are ferromagnetic. The  $4f$  level in gadolinium and other rare earths also is expected to produce the same effect; however, thermal vibrations prevent a complete alignment of spin moment. In the group behavior of moments, therefore, a strong interaction between adjacent atoms or ions couples their moments parallel to each other in spite of thermal agitation, and ferromagnetism is produced. The nature of this interaction is discussed in the theories of Heisenberg, Zener, and in the domain theory of Néel. The scope of this work does not permit even a brief discussion of these theories.

### Antiferromagnetism

In a few compounds such as titanium sesquioxide, the induced magnetism increases with temperature up to a critical point called the "antiferromagnetic Curie temperature" or Néel point, beyond which the compounds behave like normal paramagnetics (Figure 5). This phenomenon is called antiferromagnetism and is treated as a special case of ferromagnetism.

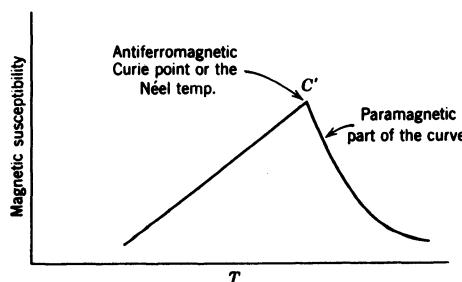


Fig. 5. Magnetic behavior of a typical antiferromagnetic.

## BASIC LAWS

The fundamental inverse square law of Coulomb, which forms the basis for defining magnetic parameters, and the Curie-Weiss law, describing para- and ferromagnetic behavior, were considered in the previous sections. Other laws of a more direct practical application will be discussed here.

### Additivity of Atomic Constants

P. Pascal studied a large number of gases and organic, metalloid, and complex compounds. The data he collected and the constants he derived for atomic susceptibilities on the assumption of an additivity law indeed stand out as a pioneering contribution to magnetochemistry. According to him, the molecular susceptibility  $\chi_M$  of a compound could be expressed by

$$\chi_M = \sum n_A \cdot \chi_A + \lambda$$

where  $n_A$  is the number of atoms of susceptibility  $\chi_A$  in the molecule and  $\lambda$  is a constitutive correction constant depending on the nature of the chemical binding between the atoms. For ions, it is assumed that

$$\chi_M = \chi_{\text{cation}} + \chi_{\text{anion}}$$

which invalidates the necessity of a correction constant.

It must be pointed out that the derivation of Pascal's constants is purely empirical and is the result of a judicious mathematical juggling of numbers. Several attempts<sup>50,51</sup> have been made to attach a theoretical significance to the constitutive correction constant, and although some of these<sup>52</sup> have been partially successful, they have failed to change the empirical nature of Pascal's constants.<sup>53</sup> Nevertheless, their usefulness lies in obtaining diamagnetic susceptibility corrections which cannot otherwise be estimated for paramagnetic systems such as the free radicals, ions in solution, etc., and in allowing comparisons between the theoretical and experimental values of susceptibilities of atoms, ions, etc.

The values for susceptibilities of atoms and the constitutive corrections constants are compiled in many books.<sup>32,37,43</sup> This author<sup>49</sup> showed conclusively that the magnitude of the susceptibility of a cation depends on the nature of the anion and vice versa.

### Wiedemann's Law

The mass susceptibility  $\chi$  of a mixture of components with susceptibilities  $\chi_1, \chi_2, \dots, \chi_n$  and weight fractions  $p_1, p_2, \dots, p_n$  may be expressed by

$$\chi = \chi_1 p_1 + \chi_2 p_2 + \dots + \chi_n p_n$$

The law is obeyed quite closely by mechanical mixtures and solutions of diamagnetic substances in which little or no interaction takes place either between molecules or ions of the components or between these and the solvent. This stipulation makes it imperative that caution be exercised in deducing the susceptibility of a solute from that of the solution. The application of the law to solid or liquid solutions containing paramagnetic ions becomes even more difficult as the interactions among ions or between ions and the solvent become quite pronounced. In such cases it is, therefore, necessary to ascertain that the system is "magnetically dilute."

### MEASUREMENT OF MAGNETIC SUSCEPTIBILITY

Techniques for the measurement of magnetic susceptibility have been described in many texts.<sup>3,5,17</sup> A detailed description of these techniques which should be of special interest for biomagnetic work is given by this author elsewhere.<sup>43</sup> An extensive review of modifications of the classical methods and of some new methods is also given by this author.<sup>35,36</sup>

### ACKNOWLEDGMENTS

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## *Chapter 4*

# The Vector Character of Field and Gradient and Its Possible Implications For Biomagnetic Experiments and Space Travel

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In the Introduction, a survey was made of the different physical phenomena which through their cumulative physical effects could be considered as precursors of biomagnetic effects. As we have seen there, the overwhelming majority of these cumulative effects is reversible, meaning that a change in the direction of the field or gradient vector relative to the coordinate system of the specimen (organ, cell, molecule) will entail a change in the direction or a reversal of the sign of the cumulative effect.

Some of the enumerated irreversible cumulative effects can be easily discounted. For example, the average traveling speed of a laboratory mouse seldom exceeds 10 cm/sec and its acceleration 20 cm/sec<sup>2</sup>. In a homogeneous field of 10 kOe, the polarization current would be of the order of  $10^{-8}$  A/cm<sup>2</sup>; and in an inhomogeneous field with a gradient of 1000 Oe/cm the conduction current would be of the order of  $10^{-6}$  A/cm<sup>2</sup>. These currents will merely persist during displacement and acceleration, respectively. Considering the well-known biological effects of distributed electric currents, it is highly improbable that currents of such low specific intensity and short duration should produce biological effects through the Joule heat they generate. Similarly, the adiabatic temperature increase due to the magnetocaloric effect in, say, ferrohemoglobin when introduced in a field of 10 kOe is of the order of  $10^{-2}$ °C. It is several magnitudes lower for most other paramagnetic substances found in the body. Ferromagnetic substances are normally not present in biological systems. It is unlikely that such small temperature changes would have any biological effect on organisms, the temperature of which is subject to a much wider variation.

It is reasonable to assume that cumulative physical effects have to reach a certain magnitude before they can trigger a biological process. Whenever the cumulative physical effect is reversible, this require-

ment can be fulfilled only if the specimen (organ, cell, molecule) remains in an unaltered position relative to the field or gradient vector. At first glance it would seem, therefore, that to obtain biomagnetic effects in living organisms, animals would have to be restrained during magnetic treatment and other motile specimens (e.g., bacteria) immobilized. This is, however, not always necessary.

In a cage with smooth walls and ceiling, the motility of unrestrained laboratory animals, particularly that of mice, rats, and guinea pigs, will be restricted to movements in the horizontal plane and rotations around a vertical axis. Mice never rest or sleep in a supine position. Other animals (squirrel monkeys) may rest in dorsal position, but seldom for a long time. It is, therefore, sufficient to place unrestrained animals in a magnetic field with vertical field and gradient vectors in order to ensure that the animal remains in a constant position relative to the field and gradient vector. Hence we should expect a difference between the results of experiments executed in vertical and in horizontal magnetic fields.

In the literature only a few experiments can be found where unrestrained animals were exposed to strong horizontal fields. The experiments described in Part II, Chs. 5-6 gave positive results, seemingly contradicting our argument. But we have to note that in these experiments the mice were housed in cages which had a floor area of only  $1\frac{7}{8} \times 1\frac{7}{8}$  in. The length of an adult mouse is about 3.5 to 4 in. Although a mouse can turn around in such a small cage, it presumably was motionless most of the time. Furthermore, in my experience mice confined to such small quarters shortly become accustomed to resting always in the same position, determined by light, air currents, place of food, water supply, etc.\*

In the U. S. Naval School of Aviation Medicine a squirrel monkey is being treated in a large electromagnet with horizontal field direction;<sup>1</sup> up to now no results have been reported.

In the Lawrence Radiation Laboratory of the University of California a large number of mice were treated in the 14-kOe homogeneous magnetic field of a large water-cooled solenoid.<sup>2</sup> These experiments were intended to repeat our results.<sup>3</sup> They could not find any biomagnetic effect on ascites tumor growth *in vivo*, red and white blood cell count, and growth rate of young mice. We wish to note that for Ehrlich ascites tumors we do not have comparable experiments and that the erythrocyte count does not change in homogeneous fields. But I think that the decrease in the leukocyte count and the retardation of the growth rate belong among the best-established biomagnetic effects (see Part II, Chs. 1 and 3). The authors of the above paper<sup>2</sup> do not mention any-

\*I used this peculiarity of mice when I treated the spontaneous mammary gland carcinoma of the C3H strain. The special pole caps had to be positioned accurately with respect to the tumor. To provide some freedom of motion for the animal during the long treatment, the mouse was placed in a horizontal cylinder  $1\frac{1}{2}$  in. in diameter and  $3\frac{1}{2}$  in. long, in which it could turn around. Nevertheless, since light and air current came from one side of the tube, 99% of the time it remained in the same position, facing the same end of the tube.

where in the text the direction of the magnetic field; but one can see from the picture of the magnet that it was horizontal. The use of a horizontal magnetic field seems to have been rather accidental.

A comparison of the negative results of the Lawrence Laboratory group obtained by using a horizontal field with our positive results obtained in vertical fields (both made on unrestrained mice) indicate that the retardation of the growth rate and the change in the leukocyte count are biomagnetic effects initiated by a reversible physical precursor, belonging in the phenomenon group (a), (b), or (c), but not in group (d). (The letters refer to the classification of physical phenomena as given in the Introduction.)

A closer scrutiny of the data of the Lawrence Laboratory group reveals that around the 11th day of residence in the field, the weight of the mice in the treated group was also less than that of the mice in the control group. The difference in weight (using the 10th, 11th, and 12th day weight averages) is  $-0.54 \pm 0.45$  g in the experiment with 40 mice, and  $-0.70 \pm 0.83$  g in the experiment with 20 mice. Although the weighted average  $-0.59 \pm 0.08$  g is significant only on a probability level of 1:12, it suggests that the effect of growth retardation with a maximum weight difference around the 11th day was present, but attenuated by a factor of about three.

Similarly, we see that the leukocyte count on the 16th day was significantly higher than on the following days. M. F. Barnothy found a transient maximum in the leukocyte count around the 16th day of residence in the field (see Part II, Ch. 3). Unfortunately, no data on leukocyte count are given by the Lawrence Laboratory group for the time preceding the 16th day of residence in the field; but the high value on the 16th day suggests that a change in the leukocyte count was also present there, but attenuated.

It is easy to understand that one obtains with unrestrained animals in a horizontal field not an annulment, but merely an attenuation of biomagnetic effects. The motion of animals is quite random; thus opposite orientations will not prevail for the same length of time and will not completely cancel out reversible cumulative effects. It is also reasonable to assume that some reversible effects may develop to a magnitude necessary to trigger biological effects during time intervals which are short compared to the intervals during which the specimen changes its position relative to the field vector. These considerations would imply that two critical frequencies would exist regarding the rate of directional changes: one frequency above which no biological effect could develop because the cumulative reversible effects annul each other before reaching the necessary magnitude; and another, lower critical frequency below which no attenuation of the biological process will occur because the reversible effect had sufficient time between two directional changes to reach a magnitude to trigger biological functions. Between these two frequency limits an attenuation of biomagnetic ef-

fects could be expected. Should the attenuation be governed by statistical laws, it should be proportional to the square root of the number of complete directional changes.

Of course, a further complication could arise if the triggered biological effect itself is reversible, or if it changes its sign during a sustained influence of a constant physical effect, or if it occurs only if the physical effect has a definite direction.

The experiments described in Part III, Ch. 3 likewise seem to support the reversible precursor assumption. Bacterial cells in culture broth constantly change their position as a result of Brownian motion and also because of their own motility. However, when the bacterial cell is para- or diamagnetic relative to its surrounding—as it most probably is—and at the same time the cell is not spherical, but rod-shaped, then the homogeneous part of the field vector could orient the cells and keep them in constant position relative to the gradient vector, or at least dampen their random rotations. Indeed, it was found that the inhibiting effect of the field was observed on the rod-shaped bacteria at a far lower paramagnetic strength than on the spherically shaped bacteria. This result would indicate that the physical phenomenon which caused a reversible precursor must belong among the group (d) phenomena.

From the foregoing considerations it follows that changing the direction of the field vector or the direction of the gradient vector independently of each other would offer us a possibility of distinguishing between precursors belonging in the (a), (b), (c), or in the (d) group, and to distinguish between reversible or irreversible physical precursors. Finally, by changing the rate of direction changes we could estimate the time necessary for the cumulative physical effect in question to develop to a magnitude adequate to initiate the biological process.

In an electromagnet the polarity of the field vector can be easily reversed by reversing the current direction.

In an inhomogeneous field, the direction of the accelerating force acting on elementary dipoles and para- and diamagnetic particles is determined by the direction of the gradient vector alone and is independent of the direction of the field vector. The direction of the gradient vector is determined by the shape of the pole caps. It is not reversed when the field polarity is reversed. To change the direction of the gradient vector we therefore have to exchange the pole caps. This can be done either manually or, when higher reversion rates are desired, by mounting the two pole caps on a cylindrical nonmagnetic support, which is automatically turned at prechosen times. To facilitate turning, the field could be temporarily reduced to zero, but would otherwise maintain its polarity and strength during the experiment.

The field produced by a coil or solenoid is the same in strength and direction at the symmetrical points situated on the axis on opposite sides of the center of the coil. The magnitude of the gradient vector is

also the same, but, since its direction is always toward the center of the coil, it is opposite on the two sides. By switching the location of a specimen from one side of the coil to the symmetrical position on the other side (either by changing the relative position of coil and specimen, or by using two coils situated symmetrically on the two sides of the specimen but energizing them alternately) the direction of the gradient vector can be reversed without changing the magnitude and direction of the field vector.

A continuous change in the direction of the gradient vector can be achieved in an electromagnet with a gap which gradually narrows from one side to the other and produces a gradient perpendicular to the field vector. When now the pole caps are turned around an axis parallel to the field vector, the direction of the gradient vector will also rotate around the field direction, whereby in the middle portion of the gap the magnitude of the field will remain unchanged.

#### APPLICATIONS TO SPACE TRAVEL

In space travel the astronaut may be exposed for long periods to strong extraterrestrial magnetic fields. If the spaceship is propelled by ion-jet engines, the astronauts will be exposed to the strong stray fields of the magnets necessary to confine the plasma. The reversible precursor theory suggests that the possible detrimental effects of long-term exposures to magnetic fields can be overcome by periodically changing the positions of the astronauts relative to the external or internal magnetic field and gradient:

1. If only an external magnetic field is present, a slow rotation of the entire spaceship, or space station, around an axis perpendicular to the direction of the external field would suffice. The axis of rotation should be oriented with the help of a servosystem, activated by suitable sensing devices, monitoring the direction of the external field.
2. If the magnetic field is produced in the spaceship or the space station, the astronaut, or the capsule in which the crew lives, should be slowly rotated around an axis perpendicular to the internal field.
3. The gradient of any external field will presumably be extremely small. But, if the paramagnetic strength of the internal field exceeds 1 par, the astronauts should also be rotated around an axis perpendicular to the gradient vector.

From a comparison of the results of animal experiments on growth rate and leukocyte count made in the laboratory of the Biomagnetic Research Foundation and the Lawrence Radiation Laboratory of the University of California, respectively, it can be estimated that a rotation of 10 to 1000 revolutions/day should suffice to attenuate biomagnetic effects to a harmless level. Such a slow rotation will not induce appreciable polarization currents, nor should it cause dizziness. Due to

the weightlessness in the spaceship or space station, the rotation would remain unobservable to the astronauts.

\* \* \*

In space travel of extended duration, the discomfort caused by weightlessness, as has been experienced by astronauts in satellites, will constitute a serious problem. The discomfort is, of course, the result of several factors. But as presently assumed, it is largely due to the abnormal sensations in the balance organ.

The gravity sensory organs in the inner ear are the macula utriculi and the macula sacculi. They consist of a layer of gelatinous substance which is penetrated by hair tufts representing nerve endings. The jelly beyond the hair ends contains a multitude of microscopic crystalline bodies, so-called otoliths, consisting of calcium carbonate (aragonite), proteins, and some magnesium salts. The specific gravity of these otoliths is almost three times greater than that of the surrounding jelly in which they are floating. Through their inertia they convey to the nerve endings the information regarding the state of acceleration of the body. They also convey, therefore, the direction of the gravitational acceleration, the information of the "downward" direction.

Investigating the growth of the spontaneous mammary gland tumor of the C3H strain mice in fields of high paramagnetic strength, I was forced to restrain the animals in a glass tube. The axis of the glass tube and the gradient vector were perpendicular to each other and both were horizontal. The mice always assumed a position such that their chest and abdomen were slightly turned from the downward position toward the direction of the gradient. They remained in this position, or shortly resumed this position, whenever I turned the glass tube around its horizontal axis. In these experiments I always used mice which had the spontaneous carcinoma on the right or on the left side of the neck. As a consequence, the animal's head, with the balance organ, was also in the high-gradient field.

One explanation for this peculiar behavior is the following. Composite crystals, such as otoliths, are sometimes highly paramagnetic. In a magnetic field with a horizontal gradient vector, the resulting force acting on the otoliths will be the resultant of the gravitational force and the magnetic force. Its direction will, therefore, more or less deviate from the usual downward direction. An animal whose balance organ is exposed to a magnetic field with a horizontal gradient will thus take up a position in which "downward" is the direction of the resultant. In the circular glass tube, where the mouse was fairly evenly supported in all directions, other factors such as the stress of standing on its feet did not interfere with the assumed sideward position.

I suggest, therefore, that an inhomogeneous magnetic field which produces an accelerating force upon the otoliths be used in spaceships and space stations to create the sensation of a gravitational field acting upon the balance organ. Of course, the feasibility of this proposal de-

pends on the paramagnetism of the otoliths. Should the susceptibility of the otoliths be of adequate magnitude, a small permanent magnet placed in close proximity to and below the inner ear could probably produce the desired effect and simulate the sensation of a gravitational field. Considering the present highly sophisticated medical techniques, it is perhaps not entirely impossible to increase the paramagnetic susceptibility of the otoliths.

In the experiments mentioned (mice in a horizontal glass tube in a magnetic field with horizontal gradient), the "downward" direction of the mice deviated by about 20° from the vertical toward the horizontal. At the location of the inner ear the paramagnetic strength of the field was about 100 par (1 par =  $10^6$  Oe<sup>2</sup>/cm). If the observed effect is caused by the accelerating force of the field on the otoliths, the paramagnetic susceptibility of the otoliths should have been of the order of  $10 \times 10^{-6}$ —not an impossible value.

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## *Chapter 5*

# Rotational Diffusion in a Magnetic Field and Its Possible Magnetobiological Implications

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In trying to interpret old and new experiments on the action of magnetic fields upon biological growth and metabolic processes, as well as in order to design new experimental research and to predict possible results, several physical-chemical concepts and mathematical developments have to be taken into account.

An analysis of the biochemistry of systems which are in a magnetic field implies at least an investigation of the possibility and predictability of changes in the concentration of the substances which constitute the system, changes in the free energy of activation, and, therefore, changes in the specific rate constants of the chemical reactions which occur in the system.

The mechanism of molecular collision is essential for the understanding of how the rate constant is built up.

Our purpose is to explore in this paper a very special aspect of chemical kinetics, closely related to the velocity of reactions, which might have some fruitful implications from the magnetobiological viewpoint. This aspect refers to rotational diffusion. Since some numerical estimates of the influence of a magnetic field upon the reaction rate through changes of concentration and of free activation energy yield results which would practically be of little importance, we have considered that an effect due to modifications of the rotational diffusion of paramagnetic molecules caused by magnetic fields could account for some experimental observations.

Some theoretical investigations show that Brownian rotation of molecules, that is, rotational diffusion,<sup>1</sup> may be important with respect to chemically effective collisions when the molecules involved possess specific reactive sites.<sup>2,3</sup>

This subject constitutes an aspect of the general problem of biological growth inhibition by magnetic fields, which the author studied during his stay as a visiting professor at the University of Chicago, Committee on Mathematical Biology, in 1962.

It has been pointed out that interpretations based on a simple collision mechanism between molecules which possess active parts do not give reaction rates in agreement with actual biochemical reactions. This agreement improves if rotation of molecules is considered in the analysis.

Setlow and Pollard<sup>3</sup> state that, "If we suppose that at collision the colliding molecule is held very momentarily by the operation of the London forces, and that the molecule is also free to rotate while it is held, then the opportunity for the reactive part of the molecule to be presented to the specific surface on the enzyme or ribosome will be increased." Some numerical calculations carried out by these authors (for which we refer the reader to the original publications) lead to the conclusion that molecular rotation speeds up the reaction process by a factor of at least ten.

If such molecules with specific reactive sites are paramagnetic, there exists the possibility that an externally applied magnetic field may either slow or stop their rotation, a fact which would imply a decrease of the probability of effective collisions, and therefore a retardation of the corresponding biochemical reaction. We know that there are intermediate molecules<sup>4</sup> (molecules in an activated or excited state, more precisely, in a triplet state; free radicals, for example) which fulfill those conditions.

We have to outline an approach which would only have a theoretical and speculative value. In order to elaborate a treatment closer to physical and magnetobiological reality, we need better experimental information and better mathematical developments.

## I. DEFINITION OF A REDUCING FACTOR FOR ROTATIONAL DIFFUSION

We consider that the magnetic moment  $\vec{\mu}$  created by unpaired electrons is rigidly bound to the molecule. Thermal agitation of the medium in which the paramagnetic molecule is contained determines component rotations around the three reference molecular axes<sup>5</sup> X, Y, Z, from which a displacement of the direction of  $\vec{\mu}$  derives.

If an external magnetic field  $\vec{H}$  is applied to the system where the paramagnetic molecule is located, one feels inclined to think that thermal molecular rotation would be hindered because  $\vec{\mu}$  becomes oriented by the field and it is rigidly bound to the molecule.

According to quantum physics,  $\vec{\mu}$  is oriented with respect to the direction of  $\vec{H}$  following well-established conditions. Each direction defines a quantum level, and there exists a number of quantum levels determined by the resultant spin quantum number  $S$ .<sup>6</sup> In fact, if  $M_z$  expresses the magnetic quantum number, it has  $(2S + 1)$  possible values:

$$+ S, + (S - 1), + (S - 2), \dots, 0, \dots, -(S - 2), -(S - 1), - S \quad (1)$$

The population  $N_{M_z}$  of the quantum level corresponding to  $M_z$ , that is, the number of molecules with  $\vec{\mu}$  oriented according to the direction fixed by  $M_z$ , turns out to be<sup>7</sup>

$$N_{M_z} = \left(1 + \frac{M_z g \beta H}{kT}\right) \frac{N}{2S+1} \quad (2)$$

where  $\gamma$  is the Landé splitting factor,  $\beta$  is the Bohr magneton,  $k$  is Boltzmann's constant,  $N$  is the number of paramagnetic molecules per gram, and  $T$  is the absolute temperature.

We can separate the total set of quantum levels in two groups: one group of levels with energies greater than the zero-field value, another group of levels with energies less than the zero-field value. The first group corresponds in (1) to

$$0, \dots, -(S-2), -(S-1), -S \quad (3)$$

The second group corresponds in (1) to

$$0, \dots, +(S-2), +(S-1), +S \quad (4)$$

The molecules with their magnetic moments at this second group of levels are more stable than the molecules which have their magnetic moments oriented according to the first group of quantum levels, since the corresponding energies are smaller than the zero-field value. We assume, consequently, that the paramagnetic molecules of the population of these quantum levels would less probably diffuse by thermal rotation from the direction they have reached under the action of the external magnetic field. In other words, the total number of molecules which likely belong to this group of less energy and greater stability should determine a decrease of the chemical rate if the paramagnetic molecules possess specific reactive sites.

Following this line of reasoning, we want to introduce the hypothesis that the chemical reaction velocity diminishes in proportion to the quotient of the total number of paramagnetic molecules existing at the levels of smaller stability and the total number of paramagnetic molecules existing at the levels of greater stability.\* We will express such

\*A discussion with Dr. Hugo Murua Martinez resulted in some observations concerning several points of this paper. In order to make clearer the basis of the hypothesis that "the chemical reaction velocity diminishes in proportion to the quotient of the total number of paramagnetic molecules existing at the levels of smaller stability and the total number of paramagnetic molecules existing at the levels of greater stability," it should be kept in mind that the magnetic field, since it imposes a preferred orientation on the paramagnetic molecules, restrains the molecules from rotating freely, but this does not at all imply a removal of every degree of freedom. Therefore, the case  $\gamma=0$  must be considered as an ideal extreme situation leading to equation (30), whose purpose is only to get a tentative numerical estimate of the field intensity which might completely stop this type of chemical reaction. This equation contradicts, in the rigor of logic, the assumption that  $g\beta H/kT \ll 1$ , under which formula (2) is obtained from the Boltzmann distribution. A consistent development demands the use of unapproximated expressions, or that terms of higher order be taken into account in the expansion of the exponential. A similar clarification holds with respect to equation (53). According to Dr. Martinez, a more general way of formulating the reducing factor for rotational diffusion would be the introduction of an increasing function of  $\gamma$ ,  $f(\gamma)$ , such that  $f(\gamma)=1$  for  $\gamma=1$ ; for example,  $f(\gamma)=1-a(1-\gamma)$  reduces to  $f(\gamma)=1$  when  $\gamma$  does not differ too much from unity, but one should have to know the value of the constant  $a$  (perhaps experimentally) before predictions can be made about how much the reaction velocity will be reduced for a given value of  $\gamma$ .

a quotient by

$$\gamma = \frac{\sum N_{-M_z}}{\sum N_{+M_z}} \quad (5)$$

The terms of (5) have to be calculated with formula (2). If  $(2S + 1)$  is an even number, the number of levels in the groups (3) and (4) turns out to be  $(2S + 1)/2$ , with  $+1/2$  or  $-1/2$  for the first value of  $M_z$ , and  $+S$  or  $-S$  for the last value of  $M_z$ . If  $(2S + 1)$  is an odd number, the number of levels equals  $(S + 1)$ , with 0 for the first value and  $+S$  or  $-S$  for the last value of  $M_z$ . Therefore, from (2) we have

for  $(2S + 1)$  even:

$$\sum N_{-M_z} = \frac{N}{2} + \frac{N}{(2S + 1)} \frac{g\beta H}{kT} \sum_{-\frac{1}{2}}^{-S} M_z = \frac{N}{2} - \frac{N}{(2S + 1)} \frac{g\beta H}{kT} \sum_{\frac{1}{2}}^S M_z \quad (6)$$

$$\sum N_{+M_z} = \frac{N}{2} + \frac{N}{(2S + 1)} \frac{g\beta H}{kT} \sum_{\frac{1}{2}}^S M_z \quad (7)$$

for  $(2S + 1)$  odd:

$$\sum N_{-M_z} = \frac{N}{(2S + 1)} \left[ (S + 1) + \frac{g\beta H}{kT} \sum_0^{-S} M_z \right] = \frac{N}{(2S + 1)} \left[ (S + 1) - \frac{g\beta H}{kT} \sum_0^S M_z \right] \quad (8)$$

$$\sum N_{+M_z} = \frac{N}{(2S + 1)} \left[ (S + 1) + \frac{g\beta H}{kT} \sum_0^S M_z \right] \quad (9)$$

Since  $\sum_{\frac{1}{2}}^S M_z$  and  $\sum_0^S M_z$  are arithmetical progressions with  $(2S + 1)/2$  and  $(S + 1)$  terms, and  $1/2$  and 0 as first terms, respectively, we obtain:

$$\sum_{\frac{1}{2}}^S M_z = (2S + 1)^2/8 \quad (10)$$

$$\sum_0^S M_z = (S + 1)S/2 \quad (11)$$

In consequence, (6), (7) and (8), (9) transform into

$$\Sigma N_{-M_z} = \frac{N}{2} \left[ 1 - \frac{g\beta H}{kT} \frac{(2S+1)}{4} \right] \quad (12)$$

$$\Sigma N_{+M_z} = \frac{N}{2} \left[ 1 + \frac{g\beta H}{kT} \frac{(2S+1)}{4} \right] \quad (13)$$

$$\Sigma N_{-M_z} = \frac{N(S+1)}{(2S+1)} \left[ 1 - \frac{g\beta H}{kT} \frac{S}{2} \right] \quad (14)$$

$$\Sigma N_{+M_z} = \frac{N(S+1)}{(2S+1)} \left[ 1 + \frac{g\beta H}{kT} \frac{S}{2} \right] \quad (15)$$

With these expressions,  $\gamma$  becomes

$$\gamma = \frac{1 - g\beta H(2S+1)/4kT}{1 + g\beta H(2S+1)/4kT} \quad (16)$$

for  $(2S+1)$  even, and

$$\gamma = \frac{1 - g\beta HS/2kT}{1 + g\beta HS/2kT} \quad (17)$$

for  $(2S+1)$  odd.

As numerical illustrations, let us take molecules with  $n=1$ ,  $n=2$ ,  $n=3$ , and  $n=4$  unpaired electrons. We should obtain:

$$S = n/2 = \frac{1}{2}; (2S+1) = 2 \text{ (even)}; \quad \gamma = \frac{1 - g\beta H/2kT}{1 + g\beta H/2kT} \quad (18)$$

$$S = 1; (2S+1) = 3 \text{ (odd)}; \quad \gamma = \frac{1 - g\beta H/2kT}{1 + g\beta H/2kT} \quad (19)$$

$$S = \frac{3}{2}; (2S+1) = 4 \text{ (even)}; \quad \gamma = \frac{1 - g\beta H/kT}{1 + g\beta H/kT} \quad (20)$$

$$S = 2; (2S+1) = 5 \text{ (odd)}; \quad \gamma = \frac{1 - g\beta H/kT}{1 + g\beta H/kT} \quad (21)$$

## II. APPLICATION OF THE ROTATIONAL DIFFUSION REDUCING FACTOR

Now we wish to apply the hypothesis formulated with respect to the operation of  $\gamma$ .

A way of obtaining an understanding of the mechanism of inhibitory effects of magnetic fields upon metabolizing systems and, of course, upon growth would be through the theory of the absolute rate constants. It has been established that the factor  $A$  of the classical equation<sup>8</sup>

$$K = A e^{-E/RT} \quad (22)$$

which gives the specific rate constant of a chemical reaction, has the meaning of the frequency with which the reacting molecules collide. The new form of (22) is the following:

$$K = (kT/h)e^{-\Delta F^*/RT} \quad (23)$$

where  $k$  is Boltzmann's constant;  $T$  is the absolute temperature;  $h$  is Planck's constant;  $R$  is the gas constant; and  $\Delta F^*$  is the change in free energy corresponding to the formation of the activated state from the reactants. If  $K$  deviates from the ideal behavior predicted by the simple collision theory, as it occurs in some biochemical reactions,<sup>3</sup> the introduction of a factor  $G$  in order to account for the real situation would be justified. Therefore, we would write for the actual frequency factor

$$\phi = G kT/h \quad (24)$$

or

$$K = \phi e^{-\Delta F^*/RT} \quad (25)$$

Now, the velocity of a chemical reaction is defined by

$$v = KC_A C_B \dots \quad (26)$$

where  $C_A, C_B, \dots$  are the concentrations of the reactants. In a magnetic field, considering only the effect of it on the rotational diffusion, we should have

$$v_H = \gamma KC_A C_B \dots \quad (27)$$

or

$$v_H = \gamma G(kT/h)e^{-\Delta F^*/RT} \quad (28)$$

In other words,  $\gamma$  changes the value of the rotational diffusion factor  $G$ .

For the sake of illustration, let us consider a biochemical reaction in which a free radical with  $n=1$  participates. With (18), and  $g \approx 2$ ,  $\beta \approx 9.3 \times 10^{-21}$  erg/Oe,  $H=5000$  Oe,  $k \approx 1.4 \times 10^{-16}$  erg/ $^{\circ}\text{K}$ , and  $T=300^{\circ}\text{K}$ , we get

$$\gamma = 0.88 \quad (29)$$

In this case, the rotational diffusion factor  $G$  and, consequently, the reaction velocity should decrease as much as 12%.

Within this approach, a reaction should be stopped completely if  $\gamma=0$ , that is, the condition

$$1 - g\beta H/2kT = 0 \quad (30)$$

must be fulfilled. The magnetic field necessary to attain such an effect should have the value

$$H = 2kT/g\beta = 45 \times 10^5 \text{ Oe} \quad (31)$$

### III. CONTINUOUS DISTRIBUTION FUNCTION FOR THE PARAMAGNETIC MOLECULES ORIENTED BY A MAGNETIC FIELD

Formula (2) describes, as a function of the magnetic quantum number  $M_z$ , the space distribution of the oriented molecular magnetic moments. Now we wish to omit quantized orientation and to analyze the orientation of paramagnetic molecules by a magnetic field in a continuous way. We will closely follow the theory developed for the study of orientation of molecules by a velocity gradient.<sup>5</sup>

Let  $X, Y, Z$  represent, as in Section I, the three axes of molecular rotation. If elementary rotations  $d\theta_X, d\theta_Y, d\theta_Z$  due to thermal agitation occur around  $X, Y, Z$ , respectively, the magnetic moment  $\vec{\mu}$  experiences a resultant change in orientation from  $\vec{\mu}'$  to  $\vec{\mu}''$  with respect to a fixed direction, which is measured by the angle element  $d\theta$ , on any plane passing through the chosen direction. We choose the direction of the magnetic field as such reference.

The change per unit volume in the number of molecules between  $\theta$  and  $\theta + d\theta$  produced by thermal agitation is determined by the following differential equation:

$$\frac{\partial f(\theta)}{\partial t} = \Theta \frac{\partial^2 f(\theta)}{\partial \theta^2} \quad (32)$$

$f(\theta)$  represents a distribution function, and  $\Theta$  expresses the rotational diffusion coefficient.<sup>5</sup>

The rotational diffusion process is opposite to the orientation process caused by the magnetic field. The field operates upon  $\mu$  and imposes on it a direction change which takes place with the angular velocity

$$\omega = \frac{d\theta}{dt} = \frac{m_H}{8\pi\eta r^3} \quad (33)$$

where  $m_H$  is the mechanical moment of the force  $F_H$  due to the applied magnetic field;  $\eta$  is the viscosity coefficient of the liquid in which the molecule moves; and  $r$  represents the radius of the molecule.

Let  $d$  be the distance of the application point of  $F_H$  from the axis passing through the rotation center of the molecule, parallel to the field direction. We have

$$m_H = F_H d \quad (34)$$

$$d = r \sin \theta \quad (35)$$

Thus

$$m_H = F_H r \sin \theta \quad (36)$$

Therefore, (33) becomes

$$\omega = (F_H \sin \theta) / 8\pi r^2 \quad (37)$$

By introducing the relation:

$$F_H = -mH \quad (38)$$

which implies that the attracting force generated by the magnetic field on the surface of the molecule equals the "magnetic mass"  $m$  times the field intensity, and defining the "magnetic mass" as

$$m = \mu / 2r \quad (39)$$

we get from (37):

$$\omega = -(\mu H \sin \theta) / 16\pi\eta r^3 \quad (40)$$

For both processes (thermal disorientation and magnetic orientation) occurring simultaneously, the differential equation, as for the case of molecular orientation by a velocity gradient<sup>5</sup> reads as follows:

$$\frac{\partial f(\theta)}{\partial t} = \Theta \frac{\partial^2 f(\theta)}{\partial \theta^2} - \frac{\partial [f(\theta)\omega]}{\partial \theta} \quad (41)$$

Once the system has reached a steady state, the condition

$$\frac{\partial f(\theta)}{\partial t} = 0 \quad (42)$$

holds. With this condition, and (40), equation (41) yields

$$\frac{\partial^2 f(\theta)}{\partial \theta^2} - \frac{\mu H}{16\pi\eta r^3 \Theta} \frac{\partial [f(\theta) \sin \theta]}{\partial \theta} = 0 \quad (43)$$

A first integration leads to

$$\frac{\partial f(\theta)}{\partial \theta} + \frac{\mu H \sin \theta}{16\pi\eta r^3 \Theta} f(\theta) = C_1 \quad (44)$$

The rotational diffusion coefficient  $\Theta$  is given by the following formula:<sup>1-3,5</sup>

$$\Theta = kT / 8\pi\eta r^3 \quad (45)$$

By substituting (45) into (44) we obtain

$$\frac{\partial f(\theta)}{\partial \theta} + \frac{\mu H \sin \theta}{2kT} f(\theta) = C_1 \quad (46)$$

The solution of this equation furnishes a function which gives the probability that the molecular magnetic moment is oriented at a certain angle  $\theta$ .

Since  $f(\theta)$  depends only on  $\theta$ , equation (46) can be solved by well-known methods.<sup>9</sup> Considering, furthermore, that  $\mu H / 2kT$  is small, we expand the exponentials of the integrands in the general expression of  $f(\theta)$  in series, and then we integrate term by term. As a good approximation, it suffices to keep only the first two terms of the series. The constants turn out to be

$$C_1 = 0 \quad (47)$$

$$C_2 = 1/\pi \quad (48)$$

since for  $H = 0$ ,  $C_1 = \partial f(\theta) / \partial \theta = 0$  [condition for the maximum of  $f(\theta)$ ], and  $\int_0^\pi f(\theta) d\theta = 1$  (certainty condition). In consequence, the solution of (46) is

$$f(\theta) = \frac{1}{\pi} \left( 1 + \frac{\mu H}{2kT} \cos \theta \right) \quad (49)$$

If  $N$  is the total number of paramagnetic molecules per gram in the system, the number of molecules oriented at the angle  $\theta$  will be

$$N_\theta = \frac{N}{\pi} \left( 1 + \frac{\mu H}{2kT} \cos \theta \right) \quad (50)$$

The definition of  $\gamma$  becomes, with (50),

$$\gamma = \frac{\int_{-\pi/2}^{\pi} N_\theta d\theta}{\int_0^{\pi/2} N_\theta d\theta} = \frac{\pi - \mu H/kT}{\pi + \mu H/kT} \quad (51)$$

Let  $\mu = 54 \times 10^{-21}$  erg/Oe, which is a possible value for the molecular magnetic moment<sup>10</sup> (for example, ferrihemoglobin). In a field  $H = 5000$  Oe, at  $T = 300^\circ\text{K}$ , we should find

$$\gamma = 0.99 \quad (52)$$

that is, 1% of decrease in the velocity of reaction. Remembering the condition (30), we obtain

$$H = \pi kT/\mu \quad (53)$$

With the numerical values just mentioned, we find that

$$H = 8 \times 10^5 \text{ Oe.} \quad (54)$$

is the field necessary to stop the reaction completely.

#### IV. DISCUSSION

The content of this paper has only, as we said above, the value of a theoretical outline. It gives viewpoints which may encourage better, more extensive, and deeper research. It would be necessary to deal with well-known enzymatic reactions in an attempt to apply the foregoing concepts and to obtain quantitative information about chemical velocities in a magnetic field.

Formulas (16), (17) and (51) show that a reinforcement of the inhibitory effect should be attained by decreasing the temperature and by increasing the magnetic field intensity and the value of the molecular magnetic moment. If the temperature is increased, the inhibitory effect diminishes.

In order to apply the ideas and relationships expounded in this paper in an analysis of growth inhibition by magnetic fields, it would be necessary to introduce the factor  $\gamma$  into the anabolic and catabolic term which could be made to appear in the differential equation of biological growth. Due to the intricacy of the chemistry of living systems, those terms should describe the chemical complexes corresponding to anabolism and catabolism as a whole by means of an average method following definitions as stated by Rashevsky.<sup>11</sup>

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## *Chapter 6*

# Distortion of the Bond Angle in a Magnetic and Its Possible Magnetobiological Implications

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Various theories of the biological effect of magnetic fields have been offered in the past. The most common theory offered is that the effect originates in the molecules containing iron, such as hemoglobin and the cytochromes. These theories are proposed because of the well-known paramagnetic properties of iron.

Cobalt is a necessary trace element for plant growth. Mericle et al.<sup>1</sup> studied cobalt distribution via radioactive tracers in plant leaves and found that magnetic fields do affect the distribution. These experiments were undertaken, the authors state, because cobalt is one of the magnetic elements in the iron transition series of the periodic table. The valence state of the cobalt in the leaves was not determined by these investigators. Selwood<sup>2</sup> states that the cobaltic ion is diamagnetic while the cobaltous ion is paramagnetic.

Gerencser, Barnothy, and Barnothy<sup>3</sup> concluded that, since the effect of temporary inhibition of the growth in bacterial cultures could be observed only in highly inhomogeneous fields, the inhibition may be due to a paramagnetic phenomenon. They assume that later generations adjust to the detrimental factors, but do not propose any mechanism for this response.

Gross, Gottfried, and Smith<sup>4</sup> outlined a theory that biomagnetic effects may be due to an inhibition of enzyme activity, caused by distortion of the bond angle, or orbital, of the paramagnetic molecules by a magnetic field. This theory is presented in greater detail below.

Our findings<sup>5</sup> (see Part II, Chs. 5 and 6), and corroborative evidence of other investigators noted earlier, indicate a generalized inhibition or delay of biochemical processes. Our observations that fibroblast proliferation and antibody production are significantly delayed lead us to postulate that the biosynthesis of large molecules is interfered with in the presence of magnetic fields. For reasons stated later, this interference should be more pronounced the larger the reacting moieties.

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The elaboration of these products of biochemical reactions proceeds under the control of enzymes, themselves large molecules. In order for a reaction to proceed, the structure of an enzyme and the product of the reaction require a close fit in three dimensions. The magnetic field will act on these molecules to interfere with chemical reactions far more strongly if they are paramagnetic than if they are diamagnetic.

The chemical intermediates of these biological processes will in some cases be free radicals, which are paramagnetic.

The external magnetic field causes an unpaired electron to assume a preferred orientation with respect to the magnetic field, which inhibits the rate of chemical reaction. Is the energy relationship such that this constraint on an unformed bond affects the course of the chemical reaction?

Organic chemists have found experimentally that the energy in kilocalories/mole required for deformation of the tetrahedral bond angle in carbon<sup>6</sup> is

$$E_\theta = \frac{1}{2} k_\theta (\Delta\theta)^2 \quad (1)$$

where  $k_\theta$  is a constant equal to 0.035, and  $\Delta\theta$  is the angular distortion in degrees from the tetrahedral bond angle  $109^\circ 28'$ . The energy required to bend the bond angle  $1^\circ$  is 0.0175 kcal/mole. This low energy requirement accounts for the comparative ease of formation of strained cyclic compounds such as cyclopropane and cyclopropene.

The unpaired electron of a free radical is not localized and may be in an s or p state. There is a transition period during which this electron becomes localized in the formation of an  $sp^3$  hybrid to form a covalent bond with a reacting atom. It is during this transition period that the electron will have its orbital distorted, according to our argument, and a chemical reaction of which it might become a part is prevented from proceeding promptly. If this subtracts a small amount of energy from the total amount of energy of activation available, it may be that the magnetic field will prevent the new molecules from forming. This, then, is the mechanism postulated for the interference of the magnetic field with chemical reactions in biological systems.

If we accept the concept that magnetic field energetics are sufficient to disturb bond angle orientation, then we must consider whether this change of orientation is sufficient to influence energy transfer in chemical reactions. Foerster<sup>7</sup> calculates that the probability of energy transfer is proportional to the square of the interaction energy. His quantitative treatment yields the following expression for the rate constant of the transfer process:

$$\eta_{S \rightarrow A} = \frac{9000(\ln 10) \kappa^2}{128 \pi^6 \eta N \tau_S R^6} \int_0^\infty \frac{f_s(\nu) \epsilon_A(\nu)}{\nu^4} d\nu \quad (2)$$

where  $\nu$  is the wave number,  $\epsilon(\nu)$  is the molar decadic extinction coefficient,  $f(\nu)$  is the spectral distribution of fluorescence,  $N$  is Avogadro's number,  $\tau_s$  is the intrinsic or radiative lifetime of sensitizer,  $\eta$  is the refractive index of solvent,  $R$  is the distance between molecules, and  $\kappa$  is an orientation factor. The significance of the quantities in this equation is given fully in the original reference. We are most concerned here with the author's need to introduce an orientation factor into this expression to obtain a quantitative statement for the rate constant of the process. The orientation factor  $\kappa$  is given by the expression

$$\kappa = \cos \phi_{SA} - 3 \cos \phi_S \cos \phi_A \quad (3)$$

where  $\phi_S$  is the angle between the transition moment vectors of both molecules,  $\phi_S$  is the angle between  $\vec{S}$  and  $S \rightarrow A$ , and  $\phi_A$  is the angle between  $A'$  and  $S \rightarrow A$ . The average value of  $\kappa^2$  for random distribution is  $\frac{2}{3}$ . We argue that the magnetic field restricts orientation and thereby decreases the value of  $\kappa^2$  below  $\frac{2}{3}$  and reduces the reaction rate. A change of 0.001 in  $\kappa^2$  or 0.03 in  $\kappa$  would be sufficient to account for the observed biomagnetic effects.

Will thermal agitation erase the effects of the magnetic field on the orientation factor and bring that factor closer to the average value for random distribution? This would most likely occur for small paramagnetic molecules of mass close to that of the solvent molecules. When the masses are equal, energy transfer is a maximum. In this event, the colliding molecules would be so buffeted that the orientation of an electron with respect to the external magnetic field would be effectively cancelled.

However, when the paramagnetic molecule is of large mass compared to the solvent molecules, the position and orientation of the large molecule would slowly change, and the average value of the orientation factor for the electron in the magnetic field would then differ appreciably from the average value for random orientation. Consequently, it is in the larger molecular aggregations that a chemical effect is probable when an external magnetic field is applied. Such biological processes as the production of enzymes and other polymers should be affected and the rate at which they are produced altered.

In electron paramagnetic resonance, an effect is detectable under these circumstances even though the amount of transferred energy is small. A small percentage of the affected molecules are raised to the next higher energy level. According to the Boltzmann distribution, the number of molecules is given by

$$N_1 = N_0 e^{-E_1/kT} \quad N_2 = N_0 e^{-E_2/kT} \quad (4)$$

where  $N_0$  is the total number of molecules,  $N_1$  is the number of molecules in the lowest energy state and  $E_1$  the corresponding energy, and  $N_2$  is the

number of molecules in the next higher energy state and  $E_2$  the corresponding energy. Then

$$\frac{N_1}{N_2} = \frac{N_0 e^{-E_1/kT}}{N_0 e^{-E_2/kT}} = e^{(E_2 - E_1)/kT} = e^{\Delta E/kT} \quad (5)$$

and, expanding the exponential in a series and neglecting higher terms,

$$\begin{aligned} N_1/N_2 &= 1 + \Delta E/kT \\ &= 1 + (0.7/0.6) \times 10^{-3} \cong 1 + 10^{-3} \end{aligned} \quad (6)$$

It is this small difference in population that provides an EPR signal. The small population difference between two energy states that provide an EPR signal is relevant to these considerations only in that, as will be recognized, an equally small number of molecules may be involved in producing a biomagnetic effect.

On the average, the magnetic field will remove a small percentage of the enzyme or substrate molecules from the process of the reaction. The amount of the product is given by

$$A = A_0 e^{-Kt} \quad (7)$$

where  $K$  is the reaction rate in reciprocal seconds,  $A$  is the amount of final product, and  $A_0$  is the amount of initial material.

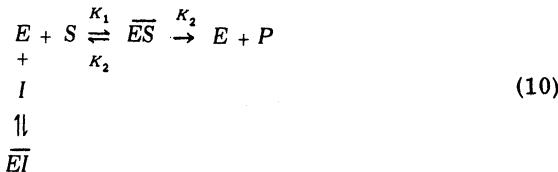
Our experimental results on wound healing and on the decrease of antibody titer, the plant growth experiments of Mericle et al., and the experiments of other investigators indicate a difference after 3 days of magnetic treatment of approximately 10% compared to a control group. We may then compute the difference in reaction within and outside of the magnetic field, with the magnetically treated product indicated as  $A_1$ :

$$\begin{aligned} \frac{A}{A_1} &= \frac{A_0 e^{-Kt}}{A_0 e^{-K_1 t}} = e^{-(K - K_1)t} \\ &= e^{-\Delta K t} = 1.1 \end{aligned} \quad (8)$$

$$\begin{aligned} \Delta K &= -(\ln 1.1)/t = -\frac{2.3 \times 0.042}{2.6 \times 10^5} \\ &= -4 \times 10^{-7} \text{ sec}^{-1} \end{aligned} \quad (9)$$

An experimental model for detection of this effect requires a system wherein a difference in rate of the order of  $10^{-8} \text{ sec}^{-1}$  can be detected.

The argument just offered for reduction in the reaction rate implies that the magnetic field produces an inhibition of the reaction. The equation for the reaction can be written as



where the symbols refer to the concentrations of the enzyme ( $E$ ), substrate ( $S$ ), enzyme-substrate complex ( $\bar{E}S$ ), inhibitor ( $I$ ), enzyme inhibitor complex ( $\bar{E}I$ ), and product ( $P$ ), and  $K$  with the appropriate subscripts represents the reaction rate.

The Michaelis-Menten equation in its integrated form<sup>8</sup> for the normal reaction (in our case, in the absence of the magnetic field) may be written as

$$\frac{1}{K_{\overline{E}\overline{S}}} = \frac{K_m}{K_{\overline{E}}} \frac{1}{S} + \frac{1}{K_{\overline{E}}} \quad (11)$$

The Michaelis constant is

$$K_m = (K_2 + K_3)/K_1 \quad (12)$$

and the variables are  $1/K_3 \bar{ES}$  and  $1/S$ .

When a reversible or competitive inhibitor is present, the Michaelis-Menten equation has the form

$$\frac{1}{K_i E S} = \frac{K_m}{K_i E} \frac{1}{S} \left( 1 + \frac{I}{K_i} \right) + \frac{1}{K_i E} \quad (13)$$

where the term  $IK_m/K_iK_e$  represents the effect of the inhibitor in removing enzyme from the desired reaction. In our case,  $I$  is the action of the magnetic field and  $\bar{E}_1$  is then the concentration of enzyme molecules diverted from the main reaction.

When the inhibiting factor is the magnetic field, a family of lines results when  $1/K_c \bar{E}S$  is plotted against  $1/S$ . If kinetic determinations are made in various magnetic fields we suggest that a family of lines will result as shown in Figure 1. The larger the inhibition the steeper should be the slope of the line.

The proposed mechanism for the observed biomagnetic effect would be confirmed if such an *in vitro* test were to be demonstrated. One such proposed system could be the production of DNA or RNA by polynucleo-

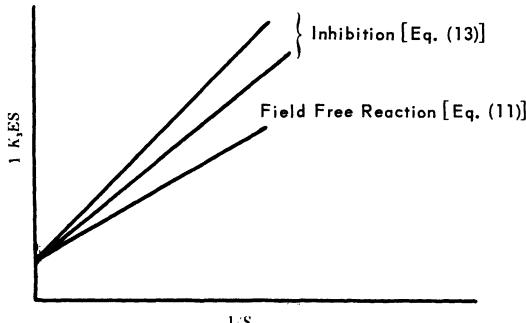


Fig. 1

tide phosphorylase. The method of Singer and Guss<sup>9</sup> is suggested. Perhaps a more detectable result could be obtained were a chain of events, several of them disturbed by external magnetic fields, to occur. A test system then would be the rate of production of synthetic proteins, as for instance, polyphenylalanine produced by the protein-synthesizing enzyme systems of Nirenberg and Matthaei.<sup>10</sup>

It might also be possible to demonstrate more readily the influence of magnetic fields on chemical reactions with free radical intermediates in situations where the reacting moiety could be constrained and held in preferred alignment with the externally applied field. Such a condition may obtain in solid state reactions when isomerization is triggered by radiation<sup>11</sup> and the radiation-initiated polymerization of monomer crystals.<sup>12</sup>

Experimental investigation of these reactions is in progress at our laboratory.

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## *Chapter 7*

# A Possible Effect of the Magnetic Field Upon the Genetic Code

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A book on biomagnetism would be incomplete if it did not mention—at least in a qualitative way—a further mechanism and its relation to biomagnetic phenomena. P.O. Löwdin<sup>1</sup> has drawn attention to the fact that the quantum mechanical effect of proton tunneling may show up in some processes in which biological amplification plays a role.

In ordinary chemistry, the tunnel effect has so far been of little importance. In chemical kinetics one usually considers only processes which have sufficient energy to take particles above the potential barrier between two states and the effect of quantum tunneling can be neglected. In the following we will attempt to investigate the influence of a magnetic field upon the tunneling of protons in DNA.

It was Delbrück's<sup>2</sup> idea in 1930 that the immense stability of the hereditary substance in chromosomes over thousands of years may indicate that it is nothing but an immense molecule in a stationary state and that mutations correspond to quantum jumps between isomeric forms of that molecule. Chromosomes consist mainly of three kinds of substances: protein, deoxyribonucleic acid (DNA), and ribonucleic acid (RNA). Proteins are made of 20 different kinds of amino acids strung together in long polypeptide chains, sometimes containing several hundred amino acids. The nucleic acids are also long chain molecules, but made up usually of only four nucleotide bases. DNA is the custodian of the genetic code. A kind of RNA, called the messenger-RNA, picks up the code information from the DNA and carries the message from the nucleus of the cell to the surrounding cytoplasma, where with the help of ribosomes the proteins are mainly synthesized. The coding contained in the DNA molecule determines the sequence of the 20 different amino acids in the protein molecules.

According to the Watson-Crick Model,<sup>3</sup> the DNA molecule consists of two very long chains made up of alternate sugar and phosphate groups. The sugar is always the same, deoxyribose, and is always joined to the

phosphate in the same way, so that the long chain is perfectly regular, repeating the same sugar-phosphate sequence over and over, sometimes several hundred thousand times. But each sugar has a base attached to it and these bases are not always the same.

Four different types of bases are found in DNA. Two are purines, adenine and guanine, and two are pyrimidines, thymine and cystosine. The three molecules sugar, phosphate, and base form a nucleotide. The two sugar-phosphate chains form a double helix, with the two strands joined at regular intervals, like rungs in a ladder, by hydrogen bonds between complementary pairs of nucleotide bases. A purine-type base is always linked by two or three H-bonds with the complementary pyrimidine-type base. The double helix has five base-pairs per half-turn.

The hydrogen bond between complementary bases is formed by a proton shared by two lone electron pairs situated on the nitrogen or oxygen atoms of the bases. The attractive force exerted by the two lone electron pairs on the shared proton can be represented as a double-well potential separated by a potential barrier. The wells represent possible equilibrium positions of the proton.

In quantum mechanics the proton is a wave packet, which may have nonzero amplitude even in regions classically forbidden. The square of the proton wave function describes the probability that the proton is attached to either one of the lone electron pairs of the complementary bases. If the double-well is asymmetrical, the proton will be comparatively stationary in the lower-lying energy level of the deeper well and tunneling will occur only rarely. The fact that the genetic code is transmitted intact in millions of multiplications implies that the double-well has to be highly asymmetrical.

Cell division is preceded by a replication of the DNA molecules. The two strands of the double helix sever their H-bonds, uncoil, and separate. Nucleotide bases floating loosely within the cell begin to attach to each of the single strands, building two new complementary strands. When the proper bases are joined, two new double helices are formed; thus two complete DNA molecules, with the same base sequence as the original, are produced. The synthesis of the complementary chains begins as soon as the two original chains start to unwind, so that only a short stretch of the chain is ever really single. For every H-bond that has to be broken, two new ones will be formed. The total length of all DNA molecules in a single chromosome can be something like 4 cm, which means that there must be more than 100 million H-bonds in one chromosome.

If the complementary bases have equal charge, the tunneling of a proton in one direction induces a tunneling of the proton in the second H-bond between the same base pair in the reverse direction, whereby the original bases are transformed into tautomeric bases. A tunneling occurring at some later time in the reverse direction may restore the

original state. If in a replication the breakup of H-bonds occurs before the original state is restored, we will have, instead of the original bases, tautomeric bases, which can no longer combine with their normal complementary bases. Different complementary bases will be attached, thus changing the base sequence and introducing an error in the code. We can see that a code error will be multiplied in each replication process by a factor of two and, hence, in the  $n$ th replication by a factor of  $2^n$ .

Should one of the bases of a pair obtain an extra charge, for example through electron donor-acceptor reactions from surrounding molecules or through the circumstance that a proton tunneling was not accompanied by a reverse tunneling of another proton of the base pair, the change in the charge distribution will greatly alter the shape of the double-well potential. For such so-called ionic tautomeric forms there does not exist a normal nucleotide base which could combine with the positive ionic forms of adenine and thymine; hence "deletion" will occur in the complementary sequence, while the negative ionic forms of these bases lack any specific code and may combine with all four normal bases. Such changes or mutations will, therefore, be irreversible.

L. Szilard<sup>4</sup> has proposed that the elementary step in the process of aging is an aging "hit" which destroys a chromosome of the somatic cell, in the sense that it renders all genes carried by that chromosome inactive. He assumes that aging hits are random events and that the probability that a chromosome of a somatic cell suffers such a hit per unit time remains constant throughout life. On the basis of this theory the frequency with which such random events occur in men is found to be about one chromosome of the haploid chromosomal set of the somatic cells in 12 years. The agent causing the hit is not discussed in detail by Szilard, but the assumption that a chromosome suffers a total loss of function in a single random event is suggestive of an external agent of high energy, that is, a high-energy photon or particle.

P. O. Löwdin proposes that the phenomenon of aging of individual organisms depends essentially on the discussed quantum mechanical tunneling and is in effect a corrosion of genetic messages, which accumulates with age.

The basic difference between these two views is that Szilard assumes that genes are subject to random "aging hits" caused by an external agent, while Löwdin assumes that on account of the tunnel effect a "pure" genetic message does not exist at all and errors occur without "hits" and will be multiplied.

Löwdin furthermore suggests that the occurrence of tumors may depend on the fact that the accumulation of errors has passed a certain limit in a particular direction and upset the balance between enhancing and controlling enzymes in the growth cycle. One should expect, as is the case, that this will happen more often at advanced age.

From the point of view of biomagnetism we should try to see what effect an external magnetic field could have on proton tunneling in the DNA molecule. The magnetic field may affect the spin orientation of the proton, a possibility already suggested by Löwdin. A second possibility would be that the proton, endowed with a magnetic moment, experiences in an inhomogeneous field an accelerating force which enhances or retards its tunneling in one direction.

The third, in our opinion the most probable, effect of the magnetic field would be that, as in the Zeeman effect, it changes or splits the energy levels of the nucleotide bases. This would change the depth of the potential wells and hence change the tunneling probability, thus increasing or decreasing the stability of the genetic code.

The synthesis of DNA occupies only about 5% of the full time of the growth and division cycle of a cell. We can speculate, therefore, that only alterations in the tunneling probability prevailing during this short time interval will produce an effect. This circumstance could be the subject of an experimental verification. In synchronized cell division we should expect that a magnetic field has the same effect whether applied continuously or only for a small fraction of the generation time, should this fraction coincide with the DNA replication period; but no effect should be observed if the application of the field does not coincide with the DNA replication period.

It should be mentioned that the polypeptide chains of proteins are also linked together by H-bonds in which proton tunneling may occur. It is true that in this case a change in the protein will not be multiplied through replication, as in the case of DNA, and one proton tunneling will hardly entail any observable consequences. But if the external magnetic field simultaneously causes the proton in a large number of protein molecules to prefer a position in variance to its normal position and these protein molecules happen to be enzymes, then the enzyme balance could become upset enough to lead to observable effects.

The mathematical treatment of proton tunneling and the computation of the change of the energy levels due to the magnetic field is complicated. There are at least two double-wells to be considered, a proton tunneling will entail a change in the charge distribution, the motion of the proton will polarize the electron cloud, etc. At the present stage we cannot attempt a quantitative computation, but will rather consider if some experimental evidence indicates that the magnetic field renders the genetic code more stable or more labile.

I would like to discuss briefly here two experiments, the results of which could be interpreted as an effect of the magnetic field on the genetic code.

J. M. Barnothy<sup>5</sup> has performed some experiments which indicate an influence of treatment in a magnetic field on aging and on the occurrence of spontaneous cancers. In one experiment he subjected 10 female C3H-strain virgin mice 70 days old for 4 weeks to a homogeneous field

of 4200 Oe strength. Five were kept in identical dummy magnets and 25 in standard plastic cages during this time. After removal from the field and dummy magnets, respectively, the activity of the mice (10 magnet, 5 dummy, 15 control) was continuously recorded from the age of 320 days to 509 days, at which time 50% of the treated and 60% of the controls were dead or had developed spontaneous cancers. The magnet and dummy cages used for treatment were 3 in. in diameter and  $1\frac{1}{2}$  in. high, with food and water supply. The pole caps were simulated on the dummy cages with 1-in.-thick brass discs (brass has a heat conductivity, as well as radiation length for cosmic ray showers, similar to that of iron).

The activity measurements were made in standard plastic cages  $11 \times 7 \times 5$  in. high, divided by a wall into two equal compartments, with water in one and food in the other compartment. At a height of 3 in. the dividing wall had an opening through which the mice could cross from one compartment to the other. While crossing they tripped a micro-switch, which electrically recorded the crossing. An average of 100 to 200 crossings was recorded per day and animal, the majority of the crossings occurring during the early morning hours. The cages were cleaned once a week; the first 3 hr thereafter was not used for activity recording, since the new bedding incited all animals to a temporarily higher activity.

The experiments were started with six activity cages with five mice per cage. Since initially he had ten more control mice than used in the activity experiment, this reserve group was kept in two cages which had similar dividing walls, but was without electric switches. Whenever a mouse which was kept in a dummy cage during treatment or one of the controls (kept during treatment in standard cages) died in one of the activity cages or developed cancer, it was replaced from the reserve group. Whenever one mouse of the group treated in the magnet died or developed cancer, the number of mice per cage was also reduced in all other five activity cages. Hence the number of mice per activity cage was gradually decreased from five to two mice per cage.

Table I lists the observed activities from the age of 320 days to 647 days. The data are reported in six age periods in such a way that within each period the number of mice per activity cage remained the same. Columns 2, 3, and 4 show the covered age period, the number of recorded days, and the number of subintervals ( $N$ ) within each period. Columns 5, 6, and 7 list for magnet, control, and dummy mice, respectively, the number of used activity cages times mice per cage and the average crossings per day and mouse.

Since it might be surmised that the increased activity of the magnet group could be related to a younger biological age, my husband checked for any difference in their ability to reproduce. At the age of 509 days, two male mice were daily alternated between the six activity cages for a period of 2 weeks. One magnet and three control mice became preg-

TABLE I

No.	Age period	Days	N	Magnet	Control	Dummy
I	320-361	41	6	2 X 5 168	2 X 5 171	1 X 5 122
II	361-404	43	5	2 X 5 184	3 X 5 136	1 X 5 144
III	404-444	40	6	2 X 4 192	3 X 4 143	1 X 4 171
IV	444-488	44	5	2 X 3 164	3 X 3 118	1 X 3 127
V	488-509	21	3	2 X 2 198	3 X 2 121	1 X 2 126
VI	600-647	47	6	1 X 2 72	3 X 2 92	— —

nant and gave birth to very small litters. The offspring were generally weak and died shortly after birth. The frequency of pregnancies does not show any difference between treated and untreated mice. Due to this interlude, the activity data between the ages of 509 and 600 days were not used in the present paper and the activity recording was resumed 3 months later (period VI).

Table II lists the difference between the activity of the treated and of the untreated mice for the six age periods together with their standard errors, the degree of freedom, the *t*-value, and the probability level. As we can see, up to the age of 361 days the activity of the treated mice did not differ from the activity of the untreated mice. However, a significantly greater activity is manifested during the age periods from II to V. After the age of 600 days, however, the treated group shows a significantly lower activity.

The mean standard deviation between the weekly average activities of the cages was 11% for the magnet and 18% for the untreated and was independent of the number of mice per cage. We may note that this corresponds to an individual standard deviation of 28% in the weekly average activity of the C3H-strain mice under the given conditions.

The mean activity of the magnetically treated mice from the age of 361 days to 509 days was  $(36.3 \pm 4.5)\%$  higher than that of the untreated mice and  $(28.2 \pm 6.4)\%$  higher than that of the dummy mice alone.

TABLE II  
Activity Difference of Treated and Un-  
treated Mice

Effect	Degree of freedom		Probability level
	I	II	
I	12.7 ± 11.6	5	1.09
II	47.5 ± 11.0	4	4.30
III	42.5 ± 15.3	5	2.78
IV	42.6 ± 4.1	4	10.40
V	74.8 ± 8.6	2	8.70
VI	-19.9 ± 4.2	5	4.75

In the later course of the experiments the weekly food consumption (Rockland Mouse Diet pellets) was also recorded. Between the ages of 430 and 509 days the food consumption was  $(26 \pm 1)\%$  lower for the treated group. This finding, together with the higher activity, would suggest a better energy metabolism of the treated mice.

The cause of these two effects, higher activity and lower food consumption, is perhaps indicated by the picture in Figure 1, which shows one of the treated and one of the dummy mice at an age of 400 days. The appearance of the treated mouse is far younger, its fur is smooth, and no wrinkles are seen. The difference in appearance and in movements at this age was so striking that when the treated were mixed with the untreated, any person even unfamiliar with mice could correctly separate them. During the next 3 months the difference in appearance gradually decreased and eventually vanished.

In the spirit of the discussed theory this experiment would indicate that magnetic treatment has a stabilizing effect on the genetic code.

In a second experiment my husband treated virgin female mice of the same strain and for the same duration, but began treatment at an age of 270 days. No difference between treated and control groups, either in appearance, in activity, or in food consumption, was observed

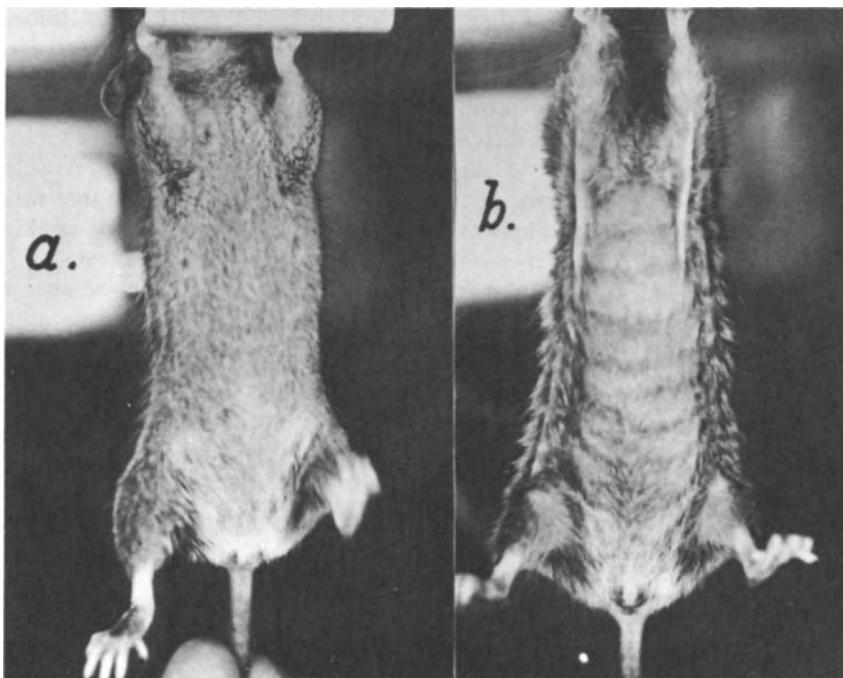


Fig. 1. C3H-strain female mice at an age of 400 days. (a) Treated 11 months earlier for 4 weeks in a magnetic field of 4200 Oe; (b) control (dummy) of the same age. Reprinted from Medical Physics, Vol. 3, p. 63, O. Glasser, ed., Year Book Medical Publishers, Inc.

up to their highest age. This experiment would indicate that treatment at a full-grown age, contrary to treatment during youth, does not have an effect upon the accumulation and total number of genetic code errors. It is tempting to explain this difference through the circumstance that during the vigorous growing stage of a biological organism, when the rate of mitosis and therefore the replication rate of DNA molecules is high, an error—but in the same way the nonoccurrence of an error—will be greatly amplified and thus animals treated in their youth in a magnetic field reach their old age with a far lower number of genetic errors. A treatment during advanced age, when the errors are more or less incorporated and the replication rate is lower, will be less effective.

C3H-strain females have a very high incidence of spontaneous mammary gland carcinomas. Of the ten mice treated in their youth in the above-discussed first experiment, four, that is 40%, had an observable tumor at the time of their death. Of the 30 controls, 22 (73%) had palpable tumors at the time of death. The difference, although significant only on a 5% confidence level, again suggests a stabilizing effect of the magnetic field on the genetic code.

Further experiments which I would like to mention in this connection were made by I. Sumegi, J. M. Barnothy, and M. F. Barnothy.<sup>6</sup> We kept young 80-day-old virgin female mice for 35 days in a homogeneous field of 4200 Oe (the cages and magnet are described in Part II, Ch. 3) and sacrificed them 200 days after removal from the field. Sections were prepared from nine main organs and inspected by I. Sümegi of the Pathological Department of the Karolinska Institutet in Sweden. In this experiment, 20 mice were treated in the magnets, 20 were kept in dummy magnets, and 20 in standard large plastic cages. Abnormalities were found in the spleen, liver, adrenal, and bone marrow.

The changes found in the spleen correspond to reactive reticulosis, manifested in the increase in the number of reticular cells in the pulpa and the presence of large number of megakaryocytes. The circumstance that of the inspected 16 dummy mice eight showed the same abnormality to a greater or lesser degree would suggest that this might be a more general organismic reaction to a stress factor and a consequence of some irritation of the reticulo-endothelium. Since all 16 of the treated animals inspected showed this abnormality to a large degree, the difference in the frequency of occurrence in magnet versus dummy group is significant on a 5% confidence level.

Abnormalities found in the liver indicate that the livers suffered some kind of lesion and the liver is in the process of regeneration. This process is characterized by a greater number of nuclear divisions, increase in the number of cells with two nuclei, and a large number of liver cells with large nuclei. In the latter, the nucleoli are on the periphery of the nucleus. Cytoplasm is less basophil and more red on eosin staining. One sees similar abnormalities in the case of chloroform, trichloreethylene, and amino nucleoside poisonings.<sup>7</sup> It seems

that the lesion caused a disturbance in the protein metabolism and in the oxidative processes in the cytoplasm of liver cells. In consequence of these processes, fatty infiltration is visible in the cells. From the magnet group, 16 out of 18 showed these strong regenerative processes, while from the dummy group only four out of 14 showed similar regenerative processes. The difference in the frequency of occurrence of this effect between magnet and dummy group is significant on a probability level of 1:1500.

The abnormalities in the adrenal are very interesting. The zona glomerulosa is slightly narrower and the zona fasciculata considerably narrower, in some instances entirely missing. The cell distribution is disorganized. There was no change in the zona reticularis. In humans such abnormalities occur after some shocks, infections, and in response to some toxic agents. None of the dummy or the standard cage group showed any of these abnormalities. From the magnet group only three out of 18 had normal adrenals. This effect is significant on a probability level of 1:4 million.

There is some evidence that glucocorticoids are produced in the zona fasciculata. A loss of the zona fasciculata may lead to a depletion of the carbohydrate stores and thus to a lower resistance against insulin and stress.

In the bone marrow of the magnet group the number of megakaryocytes was found to be lower. Their number per microscope field is

for mice in the standard cages	$7.43 \pm 0.23$	dummy - standard	$0.12 \pm 0.46$
for mice in the dummy cages	$7.55 \pm 0.40$	magnet - dummy	$-2.35 \pm 0.42$
for mice in the magnet cages	$5.20 \pm 0.15$		

Whereas between standard cage and dummy group there is no difference, the number of megakaryocytes in the bone marrow of the magnet group is 31% lower. This effect is significant on a probability level of 1 to 50,000.

We may now raise the following question: are the found abnormalities consequences of stress effects caused by the magnetic field, or are they due to a change in the genetic code? We should expect that stress effects would show up immediately after treatment and genetic changes only after the lapse of a certain time, when the amplification through the multiplication of the code errors has become manifest. In the next experiment we therefore sacrificed the mice immediately after treatment.

In the livers of the treated mice strong signs of lesions were visible, characterized by centrolobular necrobiosis and appearance of small liver cells with pycnotic nuclei. The number of nuclear divisions was much higher than when the mice were sacrificed 200 days after termination of treatment. This finding could probably mean that the pathological changes in the liver are a consequence of the stress effect of the magnetic field and, as expected, the liver shows gradual recovery.

On the other hand, the adrenals of the magnet group did not show any abnormality and the zona fasciculata was not missing. This could indicate that the observed abnormality in the adrenals could be a genetic code effect.

It should be emphasized that both of the reported experiments (postponement of aging and pathological changes in the adrenal) used here to support the proposed theory can be, in spite of the high statistical significance of the results, considered only as preliminary investigations, requiring many further observations until a definite connection of the magnetic field with the genetic code can be established.

In summary, an effect of the magnetic field upon the tunneling probability can theoretically be expected. Hence, should tunneling be the major cause for alterations in the genetic code, the magnetic field could be a powerful tool in the further investigation of the code.

#### ACKNOWLEDGMENT

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*Part II*

**Effects of Strong Magnetic Fields  
on Specimens *in vivo***

## *Chapter 1*

# Development of Young Mice

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Experiments on the influence of static magnetic fields upon the development of young mice were first made in 1948 at the University of Budapest. Half of a litter of six 5-week-old mice were kept in the vertical field of an electromagnet, the other half of the litter in identical dummy magnet cages (pole pieces simulated; same temperature, light, and ventilation), for a period of 4 weeks. The aluminum cages were 38 mm in height and 75 mm in diameter with hard-rubber bottoms, surrounded by an open tray where food and water were provided. The design of the electromagnet, heat insulation, and cooling fins used prevented any observable increase of the temperature of the cage walls. A forced air blow prevented the mice from staying for long periods outside their cage. They never attempted to escape, thus proving the adequacy of their housing conditions.

The two groups (one male and two females in each) were conditioned for 4 days to the confinement before the magnet was energized. The field strength at the center of the cage was 5900 Oe with an average gradient of 100 Oe/cm. The weight of the mice was measured daily around noon. As can be seen from their weight curve, shown in Figure 1, the field retarded the growth of the mice. We note a sharp minimum on the third day, which seems to be the result of a shock caused by the field, because they recovered rather rapidly. If we disregard this minimum, the weight of the mice in the field started to lag behind the weight of the controls from the fifth day on. Thereafter, the mice in the magnet developed significantly more slowly; one female showed practically no weight increase during the further 3 weeks in the field. The male started to lose weight on the 11th day in the field and died shortly thereafter. This "lethal effect" of the magnetic field on males was observed several times since then<sup>1</sup> and is a phenomenon worth further investigation. The females did not exhibit any adverse symptoms. Removed after 4 weeks residence in the field and mated, they gave birth to healthy litters 20 days later; this means that they must have become pregnant immediately, since 20 days is the normal gestation period of mice.

Supported by the Biomagnetic Research Foundation.

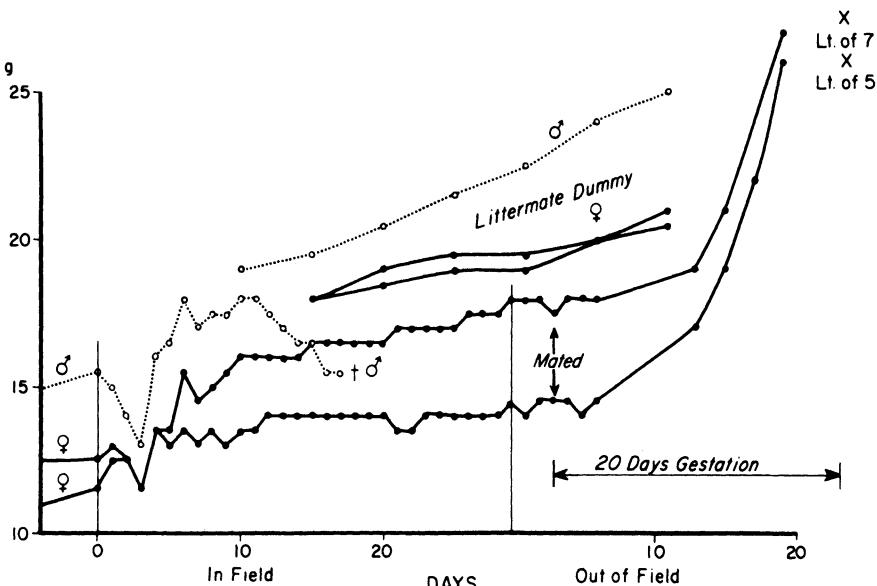


Fig. 1. Growth of 5-week-old albino mice in a vertical field of 5900 Oe. (Reprinted from Nature, Vol. 200, 1963.)

As is well known, there are many factors which can influence the development of mice. It is conceivable that the magnetic field acts via one or another of these factors, but it can not be excluded that a magnetic field slows down mitotic activities in general. In the latter case, we should expect that the rate of retardation should increase with the mitotic activity of the tissue or organ in question, as is the rule with other external inhibiting agents. For this reason, in 1948 and since then I have investigated the effect of the magnetic field upon the development of embryos in the uterus of mice and upon the growth of transplanted and spontaneous tumors in mice (see the subsequent chapter). These observations, together with the effect of the field upon hemopoiesis (Part II, Ch. 3), support the assumption of a general mitosis retardation.

The effect of the magnetic field upon the growth rate of young mice is an effect which is far less sensitive to environmental conditions than most other effects, if comfortable housing conditions are provided and the position of the animals does not change relative to the magnetic vectors. The importance of the latter is discussed in Part I, Ch. 4.

Further experiments were performed in the laboratory of the Biomagnetic Research Foundation in Evanston, with ten alnico permanent magnets of 275 lb each. Two types of field were used: in one the field strength was 4200 Oe with an average gradient of 80 Oe/cm (called in the following homogeneous); in the other the field strength averaged 3600 Oe and the gradient at the center of gravity of the mouse 650 Oe/cm (called in the following inhomogeneous). The cages were

3 $\frac{7}{8}$  in. in diameter and 1 $\frac{7}{16}$  in. high, with plastic bottoms and food and water supply, and each was connected to an inclined exercise gallery. The identical dummy cages were 2 feet above the magnet cages, but shielded against the stray field of the magnets. The dummy magnets were simulated by 2-in.-thick iron pole pieces to avoid effects caused by the biological effect of cosmic radiation.<sup>2</sup>

For weighing, the two or three mice housed in the same cage were transferred to a plastic cup and weighed with the cup. The error in weight due to loss in feces and urine is negligible even if weighing is made without a cup. One droplet of a full-grown mouse weighs 60 to 70 mg, and well-treated mice, used to the person handling them, never urinate during the weighing process.

A considerable error can, however, be introduced if weighing is made during or around the time when mice normally feed. In the morning the weight of a mouse can be 2 g higher than in the evening. Mice have the habit of feeding in groups, with the consequence that those in some of the cages might be before and those in others after feeding. I have, therefore, investigated the weight variation of mice over a 24-hr period and also recorded their activity and feeding habits with electric devices. Their main feeding time occurs between 11 PM and 5 AM, and young mice have a second snack between 8 AM and 11 AM. However, in the afternoon, between 2 PM and 6 PM, the mice rest. Measuring the weight of a larger group of mice individually day after day around 4 PM revealed that the daily weight fluctuation of individual animals does not exceed 0.55 g for young mice and 0.49 g for old mice. This fluctuation follows a strictly Gaussian distribution, implying that in an experiment where 30 animals are treated and 30 are used as controls, with these precautions the difference in the growth rate can be established with an accuracy better than 0.20 g.

Table I shows the results of one of the experiments in which 30-day-old albino female mice were treated in a homogeneous field. The first column gives the total weight of 3 mice per cage before placing them in the field; the second and third columns give their weight on the 10th and 12th day in the magnet and the fourth column the weight difference up to the 11th day. Columns five through eight contain the same data for the dummy group. We can see that the average growth was 5.74 g for the mice in the magnets and 8.33 g for the mice in the dummy magnets. The difference in growth rate during the first 11 days is, therefore,  $2.59 \pm 0.68$  g, the effect being significant on a probability level of 1:1560.

We see furthermore that the individual standard deviation of the magnet group is almost twice that of the standard deviation in the dummy group, indicating that the magnetic field affects individual mice to a different degree. For instance, the three mice in the fourth row seemed not to have been affected, while the effect was twice the average in the group of the sixth row.

TABLE I

Swiss 30-Day-Old Female Mice; Experiment S. Weight  
of 3 mice per cage

In 4200-Oe field				In dummy magnets			
$W_0$	$W_{10}$	$W_{12}$	$\Delta W_{11}$	$W_0$	$W_{10}$	$W_{12}$	$\Delta W_{11}$
38.5	58.5	64.0	22.8	42.0	64.0	67.0	23.5
41.5	47.5	56.0	10.2	42.0	71.0	75.0	31.0
45.0	65.0	66.5	20.8	43.0	61.0	64.0	19.5
45.0	69.5	74.5	27.0	45.0	70.5	72.0	26.2
47.0	64.5	68.0	19.2	45.0	71.0	72.0	26.5
47.0	55.0	57.0	9.0	46.0	69.5	73.0	25.2
48.5	61.5	64.0	14.2	46.0	70.0	71.0	24.5
49.0	67.0	70.5	19.8	47.5	70.0	72.5	23.8
51.5	65.5	66.5	14.5	52.5	74.0	76.5	27.8
53.5	67.5	69.0	14.8	53.5	75.5	75.5	22.0
<hr/>				<hr/>			
46.6		17.23		46.2			25.00
<hr/>				<hr/>			
Average growth:		5.74 g				8.33 g	
Standard deviation:		1.88 g				1.03 g	
Growth difference between magnet and dummy:				-2.59	$\pm$ 0.68 g		
$t = 3.81$ ; $n = 18$ ; P.L.: 1:1560							

Twelve similar experiments were conducted with a total of 680 female mice. In each experiment the animals were kept after arrival at least for 1 week in standard cages, then divided into five weight groups, the extremely heavy or light animals eliminated, and the remainder divided evenly between magnet and dummy cages, with three mice per cage. The results are summarized in Table II according to strain, age, and field used. The growth differences up to the 11th day of residence in the field are tabulated together with their standard errors.

We can see that the total weight difference of young mice is larger than that of older mice. The relative decrease of the growth rate, that is, the difference in growth rate divided by the growth rate of the controls, is greater for the older mice. Young mice do not stop growing in fields of such magnitude, whereas older mice sometimes may lose weight.

TABLE II

Growth Differences up to the 11th Day (g)

Experiment	Strain	Age	M/D	Field	$\Delta W_M - \Delta W_D$
T	Swiss	60	30/30	hom.	$-1.66 \pm 0.50$
P, S	Swiss	30	50/50	hom.	$-2.21 \pm 0.46$
C, D, E	RF	60	70/90	hom.	$-0.97 \pm 0.33$
K, L, M, N	Swiss	60	120/120	inh.	$-0.48 \pm 0.17$
Q, R	Swiss	30	60/60	inh.	$-0.80 \pm 0.38$

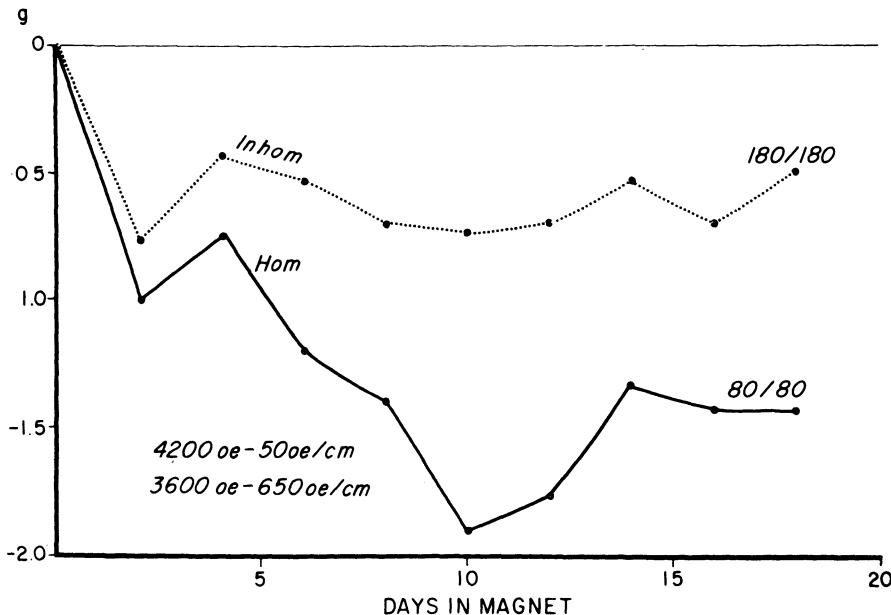


Fig. 2. Growth retardation (weight difference between treated and controls) of Swiss female mice in homogeneous (full line) and inhomogeneous (dotted line) magnetic fields. (Reprinted from Nature, Vol. 200, 1963.)

Figure 2 shows the daily weight change of the pooled results of nine experiments in which Swiss female mice were treated in homogeneous and inhomogeneous fields, respectively. We can see that the effect of the homogeneous field is far greater than that of the inhomogeneous field. In both cases the minimum occurs around the 10th day of residence. We note the minimum around the second day and following recovery, as already noted on the weight curve in Figure 1.

To investigate this second-day weight minimum, which seems to be a shock effect of the magnetic field, six 38-day-old female ICR strain mice were, in 14-day intervals, repeatedly exposed to a homogeneous field of 9400 Oe produced by a Varian 4-in. electromagnet. The mice were housed in  $3\frac{7}{8}$ -in.-diameter and  $1\frac{7}{16}$ -in.-high cages, identical to those used in the permanent magnets, but with the difference that the cages did not have an exercise gallery and the floor of the cages was a copper plate, covered with Mylar, thermally insulated from the lower pole (or the simulated pole of the dummy magnet), and kept at 28°C by means of a thermocirculator. When the electromagnet was energized, it produced a field of 9400 Oe in the center of the cage and 8900 Oe  $\frac{1}{2}$  in. from the cage wall. Water-cooling of the electromagnet kept the magnet poles 2° below the room temperature (23°C). The dummy cages, situated 2 ft from the magnet, had identical lighting and ventilation conditions (see Figure 3).

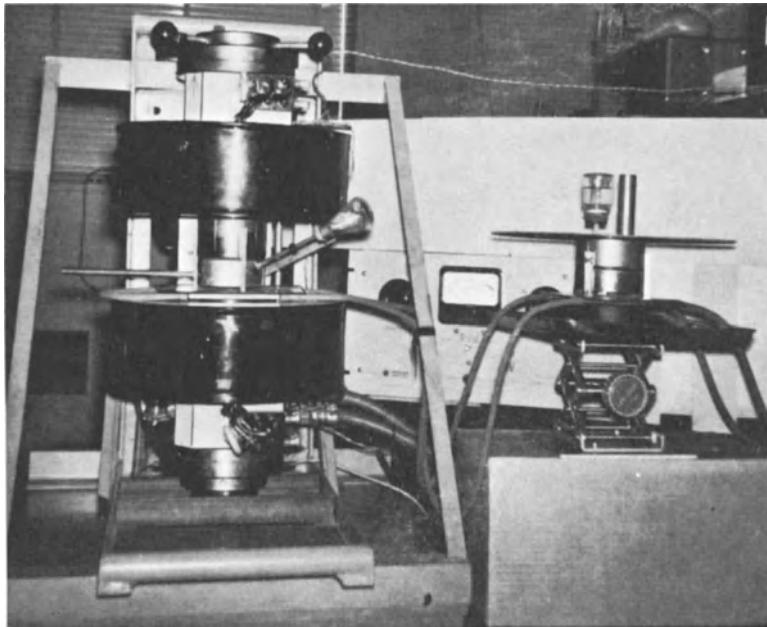


Fig. 3. Varian 4-in. electromagnet with cage between pole caps and dummy magnet.

The six animals were divided into two groups and conditioned for 18 days to the confinement, with three mice per cage. Thereafter the following sequence was adopted: The magnet was energized from 4 PM Monday to 4 PM Friday, when the magnet group was similarly placed in a second dummy magnet cage. On the following Monday, the previous dummy group was placed in the field from 4 PM Monday to 4 PM Friday and the sequence repeated. The weight of the mice was individually measured to 0.1 g every day at 4 PM, when the cages were cleaned.

Following this schedule, the same group was treated every 14 days for 96 hr in the field. The 14-day cycle was repeated five times and covered an age period from 5 weeks to 16 weeks. Group I mice started and finished the five cycles 1 week earlier than group II mice; hence their average age and weight is less, and their average growth rate slightly higher. Figure 4 shows, for each group separately, the average weight variation during the 14-day cycle. It can be seen that the second-day minimum is definitely present. The average weight difference between 0 and 48 hr in the field is  $-0.95 \pm 0.29$  g, and the difference between 48 hr and 96 hr in the field is  $+0.99 \pm 0.37$  g (both corrected for average daily weight gain). The existence of the second-day minimum is thus established on a probability level of 1:3000.

The magnitude of the minimum does not decrease in subsequent cycles, proving that, if repeated every 14 days, the mice do not get

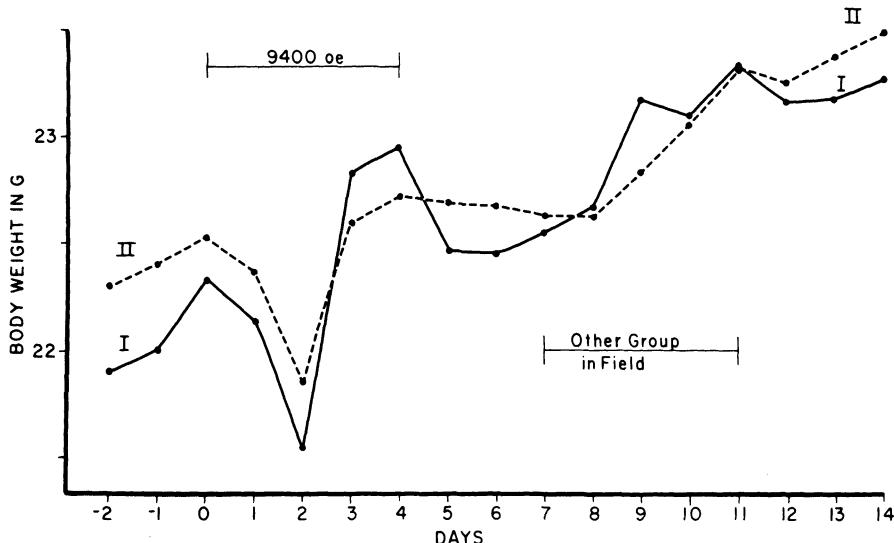


Fig. 4. Average growth curve of two groups of mice alternately exposed each 14 days for 96 hr to a vertical field of 9400 Oe. (Reprinted from Nature, Vol. 200, 1963.)

adapted to the "shock" of the magnetic field. It seems that an after-effect of the field prevails for 4 days after removal, during which time the normal growth is arrested or, as in the case of group I, a weight loss occurs.

#### ACKNOWLEDGMENTS

The electromagnet used in the last part of the experiments was purchased by Graduate Research Grant No. 56-92-36 of the University of Illinois. It is a pleasure to thank my wife for her help in conducting the experiments and for many constructive discussions.

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## *Chapter 2*

# Rejection of Transplanted Tumors in Mice

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In 1932 Julia Lengyel of the Pathological Institute of the University of Budapest came to the Institute for Experimental Physics and requested from me permanent magnets and advice on how she could produce strong magnetic fields, in which she wanted to grow chick heart tissues *in vitro*. Her results,<sup>1</sup> the deformation and retardation of the development of the argyrophil fiber system and the appearance of a large number of multinucleated giant cells, would probably require further confirmation. But I believe that her experiments were the first scientifically conducted investigations of the biological effects of static magnetic fields on tissues. A few years later, T. Huzella, Director of the Pathological Institute, showed me some of his microkinematographic pictures on which cancer cells migrating from malignant tissues into healthy tissues, following the path of the fibers, could be seen. These two observations suggested to me that malignant tumors could perhaps be isolated by subjecting the tumor and its vicinity to a strong magnetic field, thus retarding the development of the fiber system, serving as guiding path for migrating cancer cells. Even if this hypothesis should prove to be erroneous, it induced me to start experiments which showed that the growth of malignant tumors can be influenced by strong magnetic fields.

To avoid complications due to the stress caused by restraining animals, I used unrestrained mice. To keep the animals in constant position relative to the field vector, vertical magnetic fields were used (see Part I, Ch. 4). First I experimented with healthy mice to find how well they can tolerate fields of several thousand oersted strength extending over their full body. These experiments, described in the preceding chapter, revealed a new effect of the magnetic field, namely that the field retards the growth of young mice and lengthens the gestation period of pregnant mice. It was subsequently interpreted as a retardation of the mitotic rate.

These researches were initiated through the untimely death from cancer of Elisabeth Fabiny, our co-worker in investigations of the biological effect of cosmic radiation.

The growth of isolated tumors depends basically on cell proliferation by duplication of cells. In normal tissue the increase in number of cells is balanced by destruction of cells. If proliferation exceeds destruction, tumors develop. Recently, Eversen and Iversen<sup>2</sup> suggested that tumors could result also from a decrease in destruction. But this would not account for fast-growing tumors. At the time of my first experiments (1948), it was generally assumed that the rate of proliferation is more rapid in tumors than it is in normal tissues.

It is well known that external agents such as X rays, chemicals, etc., affect the fast-growing tissues more than tissues with a lower rate of mitosis. This is the basis of X-ray treatment of malignancies. I surmised, therefore, that the treatment of tumor-bearing animals would entail three beneficial effects: it would isolate the tumor and prevent a spreading through migration of tumor cells; it would retard or arrest the growth of the tumor at a field strength which is not harmful to healthy tissues, and, finally, by increasing the lifespan it would provide time for the probably existing defense mechanisms of the body to fight the tumor.

For the first experiment the T 2146 adenocarcinoma of the British Cancer Institute was chosen; it is very virulent, can be readily transplanted in different genotypes, and kills the host within 3 weeks. I am indebted to G. Klein\* for his advice in choosing the tumor and introducing me to tumor-transplantation techniques. The donor mice were shipped by air from England and the tumors transplanted in albino randombred mice. (After the siege of Budapest, inbred strains were not available.) The transplantation was performed in a glass box illuminated by a micromercury lamp, which helped to keep the box germ-free; but the operation area was shielded against the short-wave part of the spectrum.

After the donor mouse was killed with chloroform, the tumor, of about  $\frac{1}{2}$  cc volume, was peeled from under the skin. On the flank near the hind leg of the prospective host a small area was sterilized with 2% tincture of iodine and, while still wet, the fur was parted and a 5-mm-long incision made through the skin. From the surface part of the tumor a cube of about 10 mm<sup>3</sup> volume was snipped and loaded in the needle of the trocar; the trocar was introduced through the incision and pushed forward under the skin to the suprascapular region, where the tumor piece was forced out with the stylet. The trocar was designed to prevent contact of the tumor piece with the host tissues while introduced into the body. This precaution ensured that the host developed only one, fairly spherically shaped tumor, the size of which could be well determined through palpation. All mice, controls and treated alike, obtained transplants from the same tumor piece. All transplants were taken.

\*Presently Director of the Department of Tumor Biology of the Karolinska Institute in Stockholm.

Three air-cooled electromagnets were at my disposal; they were fed from the direct-current power line of the Physics Institute. One was a large Faraday-type electromagnet, adjusted to produce between cylindrical pole caps of 5-cm diameter a field of 4000 Oe in a gap of 4 cm, with a paramagnetic strength of  $1.6 \text{ MOe}^2/\text{cm}$ . The two smaller were DuBois-type magnets and produced between cylindrical polecaps of 4-cm diameter fields of 3000 Oe in gaps of 4 cm, with a paramagnetic strength of  $1.8 \text{ MOe}^2/\text{cm}$ . The field strength was measured by the ballistic method as well as with a Hall-type gaussmeter. All data refer to the field at the place where the mice usually dwelt: 2 cm from the wall of the cage and  $1\frac{1}{2}$  cm above the lower polecap. The cages were 7.8 cm in diameter and 3.8 cm in height, made from aluminum, had hard rubber bottoms, and were surrounded by open trays where food and water were provided. The mice never attempted to escape, thus proving the adequacy of their housing conditions.

To keep the temperature of the polecaps at room temperature, they were insulated from the iron core of the magnet through alternating layers of thin mica sheets and copper plates, the latter extending out in large cooling fins, through which forced air was blown. The fans (for magnet and dummy cages alike) served also to prevent the mice from staying for long periods outside their cages. The dummy cages, located 3 ft from the magnets, were identical to the magnet cages, with the pole pieces simulated by 1-in.-thick iron discs to avoid differences in biological effects of cosmic radiation showers.<sup>3</sup>

One of the investigated parameters in these experiments was the growth of the tumor. The following procedure was used to determine the tumor size. Steel balls, numbered from 1 to 6 and increasing in size from 0.3 to 1.7 cm in diameter, were embedded under a preserved mouse skin. As mentioned, the tumors were transplanted with a technique which resulted in single tumors of spherical shape. The tumors were compared by palpation with the steel balls under the mouse skin and the ball closest in size (or the half-number between two balls) noted. This procedure was daily repeated by a person who did not know which was a treated and which a dummy mouse. To determine the accuracy of the method, I let two of my graduate students determine the tumor size numbers several times. Between ten pairs of size estimations there never was more than one which deviated by a half-number from the other.

On the graphs of Figure 1 the abscissa is the time in days after transplantation and the ordinate the number of the ball equal in size to the tumor. Since the diameters of the balls were chosen in a sequence to increase as the square root of two, the ordinate is proportional to the logarithm to the base two of the tumor surface.

In the first experiment, 10 mice were inoculated from the British donor mouse. Two were placed in the large Faraday magnet and three (together with a mouse which had a No. 5 size tumor on its neck, chemi-

cally induced with benzpyrene) in the two DuBois magnets; four were placed in two control cages and one was used for further transplants. In each cage there was one male and one female mouse.

All three homotransplants in the DuBois magnets rejected the tumor in 12 to 46 days. (The mouse with the chemically induced tumor did not show either regression or growth of the tumor during its 16 days of residence in the magnet and was thereafter removed.) From the two mice in the Faraday magnet, the male died before tumor rejection; the female lost the tumor after 51 days in the field and died 3 days later still in the field. The reason for death could have been an infection of the very large wound. We know now that the white blood cell count is considerably lowered during residence in the magnetic field (see Part II, Ch. 3), which would lower the resistance of the animal against infections. Another reason could be, as L. Gross has shown (see Part II, Ch. 6), that wounds do not heal in a normal manner in fields of 4500 Oe.

The graphs of Figure 1 show some typical case histories. The most common form of rejection is shown in Figure 1A (full line). After transplantation, the mice were immediately placed in the magnet and dummy cages, but the magnets were only energized on the fifth day,

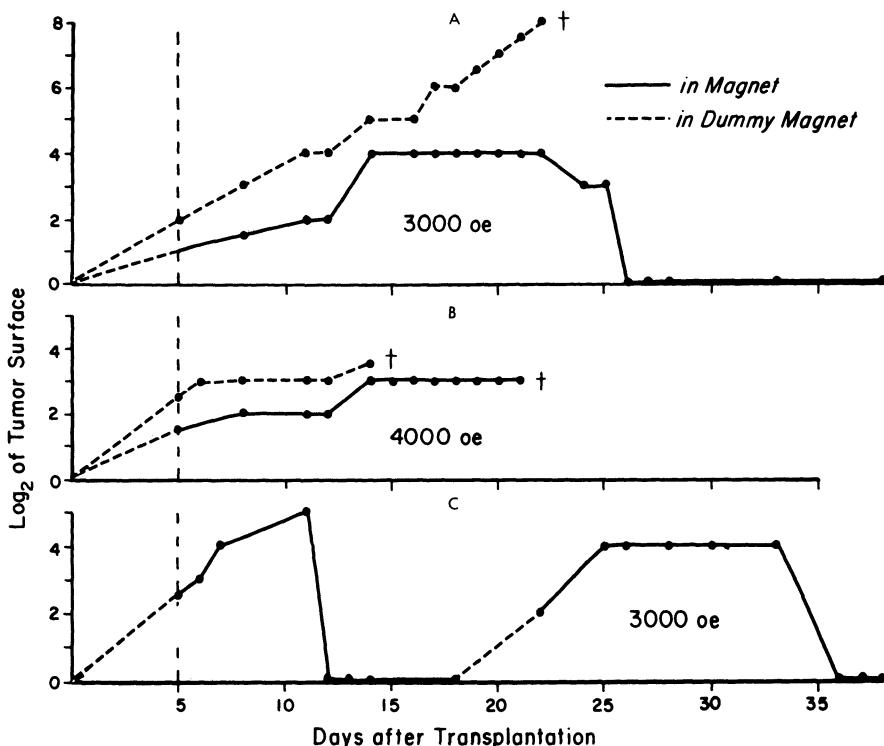


Fig. 1. Typical cases of tumor growth and rejection in magnetic fields.

when the tumors were already palpable. The tumor growth in the field was usually somewhat slower than that of the controls. After 5 to 10 days in the field, the growth stopped and the tumor size remained unchanged for another 5 to 10 days. Thereafter the tumor was rejected. In most instances the entire tumor was rejected overnight and in the morning a plain large wound was visible and some remnants of hairy skin were on the floor of the cage. In a few cases only a part of the tumor was rejected and the other part 1 to 2 days later. If the mice were removed from the magnet, the wound healed without complications and 2 months after rejection the animal could not be distinguished from other healthy mice.

Figure 1B shows the tumor growth of the mouse which died in the Faraday magnet. Figure 1C shows a case where the tumor was rejected on the seventh day in the field. But after 10 days out of the field, a new tumor was noted on the circumference of the wound. This second tumor was then, through repetition of treatment in the field, also and finally rejected.

I wish to emphasize that, in all of these experiments with homo-transplants of T 2146 carcinoma, not one case of regression, common with homotransplants,<sup>4</sup> was observed. The tumors grew in all hosts and the effect of the magnetic field was an arrest of the growth, not followed by a subsequent size diminution, but a quite abrupt rejection of the tumor.

The tumor growth of a control is shown in Figure 1A (dashed line). It increased up to size No. 5, then spread all over the side of the animal. The numbers above No. 5 correspond to the total estimated surface of the tumor cluster. The tumor growth in the control which had the smallest tumor size at the time of death is depicted in Figure 1B. In the experiments with T 2146 carcinoma, a total of 16 controls (homo-transplants) were used. All of them were positive transplants, in which the tumor grew in most cases progressively and only in a few cases was the growth arrested before killing the host. The average lifespan after transplantation was  $20.5 \pm 2.5$  days.

The observed five rejections and four complete recoveries are significant on a probability level of 1:4400 and 1:1300, respectively.

In the second experiment, five mice were inoculated from a homo-transplant of the first experiment. Only one mouse was placed in each of the two DuBois magnet cages and three in individual dummy cages. Since in the previous experiment the only animal which died before rejecting the tumor was a male, in this experiment only females were used. Of the two mice in the field, one died 6 days after transplantation from unknown causes. The growth of the tumor of the other was arrested after it reached size No. 3. Since during the following 3 weeks no rejection occurred, the animal was transferred to the Faraday magnet, the upper pole of which was exchanged for a conical pole to produce a field of great inhomogeneity. The field strength was now 4500 Oe,

with a paramagnetic strength of  $5 \text{ MOe}^2/\text{cm}$ . After 11 days in this field, the tumor was completely rejected. The mouse was by then, however, in very bad shape, could hardly move around, and even though it was removed immediately, died within 1 week. We know now that, after more than one month's residence in the field, the low white blood count no longer rebounds after removal from the field (see Part II, Ch. 3).

From these two experiments the tentative conclusion was drawn that in fields as produced by the DuBois magnets, a rejection of the tumor can be expected only in a first homotransplant, but not in later subsequent homotransplants. I am grateful to G. Klein for drawing my attention to the possibility that the magnetic field did not affect the cancer cells, but merely increased the homograft reaction of the host. The stress caused by the magnetic field, together with the isolation of the tumor from the surrounding tissues, due to the retardation of the fiber system, is presumably enough to lead to a rejection of the tumor. In the second experiment, through the repeated transplantation in the same genotype, the tumor became more hystocompatible and the effects of the magnetic field were no longer adequate to trigger a rejection. Only by transferring the animal to a field of greater strength and paramagnetic strength could a rejection be achieved. But the stress of such strong fields may kill the animal.

Should the foregoing hypothesis regarding the "immunological" reaction be correct, we should expect that isotransplants made in the British Cancer Institute could not be rejected, even when placed in the DuBois magnets, the field strength and paramagnetic strength of which seemed to be best suited for treatment.

For this reason, in the third experiment six T 2146 tumor-bearing mice were shipped from London. Four of these isogeneous hosts were placed in the DuBois magnets and the fifth was used in a homotransplant to eight albino mice. A quadrupole polecap was designed for the Faraday magnet, with four 4.0-cm-diameter cylindrical pole pairs, similar to those of the DuBois magnets. The magnet could now accommodate four of the 7.5-cm-diameter and 3.8-cm-high aluminum cages. Through this change the maximum field strength was reduced to 2400 Oe, with a paramagnetic strength of  $1.1 \text{ MOe}^2/\text{cm}$ . All eight homotransplants were placed in the four cages of this magnet.

All isotransplants in the DuBois magnets died in  $17 \pm 2.5$  days. Of the eight homotransplants in the weaker field of the Faraday magnet, two rejected the tumor and completely recovered. Six died without rejection in  $30.5 \pm 1.6$  days; their average lifespan was 10 days longer than that of the controls ( $20.5 \pm 2.5$ ).

The two rejections out of eight has a probability level 1:10 and the effect of lifespan lengthening has a probability level of 1:700. The results support the foregoing working hypothesis: the isotransplants

were in no way affected by the stronger field, whereas the lower field strength had some effect on homotransplants.

In the fourth experiment the donor was the sixth mouse from the previous shipment. Nine homotransplants were made. Two males and two females were placed in the two DuBois magnets on the sixth day after transplantation. Four females were placed in two dummy cages. The two males in the DuBois magnets died on the 32nd and 36th day; the two females rejected the tumors and completely recovered; all four controls died.

Summarizing all results with homotransplants treated in the stronger fields, we see that of the total of ten so-treated mice all five females rejected the tumor, while only two males rejected and three males died without rejection. This seems to indicate a sex preference for tumor rejection in magnetic fields.

In the fifth experiment the donor was a homotransplant of the previous experiment. None of the seven second-step homotransplants rejected the tumor until the 15th day. This experiment had to be terminated due to our departure for the United States.

The experiments were not resumed during the next 6 years. In 1954, through the good offices of L. Meduna and the generosity of the Dorothy Shattuck-Stensland Foundation, I was able to continue the experiments started in Budapest.

Ten alnico permanent magnets of 275 lb each were installed in the Pathological Department of the St. Francis Hospital in Evanston, Illinois. Cages of 4-in. diameter and 1½-in. height with automatic water and food supply were used. The field strength of 4200 Oe in the cages was somewhat higher, but the paramagnetic strength was  $0.12 \text{ MOe}^2/\text{cm}$ , considerably lower than in the DuBois electromagnets. To eliminate the uncertainty of immunological reactions in homotransplants, only isotoners were used.

From Roscoe B. Jackson Memorial Laboratory 1½-to-3-month-old C3H strain male mice were shipped, inoculated with the C3HBA mammary gland carcinoma, which kills the host in 4 weeks. All treatments were started 4 to 10 days after transplantation. A total of 40 mice were used, supplied in three shipments, scheduled to allow maximum use of the magnet space. One mouse was placed in each of the magnet and dummy magnet cages.

None of the treated mice rejected the tumor, but their average lifespan after transplantation was 35% longer than that of the controls. The tumor growth was not retarded. Due to the longer lifespan, the treated mice sometimes were running around, seemingly undisturbed, with tumors equaling their body size. Pathological investigation, performed by Dr. W. Henry of St. Francis Hospital, revealed no observable differences in the tumor tissues, with the exception that the tumors of the treated were in a higher percentage necrotic. No metastases were found in the main organs of the treated animals. The average tumor

TABLE I

Lifespan, days		Tumor weight, g	
Magnet	Dummy	Magnet	Dummy
54	40	10.0	2.8
41	28	5.6	0.5
41	20	6.0	0.1
36	16	3.0	0.2
54		12.0	
57		11.0	
56		11.5	
23	36	0.7	4.0
22	23	2.5	0.5
23	23	1.0	0.2
22	23	4.0	0.5
41	29	2.6	0.5
33	28	2.5	3.5
32	30	2.5	4.0
37	29	2.0	2.0
32	27	4.0	1.9
38	32	2.0	0.5
32		2.5	
30		4.0	
$37.0 \pm 2.6$		$27.4 \pm 1.7$	$4.7 \pm 0.8$
Difference	$9.6 \pm 3.1$ days		$3.2 \pm 0.9$ g
$t = 3.1; n = 31; P.L.: 1:500$		$t = 3.45; n = 31; P.L.: 1:2000$	

weight at the time of death was 214% greater for the treated group than for the dummy group. Table I shows the lifespan and tumor weight of 19 treated and 14 dummy mice. Seven of the treated did not develop tumors; since all of the dummies developed tumors, this difference is significant on a probability level of 1:28. The difference in lifespan is significant on a probability level of 1:500; the difference in tumor weight, on a probability level of 1:2000.

Although this experiment seems to support anew the assumption that the rejection of tumors in magnetic fields is connected with immunological reactions of the host, it cannot be entirely excluded that fields with paramagnetic strength below  $1 \text{ MOe}^2/\text{cm}$  do not exert an adequate action to trigger a rejection of the tumor.

From 1957 on, further experiments were carried out in the laboratory of the Biomagnetic Research Foundation in Evanston, Illinois with the same ten permanent magnets. All experiments were made with the inbred C3H strain mice. The following carcinomas were used:

A mutant of the C3HBA, which kills the host in 6 to 7 weeks. Iso-transplant. Field and cages same as used in the St. Francis Hospital experiments. No observable effect.

H2712 mammary gland carcinoma, which kills the host in 14 to 22 days. Isotransplant. Field and cages as above. No observable effect.

Spontaneous mammary gland carcinoma of the C3H strain females. Appears at early age and kills 50% of the females before 9 months. Incidence 98%. Field and cages as above. No observable effect.

H2712. Field 4300 Oe with 5.6 MOe<sup>2</sup>/cm paramagnetic strength, produced with additional ring-type alnico magnets in special ring cages. Of 12 treated mice, two rejected the tumor under same circumstances as described in the Budapest experiments and recovered completely. All of the 14 controls died. Rejection and recovery is significant on a probability level of 1:120.

#### SUMMARY

In fields of 2400 to 4500 Oe and of not less than 1 MOe<sup>2</sup>/cm paramagnetic strength, a rejection of homotransplants can be achieved in 20 to 80% of the cases. This effect is established on a probability level of 1:1000.

A rejection of isotransplants was observed only in fields with more than 5 MOe<sup>2</sup>/cm paramagnetic strength.

In fields of 4200 Oe with 0.12 MOe<sup>2</sup>/cm paramagnetic strength a lengthening of the lifespan of mice with isotransplants was observed. This effect is significant on a probability level of 1:500.

Every cancer research project has the ultimate aim of finding a cure for human malignancies. Since human cancers are not transplanted tumors, we cannot hope for any immunological reaction to occur, the amplification of which through the magnetic field would lead to a cancer rejection. For this reason, greater importance has to be attributed to research on cancers which are spontaneous in animals. The experiments with the spontaneous mammary gland carcinoma of the C3H strain which are now in progress in the College of Pharmacy, University of Illinois, raise the hope that in the foreseeable future the developed treatment methods will become applicable to human patients.

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## *Chapter 3*

# Hematological Changes in Mice

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In 1948 J. M. Barnothy concluded from experiments in which he placed mice in strong magnetic fields that the main biological effect of a magnetic field is, most probably, a general retardation of mitosis and, furthermore, that this effect, as is the case with other external agents, is greater in rapidly dividing cells.<sup>1</sup> Prompted by these considerations, I. Boszormeny-Nagy suggested in 1956 that we should investigate the effect of magnetic fields upon hemopoietic organs. The lifespan of leukocytes in the circulating blood is short, of the order of one day.<sup>2</sup> Should the magnetic field affect the leukocyte-producing organs, the leukocyte number would change and provide a suitable test of the retardation of mitotic activity.

The first experiments were made in the laboratory of the Biomagnetic Research Foundation in 1956. They revealed that a homogeneous vertical magnetic field of 4200-Oe does indeed decrease the number of circulating leukocytes of the mouse during the first week of residence in the field, but does not observably affect the erythrocyte count. After removal from the magnetic field a recovery sets in, during which the leukocyte count overshoots the base line.<sup>3</sup> Several further experiments performed since then have confirmed the previous results.

In 1958 I tried to use this magnetically induced leukocytosis to counteract leukopenia following X- or gamma-ray irradiation.<sup>4</sup> These experiments, which are discussed in the following chapter, showed that magnetic treatment of animals prior to their irradiation with large doses of gamma rays does indeed decrease the deathrate. In 1960 L. Gross<sup>5</sup> successfully utilized the lymphocytosis following removal from the magnetic field for treatment of transplanted tumors in mice (see Part II, Ch. 5).

In the following, the results of three experiments are reported in which the cells were counted with a Coulter electric particle counter.

The Coulter counter operates on the principle of electrical gateing. The electric resistance of a small ( $100 \mu$  in diameter) orifice changes Supported by Graduate Research Grant No. 56-92-11 of the University of Illinois and by the Biomagnetic Research Foundation.

every time a blood cell passes through the orifice. The resulting pulse, which is proportional to the volume of the cell, is electronically counted.

One-half cubic centimeter of blood diluted with saline and containing 5000 to 15,000 white blood cells is drawn through the orifice and counted. This number is 50 times as much as usually counted in four primary squares of a hemocytometer. Furthermore, the electrical method avoids the two chief errors of the hemocytometer method: the uneven mixing of cells and diluting fluid, and the settling of the cells by chance on the ruled field of the counting chamber. The standard error of the Coulter method is, according to several investigators,<sup>6-9</sup> about 2.8% for successive determinations by the same worker and 1.2% for repeated counting of the same cuvette; the comparable figures for the hemocytometer method are 10 and 7%, respectively. With the Coulter counter, counting, recording the data, and exchanging samples requires only 30 sec per sample. In experiments with mice, where the variance of the leukocyte count of individual animals may amount to 25-35%, the advantage of evaluating a large number of samples is of particular importance.

The number of leukocytes is extremely sensitive to a large variety of factors: muscular activity, digestion, temperature, stress, traumatic shock, wounds, infections, etc., and also shows seasonal variations. For these reasons we have tried to provide good housing conditions for the animals in the magnets, used females who do not fight, and applied a bleeding technique which is as gentle as possible.

#### HOUSING OF THE ANIMALS

Figure 1 shows the ten permanent alnico magnets and the row of ten dummy magnets above them. The magnets weigh 275 lb each and have 4-in.-diameter poles with conical 3-in.-diameter pole caps. The cylindrical cages are  $3\frac{7}{8}$  in. in diameter and  $2\frac{3}{16}$  in. high, and have plastic floors and automatic food and water supply. Through a circular opening between wall and floor, feces and food remnants are swept from the cage area by the tail movement of the mice. The circumstance that the cage wall does not restrict the tail position of the mice provides the illusion of a larger cage.

In experiments X and XI, the cages were connected to inclined exercise galleries; in experiment XII, the bottom of the cages was kept at a constant temperature of 80°F with the help of a thermocirculator. Horizontal connecting galleries, cooled to 50°F, provided passage between the five cages within each group. This arrangement is shown in Figure 1. The mice did not stay either in the inclined exercise galleries or in the cooled connecting galleries for any considerable time. But both devices allowed the animals some freedom of motion and exercise. Through the horizontal connecting galleries, the mice distributed themselves quite at random in the five cages; but during the main rest

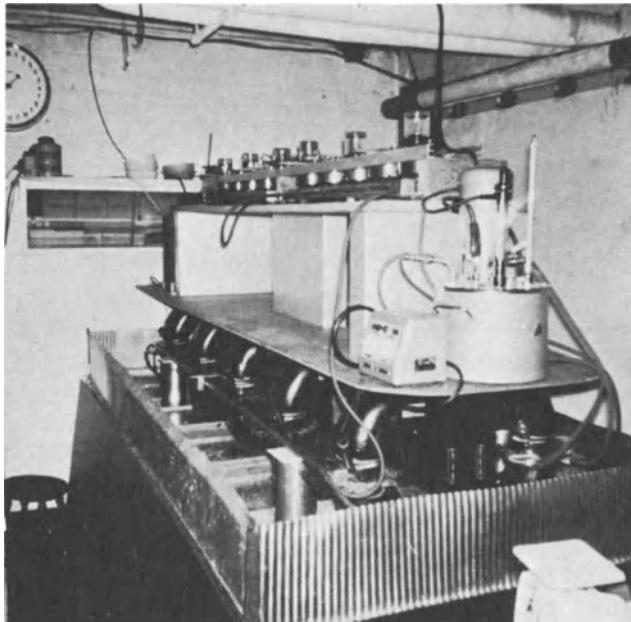


Fig. 1. Ten permanent magnets, producing a homogeneous field of 4200 Oe (left and right, lower row), and ten dummy magnets (upper row). Thermocirculators provide a constant floor temperature in the cages and cool the connecting galleries.

periods, six mice often crowded into one cage. Every second day the cages were sterilized in an automatic washer. Rockland Mouse Diet was used throughout the investigation. A large number of properly distributed small fans provided an almost unnoticeable air current through the cages; this helped to keep the cage floors dry.

The control-cage arrangements were exact replicas of the magnet cages, with the magnet poles simulated by 2-in.-thick iron discs. The thickness of the latter was chosen to avoid differences between magnet and dummy groups due to the biological effects of cosmic radiation showers.<sup>10</sup> The dummy magnets were only 3 ft above the magnets, but shielded from the stray field of the magnets.

The room had its own heating unit, the temperature regulated to 75°F. The humidity was kept between 40% and 60% with the help of a dehumidifier or air conditioner. Daylight entered the room through windows on the west side; the windows never received direct sunlight. Since the cage walls were opaque, only diffuse light entered the cages.

The magnetic field was 4200 Oe in the center of the cage. The values of field strength and gradient in the cage area are given in Table I. The field is not entirely circularly symmetric around the center; it decreases slightly faster in the direction of the yoke. The values given in the table are averages, measured with a Hall probe (Dyna Empire gaussmeter) in the four main directions at a height of  $\frac{1}{2}$  in. above the

TABLE I  
Field Strength and Gradient in Cage,  $\frac{1}{2}$  in. above Floor  
Level

Radial dist., in.	Field strength, Oe	Gradient, Oe/cm	
		horiz., inward	vert., downward
0	4200	0	0
$\frac{1}{2}$	4192	8	13
1	4120	140	110
$1\frac{1}{2}$	3660	605	254
2	2420		

floor level. The cages had metal walls, and the cool touch of its surface prevented the mice from leaning against the wall. The centers of gravity of their bodies were, therefore, never more than  $1\frac{1}{2}$  in. from the center. The paramagnetic strength of the field is on the average  $\frac{1}{2}$  MOe<sup>2</sup>/cm; thus the field can be considered as a homogeneous field. Through the random motion of the unrestrained animals the horizontal gradient vector is frequently changed in its direction relative to the animal and its organs. The field and vertical gradient vector directions are, on the other hand, constant relative to the animal, since mice in such low cages never assume supine positions. The north pole was the upper pole in the five magnets of the front row, while it was the lower pole in the five magnets of the rear row.

#### BLOOD SAMPLING

Blood samples were taken from the tail vein. Performing the incision with a sharp razor, one can bleed the animal without its being aware of the bleeding. This type of bleeding leads to the smallest amount of total blood loss (about 40 mg). Ventricular puncture would have been cumbersome and rather risky in my case, when several hundred blood samples had to be taken per experiment and when it was important to take successive samples from the same animal. The method of eye bleeding<sup>11</sup> was rejected because it is connected with a strong traumatic shock, repeated bleeding may lead to the blinding of the animal, and the total blood loss is rather excessive (about 400 mg).

The erythrocytes and the leukocytes of the mouse are similar in shape to those of other mammals. The erythrocytes are somewhat smaller and more numerous. The main difference compared to human blood is that  $\frac{2}{3}$  of the leukocytes in human blood consist of polynuclear cells, while  $\frac{2}{3}$  of the mouse leukocytes are lymphocytes. Accordingly, a leukocytosis of the mouse is practically a lymphocytosis.

#### EXPERIMENTAL PROCEDURE

With the help of an automatic pipette 10 ml of isotonic saline solution was introduced into each of several 20-ml glass vials closed with

plastic caps. Saline solutions were obtained from the Manufacturing Pharmacy Department of the College of Pharmacy, University of Illinois. The background count of saline at normal instrument setting was less than 200 for erythrocyte counting and less than 50 for leukocyte counting; these values are negligible in all cases.

For bleeding, the mouse was placed in a restraining cage, with a dark cover and a receptacle to collect the feces. Under a magnifying glass and good illumination, a small incision was made with a razor across the tail vein, in the middle  $\frac{1}{3}$  length of the tail. The free-flowing blood by capillary action filled a heparinized capillary tube, the weight of which was previously determined on a torsion balance to 0.1 mg. The capillary was again weighed and the blood washed with an automatic pneumatic device into the 10 ml of saline. A pressure tourniquet of tissue paper was applied to the wound until bleeding stopped. Thereafter the mouse was returned to a cage with clean Sanicell bedding, where it remained for  $\frac{1}{2}$  hr before being replaced in its experimental cage. The total time which mice spent outside of the magnet or dummy cages was less than 1 hr.

On all occasions when the hemoglobin content of the blood was also measured, two preweighed capillaries were filled in succession, using the same incision, and the second emptied into a solution of 5 ml of 0.04% ammonium hydroxide.

Usually 15 to 25 mg of blood could be taken with one capillary without massaging the tail. For leukocyte counting, 0.1 ml of a 0.5% saponin solution was added to stromatolyse the red cell membranes. For erythrocyte counting, 0.1 ml of the original blood solution was transferred to a second vial filled with 10 ml of saline. For blood smears, the first drop forming on the wound was touched to a glass slide and spread with a semiautomatic slider.

Blood samples were taken from the same animal at intervals of 4 to 10 days, alternately from the left or right tail vein; the incisions overlapped each other a distance of  $\frac{1}{8}$  in. and came successively closer to the body. The incisions healed in all cases without infections, and after the lapse of 10 days they were hardly noticeable.

To see whether a difference in the leukocyte number might arise depending on whether the blood is taken from the right or left tail vein, on a larger number of animals we made incisions on both sides of the tail at the same time. The difference, if any, is less than 0.6%.

To avoid any influence on the leukocyte count due to digestive functions, all samples were taken in the afternoon, during the rest period of the mice. Whenever the samples were taken from all 50-60 animals used in one experiment, from one half of the magnet and one half of the dummy groups the samples were taken between 3 PM and 4 PM, and from the other half between 7 PM and 8 PM. The front-magnet-row group was always paired with the same group of dummies, and similarly

the rear-magnet-row group always with the other dummy group. Although fluctuations of the leukocyte count occur during a single day, as well as from day to day, the suggestion that these follow a characteristic hourly rhythm has not been confirmed.<sup>12,13</sup> In several instances we have switched the mentioned magnet-dummy group pairs between early and late afternoon hours and did not find a significant difference, although there was a slight indication that the late afternoon hour counts were a few percent lower.

When mice are removed from their experimental cages and placed in large standard cages with fresh bedding, the larger space available and the new smells in it excite the animals to higher activity during the first half hour. It is known<sup>14</sup> that intense activity may increase the number of polynuclear cells, leading to a leukocytosis in man, where  $\frac{2}{3}$  of the cells are polynuclear. In mice, where they constitute only  $\frac{1}{3}$  of all leukocytes, the effect should not be prominent. We have compared the leukocyte count of freshly removed groups with groups which were removed several hours earlier. No significant difference was found.

The measurement of leukocytes with the Coulter counter necessitates some precautions. The number of counted leukocytes may change because (a) the threshold between noise level and lowest leukocyte pulse is not properly set; (b) some of the leukocytes settle; (c) the agent used to stromatolyse the red cell membranes may cause deterioration of the leukocytes. To minimize these errors:

a. The Coulter counter was monitored with a second self-triggering oscilloscope with photographic camera. The films, scanned with a photometer, showed the pulse height distribution. The pulse height distribution was also determined with a single-channel analyzer, using a channel width which gave a pulse height distribution equal to 20 channels. The photographic method could provide a pulse height distribution from each sample during its normal counting time of 15 sec; the single-channel analyzer required a blood amount pooled from 20 samples. Erythrocytes show one well-characterized pulse size group, the leukocytes two slightly differentiated groups.

The average volume of mouse lymphocytes is about  $\frac{1}{3}$  of the average volume of polynuclear cells. Since in the Coulter counter the pulse size is proportional to the cell volume, we could attempt to associate the lower pulse height group with lymphocytes and the higher pulse size group with polynuclear cells and discriminate the two type of leukocytes through a proper threshold setting. Unfortunately, the pulse size of large lymphocytes overlaps the pulse size of small polynuclears. A trial in which pulse size discrimination was compared with classification on blood smears proved that a discrimination between lymphocytes and polynuclear cells based on their pulse height difference is unreliable.

b. Repeated counting of blood dilutions without shaking the vials anew showed that the settling of the leukocytes decreases the count in 1 hr by 6%. To avoid counting errors due to settling differences in the

individual vials, the vials were shaken by hand in a sequence, so that each sample was measured exactly  $3 \pm \frac{1}{4}$  minute after the last shaking. This time difference between shaking and counting ensured the settling of large debris, but did not lead to an appreciable settling of the leukocytes themselves. The diluted blood samples of the mice treated in the magnets and in the dummy magnets were counted in one sequence without interruption; after counting the last sample, the shaking sequence and counting was continued and the first two samples counted anew. The second measurement of the same sample was on the average 0.5% lower, but the difference never exceeded 2%.

c. After saponin was added, the blood solution became clear within 2 min. On any one occasion only 10-15 samples were injected with saponin; the next 10-15 samples, 5 min later. This ensured that each sample was measured not earlier than 10 and not later than 15 min after the addition of saponin. Repeated counting of the same samples revealed that the cell count did not drop by more than 1% within 1 hr after saponin was added.

Heparin does not alter the size of the corpuscular constituents of the blood. However, since its physiological actions may depend on its strongly acid property, it cannot be excluded that it may affect electrical gating methods by altering the electric resistance of the cell membrane. I have, therefore, taken blood with heparinized and unheparinized capillaries from the same drop; differences, if any, are less than 0.5%.

All results reported in this paper are (with the exception of the curve in Figure 2) relative values between magnet and dummy groups. The relative counting errors between individual samples are: 0.2% in the saline volume; 1% in blood weight; 0.8% due to the deterioration of leukocytes through saponin; 0.05% due to settling of leukocytes. The counting loss due to the simultaneous passage of two cells through the orifice was always corrected and is generally negligible in leukocyte counting. Clogging of the  $100-\mu$ -diameter orifice did not occur more often than once in 200 samples and was always immediately noted and corrected.

The proper setting of the threshold level was determined on the basis of the pulse height analysis as described above (for erythrocytes: aperture current 6, threshold 20, amplification 4; for leukocytes: aperture current 4, threshold 15, amplification 4). To avoid changes in the amplification, threshold, etc. due to line voltage variations, the instrument was fed from a Sola constant-voltage transformer.

Random-bred Swiss female mice of equal age were obtained from animal breeders in the Chicago area. They were kept for at least 1 week in standard cages prior to their use in the experiments. The animals were individually weighed and those on both ends of the weight distribution, i.e., too heavy or too light, rejected. The remaining fairly uniform group was further subdivided into five weight groups and each subgroup evenly distributed between magnet and dummy cages. The

difference in the mean starting weight of magnet and dummy group mice was less than 0.3 g.

Data on mice which developed bite infections around the anus (3 standards), leukemia (1 magnet), cancer (1 magnet) during the investigations, and all which died during the experiment from unknown causes, were eliminated from the final results. From 164 mice used in these three experiments, in a total of 13,000 mice-days, one mouse died in the dummy cages, none in the standard cages, but five (three in experiment X and one in each of experiments XI and XII) died in the field. This mortality difference is not significant, but noteworthy.

The standard deviation of the leukocyte count of an individual mouse is 25-35%. Leukocyte counts which deviated by more than 60% from the mean value of the group, and at the same time deviated by a factor of two from the value obtained on the preceding as well as on the following occasion for the same animal, were discarded as abnormal for unknown reasons. Based on this criterion, a total of 13 data were discarded from a total of 2100 blood counts.

## EXPERIMENTAL RESULTS

### *Experiment X*

In this experiment twenty-seven 250-day-old female mice were conditioned for 2 days to the small cages and treated for 59 days in a 4200-Oe field. Twenty-seven were kept in dummy magnets. A total of 740 leukocyte counts and 440 erythrocyte counts were made; 110,000 leukocytes were classified on 220 blood smears and the hemoglobin content of the blood was determined on 270 samples.

The confinement of the mice to small cages seems to cause a drop in leukocyte count of the magnet and dummy groups alike (see Figure 2), but while the dummy group remained for the next 30 days nearly constant at a level between 6000 and 7000 WBC/mg, the magnet group dropped further to 5400 WBC/mg. This low level was followed by a sharp increase in the leukocyte count above that of the dummies, with a peak on the 16th day of treatment. But thereafter an even more drastic drop occurred, reaching the second minimum on the 31st day of treatment (probably earlier). The differences from the dummy values are significant, in both minima and the maximum, on a probability level less than 1:1000. Table II shows an example of the original data on the 31st day of treatment.

The first column in Table II indicates the number of the mouse, the second the amount of blood taken in milligrams, the third the instrument count, and the fourth the WBC count per milligram. Columns 5 through 8 contain the same data for the magnet group. The data of dummy No. 1 (15,700) and magnet mouse No. 17 (10,750) were discarded as abnormally high using the criterion mentioned earlier. Magnet mouse No. 2 escaped and, although later found, was not replaced for

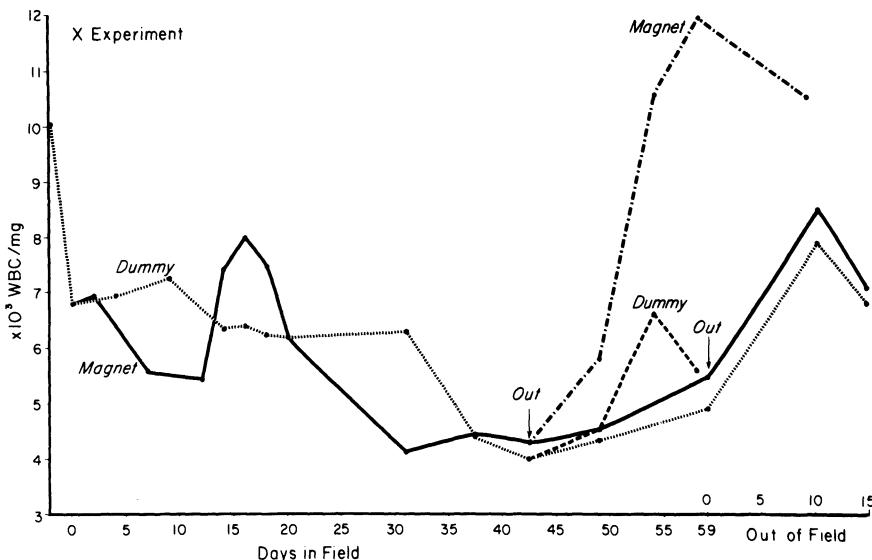


Fig. 2. Leukocyte count per milligram for 27 mice in magnets and 27 in dummy magnets, treated for 59 days. A small group was removed on the 42nd day of treatment (dashed and dash-dot lines). Experiment X.

treatment. Mice Nos. 13, 14, and 15 are the three that died in the field. The difference between magnet and dummy group at the time of the second minimum is significant on a probability level of 1:60,000.

The amount of blood taken was determined by weighing and not, as usual, by volume. The blood counts given in the table, or anywhere else in this paper, are expressed in cell number per milligram of blood. To change these values to cell number per cubic millimeter, one has to divide them by 1.055, the specific gravity of blood. Furthermore, since 0.10 ml of saponin and about 0.02 ml of blood was added to the 10 ml of saline in the vial, the correct absolute count per milligram is obtained if the values are increased by 1.2%.

After the 33rd day of confinement, the leukocyte count of the dummy group dropped for unknown reasons to the level of the magnet group (4000 WBC/mg), and the two groups remained for the rest of the investigation almost equal.

On the 42nd day of treatment, four mice from the magnet and five mice from the dummy group were removed and placed in standard cages. The leukocyte count of the four magnet mice increased in 2 weeks from 4300 to 11,900 WBC/mg, thus surpassing the base line (10,000 WBC/mg) by 1900 WBC/mg, or 19%. The leukocyte count of the five dummy mice increased, on the other hand, merely from 4000 to 6600 WBC/mg, thus to a level below the base line, and remained below the base line for the rest of the investigation (50 days). The count of the four magnet mice dropped gradually to the base line.

The main group—10 in the front, 10 in the rear-row magnets and 20

TABLE II

DUMMY				MAGNET			
No.	Blood, mg	Instr. count	WBC/mg	No.	Blood, mg	Instr. count	WBC/mg
2	16.2	6350	7820	1	24.1	5740	4750
3	24.5	11200	9190	3	27.9	4667	3350
4	20.3	5995	5900	4	22.4	4087	3650
5	22.9	9577	8360	5	25.0	10400	8350
6	22.7	5557	4900	6	25.2	3502	2780
7	16.4	4783	5840	7	21.6	5114	4750
8	26.9	9406	7000	8	16.9	5060	6000
9	22.1	6484	5860	9	20.6	1973	1920
10	19.2	4400	4600	10	19.9	2528	2550
11	22.4	8556	7650	11	27.8	2843	2040
12	19.4	4100	4230	12	23.8	4060	3400
13	25.1	6010	4790	16	19.3	6770	7010
14	22.7	9940	8760	18	27.8	5292	3810
15	23.5	5190	4420	19	22.3	4266	3830
16	23.9	4990	4180	20	19.3	5438	5640
17	23.4	8050	6860	21	24.7	3268	2640
18	19.9	6350	6390	22	22.4	4140	3690
19	16.0	7940	9880	23	18.8	3658	3900
20	21.6	6200	5750	24	16.7	2982	3580
21	19.6	5160	5260	25	27.1	7106	5250
22	20.1	8564	8500	26	19.1	4596	4810
23	21.1	5527	5250	27	20.2	4124	4090
24	23.7	7110	5980				
25	19.5	3255	3390				
Average:				$6280 \pm 360$			
Standard deviation:				$\pm 1770$			
Difference (magnet - dummy):				$-2110 \pm 490$			
$t = 4.3$				P.L. = 1 : 60,000			

in the dummy cages—were removed on the 59th day to standard cages. Both dummy and magnet groups increased their leukocyte count in 2 weeks from 4000 counts to 8000 WBC/mg and both groups settled thereafter to a level of 6500 WBC/mg. Five months after removal from the field and dummy cages, respectively, the WBC count of the magnet group was 5600 WBC/mg and that of the dummy group 5900 WBC/mg; since the error of each value is  $\pm 450$  WBC/mg, the two can be considered as equal.

The drop in the leukocyte count during exposure to a magnetic field is in accordance with our earlier findings.<sup>3</sup> A new feature is, however, the transient maximum in the leukocyte count around the 16th day in the field. In 1956 the 18 C3H female mice had on the 7th, 17th, and 29th day of their residence in the field a leukocyte count of  $11,590 \pm 850$ ,  $12,930 \pm 740$ , and  $10,350 \pm 960$ , respectively. The maximum was here similarly indicated, although its significance is only on the 5% confi-

dence level. A shortcoming of these earlier experiments was that in the first experiment none and in the second only four controls were housed in dummy magnets.

We may note that in experiment X the mice removed on the 42nd day of their treatment time showed a sharp increase and overshoot after removal, in accordance with our earlier findings,<sup>3</sup> while those removed on the 59th day did not.

The peculiar behavior of the dummy group, that is, the drop in the leukocyte count after the 32nd day to a level of 4000 WBC/mg, prompted me to investigate this question in experiment XI. Sixty female mice 70 days old were divided into three groups: 20 for magnetic treatment, 20 as controls in dummy magnets, and 20 remained in standard plastic cages. Blood was taken on all occasions from all 60 animals.

Figure 3 compares the leukocyte count of the dummy and the standard-cage groups. We note the surprisingly parallel trend of the dummy and standard groups. The difference between their WBC count remained always within the limits of error. This parallel trend is observable for the 35 days the dummy group was in small cages, as well as during the following 60 days, when the dummy group was returned to standard cages.

This parallel trend is the more surprising because, during the 90 days of observation, the leukocyte count underwent a very considerable

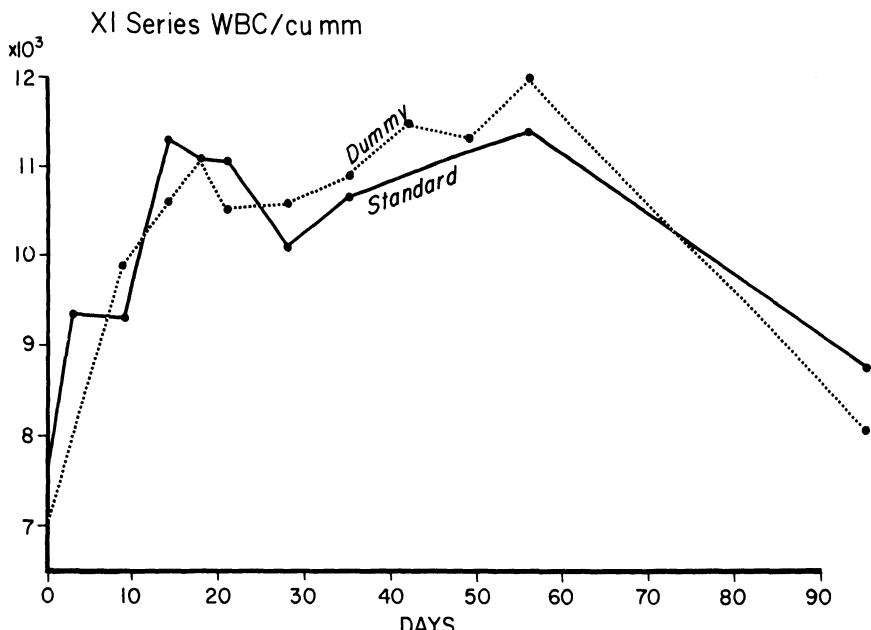


Fig. 3. Leukocyte count per milligram for 20 mice in dummy cages ( $3\frac{1}{8}$  in. in diameter,  $2\frac{3}{16}$  in. high) and 20 mice in standard cages (11 x 7 x 5 in.).

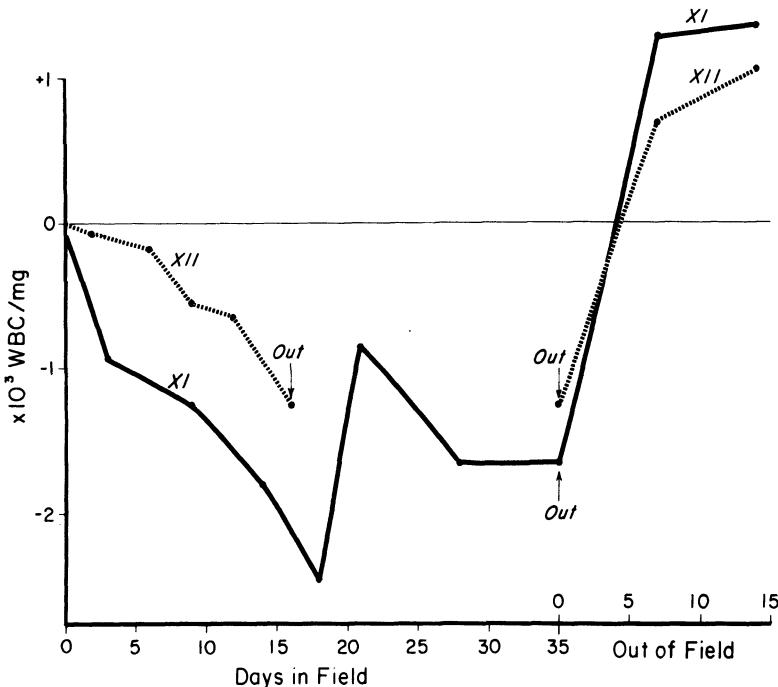


Fig. 4. Leukocyte count difference (magnet minus dummy group) during treatment and after removal from the field. Full line—experiment XI (treated for 35 days); dotted line—experiment XII (treated for 16 days).

increase and decrease. Without going into speculations regarding the causes for this large variation, we can draw three conclusions:

1. The unknown factor causing the large change in the WBC count is independent of the housing in small or large cages and should therefore be present also in the treated group.
2. In investigations which aim to determine effects of external factors on the WBC count and which are conducted over a longer period, only differences between the treated and dummy groups are meaningful.
3. Leukocyte counts listed in handbooks based on a single determination may have an intrinsic error of 30%. This is true even if the average is obtained from a large number of mice and the variance is small.

#### *Experiment XI*

In this experiment forty mice were conditioned for 1 week to small cages in dummy magnets, then equalized with respect to weight and distributed evenly in ten magnet and ten dummy cages. They were treated for 35 days in the homogeneous field of 4200 Oe, then removed together with the dummy group to standard cages and their leukocyte

count followed up for a further 60 days. A total of 690 leukocyte counts and 39 erythrocyte counts were made and 100,000 leukocytes classified on 200 blood smears. Figure 4 shows the difference in the leukocyte count of magnet and dummy mice.

We see anew the pattern of the minimum-maximum-minimum triad and the overshoot after removal from the field. They were removed around the time of the second minimum.

The individual numbering of the animals enabled us to plot the leukocyte differences for each individual magnet mouse relative to the average of all dummy mice. The individual curves thus obtained reveal that while the triad pattern is manifested in each of them, the location of the maximum coincides only in 70% of the cases with the time of the maximum as shown in Figure 4, that is, the 21st day in the field. In 30% of the cases it occurs either a few days earlier or later. Since, on the other hand, the two minima precede and follow the maximum rather closely, the maximum in the leukocyte number of one mouse may coincide with the first or second minimum of another mouse. This overlapping of minima with maxima will tend to decrease the amplitude of the triad pattern. It would seem, therefore, more appropriate to determine the actual pattern from the individual curves. We have refrained from this procedure, because with the present small number of points on the curve the individual curves are not smooth enough to safeguard against a subjectively biased selection of the times of maxima and minima.

#### *Experiment XII*

In this experiment the temperature of the floor of the cages was kept constant at 80°F by means of a thermocirculator, and the five cages within each group were connected with horizontal galleries cooled to 50°F (see Figure 1). Fifty 70-day-old female mice of the ICR strain were conditioned to the small cages for 1 week, as before, then equalized not only with respect to weight, but with respect to equal average leukocyte count. They were distributed evenly among the five front-row, five rear-row magnet cages, and the two groups of five-five dummy cages. The cages of each group were interconnected through the horizontal galleries, but not connected with the other groups. A total of 440 WBC counts were made.

The aim of this experiment was to remove the animals at the time of the first minimum; hence they were treated for only 16 days. At that time, all magnet and dummy mice were removed to standard cages and their leukocyte count followed for a further 3 weeks. The dotted line in Figure 4 shows the results of this experiment. The part of the curve containing the data after removal from the field is shifted by 19 days and plotted with the same removal date as experiment XI. Of course, this curve shows only the first minimum and the overshoot after removal.

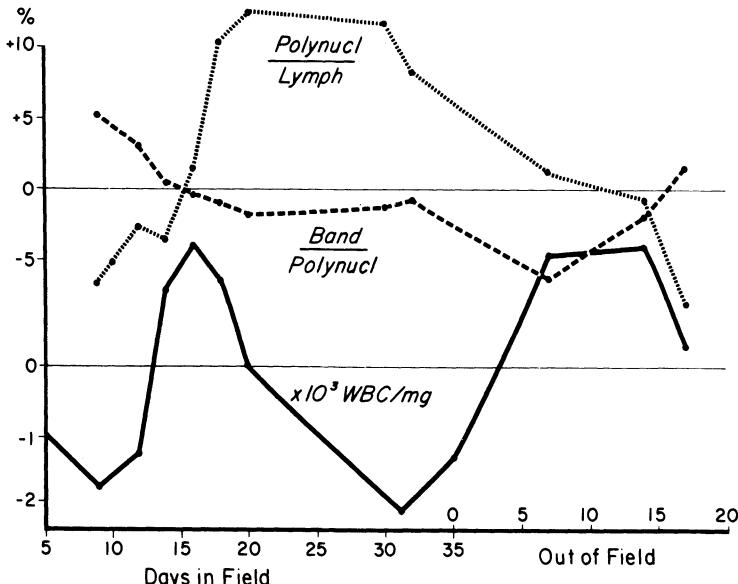


Fig. 5. Difference in the percentage ratio of the polynuclears to lymphocytes (dotted line) and of band forms to polynuclears (dashed line). The lower curve (full line) gives the difference in the total leukocyte count. All differences are magnet minus dummy values.

#### Differential Counts

In experiments X and XI, 420 blood smears were taken [and in an earlier experiment (III), 140 blood smears] during and after magnetic treatment. A total of more than a quarter of a million leukocytes were classified on these 560 smears by Miss Martha Walker, Supervisor of the Hematological Laboratory of St. Francis Hospital in Evanston, Illinois. The slides were given to her with a coding system, which did not disclose whether they were from magnet or dummy mice.

A significant deviation in the percentage of polynuclear cells (neutrophils and eosinophils) was observed in the treated group relative to the dummy group. The curves shown in Figure 5 illustrate the difference in the ratio of polynuclear cells to lymphocytes and the difference of the ratio of band forms to polynuclear cells. On the bottom of Figure 5 we have plotted, for the sake of a better interpretation, the average pattern of the total leukocytes count differences.

#### Erythrocyte Counts

On 17 occasions in experiment X and one occasion in experiment XI the erythrocytes from all animals were counted. The average number of erythrocytes ( $7.5 \times 10^6$  RBC/mg) remained surprisingly constant during and after treatment, with a standard deviation of only 0.8% from the mean of all data.

One explanation of this constancy could be perhaps that erythrocytes have a long lifetime (70 to 100 days) and a longer exposure to a magnetic field would be necessary to produce an effect. On the other hand, experiments made in an inhomogeneous magnetic field of  $2.3 \text{ MOe}^2/\text{cm}$  paramagnetic strength (experiment R) revealed a 60% increase in the erythrocyte number within 13 days. It is therefore possible that homogeneous magnetic fields affect neither the lifespan of erythrocytes nor the organs in which they are produced. The latter would be rather surprising in view of the fact that the erythrocytes are produced in the same organs as the granulocytes. This question will be further investigated using strongly inhomogeneous fields.

#### Hemoglobin Determinations

On 15 occasions in experiment X the hemoglobin content of the blood was determined. Ten to fifteen milligrams of blood was taken from the same incision as for leukocyte counting and diluted in 5 ml of 0.04% ammonia. The optical density was measured at a wavelength of 5400 Å with a Bausch and Lomb spectrophotometer. A significant difference was found between magnet and dummy groups in the 4th-to-16th-day treatment period. From the 163 determinations made during this period, the hemoglobin content of the magnet group was found to be  $5.4 \pm 1.0\%$  lower than that of the dummies. The effect is significant on a probability level of 1 : 6000.

In experiment X we measured the oxygen consumption of magnet and dummy groups on 25 occasions. The 27 mice of the magnet group (or of the dummy group) were placed on trays under a bell jar 13 liters in volume. Air, washed through KOH solution, dried with calcium carbonate, and cooled with ice, was pumped through the bell jar at a constant speed of 3 liters/min. The exhaust air was washed in a column of 1200 cc 10% KOH solution, and the amount of absorbed  $\text{CO}_2$  determined through titration. Each measurement took 60 min, during which time the temperature under the jar did not increase by more than  $0.8^\circ\text{C}$ . The measurements were made always at the same hour of the day<sup>15</sup> on days when no blood samples were taken.

The oxygen consumption of the treated was  $16 \pm 9\%$  lower than that of the dummy group up to the 16th day. Although this difference in oxygen consumption is not significant, it is noteworthy that it coincides with the lower hemoglobin content of the blood, which latter would imply lower oxygen-carrying capacity of the blood.

#### Right-Left Difference

As already mentioned, for the five magnets of the front row, the north pole was the upper pole, while for the five magnets of the rear row it was the lower pole. Mice were never exchanged between front and rear magnet rows. Since in low cages mice never assume supine positions, their orientation with respect to the field vector always re-

mains, in spite of their motion, the same. The importance of this factor is discussed in Part I, Ch. 4. The physical phenomena responsible for triggering biological effects are vector phenomena, the direction of which, relative to the organs of the animal, depends on the position of the field vector relative to the animal. If, say, some of the phenomena occurring in the front row are right-handed in the coordinate system of the animal, the same phenomena will be left-handed in the rear row, and vice versa. Most, if not all, biological organisms have a right-left asymmetry. One would expect therefore, that the processes occurring in the blood-producing organs would also display a right-left asymmetry and would be, therefore, differently affected by a right-handed or left-handed physical phenomenon.

With this in mind, we have inspected our data to determine whether the leukocyte count in the front row was affected differently from that in the rear row.

In experiment X, with the exception of initial and maximum values, the leukocyte count of the rear row was always higher in the field and after removal from the field; but they became equal 2 months after removal. However, the diffuse light inside the cages during the daytime, although equal in the front-row and dummy cages, was slightly less in the rear row. Mice are not known to be light-sensitive, and albino mice have poor vision; they are most active during night and mostly sleep during the day. But, to eliminate the slight illumination difference during the daytime, from the 44th day of treatment exactly equalized artificial light was used instead of daylight. It did not produce any difference; the leukocyte count of the rear row remained persistently higher.

In experiment XI, where daylight was used as before, the front row had the higher leukocyte count; the difference vanished 2 months after removal.

In experiment XII, the daylight illumination difference between front and rear row was eliminated with the help of opaque and semitransparent baffles and reflectors. The leukocyte count, initially equal, was higher in the front row during treatment; after removal, the difference reversed.

From these observations, no clear decision can be made regarding the existence of a right-left difference. Experiments are in progress in stronger fields to check the existence of a right-left difference on the growth rate of mice. This effect is far less sensitive to other uncontrollable factors than the effect upon leukocyte count.

#### DISCUSSION

To summarize the results on the leukocyte count, we can say that a vertical homogeneous magnetic field of 4200 Oe decreases the number of circulating leukocytes in virgin female mice within the first 2 weeks by 20 to 40%. This minimum is followed by a temporary increase in the

leukocyte count, which may reach the base line. It is followed by a second drop, reaching a minimum around the 30th day of residence in the field. The time of the first minimum and that of the maximum seems to occur in older mice a few days earlier (12th and 16th day, respectively) than in younger animals (18th and 21st day, respectively). If the mice are removed from the field at the time of the first or second minimum, the number of leukocytes drastically increases and in about 2 weeks overshoots the base line by 20%. But this increase after removal is missing if the mice are left in the field for a prolonged time after the second minimum.

I would like to suggest tentatively the following explanation for this change in the leukocyte count. As the investigations of Patt and Maloney<sup>16</sup> indicate, the number of myelocytes in the bone marrow capable of maturing is greater than the number which actually enters the blood stream. I assume that the magnetic field shortens the lifespan of circulating leukocytes, lymphocytes, and granulocytes alike, causing the first minimum. The lowered number of granulocytes enhances the maturation of the stored myelocytes, and young granulocytes are now ejected into the blood stream. This would explain the greater ratio of young forms (bands) up to the 16th day. At the same time, the lymphocytes which are stored in lung, liver, spleen, and other organs in close proximity to the smaller blood vessels are mobilized and, together with the ascension of stored granulocytes, lead to the maximum. However, at this time the inhibition of all blood-forming organs becomes manifest and, after the exhaustion of the stored reserves, the total number of circulating leukocytes decreases gradually to the low level found in the second minimum. It seems that the lymph nodes are slightly more inhibited than the bone marrow, as indicated by the increased ratio of polynuclear cells to lymphocytes. After removal from the magnetic field, the regeneration of the blood-forming organs sets in, which, as usual, leads to an over-production and temporary overshoot above the base line. The lymph nodes seem to recover somewhat faster (as indicated by the decrease of the ratio of polynuclear cells to lymphocytes to normal and even below the normal level). The average lifespan of the polynuclear cells returns to the normal level, leading to a temporary depression of young forms below normal, until the bone marrow resumes its usual operation.

To summarize briefly the proposed mechanism: the first minimum is caused by the shortening of the lifespan of leukocytes; the maximum is the consequence of the mobilization of the stored cells; at the peak of the maximum the storage is depleted; the second minimum is caused by the inhibition of the leukocyte-producing organs. This explanation shall be further investigated using radioactive tracers.

#### ACKNOWLEDGMENT

The experiments were performed in the laboratory of the Biomagnetic Research Foundation in Evanston, Illinois. It is my pleasure to

express thanks to my husband for his help in the experiments and for many constructive discussions.

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## *Chapter 4*

# Reduction of Irradiation Mortality Through Pretreatment

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The effect of whole-body irradiation on rodents with lethal doses of X rays or gamma rays is one of the most widely investigated phenomena. The mortality depends on the total dose, intensity at which this dose is delivered, strain, sex, and age of the animal. The first observable symptom is a drastic drop in the number of circulating leukocytes. This drop is a direct consequence of bone marrow destruction. Both serial histologic studies and direct counts demonstrate the rapid loss of nucleated cells from the bone marrow. The animal's failure to replenish the granulocytes, platelets, and erythrocytes from the circulating blood is primarily responsible for the death.

Bacteria invade the mucous membranes and the blood stream and in the absence of leukocytes the resulting septicemia is an important cause of death in irradiated animals. When platelets reach critically low levels hemorrhages occur; these may be so extremely severe as to play a major role in causing death. Anemia may be severe in the presence of hemorrhage, but is also caused by lack of erythropoiesis in the bone marrow.

The experiments described in the previous chapter demonstrate that treatment in homogeneous magnetic fields is followed—after removal from the field—by leukocytosis, reaching its maximum between the 7th to 14th day. This observation gave me the idea that this artificially induced leukocytosis could be utilized to counteract the drastic decrease of leukocytes after irradiation and reduce the lethal effect of irradiation, even if the other implications of the bone marrow destruction, namely drop in number of platelets and erythrocytes, could not be avoided.<sup>1</sup>

In the first experiment sixty 60-day-old virgin female RF strain mice from the Roscoe B. Jackson Memorial Laboratory were used. A sample of ten mice of average weight distribution was selected and

placed for a period of 14 days in a homogeneous, vertical magnetic field of 4200 Oe. The mice were individually housed in 3-in.-diameter,  $1\frac{7}{16}$ -in.-high cages in ten permanent magnets. The cages had plastic bottoms and automatic food and water supply. The average vertical gradient of the field within the cage was 40 Oe/cm. The remaining 50 mice were kept in standard plastic cages with five mice in each cage. Rockland Mouse Diet and water were supplied ad libitum to both groups. No controls in dummy magnets were used, which is a shortcoming of this experiment.

At the end of the 2-week residence in the field, the treated mice were placed for 3 days in standard cages; thereafter whole-body irradiation was given to all 60 mice with the cobalt therapy unit of the Cook County Hospital in Chicago. All animals were deprived of food 12 hr prior to irradiation to minimize intestinal syndromes.<sup>2</sup>

During irradiation the mice were placed in  $8 \times 8 \times 2$ -in.-high aluminum trays, at a distance of 80 cm from the cobalt source. The slight 0.8% difference in dose between center and side of the tray was equalized through the constant milling of the mice in the tray. The mice were irradiated in three groups, 20 mice per group, and the treated and controls were intermixed. A total dose of 750 r was administered to each group in 790 sec at an intensity of 57 r/min. The automatic shutter control with 1-sec accuracy ensured equal doses for each of the three groups irradiated.

In the following the radiation dose is always given for cobalt gamma rays (1.17 and 1.33 MeV). In order to convert this dose to a 250-KeV X-ray dose of equal biological effectiveness, the values given should be divided by a factor<sup>3</sup> of 1.33. The expression "control" refers throughout the paper to animals irradiated but not treated previously in a magnetic field, and the expression "treated" refers to animals which were first treated in a magnetic field and irradiated thereafter. The animals were under observation for 30 days after irradiation and mortality values refer to this interval.

In the first experiment the mortality of the control group was 30% (15 mice), but none of the treated died. The difference in mortality is significant on a probability level of 1:23.

The body weight of the mice was measured daily. Figure 1 (curve I) shows the weight difference between the magnetically treated and control groups. During magnetic treatment, as could be expected (see Part II, Ch. 1), the mice in the field lost weight relative to their controls. Removed from the field, they resumed their development in spite of the irradiation, while the control group did not grow further and actually lost weight as a consequence of the irradiation. Even the weight of the survivors in the control group remained below the weight of the treated group up to the 35th day.

The 45 survivors from the first experiment were used in a second experiment. The same 10 mice were treated anew for 14 days in the

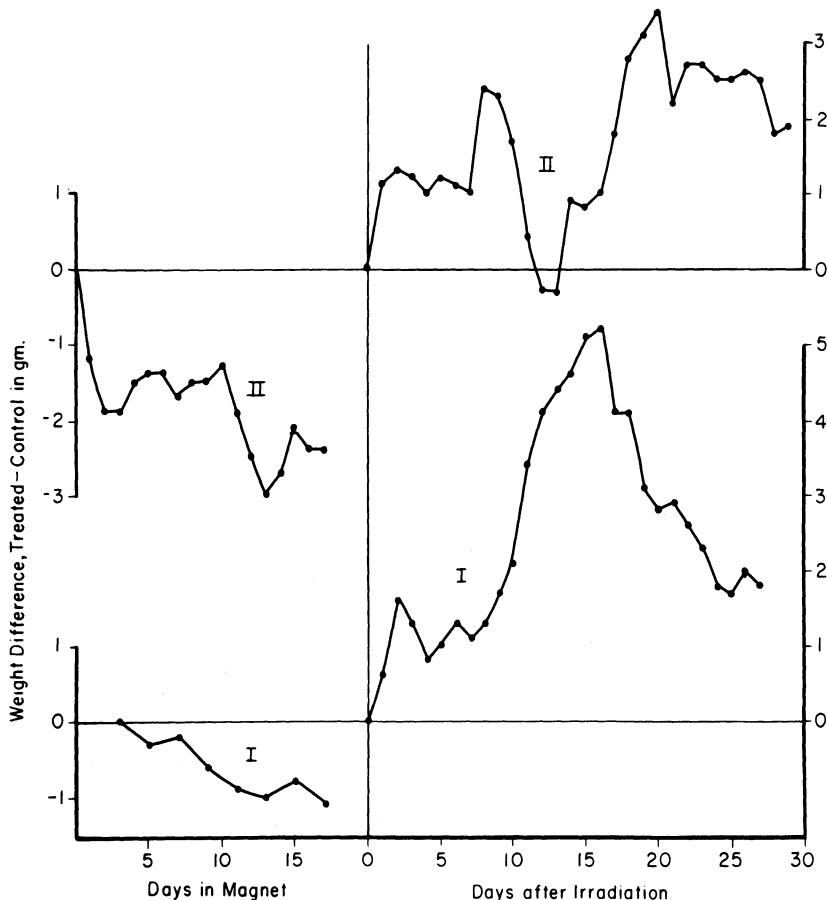


Fig. 1. Weight difference between magnetically pretreated and control groups during residence in the field and after irradiation. Although the groups magnetically pretreated were at the time of irradiation lighter than the controls, they showed a smaller weight loss and more rapid recovery from irradiation injuries than untreated survivors.

magnetic field and, after a delay of 3 days in standard cages, all 45 mice were irradiated with 850 r. The second irradiation was administered 44 days after the first.

From the control group 89% (31 mice) died, while from the treated group 80% (8 mice) died. All dead animals were immediately autopsied. The spleen weight of the treated group was normal,  $73.1 \pm 4.6$  mg; that of the controls was considerably heavier,  $127.8 \pm 8.2$  mg. The difference in spleen weight is significant on a probability level of 1:40,000. It indicates that the treated animals were not affected by their first, low lethal irradiation 44 days earlier and, therefore, their spleens did not pass through a transitory hyperplasia as a consequence of the recovery process from leukopenia,<sup>4</sup> as did the spleens of the controls.

In this second experiment, the mice were 4 months old and had passed their development stage. It is not surprising that after irradia-

tion the weight of both groups dropped; but the magnetically treated group lost less weight (Figure 1, curve II). The group magnetically treated also recovered earlier, around the 18th day, while the control group regained its original weight—its weight at the time of irradiation—only after 35 days.

In the course of later investigations, 17 further experiments were performed, using a total of 920 mice. Mice of different age and strain were treated in homogeneous and inhomogeneous fields and various time intervals were interposed between termination of magnetic treatment and irradiation; magnetic treatment after irradiation was also tried. In these experiments the larger cages and dummy magnets, described in the previous chapter, were used. In most instances 30 mice were treated in ten permanent magnets and the same number were housed during that time in the ten dummy magnets. The total dose was always 800 r, delivered with an intensity between 33 r/min and 100 r/min, producing a mortality of 30 to 100% in the control group.

After irradiation the animals were placed in standard cages, three treated and three controls to the same cage to compensate for possible cage effects. The body weight was measured daily; the dead were removed within 4 hr after death, even during the night. They were immediately autopsied, the liver and spleen weight were measured, and from a general inspection of the organs the possible cause of death was noted. In three experiments (one in homogeneous, two in inhomogeneous fields) blood samples were taken on several occasions after irradiation from the tail vein of control and treated animals, and leukocytes and erythrocytes were counted and blood smears investigated.

These extended investigations revealed many interesting features, but in most instances they can be considered only as preliminary results which need further investigation. Two significant results emerged.

At high lethal irradiation doses, magnetic pretreatment of mice in a homogeneous 4200-Oe magnetic field, and with less than a 3-day

TABLE I

Magnet	Dummy	Relative mortality difference, %
70.0	90.4	-22.6
66.7	100.0	-33.3
45.0	70.0	-35.7
60.0	76.7	-21.8
53.4	66.7	-19.9
		$-26.7 \pm 3.2$

$t = 8.3$ ;  $n = 4$ ; P. L. = 1 : 800.

delay between the termination of the treatment and irradiation, decreases the death rate by 23 to 30%. The results of five such experiments performed under the described conditions are given in Table I. The difference in mortality rate is significant on a probability level of 1 : 800.

The leukocytosis following the magnetic treatment does not compensate the leukopenia caused by the irradiation; it merely diminishes it slightly. On the 10th day after irradiation the leukocyte count of the treated group was 13% of normal, while that of the controls was 9%. Nevertheless, it seems that even this small difference is adequate to prevent death in some cases. The lowering of the deathrate is most pronounced from the 7th to the 14th day after irradiation, that is, during the intestinal and bone marrow mode of the radiation syndrome. This interval coincides with the time when the maximum of the leukocytosis is observed after magnetic treatment (see preceding chapter).

Treatment in inhomogeneous fields affects not only the leukocyte count, but also the erythrocyte count and could therefore influence irradiation anemia. But due to the simultaneous variation of two blood elements (probably also platelets) in experiments with inhomogeneous fields, the interpretation of the mortality curves is too complex to be attempted at present.

The experiments were performed in the laboratory of the Biomagnetic Research Foundation; I wish to express my gratitude to Dr. M. F. Magalotti for making available the irradiation facilities of the Cook County Hospital. It is my pleasure to thank my husband for his help in carrying out these experiments.

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## *Chapter 5*

# Lifespan Increase of Tumor-Bearing Mice Through Pretreatment

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In 1956, Barnothy, Barnothy, and Boszormenyi-Nagy<sup>1</sup> reported the observation of leukocytosis in mice subsequent to a period of residence in magnetic fields of about 4000 Oe. Murphy<sup>2</sup> in 1926 observed that the induction of lymphocytosis by a variety of agents such as low-level X-irradiation, dry heat, and injection of oils was accompanied by an extended survival and, in some cases, regression of an implanted tumor. That the lymphocyte is the active agent in this process is also confirmed by the results of Algire, Weaver, and Prehn,<sup>3</sup> who found that transplants are not rejected when put in porous chambers in the peritoneum of mice. The chambers have barriers too small for lymphocytes or other cells to enter, but large enough to permit serum and fluid to pass.

We presumed that the leukocytosis produced by exposure to magnetic fields would increase life expectancy or cause tumor regression in a manner similar to that observed by Murphy, since lymphocytes comprise approximately 75% of the white blood cells in mice.

Alnico magnets\* were used to produce the magnetic fields. These magnets weigh 35 lb and have a field intensity of approximately 3000-4000 Oe with variation across the pole face of 200 Oe/cm. The gap between poles is 2 in. and the pole face is 1½ in. in diameter. Twenty-four such magnets were used. Figure 1, a photograph of a typical magnet assembly, shows the cage, feeder, and water bottle. The field vector was horizontally directed. Measurements of field intensity were made with a Hall-effect probe (Radio Frequency Laboratories gaussmeter). Figure 2 indicates the field intensities at various points in the gap. Dummy magnets were made of brass, shaped in the form of the pole pieces; each dummy pole piece weighed approximately 15 lb.

The mice under test are individually housed in cube-shaped cages constructed of nonmagnetic stainless steel wire mesh approximately 2 in. on each edge and placed between the poles of the alnico magnets. Supported in part by National Institutes of Health Grant No. C-4561.

\*These magnets were loaned to us for these studies by the Raytheon Manufacturing Company.

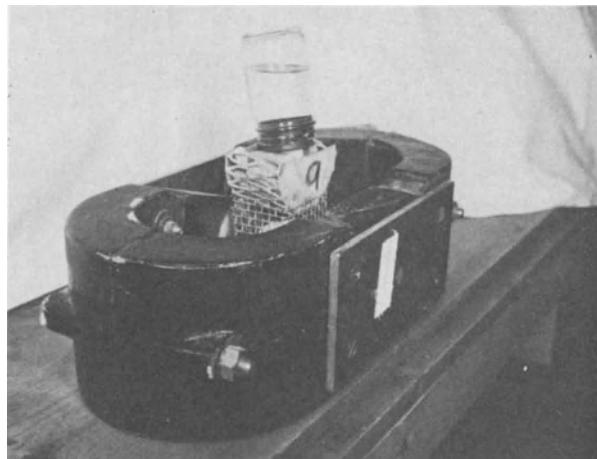


Fig. 1. Magnet with cage, feeder, and water bottle.

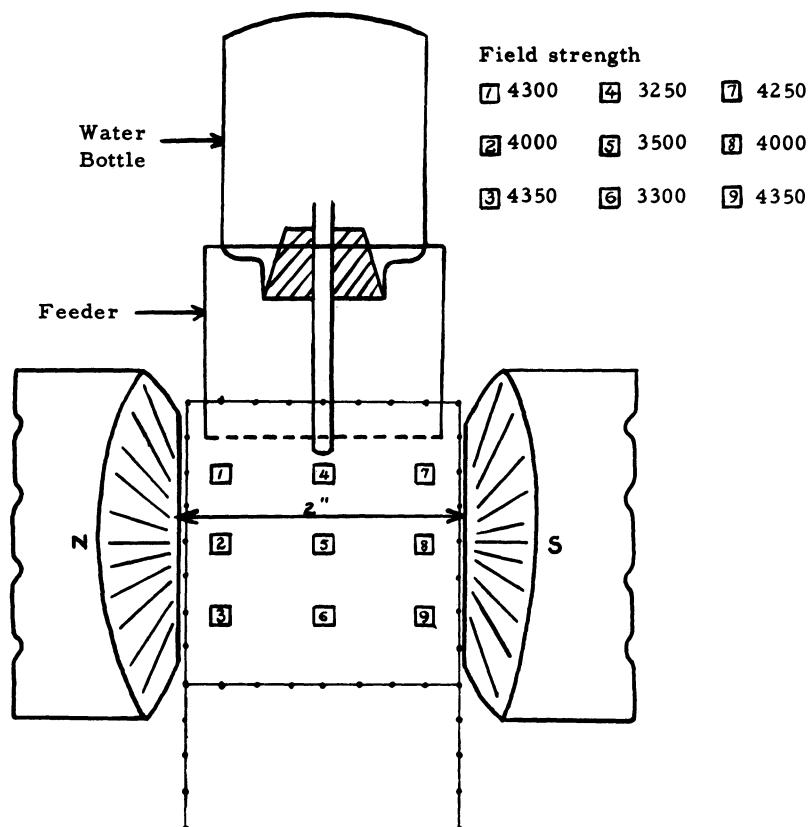


Fig. 2. Field intensities in the cage area in oersteds at the points indicated.

or between the poles of the dummy magnets. The animals used were 3- to 4-month-old mice of A/He, DBA/1, DBA/2, C3H, BALB/c, and C57BL/6 inbred strains from the colony at the Waldemar Medical Research Foundation.

In this experiment, tumor-free mice were individually housed in magnetic fields and maintained there continuously for 30 days. A group of control animals was individually housed in dummy magnet cages outside of magnetic fields. Another group of animals was normally housed. All groups were implanted with tumors 7 to 10 days after the test mice were removed from magnetic fields, by which time lymphocytosis had developed. The growth pattern of these tumors and the fates of their hosts was observed. The tumors and strains of mice for these tests were:

<u>Tumor</u>	<u>Mouse strain</u>
dbrB mammary adenocarcinoma	DBA/1
Sarcoma I	A/Jax
H2712 mammary adenocarcinoma	C3H
C4461 pulmonary adenocarcinoma	A/He
L1210S lymphoid leukemia	DBA/2
Ehrlich's ascites adenocarcinoma (EAC)	C3H

Solid tumors were obtained from the appropriate host and a mince was made.

Implantation was made subcutaneously in the mucosal region with a 5-in. 13-gauge trocar inserted near the tail and passed subcutaneously along the animal's body to the site of implantation. Both tumor mince and implantations were made under rigidly aseptic conditions. Cultures and thioglycollate tests were made of all tumor minces as well as of the lyophilized tissue in order to assure freedom from bacterial contamination. The animals receiving tumor implants were cleanly shaven on the tail area, which was prepared with alcohol-hyamine (70% isopropanol with 1:5000 hyamine) to sterilize the region of the skin through which the trocar would be inserted. All tumors were observed daily for first appearance of tumor. Records of tumor size by palpation and measurement with calipers of two perpendicular diameters were recorded three times weekly.

Ascitic tumors were taken by paracentesis from their hosts. An aliquot was taken and cell concentration was determined by hemocytometer count. The ascitic fluid was diluted with saline to a concentration of 50 million cells per milliliter and 0.25 ml was injected into each animal.

Our initial test was with DBA/1 mice in which we implanted a dbrB tumor. The dbrB tumor<sup>4</sup> is derived from a mammary gland tumor maintained in serial passage since 1918, with 100% takes. It metastasizes to the liver and lungs and kills the host in 15 to 20 days. It is

transplantable in 7 to 10 days. Control mice lived an average of 16.7 days. The average lifespan of the animals that have resided in the magnetic field is 23.3 days, an increase in life expectancy of 30% while carrying the tumor. There does not appear to be any difference in the onset or rate of growth of the tumor, but the animals that are treated survive with larger tumors and appear less debilitated than their controls. A second test was begun using A/Jax mice and Sarcoma I. The results of this trial are statistically not significant, with the average life of the animals treated in the magnetic fields 19.1 days as compared to 18 days for the controls. The increase in life expectancy is 5½% with a P of 60%.

Of the six tumors used, only one, the dbrB, strain specific for DBA/1 mice has a narrow range of time of death for the controls (see Table I). Since time of death is the sole parameter measured for this effect, this variance in time of death for this particular tumor is the factor that produces statistically significant results. Computation of the confidence limits were made by the Student *t* test wherever applicable and closely follow procedures given by Bailey.<sup>5</sup> Where a deviation is given, as in Table I, it is the standard error or the square root of the variance as defined by Bailey. The variance,  $S^2$ , is given by the expression

$$S^2 = \frac{1}{N - 1} \sum D^2 \quad (1)$$

where  $D$  is the difference of each measurement from the mean and  $N$  is the total number in the group. The summation is divided by  $(N - 1)$  to correct for small groups. The significance  $t$  is given by the formula

$$t = (M_c - M_e) / \sqrt{S_c^2/N_c + S_e^2/N_e} \quad (2)$$

where subscripts  $c$  and  $e$  refer to control and test groups and  $M$  is the mean of the group.

As can be seen from the formula for the Student *t* test, a small scatter in the normal or control animals permits a wider scatter in the experimental mice for the purpose of arriving at a significant result. The dbrB in DBA/1 mice was therefore selected for two further trials. The raw data and the derivation of  $P$  via the Student *t* test are given (Table II). The results summarized at the end of Table II indicate that for the host-tumor system used (dbrB in DBA/1 mice) the magnetic pre-treatment does delay time of death with tumor, with a value of  $P$  that renders the results statistically significant. The experiments on biomagnetic effects reveal a pattern of delay in response and inhibition of growth or function. Magnetic field exposure apparently inhibits the reticulo-endothelial (RE) system (see Part II, Ch. 6). The response of the mouse to this interference is the production of increased numbers of

TABLE I  
Partial Summary of Tests for Increase of Lifespan after Residence in Magnetic Fields

Experi- ment	Strain	Tumor	No. of magnet	No. of control	Time in magnets	Life-span exptl. (days)	Life-span controls (days)	Effect of magnet on life- span (days)	%	P (%)
			animals	animals	(days)	(days)	(days)	t		
1	DBA/1	dbrB	11	23	30	23.3 ± 4.3	16.7 ± 3.5	+ 6.6	+ 39.0	4.5 0.1
2	A/Jax	Sa L.	10	11	30	19.1 ± 3.5	18.1 ± 4.2	+ 1.0	+ 5.5	0.5 60
3	C3H	H2712	5	12	30	17.4 ± 11.3	17.3 ± 6.8	+ 1		0.1 79
			6	12	22	18.5 ± 4.1	17.3 ± 6.8	+ 1.2	+ 7.1	0.4 70
			6	12	14	14.5 ± 2.9	17.3 ± 6.8	- 1.8	- 16.0	0.5 70
			5	12	7	17.2 ± 5.8	17.3 ± 6.8	+ 0.1	1	0.1 90
4	DBA/1	dbrB	12	8	30	19.4 ± 5.3	16.9 ± 2.9	+ 1.6	+ 9.5	0.9 30
5	DBA/2	L1210S	23	47	30	9.0 ± 0.78	8.8 ± 0.63	+ 0.2	+ 2	0.1 90
6	C3H	EAC	24	46	30	14.1 ± 3.8	14.0 ± 3.6	+ 0.1	-	- -

Note: In columns 7 and 8, standard deviations, not standard errors, are indicated.

TABLE 2

Data for dbrB Tumor in DBA/1 Mice after Residence in Magnetic Fields

Control		Exptl.		Control		Exptl.		
No. of animals	Survival (days)	No. of animals	Survival (days)	No. of animals	Survival (days)	No. of animals	Survival (days)	
Experiment 1, 3-59								
11	14	4	19	9	14	3	16	
5	16	2	22	4	15	2	17	
2	18	1	24	5	16	2	18	
2	22	1	25	1	17	3	19	
2	23	2	28	3	19	1	21	
1	24	1	31	5	20	1	24	
Experiment 4, 8-59								
2	13	2	12	1	21	2	25	
1	16	1	13	2	24	1	26	
2	17	2	17	1	25	2	28	
2	18	3	18	1	26			
1	22	1	21	Experiment 8, 7-60				
				6	13	2	15	
				7	14	3	17	
				5	15	2	18	
				9	16	4	19	
Experiment 7, 4-60								
				5	17	2	21	
				5	18	1	22	
				3	20	3	23	
				4	21	2	25	
8	12	1	14	1	25	1	27	
4	13	1	15	2	27	2	29	
						1	32	
Experiment								
	$N_c$	$N_e$	$M_c$ :	$M_e$	$S_c^2$	$S_e^2$	$t$	$P(\%)$
1	23	11	16.7	23.3	12.5	18.5	4.5	0.1
4	8	12	16.8	18.6	8.5	28.0	0.97	30
7	44	23	16.1	18.8	15.2	20.1	2.25	0.2
8	47	23	16.9	21.4	7.7	22.8	4.2	0.1
Control								Exptl.
No. of mice in group								$N_c$
Mean survival time (days)								$N_e$
Difference between individual survival time and mean								$M_c$
survival time (days)								$D_e$
Variance, $S^2 = (\sum D^2)/N - 1$								$S_e^2$
Significance test factor (Student's $t$ )								$t$
Probability that $t$ has occurred by chance (%)								$P$

white blood cells upon termination of residence in the magnetic field. Normal mouse blood contains 75-80% lymphocytes, so this leukocytosis is effectively a lymphocytosis. The existence of a lymphocytosis produced by prior magnetic field treatment functions to delay the lethal effect of a transplanted tumor.

Murphy (1926) had shown that a lymphocytosis induced by such methods as mild X-irradiation, oil injection, and application of dry heat would also materially increase the life expectancy of mice bearing transplanted tumors. The lymphocytosis produced by magnetic fields is smaller in magnitude than that produced by such stimuli as dry heat, and its effect on tumor growth, while not as great as in the cases reported by Murphy, was nevertheless in the same direction. The extension in life expectancy was increased for most host-tumor systems tested, but statistical validity was obtained only with the dbrB tumor in DBA/1 mice. Of all the transplantable tumors used, the characteristics of the dbrB tumor were peculiarly suited for this test. The standard deviation for time of death of the mouse after tumor implantation is small and the growth rate of the tumor is slow in its initial stages. This tumor consequently was selected for further study with larger numbers of animals. An examination of equation (2) for  $t$  shows that where the difference between the means of the experimental and the control groups is small, the variance  $S^2$  must be small to provide statistical significance. If this is not the case, then  $N$ , the number of mice in the group, must be large. A limit on  $N$  is set by our equipment, and that limit is the number of mice in any one experiment that can be housed in magnetic fields. Four tests were run on the dbrB tumor in DBA/1 mice and the significance of each test is given at the end of Table II. Treating all data as a whole, the confidence limit  $P$  is less than 0.1%. These experiments prove the existence of a biomagnetic effect with a high statistical validity.

An attempt to determine the minimum period of magnetic field exposure to produce leukocytosis subsequent to removal of mice from the field was inconclusive because of the choice of host-tumor system and the small number of mice in each group. This experiment was not pursued further because the length of time to mount a valid test was deemed excessive. In any event, a shortened residence period would not materially shorten the projected test program.

We have demonstrated with high statistical significance that the life expectancy of a tumor-bearing host is lengthened if the host is exposed to a magnetic field prior to the transplantation of the tumor. This effect is apparently related to the leukocytosis which follows magnetic treatment.

#### EFFECT OF MAGNETIC FIELDS ON TUMORS IMPLANTED DURING RESIDENCE OF THEIR HOSTS IN MAGNETS

An experiment was performed to test the effect of magnetic fields directly on tumor growth. Such experiments were performed by others, some with positive, others with no results (see Part II, Ch. 2).

Since the measured parameter was the lifetime of the mice, we chose the C4461 tumor in A/He mice because of its slow rate of growth.

This tumor<sup>3</sup> kills the host in 42 to 50 days. Tumors were implanted at the same time that mice began their period of residence in the magnetic field. Nine were magnetically treated and 11 were controls in dummy magnets; the lifespan of the treated group was  $70.0 \pm 33.1$  days, while that of the controls was  $49.4 \pm 19.7$  days. The difference is statistically not significant.

The prime characteristic required for a test is a narrow range of variance of the test parameter, to limit the number of animals necessary in the group and still obtain statistical validity. None of the other slow-growing tumors carried in serial transplants meet this criterion. Consequently, experiments of this kind were not pursued further.

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## *Chapter 6*

# Wound Healing and Tissue Regeneration

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We sought a means of evaluating the effect of magnetic fields on growth processes that would yield data in a shorter time period than could be achieved with observation of processes such as tumor growth. We therefore chose to study wound healing and tissue regeneration.

## WOUND HEALING

At our laboratory we have acquired considerable experience with a technique of detecting reparative differences in skin wounds of mice.<sup>1</sup>

Mice were the animals selected. Alnico magnets\* were used to produce the magnetic fields. These magnets weighed 35 lb and had a field intensity of approximately 3000-4000 Oe with variation across the poleface of 500 Oe/in. The gap between poles is 2 in. and the poleface is  $1\frac{1}{2}$  in. in diameter. Measurements of field intensity were made with a Radio Frequency Laboratories gaussmeter and Hall-effect probe. Details of the magnet assembly, field intensities, and dummy magnets will be found in the preceding chapter.

The mice under test are individually housed in cube-shaped cages constructed of nonmagnetic stainless steel wire mesh approximately 2 in. on each edge and placed in the horizontal field between the poles of the alnico magnet. Control animals were individually housed outside of magnetic fields under identical conditions, but in dummy magnets. To check on cage effect, an equal number of mice normally housed served as an additional control group. In all cases, mice individually housed in dummy magnets gave results similar to those of normally housed mice, and, in the interests of achieving better statistics, both controls were grouped together.

The animals used were 3- to 4-month-old mice of C57BL/6 inbred strain from the colony at the Waldemar Medical Research Foundation.

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\*The magnets were loaned to us for these studies by the Raytheon Manufacturing Company.

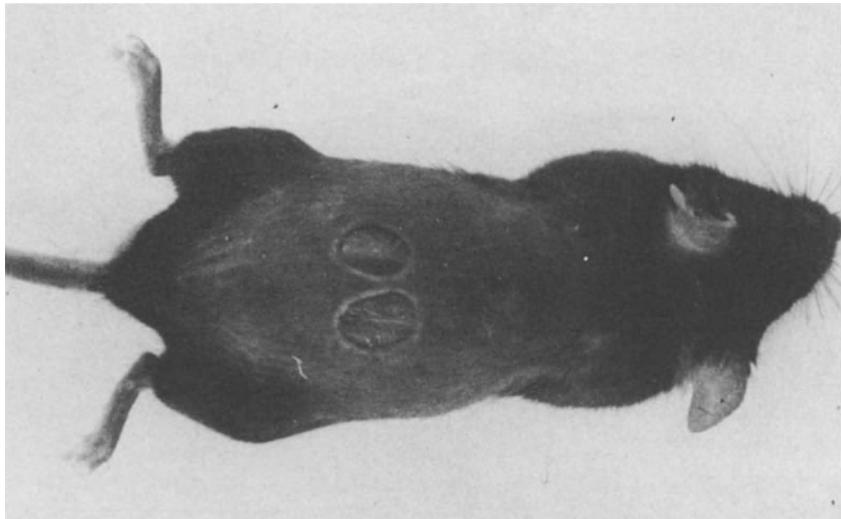


Fig. 1. Skin wounds on dorsum of C57BL/6 mouse.

Prior to wounding, the animals were closely shaved over a wide area of the dorsum to eliminate any inflammation or irritation from particles of hair. Two identical wounds  $\frac{1}{4}$  in. in diameter and  $\frac{1}{2}$  in. apart were inflicted with a punch to the dorsum of the mouse (Figure 1).

The experimental animals are grouped as follows:

Group	Treatment
I	Housed in a magnetic field for 1 month, then wounded and taken out of the magnetic field.
II	Housed in a magnetic field for 1 month, then wounded and replaced in the magnetic field.
III	First wounded and then placed in a magnetic field.
IV	Wounded and normally housed, and wounded and individually housed in dummy magnets.
V	Housed in a magnetic field for 1 week, then wounded and replaced in the magnetic field.

At each time, eight C57BL/6 mice were sacrificed from each group, providing a total of 16 wounds for histologic examination for each group. When an animal was sacrificed, an area of skin containing the wound with underlying fascia was removed. Transverse interrupted serial sections through the wounds were examined after staining with hematoxylin and eosin. The animals were sacrificed 5 and 9 days after wounding. These times are optimal in detection of reparative differences.

TABLE I  
Evaluation of Wound Healing in Magnetic  
Fields

(Numbers are based on a 1 to 4 plus scale)

Group No.	I	II	III	IV (controls)	V
at 5 days	1.4	1.3	2	2.3	1.3
at 9 days	2.9	3.2	3	3.8	2.9

The histologic reparative state can be designated by the characteristics listed below. The examiner was unaware of the source of the specimen he was examining. The characteristics were evaluated on a 1 to 4 plus scale:

1. Degree of epithelization—from no cover to completely covered.
2. Degree of exudation—cellularity, leukocytes, edema, hemorrhage, etc.
3. Amount of granulation-tissue formation—new capillaries and stroma.
4. Amount of collagen formation.

The data (Table I) show a consistent and noticeable effect of the magnetic field on wound healing. Microscopic evaluation also revealed a consistent and marked reduction in fibrosis due to magnetic field treatment.

Note that Group II, with 1 month prior residence in the magnetic field, gives data almost identical with that of Group V with 1 week prior residence in the magnetic field, indicating that any delay in healing or growth is probably due to the effect of the field and not to any cumulative effect of prior treatment. Note also that some prior residence is effective since Group III with no prior residence showed an effect intermediate between Group I and the controls, Group IV.

The data represent two experiments which gave essentially similar results of approximately a 20% delay in wound healing, i.e., the ratio of (IV - III)/IV at 9 days is  $(3.8 - 3.0)/3.8$ , which equals 21%. The data are grouped together to achieve greater statistical significance.

Coded slides were evaluated separately by two experimental pathologists to assure freedom from subjective bias. Both had had prior experience in grading the histologic process of wound healing. Both found that fibroblast proliferation and fibrosis were markedly delayed.

#### TISSUE REGENERATION

The influence of magnetic fields on tissue regeneration was studied via inflammation and repair following turpentine injection. The animals

were closely shaved over chest and upper abdomen and 0.25 ml of turpentine was injected intracutaneously just above the xiphoid process. When the animal was sacrificed, an area of skin was taken from the injected region including underlying fascia down to the sternum, and transverse interrupted serial sections through the regenerating area were examined after staining with hematoxylin and eosin. Experience with this type of histologic examination was noted in the preceding section.<sup>1</sup> Animals were sacrificed at 24, 48, and 72 hr following turpentine injection. These times represent optimal periods for detection of inflammation and regeneration.

The state of regeneration was evaluated on a 1 to 4 plus scale with the examiner unaware of the source of the specimen.

The experimental animals were grouped as follows:

- |           |                                                                                                           |
|-----------|-----------------------------------------------------------------------------------------------------------|
| Group I   | Housed in a magnetic field for 1 month, then injected and taken out of magnetic field.                    |
| Group II  | Housed in a magnetic field for 1 month, then injected with turpentine and replaced in the magnetic field. |
| Group III | Injected with turpentine and then placed in a magnetic field.                                             |
| Group IVA | Control group— injected with turpentine and individually housed.                                          |
| Group IVB | Control group— injected with turpentine and normally housed.                                              |
| Group V   | Housed in magnetic field for 1 week, then injected with turpentine and replaced in the magnetic field.    |

A study of the slides of the turpentine-induced inflammation and regeneration gave no indication of modification of growth or repair for mice in magnetic fields as compared with the controls. The scatter of observations was much wider due, in part, to leakage of turpentine to surrounding tissue rather than its retention intracutaneously. A second cause of scatter is the ulceration and abcess formation in a considerable number of the animals. Both factors interfere with a clear pattern of tissue regeneration. Consequently, these experiments were not pursued further.

Using a different method, we attempted to study tissue regeneration in liver damaged by carbon tetrachloride. The experimental groups of animals were selected and treated with magnetic fields in the same manner as those used for the experiment with turpentine inflammation. Mice were injected intraperitoneally with 0.025 ml carbon tetrachloride. Animals were sacrificed 2 days and 5 days after injection.

Transverse interrupted serial sections through the lobes of the liver were examined after staining with hematoxylin and eosin, and the state

of regeneration evaluated on a 1 to 4 plus scale with the examiner unaware of the source of the specimen.

All groups sacrificed at the same time showed the same state of regeneration. It was therefore not possible to distinguish any effect of magnetic fields on the process of tissue regeneration after damage with carbon tetrachloride.

#### EFFECT OF FIELD ON OTHER ORGANS AND TISSUES

The effect of magnetic fields on other organs and tissues was investigated via histologic examination and gross observations. Mice were sacrificed at intervals during a 30-day period of residence in the magnetic field and at intervals after removal from the magnetic field. Equal numbers of mice were sacrificed in the control groups. The spleens, adrenals, livers, bone marrows, and lymph nodes were examined for gross appearance. Slides of these organs in interrupted transverse serial section were prepared with hematoxylin and eosin for histologic examination. Portions of the jejunum were removed and prepared in like manner for determination of the mitotic index.

Histologic examination of spleen, adrenals, liver, bone marrow, and lymph nodes revealed no hyperplastic changes, nor were any gross abnormalities observed; there were no perceptible differences in weight, color, or appearance between specimens taken from magnetically treated mice and their controls, regardless of the time the samples were taken. Counts of mitoses in the pyramidal cells of the villi of the jejunal mucosa were taken. The mitotic index was the same for all groups of animals. The magnetic field had no perceptible effect on these latter factors.

#### DISCUSSION

In our studies on wound healing we obtained independent confirmation of the effect from two pathologists who examined coded slides. Both pathologists concurred that fibroblast proliferation and fibrosis are reduced in magnetic fields. There was a more distinctive difference between treated and control animals for this factor than for the other parameters in wound healing. The reduction in fibroblast proliferation led us to postulate an interference by magnetic fields with the production of large molecules such as proteins, ribonucleic acid (RNA), and deoxyribonucleic acid (DNA). This might be the common factor in reports that magnetic fields produced more embryological anomalies,<sup>2,3,4</sup> and reduced plant<sup>5</sup> and bacterial growth.<sup>6</sup> (See also Part II, Chs. 7 and 9, and Part III, Ch. 3.)

Our inability to observe magnetic field effects in tissue regeneration after turpentine injection can be ascribed to experimental difficulty in obtaining tissue sections free of ulceration. Liver regeneration after

carbon tetrachloride poisoning is quite rapid, so that recovery of the host both in the presence and absence of magnetic fields occurred before any appreciable histological differences could be observed. Lacking such evidence, we did not undertake chemical assays.

For the same reason, further studies in mitotic index and observations of processes in other organs were not made.

Our observation that fibroblast proliferation in wound healing is markedly delayed by magnetic fields led us to postulate that the enzymatic production of large protein molecules and other biological polymers are inhibited by magnetic fields. This was tested in part by showing that magnetic fields reduce the peak antibody titer as compared to untreated controls.<sup>7</sup> This concept was extended to a proposed theoretical mechanism as discussed in Part I, Ch. 6.

#### ACKNOWLEDGMENT

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## *Chapter 7*

# Effect on *Drosophila melanogaster* and S-37 Tumor Cells; Postulates for Magnetic Field Interactions

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Since ancient times man seems to have been fascinated by the mysterious powers of a magnetic field over life, but a true scientific interest in the study of its effects on living matter was not aroused until the nineteenth century. Since then, several reports on the effects of magnetic fields on life from bacteria to man have appeared from time to time. In the last decade this interest has intensified and several studies have been published in many journals.

This paper reports some studies on *Drosophila melanogaster* *in vivo* and Sarcoma-37 mouse tumor cells *in vitro* along with a summary of related early work. Here we have also included a résumé of magnetic field effects on chemical reactions carried out by other workers and our postulates for explaining the interaction between magnetic fields and biological systems. It must be pointed out at the outset that the general nature of our studies should be regarded as being exploratory.

## I. STUDIES ON *Drosophila melanogaster*

### Early Work

One of the early reports on the influence of a magnetic field on *Drosophila melanogaster* was published by Chevais and Manigault.<sup>1</sup> In their experiments, extremely inhomogenous fields obtained from permanent magnets with very narrow gaps were used. They reported that they were able to obtain a field gradient ( $HdH/dx$ ) in millions of cgs units in certain regions of the narrow pole gap. The *Drosophila* eggs were subjected to the magnetic field for 24 hr at 25°C, after which the flies were allowed to develop outside the field. The emerging flies were then tested for genetic changes (mutations) by using classical methods

including Clb (Müller) test; some visible wing and lethal mutations were observed in exposed flies but none in the controls. Some experiments were also conducted with fluctuating magnetic fields, but no mutations were observed. They also commented that their experiments were preliminary in nature and more precise experiments on *Drosophila* eggs, adults, and even isolated chromosomes were to follow. A search of the literature failed to show further work on *Drosophila* by these investigators.

In recent years Beischer studied the action of an extremely high magnetic field on *Drosophila*. His studies are reported in Part II, Ch. 11.

### Studies by the Authors

The purpose of these studies was to determine if a magnetic field has any effect on living matter and, if so, to study the effect quantitatively as far as possible.

#### *Production of Magnetic Fields and Their Measurement*

Several alnico horseshoe and ram-horn type permanent magnets producing fields up to 8000 Oe were used in our experiments; a variation in the intensity was obtained by attaching or removing steel pole-faces to the magnets. The use of electromagnets was avoided for obtaining fields over this range, because these invariably produce undesirable heating effects unless special precautions are taken to cool the coils. Special electromagnets with cooling devices will be used for future work involving the use of very intense fields. In some experiments, inhomogeneous fields were employed by adapting pole pieces of special design; however, no attempts were made to obtain extremely high field gradients. In all experiments the position of the poles was always kept vertical with the south pole at the base. Figures 1 and 2 show some details of the magnetic setup.

The intensity of the magnetic field was measured by using a Hall-type gaussmeter system.<sup>2</sup> The probe of the meter was only 1 mm thick, 3 mm wide, and about 4 cm long. The measurements were made at

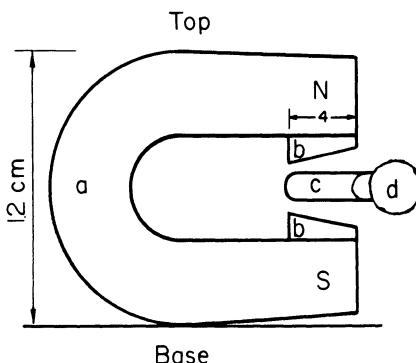


Fig. 1. Horseshoe magnet with a vial containing *Drosophila melanogaster* culture in the pole gap. (a) Magnet; (b) pole faces; (c) culture vial; (d) cotton plug; (N) north pole; (S) south pole.

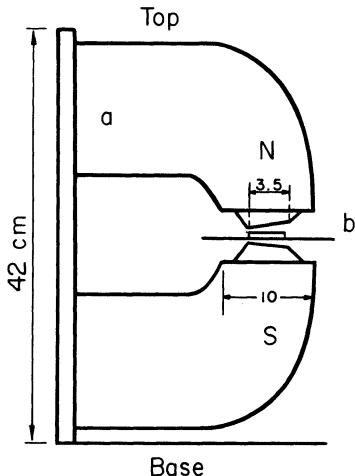


Fig. 2. Ram-horn magnet with a culture slide in the pole gap. (a) Magnet; (b) culture slide; (N) north pole; (S) south pole.

different points in both vertical and horizontal directions, and the field gradient was calculated. The field intensity is expressed in oersteds, and the field gradient as oersteds per centimeter.

#### *Experimental Procedure*

A pure culture of *Drosophila melanogaster* of the wild type (normal type), procured from General Biological Supply House, was grown on banana agar medium for at least three generations before being subjected to magnetic fields.

In this experiment *Drosophila* organisms were exposed to magnetic fields of different intensities (100 to 4400 Oe) over periods varying from one to three generations.

Five pairs of very young males and virgin females (which had just emerged from the pupae) were placed in a small glass vial partially filled with banana agar medium. The vials were selected to fit in the pole gaps of different magnets. The vials containing the flies were immediately placed in a horizontal position in the pole gap. Several vials were used for each group. The field intensities are shown in the first column in Table I. The males and females were allowed to mate in the field, after which the females laid eggs on the surface of the medium. After 72 hr (when enough eggs were laid) the parents were removed from the vials and the eggs were left in the field. Special precautions were taken to keep the eggs, developing larvae, pupae, and the newly hatched flies exposed to the magnetic field at all times, except when removed for very short intervals for examinations. The vials were also daily inspected for moisture content and for possible contamination, etc. The entire life cycle of *Drosophila* was allowed to take place in the magnetic field. The newly hatched flies were examined daily till all the flies were hatched. This examination was carried out by first making the flies motionless with ether and then observing them under a micro-

scope. Each fly was examined for any abnormalities in the external characters such as sex, color, and size and shape of eyes, head, thorax, abdomen, wings, bristles, and the total body as a whole. The frequency of each class of abnormality and the total frequency of the flies were noted and tabulated.

Flies from this first generation, some with normal external characters, were again mated in the magnetic field to obtain a second generation. The same procedure was followed for the second and the third generation. Controls from each generation were treated exactly as the exposed. The control vials were placed between two sheets (about 0.5 cm in thickness) of unmagnetized iron to simulate the conditions of the experimental samples. Both the control and the experimental samples were placed on the same table and were exposed to the same experimental conditions such as moisture in the vials, exposure to light, temperature, etc. The examination of the experimental and the control flies was carried out at approximately the same time of the day. Separate controls were kept for each set of experiments. All the experimental procedures were carried out at a room temperature of about 25°C.

The *Drosophila* flies showing the abnormalities were further tested to determine whether the abnormalities were genetic or nongenetic in nature. The procedures followed were the classical ones used by Morgan and other geneticists.<sup>3,4</sup> Possibilities for dominant, recessive, recessive lethal, sex-linked recessive, and sex-linked recessive lethal mutations were checked and ruled out. Two different series of experiments were conducted during different periods of the year and the results were pooled.

#### *Results and Discussion*

The results of these tests seemed to show that all these deformities (abnormalities) were nongenetic in nature.

All the observed deformities persisted through the entire life of the fly.

Figure 3 shows a wild type (or normal) *Drosophila melanogaster* fly.

Wrinkled wing: one or both wings were found to be wrinkled. Figure 4 shows a fly with wrinkled wings. Rudimentary wing: both the wings were very small and underdeveloped. Bean eye: one or both eyes were found to be bean-shaped instead of their normal oval shape.

Protruding eye: a small portion of one or both eyes was found to be sticking out of the eye like a thorn. The color of this portion was usually yellowish gray in contrast with the normal red color of the eye. Figure 5 shows a fly with protruding eye (right-hand corner) and a fly with normal eye (in the left-hand corner). Black back: the color of the dorsal part of the thorax was black instead of the normal yellowish-gray color. Beady eye: eye glistened like a bead.



Fig. 3. Drosophila melanogaster fly—normal (wild type).

All the quantitative data on *Drosophila* and S-37 tumor cells were analyzed by the Medical Computer Center, University of Cincinnati, using the Z test. The level of significance used in all the deformity studies was 0.05.

Table I summarizes the results obtained for the first generation. It will be seen that a small number of deformities were observed both in the flies exposed to different magnetic field intensities and in the con-

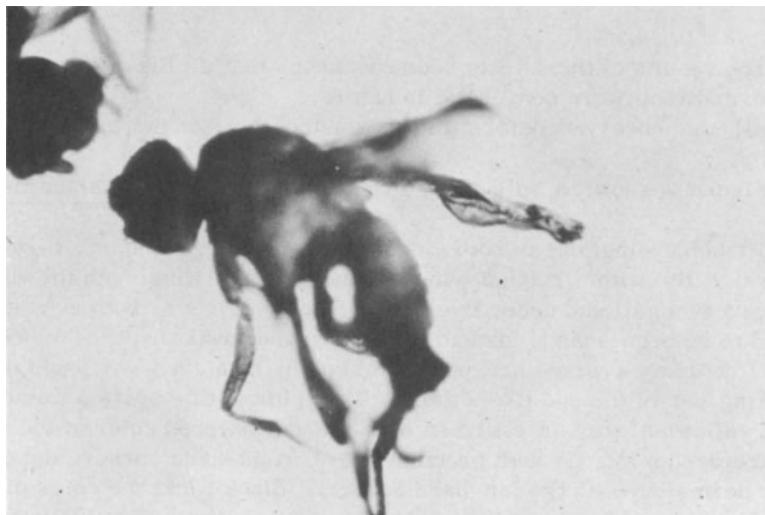


Fig. 4. Drosophila melanogaster fly with wrinkled wings.

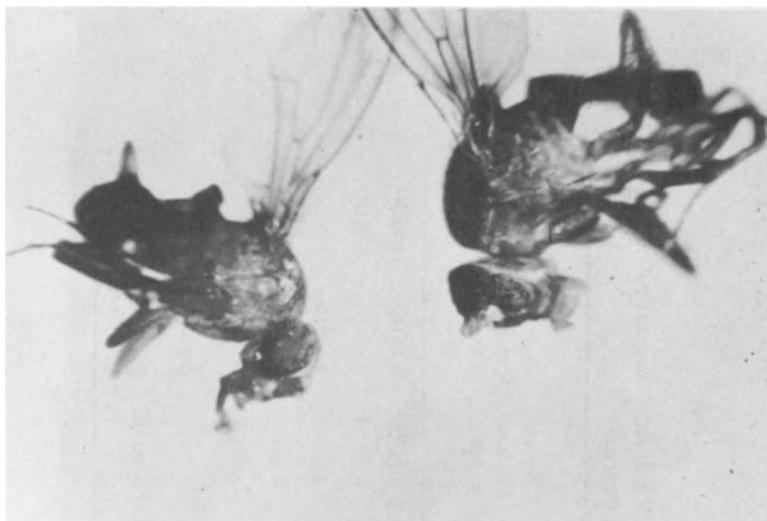


Fig. 5. Drosophila melanogaster. Left-hand corner—normal eye. Right-hand corner—protruding eye.

trols. The frequency of various deformities ranges from 0 to 0.3%, and no statistically significant difference was observed between the exposed and the control samples. It is well known that in a natural population of any species of organism, either plant or animal, a very small number of genetic and nongenetic deformities occurs under natural conditions. These are attributed to different reactions of different individuals to environmental conditions which cannot be completely controlled even in a laboratory. Therefore, when an external agent is tested to observe its ability to cause such changes, one usually does not expect an "all" or "none" effect but a difference in the frequency of changes observed between control and treated population. Analysis of Table II shows that the percent deformities observed in the second generation at 3000 and 4400 Oe are much higher than the control groups. This increase in the total number of deformities is statistically significant. Among the individual groups the increase in the wrinkled wing, rudimentary wing, and black back are statistically significant, but protruding eye, bean eye, and beady eye are not. The exposure at 100, 600, and 1500 Oe did not show significant increase in percent deformities.

The results for the third generation as summarized in Table III also show that the exposure of flies to 3000 and 4400 Oe increased the total number of deformities, whereas exposures to low intensities of 100, 600, and 1500 Oe did not show any significant increase. Among the individual deformities, wrinkled wing, rudimentary wing, protruding eye, and bean eye showed statistically significant increases at 3000 and 4400 Oe, whereas black back and beady eye did not.

TABLE I  
Effect of Magnetic Fields of Different Intensities on the External Characters of *Drosophila melanogaster* in the First Generation

Magnetic field	Maximum intensity, Oe	Maximum gradient, Oe/cm	Total frequency of flies	Percent frequency of wrinkled wing	Percent frequency of rudimentary wing	Percent frequency of protruding eye	Percent frequency of beady eye	Percent frequency of black back	Percent frequency of black eye
100	20	334	0.3 (N)	0.00 (N)	0.00 (N)	0.3 (N)	0.00 (N)	0.00 (N)	0.00 (N)
600	100	399	0.25 (N)	0.00 (N)	0.00 (N)	0.25 (N)	0.00 (N)	0.25 (N)	0.00 (N)
1500	300	794	0.12 (N)	0.24 (N)	0.00 (N)	0.12 (N)	0.00 (N)	0.00 (N)	0.00 (N)
3000	650	671	0.30 (N)	0.15 (N)	0.00 (N)	0.00 (N)	0.00 (N)	0.15 (N)	0.00 (N)
4400	800	634	0.30 (N)	0.00 (N)	0.15 (N)	0.15 (N)	0.00 (N)	0.00 (N)	0.00 (N)
Control (unexposed)	—	1231	0.24	0.08	0	0.24	0.00	0.08	

N—the difference between control and exposed not significant as determined by using the Z test at a 0.05 level of significance.

TABLE II  
Effect of Magnetic Fields of Different Intensities on the External Characters of *Drosophila melanogaster* in the Second Generation

Magnetic field	Maximum intensity, Oe/cm	Total frequency of flies	Percent frequency of wrinkled wing	Percent frequency of rudimentary wing	Percent frequency of protruding eye	Percent frequency of bean eye	Percent frequency of beady eye	Percent frequency of black back
100	20	493	0.20(N)	0.20(N)	0.00(N)	0.00(N)	0.00(N)	0.00(N)
600	100	322	0.30(N)	0.00(N)	0.00(N)	0.30(N)	0.00(N)	0.30(N)
1500	300	446	0.22(N)	0.22(N)	0.00(N)	0.22(N)	0.00(N)	0.22(N)
3000	650	807	0.99(S)	0.49(S)	0.12(N)	0.61(N)	0.00(N)	0.37(S)
4400	800	935	1.00(S)	0.55(S)	0.85(N)	0.64(N)	0.00(N)	0.32(S)
Control (unexposed)	—	2163	0.23	0.05	0.00	0.23	0.00	0.05

S—the difference between control and exposed groups significant as determined by using the Z test at a 0.05 level of significance.

N—the difference between control and exposed groups not significant as determined by using the Z test at a 0.05 level of significance.

TABLE III  
Effect of Magnetic Fields of Different Intensities on the External Characters of *Drosophila melanogaster* in the Third Generation

Magnetic field		Total frequency of flies	Percent frequency of wrinkled wing	Percent frequency of rudimentary wing	Percent frequency of protruding eye	Percent frequency of bean eye	Percent frequency of beady eye	Percent frequency of black back
Maximum intensity, Oe	Maximum gradient, Oe/cm							
100	20	1125	0.26(N)	0.17(N)	0.00(N)	0.08(N)	0.00(N)	0.17(N)
600	100	1532	0.19(N)	0.065(N)	0.00(N)	0.19(N)	0.00(N)	0.06(N)
1500	300	1530	0.13(N)	0.13(N)	0.065(N)	0.13(N)	0.00(N)	0.00(N)
3000	650	1371	0.80(S)	0.29(S)	0.73(S)	0.51(S)	0.00(N)	0.29(N)
4400	800	1229	1.50(S)	1.30(S)	0.65(S)	0.48(S)	0.08(N)	0.24(N)
Control (unexposed)	—	4852	0.18	0.06	0.00	0.18	0.00	0.06

S—the difference between control and exposed groups significant as determined by using the Z test at a 0.05 level of significance.

N—the difference between control and exposed groups not significant as determined by using the Z test at a 0.05 level of significance.

The percent frequencies of the deformities in the control and lower-intensity exposure groups are comparable. The following conclusions may be drawn from the results described above:

1. When *Drosophila melanogaster* organisms were exposed to magnetic fields of 3000 and 4400 Oe for more than one generation, the frequency of deformities increased.
2. Exposure to lower intensities, 100, 600, and 1500 Oe, even for more than two generations, did not increase the frequency of deformities.
3. A very small frequency of most deformities was observed in the control, but a specific "protruding eye deformity" was not seen in the control group. Therefore, a search was conducted among large control populations of *Drosophila* grown in a large bottle. After a prolonged search of about 500,000 flies, one fly with a small protrusion of the eye was observed in the control.
4. During this exploratory stage of the investigation it is not possible to state whether the observed deformities were due to high intensities alone or due to the combined effect of the high gradient and the high intensities; unless experiments are conducted with different gradients for each intensity, no conclusion concerning this effect can be drawn.
5. During the present studies, the highest intensities that could be obtained with the available equipment for the required size of the pole gap were used. It may be pointed out that, in future studies, use of higher intensities (with different gradients) may be useful to obtain quantitative information on this phenomenon. Similarly, use of dummy magnets of identical unmagnetized materials to equalize the environmental conditions with respect to cosmic radiation seems advisable.

## II. IN VITRO STUDIES ON S-37 MOUSE TUMOR CELLS

### Early Work

Since the advent of tissue culture, this tool has been used in several ways to study the action of external agents on living cells and tissues.

In the thirties considerable progress had been made in tissue culture techniques and some workers studied the action of magnetic fields on tissues and cells *in vitro*.

Julia Lengyel<sup>5,6</sup> in 1933 reported some very exciting results; retardation in the growth of chick heart cells was observed due to a magnetic field of about 1000 Oe obtained from a permanent magnet. She also observed an abundant formation of abnormal giant cells in the exposed culture.

Later, Huzella<sup>7</sup> (1934) studied the action of high-intensity alternating magnetic fields on embryonic chick heart cells. He was able to observe some changes in the shape and orientation of the individual cells and their cultured characteristics.

During the next few years, DeLorenzi<sup>8,9</sup> conducted similar experiments. He, too, studied the action of both direct and alternating field on heart, blood vessels, and spinal ganglia. He observed a marked decrease in the mitotic coefficient, but did not notice any changes in the orientation of the cells or fibers. He further stated that when the cells were exposed to low-intensity fields for short periods an inhibition in the formation of equatorial constriction and prolongation in the anaphase resulted. As the original reference was not available to the authors, a detailed description of the exact field intensities, etc., cannot be given here.

In 1936 Ruby Payne-Scott and her associate<sup>10</sup> reported their results on the influence of a magnetic field of 5000 Oe on embryonic heart tissue. Hanging-drop cultures were prepared from 7- to 9-day-old embryos, and after the first subcultivation the culture chosen for the treatment was placed in a small incubator between the poles of a magnet. The culture slide was placed in a vertical position in the pole gap. It was allowed to remain in the field for 3 to 6 hr and immediately fixed and stained. In some cases the slide was incubated outside the field for several hours before staining. Adequate controls were furnished. The exposed cultures did not show any chromosomal abnormalities in the dividing cells, but a slight tendency of protoplasmic disintegration was observed in some resting cells. However, the workers reported that they were unable to establish this effect in all the cultures.

Perakis, who had earlier conducted some studies with sea urchin eggs, also carried out some *in vitro* experiments<sup>11</sup> with chick heart fibroblasts. The cultures were exposed to inhomogenous magnetic fields of low intensities. The average intensity was 200 Oe and the maximum intensity was 490 Oe. The average time of exposure was 68 hr, 23 min. The growth of the tissues in the exposed and control cultures was measured and the results were analyzed statistically. This indicated that the inhomogenous field had a stimulating effect on the growth of the chick fibroblasts, the average increase being about 24%.

In recent years (1961) Butler<sup>12</sup> (St. Luke's Hospital, Phoenix, Arizona) observed some inhibitory effects of magnetic fields on K.B. cells (human epidermoid carcinoma) in tissue culture.

#### Studies Conducted by the Authors<sup>13</sup>; Effects of Magnetic Fields on Sarcoma-37 (S-37) Ascites Tumor Cells

##### *Experimental*

For cellular studies, Sarcoma-37 ascites (fluid) mouse tumor was chosen. Over the years, this material has been found to be useful for cellular observations, because the tumor cells stay suspended singly in the ascites fluid and can be observed for structural details under the microscope. Although a freshly drawn ascites tumor may contain a few other cells such as leukocytes, red blood cells, fibroblasts, etc., the

S-37 tumor cells predominate, and for all practical purposes it can be regarded as made up almost entirely of such cells. Under unusual circumstances, due to some change in the host or the tumor itself, the population of other cells such as fibroblasts may increase. In such an event it is preferable to use fresh material and discontinue the use of such a tumor. The S-37 ascites tumor cells can be grown in a hanging drop in their own ascitic fluid without adding any external nutrients; that is, the ascitic fluid itself acts as nutrient medium. Excellent hanging-drop culture slides can be made from the tumor and may be observed during all stages of development. Most of the morphological observations can be made without staining the cells. The cells can also be stained for specific or detailed observations. The cells start differentiating within a few minutes after the preparation of the hanging-drop cultures and continue to grow and differentiate for several hours when incubated at 37°C under proper conditions. The cells also differentiate at lower temperatures, but at a slower rate. Few cells undergo mitosis and different stages of mitosis can be observed (with proper light arrangement of the microscope) in progress. Most cells do not undergo mitosis but merely differentiate and grow in size when kept in a hanging drop. During the first 15 to 30 min (at 37°C) the cells show spikelike projections on the spherical surface and in a few hours attain the normal characteristic spindle shape. These cells survive for more than 24 hr without additional nutrient media. The cultures can be kept alive for a long time with the addition of fresh nutrient medium. When the cells are kept in hanging-drop cultures without replenishing the original ascitic fluid medium or without the addition of new media, the nutrients are gradually used up, toxic materials accumulate, and, as the available oxygen is depleted, the cells gradually start degenerating after about 24 hr. They go through different degenerating phases such as rhesis, pycnosis, etc., depending on the conditions; finally, complete degeneration or cytolysis of the cells takes place. Different stages of differentiation, cell growth, degeneration, and cell division can be studied in great detail in the first 24 hr under proper experimental conditions. In the present study we investigated the effect of magnetic fields of different intensities and gradients on differentiation and degeneration of the S-37 ascites tumor cells *in vitro*.

The tumor was grown in 11- to 12-week-old mass-inbred Rockland-type mice by injecting 0.1 ml of diluted S-37 ascites tumor (one part ascites tumor and three parts Tyrode's solution) in the peritoneal cavity. When the tumor was 6 to 8 days old, the mouse was decapitated and the fluid was withdrawn from the peritoneal cavity (with a Pasteur pipette). The ascites cells were first examined under a microscope for the general condition of the tumor and a cell count was made. Whenever the tumor was found to be hemorrhagic it was discarded. After the preliminary examination the fluid was immediately diluted with Tyrode's solution to give approximately the same number of cells per unit volume

TABLE IV

The Effect of Magnetic Fields on Sarcoma-37  
Ascites Tumor Cells in vitro

Magnetic field Intensity, Oe	Gradient, Oe/cm	Percent degeneration of exposed cells	Percent degeneration of control cells	Statistical significance* of the effect on tumor cells
100	20	17	18	Not significant
600	100	10	9	Not significant
1500	300	18	19	Not significant
2000	300	10	10	Not significant
4400	800	25	3	Significant
7000	1000	98	10	Significant
8000	1000	82	8	Significant

\*Statistical significance determined by the Z test at a 0.05 level of significance.

in all the culture slides in different experiments. The hanging-drop culture slides were prepared according to the technique used by Paul<sup>14</sup> and under aseptic conditions. The slides to be exposed were placed in the pole gaps of different magnets kept in a constant-temperature incubator at 37°C. The description of the magnetic setup is given in the earlier section on *Drosophila*. The intensities and the gradients of the magnetic fields are given in the first column of Table IV. All the slides were examined microscopically just before they were placed in the incubator. During the preliminary experiments, the slides were examined every 2 hr for 24 hr, but later such frequent examinations were discontinued so as not to move the slides frequently from the magnetic field. The microscopic examination of the culture slides during the first 18 hr was conducted without the aid of any stain, but after this period the final detailed observations were made by staining the slides with acetocarmine. A 1% carmine solution in 45% acetic acid was used. Photomicrographs of some typical stained slides were prepared. The control samples were shielded from all stray magnetic fields and were kept under the same conditions as the experimental slides. The control slides were rested on unmagnetized iron pieces to simulate the experimental conditions. Each group contained 4 slides.

#### *Results and Discussion*

When the S-37 tumor culture slides were examined after the first 2 hr, both control slides and those exposed to different intensity magnetic fields showed the usual normal spikelike growth on the spherical surface

TABLE V

Detailed Information of the Studies on the Effect of Magnetic Fields (of 8000 Oe intensity and 1000 Oe/cm gradient) on Sarcoma-37 Ascites Tumor Cells

Experiment No.	Percent* degeneration of exposed tumor cells	Percent* degeneration of control tumor cells
1	95	1
2	82	2
3	85	24
4	80	1
5	93	9
6	85	17
7	99	3
8	37	10

\*2000 cells counted in each experiment. Average percent degeneration of exposed cells is 82. Average percent degeneration of control cells is 8;  $t = 9.83$ ; probability level = 1:100.

of the cells. After 4 to 8 hr this growth continued and the initial spikes elongated further. Mitosis was also observed in one or two cells. At this time, no difference was observed in the differentiation of cells between control slides and those exposed to magnetic fields. When the

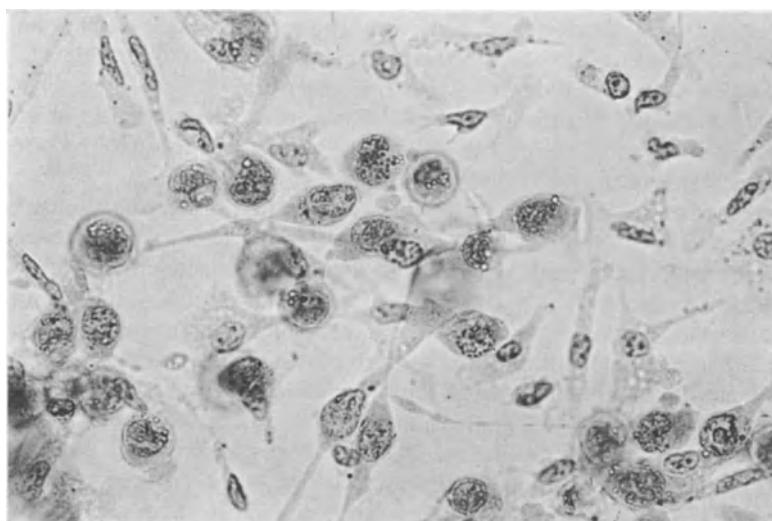


Fig. 6. Sarcoma-37. Ascites tumor cells after 18 hr of normal growth in vitro at 37°C.

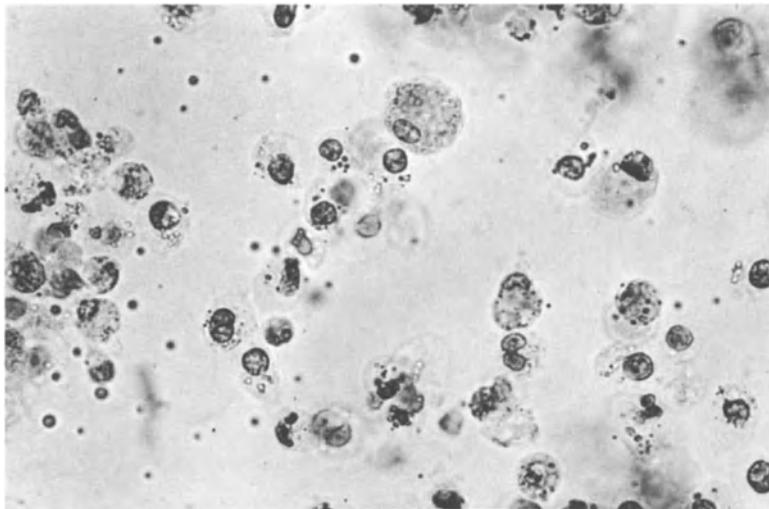


Fig. 7. Sarcoma-37. Ascites tumor cells after 18 hr of growth in vitro at 37°C in a magnetic field (of 8000 Oe intensity and 1000 Oe/cm gradient).

slides were again examined after 18 hr, most cells in the control cultures showed the normal, typical spindle-shaped structure which represented normal differentiation. A small number of cells, as shown in Table V, were seen to be degenerating at the periphery. Figure 6 shows the cells from the control group. In the group exposed to magnetic fields of high intensities (4400 to 8000 Oe), a larger number of cells showed different stages of degeneration. Pycnosis (shrinkage and darker staining of the nucleus), rhaxis (dark staining and breakage of nucleus into several pieces), and cytolysis (scattering of the nucleus outside the cell and a complete breakdown of the cell membrane) were observed frequently. Figure 7 shows some of the exposed cells in different stages of degeneration of the cells. Tables IV and V give the data on percent degeneration, etc.

The studies on the effect of magnetic fields on ascites tumor cells was spread over a period of about a year and a half. During the early part of the investigation, the results were quite reproducible, but during the latter part a change in the tumor material and the host mice was observed. The tumor became hemorrhagic; an increase in the leukocyte and fibroblast content of the tumor was also observed. The reproducibility could not be obtained. Ascites tumor from another source as procured and a new series of transplanations was started in Rockland-type mice and in pure inbred Swiss mice. The exposures to magnetic fields were continued, but the original reproducibility was not regained. It may be emphasized that the experiments reported here are exploratory in nature. Further elaborate work with pure strains and under rigorously controlled conditions is in progress.

These studies seem to indicate the following trends:

1. Magnetic fields of high intensities (4400 to 8000 Oe) seemed to produce some degeneration of S-37 tumor cells at a temperature of 37°C after about 18 hr.
2. Magnetic fields of low intensities (100-2000 Oe) did not show any visible effects after the same period.
3. The observed effect seems to be very specific or dependent on certain precise conditions of tumor and the magnetic field. The basic condition of the tumor seems to play a very important part.

### Studies on S-37 Solid Tumor

#### *Experimental*

This experiment was conducted to observe the effect of magnetic fields on the growth of S-37 solid tumor and to compare the results with those obtained with the ascites cells. A solid S-37 tumor can be produced by injecting the S-37 tumor cells subcutaneously instead of intraperitoneally. The solid tumor, in contrast to the ascites tumor, spreads slowly and is confined to a small area in the beginning. Although both tumor types may be derived from the same source and are interconvertable, the solid tumor is made up of a complicated stroma. An ascites tumor, on the other hand, consists mostly of cells suspended in the ascites fluid. The solid tumor needs an additional medium such as plasma for growth in tissue culture. The solid and ascites tumors differentiate and grow differently in tissue culture. In the ascites form the cells differentiate into spindle-shaped cells and can be analyzed individually, whereas in the solid form the cells are held together. The detailed examination of individual cells in the solid tumor (by hanging-drop technique) is not possible; only the outgrowth, increase in size, and superficial examination of cells is possible.

The S-37 solid tumor was obtained by injecting S-37 cells subcutaneously in 11- to 12-week-old Rockland-type mice. The same dilution procedures as for ascites tumor were used. When the tumor was about 5 to 8 mm in diameter, the animal was decapitated and the tumor was removed. The extraneous material was separated and the inner homogeneous material was cut into pieces 0.5 to 1 mm in diameter. Plasma-clot culture slides were prepared by using the technique described by Paul.<sup>14</sup> Aseptic techniques were used during the entire procedure. Chick plasma was procured from Difco laboratories. The culture slides were immediately placed in a horizontal position in the center of the pole gaps of magnets kept in constant-temperature incubators at 37°C. The same magnetic fields as described for ascites tumor experiments were used. The cultures were examined microscopically every 12 hr for 96 hr. The control cultures were kept under identical conditions between the pieces of unmagnetized iron.

*Results and Discussion*

Both control and exposed tissues showed outgrowth at the end of 32 hr; subsequent to this the projections of the outgrowth elongated and increased in size. After 96 hr, degeneration of the tissue was seen in both the control and exposed cultures. During the experiment the nutrient medium was not replenished. No difference in the growth pattern of control and exposed tissues was observed. The degeneration of cells in both groups started at the same period. These results suggest that the magnetic field within the range used did not have any visible effect on S-37 solid tumor tissue *in vitro* under these experimental conditions. These investigations were carried out during the period when results with ascites tumor were reproducible.

The results of several experiments conducted to study the effect of a magnetic field on cells and tissues indicate the following trends:

1. Only magnetic fields of high intensities (4400 to 8000 Oe) seemed to have a degenerative effect on S-37 ascites tumor cells under certain conditions. At the same time, fields of lower intensities did not have any effects on these cells under similar conditions.
2. S-37 solid tumor, derived from the same origin as the S-37 ascites tumor, was not visibly affected by the same range of magnetic field.
3. These results indicate that the magnetic fields in the range of 4400 to 8000 Oe have preferential action on certain cells.

We wish to point out that the reproducibility of the results seemed to depend on a number of parameters such as (a) the nature of the tumor and (b) environmental conditions such as temperature. On some occasions we were perplexed by considerable variation in the results, but a careful analysis of the situation revealed that the tumor itself had undergone a change.

While such variation in the visible effects was noticed, it occurred to us that the use of a more sensitive physiological technique would possibly indicate the existence of a minute biomagnetic change. It is well known that the visible effects observed in an individual are the final result of several consecutive and/or simultaneous reactions. Most of these reactions depend on the minute amounts of biological catalysts (or what are known as enzymes). These reactions may proceed for several hours, days, or even years before one notices the final result or the visible effects. Quite often small changes in these reactions (which may be produced by external agents, such as electromagnetic radiation, magnetic fields, etc.) do not result in any visible effects at all, and such changes go unnoticed. The intermediate reactions can be studied with precision by biochemical and biophysical methods. Such studies with pure strains of cells, under rigorously controlled environmental conditions, are in progress in our laboratories.

### III. EFFECT OF MAGNETIC FIELDS ON CHEMICAL REACTIONS

#### Possible Types of Magnetic Interaction

The purpose of this survey is to understand the magnetic interactions with biological systems through a correlation with the effects of magnetic fields on chemical reactions. The underlying basis for this stems from the fact that life processes themselves represent a delicate balance between several interdependent biochemical reactions. Here one may consider three different approaches and evaluate each in terms of our existing knowledge of chemical and physical processes. These are:

1. The direct influence of magnetic fields on equilibria and rates of chemical reactions. This aspect will be discussed below (under Survey of Chemical Reactions).
2. The direct influence of magnetic energy on chemical bonding.
3. Indirect effects of magnetic fields resulting from alteration in the physical forces in a system.

These last two aspects are discussed in Section IV.

#### Survey of Chemical Reactions

A survey of literature shows that the effect of magnetic fields on chemical reactions of diverse types has been studied over the past 50 years by many workers. Some reports are quite conflicting, which again suggests a need for a careful examination in this area. Bhatnagar and Mathur<sup>15</sup> have reviewed work up to 1935 including their own. Another review, by Müller,<sup>16</sup> appeared in 1937. The most recent one, with about 54 references, is by Delhez.<sup>17</sup> Unfortunately, limitations of space do not allow a detailed review of this fascinating area; however, a brief account given below shows the diverse types of chemical reactions that have been studied so far.

##### *Reactions in the Liquid State*

Bhatnagar and Mathur<sup>15</sup> mainly studied oxidation-reduction-type reactions involving a paramagnetic ion of some type. Reduction of potassium permanganate and chromic acid showed accelerating effects. A retarding effect in the reduction of ferric chloride was reported, while some reactions of Fe(II) and Co(II), esterification, and nitration did not show any effects. An excellent summary of their work is given in their book on magnetochemistry.<sup>15</sup> Parker and Armes<sup>18</sup> reported accelerating effects for some reduction reactions in the absence of stirring. Reference may be also made to the work of Cegielsky<sup>19</sup> and Heimrod,<sup>20</sup> who refuted Rosenthal's<sup>21</sup> observations on hydrolysis of starch solution in a fluctuating magnetic field, and of Berndt<sup>22</sup> and Nichols.<sup>23</sup> Leffler<sup>24</sup> found no effect on the decomposition rate of benzoyl peroxide. Tyutyulov<sup>25</sup> also studied some reactions in this general area.

*Gaseous Reactions*

Kornfeld and Klingler<sup>26</sup> and Henglein<sup>27</sup> did not observe any effects for reactions of nitric acid with oxygen and chlorine. On the other hand, a patent by Reeves<sup>28</sup> claims 5% increase in the conversion of feed gas C<sub>3</sub>H<sub>8</sub> to high molecular weight compounds.

*Polymerization*

Schmidt and co-workers<sup>29</sup> observed retardation in the polystyrene process from 4.9 to 0.56% and attributed this to the orienting effects of the molecules and the resulting restrictions on the orientation of molecules. This work has been refuted by Breitenbach and Richter.<sup>30</sup> Leffler and Sienko<sup>31</sup> pointed out that since the organic free radicals are in a  $\Sigma$  state, in which the coupling between the spin moment and the molecule can be neglected, it is more likely that an external magnetic field orients only the spin moments of the electrons. Subsequently, Collins and Bryce<sup>32</sup> reported that no significant change in the rate of polymerization of styrene (catalyzed or uncatalyzed) was observed in fields of 12,000 Oe at 80°C. Similarly, no changes in the rate of decomposition of benzoyl peroxide or hydrogen peroxide were observed. Wojtczak<sup>33</sup> observed a decrease in the polymerization rate of acetaldehyde in a steady field and an increase in an alternating field.

*Catalysis*

A negative influence (retardation) was found by Krause and Bin-kowna<sup>34</sup> for the catalytic action in the system H<sub>2</sub>O<sub>2</sub>—HCOOH; Ogawa<sup>35</sup> concluded that the action of reacting molecules on spins of valency electrons in the presence of ferromagnetic catalyst is retarded by the magnetization of ferromagnetic catalysts. A patent assigned to Krum-holtz<sup>36</sup> describes a magnetic field control of heterogeneous catalysts. A uniform and constant field above 5 to 10 Oe is said to produce retarding effects and a strength below 5 Oe is said to produce accelerating effects on the activity of heterogeneous catalysis. Siemens and Halske A.G.<sup>37</sup> studied gas reactions involving free radical mechanisms such as the formation of chlorosilanes from vaporized alkyl halides and silicon in the presence of an activating catalyst like copper and a magnetic field. They suggest that cobalt or nickel undergo increase in susceptibility and thereby increase the migration of free radicals to the silicon particles.

The oxyhydrogen gas reaction, the reaction of nitrous oxide with hydrogen, and the ortho-para hydrogen conversion were investigated extensively by Schwab and Kaiser.<sup>38</sup> They did not detect the external magnetocatalytic effect and found no alteration in the activation energy in the paramagnetic range for the parahydrogen conversion. They could not confirm the earlier results of Vieth<sup>39</sup> relating to nickel catalysts and suggested that no interrelation exists between the magnetic state and the catalyst activity.

*Electrochemical Reactions*

Shchukarev<sup>40</sup> investigated, among others, electrochemical reactions in transverse and longitudinal magnetic fields varying between 2000 and 7000 Oe; most of the work was reported during the years from 1916 to 1926. From time to time he tried to correlate valence of ions, their migration, and a number of other parameters with the magnetic field. It is important to note that after finding that the phenomenon of the action of a magnetic field on chemical reactions was more complicated than he had originally suspected and that they were too complex for individual observation, he decided to give up his study of these phenomena and to retract his former statements.

Among the many chemical reactions studied in a magnetic field, the electrochemical reactions are indeed the most complicated, since here an interaction between the electrical currents used for electrolysis and the external magnetic field occurs; this makes it difficult to attribute the observed effects, if any, to the magnetic field. Sisoev<sup>41</sup> was not able to establish experimentally any effect of a magnetic field on the course of chemical reactions. He suggested that Shchukarev's magnetochemical effect on electrolysis is of the nature of polarization at the electrodes and not a magnetic effect. The only corroborative work in this field seems to be that of Kilgus<sup>42</sup> and Ehrenhaft.<sup>43</sup> They first reported that the production of gases by the chemical or electrolytic decomposition of liquids increases in a magnetic field. Ehrenhaft also observed an increase by as much as 8.3% in the volume of gases evolved during the decomposition of water when carried out in a magnetic field.

*Theories Proposed for the Influence of Magnetic Fields on Chemical Reactions*

Among the early reports, Kuczynski's<sup>44</sup> work may be mentioned. According to him, the effect of an electric or magnetic field on a reaction will be greater for greater changes in the dielectric constant or magnetic permeability and in volume. The following quantitative relationship was given:

$$\ln K_2/K_1 = (H^2/8kT) (V_2E_2 - V_1E_1)$$

where  $K_1$  is the normal state of equilibrium,  $K_2$  is that under the action of the electric field,  $E_1$  and  $E_2$  are the corresponding dielectric constants,  $V_1$  and  $V_2$  are the volumes of the medium, and  $H$  is the electric field potential. Kuczynski<sup>44</sup> stated that a similar expression can be derived for the influence of the magnetic field by substituting the appropriate symbols for magnetic quantities in the right-hand side of the above expression.

Kaneko<sup>45</sup> also gave a theoretical treatment of the influence of a magnetic field on chemical reactions in terms of the thermodynamic functions. The most extensive treatment given in the thirties is that due to Bhatnagar and Mathur,<sup>15</sup> who list several references. They con-

cluded that the homogeneous reactions are accelerated or retarded depending on whether the sum of the molecular susceptibilities of the final products ( $\sum_f \chi_M$ ) is greater or less than the corresponding sum ( $\sum_i \chi_M$ ) for the initial products.

#### IV. POSTULATES FOR MAGNETIC FIELD INTERACTION WITH BIOLOGICAL SYSTEMS

##### Magnitude of Magnetic Energy and Its Possible Influence on Chemical Bonding

The energy of interaction  $E$  of a substance of molar susceptibility  $\chi_M$  placed in a magnetic field  $H$  is given by

$$E = \frac{1}{2} \chi_M \cdot H^2$$

For diamagnetics,  $\chi_M$  is of the order of  $10^{-4}$ ; hence the energy corresponding to a field of 10,000 Oe is of the order of  $5 \times 10^3$  ergs/mole. The thermal energy  $RT$  corresponding to room temperature ( $\sim 25^\circ\text{C}$ ), where  $R$  is the gas constant, is found to be  $2.5 \times 10^{10}$  ergs/mole. Hence, one cannot expect magnetic energies to have significant effects on thermodynamic properties. Most organic molecules comprising biological matter are indeed diamagnetic. Even for paramagnetics, taking a value of  $\chi_M = 10^{-2}$  (some metal-proteins, e.g., for hemoglobin  $\chi_M = 1.4 \times 10^{-2}$ ) as an upper limit, the magnetic energy in a field of 10,000 Oe will be about  $5 \times 10^5$  ergs/mole and will still be considerably less than the thermal energy  $RT$ . Even the energy of a very weak bond, such as the hydrogen bond, of the order 5 kcal is many times greater than the magnitudes of magnetic energy mentioned above. Hence, one cannot expect the magnetic energy to alter the nature of chemical bonding in general and in biological systems in particular.

##### Indirect Action of Magnetic Fields

The above considerations suggest that any explanation for biomagnetic effects should be free of assumptions involving a "direct" interaction of energy with a biological system. Hence the following postulates were proposed to explain some effects of magnetic fields on biological systems.<sup>13</sup> These may be referred to as the "redistribution of dia- and paramagnetism hypothesis."

The chemical components in the cells have varying magnitudes of magnetic susceptibility, representing the diamagnetic and the permanent and any transient paramagnetic contributions which may result from enzymatic reactions vital to the living systems. Hence the permanent and any transient components will experience varying forces under the influence of the magnetic field. Although these forces are small from a macroscopic point of view, they may be appreciable enough to overcome

weak intermolecular forces and thus to upset the delicate balance (chemical or other equilibria, inside and outside the cell) necessary for the sustenance of a living system. Such changes are assumed, at least in part, to produce the degeneration of the cells, as indicated in the tumor cell experiments. It is further conceivable that, in a totally diamagnetic system, preferential orientation effects may occur due to the "diamagnetic currents" arising from a circulation of the  $\pi$  electrons in certain molecules. The significance of this is discussed below.

### Significance of $\pi$ -Electron Distribution

Important aspects of magnetic susceptibility studies on components of blood and carcinogenic compounds, magnetokinetic studies on certain enzymes, and various aspects of nucleic acids have been reviewed briefly by us.<sup>46</sup> The differences observed in the magnetic susceptibilities of normal and cancerous tissues also call for an explanation. In this connection it would be appropriate to mention here that, in our opinion, a large spread of  $\pi$  electrons (mobile electrons, not participating in a carbon-carbon sigma bond) may conceivably control the observed magnetic properties in the biological and biochemical systems. For example, in proteins, which may be pictured as polymerized amino acids, a certain spread of  $\pi$  electrons seems possible within an individual amino acid component and probably over the different components. Indeed, the higher the degree of unsaturation and especially of conjugation, the more pronounced will be the effect of  $\pi$ -electron distribution on the magnetic susceptibility of such systems.

In general, the magnitude of the diamagnetism of a system will depend on the area ("orbit") over which the  $\pi$  electrons are distributed and on their number. Now, during the degradation of such a system (for example, in the denaturation of proteins), the "orbits" confining  $\pi$  electrons will certainly decrease and so will the number of electrons in each such "orbit." Thus a large-scale decrease in diamagnetism can very well appear as an apparent "paramagnetic" effect during any disintegration of a polymeric system, even though the system may not initially contain any unpaired electrons. Conversely, increase in diamagnetic susceptibility can be explained on the basis of "a polymerization process" in certain systems.

We also wish to point out that the bonding between the metal atom and the ligands in chemical complexes, on the one hand, and that between the metal atom and proteins, on the other, occurring in biological systems (for example, in hemoglobin) has been so far visualized only in terms of "covalent" or "ionic" bonding, and the interpretations of the magnetic susceptibility data are mostly carried out along these lines. It is interesting to note that the recent work of Earnshaw and Lewis<sup>47</sup> on polymeric Cr(III) complexes suggests the possibility of  $\pi$  bonding between the paramagnetic metal ion with unfilled *d* orbitals

and the neighboring oxygen atoms. It would indeed be desirable to take a new look at biological systems in terms of the possibility of such bonding.

Some of the ideas stated above were first put forward by us at the First Biomagnetics Symposium.<sup>48</sup>

#### ACKNOWLEDGMENTS

Early work on biomagnetic studies was started in 1953 independently by one of us (I.L.M.) in the magnetochemical laboratories of Prof. P. W. Selwood at Northwestern University. We wish to express our gratitude to him for the use of laboratory facilities. Subsequently, some work was done at Harvard University (1955-57) and was continued at laboratories in Cincinnati (Institutum Divi Thomae and University of Cincinnati). We wish to thank several colleagues, too numerous to be listed here, for their encouragement and for providing various facilities. We are indeed thankful to the American Cancer Society for providing grants (T-250) for our research since 1962.

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## *Chapter 8*

# Magnetotropism

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The experiments we are about to describe started<sup>1</sup> as a new approach to an old problem—the mechanism of gravity perception in plants. All higher plants are sensitive to gravity; indeed the oriented growth of organs such as roots, shoots, and leaves is governed primarily by it. If a plant organ is by any means displaced from its normal direction of growth, certain regions of the organ sensitive to gravity will perceive the change and then, by a hormone-mediated mechanism, will induce one side of the organ to grow faster than the other, producing a curvature tending to restore the normal orientation to the growing part of the organ. The sensitive areas are usually the extreme apical regions.

The mechanism of graviperception is still a mystery. The oldest and still the most convincing theory postulates that certain protoplasmic bodies (amyloplasts) containing starch grains, which sediment rapidly under gravity in gravity-sensitive cells, are the perceptor bodies. Such bodies, which have been termed statoliths, occur in almost all gravity-sensitive organs, but do not occur in insensitive ones and the main strength of the theory comes from this very close correlation between sensitivity and the statolith occurrence. So far, experimental support for this theory has come solely from techniques which bring about displacement of these amyloplasts by virtue of their mass, that is, by the use of gravitational or centrifugal forces. Experiments of this type can do no more than check the strictness of the correlation between amyloplast sedimentation and the gravity-induced growth curvatures in sensitive plant organs; they can never prove that the sedimentation induces the curvature. (For a recent analysis of graviperception, see the paper by Audus, 1962.<sup>2</sup>) In an attempt to break this somewhat vicious circle, it was hoped that by exposing such organs to a steep magnetic gradient some lateral migration of amyloplasts across the cell might be induced by virtue of the difference between the diamagnetic susceptibility of starch grains and that of the surrounding cytoplasm of the cell. If this could be done under conditions where the amyloplasts were prevented from sedimenting under gravity and where, therefore, sensitive organs

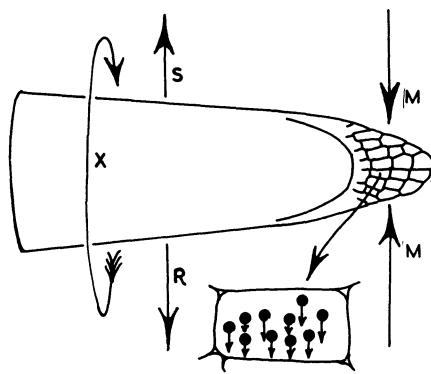


Fig. 1. Diagram to illustrate the proposed use of the diamagnetic properties of starch grains to check the statolith theory of graviperception in plants. The diagram is a representation of a sensitive plant organ with the mobile statoliths in the tip cells. The arrow  $M$  marks the direction of the maximum negative gradient of magnetic field through the sensitive tip and perpendicular to the organ's main axis, which is kept horizontal. The organ and magnetic field are rotated slowly together (arrow  $X$ ) about this axis. Arrows  $S$  and  $R$  represent the expected directions of shoot curvature, respectively, if statolith movement in the sensitive cell under the magnetic forces were in the direction indicated in the magnified drawing of a single cell.

cannot show gravity-induced growth curvature, then any movement of these amyloplasts in the magnetic gradient correlated with an appropriate growth curvature would go a long way toward establishing that movement as the real cause of the curvature. This approach is shown pictorially in Figure 1.

The force involved in this movement in a magnetic field is given by the following formula

$$(K_1 - K_2) \cdot vH \cdot dH/dx$$

where  $K_1$  and  $K_2$  are the volume susceptibilities of the starch grain and the surrounding medium, respectively,  $v$  is the volume of the particle,  $H$  is the magnetic field strength and  $dH/dx$  is the field gradient. Unfortunately, no direct measurements of  $K_1$  or  $K_2$  have been made, as far as we are aware, but by the use of the Pascal constants it has been calculated that forces of the order of  $1/200$  of that due to gravity might be expected from the kind of magnets available to us. Since  $K_1$  and  $K_2$  are both negative (diamagnetic) and  $K_1$  is likely to have a higher negative value than  $K_2$ , the net force on the starch grain would be expected to be in a direction down the gradient, i.e., from high to low field strengths. These calculations have been set out in detail elsewhere.<sup>1</sup>

## TECHNIQUES

The basic design of apparatus is outlined in Figure 2. A large permanent horseshoe-type magnet was provided with beveled pole pieces of the shape illustrated ( $A$ ) and giving distribution curves of field  $H$  and force  $H(dH/dx)$  as shown at  $B$ . The field strength distributions were directly measured using the Quincke method. The seedling plant, having been grown on agar or other suitable moist medium in a thin glass cell, and normally oriented to gravity, was so placed between the poles of the magnet that its long axis was parallel to the gap and in the region of the greatest force, which coincided approximately with the plane of the

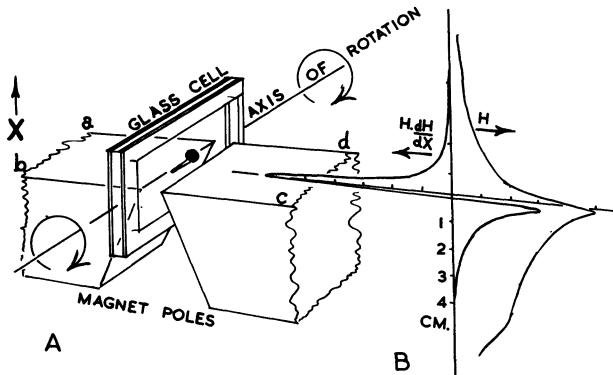


Fig. 2. A—Diagram showing the placement of the experimental glass cell and germinating seedling between the poles of the permanent horseshoe magnet. Only the poles have been drawn. The axis of rotation indicated is horizontal. B—Graphs showing the distribution of field  $H$  and the parameter  $H(dH/dx)$  along the central axis midway between the poles and perpendicular to the plane  $abcd$ .

side  $abcd$  of the magnet. At this point the field strength was approximately 4000 Oe and the gradient over 5000 Oe/cm. The whole magnet-cell system, supported in a brass cradle, was rotated slowly at a speed of 1 revolution per 100 sec. It must be emphasized at this point that this rotation is an essential part of the technique, since it is this which eliminates any effect of gravity on amyloplast distribution and prevents any growth curvatures which might be caused by gravity and which would undoubtedly swamp any responses to the magnetic gradient. For example, placing the root of a cress seedling, one of the organs used in these studies, in a horizontal position for only 15 sec brings about a detectable sedimentation of the amyloplasts in the cells of the tip; this, or slightly greater exposures, would induce small growth curvatures in this organ. Observations on the subsequent behavior of the sensitive organ in the magnetic gradient were made by photography on 35-mm film, using low-intensity electronic flash lighting, latterly using a deep red filter, without stopping the rotation. Otherwise the whole series of experiments was carried out in complete darkness and at 25°C in a constant-temperature room. One flash is equivalent to about 1000 ft-c-sec. Assuming a difference of 10% between the illumination of magnet and control samples, one flash per day would be equivalent to a continuous light difference of  $\frac{1}{1000}$  ft-c during 24 hr.

#### GROWTH RESPONSES

In all organs so far studied, namely roots of Lepidium sativum, Phleum pratense, and Dahlia sp., coleoptiles of Avena sativa and Poa nemoralis, hypocotyl of Helianthus annuus, and sporangiophores of Phycomyces, the results of the above treatment have been a growth curva-

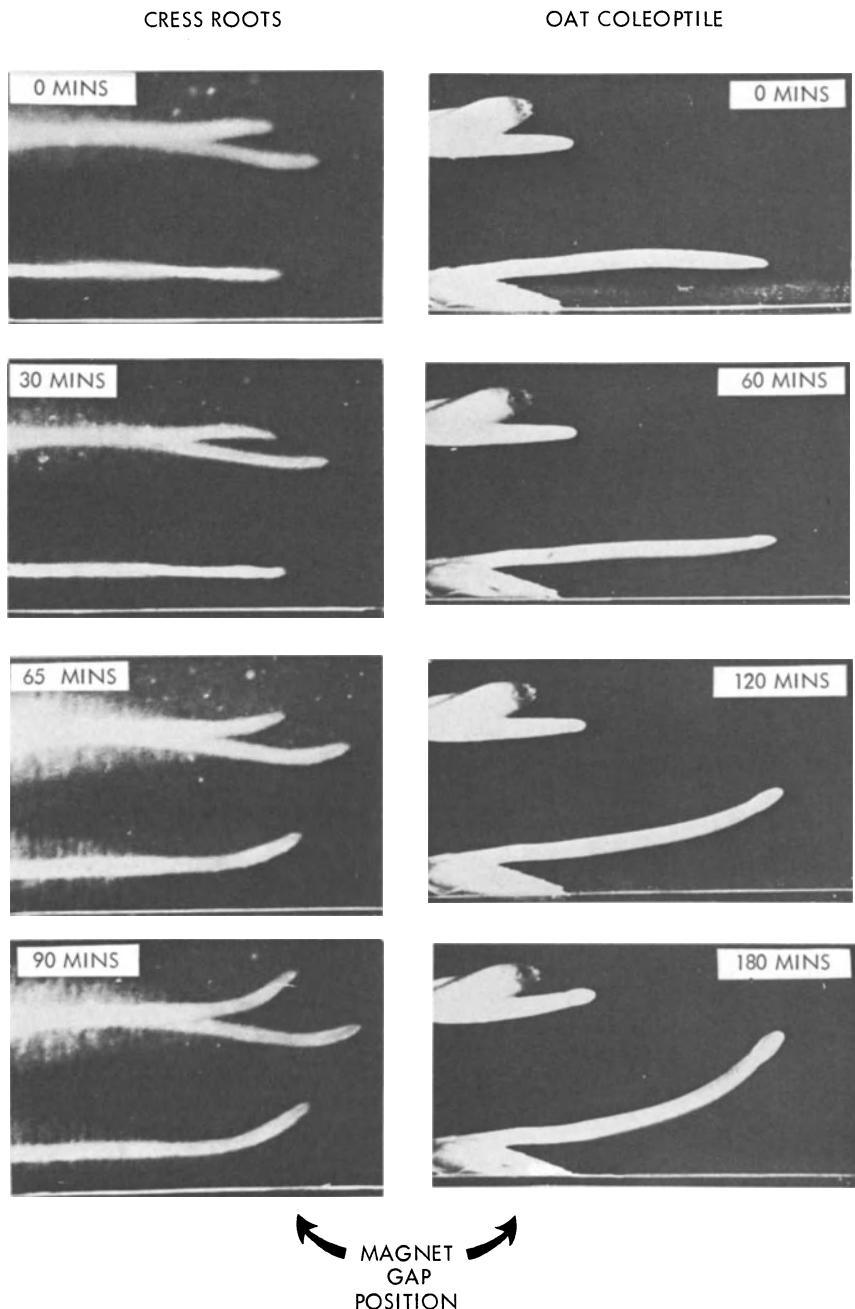


Fig. 3. Records of typical growth curvatures observed with cress (*Lepidium sativum*) roots and oat (*Avena sativa*) coleoptiles in the magnetic gradient. The bottom white line in each frame marks the position of the magnet gap.

ture of the organ down the magnetic gradient, that is from regions of high to regions of low magnetic flux. Two kinds of organ have been studied extensively: the roots of Lepidium sativum and the coleoptiles of Avena sativa. Figure 3 shows photographic frames selected from two series of experimental records, one for each species, where the progress of typical responses is clearly seen. In all these experiments great care has been taken to eliminate the possibility of curvatures due to other physical gradients inherent in the construction of the experimental apparatus. For example, it has been suggested that a large mass of metal on one side of the experimental cell might afford a unilateral screening action on cosmic rays, which might affect the uniformity of growth of the experimental organ. To obviate all these sources of artifact, strict control experiments have been carried out in almost all cases, using organs placed between exactly similar but non magnetized pole pieces also rotated on the same axis but well away from the magnet. In no instance have consistent curvatures been recorded in these controls. Such controls automatically allow for any responses to light stimuli arising during the flash photography.

These results are at one and the same time gratifying and disappointing. They are gratifying in that, as far as we can ascertain, this is a completely new phenomenon. It has been christened "magnetotropism;"<sup>1</sup> they are disappointing in that they are not what one would have expected on the basis of amyloplast migration. Thus, under gravity these amyloplasts always sediment to the lower part of the cell, but associated growth curvature responses are opposite in roots and in coleoptiles. Roots curve downward, that is, in the direction of amyloplast sedimentation, whereas coleoptiles curve upward, in a direction opposite to this sedimentation. But in these magnetic field experiments all organs studied, including both root and shoots, curve in the same direction. This would imply either that these magnetotropic curvatures have very little in common with geotropic curvatures, or that amyloplasts migrate in the magnetic gradient in opposite directions in roots and in coleoptiles. The next logical step therefore was to look for evidence of such migration.

#### AMYLOPLAST MOVEMENT

Great care was needed in these investigations of starch grain distribution to avoid any possibility of experimental artefact due to gravity itself. We have already mentioned that migration under gravity can be detected within a few seconds in cress root tips and so it was necessary to fix the material rapidly without stopping the rotation or removing it from the magnetic field. This was done by enclosing the glass cell in a small polythene bag at the commencement of the experiment and then running in strong formalin solution after an appropriate experimental period during the rotation. Thoroughly fixed organs were then washed

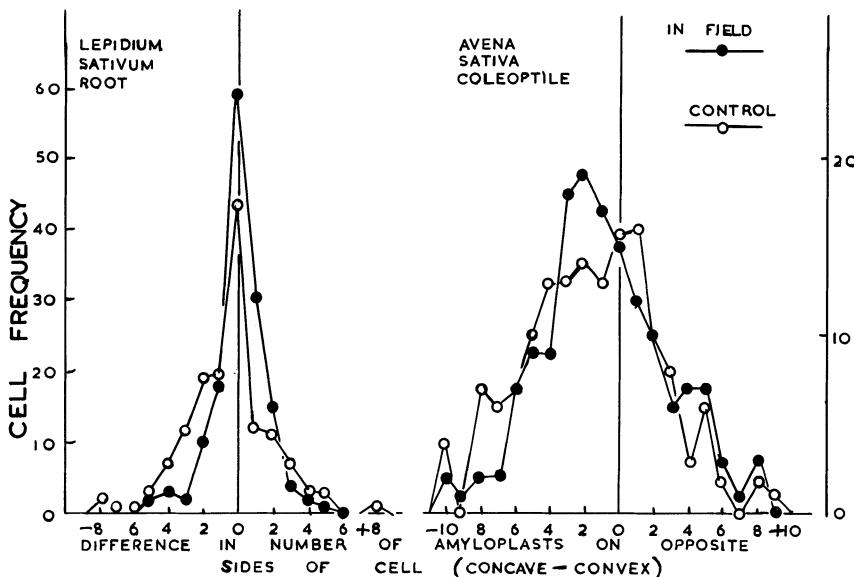


Fig. 4. Cell frequency curves for a root tip of cress (*Lepidium sativum*) and coleoptile tip of oat (*Avena sativa*) for amyloplast distribution between the two sides of the cell in organs rotated in a magnetic gradient and at a point far removed from the magnet, respectively.

and carefully oriented in a film of sodium alginate blackened with India ink to facilitate precise sectioning in the right plane. The film was then gelled and the organ embedded in it by flooding with calcium chloride solution. After dehydration and embedding in wax, a complete series of longitudinal 8- $\mu$  microtome sections could then be cut through the organ in a plane parallel to the direction of the maximum magnetic gradient. All the cells of the organ tip were subsequently surveyed and movable amyloplast distributions recorded by counting the number on the two sides of the cell toward the convex and concave sides respectively of the curved organ. For comparison, observations were made in each experiment on the control organs rotated at the same speed and under the conditions described above.

Figure 4 shows the result of two such sets of observations, one on a root of *Lepidium sativum* and the other on a coleoptile of *Avena sativa*. These are cell frequency diagrams in which the discontinuous variate is the difference in the number of starch grains between one side of the cell and the other. For organs in the magnetic gradient the variate is the difference between the sides of the cell toward the concave and convex sides of the root, respectively. For the controls, it is the difference between one arbitrarily selected side of the organ and the other. For the cress root tip with a total number of amyloplast-containing cells of about 150 and an average amyloplast content per cell section of just about 7, it will be seen that for both experimental and control roots

the mode falls at zero, and there is no significant effect of the magnetic gradient on amyloplast distributions. In the tip of an *Avena* coleoptile, which has about the same number of amyloplast-containing cells and an average content per cell section of approximately 10, the mode for experimental tips falls at -2, showing a significant excess of amyloplasts on the convex side of the curving organ. On the other hand, the control tip also shows a similar asymmetric distribution, due presumably to some experimental variable not yet eliminated. These two sets of results are typical of a large number of observations of this sort. Thorough statistical analyses show that in the majority of instances there is no significant migration of amyloplasts across the cell in the magnetic field. The occasional observation of a significant asymmetry of distribution is no more frequent in experimental organs than it is in control organs. Moreover, the small movements are not consistent in direction, being sometimes toward the concave and sometimes toward the convex side of the curving organ.

It must therefore be concluded that the magnetotropic curvatures induced cannot be mediated by amyloplast migration in the magnetic gradient and are of little help to us in our study of graviperception. However, we now have on our hands a new phenomenon that is equally intriguing, though hardly a natural one.

A varied number of preliminary experiments have been carried out to elucidate the mechanism of these growth curvatures.

#### CURVATURE INTO AGAR

The organ itself, being diamagnetic, will experience, as it grows in moist air, a small force down the magnetic gradient and, depending on its rigidity, will be bent very slightly in that direction. Such mechanical effects on the organ as a whole will be very small and have not in fact been observed, but it is not impossible that small tensions and compressions set up on opposite sides of the organ may be the cause of the growth responses and the subsequent growth curvatures. This possibility has been checked by arranging for coleoptiles to grow along the surface of a block 2% agar placed perpendicular to the magnetic gradient and on the side of the organ away from the magnet. Any tendency of the coleoptile to curve will immediately be countered by the elastic resistance of the agar gel, which, even for small tip displacements, would produce a bending moment in the reverse direction far greater than any produced by the magnetic gradient. Under these experimental conditions, we have observed consistent curvatures of *Avena* coleoptiles down the magnetic gradient, causing thereby an actual penetration of the gel by the tip. A result of this kind is illustrated in Figure 5. Mechanical strains set up in the growing organ by the magnetic gradient cannot, therefore, explain the growth curvatures produced.

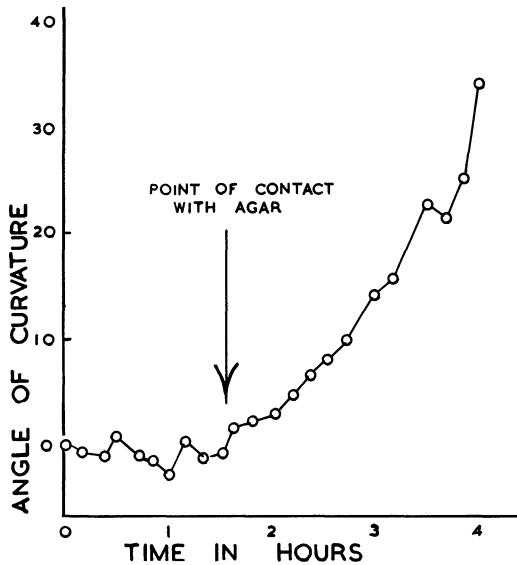


Fig. 5. Time course of curvature of a coleoptile of oat down a magnetic gradient and against the mechanical resistance offered by a 2% agar gel.

#### QUANTITATIVE RELATIONSHIPS

It is a characteristic of the response observed that, like responses to other stimuli, a considerable period elapses between the initiation of the stimulus and the commencement of curvature. This period has been called the reaction time and is an inverse measure of the rate of change of cell equilibrium which culminates in the subsequent growth curvatures. It can thus be used as a measure of the intensity of response of the "detecting mechanism" to the actuating stimulus, here the magnetic gradient.

A typical progress curve is shown in Figure 6. An objective estimate of the reaction time can be obtained by taking the points for the linear part of the curvature progress curve, calculating from them a regression line, and finding the point at which it cuts the zero-response axis. From the slope of the regression line, the initial rate of curvature can also be objectively determined. A considerable number of experiments have been done with cress roots and oat coleoptiles starting with the organ at different distances down the magnetic gradient from the narrowest part of the gap, which corresponds roughly to the region of the maximum value of  $H(dH/dx)$ . Reaction times have been calculated from all these experiments and have been plotted as reciprocals against initial distance from the magnet gap in Figure 7.

The upper results are for *Avena* coleoptile. It will be seen that the rate of response (reciprocal of the reaction time in minutes) falls away rapidly with increasing distance from the magnet. This decrease is

statistically significant at the 1% point. The results for cress roots in the lower graph show a greater scatter, but here again there is a significant fall in the rate of response which just reaches the 5% significance point.

If these responses were due to the movement of, or pressure on, some diamagnetic component of the responding cell, one would have expected the rate of reaction to have been proportional to  $H(dH/dx)$  and to have declined in the same way with distance from the magnet. However, the reaction rate falls with distance much less steeply. Calculations from the calibration curve of the magnet have shown that, within the limits of the rather large experimental errors involved, the decline in reaction rate more nearly follows the change in the gradient itself ( $dH/dx$ ), as shown by the continuous curves in Figure 7. This strongly suggests that the growth curvatures may result from a direct modifying effect of the magnetic field on the extension growth of the cell, causing an acceleration which is proportional to the magnetic flux passing through it, which in these experiments is transverse to the direction of its extension. The existence of this strong flux gradient across the organ would cause differential effects on the growth rates of the two opposite sides, and this would necessarily result in growth curvatures proportional to the flux gradient. The next step, therefore, is to make observations on the effects of strong uniform magnetic fields on the growth rates of these organs, so that these possibilities can be directly checked.

Calculations of initial curvature rates have revealed no consistent changes with initial position in the field, the scatter of points being very large. Since the stimulus changes both in direction and intensity as the organ responds, these results are very difficult to interpret. Further speculation must await less variable results from improved techniques and more uniform plant material.

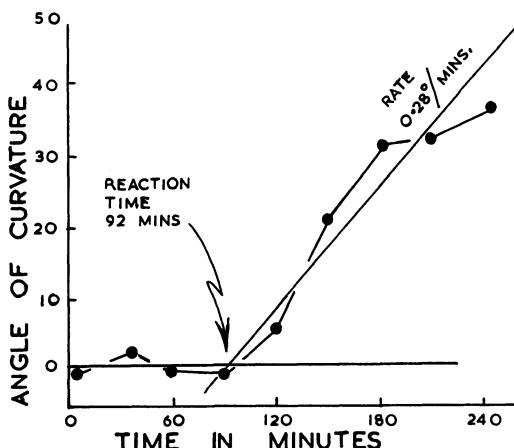


Fig. 6. Time course of magnetotropic curvature in a root of cress showing the fitted regression line and the method of calculating the reaction time and the curvature rate.

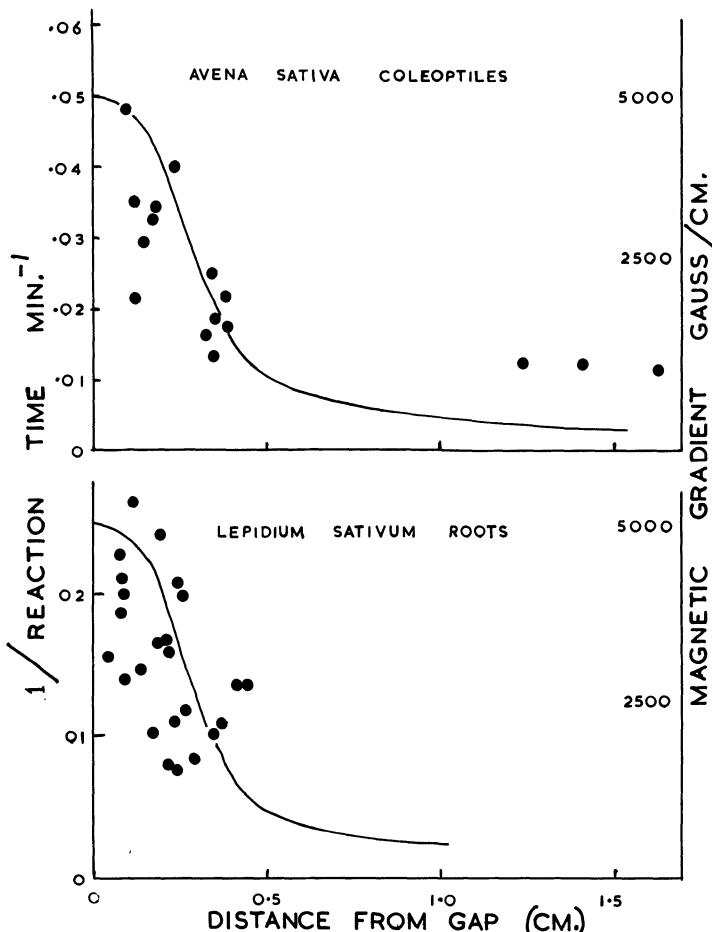


Fig. 7. Diagrams showing the relationship of response rate (reciprocal of the reaction time) to initial position of organ in the magnetic gradient (distance from gap). The smooth curves of field gradient distribution were calculated from direct measurements of the flux distribution using the Quincke method.

#### MAGNETIC FIELDS AND PLANT GROWTH—A BRIEF SURVEY

Naturally, the discovery of this new response has prompted a thorough search of the literature for evidence of similar or related effects in earlier work. Scattered through the biological journals of the last half century are reports of some score or so investigations on plants, most of them purely empirical in concept.

Microorganisms such as bacteria and yeasts have received some attention.<sup>3-6</sup> Homogeneous fields, even of intensities of the order of 11,000 Oe, have no observable effects on various aspects of growth, but heterogeneous fields appear to retard the growth of tumors induced on *Pelargonium* sp. by *Agrobacterium tumefaciens*<sup>6</sup> and to reduce by 20-

30% the rate of budding of yeast cells growing on agar. The latter work involved a comprehensive series of observations from which the reality of the effects claimed are statistically not in doubt. However, the paucity of suitable control experiments to eliminate the possibility of artefact from associated gradients (e.g., of metabolic staining products on the plate) and the fact that the fields varied across the experimental plates in both strength and direction in some undetermined manner make it very difficult to visualize the physiological implications of these effects.

The phenomenon of protoplasmic streaming in the cells of higher plants, involving the circulation of charged organelles and other smaller cytoplasmic particles around the cell, has been the obvious choice of several physiologists in their search for interactions with magnetic fields. The early work of Bequerel, Dutrochet, and Reinke<sup>7</sup> yielded no positive responses, but the studies of Ewart<sup>8</sup> and the more recent and extensive work of Ssawostin<sup>9</sup> have claimed significant, albeit inconsistent, effects. The latter worker, using uniform electromagnetic fields of up to 7000 Oe and careful control of temperature, demonstrated statistically significant retardations of streaming rate when the flow of cytoplasm was parallel to the direction of the field and either retardation or acceleration when it was perpendicular to the field. The effects occurred either during the 6-min exposure to the field or subsequently when it had been switched off. Although the effects in individual experiments can be established by statistical analysis of the data, lack of consistency of behavior between experiments demand careful repetition of this work. A discussion of the physiological implications of these results would therefore be premature.

Little serious systematic study has been made of the effects of magnetic fields on the germination of seeds of higher plants and the subsequent growth of seedling roots and shoots. A few casual observations by Bayliss<sup>10</sup> could demonstrate no growth responses of seedling roots of Pisum sativum (garden pea) or Vicia faba (broad bean) to uniform and intense magnetic fields. However, she claimed that roots horizontally disposed "just above one pole" responded more to gravity than similarly disposed roots below the pole (no measurements or further data given). This interesting observation led her to suspect a possible induced movement of the starch grain statoliths in the tips of these roots, but anatomical investigations failed to reveal any such action (again no experimental details or numerical data are given).

Ssawostin,<sup>9</sup> in a very poorly documented paper, claimed that uniform fields greatly stimulated the extension growth of the seedling shoots (coleoptiles) of wheat for fields both perpendicular to and parallel to the main axis of the organ. No curvature responses were observed toward either pole. An even vaguer report by Murphy<sup>11</sup> claimed an acceleration of the germination of several seeds by unspecified artificial magnetic fields.

Perhaps the most striking claims of magnetic effects on seedlings are those of Krylov and Tarakanova<sup>12</sup> and Krylov.<sup>13</sup> Working with various seeds, particularly wheat, they claimed that, during the phase of water imbibition only, the orientation of the embryo relative either to the earth's magnetic field or to the field of small permanent magnets greatly influences the subsequent growth of root and shoot in the developing seedling. Thus, with seeds oriented with the embryonic root directed toward the earth's south pole or the south pole of the permanent magnet, the subsequent growth of both root and shoot was much greater (2-5 times) than with seeds oriented with their roots toward the north pole. Furthermore, in seedlings of maize, roots initially pointing north bent around and grew toward the south pole, a phenomenon they christened "magnetotropism." Since no responses were observed from seeds fully imbibed previous to orientation in the magnetic field, the action is postulated to occur only on biochemical processes when they are being initiated during imbibition and when (biochemical) polarity is being established.

Although these authors claim that these experiments were repeated many times, each with the same result, the numerical data recorded are meager in the extreme, being merely the average lengths of shoots and roots of samples of 100 seedlings for one terrestrial magnetism experiment and of an unprescribed number for one permanent magnet experiment. The articles afford no clue to the variability of the material and hence to the significance of the responses claimed.

In view of these serious deficiencies and because of the magnitude of the effects claimed for such a weak field as that of the earth, the senior author has repeated these experiments as closely as possible using wheat (var. Eclipse), full attention being paid to appropriate replication to enable precise statistical evaluation of the results. In a series of experiments in the earth's magnetic field and in a reasonably homogeneous field of a large permanent magnet ( $1\frac{1}{2}$ " gap,  $1\frac{3}{4}$ "  $\times$   $1\frac{3}{4}$ " pole pieces, 1700-Oe field), no consistent effects of orientation could be established on germination rate or root and coleoptile growth rates over the first three days from soaking. Occasionally, apparent effects of orientation have been observed in individual petri dishes, which have been used as germination vessels in the terrestrial magnetism experiments. A typical result is shown in Table I for an experiment involving 240 grains. Thus, in two dishes (3 and 5) there were significant effects associated with orientation, but these differences were not consistent, being in favor of the north-pointing roots in dish 5 and of the south-pointing roots in dish 3. These differences are most likely related to some unknown gradient in the petri dish (e.g., nonuniform filter paper on which the grains were soaked and germinated) favoring more growth on one side than on the other.

In our opinion some such unsuspected gradient, correlated with orientation, must have been the cause of the growth differences re-

TABLE I

Mean Root Growth per Seedling (mm) over the 46-69 hr Period

	Dish number					
	1	2	3	4	5	6
Root end N-pointing	35.5	43.3	36.2	51.5	49.4	49.9
Root end S-pointing	41.1	47.7	47.9	44.6	39.4	43.3
Difference (N-S)	-5.6	-4.4	-11.7	+ 6.9	+ 10.0	+ 6.6
Least significant difference (5%)	9.23	8.57	9.08	9.66	9.00	9.13

corded by Krylov, although it is extremely difficult to imagine how growth differences of the order of 150% could have arisen. In further contrast to the results of Krylov, no curvatures of either root or coleoptile relative to orientation were observed in these experiments.

## GENERAL CONCLUSIONS

The general conclusions that can be drawn from this summary is that relatively large effects of magnetic fields on growth rate of higher plant organs are most unlikely. However, the magnetotropic curvatures in strong magnetic gradients observed in our own experiments are consistent with a very small growth-accelerating effect of fields transverse to the long axis of the organ but, since growth curvatures are extremely sensitive indicators of small differences in growth rate, it is unlikely that small induced changes of this kind would ever be shown up by the relatively coarse methods involving direct measurements of growth rates of sample.

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## *Chapter 9*

# Plant Growth Responses

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The question of whether magnetic fields may exert an influence on biological processes in plants is controversial. We are confronted on the one hand by a number of reports which purport to demonstrate effects of magnetic fields on one or more phases of plant growth and development and, on the other hand, with experiments where the magnetic field had no effect.

Tolomei<sup>19</sup> and Murphy,<sup>13</sup> for example, found that the presence of magnetic fields had an acceleration effect on seed germination of several species, whereas Favret et al.<sup>7</sup> sometimes observed complete inhibition of germination in cabbage. More rapid growth of shoots and roots of pepper grass has been found by Whish;<sup>20</sup> Sswostin<sup>18</sup> used magnetic fields to produce as much as 100% increase in the rate of elongation of wheat seedlings; and Favret et al.<sup>7</sup> report some biomagnetic experiments in which they obtained onion roots 10 times the length of those of controls. Tropistic responses in which the direction of curvature is away from the center of the field were observed as long ago as 1893 by Tolomei<sup>19</sup> and have been studied more recently in considerable detail by Audus.<sup>1‡</sup> Orientation of bean roots in one direction in a magnetic field has been found by Puma<sup>15</sup> to produce complete growth inhibition, whereas orientation in other directions was without effect. Retardation effects have also been observed by Kimball<sup>9</sup> on budding of yeast, by Cluzet and Ponthus<sup>5</sup> on yeast development, and by Magrou and Manigault<sup>10</sup> on growth of crown gall tumors.

In contrast to the above reports, neither Reinke<sup>16</sup> nor Baten<sup>2</sup> were able to demonstrate any clear-cut effects of magnetism on overall plant growth; D'Astre<sup>6</sup> observed no effects on seed germination, Jennison<sup>8</sup> none on yeast; Sswostin<sup>18</sup> could not confirm Tolomei's results on germination; and Favret et al.<sup>7</sup> felt their own results to be uncertain.

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‡See also the preceding chapter.

In the hope of contributing toward the eventual resolution of the question of biomagnetic effects on plants, the experiment described below was undertaken. We wished to determine whether a steady magnetic field continuously applied over a period of days could induce effects upon the growth of higher plants which would be both easily measurable and statistically significant; and if so, to discover the means by which such effects were produced. Our choice of experimental material and the methods employed were governed throughout by attempts to avoid many of the objections that could be raised against some of the earlier experiments directed to the same end.

#### MATERIALS AND METHODS

In experiments where the effects to be observed are expected (in view of the long controversy about their existence) to be something less conspicuous than gross morphological alterations, the use of experimental material having high genetic uniformity and consistency of growth pattern is of utmost importance. For the studies presented here, barley seed (Hordeum distichum L. emend. Lam. cv. Hannchen, C.I. 531) from a highly-selected, long-inbred line (constituting the control stock used extensively by two of the authors, R.P.M. and L.W.M., in radiation studies<sup>12</sup> during the past 10 years) was chosen as our experimental plant material, primarily because of its inherent stability and high reproducibility of induced changes in growth habit.

Our decision to use permanent magnets (alnico  $5 \times 4 \times 1\frac{1}{4}$  in. horseshoe) was based largely upon the freedom we would achieve from accompanying thermal effects. The chief disadvantage lay in the relatively low strength of the magnetic field achievable with the magnets we had available at the time (maximum field intensity of  $\sim 1200$  Oe). This shortcoming we felt might be offset, however, by the fact that use of these permanent magnets permitted the plant materials to be continuously exposed over a period of days and also provided inhomogeneous magnetic fields (claimed by some<sup>9,10</sup> to be the only ones effective).

Barley grains (ten per group) were germinated in hollow rolls of double-layered brown-paper toweling (Steiner), each roll standing upright with the lower end in tap water. The grains were positioned in the toweling by means of a template so they were equidistant along a straight line, and after being rolled were oriented in the same horizontal plane within the roll, with their long axes parallel and vertical (radicle down). During the course of this experiment, 90 grains in all (contained in nine replicate rolls) were germinated in the presence of an externally applied magnetic field, while an equal number of replicates were germinated concurrently in the absence of the magnetic field to serve as controls.

For exposure to the magnetic field, individual rolls containing grains were placed 3-4 mm in front of the face of the magnet (one roll per

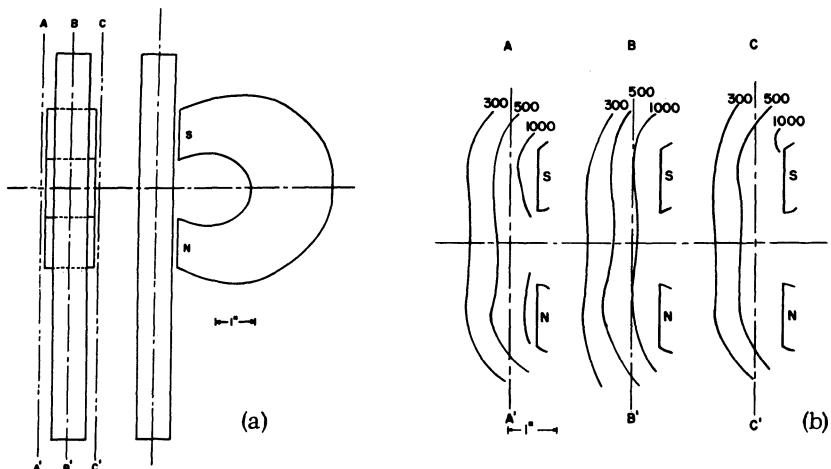


Fig. 1. (a) Schematic diagram of experimental setup showing toweling roll in the field of the horseshoe magnet. Lines A—A', B—B', and C—C' represent sections perpendicular to the pole faces and along which the magnetic field was mapped as shown in (b). The grains were located in the horizontal center plane with their longitudinal axes parallel and vertical. (b) Lines of equal magnetic field intensity for sections indicated in (a).

magnet) (Figure 1). In each case, the plane of the horseshoe magnet was oriented vertically with the south pole uppermost and the pole faces directed toward the north pole of the earth. The grains were located in the horizontal plane bisecting the line joining the poles. Accordingly, the field was vertical, and the magnetic field gradient differed from zero only at right angles to the main magnetic lines of force. Above and below the grains a less steep gradient extended vertically, so to speak, paralleling the main lines of force and the long axes of the grains.

The experiments were carried out in a small, isolated laboratory formerly used for tissue culture work. Overhead ceiling fluorescent lights remained on continuously. Ambient room temperature was 24–25°C and 17–18°C within the rolls of germinating grains (determined at frequent intervals each day by a mercury thermometer inserted into the rolls). At no time did we discern by this means any temperature difference as great as 0.5°C, between any roll in the magnetic field and its corresponding control roll.\*

At intervals following initiation of the experiment, rolls from the magnetic field and respective control groups were opened and measurements made of the length of the shoot and of the longest root. It has been ascertained in other studies<sup>4</sup> that in our strain of barley the length of the longest root provides a reliable index of total root growth and has the substantial advantage of being both more rapid and requiring less

\*These experiments were carried out in four "runs" in time. One run consisted of one magnet roll and its control, two runs consisted of two magnet rolls and the respective control for each, and the fourth, a run of four such pairs. In each case, the respective control roll for a particular magnet roll was designated initially and located within a few feet of the magnet roll.

handling of the seedlings during times when measurements are being made. All experiments were terminated by the 8th day.

Measurements of shoot and root lengths were analyzed using the Student's *t* test for significance of differences in mean length, and average growth rates were calculated for each time interval between measurements. In addition, each seedling in the magnetic field was paired with its counterpart among the control seedlings, both as to experimental "run" and as to position within the roll. Analysis of mean pair differences was made, again by use of the Student's *t* test, for significance of the difference between paired measurements.

## RESULTS AND DISCUSSION

### Growth Rate

Although the rate of germination seemed unaffected, readily detectable differences between test seedlings growing in the magnetic field and control seedlings were apparent when shoot and root growth were observed over a period of days following emergence. At each of the seven times of measurement, the lengths of roots and shoots were significantly greater for seedlings in the magnetic field. This effect was evident whether comparison was made on the basis of differences in group means or mean pair differences. As may be seen in Tables I and II, the differences were significant at the 0.1% level in all but three instances (root measurements at 140.5, 164.5, 184.7 hr); in these, the effects were significant at the 5% level or less.

TABLE I

Comparison of the Mean Shoot and Root Lengths (mm) of Barley Seedlings Growing in the Presence or Absence of Magnetic Fields (Maximum Field Intensity, 1200 Oe), Using Student's *t* Test for Significance of the Difference in Sample Means

	Time, hr	Magnetic Field			Control			<i>t</i>	P. L.
		N	$\bar{x}$	$s_{\bar{x}}$	N	$\bar{x}$	$s_{\bar{x}}$		
Shoots	92.9	90	10.61	0.28	90	7.78	0.29	7.03	< 0.001
	113.8	30	23.77	0.51	30	18.07	0.63	7.05	< 0.001
	140.5	90	47.73	0.67	90	38.93	0.70	9.14	< 0.001
	164.5	70	77.70	0.77	70	64.40	1.08	10.03	< 0.001
	184.7	30	96.23	1.26	30	80.23	1.20	9.20	< 0.001
Roots	43.7	90	8.86	0.38	90	6.88	0.35	3.83	< 0.001
	68.5	50	28.56	0.64	50	24.22	0.73	4.48	< 0.001
	92.9	90	73.92	1.37	90	60.71	1.56	6.33	< 0.001
	113.8	30	103.97	2.09	30	91.93	2.27	3.88	< 0.001
	140.5	90	133.48	1.21	90	129.39	1.55	2.08	< 0.05
	164.5	70	150.43	1.07	70	145.61	1.61	2.53	< 0.02
	184.7	30	159.67	2.69	30	149.63	1.91	3.04	< 0.005

When the average growth rates (mm/hr) were calculated for shoots and roots of control seedlings and those growing in the magnetic field, the results shown in Figure 2 were obtained. Of particular interest is the fact that the roots of seedlings developing in the magnetic field present a different pattern of changing growth rate than that of the controls, whereas the patterns exhibited by the shoots are similar. Although the roots in the magnetic field initially showed increased growth rates over the controls, their growth rate subsequently dropped sooner and more abruptly than that of the controls, then eventually rose again. The shoots, on the other hand, showed consistently higher growth rates, but in a pattern of rise and fall that closely paralleled the controls. In the data of Tables I and II, therefore, the greater length of the shoots in the magnetic field resulted from continuing higher growth rates. In the last three root measurements, however, the greater length of the roots in the magnetic field was largely due to a carry-over of size advantage from earlier growth stimulation.

On the basis of the above analyses, we could state that the presence of the magnet did indeed exert an effect on the growth of both shoots and roots in barley seedlings, in a manner which was readily measurable and statistically significant. The points remaining to be answered lay, first, in determining whether the effects observed, including both those on growth rate and the pattern of change in rate, were actually the result of the magnetic field produced by the magnet or whether they could be simply the result of the physical presence of an object the same size, shape, and composition as the magnet; and, second, if the effects are attributable at least in part to the magnetic field per se, by what means they could be mediated.

TABLE II

Comparison of Shoot and Root Lengths (mm) of Barley Seedlings Growing in the Presence or Absence of Magnetic Fields (Maximum Field Intensity, 1200 Oe). Based on Pair Analysis Using Student's *t* Test for Significance of the Difference between Paired Measurements

	Time, hr	N	$\bar{d}$	$s_{\bar{d}}$	<i>t</i>	P.L.
Shoots	92.9	90	+2.83	0.39	7.26	< 0.001
	113.8	30	+5.70	0.75	7.60	< 0.001
	140.5	90	+8.80	0.71	12.39	< 0.001
	164.5	70	+13.30	1.17	11.37	< 0.001
	184.7	30	+16.00	3.34	4.79	< 0.001
Roots	43.7	90	+1.98	0.40	4.95	< 0.001
	68.5	50	+4.34	0.94	4.62	< 0.001
	92.9	90	+13.21	1.92	6.88	< 0.001
	113.8	30	+12.03	2.91	4.13	< 0.001
	140.5	90	+4.09	1.80	2.27	< 0.05
	164.5	70	+4.77	1.91	2.50	< 0.02
	184.7	30	+10.03	3.29	3.05	< 0.005

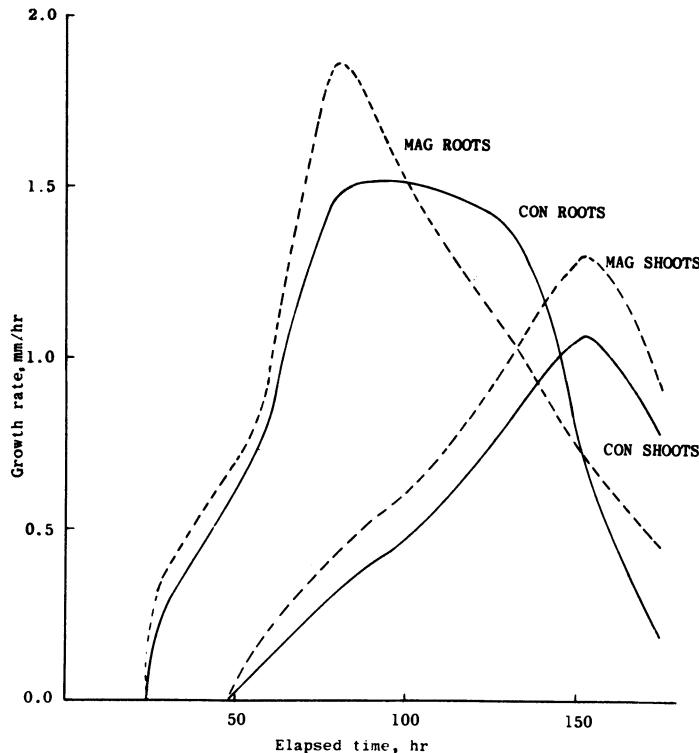


Fig. 2. Changes in average growth rate of roots and shoots of barley seedlings growing in a magnetic field (maximum field intensity, 1200 Oe) as compared with those of controls.

#### Study of Temperature Effect

We had been generally satisfied that the mere presence of the magnet would not of itself produce sufficient changes in air circulation, heat exchange, etc. to cause temperature differentials large enough to account for the growth-rate differences observed, especially since the temperature determinations during the course of the experiments had shown that any temperature differences involved were probably less than  $0.5^{\circ}\text{C}$ . Furthermore, the differences in growth rate patterns of the roots in test and control seedlings also seemed to argue against temperature as an explanation.

Because, however, we had not had "dummy" magnets available to simulate the mere physical presence of the magnet, we now undertook a somewhat more detailed study of duplicated experimental conditions with more precise measurement of temperature. The output from differential copper-constantan thermocouples was fed into a microvolt amplifier, to permit measurement of  $0.02^{\circ}\text{C}$ . It was ascertained by this means that under the conditions of the original experiments, the physical presence of the magnet probably did produce a slight increase in tem-

perature averaging about  $0.25^{\circ}\text{C}$ , but seldom reaching as much as  $0.5^{\circ}\text{C}$ . Thus, our earlier estimates of the maximum temperature differences involved were reasonable.

The observation that the average temperature differences involved were only about  $0.25^{\circ}\text{C}$  made improbable the effect of temperature as the sole basis for our observed results. Yet we did not actually know with certainty just what would be the effect of such temperature differences on the growth rate of our strain of barley, even in the absence of the magnetic field. Therefore, we carried out growth studies over a wide range of temperatures from  $15\text{--}40^{\circ}\text{C}$  (details will be published elsewhere). In these studies, precise temperature control was maintained over a period of days through the use of multiple water baths and Dewar flasks. Unfortunately, the clumsiness of this setup precludes its use in magnetic experiments.

From data obtained in the temperature studies, construction of growth-rate curves is possible for any desired temperature interval from  $15\text{--}40^{\circ}\text{C}$ . In such a graph prepared for the temperatures  $17$  and  $18^{\circ}\text{C}$ —even though differences in growth rate are apparent—little effect is evident upon the pattern of rate change in the roots (Figure 3). When the growth-rate curves obtained in the biomagnetic experiments (Figure 2) are compared with these, it is obvious that the degree of growth en-

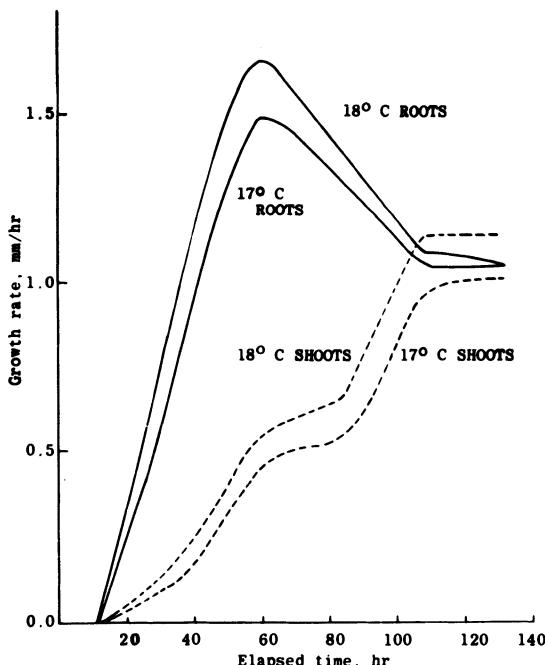


Fig. 3. Effects of different temperatures ( $17$  and  $18^{\circ}\text{C}$ ) on changes in average growth rates of shoots and roots of barley seedlings.

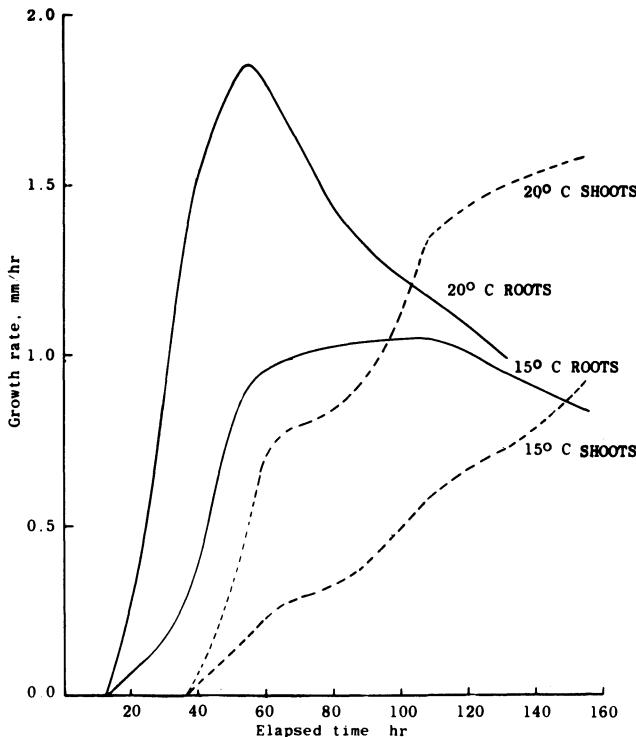


Fig. 4. Effects of different temperatures (15 and 20°C) on changes in average growth rates of shoots and roots of barley seedlings.

hancement occurring in the presence of the magnet is some four or more times that which could be attributed to temperature differences of 0.25°C alone. Furthermore, such a temperature differential will definitely not account for the differences in patterns seen for the roots.

However, with temperature differentials as great as 5°C (Figure 4), some striking changes in pattern similar to those in the biomagnetic data do appear. Here, an abrupt maximum in growth rate is reached by roots at 20°C in contrast to an absence of a peak at 15°C; but even so, there is no lateral displacement and overlap of growth-rate curves to produce the alternating enhancement and inhibition effects observed in the biomagnetic experiments.

#### HUMIDITY AND LIGHT RELATIONSHIPS

It would have also been necessary to give serious considerations to differences in humidity as a possible contributing factor in our observed results had it not been for the fact that germination and seedling growth took place within rolls of wet toweling. Although specific control of humidity within the laboratory was not attempted, the experiments were carried out during the winter while the heating system was under thermo-

static control and in a room where all of the air vents had been completely closed off when this laboratory had been used previously for tissue culture. There is no reason to believe that differences in humidity existed from one part of the room to another, let alone within the few feet distance separating the test setup from its respective control. Within the rolls the air was saturated. Under these conditions, the vapor pressure of water would differ by less than 0.25 mm Hg for the observed temperature differences of 0.25C° between magnet and control rolls.

The use of double-layered rolls of brown toweling for the germination and seedling growth also effectively reduced, and for all practical purposes eliminated, differential light as a factor in our experiments. With the use of a calibrated photoelectric cell, no differences in illumination could be detected between corresponding grain positions in control and magnet rolls, nor between any two positions within either magnet or control rolls, except at the position of the outermost grain, this grain being within only one double layer of toweling from the outside. In the magnet rolls, this outermost position was the one nearest the magnet. Here, however, shading by the magnet resulted in sufficient light reduction so that the outermost grain in the magnet rolls actually received a level of illumination (~1 ft-c) comparable to that at all of the other positions within the roll. Only at the outermost position in the control rolls was the light intensity greater (an increase of 3 ft-c over any other position). To determine whether such differences in light intensity did produce detectable effects on seedling growth, paired comparisons were made in the control rolls between the seedlings at the outermost position and those located at the three positions deepest within the toweling. At all of these latter positions, the seedlings were enclosed in three double layers of toweling both from the exterior surface and the interior surface of the rolls. Determination of the frequency with which roots or shoots of seedlings at any one position were longer (2 mm or more) or shorter (2 mm or more) than those at any of the other positions revealed no significant differences in the control rolls.

#### Interpretation of Growth-Rate Effects

With light and humidity differentials dismissed as causative factors, and temperature effects seemingly reduced to no more than a supportive role, the magnetic field indeed appears to be the most probable agent in the production of the observed growth effects in barley. In considering possible reasons for the apparent differences in behavior of roots as compared with shoots growing in the magnetic field, we might speculate that a differential magnetosensitivity exists between these organs similar to that which occurs with temperature (Figures 3 and 4), ionizing radiation,<sup>12</sup> and chemical mutagens.<sup>11</sup> If such were true, one would expect to find a difference in either magnitude or frequency of response of one region as compared with another. Yet examination of the present data

reveals that it is the shoot region which exhibits the higher frequency and magnitude of response rather than the root region, with its characteristically different growth-rate pattern.

In fact, when evidence for concordance and discordance is looked for in the pair analysis data, it may be seen in Table III that at 92.9 hr, 78% of the seedlings in the magnetic field had roots and shoots which behaved in a like manner in comparison with paired seedling controls. In 83% of these concordant seedlings, both the root and shoot of the test seedling were 2 mm or more longer than in the control seedling of the pair, within less than  $\pm 2$  mm of the control in only 4%, and 2 mm or more shorter in 13%. In only 2% of all seedling pairs were the shoot and root of the same test seedling discordant, in both cases the shoot being shorter and the root longer. In other words, at 92.9 hr, the majority of seedlings in the magnetic field tended to behave as units: in some instances responding not at all, but in most, showing stimulation of both shoots and roots.

Re-examination of these same seedlings at 140.5 hr gives a somewhat different picture (Table III). By this time, the percent of concordance had dropped to 58% and that of discordance risen 12-fold to 24%. The discordant group was made up almost entirely of seedlings in which the shoots were longer, but the roots shorter, than in the comparable controls. By tracing the source of this newly appearing discordance, it was found to have arisen almost entirely from seedlings in which the roots of the test seedling had been longer than the control seedlings of the pairs at 92.9 hr. Thus the difference in behavior of the shoots and roots in regard to growth-rate patterns comes about for the most part from seedlings in which, initially, the roots are strongly enhanced in growth rate and then inhibited, while the shoots remain in an enhanced

TABLE III

Frequency of Concordance and Discordance between Shoots and Roots of the Same Seedlings in the Magnetic Field (Measured at 92.9 hr and 140.5 hr) When Compared with Their Respective Paired Controls. Based on Differences of 2 mm or More for + or -

Time, hr	N	Complete concordance				Partial concordance or discordance				Complete discordance			
		Sh	Rt	Sh	Rt	Sh	Rt	Sh	Rt	Sh	Rt	Sh	Rt
		+	+	±	±	-	-	+	±	±	+	±	-
92.9	90	58	3	9		4	10	3	1	0	2		
		78%				20%				2%			
140.5	90	48	0	4		6	4	5	1	21	1		
		58%				18%				24%			

condition. The fact that some seedlings appear to be more sensitive to magnetic fields than others suggests the possibility of selecting for or against such characteristics.

It is highly probable that in susceptible seedlings the differential magnetic field response on the part of shoots vs. roots reflects to a large extent their differences in morphogenetic patterns of development. In monocot seedlings such as barley, the principal meristematic sites responsible for shoot elongation are located at the bases of the leaves, whereas those of the roots are near the tips. Thus, throughout the course of exposure to magnetic fields, the most meristematic regions of the shoots remained, as a whole, relatively stationary within the region of highest field strength and steeper gradients. During this time the shoots exhibited consistently increased rates of growth. In contrast, the rapidly-growing regions of the roots rather quickly grew out of the magnetic field, and hence were exposed to the higher intensities and steeper gradients for only a comparatively short time following emergence. It was apparently during this time that the initial growth-enhancement occurred, to be followed by relative inhibition—despite the fact that the basal portions of the roots yet remained within the field. These associations between meristematic location (relative to the region of highest magnetic field intensity) and the occurrence of growth enhancement strongly suggest that only meristematic portions of the seedlings possess magnetosensitivity or the capacity to react by producing easily detectable growth effects. In this respect, our results agree with those of Ssawostin,<sup>18</sup> who found that placing the bases of the shoots of wheat seedlings (morphogenetically similar to barley) within a magnetic field produced greater growth stimulation than keeping only the tips of the shoots within the field. In our data, the apparent exception to this explanation, found in the root enhancement which occurred during the final time interval, may be an abscopal effect related to the initiation of synthetic activity in the shoot region.

#### Mediation of Growth-Rate Effects

No ready and proved explanations are at hand with regard to the physicochemical means by which magnetoenhancement of growth might be mediated. Different investigators have proposed evidence of alterations in the rate of metabolic processes, by preferential slowing down (or speeding up) of chemical transformations on the basis of relative magnetic susceptibilities of reactants and end products<sup>3</sup> or by changing the activity of enzymes through bringing about greater order at either an intra- or intermolecular level.<sup>17</sup>

As an alternative, or in conjunction with any contribution to growth control made by other means, it should also be considered that the strength and inhomogeneity of the magnetic field might be sufficient, when applied to a biological system over an extended period of time, to

influence materially the distribution pattern of magnetically susceptible substances within a seedling. Even oxygen may be important in this regard, although not likely to be a limiting factor in our experiments. Magnetically susceptible nutriments such as Fe, Mn, and Co, however, are present in plants in low concentrations as trace elements. They are generally considered to be only partially mobile physiologically, yet apparently play important regulatory roles in seedling growth. Recent studies on corn<sup>14</sup> have shown that normally these microelements, originally contained within the grain, are translocated during germination and accumulate in the regions of greatest meristematic activity. If the microelement supply available for meristematic regions is a limiting factor in the seedling growth of barley, as Pedretti<sup>14</sup> suggests is true for corn, and if modification of the normal distribution of susceptible microelements can be induced by a magnetic field, we might expect to see corresponding changes in growth rates of the regions involved. Increases in growth rate could result from an augmentation of suboptimal trace element levels, and loss of enhancement or even inhibition could occur through diminution or an interference with transport. Thus, in the present experiments, the presence of the magnetic field may have enhanced a normal tendency of Fe, Mn, and Co to collect in the basal meristems of the shoots and so produced continuous stimulation. In the roots, however, the same situation would have been only temporary. Then, as these meristems began to move out of the field, the forces exerted would be in opposition to continued translocation of the micro-elements into the root meristems, thereby producing the altered growth-rate pattern exhibited by the roots in the magnetic field.

On the basis of purely theoretical calculations for molecular-magnetic-field interactions, it is indeed difficult to account for biomagnetic effects on plant growth unless one supposes either some subtle statistical phenomenon or complex biological synergisms. Our postulation that the biomagnetic effects herein described may be due to induced changes in the rate and/or pattern of translocation and accumulation of magnetically susceptible microelements is compatible with either of the above and should be capable of direct experimental verification or rejection. At present we have some suggestive evidence in this regard. Preliminary data appear to show that, for barley seedlings to attain and sustain the level of growth stimulation observed in this study, some light (even though only diffuse) and an additional source of trace elements (e.g., those contained in tap water as compared with distilled water) may be necessary. Both of these requirements, if substantiated, would argue strongly for microelement involvement, i.e., of substances which not only have partial physiological mobility but are also present in suboptimal amounts. Recent studies using radioactive Co<sup>60</sup> and Fe<sup>59</sup> permit us to draw the tentative conclusion that the presence of an inhomogeneous magnetic field can detectably influence the level of accumulation of these isotopes in the meristematic regions of actively growing leaves, but has

little if any effect on distribution patterns in leaves which have attained nearly full expansion.

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## *Chapter 10*

# Effects on the Central Nervous System

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Most of the recent works of biomagnetism<sup>1</sup> are devoted to the problem of the effect produced by a static magnetic field on the cellular and tissue levels. The aim of such investigations is to disclose the bio-physical mechanism which governs the action of a magnetic field.

But of no less interest is the physiological analysis of the influence exerted by a static magnetic field on the integral organism of vertebrates, where reactions to various stimuli are effected through the central nervous system. It has been shown that, in order to ascertain the biological action of a static magnetic field on isolated organs and tissues, it is necessary to utilize fields of greater strength than in experiments with an integral organism.<sup>2</sup> Whereas under usual conditions the action of a static magnetic field is not readily felt by man, in the state of hypnosis<sup>3</sup> or in the state of mescalin intoxication<sup>4</sup>, the magnetic field modifies the visual images. The aforementioned facts, as well as data relating to the possible orientations of animals by the terrestrial magnetic field, prompted us to set ourselves the task of studying the properties of a static magnetic field as a stimulus by applying such modern methods as conditioned-reflex and electrophysiological methods.

The experiments were carried out on fish (crucians and sticklebacks), birds (pigeons and bullfinches), and mammals (rabbits) by the conditioned-reflex method. In mammals we recorded the EEG, in fish and birds the motor activity; in addition, we ascertained the sensitivity of fish to electric current. The reactions of the animals to light and sound were utilized for control purposes.

The static magnetic field was generated by a permanent cobalt magnet, as well as by air-core and iron-core electromagnets. Any temperature and acoustic stimulations were avoided. The strength of the field varied from 1 to 800 Oe. The duration of the action of the magnetic field ranged from several seconds (in experiments with conditioned reflexes) to several hours (in experiments with recording of the motor activity).

It was found that food-seeking and electrodefensive conditioned reflexes can be produced in fish by static magnetic fields of 100 to 200 Oe strength. Food-seeking conditioned reflexes (five fish) occurred on the average after five trials; they were established (the criterion was five successive times) after 23 trials and reached 60% stability. Electrodefensive conditioned reflexes (14 fish) occurred on the average after 11 trials; they proved established after 64 trials and reached 39% stability. Thus, in fish it was easier to produce food-seeking conditioned reflexes to a magnetic field than electrodefensive reflexes; however, the production of both by a magnetic field is more difficult than by light or sound stimuli. These data prove that a magnetic field as a physiological stimulant is weaker than the above-mentioned stimuli.

But in the investigation of inhibitory conditioned reflexes to the combined stimulus of "magnet + light" it turned out that a magnetic field exerts a stronger inhibitory action than light or sound. Food-seeking inhibitory reflexes (five fish) appeared on the average after three trials; they were established after 15 trials and reached 84% stability. Electrodefensive inhibitory conditioned reflexes (seven fish) occurred after three trials, became established after eleven trials, and reached 78% stability. The inhibitory influence of the magnetic field often persisted for several minutes after it was discontinued. These results show that the action of a magnetic field is predominantly of an inhibitory character and that its influence persists, which finds its expression in consecutive inhibition.

In pigeons (seven birds) we failed to observe any positive or inhibitory food-seeking or defensive conditioned reflexes by a static magnetic field of 200 Oe; this fully supports the findings of Orgel and Smith.<sup>5</sup> However, in experiments with alimentary conditioned reflexes, the magnetic field increased the number of intertrial reactions in pigeons by a factor of 2 to 3. When alimentary conditioned reflexes were produced in pigeons by light stimulus, the magnetic field proved to inhibit them in 70% of all cases. It should be pointed out that such an inhibitory action was already observed during the first applications of the combination of "magnet + light"; its intensity did not change whether the action of these stimuli was accompanied by food or not. Sometimes the inhibitory action of the magnetic field persisted for several minutes after the magnet current had been switched off. Thus, although in pigeons the production of a conditioned reflex by a static magnetic field proved to be impossible, the magnetic field exerted an unconditioned inhibitory action on the optical conditioned reflexes and increased the number of intertrial reactions. The fact that a magnetic field has a stronger effect on fish can perhaps be explained by the peculiar structure of their central nervous system, as well as by the probable influence of the magnetic field on the water. This question, however, needs further experimental investigation.

In fish the static magnetic field also increased the number of intertrial reactions several times; it inhibited the conditioned reactions

to a signal in 85% of all cases when a light stimulus was applied and in 70% of all cases when an acoustic conditioned stimulus was applied.<sup>6</sup>

The predominantly inhibitory effect of a magnetic field was observable also when we tested the sensitivity of fish to electric current. The strength of the current which is necessary to provoke a slight quiver in a fish increased on the average by 45% under the influence of a static magnetic field of 100 to 200 Oe.<sup>7</sup>

During the action of a static magnetic field of about 800 Oe we observed on the EEG of rabbits (11 animals) a highly significant ( $p < 0.001$ ) increase in the number of spindles in the frontal region of the cerebral cortex, as well as an increase in the number of slow high-amplitude oscillations in the occipital region;<sup>8</sup> this also indicates the emergence of an inhibitory state in the central nervous system.

Furthermore, under the action of the magnetic field the interval after which the reaction of assimilation of light flashes of growing brightness appears on the EEG of a rabbit increased from  $18.4 \pm 0.4$  sec to  $20.4 \pm 0.4$  sec; this implies a decline in the excitability of the cortical end of the visual analyzer.

It must be noted that although we often observed an inhibitory effect of the magnetic field, the character of the reaction depended on the initial functional state of the central nervous system. Thus, against the background of a reduced sensitivity of fish to electric current, we observed an increase of this sensitivity in the magnetic field. Under the action of the magnetic field the EEG of rabbits exhibited acute waves which reflected a process of excitation. During the production of conditioned reflexes by a static magnetic field in fish and pigeons, the number of intertrial reactions increased, irrespective of the reaction to the given signal; this means that the conditioned reflex to the surroundings proved to be uninhibited. Moreover, a static magnetic field of 100 Oe increased the motor activity of fish, while a magnetic field of only about 1 Oe increased the motor activity of bullfinches.<sup>9</sup>

Thus, although a magnetic field produced predominantly an inhibitory effect, it may also provoke excitation in the central nervous system.

Although there exists an assumption that magnetic fields are perceived by fish with the help of an organ of the lateral line,<sup>10</sup> in our experiments after the denervation of this organ a conditioned reflex to the magnetic field could be as successfully produced as in intact animals. Our experiments with blinded fish did not confirm the assertion that the retina is highly sensitive to a magnetic field.<sup>11</sup> After a complete bilateral enucleation of fish, conditioned reflexes to a magnetic field were produced even more readily than in animals with intact vision.<sup>12</sup>

We investigated the effect of lesions of different cerebral regions in fish on conditioned magnetic reflexes and found that lesions of the forebrain, tectum opticum, or cerebellum do not modify the reaction of fish to a magnetic field, although they derange the conditioned reflexes to

light and sound. Only lesion of the diencephalon caused disturbances of the conditioned reflexes to a magnetic field. So we assumed that a magnetic field can exert direct influence on the diencephalon without participation of the sense organs.<sup>9</sup>

To verify the above assumption experimentally we investigated the EEG reaction to a static magnetic field of the rabbit's encephale isole after additional bilateral sections of the optical and olfactory nerves. It proved that the structures of the forebrain and diencephalon deprived of any nervous connections with the receptors react to a static magnetic field more often, more rapidly, and more intensively than an intact brain.

Normally, the modifications on the EEG of a rabbit occurred approximately 20 sec after the electromagnet had been switched on. Ten seconds after it was switched off a new increase in the number of spindles and of slow oscillations occurred. This reaction resembles the off-effect which is observed on the retina of the eye under the action of light. The reaction to the switching-off manifested itself less frequently and was less pronounced than the main reaction.

In the preparation of the encephale isole, the main reaction emerged approximately 10 sec after the electromagnet had been switched on, while the reaction to its switching-off remained the same as in normal animals. This fact leads to the assumption that the main reaction to a magnetic field and the reaction to its switching-off are effected by different physiological mechanisms.

Since the EEG reaction of a rabbit to a magnetic field arises with a considerable latent period and in a diffuse way, i.e., simultaneously in several regions of the cortex of both hemispheres, it may be assumed that it is the more inert structures of the diencephalon which are responsible for this reaction.

Ionizing radiation<sup>13</sup> and high-frequency electromagnetic fields<sup>14</sup> exert direct influence on the diencephalon. We have demonstrated that high-frequency electromagnetic fields and static magnetic fields produce a similar action on the conditioned reflexes of fish and on the EEG of rabbits.<sup>15</sup>

Consequently, it may be assumed that a static magnetic field exerts direct nonspecific action on the central nervous system of vertebrates. This assumption does not exclude the possibility of a reflexive effect due to magnetic fields, which is confirmed by experiments with isolated organs and tissues.

In summary, we point out several peculiar features of the physiological action of a static magnetic field as a stimulus.

1. A magnetic field is a weak stimulus. The reaction to this stimulus takes place approximately in 40 to 70% of all cases when a magnetic field is applied.

2. A magnetic field produced predominantly an inhibitory effect.
3. A reaction to a magnetic field sometimes persists even after the latter is discontinued.

4. A static magnetic field acts directly on the structures of the diencephalon and forebrain.

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## *Chapter 11*

# Survival of Animals in Magnetic Fields of 140,000 Oe

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Future developments in space travel may expose man for prolonged periods of time to magnetic fields considerably stronger than the geomagnetic field.<sup>1</sup> Such exposure may have its source in magnets used in ion propulsion devices or in magnetic shielding against cosmic radiation.

The low energy density of magnetic fields used in previous studies seems to be a main reason for a lack of easily observable bioeffects. Since the energy per unit volume in a magnetic field increases with the square of the magnetic intensity  $H$ , an increase in these values should make biomagnetic effects more obvious.

The quest for fields of higher energy is met at present by an active development of solenoids producing fields of high strength. Several laboratories with power sources of a few megawatts can produce fields of the order of 100,000 Oe. The plans of the MIT National Magnet Laboratory include motor generators capable of producing up to 8 MW, which will permit construction of a magnet with a field strength up to 250,000 Oe.<sup>2</sup> The present study used the magnet facilities of the Solid State Division of the U. S. Naval Research Laboratory, Washington, D. C. The study represents a first exploring expansion of biomagnetic research into the range of very high magnetic field strength. It will be followed later by more detailed experimentation.

## ANIMALS

The mice used in this study were young animals NIH strain with weights under 20 g to fit into the narrow tubing in the magnet core.

Drosophila melanogaster, Wild type and Oregon-R type, separated by sex and in sex mixture, were used.

<sup>1</sup>Opinions and conclusions contained in this report are those of the author and are not to be construed as necessarily reflecting the view or the endorsement of the Navy Department. This research was conducted under the sponsorship of the Office of Bio-Sciences Program, National Aeronautics and Space Administration.

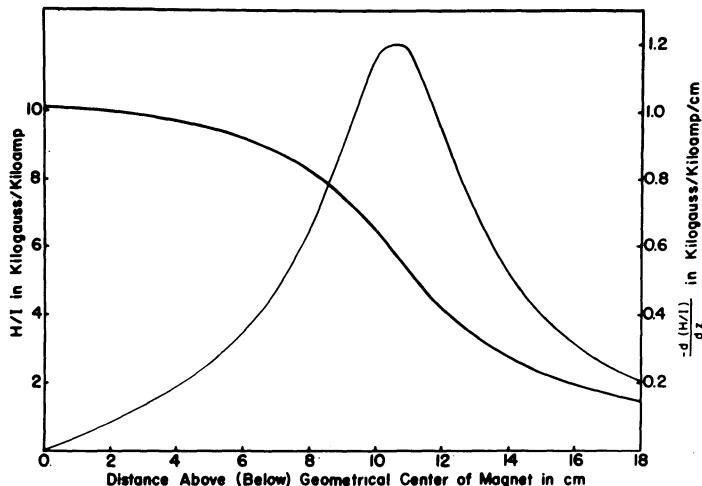


Fig. 1. Distribution of the field strength along the core of a modified Bitter magnet. [The International Electrotechnical Commission in 1930 adopted the resolution to name the unit of field intensity oersted; this unit was formerly called gauss.—Ed. note.]

Eggs and sperm of the sea urchin Arbacia punctulata were obtained from animals caught off the entrance to Pensacola Bay in the Gulf of Mexico. The eggs were filtered through cheesecloth, washed, and diluted with sea water.

Photobacterium fischeri was procured from the American Type Culture Collection, Washington, D. C. The culture medium consisted of 3 g NaCl, 0.3 cc glycerol, 0.3 g CaCO<sub>3</sub> and about 2.3 g nutrient agar in 100 cc of water.

#### APPARATUS

The solenoid of modified Bitter principle had a vertical core with a 1-in. inner diameter. The water-cooled magnet could operate at 140,000 Oe for at least 2 hr. Figure 1 shows the variation of the field strength and the gradient with the distance above or below the geometrical center of the magnet. The maximal field strength in the center of the magnet does not change by more than 1% over a length of the core of 4 cm. Maxima of the gradient are attained at a distance of 11 cm on both sides of the geometrical center of the magnet.

Since bioeffects of alternating magnetic fields may be different from the effects of constant fields, the characteristic for the generator needs to be considered. The main component of the ripple is an alternating current of 12 cps on which are superposed currents of higher frequency, but of lower intensity. The magnetic field strength due to the ripple current did not exceed about 50 Oe at the highest load of the magnet.

The animals were enclosed in a plastic tube which fit the core of

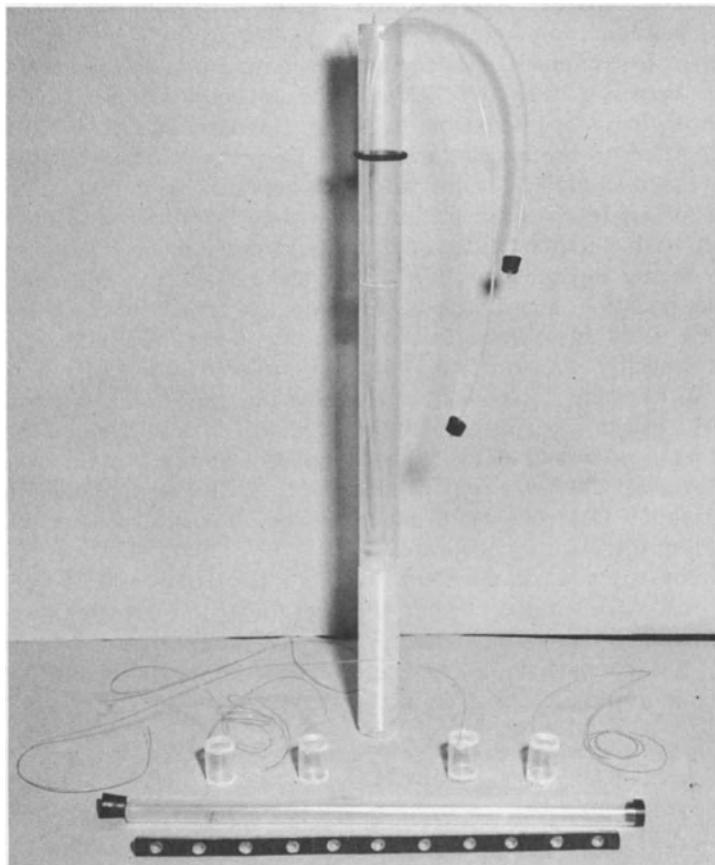


Fig. 2. Plastic-tube insert used to contain the animals. Smaller containers were used for flies and eggs, and the long slat with cups held the bacteria cultures inside the plastic tube.

the magnet (Figure 2). The lower part of the animal test tube carried a cooling jacket which also enclosed the mouse between a perforated partition wall and the bottom of the plastic tube. Tubing supplied air to the mouse enclosure. Other biosamples were housed in small plastic containers which could be lowered into the plastic tube and held at a desired distance from the center of the field. Four such containers can be seen in Figure 2. A separate tubing enclosed a plastic slat with cup-like indentations to hold bacteria-inoculated media (bottom of Figure 2).

#### PRELIMINARY TRIALS

Preliminary experiments outside the magnetic field demonstrated that mice could be held enclosed in a narrow vertical tube with the head of the animal pointed upward for at least 6 hr. Oxygen must be supplied

and carbon dioxide removed by a steady stream of air guided to the animal's head.

Other experiments showed that the temperature close to the geometric center of the core increased from room temperature to 35°C during 1 hr of operation of the magnet at 100,000 Oe. To avoid this heating effect by the magnet and to remove metabolic heat generated by the enclosed animals, a water jacket was applied around the plastic tube. A flow of 8.5 ml of water per minute with an inlet temperature of 10°C and an outlet temperature of 23°C held the thermoenvironment of a mouse during an exposure time of 2 hr close to room temperature.

The question about a possible biological effect of the alternating magnetic field superposed on the direct magnetic field was approached experimentally. An alternating current similar in frequency and strength to the alternating component of the rotating generator was applied to the coil. For this purpose a number of additional components including frequencies of 20 and 36 cycles was superposed on the main wave form of 12 cycles. The total field strength of this alternating magnetic field was about 50 Oe, roughly as much as the alternating component field strength of the rotating generator.

A mouse placed in the center of this alternating field as well as 30 fruit flies placed in the core at different distances from the geometric center showed no visible effects after 1 hr of exposure to the ripple field. It appears that the magnetic ripple field can be neglected in a discussion of the effects of the direct field.

#### FINAL TRIALS

The final procedure of exposure of animals to very strong magnetic fields was determined by the desire to use the available volume in the core as efficiently as possible. In addition, information was desired not only on the bioeffects of the homogeneous part of strong fields but also on the effects of the considerable gradient of the inhomogeneous fields bordering the center of the core on both sides (Figure 1).

In experiments with mice, the animal was enclosed in the end part of the plastic tube. It could move slightly but not reverse its position. The mouse was cooled by the water jacket and supplied with air from the outside by a pump. Usually, the plastic tube was lowered into the core of the magnet to a depth which placed the center of the animal at the geometrical center of the field. The gradient in this part of the field was about 500 Oe/cm. For exposure of the mouse to a part of the magnetic field with a high gradient, the plastic tube was lowered to the specified location in the core of the magnet.

*Drosophila* were exposed in small plastic containers holding 10 to 20 flies and lowered into the plastic tube to a specialized location in the field not containing the mouse. In experiments in which fruit flies were exposed exclusively, the small containers were staggered on top of each other along the useful length of the plastic tube.

Sea urchin eggs were exposed in containers similar to those used for *Drosophila*. The eggs were fertilized shortly before exposure to the magnetic field. Samples of luminescent bacteria on a solid medium were arranged in the form of small cups in a slat which extended nearly the whole length of the core. The slat was enclosed in plastic tubing to prevent the samples from drying out. The luminescent bacteria were photographed in their own light by contact photography before and after the experiment or the light measured with a photomultiplier.

The fully charged biosample tube was inserted into the magnet core and the field increased at a regulated rate of 700 Oe/sec. After the specified time of exposure, the field was decreased at the same automatically regulated rate. Several experiments were performed at a constant maximal field strength of 140,000 Oe. Other experiments used fields of lower intensity, but not, however below 100,000 Oe in the center of the coil.

## RESULTS

### Mice

From a total number of seven mice exposed in separate experiments to a homogeneous magnetic field of 100,000 Oe or higher, six survived. The one lethal exposure to a field of 120,000 Oe for 1 hr may be attributed to thermostress, since this was the only experiment in which no cooling of the animal was applied. Three of the remaining six animals were exposed for 10 min, 30 min, and 1 hr to a practically homogeneous field of 120,000 Oe. The animals all survived this exposure, after which they increased normally in weight and are in good health at the time this report is written, 8 months after exposure. Two other mice were exposed to 100,000 Oe for 1 and 2 hr, respectively. These animals also showed no ill effects and increased normally in weight after exposure, but the animal exposed for 1 hr died 4 months afterwards from unknown causes. However, one animal from a control series of matched unexposed animals died also at about the same time. One last mouse exposed to a high gradient of the magnetic field (7000 Oe/cm at 43,000 Oe) showed no ill effects after 1 hr of exposure. The animal's weight increased normally and it remained in good health 8 months after exposure. No changes in numbers of neutrophils, lymphocytes, or monocytes were noticed in any of the animals directly after exposure and also none 1 day later.

### *Drosophila*

Eggs (age 4½ hours), larvae (6 days), pupae (8 days), and adult flies of both sexes (20 days) were exposed to a homogeneous field of 140,000 Oe (1% gradient) for 1 and 2 hr in separate experiments. The post-exposure development was not influenced by the magnetic field: the per-

centage of hatching and the sex ratio in the P-1 and F-1 generation were the same as in control samples and no mutants or abnormalities were observed in the F-1 generation.

Similar exposures for 2 hr in those parts of the field with the highest gradient of about 10,000 Oe/cm also did not show any influence on the development of *Drosophila*. A total of 800 eggs, larvae, pupae, and imagoes were exposed during these experiments in homogeneous and heterogeneous strong magnetic fields.

In a test of the cumulative effect of the magnetic field, a sample of flies was exposed to a field of 75,000 Oe with a gradient of 11,000 Oe/cm for two periods each of  $\frac{1}{2}$  hr duration. A field-free interval of  $\frac{1}{4}$  hr separated the exposure periods. The effect of this cumulated exposure was not lethal.

A study of survival was not the exclusive goal of the experiments with *Drosophila*, but an attempt was also made to answer some genetic questions. Two additional experiments were made for this purpose by Close and Beischer<sup>3</sup> as follows:

1. The "Muller 5" strain of *Drosophila melanogaster* contains a recessive "yellow body" gene on the X-chromosome. The trait is observed in females receiving a Wild-type homolog from the male parent and becomes manifest in the F-1 females in the event of mutation at this locus in the exposed male parent.

Male Wild-type *Drosophila* were placed for 30 min in a homogeneous field of 100,000 Oe. Three days later the male flies were crossed with virgin "Muller 5" females. Only one yellow-body female was observed out of 250 female F-1 flies with normal body color, while no mutants were observed among 218 female F-1 controls. As the number of animals observed was small, these results are not very conclusive insofar as deviation from normal recessive mutation rate at this locus is concerned. It should be pointed out, however, that all the F-1 flies examined, with the one noted exception, appeared normal and were fertile.

2. Alterations in the sex ratio may also provide an indication of mutation. Since mutations are nearly always harmful, males exposed to mutagenic agents such as radiation produce fewer female offspring and a higher proportion of male in the progeny results.

When both parents are exposed to mutagenic agents to the same extent, distortion in the sex ratio may also occur. Fewer male offspring will be observed in this case as a manifestation of sex-linked lethals in the exposed female parent.

Out of 433 flies of the F-1 progeny of the male Wild-type flies originally exposed for 30 min to a homogeneous field of 100,000 Oe, 43% were male, while for the controls 45% of the F-1 progeny were male. The difference is not significant and the deviation is opposite that expected if the paternal X-chromosome bears an increased load of mutations.

In an experiment in which male and female Wild-type were exposed to a homogeneous field of 100,000 Oe for 2 hr, 44.3% of 289 F-1 flies were male, whereas in a control experiment outside the field 49% of 181 flies were male. The difference, even though in the direction of a mutagenic effect, is not significant. All of the F-1 flies examined in connection with this experiment appeared normal in appearance and fertility.

The results of this genetic study can be summarized by stating that no temporary infertility of the male *Drosophila*, Wild-type, or sex-linked chromosomal changes occurred with high frequencies in animals exposed for short time intervals to high-intensity magnetic fields. It was also observed that the life cycle of animals born from exposed females was not disturbed and that hatching times and the sex ratio were not affected in such animals.

#### Sea Urchin Eggs, Luminescent Bacteria, and *Neurospora*

Early cleavage of ova of the sea urchin *Arbacia punctulata* was retarded significantly by exposure of the eggs for 2 hr after fertilization to the homogeneous and heterogeneous part of a magnetic field with a maximum strength of 140,000 Oe. Normal plutei formed in the same percentage of the original eggs as in the control samples. A 2-hr exposure of *Neurospora crassa* conidia to homogeneous and heterogeneous portions of a 140,000-Oe field produced no mutants. The light emission from *Photobacterium fischeri* measured by photomultiplier tube was not changed by a 1-hr exposure to magnetic field conditions as used for *Neurospora*.

#### DISCUSSION

In this study a first attempt has been made to extend the limits of information on the biological effects of very strong magnetic fields. The maximal field strength applied in previous biomagnetic experiments, to our knowledge, was 43,000 Oe,<sup>4</sup> and most experiments in this field have been executed at a field strength below 20,000 Oe. One of the main reasons for increasing the field strength in biological experiments is the steep increase of energy density with increase of field strength. The energy density per volume of a magnetic field increases with the square of the field strength. The energy density at 125,000 Oe is about 40 times greater than at 20,000 Oe.

However, even in the strongest magnetic fields which can be realized technically, the magnetic moment of the electron multiplied by the field strength is small compared with the kinetic energy  $kT$ . Only in a field of several million oersted would the magnetic moment of the electron spin ( $0.917 \times 10^{-20}$  erg) multiplied by the field strength equal and surpass the kinetic energy  $kT$  at room temperature ( $4.14 \times 10^{-14}$

erg). These considerations make it appear most unlikely that the strongest available homogeneous magnetic fields will elicit purely magnetic effects in biological material. The survival of mice and flies demonstrated in this study at 120,000 Oe is plausible in the light of this consideration.

The results of the exposure of animals to strong magnetic fields with a high gradient do not differ noticeably from the results obtained with strong homogeneous fields. The physical effects of such fields on living matter seem to be within tolerable limits for the applied exposure time.

The results of this study even in their present rudimentary form have valuable implications on the exposure of man to strong magnetic fields. The fact that a mammal survived prolonged exposure to a magnetic field of 120,000 Oe increases to a certain degree confidence in the safety range in human exposure. It was reported previously<sup>5</sup> that no harmful effects were observed in men exposed to magnetic fields up to 20,000 Oe for a duration of 15 min. Further animal experiments at very high field strength will substantially contribute to the appraise-ment of possible physiological and psychological effects in man if he should be exposed to strong magnetic fields in connection with space travel.

#### ACKNOWLEDGMENTS

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Mr. C. S. Ezell contributed greatly to the success of this study in preparing special equipment and by zealous participation in the ex-periments.

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*Part III*

Effects of Strong Magnetic Fields  
on Specimens *in vitro*

## *Chapter 1*

# Tissue Respiration

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The effect of magnetic fields on normal and malignant cells has been a subject of controversy for many years. Among those workers reporting positive effects are Payne-Scott and Love,<sup>1</sup> who described protoplasmic degeneration in cultures of embryo chick heart following exposure to a field of 5000 Oe. In our laboratories, I. L. Mulay<sup>2</sup> noted degeneration of S-37 ascites cells (*in vitro*) within 18 hr of exposure to a field of 8000 Oe. Control cells showed normal growth and active differentiation at corresponding times (see Part II, Ch. 7).

We have studied the effects of magnetic fields on cell respiration. It was felt that if quantitative effects were observed, it would then be possible to make comparative studies of the response of various types of tissue to the influence of magnetic fields. We have conducted such studies on normal and malignant tissues, on adult and embryo tissues, and on yeast.

The experimental apparatus consisted essentially of a permanent magnet and a microrespirometer designed to maintain the experimental tissue within the field throughout the observations. A similar respirometer, containing the same type of tissue, was held outside the field as the control.

The constant-pressure respirometer, designed by J. C. Fardon of our staff,<sup>3</sup> consists of two gas chambers of approximately equal volume connected through a capillary U tube containing an index drop of odorless kerosene colored with Sudan III. An adjustable mercury reservoir is connected by a calibrated capillary to the gas chamber containing the tissue. A quantity of distilled water contained in the "control" arm of the respirometer allows adjustment of the gas volume and equilibration of the vapor pressure between the two sides of the instrument. As oxygen is removed from one side of the system, the index drop moves in relation to an index line inscribed on the magnifying lens system used to observe the drop. At the chosen time interval the index drop is restored to the original relationship with the index line by adjusting the

mercury level in the capillary connected to the reservoir. This re-establishes the equilibrium which originally existed between the two sides of the respirometer. Since the expired CO<sub>2</sub> is absorbed by 20% KOH contained in the small side-arm of the tissue flask, the difference in successive mercury column levels indicates the amount of oxygen removed from the system by the tissue, which is contained in a glass "boat." With this instrument it is possible to measure the oxygen uptake of tissue of the order of 1-4 mg dry weight.

The permanent magnet used in all experiments produced a field of 7300 Oe in an air gap of 1.5 cm. The field strength was determined in a plane equidistant from the pole caps at several positions on the X and Y axes. Under these conditions the field showed no gradient measurable with the instrument used, with the exception of a sharp increase of 700 Oe noted at the extreme edges of the 2.3-cm-diameter pole caps. The instrument used was a Model A-2 gaussmeter fitted with probe FA-21, produced by the G. R. H. Halstell Company. Comparative measurements with a Model 150 gaussmeter, manufactured by F. W. Bell, Inc., Columbus, Ohio, were in agreement within the limits of a Hall-effect generator. Since at this stage of our experiments we were merely trying to establish conclusively if an effect existed, we were not concerned with such possible variables as parallelism of pole faces and homogeneity of field strength.

The sensitivity of the respirometer dictated the type of temperature control necessary. The entire experimental apparatus, including the magnet and the experimental and control respirometers, was immersed in a waterbath held at 37°C ± 0.01°. Temperature control was by means of a mercury thermoregulator in the bath which, acting through a relay, switched an infrared lamp used as the heater. Localized heating was minimized by constant agitation of the water. The tissue was protected from any infrared effects by shielding the respirometer with aluminum. The field at the control position was read as 0 on the most sensitive scale of the instruments, and so it was assumed to be that of the earth at that point. Simultaneous oxygen-consumption measurements were taken with the "experimental" tissue continuously exposed to the field while the "control" tissue was at the 0-Oe point. The gas phase in all studies was air. Moving the experimental respirometer to positions at various distances from the magnet made it possible to reduce the field to which the experimental tissue was exposed. Reproducible distances were assured by the use of plastic gauge blocks.

After it was established that an effect existed on tumor cells, the oxygen uptake of the Sarcoma 37 (S-37) and the Ehrlich adenocarcinoma was compared to that of normal embryonic and adult mouse kidneys. The tumors were used in their ascites forms at 5-8 days following serial, intraperitoneal implantation in Boontucky and Swiss mice, respectively. The embryo and adult kidneys were from Boontucky females. The indicator, in Hanks balanced salt solution, which was used as the

medium, allowed observation of gross pH changes. Readings were taken every 15 min and QO<sub>2</sub> values calculated at hourly intervals for either 3 or 4 hr, depending upon the tissue being studied.

In a number of preliminary experiments, some of the results of which have been published elsewhere,<sup>4</sup> the tumor cells were suspended in Tyrode solution. Aliquots of the tumor suspensions were placed in both the experimental and control respirometers from the same pipette. Each of the flasks was charged with 0.05 ml of a 1:1 dilution of the Ehrlich or 0.05 ml of a 1:4 dilution of the S-37 tumor. The percent change in oxygen uptake was calculated at hourly intervals for 3 hr. In four experiments with the Ehrlich tumor, a mean depression of oxygen uptake of 34.4% was found.

An attempt was made at this time to discover whether any relation existed between temperature, field strength, and respiration. For this purpose, four experiments with the S-37 were conducted at 32°C. A mean depression of 37.9% was noted. Compared with controls which were maintained at 37°C, the data indicated that the effect of the field is dependent upon temperature. In the case of the Ehrlich tumor, there was little difference in the hourly values, while the S-37 showed the greatest change during the first hour of exposure.

During these preliminary experiments the tumor suspension was introduced directly into the flasks, which were then attached to the respirometers. This procedure prevented quantitative removal of the tissue for dry-weight determinations needed for QO<sub>2</sub> calculations. The use of Tyrode solution precluded observation of pH changes. To further refine the experimental conditions it was decided to use tared "boats" to contain the tissue and Hanks solution as the medium in subsequent experiments. At no time during the experiments was a pH change indicated by the phenol red indicator in the solution.

The S-37 tumor was used in ten experiments at 37°C. The same procedure of pipetting was used to deliver the tumor suspension to the "boats" as had been previously used to deliver the suspension directly to the flasks. Close agreement of the dry weights of the experimental and control tissues (which could now be determined) added validity to the preliminary experiments in which the same pipetting procedure had been used. There was a mean hourly decrease in QO<sub>2</sub> of 29.3% (Table I), with the greatest change again taking place during the first hour of exposure to the field. Standard errors of the means of the experimental and control QO<sub>2</sub> values were calculated and are also shown in Table I.

The effect of the magnetic field on normal mouse tissue was next investigated. Embryo and adult mouse kidneys were sliced so as not to exceed 0.3 mm thickness to allow optimum gaseous exchange. Each kidney pair from a single mouse was divided between the experimental and control respirometers to minimize differences between individual animals. Readings were taken and QO<sub>2</sub> values calculated as was done for the tumors.

TABLE I

Effect of a Magnetic Field of 7300 Oe on the  $\text{QO}_2$  of  
Sarcoma 37 at 37°C  
(Average of ten experiments)

	Exposure time, min			
	60	120	180	240
Control	3.60	2.95	2.54	2.36
Experimental	2.22	2.16	1.89	1.83
% Depression	38.3	26.7	21.7	22.4
Mean $\pm$ S.E.				
Cont. $2.87 \pm 0.14$				
Exptl. $2.03 \pm 0.11$				
$N = 37$	$t = 4.67$		$P < 0.001$	

The curved-crown-rump length of the embryos was taken as a relative criterion of embryo age.<sup>5</sup> In 12 experiments with the embryo kidney, a progressive relationship with age was found to exist, ranging from a maximum depression of oxygen uptake of 93.5% for the very young (15 mm) embryo kidney to a stimulation of 5.9% as the embryos approached full term (Table II). The degrees of depression can be arranged in groups of like quantitative effects related to embryo length. Efforts are being made to correlate these groups with the embryological

TABLE II

% Depression of  $\text{QO}_2$  of Embryo Mouse Kidney Sub-  
jected to a Magnetic Field Strength of 7300 Oe

Embryo length, mm	Exposure time, min			
	60	120	180	240
Percent depression				
15	86.6	86.6	86.0	86.4
15	81.5	90.6	93.5	88.3
17	33.9	37.6	37.7	37.5
19	49.3	24.0	13.2	32.2
20	47.2	42.1	39.8	36.2
20	47.0	42.5	29.4	40.4
23	21.5	25.1	30.1	25.9
23	5.9*	6.0	7.8	2.9
24	4.2*	0.0	1.5	0.7*
25	10.1	11.9	6.0	9.2
27	2.4	12.8	15.5	10.3
$N = 33$	$t = 4.84$		$P < 0.001$	

\*Stimulation.

development of the mouse kidney. From Table II it can be seen that at the 23-mm level there is a marked difference between the effect as seen in the two experiments. This suggests that some sort of threshold has been reached. Above this length, the results are variable, as is the picture with adult kidney. It is in this length range that the embryos approach full term.

An attempt was made to discover any correlation of effect with kidney age. Adult kidney was used in nine experiments. For this purpose, kidneys were chosen from female mice of age 1 day, 2 weeks, 5-6 weeks, 15-16 weeks, and 18-19 weeks. With the exception of a single experiment run on kidney at the 1-day-age level, the experiments were duplicated (with respect to kidney age) at different times. A wide variation in effect was found which could not be correlated with age or any other known factor (Table III). Results ranged from a stimulation of 25% to a depression of 14%. This picture of variability was similar to that noted for embryo kidneys approaching full term. This type of variation may be responsible for some of the conflicting results reported in the literature.

In one additional trial, adult kidney was held in the control (0 Oe) position for 2 hr, then moved to the 7300-Oe position, continuous measurements being taken. There was no alteration in the respiratory pattern following the transposition.

A short series of experiments was conducted with the S-37 in an attempt to correlate the degree of effect with the field strength. The limited amount of data available indicates a direct quantitative relationship between cause and effect. Comparable points, selected from single experiments, indicate this relationship (Figure 1).

In yet another short series, yeast was used. Many studies of yeast respiration have been conducted in our laboratories using this type of microrespirometer, so that results obtained in this series served a dual purpose: investigation of the effect of the field on this type of

TABLE III

Effect of a Magnetic Field of 7300 Oe on the  $\text{QO}_2$  of  
Adult Mouse Kidney. Age Range of Mice—1 Day  
to 19 Weeks  
(Average of nine experiments)

	Exposure time, min			
	60	120	180	240
Control	2.5	2.7	2.6	2.7
Experimental	2.6	2.6	2.5	2.7
Mean $\pm$ S.E.				
Cont. $2.6 \pm 0.14$				
Exptl. $2.5 \pm 0.16$				
$N = 33$	$t = 0.524$	$P > 0.6$		

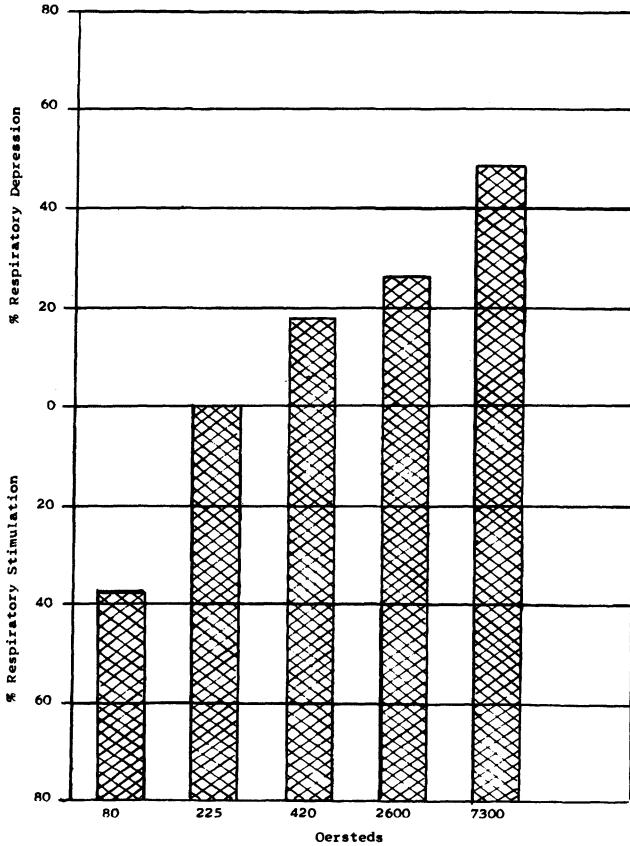


Fig. 1. The effect of magnetic fields of varying strength on the respiration of Sarcoma 37 cells.

tissue and a check of the reliability of the instrumentation by comparison with data obtained in previous studies. In five experiments the effect was uniformly one of stimulation of oxygen uptake.

It should be pointed out that experimentation by these methods is quite tedious and time-consuming. The sensitivity of the respirometers is such that they are susceptible to the slightest deviation in procedure. For these reasons it was necessary to conduct many preliminary experiments to determine optimum conditions for each type of cell. As an example, some 15 preliminary experiments were conducted to determine optimum conditions for the S-37 tumor. The qualitative effect of the magnetic field in all these trials was as reported here. It was only after standard conditions had been determined that the duplicate experiments discussed here were considered quantitatively reliable. As can be seen, considerable effort has been devoted to attempts to increase both the qualitative and quantitative accuracy of the investiga-

tions. As is to be expected, control of the inherent biological variables (such as the ratio of tumor to other types of cells in the ascites tumors) is difficult, and it is in this area that there remains considerable room for improvement.

To determine the statistical significance of the results analysis-of-variance and *t* tests were conducted on the data for the S-37 tumor and the embryo and adult kidneys. The data were so arranged that all hourly  $QO_2$  values were given equal weight. The difference between the means of the experimentals and the corresponding controls was 0.84 for the S-37, 0.92 for the embryo kidney, and 0.1 for the adult kidney. Standard errors of the difference between the means were 0.18, 0.19, and 0.21, respectively. The difference between the means of the samples of the S-37 and the embryo kidney is greater, in both cases, than 4 standard errors, indicating a greater than 99.9% probability that the effect is not due to chance. This is not the case with the adult kidney; here the variation between the samples is such that no significance can be shown. The values of *N*, *t*, and *P* are shown in the respective tables.

Results with the many types of cells studied would indicate that both the qualitative and quantitative effects of a magnetic field on tissue respiration are correlated with several biological factors in addition to those pertaining to the field itself. As a result of these experiments, it can be concluded that a magnetic field has an effect on cellular metabolism which is related to the type and age of the tissue. It can be further stated that this effect appears to be correlated with field strength and temperature. To be further investigated is the quantitative relationship between the effect of a magnetic field and some aspect of the cellular division mechanism. A more detailed study of this phenomenon has been undertaken using tritiated thymidine as a tracer.

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*Chapter 2*

## Agglutination of Human Erythrocytes

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Our motivation in searching for effects of magnetic fields on immunochemical reactions was twofold: first, an unequivocal demonstration of the role of magnetic fields in influencing biochemical reactions might be provided; second, information bearing on the mechanism of the immune reaction might be obtained.

On the first point, the delicacy of the response in certain of the antigen-antibody reactions (see, e.g., references 1, 2) makes it likely that any magnetic response would be easily discerned. It is a striking characteristic of the agglutination reaction that it permits unequivocal detection of specific substances in amounts of hundredths of a microgram. Now the kinds of matter making up erythrocytes are perhaps colloidal or even mesomorphic. The molecules of such matter exhibit many cooperative effects which permit the disordering thermal energy to be overcome at ordinary temperatures. Since the energy of interaction of individual molecules with realizable magnetic fields is very small, it is likely that cooperative effects will be necessary to surmount the disordering effect of thermal agitation. Hence the agglutination reaction seems worth studying for possible influence by magnetic fields. On the other hand, we might suspect that the extreme specificity of immunochemical processes portends failure of the small perturbing force of magnetism to influence the reaction. Immunochemical methods permit clear differentiation between large molecules differing only in small regions, which may be merely cis-trans isomeric or enantiomorphic or which may contain amino acids in only slightly differing sequence. One could argue that if the molecular configurations are so rigid as to reject a nonreacting molecule, they are not likely to be influenced by a weak perturbation. Hence, magnetic fields would not be capable of influencing the reaction.

On the second point, we need only note how exciting a frontier immunology has become in general biology. The past decade has shown that immunological phenomena are surely key mechanisms in the evolution of man and other animals. This work was supported in part by Research Grant GM 08967 from the National Institutes of Health, and by Research Grant G6208 of the National Science Foundation.

tion and growth of living bodies, evidently linked to the genetic system itself. The mode of formation of antibodies is a cardinal question in biology, and the history of science leads us to suspect that it will not be answered without detailed experimental findings on the composition and interaction of antibodies and antigens. If a magnetic field, then, has demonstrable effects, it may afford an invaluable probe to supplement traditional ones in the attack on a critical problem in modern biology.

In our laboratory, only *in vitro* immunological experiments were feasible. We chose as the principal subject of study erythrocyte agglutination by human anti-Rh antibodies. In choosing the agglutination reaction in preference to the precipitation reaction, we have sacrificed known quantitative behavior for increased sensitivity. As a consequence, we have encountered a major problem in setting up a quantification scheme for the agglutination reaction. Temporarily we have adopted the Race-Sanger visual scoring system,<sup>3</sup> at the same time pursuing the development of instrumental methods based on rate of sedimentation and on electronic counting of particles. In spirit, our investigation does not differ much from classic work such as that of Eagle,<sup>4</sup> who used time of reaction with various antibody-antigen phenomena to elucidate the mechanism of the interaction.

## EXPERIMENTAL CONSIDERATIONS

### Materials

Blood. The samples of blood were taken from various individuals in our laboratory. Capillary blood was withdrawn from the finger tip with the aid of a lancet and collected in a test tube containing physiological saline. The cells were centrifuged and washed three times with saline and made into a 2% suspension. The blood was used within 3 hr after withdrawal from the donor.

Antisera. The antisera were obtained from Knickerbocker Biologicals, Inc., and from Ortho Pharmaceutical Corp.

Ancillary Materials. The saline solution, made up from distilled water and reagent-grade salt, was filtered through Whatman No. 2 paper to remove particulate matter. It was never more than 24 hr old at the time of use. In all visual experiments and most counter experiments the blood suspension was not buffered. In some counter experiments a  $\text{Na}_2\text{HPO}_4$ - $\text{NaH}_2\text{PO}_4$  buffer was used, with no significant change in results.

### Procedures

Treatment of Cell Suspension and Antiserum. The antiserum was serially diluted with saline in ten steps resulting in concentrations from full strength to  $\frac{1}{512}$  ( $1/2^0$  to  $1/2^9$ ). To a  $10-\mu\text{l}$  sample of a given dilution of antiserum in a  $5 \times 50$  mm test tube, a  $10-\mu\text{l}$  sample of the cell suspension was added. The mixture was shaken by hand, placed in a water bath

maintained at  $37.5 \pm 0.1^\circ\text{C}$ , and incubated over various periods of time from 5 min to 2 hr. At the conclusion of the incubation period, the mixture of cells and antiserum was withdrawn with a pipette and deposited onto a slide for the visual examination, or into a beaker of saline for the particle-sizing experiments.

### Magnetic Fields

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, and the samples set in the gap between the other pair of faces. For homogeneous fields ferromagnetic material was excluded from the gap. The faces were  $1 \times 3$  in. rectangles, the gap being typically 1 in. Under these conditions the field was about 3000 Oe, with gradients less than 200 Oe over a region about  $\frac{3}{4}$  by  $2\frac{1}{2}$  in. For inhomogeneous fields, suitably-shaped soft-iron pole pieces were fastened to the faces at the gap. With an iron-wedge pole piece, the field could be increased to 7000 Oe at the apex, falling to 3000 Oe at the other side of the gap, to give gradients of about 3000 Oe/cm and field strengths of about 4000 Oe at the location of the samples under study.

Electromagnets. Two kinds of electromagnets were available for the study. A pair of smaller ones (Model R3, Modern and Classical Instruments Corp., Livermore, California) had  $1\frac{1}{2}$ -in. circular pole faces and a gap adjustable from 0 to 8 in. The coils were water-cooled and would set up 15,000 Oe in a  $\frac{3}{4}$ -in. gap when fed with 5A at 100 V d-c. A larger electromagnet (Model L128, Harvey-Wells Corp., Framingham, Massachusetts) had 12-in. circular pole faces and a fixed 2-in. gap. Its water-cooled coils set up a field of 12,000 Oe when fed by 50 A at 100 V d-c.

The field intensity  $B$  was measured by Hall-effect devices (Instrument Systems Corp., Model A-102) and by flip-coil instruments (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 in. 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 1 and 2 show part of the results obtained.

### Visual Scoring

For the visual scoring, the sample was removed from the incubation tube with a Pasteur pipette and spread on a microscope slide for examination in white light at 100 $\times$ . The extent of agglutination was assessed according to the Race-Sanger symbols<sup>3</sup> as follows:

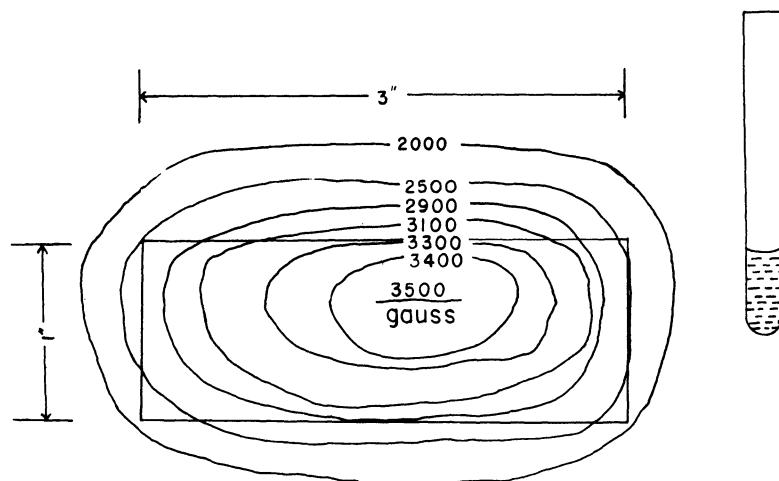


Fig. 1. Map of magnetic field at the position of test tubes for agglutination studies. The contour lines are loci of constant field strength as labeled. The source of the field was a pair of permanent magnets.

Symbol	Description	Point value
+++	Agglutination clearly visible to the naked eye	10
++	Very large agglutinates seen microscopically	8
+	Large agglutinates seen microscopically	5
(+)	Smaller agglutinates seen microscopically	3
w	The smallest definite agglutinates	2
-	No agglutination and cells evenly distributed	0

The point values are arbitrary numbers as assigned by Race and Sanger for the purpose of obtaining semiquantitative comparisons between antisera.

#### Particle-Counting Methods

To count erythrocytes, the Coulter counter is by far the fastest device. With it as many as 100,000 particles can be counted and their volume distribution obtained in less than 2 min. Its operation is based on the increase in resistance across a capillary orifice immersed in a conducting liquid when a nonconducting particle passes through it. When a suspension of erythrocytes (mean diameter  $\sim 7 \mu$ , mean volume  $\sim 85 \mu^3$ ) is drawn through the orifice with simultaneous passage of a constant electrical current, each particle produces a voltage pulse whose height is

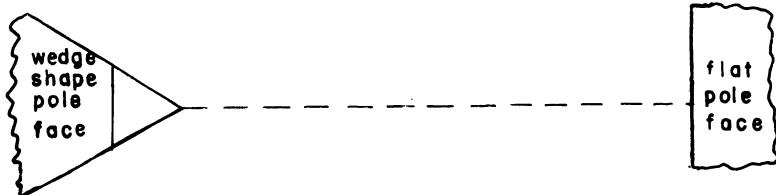
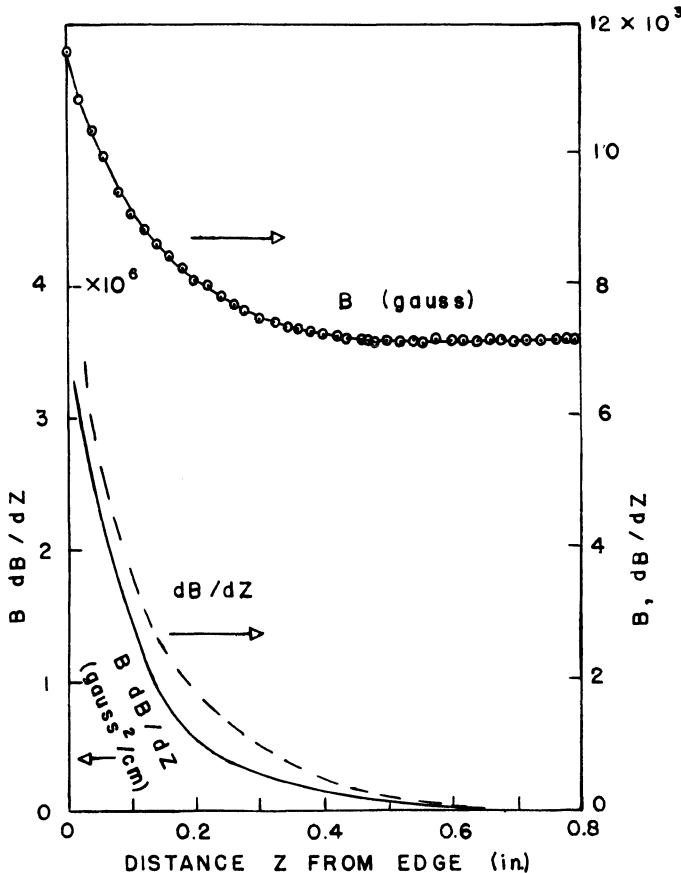


Fig. 2. Plot of magnetic field quantities as a function of distance  $z$  from apex of iron wedge placed in gap to produce inhomogeneous field. The magnetic field intensity  $B$  determines the orienting influence of the field; the field gradient  $dB/dz$  determines the force on permanent dipoles in the field; and the product  $B(dB/dz)$  determines the force on induced dipoles in the field.

supposed to be proportional to the volume of the particle. An electronic pulse-height analyzer and an electromechanical plotter reduce the data to a convenient form.

The Coulter counter was patented in 1953 and first described in the scientific literature in 1956.<sup>5,6</sup> It was developed initially for erythrocyte counting, as described in the references just cited. It was subsequently utilized for counting leukocytes and platelets. Its use for determining

erythrocyte size distribution was initiated by Mattern et al.<sup>7</sup> with the Model B counter—which permits determination of only the total number of particles having volumes above a certain threshold fixed by the operator—through the scheme of running different portions of the same sample with the counter set at different thresholds. The first use of the counter in studying agglutination was made by Halloran et al.,<sup>8</sup> who noted essentially that total count was decreased sharply by the existence of agglutination. It is true that in this study the threshold was adjusted to three different levels, but no quantitative deductions with respect to distribution were attempted. A related study was reported by Goodman.<sup>9</sup> The first work with the Coulter counter describing the changes in distribution occurring during agglutination\* was reported by Brecher et al.<sup>11</sup> These investigators found rises in the peaks at multiples of the single volumes, but they did not attempt to give quantitative expression to their findings.

### Sedimentation Methods

Upon agglutination of two or more erythrocytes to form a single assembly, the total weight remains the same, yet the perimeter of the assembly is less than that of the cells individually. Hence the terminal velocity of the assembly when falling through a viscous liquid will exceed that of the individual particles. That this effect can be used for studying agglutination was shown twenty years ago by Hirst and Pickels<sup>12</sup> (see also references 13-17). In the present work, where the effect sought may be small, the apparatus must be refined and enlarged to 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 from it.

## RESULTS

### Visual Scoring

Effect of Concentration. With anti-D (anti-Rh<sub>D</sub>) an enhancement of agglutination in a magnetic field was observed. Table I shows the results of a typical experiments with anti-D serum and D-positive cells incubated for 1 hr at 37<sup>1/2</sup>°C. At the highest concentrations, the agglutination was maximal on the Race-Sanger scale and no difference was scored, even though greater clumping was apparent for the sample incubated in the field. For intermediate and low concentrations, a difference was scored. The overall results on the Race-Sanger scales gave a total score of 74 to 56, an enhancement of 32% on this arbitrary scheme.

Effect of Field Strength. Similar runs were made in more or less

\*The use of particle-counting methods other than electronic for quantitating the agglutination reaction has, of course, a longer history. Cf., e.g., Wilkie and Becker.<sup>10</sup>

TABLE I

Effect of Antiserum Concentration on Anti-D Reaction with D-Positive Cells (Field strength = 2000 Oe)

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

TABLE II

Enhancement of Agglutination by Magnetic Fields (anti-D serum, D-positive cells)

Field strength, Oe	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

homogeneous fields, the average strengths running from about 20 to 5000 Oe. The results are shown in Table II. Subsequent experiments, at fields up to 16,000 Oe, produced by the 12-in. electromagnet, corroborated results obtained at lower field strengths with the permanent magnets. The effects at higher serum concentrations were extremely pronounced.

Effect of Cell Type. When D-negative cells were incubated with anti-D serum, no reaction was observed at any of the field strengths used,

thereby indicating that the magnetic field does not produce nonspecific agglutination.

Effect of Antiserum Type. Similar results were obtained with cells of various D-positive genotypes against anti-D serum. Anti-C (anti-rh') and anti-E (anti-rh") sera against C-positive and E-positive cells, respectively, yielded essentially the same pattern as anti-D serum with D-positive cells. Anti-c (anti-hr') and anti-e (anti-hr") have not yet been tested. When the antibodies of the ABO and MN systems were tested in analogous experiments, no enhancement by magnetic fields was observed.

Effect of Incubation Period. For antigen-antibody reactions where enhancement was observed, the incubation period at the normal temperature was varied to  $\frac{1}{2}$  and 2 times the normal period. As a rule, increasing the period increased the degree of agglutination slightly, but definite quantitative relations could not be established.

Effect of Incubation Temperature. Incubation at temperatures  $1\frac{1}{2}^{\circ}$  above and below  $37\frac{1}{2}^{\circ}\text{C}$  showed negligible differences.

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. In one test, the usual series of ten twofold dilutions was used, with a result similar to that found for homogeneous fields. Nevertheless, it is impossible to state decisively that inhomogeneity is a determining factor in enhancing the agglutination.

Results of Other Workers. Foner<sup>18</sup> at the Boston University Medical School has confirmed the enhancement of agglutination in the anti-D reaction and has found enhancement in the anti-A reaction as well. She found no strong effect of field inhomogeneity.

#### Particle-Counting Method

Detailed studies with the electronic counter were made on the distribution of erythrocyte volumes in both agglutinated and unagglutinated blood. The effects of antiserum concentration, incubation period, and incubation temperature were studied. In lengthy experimentation we could detect no effect on agglutination in a strongly inhomogeneous field of maximum strength 16,000 Oe and magnetic gradient  $\sim 1500$  Oe/cm. A full account of the work is available in the Ph. D. thesis of Adolph E. Smith, "A Search for Biological Effects of Magnetic Fields," Michigan State University, 1963.

#### 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 in order to obtain quantitative expression of the enhancement and to see how field strength and field gradient affect the agglutination at varying concentration of antiserum and at varying period 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 by the Coulter counter, on the other hand, show no signs of 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 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 noticeable 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 kinds 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 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  $10^4$  Oe 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 20-22). 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.<sup>19,23</sup> Hence it is plausible 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 will detect agglutination, but only that resulting in formation of sturdy aggregates.

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### *Chapter 3*

## Inhibition of Bacterial Growth in Fields of High Paramagnetic Strength

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The results of investigations of the effect of magnetic fields on the growth of bacteria have so far been inconclusive. Leusden<sup>1</sup> found that the growth of coliform organisms and staphylococci was not affected by exposure to magnetic fields. Jennison<sup>2</sup> grew 25 species of bacteria, yeasts, and molds on solid media between the poles of an electromagnet which provided a homogeneous field of 3000 Oe. After 48 hr of exposure, he checked the cultures for size of colony, staining reactions, pigment production, and spore formation. He found no difference between magnet cultures and controls. However, Magrou and Manigault<sup>3</sup> showed that tumor production by Bacterium tumefaciens on a strain of Pelargonium zonale was retarded by magnetic fields.

### EXPERIMENTAL RESULTS

The work reported here was begun by growing a nutrient broth culture of Serratia marcescens between the tapered, constant-gradient polecaps of a Varian 4-in. electromagnet. The average field strength was 15,000 Oe, with a constant gradient of 2300 Oe/cm throughout the culture medium. The paramagnetic strength of this field was 34.5 MOe<sup>2</sup>/cm or 34.5 par.

Standard culture tubes containing 5 ml of nutrient broth were inoculated with one drop of a 24-hr broth culture from a 1-ml pipette.

TABLE I

Serratia marcescens

15,000 Oe, 34.5 par. Average of two to four plates. Number  $\times 10^5$  = organisms/ml.  
 Relative growth rate differences  $\Delta = \delta M/M - \delta C/C$ .

Date	3 - 3 - 62			3 - 21 - 62			5 - 26 - 62		
Incub. time, hr	M	C	$\Delta$	M	C	$\Delta$	M	C	$\Delta$
0	83	88		110	138		285	305	
3	183	157	-1.4	275	262	4.6	406	350	5.8
4	(430)	(375)	0.0	(500)	(455)	5.5	(880)	(710)	3.8
5	1040	910		933	800		2000	1550	
6	(1160)	(1400)	-31.6	1917	1343	18.8	1788	2000	-36.5
			<u>-31.3</u>			<u>-38.7</u>			<u>-24.5</u>
7	1310	2175	27.5	1718	1777	-51.2	1850	2650	7.8
8	(1950)	(2420)	23.0	1900	3340	-5.1	1925	2550	15.8
9	(2800)	(2780)	26.2	2113	3913	36.0	2387	2700	
10	(4100)	(3120)		3550	4550		3137	2575	32.0
$10\frac{1}{2}$	4880	3320							
	$\Delta, \%$			<i>t</i>	<i>n</i>	P.L.			
Minimum	$-35.7 \pm 3.7$			9.6	4	1: 1800			
Overshoot	$+31.4 \pm 2.8$			11.0	2	1: 120			
Difference	$-67.1 \pm 4.7$			14.3	6	$1: 2 \times 10^6$			

One tube was incubated in the magnet and the other, serving as a control, was incubated outside the magnet. Both culture tubes were surrounded by aluminum blocks, the temperature of which was kept at 27°C by means of a thermocirculator. The aluminum block with the culture tube in the magnet was heat-insulated from the pole pieces. The temperature of the latter was kept at  $27 \pm 1^\circ\text{C}$  by the cooling pump of the magnet.

Great care was taken during sampling that mixing and aerating of the two cultures (magnet and control) should be alike. The culture tubes were lifted from their thermostat blocks and the medium mixed as usual by letting the liquid drawn in the pipette reflow five times before the sample was taken. The tubes were thereafter immediately replaced. This procedure lasted about 50 sec for each tube; during this time, once each hour, the magnet culture was out of the field.

At hourly intervals, 0.1-ml samples were taken and diluted in peptone water by serial dilutions; 0.1 ml of the last two dilutions was surface-plated on nutrient agar in duplicate. The plates were incubated at 27°C for 48 hr and the resulting colonies counted. In this way, we had for each incubation time two, in some cases four, plates on which colonies could be counted. The procedure is illustrated by the following two examples. In the first experiment with Staphylococcus aureus (Table II), after 7 hr of incubation of the magnet culture we counted 66 and 62 colonies on two plates with  $5 \times 10^6$  dilution and 30 and 30 colonies on the two plates with  $10^7$  dilution. Their average gives  $3100 \times 10^5$

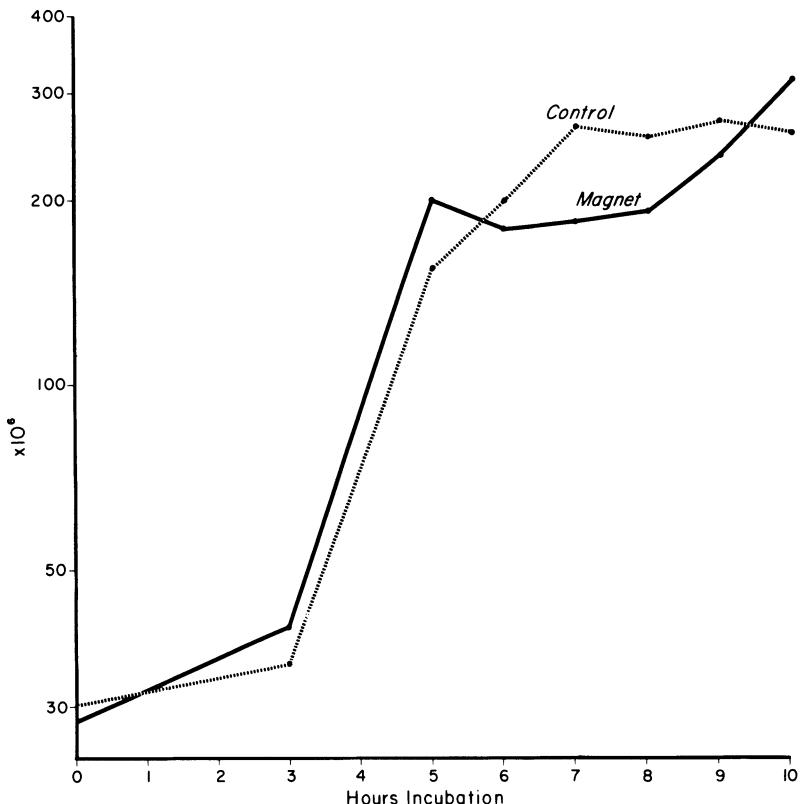


Fig. 1. Growth curve of *Serratia marcescens* culture (number of viable cells per ml) incubated in a magnetic field of 15,000 Oe strength and 2300 Oe/cm gradient (full line) and control culture (dashed line).

cells/ml. In the second experiment with *Serratia marcescens* (Table I), after 6 hr of incubation of the magnetic culture we counted 208 and 152 colonies on two plates with  $10^6$  dilution and 27 and 16 colonies on two plates with  $10^7$  dilution. To find the average number of cells in the culture, we assigned double weight to the data obtained with the lower dilution, thus yielding  $1917 \times 10^5$  cells/ml.

Table I lists the experimental data obtained in the three measurements made with *S. marcescens*. The first column indicates the incubation time in hours, the second and third the average number of cells as found from the colony counts in the magnet (M) and in the control culture (C), respectively. To obtain the number of viable cells per milliliter, the listed numbers should be multiplied by  $10^5$ . The parentheses in the table indicate counts which were not actually counted, but obtained through interpolation from the growth curve. The fourth column lists the relative difference in growth rate per hour between magnet and control cultures ( $\Delta$ ). Columns five through ten contain the same data for the two other experiments.

Figure 1 illustrates one of the actually measured growth curves of

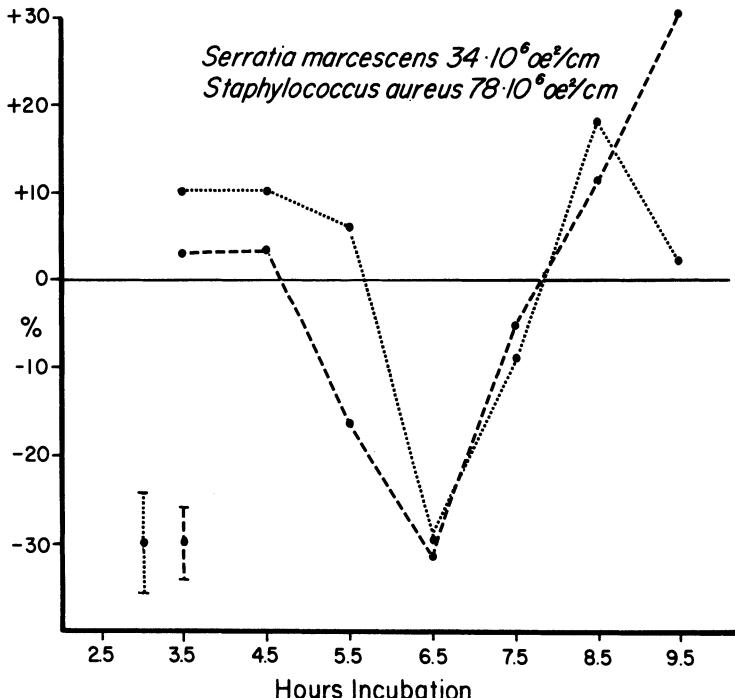


Fig. 2. Difference in the relative hourly growth rate between magnet and control culture. Dashed line—*Serratia marcescens*; dotted line—*Staphylococcus aureus*. Standard errors are indicated.

magnet and control cultures (experiment 5-26-62). The magnet and control cultures were found to have approximately the same number of cells and the same growth rate up to 6 hr. At 7 hr the magnet culture showed a lower plate count than the control. This difference was greatest at 8 hr and diminished thereafter up to 10 hr, when magnet and control cultures again had approximately the same number of cells.

We may note that in all three experiments the growth rate of the magnet culture significantly decreased for a duration of 2 hr below the growth rate of the control culture. However, this decreased growth rate occurs in the first and third experiments between the 5th and 7th hour, while in the second experiment it occurred between the 6th and 8th hour. After this minimum, in all three experiments the growth rate of the magnet culture rebounds and surpasses the growth rate of the controls.

If we average all three experiments, we find a highly significant minimum of 36%, which is followed by a significant 31% overshoot. The difference between minimum and overshoot is significant on a probability level of  $1:2 \times 10^6$ . In Figure 2 we have plotted (dashed line) the relative growth-rate difference between magnet and control cultures; it has a sharp minimum around 6.5 hr of incubation. The points in Figure 2 are the average values of the three experiments; but, to

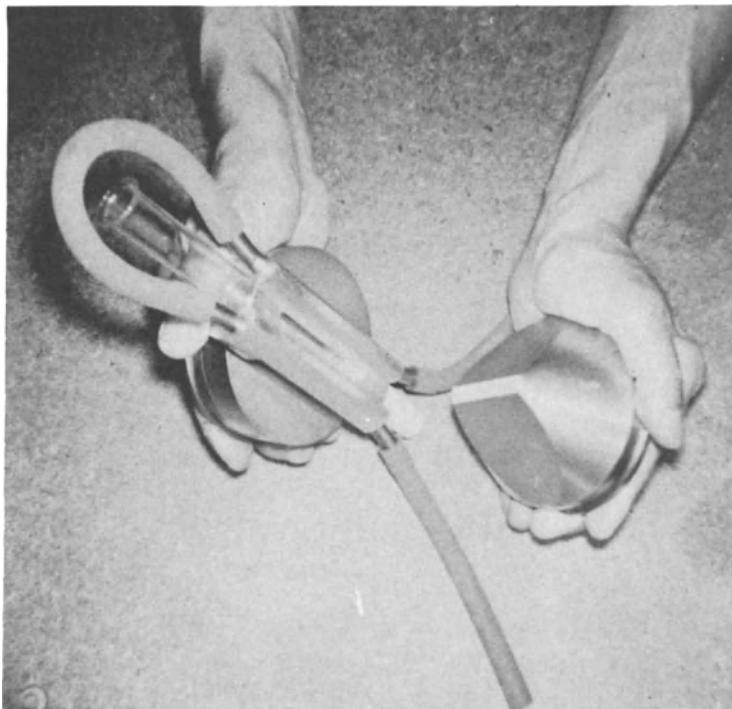


Fig. 3. Polecaps used to produce a field of  $78 \text{ MOe}^2/\text{cm}$  paramagnetic strength, showing test tube in thermostat.

smooth the curve, an overlapping weight function of (1-2-1) was applied. This procedure somewhat decreases sharp minima and maxima, but increases the reliability of the curve form. The applied weight function is the first approximation of a normal distribution.

A similar procedure was followed with a culture of S. aureus, except that the incubation temperature was  $37^\circ\text{C}$ . Using the same constant-gradient polecaps as before, no effect on the growth rate was observed. The experiments were thereafter repeated using special polecaps. These polecaps, shown in Figure 3, consist of a wedge opposite a concave piece. In the test tube, a field of the same average strength is produced as before (15,000 Oe), but with an average gradient of 5200 Oe/cm. The gradient in the culture medium was nowhere less than 3000 Oe/cm and reached 8000 Oe/cm on the side closest to the wedge. The average paramagnetic strength of the field was 78 par, or more than twice as strong as the one previously used.

The results of the two experiments made with S. aureus are listed in Table II, where again the average was obtained from the colonies counted on the two or four plates; the relative growth-rate differences ( $\Delta$ ) are indicated as in Table I.

A difference in the number of cells was evident from the 3rd to 6th hour, when the magnet culture showed a higher plate count and higher

TABLE II

*Staphylococcus aureus*

15,000 Oe, 78 par. Average of two to four plates. Number  $\times 10^5$   
 = organisms/ml. Relative growth rate differences  $\delta M/M - \delta C/C$ .

Date Incub. time, hr	7 - 7 - 62			7 - 20 - 62		
	M	C	$\Delta$	M	C	$\Delta$
0	80	76		50	47	
3	1345	1237	6.1	900	1017	14.0
4	(2080)	(1800)	7.7	(1290)	(1220)	12.3
5	3300	2637	3.5	1830	1525	28.0
6	3112	2400	-43.0	2880	1800	-41.1
7	3100	3700	-7	2087	1975	-17.3
8	4862	5850	16.8	2875	3250	19.1
9	5075	5162	7.6	4395	3700	-3.3
10	5225	4925		4725	4112	
	$\Delta\%$	<i>t</i>	<i>n</i>	P.L.		
Minimum	-42.0 ± 1.0	44.4	1	1:66		
Overshoot	+18.0 ± 1.1	15.7	1	1:23		
Difference	-60.0 ± 1.5	40.0	2	1:2400		

growth rate than the control. However, at 6 hr an inhibition of this growth began and reached its maximum at 7 hr, when the plate count of the magnet culture fell below that of the control. This difference gradually diminished up to the 9th hour, when the magnet and control cultures had approximately the same number of cells. Thus, *S. aureus* was maximally inhibited around the 6th-7th hour, as was *S. marcescens*. Between the 6th and 7th hour the difference in the relative growth rate was 42%, which was followed by an 18% overshoot. The difference between minimum and overshoot is significant on a probability level of 1:2400.

The dotted line of Figure 2 shows the relative growth-rate difference between magnet and control cultures for *S. aureus* (it is the average of the two experiments and again the same weight function was used to smooth the curve).

The higher growth rate for the hours preceding the minimum is significant for *S. aureus*, but not significant for *S. marcescens*, whereas the higher growth rate shown for the hours following the minimum is significant for both cultures and is a prerequisite for the magnet culture to achieve the same height of the maximum stationary phase as the control culture.

The results indicate that magnetic fields of the paramagnetic strength used do affect the growth of bacteria. The fact that the inhibition was observed in a highly inhomogeneous magnetic field, and particularly that the inhibition of *S. aureus* was found in a field of the same average strength as with *S. marcescens*, but only at a higher paramagnetic

strength, indicates that the inhibition has to be attributed to a paramagnetic phenomenon in which magnetic dipoles play a role. The results reported in the following chapter indicate that homogeneous fields can also affect the growth curve of bacterial cultures; but, in homogeneous fields, inhibition sets in only in the maximum stationary phase (not followed up in this paper), when other detrimental factors become manifest and growth rate is compensated by deathrate.

We could ask the question: why did S. aureus require a higher paramagnetic strength? Of course, there may be many reasons, but the answer could probably be found in the reversibility of magnetic effects, a consequence of the vector character of magnetic field and gradient. This question and its implications are discussed in Part I, Ch. 4 of this volume.

Bacteria do change their position relative to the magnetic field and gradient vectors due to their inherent motion, as well as due to their Brownian motion. This would tend to compensate reversible physical effects, to which class the great majority of all magnetic effects belong. An exception would be if the bacterium as a whole were oriented by the magnetic field and thereby be forced to keep its position not only relative to the field vector, but also relative to the gradient vector. This situation could occur if two prerequisites are satisfied: the bacterial cell is para- or diamagnetic relative to the medium (it most probably is), and its shape is nonspherical. S. marcescens is a rod, its orientation plausible; S. aureus is, on the other hand, spherically shaped, its orientation unlikely.

#### MATHEMATICAL ANALYSIS

Although the physiological mechanism which is the cause of the observed inhibition of bacterial growth is yet unknown, we may try to reach a better understanding through a mathematical analysis of the growth curves.

The number of viable cells during the logarithmic growth period can be described by

$$N = N_0 e^{At/i}$$

Here  $N_0$  is the number of viable cells at the end of the lag period, and  $t$ , the incubation time, is counted from the end of the lag period. The dimensionless number  $A$  determines the cell number in the maximum stationary phase and  $i$  approximately equals the duration of the logarithmic-increase phase;  $A/i$  represents the slope of the increase in the number of cells if plotted on semilogarithmic paper. This formula would, of course, lead to an exponential increase without limit. To take into consideration the fact that the available volume and nutrient material limit this number, we can alter our formula by writing in the exponent, instead of  $At/i$ , the expression  $A(1 - e^{-t/i})$ . As is evident, this formula

gives  $N = N_0$  for  $t = 0$ . For small values of  $t$ ,  $e^{At/i}$  can be approximated by  $1 - At/i$ ; thus we get the exponential increase. But, for values of  $t$  large compared to  $i$ , the exponential term will decrease to zero and the expression will asymptotically approach the constant value  $N_0 e^A$ , the cell number in the maximum concentration of the stationary phase.

The formula has still to be adapted if it is to explain the death phase. This can be done by adding a further term to the exponent:

$$N = N_0 \exp \{ A [1 - e^{-t/i} - e^{(t-\delta)/d}] \} \quad (1)$$

Here,  $d$  in the last term is a parameter determining the slope of the logarithmic death phase; it is a measure of all detrimental factors appearing during the further history of the culture, toxic products, pH change, etc.;  $\delta$  is the total length of the growth curve, the time interval during which the number of viable cells will be again reduced to  $N_0$ . Since in most cases  $\delta$  is several weeks and we have conducted our measurements only up to 10 hr of incubation, the third term in the exponent will not play a role in our case. But we still see that it will help us to explain the behavior of the magnet culture. We can well approximate the growth curve of S. marcescens by using  $A=4$  and  $i=4$  hr. The division time of cells is  $\ln 2i/A = 40$  min.

The magnetic field can be considered either as a factor which decreases the fission rate or as a factor which increases the deathrate of the cells. The first assumption can be ruled out, since in the beginning of the logarithmic-increase phase the magnet culture showed the same (or even an increased) growth rate. We therefore will assume that the second reason is the dominant one. But if the magnetic field can be considered as an additional detrimental factor, we may take care of this factor with a term similar to the third term in the exponent of (1), writing  $e^{(t-\mu)/m}$ , where  $m$  is the measure of the deathrate caused by the magnetic field and  $\mu$  is the time delay necessary for the biomagnetic effect to develop to a point where it is observable. If this magnetic detrimental factor is considerably stronger than the other, normal detrimental factors, then  $m$  and  $\mu$  are shorter than  $d$  and  $\delta$ ; in other words, in the magnetic field the culture would start the death phase at an earlier time and the number of viable cells would decrease to the original cell number  $N_0$  much earlier, namely, after the lapse of  $\mu$  hr. Our equation could now explain the drastic decrease in the growth rate of the magnet culture. But we still lack an interpretation for the cause of the growth-rate increase following this inhibition.

Mathematically, the only possible explanation of the further part of the growth curve is the assumption that a new generation is born which is better adapted to withstand the detrimental factors of the magnetic field. It should be noted that at the time the first indication of an inhibition of the growth rate in the magnet culture appears, the majority of the cells already belong to the 5th to 8th generation. Through the in-

creased deathrate of the nonresistant members of the population, a natural selection sets in, gradually enriching the newer generations with better-adapted members. Depending on when and to what degree in the later generations the resistance against the detrimental factors of the field is building up, their growth can more or less compensate the increased deathrate of the earlier generations. The total number of viable cells could decrease, stay constant, or increase in number, simulating a temporary inhibition, after which a resumed growth rate will manifest itself. The circumstance that after 6.5 hr of incubation the growth rate quickly returned to its original value, and that eventually the same maximum concentration value was reached with the field as without, suggests that the adaptation of the later, say 10th to 12th, generations must have been more or less complete.

Mathematically, the growth curve of these resistant generations can be described with an equation similar to that used before for the normal growth curve, that is, for the control culture, with the difference that the zero hour, the start of the logarithmic increase, is here shifted to a later time  $\nu$ , the time necessary for the resistant viable cells to reach the number  $N_0$ . If we assume that the fission rate of the resistant cells is the same as that of the original population, the total number of viable cells will be given by

$$N_m = N_0 \exp \{A[1 - e^{-t/i} - e^{(t-\mu)/m}]\} + N_0 \exp \{A[1 - e^{-(t-\nu)/i} - e^{(t-\delta)/d}]\} \quad (2)$$

The first term in this equation describes the growth curve of the original population, the second term that of the magnetoresistant population; the parameters have the same meaning as discussed above, where it was assumed that the effect of the magnetic field on the deathrate overrides all other detrimental factors.

From (1) and (2) we can compute the relative difference in growth rate between magnet and control cultures. The result of this computation is shown as the full-line curve in Figure 4. We have used for  $A$  and  $i$  the values indicated above; for  $m$  we used the value of 1 hr, which means that the original population would be halved after the lapse of  $\ln 2A/m = 10$  min; we used 5.5 hr for  $\mu$ , the time during which the number of cells in the original population would, through the increased deathrate, drop to the initial concentration  $N_0$ ;  $\nu$  was taken as 1 hr. For the sake of comparison, we have replotted in Figure 4 the curve obtained with S. marcescens; we can see it follows the theoretical curve well. The S. aureus curve shows in the beginning a somewhat enhanced growth rate in the magnet culture. This could be explained by a slightly higher fission rate of the resistant cells.

We wish to stress that the found increase in the growth rate after the minimum requires the formation of a magnetoresistant strain.

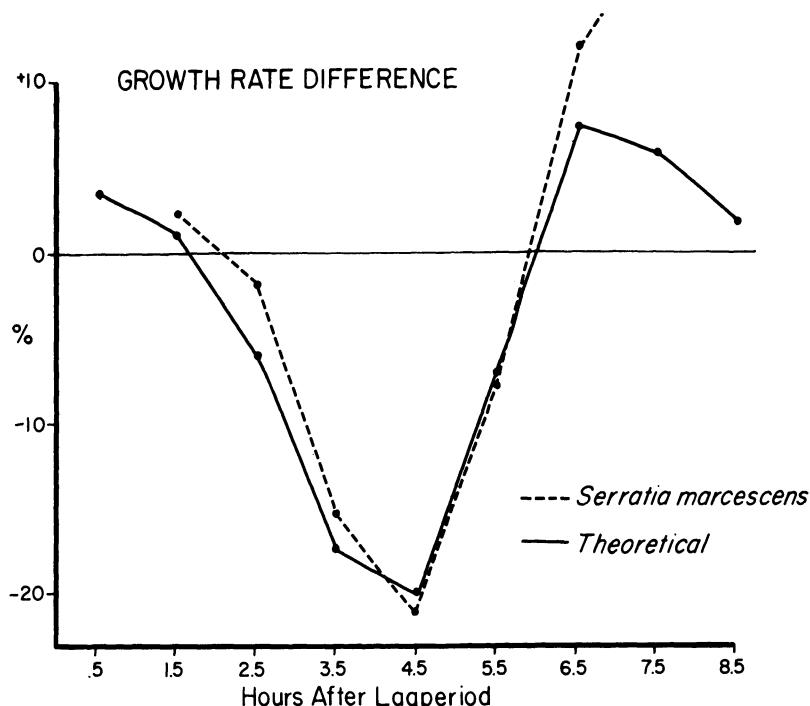


Fig. 4. Difference in the relative hourly growth rate between magnet and control culture. Full line—curve computed from equations (1) and (2); dashed line—*Serratia marcescens*.

Another possibility would be that the magnetic field does not enhance the death of the cells, but merely produces a temporary inhibition in cell division, until the cells become more adapted to the new environment, the magnetic field. This process would be similar to that occurring during the lag phase. However, the situation here is different. The first sign of inhibition is noted when the majority of the population consists of 5th to 8th generation cells. It is somewhat difficult to imagine that cells which multiplied in the field for 5-8 generations without being affected should abruptly cease to divide in order to adapt themselves to the field and resume thereafter their normal fission rate.

In our opinion the magnetic field does not affect the fission rate; it merely increases the deathrate of the original population. In the field a resistant strain starts to develop and the high mortality rate of the nonresistant population rapidly clears the biological space for the multiplication of the resistant cells. Whether or not the term "resistant population" should be understood as a mutation cannot be determined at present. No morphological changes have been found. Many further investigations are needed until a true understanding of the mechanism involved can be reached.

In all three experiments with *S. marcescens* the duration of the lag phase of the growth curve was about 3 hr and had apparently the same

length in the control as in the magnet culture. The magnet culture showed a slightly higher growth rate during this 3-hr period, the difference being significant on a 5% confidence level.

The lag phase is essentially a period in which the protoplasma of the old but still viable bacteria in the inoculum is acquiring the characteristic of young protoplasma. It is a phase of rejuvenescence in which they grow in size but do not divide, or at least the division rate is extremely low. The fact that the lag phase was not lengthened and that even a somewhat greater division rate was observed in the magnet culture would imply that the magnetic field used had no inhibitory effect upon the rejuvenation process or upon the division rate. Conversely, an inhibition was observed (in the same magnetic field) during the logarithmic growth phase, but only after the 5th to 8th generation.

The logarithmic phase differs from the lag phase in that cell division and hence DNA replication is vigorous and occurs repeatedly in the logarithmic phase. It is tempting to speculate that, while it seems that the magnetic field has little effect upon processes affecting merely the metabolism and growth of the cell, it significantly affects the division process, the period of DNA replication. It did this in spite of the fact that the time required for DNA replication constitutes merely a small, about  $\frac{1}{20}$ , fraction of the total cell-division cycle.

The circumstance that an effect upon the DNA replication period is not observed immediately, but only after the 5th to 8th generation, suggests that the effect of the magnetic field may be related to an alteration of the genetic code, since any alteration in the genetic code will be magnified by a factor of 100 to 1000 in the 5th to 8th generation. A further discussion of this possible effect of the magnetic field upon the genetic code can be found in Part I, Ch. 7.

#### ACKNOWLEDGMENTS

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## *Chapter 4*

# Inhibition of Bacterial Growth in Homogeneous Fields

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The use of magnetic fields as shielding against cosmic radiation during missions in space is presently being investigated. Such active shielding apparently requires magnetic fields stronger than the geo-magnetic field. In order to predict the possibility of a biosystem suffering a deleterious effect in constant high magnetic fields, it becomes necessary to study the responses of the system when placed in such fields. Studies of the responses of microorganisms, as the biosystem, to magnetic fields have been carried out at General Dynamics/Fort Worth for the past year.

In an early study by Jennison<sup>2</sup> on the growth of bacteria in a homogeneous field of 3000 Oe, no effect on the colony size, size and shape of individual cells, reaction to Gram stain, or pigment and spore production was observed.

Studies more recently reported by Gerencser, Barnothy, and Barnothy<sup>1</sup> have shown that microorganisms are affected when exposed to highly inhomogeneous magnetic fields. Using a 4-in. Varian electromagnet with a field of 15,000 Oe and a constant gradient of 2300 Oe/cm, they reported an inhibition of growth in Serratia marcescens when exposed for 7-8 hr which returned to the level of the control at 10 hr. Staphylococcus aureus under the same conditions showed no effect. When the field was altered so that the field strength remained the same, but the paramagnetic strength was more than twice that used on S. marcescens, the plate counts from the third to sixth hour showed higher levels than the controls. At 6 hr, inhibition began, and by 7 hr, growth was below that of the control and leveled off at 9 hr with the control. They concluded that magnetic fields of a high paramagnetic strength do affect the growth of bacteria. No morphological changes were shown. (See preceding chapter.)

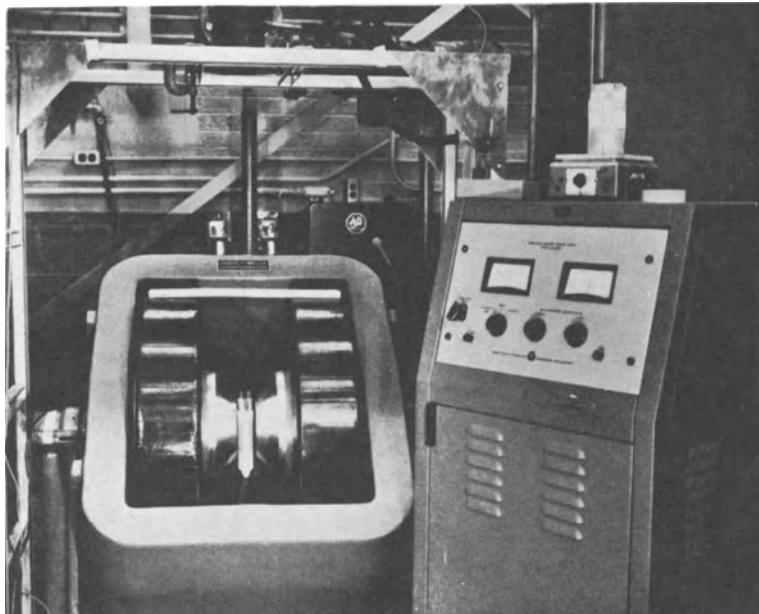


Fig. 1. Electromagnet and power supply used for exposing the microorganisms.

The purpose of this paper is to report the results of studies on the effects exhibited by selected microorganisms on a physiological or morphological basis when exposed to a constant homogeneous magnetic field.

#### MATERIALS AND METHODS

The magnets used in these studies were a Harvey-Wells Model L 128 12-in. electromagnet and Alnico V permanent magnets.

The microorganisms included Staphylococcus aureus, Sarcina lutea, and Escherichia coli, all of the General Dynamics/Fort Worth culture collection. These microorganisms were selected as possible microflora representative of a biosystem in a space vehicle. The stock cultures of the microorganisms were maintained in nutrient broth for 24 hr before being used as the inoculum for the exposed cultures.

The medium used in the growth-curve response study in the electromagnet and that used in the permanent magnet was nutrient broth. In the physiological production of gas by E. coli, the medium was 2% dextrose-nutrient broth.

In the study with the electromagnet, standard test tubes (0.78 in. OD) with 20 ml nutrient broth were inoculated with one loopful of the stock culture of the organism prior to placing the tubes in a thermostatically controlled waterbath maintained at 37°C between the poles of the magnet. This volume of media remained in the homogeneous field of the magnet. The culture tubes remained undisturbed except when sampled.

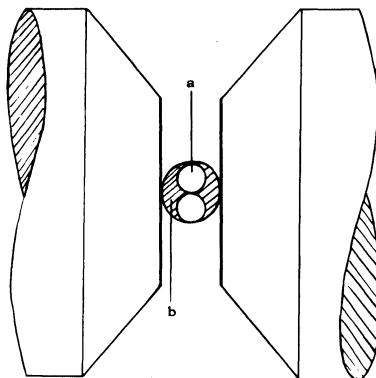


Fig. 2. Diagrammatic representation of culture tubes (a) in a constant-temperature waterbath (b) between the 6-in. pole faces of the electromagnet with a field strength of 14,000 Oe.

A control sample was prepared and held at the same temperature conditions ( $37^{\circ}\text{C}$ ) in a separate waterbath.

The electromagnet, cooling-water pressure regulators for the magnet coils, Model HS1365 Power Supply, and constant  $37^{\circ}\text{C}$  waterbaths with the experimental and the control samples in place are shown in Figure 1. The tube arrangement in the constant  $37^{\circ}\text{C}$  waterbath between the poles of the electromagnet is shown in Figure 2 from a top view. The poles of the magnet have been tapered to a 6-in. face with a 4-in. homogeneous field in the center. The paramagnetic strength over the total volume of the sample which was entirely within the 4-in. homogeneous field remained at the constant level of  $0.14 \text{ MOe}^2/\text{cm.}$

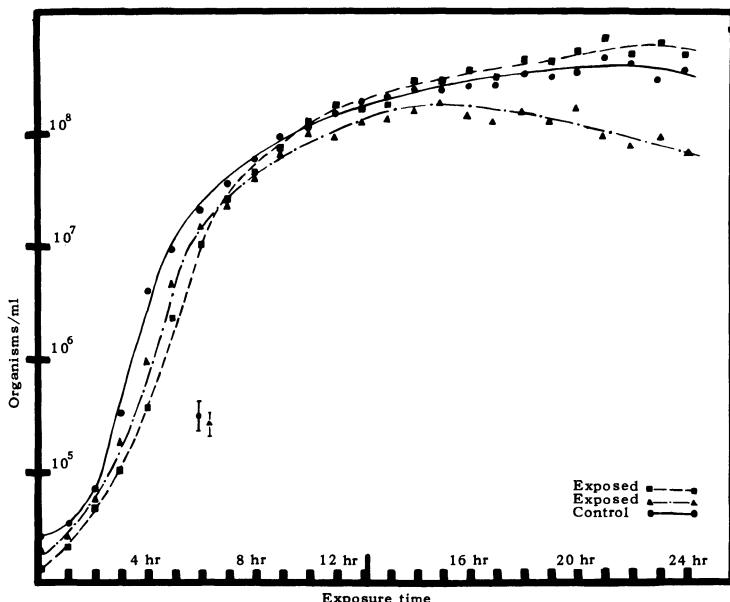


Fig. 3. Growth-curve response of *S. aureus* exposed to 14,000 Oe for 24 hr.  
Standard deviation indicated.

TABLE I

Statistical Analysis of Counts on S. Aureus

	18 h		21 h		24 h	
	Control	Exposed	Control	Exposed	Control	Exposed
Mean *	153.6	89.7	203	85	229	79
Average deviation *	48.8	37.1	72.7	36.6	64.6	36.6
Standard deviation * ( <i>S</i> )	72.5	42.6	102	54.2	89.1	46.7
Standard error * ( <i>S<sub>x</sub></i> )	27.4	16.1	38.6	20.5	33.7	17.6
Standard error of difference *		31.8		43.8		38.0
Degrees of freedom (DF)		12		12		12
<i>t</i>		2.01		2.71		3.94
Probability Level (PL)		1:15		1:55		1:400

\*Expressed in organisms/ml  $\times 10^6$ .

The response of the exposed organisms to a constant homogeneous field of 14,000 Oe was determined by running growth reponse curves on the cultures. One-milliliter samples were taken every hour, and the viable cells estimated by the serial dilution plate-count method. In order to compile data over a 24-hr exposure period, separate series of runs were conducted. Samples were taken during a 0-12 hr exposure period in a series of runs and during a 12-24 hr period in a second series. The results presented in the growth response curves are combined from the series of runs to give a continuous curve over the 24-hr period.

In the permanent magnet study, a hanging-drop slide culture of the organisms was placed between the poles of a horseshoe or round-shaped magnet. The response of the exposed organisms to a constant field of 700 Oe for 36 hr was determined by observations for morphological changes in Gram-stain preparations of the cultures. This method was also employed in observing morphological changes in the culture exposed in the electromagnet study.

The method used for determining a basic physiological response by E. coli involved the collection of the gas evolved from the fermentation of dextrose with an inverted Durham-type fermentation tube. The gas collected in the exposed and control samples was analyzed spectrometrically.

## RESULTS AND DISCUSSION

The response of S. aureus to a constant homogeneous field of 14,000 Oe is shown in Figure 3 on the curve designated as Exposed<sub>2</sub>. Under these experimental conditions, the growth of the exposed culture progressed in the same trend as the control for a period of 15 hr. At the 16th-hr sampling period, an inhibition of growth was exhibited which continued throughout the remaining period of exposure.

A statistical treatment of the counts of S. aureus at exposure times selected from the section of the growth response curve showing inhibition is presented in Table I. The averages for the control and exposed sample counts at 18, 21, and 24 hr were compared on the basis of the *t* test to determine if the two averages were significantly different. The data indicates that at the longer exposure times there is statistical justification for concluding that the two averages are significantly different and that a magnetic effect exists.

When the broth cultures were handled in a manner which exposed them to a variable field, from 14,000 Oe at the center to that found outside the magnet poles, the response of S. aureus obtained was not significantly different from the control, as shown in Figure 3 on the curve designated as Exposed<sub>1</sub>. The variation in the magnetic field was produced by removing the test tube from the field every hour when samples were taken. After the time necessary for obtaining the sample, about 3 sec, the tube was placed back in the field.

Based on the result obtained under the conditions of these experiments, it was concluded that S. aureus is affected by a constant homogeneous magnetic field of 14,000 Oe. However, it did not exhibit inhibition under conditions which exposed the culture to the same field if the exposure was interrupted hourly for 3 sec.

When S. aureus was placed in a constant homogeneous field of 700 Oe in the permanent magnet, a disarrangement of the cells from the typical grapelike clusters to single isolated cells was observed. This is shown in Figures 4a and 4b. A possible explanation of this response

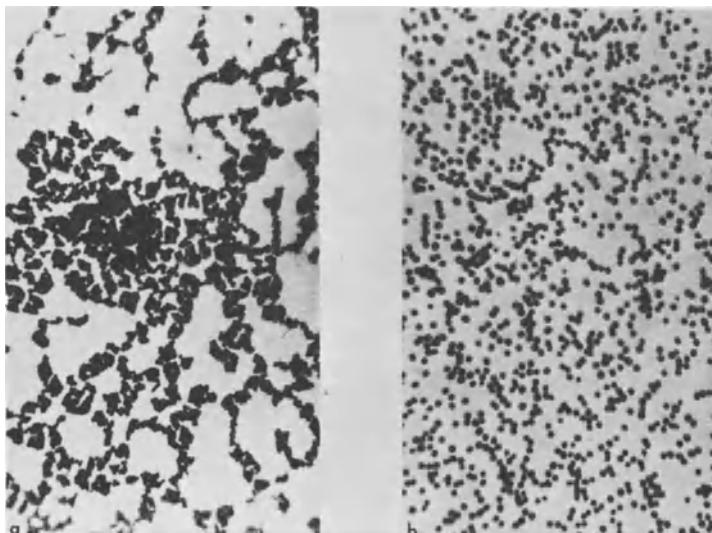


Fig. 4. (a) Photomicrograph of normal cell arrangement of S. aureus. Magnification ~1500. (b) Photomicrograph of S. aureus cells disarranged when exposed to 700 Oe in a permanent magnet.

is that the magnetic field produces a reversal of the charge on the cells, creating elemental magnetic fields which repel each other.

When S. lutea was exposed to the same constant homogeneous field used on S. aureus, no significant inhibition was observed.

With E. coli as the biosystem, no significant quantitative difference in cell level was indicated by the growth response curves. These results raised the interesting question as to whether a difference exists in the biological makeup between Gram-positive and Gram-negative micro-organisms in response to magnetic energy.

In an effort to establish a basis for a physiological response, the gas produced by E. coli when exposed for 48 hr in a homogeneous field of 14,000 Oe was analyzed for its composition. A slight difference in hydrogen-gas production was obtained in the exposed system. The slight stimulation of growth and gas production is not considered as a highly significant response of E. coli to high magnetic fields at this time. Further study is needed on this type of response.

A cybernetic analysis is currently being conducted on the data obtained in the growth-curve response experiments presented in this paper.

#### ACKNOWLEDGMENT

Aid and suggestions from members of the Bioscience Group and the Magnetic-Cryogenic Group in conducting this study, and review and support from Dr. E. L. Secrest in the Applied Science Laboratory, are greatly appreciated.

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## *Chapter 5*

### **Increase of Trypsin Activity**

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The mounting evidence of the biological effects of magnetic fields, reviewed in the other papers in this volume, and in particular the effects observed by our colleagues on the respiration of tumor and normal embryonic cells<sup>1</sup> (see also Part III, Ch. 1), made us wonder if the metabolic effects could directly involve enzymes. Since studies on the denaturation of trypsin by ultraviolet irradiation were under way in our laboratory, we decided to compare the results with the behavior of trypsin in a magnetic field.

Trypsin is a protein of modest size with a molecular weight of 23,800. A fair amount of information is available about the general structure of the molecule, and in particular it is known that histidine and serine are essential at the active site. While the native enzyme contains no free sulfhydryl groups,<sup>2</sup> it does contain six disulfide bonds.<sup>3</sup> A three-step mechanism for the hydrolyses catalyzed by trypsin has been proposed by several authors.<sup>4-6</sup> Neurath and Dixon<sup>7</sup> have suggested a modification of Cunningham's<sup>6</sup> scheme. In this scheme, hydrogen bonding between histidine and serine stabilizes the enzyme. Acylation of the enzyme by the substrate occurs at this active center. Recently, kinetic evidence has been presented for a functional carboxyl group, probably as aspartic acid.<sup>8</sup>

The inactivation of the enzyme by ultraviolet irradiation at 2537 Å has been extensively investigated (e.g., Augenstine and Ghiron,<sup>9</sup> Augenstine, Ghiron, Grist, and Mason,<sup>10</sup> McLaren and Luse<sup>11</sup>). Augenstine and Ray<sup>12</sup> have proposed a "weak-link" theory of inactivation which includes the following steps. The first step is the opening of a disulfide bond, which involves a small or negligible entropy change. The second stage, the breaking of neighboring bonds such as hydrogen bonds, is accompanied by an appreciable entropy increase. These two stages constitute reversible inactivation. The final stage of irreversible inactivation involves the rupture of all bonds constituting the weak link, including a second S-S bond.

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With this background of trypsin structure and the changes it undergoes upon ultraviolet inactivation, it is possible to make a comparison with the behavior of the enzyme in a magnetic field. Hence, we have undertaken a preliminary comparison of the response of trypsin to ultraviolet radiation of 2537 Å and to a magnetic field. Criteria have been the enzymatic activity, the changes in ultraviolet absorption, and the number of —SH groups per molecule.

#### MATERIALS AND METHODS

The trypsin used in all experiments reported in the present paper was the twice-crystallized salt-free enzyme, Lot No. 6134, from the Worthington Biochemical Corporation.

Enzyme activity measurements were made by the method of Erlanger et al.<sup>13</sup> In this procedure, enzyme activity was followed by the rate of hydrolysis of the substrate benzoyl-DL-arginine-p-nitroanilide hydrochloride (BAPA) at pH 8.2. The colored hydrolytic product, p-nitroaniline, was estimated spectrophotometrically at 410 m $\mu$  in a Beckman DU spectrophotometer. The stock solution of substrate was prepared by dissolving 43.5 mg of BAPA in 2 ml of dimethylsulfoxide and bringing the solution to 100 ml with 0.05 M Tris buffer, pH 8.2, containing 0.02 M CaCl<sub>2</sub>, or with barbital buffer of the same pH. The solution should be maintained above 26°C to avoid precipitation. It was always prepared freshly. Water (0.9 ml) was added to 5 ml of substrate stock solution and allowed to equilibrate for 5 min in a thermostatically controlled bath at 26°C for 5 min. At zero time 0.1 ml of trypsin solution was added and the reaction allowed to run for 600 sec, when it was terminated by addition of 1.0 ml of 30% acetic acid. A suitable control without enzyme was also used. The intensity of color at 410 m $\mu$  was then measured and compared with a previously made calibration curve of optical density vs. trypsin concentration. We used a microliter syringe with a capacity of 100  $\mu$ l, the experimental error not exceeding  $\pm 1\%$ .

Ultraviolet absorption spectra of trypsin solutions were measured directly in the Beckman DU spectrophotometer. The spectra of trypsin incorporated in discs, as described later, were also measured directly in the Beckman spectrophotometer by the use of a specially designed holder for the discs.

Sulphydryl groups were determined by the method of Klotz and Carver.<sup>14</sup> In this procedure the sulphydryl groups were titrated with salyrganic acid in the presence of pyridine-2-azo-p-dimethylaniline as an indicator. The titration was carried out in a special cell, described by Klotz and Carver, and was fitted directly into the Beckman DU spectrophotometer so that absorbancy could be read at 550 m $\mu$ . The titration employed a micrometric syringe buret so that near the equivalence point the mercurial could be added in increments of 0.03 ml.

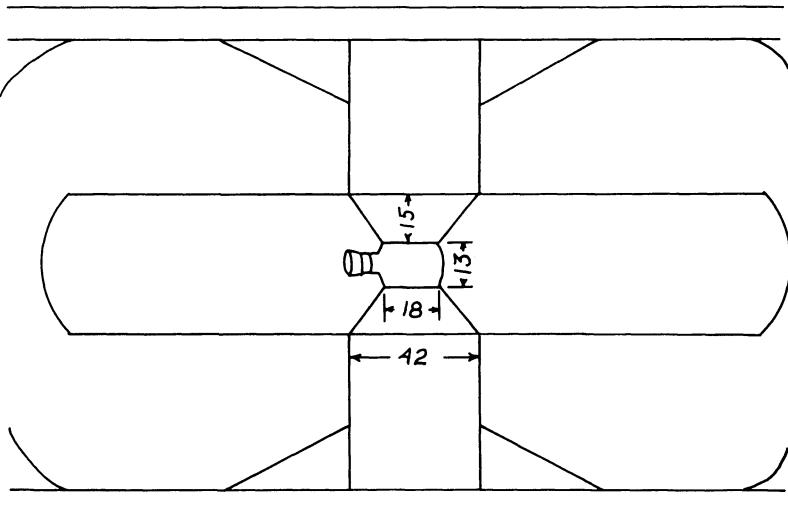


Fig. 1. 8000-Oe double-horseshoe magnet. Dimensions in millimeters.

Stock solutions were made up in 0.1 M acetate buffer, pH 5.8. The dye solutions were about  $2 \times 10^{-4}$  M (the exact concentration need not be known). Salyrganic acid was weighed accurately to give stock solutions of  $1 \times 10^{-3}$  M in acetate buffer containing  $5 \times 10^{-3}$  M sodium chloride. Dye solution may be kept for a month, the mercurial for not over 1 week. Dye solution was diluted to a volume of 9 ml, i.e., a concentration of about  $8 \times 10^{-5}$  M in the titration vessel. After deoxygenation with nitrogen, exactly 1 ml of trypsin solution was added to the titration vessel and an absorption reading was taken. Salyrganic acid was added and several readings were taken to establish a base line before equivalence was reached. After addition of each increment of salyrganic acid, nitrogen was directed into the cell to mix the solution. Buffer solution alone was placed in the reference absorption cell.

Ultraviolet irradiation of trypsin solution by exposure for 1- to 5-min increments for periods up to 20 min was accomplished with a Sperti germicidal ultraviolet lamp, which emits 90% of its energy at 2537 Å. The enzyme solution completely filled a stoppered cylindrical quartz cell (1 cm  $\times$  6 cm) which was slowly rotating (1 rpm) and which was 3 cm from the lamp and at approximately 23°C. Trypsin in KBr discs, described later, was also irradiated under similar conditions for periods up to 8 hr.

Trypsin solutions were exposed to a magnetic field of 8000 Oe, with an average gradient of about 220 Oe/cm, obtained from a double-horseshoe alnico permanent magnet with a pole gap of 13 mm. A specially constructed circular glass cell which was designed to fit snugly between the poles contained the solution. Trypsin in KBr discs could be exposed to a magnetic field of 15,000 Oe with an average gradient of about

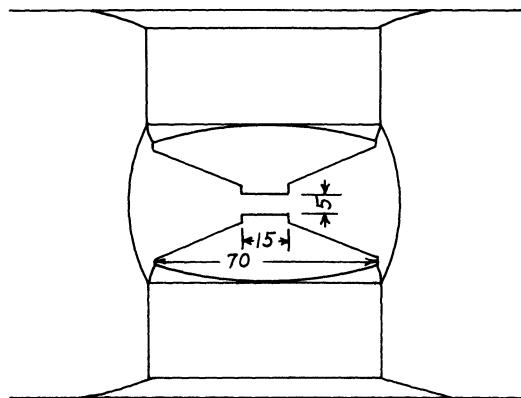


Fig. 2. 15,000-Oe quadruple-horseshoe magnet. Dimensions in millimeters.

530 Oe/cm in a quadruple-horseshoe magnet with a pole gap of 5 mm. The first field is equivalent to 1.8 par, the second to 8 par paramagnetic strength (1 par =  $10^6$  Oe $^2$ /cm). Early observations were made at room temperatures at approximately 23°C, but for all later measurements the magnets were placed in an incubator at 32° or 36°C. Unexposed control samples of both solutions and discs were also placed in the incubator but were not placed in dummy magnets. Diagrams of the magnets are shown in Figures 1 and 2.

Trypsin solutions were prepared in concentrations of 270 to 500 µg/ml in 0.001 M HCl, pH 3.3, for all work. These solutions could be used directly for enzyme activity, ultraviolet absorption, or sulfhydryl measurements. For exposure to the high-field magnet, trypsin was incorporated at a level of 0.75% in 300-mg KBr discs. The KBr and trypsin, screened through a 200-mesh sieve, were weighed and pressed into clear discs 1 cm × 0.8 mm at a pressure of 10,545 kg/cm $^2$  (150,000 lb/in. $^2$ ). In addition to fitting the pole gap of the large magnet, these discs had the advantage of chemical stability and, hence, could be exposed for periods up to 8 hr. Exposure of trypsin solutions was limited for the most part to 3 hr because even at pH 3.3 trypsin solutions undergo inactivation. Assays and spectral determinations were made within 10 min in solution. Spectra of discs were also run immediately, but redeterminations made after periods of up to 2 months showed no change.

## RESULTS

### Ultraviolet Irradiation

Table I shows the results of a typical inactivation of trypsin in solution (270 µg/ml in 0.001 N HCl) by ultraviolet irradiation at 2537 Å as a function of time at 23°C. These results are much as expected from the literature.

TABLE I

Inactivation of Trypsin Solution (270  $\mu\text{g}/\text{ml}$  in 0.001 N HCl) by UV Radiation (2537 Å)

Time, min	Optical density at 410 m $\mu$	% original activity
0	0.330	100
1	0.230	70
2	0.165	50
3	0.123	36
4	0.083	25
5	0.068	21
10	0.054	16
15	0.044	13
20	0.044	13

Figure 3 presents typical effects on ultraviolet absorption of trypsin in solution (450  $\mu\text{g}/\text{ml}$  in 0.001 N HCl) exposed to ultraviolet of wavelength 2537 Å for varying periods of time. Irradiation elevates the absorption over the whole spectrum, with the trough at 252 m $\mu$  filling in at a greater rate than the peak at 275 m $\mu$  rises, thus reducing the ratio of maximum to minimum exponentially with the time of irradiation. Longer periods of irradiation essentially eliminate selective absorption.

Native trypsin, as previously pointed out, contains no free sulfhydryl groups but possesses six disulfide bonds. Under our conditions, irradiation of trypsin solutions for 30 min with ultraviolet light at 2537 Å nearly completely inactivates the enzyme and causes the appearance of approximately  $3.0 \pm 0.5$  —SH groups per molecule. This

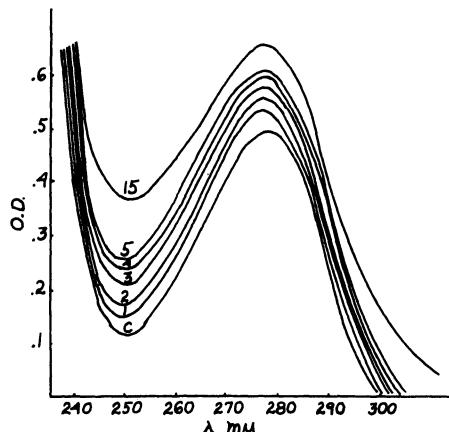


Fig. 3. Effect of ultraviolet irradiation (2537 Å) on ultraviolet absorption of trypsin (450  $\mu\text{g}/\text{ml}$  in 0.001 N HCl). Curve C is for native trypsin. The numbers on the curves designate exposure times in minutes.

can be compared with Augenstine and Ghiron's<sup>9</sup> finding of approximately three groups under somewhat different conditions and using different procedures for activity and sulphydryl determinations.

### Magnetic Field Exposure

Table II shows the results of five experiments (representative of 29 performed) on the exposure of trypsin solutions to the 8000-Oe field for periods up to 3 hr. These experiments are chosen to illustrate the use of varying concentrations of trypsin and various temperatures. Relative activity was obtained by comparing exposed samples with control samples maintained for the same period of time under the same conditions. It should be emphasized that in none of our experiments was the field found to be without effect or to cause inactivation. In all cases an increase in activity was observed upon exposure for 2 hr, with the effect leveling off or slightly decreasing after exposure for another hour. The data for 0 hr (control) of exposure were compared with the combined data for the 1, 2, and 3 hr exposures for all 29 experiments in an analysis-of-variance test, giving  $F = 14.26$ , or  $p < 0.001$ .

TABLE II  
Exposure of Trypsin in Solution to 8000-Oe Magnetic Field

Conc., $\mu\text{g}/\text{ml}$	Temp., °C	Exposure, hr	Optical density at 410 m $\mu$	Relative activity
323	36	0	0.407	100
		1	0.461	110
		2	0.468	112
		3	0.475	114
318	36	0	0.387	100
		1	0.429	113
		2	0.469	123
		3	0.457	120
427	32	0	0.518	100
		1	0.545	105
		2	0.611	118
		3	0.590	114
403	32	0	0.485	100
		1	0.518	107
		2	0.522	108
		3	0.492	102
504	Room (app. 23)	0	0.612	100
		1	0.650	106
		2	0.658	108
		3	0.657	107

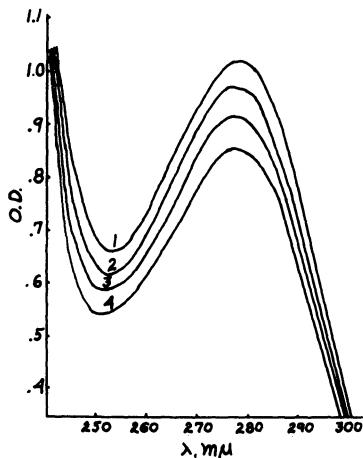


Fig. 4. Effect of exposure of trypsin (0.75% in KBr disc) to a magnetic field of 15,000 Oe. Curve 1—native trypsin; curve 2—2-hr exposure; curve 3—5-hr exposure; curve 4—7-hr exposure.

Exposure for longer intervals in solution was somewhat untrustworthy because of the spontaneous inactivation of trypsin solution over long periods of time. In our experience the trypsin solutions could be maintained for 3 or 4 hr at the temperatures used without an alteration in activity greater than 1%.

Exposure of trypsin to magnetic fields resulted in a decrease in ultraviolet absorption. Figure 4 represents the ultraviolet absorption spectra of trypsin incorporated in KBr discs which were exposed to a field of 15,000 Oe for up to 7 hr. Trypsin in discs is used for illustration because longer exposure periods can be made with discs than with solutions. Changes in solution are qualitatively similar but, of course, differ quantitatively. The ultraviolet absorption over the whole spectrum was decreased but the maximum-to-minimum ratio was increased. Discs (native, irradiated, or exposed to a field) stored for as long as 2 months in a desiccator gave the same absorption spectra as when originally measured.

Trypsin exposed to 8000 Oe in solution revealed no free sulfhydryl groups, unlike the ultraviolet-irradiated enzyme.

#### DISCUSSION

The evidence presented indicates that exposure of trypsin to ultraviolet irradiation of 2537 Å produces opposite effects to those resulting from exposure to a magnetic field in terms of enzyme activity, ultraviolet absorption, and release of —SH groups. Because of inactivation of trypsin in solution over periods of time, we do not know the permanence of the field effects observed in solution. However, some degree of permanence is suggested by the finding that trypsin exposed in KBr discs retains its ultraviolet spectral characteristics for at least 2 months.

It would be very desirable to learn whether changes can be detected by other means and, in particular, by infrared absorption. Instrumental limitations have prevented us from making such studies, but meager preliminary infrared-absorption determinations (kindly performed by Dr. Dieter Hummel) are consonant with our results. Exposure of trypsin (0.75%) in KBr discs to the 15,000-Oe field for 2 hr produced about a 5% increase in absorption at  $3\mu$ , suggesting an increase in  $-\text{NH}$  stretching vibration, but no change at  $9\mu$ . Exposure to ultraviolet for 1 hr broadens the band around  $9\mu$ , which would indicate a decrease in coupling, and decreases the absorption due to hydrogen bridges; no change was found at  $3\mu$ .

Denaturation of proteins involves disorganization of the internal structure of the molecule by cleavage of hydrogen bonds and by elimination of bonds contributing to tertiary structure. In trypsin solutions, ultraviolet irradiation causes excitation of the vibrational modes of hydrogen atoms, which "is essential for the initiation of a charge-transfer process which ultimately leads to sufficient modifications of the macromolecular conformation, so that inactivation results," in the words of Augenstine et al.<sup>10</sup> Augenstine and Ghiron<sup>9</sup> and McLaren and Luse<sup>11</sup> have demonstrated that inactivation of trypsin by ultraviolet irradiation at 2537 Å involves breakage of disulfide bonds, which is confirmed by our data on sulphydryl determination.

Augenstine's<sup>12</sup> three-stage weak-link theory of trypsin inactivation, involving the opening of a disulfide bond, breaking of neighboring hydrogen bonds, and final rupture of other bonds, including a second disulfide bond, was summarized above. Is it possible that the observed effects of magnetic fields may to some extent be the reverse, in the sense of bringing about a better-ordered array of the molecule, perhaps by affecting the polar groups which can form hydrogen bonds or can ionize? At the same time the hydrophobic interaction of nonpolar side chains might modify the strength of hydrogen bonds by restricting rotation of the side chain. These postulated alterations might be intramolecular or intermolecular. In the latter case, a dimerization of trypsin molecules through bonding of the inactive portions of the molecule to give a dimer with an active "head" and "tail" is conceivable. These changes, of course, are speculative. They might be expected to result in an entropy decrease, as suggested by Casimir<sup>13</sup> as early as 1940. Careful studies of possible enthalpy changes in a magnetic field would be difficult, but might throw light on the problem.

It should be pointed out that at present we have no evidence that inactivation by ultraviolet can be reversed by subsequent exposure to a magnetic field. As would be expected, trypsin solutions irradiated with ultraviolet for 30 min and nearly completely inactivated show no restoration of activity or reduction in the number of  $-\text{SH}$  groups after exposure to an 8000-Oe field for 1 or 2 hr. Exposure of trypsin solutions to ultraviolet for 1 min resulted in 30 to 35% loss of activity and

the appearance of approximately 0.7 to 0.8 —SH group. Subsequent exposure to the 8000-Oe field for 1 to 2.25 hr caused no reversal.

The results reported are frankly exploratory. Other means of comparison between native, ultraviolet, irradiated, and magnetically exposed trypsin should be used, including infrared absorption. Other protein properties—viscosity, sedimentation, and the like—are of interest. Obviously, the effects of impurities are important. While the trypsin employed was a twice-crystallized commercial preparation which has been used by a number of workers, more highly purified enzyme should be studied.

Field strength and nature of the field should be investigated. It is expected that fields of various intensities can be compared in the near future. Homogeneity of the field also may be important. This is suggested in biological investigations (e.g., Gerencser, Barnothy and Barnothy;<sup>16</sup> see also Part III, Ch. 3).

Our studies were conducted at a limited number of temperatures and no correlation between temperature and the effects of magnetic fields has been observed. Likewise, no correlation has been seen between concentrations and field effects. However, systematic study of temperature and concentration should be undertaken over wide ranges. The pH of the trypsin solution has been maintained in the region of maximum stability in our work. Variation of pH could be studied with due attention to pH effects. Finally, it is obvious that other enzymes and proteins should be investigated. Enzymes, of course, have the experimental advantage of an activity which can be measured.

Limited experience with trypsin from another source has indicated a variability of enzyme activity. One sample of low activity gave little or no significant response to a magnetic field. Lyophilization, in our experience, lowers enzyme activity, but if crystalline trypsin is exposed to a magnetic field before lyophilization it retains its increased activity relative to lyophilized native trypsin. These findings suggest that the prior history of trypsin may affect its behavior in a magnetic field.

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*Chapter 6*

## Magnetic Reactivation of Partially Inhibited Trypsin

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The preliminary observations by Cook<sup>1</sup> and Smith<sup>2</sup> (see also the previous chapter) of changes in the activity of trypsin following exposure to a static magnetic field have been reinvestigated. Under similar, but more precisely defined conditions, we have observed that partially inhibited trypsin preparations show a 4-12% restoration of activity. Reactivation has been observed in our experiments following deactivation by egg-white trypsin inhibitor and apparently also following deactivation by 1-3 hr autolysis at pH 7-8 in the absence of calcium ions. No reactivation was observed after deactivation by ultraviolet exposure or treatment with soy-bean trypsin inhibitor or diisopropylphosphoro-fluoridate (DPF). Reactivation of the inhibited preparation following exposure to the magnetic field in no case exceeded the usual activity level of the uninhibited enzyme. The maximum reactivation (9-12%) followed the longest magnetic exposures (1008-1106 min). An exposure of 90 min coupled with slight (4%) inhibition appears to be insufficient.

The activity of the trypsin was determined by spectrophotometric measurement of the concentration of p-nitroaniline during hydrolysis of the artificial substrate benzoyl-DL-arginine-p-nitroaniline hydrochloride (BAPA) as described previously.<sup>3</sup> The procedure used previously<sup>1</sup> was modified to reduce observational deviations in volumetric and time measurements. The procedure for exposure and analysis consisted of the addition of 40.0 mg of trypsin to 100 ml of a solution which contained 0.001 M hydrochloric acid and 0.020 M calcium chloride. This solution was divided into two parts, one of which was treated to partially inhibit activity. When necessary, the pH was readjusted to 3.0 using 0.2 M hydrochloric acid. Each of these two parts was then separated into two samples, one of which was exposed. All four samples

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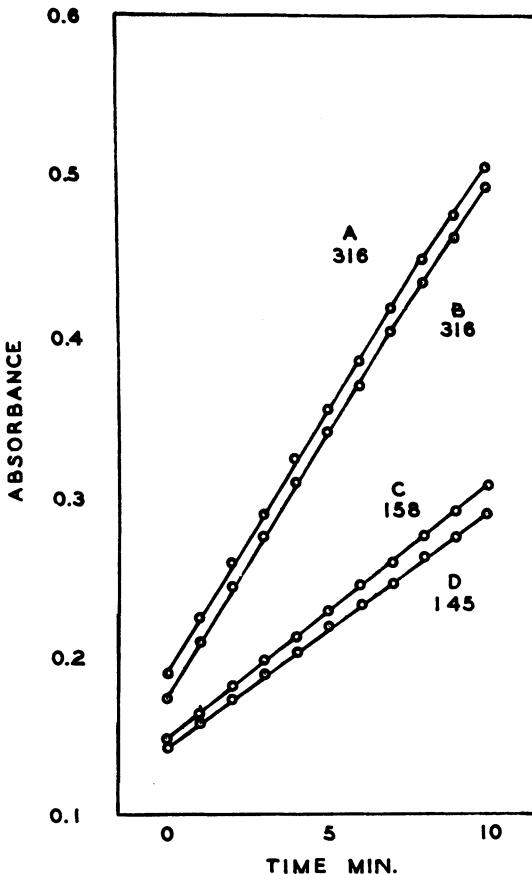


Fig. 1. Change in UV absorbance at  $410\text{ m}\mu$  with time for trypsin cleavage of BAPA at  $30^\circ$ . A—Uninhibited, exposed; B—uninhibited, unexposed; C—inhibited (egg white), exposed; D—inhibited (egg white), unexposed. Slopes are given in computer readout for least-squares regression analysis on 1-min observations.

received the same dilution and temperature treatment. After exposure, samples were chosen in a random manner for analysis and analyses on all four samples completed within 3 hr. The analysis was performed by adding 2.0 ml of solution containing  $4\text{ }\mu\text{g}$  of trypsin to 10.0 ml of buffer-substrate solution. The buffer-substrate solution had a pH of 8.15 and contained 1.0 mM BAPA, 20.0 mM calcium chloride, and 12.5 mM tris[hydroxymethyl]aminomethane. The temperature was maintained during the enzymatic hydrolysis at  $30^\circ$  to avoid solubility changes and temperature-related variations in rates. The magnetic field exposures were made at room temperature ( $27 \pm 1^\circ$ ) in a 5000-Oe homogeneous field of a 9-inch Varian Model V-3401 electromagnet equipped with VFR 2503 "Fieldial" Regulator.

The absorbance at  $410\text{ m}\mu$  was measured at 1-min intervals for a period of 10 min with a Beckman DU spectrophotometer. Illustrative data are shown in Figures 1 and 2. In Table I, the activity values for several experiments are presented. These are expressed in arbitrary values of 1000 times the increase in absorbance values over the 10-min period of the experiment. This increase was determined from the slope obtained by a least-squares regression analysis with a 1620 computer. The nonlinear terminal data were not included. The trypsin (Worthington Biochemical, 2 $\times$  Cryst., Lyophilized Salt Free, TRL-6227A; Worthington Biochemical, 2 $\times$  Cryst., Salt Free, TRSF-161; Nutritional Biochemicals, 2 $\times$  Cryst., Salt Free, Lot No. 7485) was stored at room temperature prior to use.

Inhibition was produced by exposure in a quartz vessel to ultra-violet light (Hanovia Mercury Lamp No. 30600) for 1 min, by autolysis at pH 7-8 without calcium ions for 1-3 hr at  $30^\circ\text{C}$ , by addition of  $100\text{ }\mu\text{g}$  of egg-white trypsin inhibitor (Nutritional Biochemicals Corporation,

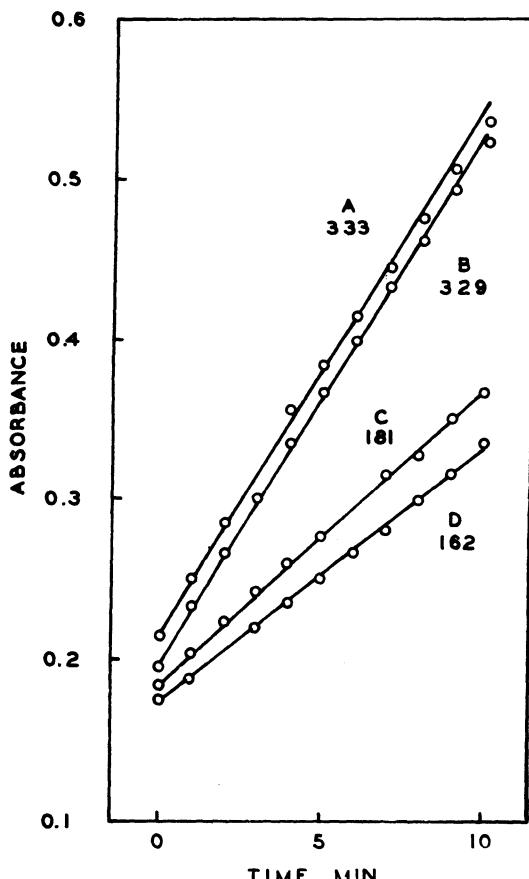


Fig. 2. Legend as in Figure 1.

TABLE I  
Combined Inhibition and Magnetic Effects

Method of inhibition	Activity (arbitrary units)				Time of 5000-Oe exposure, min	Inhibited activity ratio Exposed Unexposed		
	Uninhibited		Inhibited					
	unexp.	exp.	unexp.	exp.				
Egg White	325	326	312	316	90	1.01		
"	329	333	162	181	1008	1.12		
"	200	203	188	197	195	1.05		
"	205	201	186	193	195	1.04		
"	316	316	145	158	1008	1.09		
"	299	298	275	288	1106	1.05		
Autolysis	440		375	395	190	1.05		
Soy Bean	375		263	266	234	1.01		
Ultraviolet	288	290	110	112	180	1.02		
DPF			27.4	27.2	480	0.997		

Lot No. 5200) in 1 ml of buffer solution, and by addition of 24  $\mu\text{g}$  of soy-bean inhibitor (Worthington Biochemical Corporation, Lot No. S15490 in 0.1 ml buffer solution). The diisopropylphosphorofluoridate-inhibited sample was a commercial preparation (Worthington TDIP, no lot number given).

Variations of the activity of the enzyme from 200-440 units have been observed in control preparations. These are attributable to variations in sample lots, methods of preparation of enzyme, and concentration. These variations in the activity of the control do not alter the response to magnetic reactivation within this range. Thus, reactivation has been observed from control levels of 200-400 prior to deactivation by inhibitor.

The data now available indicate that, for certain types of deactivation, magnetic field exposures reactivate trypsin after partial loss of its activity. Presumably, the magnetic field reorganizes the enzyme at or near the active site. The effect may be attributable to the re-establishment of certain hydrogen-bonded structures or related dipolar structures. Chemical bondings are apparently not altered in this magnetic reactivation process since deactivation by processes known to involve chemical bond changes, such as with ultraviolet exposure<sup>4</sup> or treatment with DPF,<sup>5,6</sup> has not been reversed by magnetic exposure. Several variables appear to be highly critical and further experiments to evaluate these carefully are in progress.

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*Part IV*

**Effects of Very Weak Magnetic Fields**

## *Chapter 1*

# Responses of Planarians and Snails

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It is becoming increasingly apparent, especially from studies of biological rhythmicity and animal orientations in space, that living things are sensitively responsive to physical factors of their environment above and beyond those commonly recognized as effective ones. Certain readily demonstrable properties related to the timing and phasing of persistent biological rhythms and to animal homing abilities appear to defy plausible explanation solely in terms of conventional physiological responses and reactions.

These foregoing kinds of phenomena suggest that the living system is sensitive to pervasive geophysical forces with time-intensity variations related to all the major natural periods of the earth's atmosphere. The phenomena suggest, in addition, that the effective subtle forces also convey information to the organism concerning spatial orientation in its geographic environment.

It is of great interest to learn whether geomagnetism may in some manner be involved in organismic adaptation to the physical environment. Time-intensity variations of numerous parameters of terrestrial magnetism appear to possess periods reflecting the natural atmospheric rhythmic changes. Furthermore, since magnetism is a vector force, it could theoretically provide information of important adaptive significance for spatial orientation of organisms. The vector patterns of magnetism also vary over the earth's surface. It has been difficult to believe that the very small energies contained in geomagnetic fields are capable of eliciting any biological response. However, it is equally difficult to conceive biological effectiveness of the alternatively available possible pervasive geophysical forces.

Experiments were designed to determine whether a living organism could perceive differences in strength and direction of the horizontal vector of very weak artificial magnetic fields. Other experiments were designed to learn whether organisms were capable of responding to the

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earth's own magnetic field. It was presumed that any perceptive mechanism of adequate sensitivity for such responsiveness would be saturated out by the far stronger fields which have generally been employed in biological experimentation. It was further presumed that if magnetism were normally in any manner related to biological clocks or biological compass problems, responses to this force would probably vary with the natural geophysically-correlated clock-periods and with geographic orientation of the organisms. Therefore, the experiments were designed in such a manner as to permit recognition of these possibilities if they were present.

Finally, it was presumed that any observable responses to such weak magnetic fields would display characteristics of a typical organismic response to a stimulus. The magnetic-field perception would play the role of perception of a typical stimulus pattern comparable to that of more conventional ones triggering an organismic response. And as with response to many other kinds of stimuli, the nature of the response would be altered by numerous factors.

Special sensory systems of living organisms appear invariably to be a consequence of particular cells, tissues, or organs becoming highly specialized to deal with a single stimulus modality to which all cells display at least some sensitivity. Exteroceptors, dealing with external physical stimuli, are normally located adaptively at or near the body surface. Magnetism, because of its highly pervasive character, might affect directly any, or every, cell in the body of an organism. Potentially, it could influence and modify in a differential fashion not only the whole pattern of sensory inflow into the coordinating and integrating centers of the organism, but could even modify differentially in time and space the activity within these centers directly.

#### METHODS

The spontaneous orientation reactions of animals provide a simple and sensitive means for measuring biological response to weak magnetic fields. Figure 1A illustrates the kind of apparatus used to quantify such reactions in the mud snail. A flat-bottom glass crystallizing dish containing an aluminum corral and filled to a depth of 2 cm with sea water is centered over a polar coordinate grid. Mud snails are placed in the corral and are permitted to emerge through the narrow corridor. When each snail reaches the arc 3 cm from the opening of the corridor, its orientation is recorded as the number of the sector in which its largest portion is located. This simple type of apparatus has been easily modified to accommodate orientational responses in *Dugesia*, *Drosophila*, and the microorganisms *Paramecium* and *Volvox*.

In operation the apparatus is placed within a box which furnishes a constant light field (either symmetrical or asymmetrical) as shown in Figure 1B. Through a shielded opening above and behind the corral the

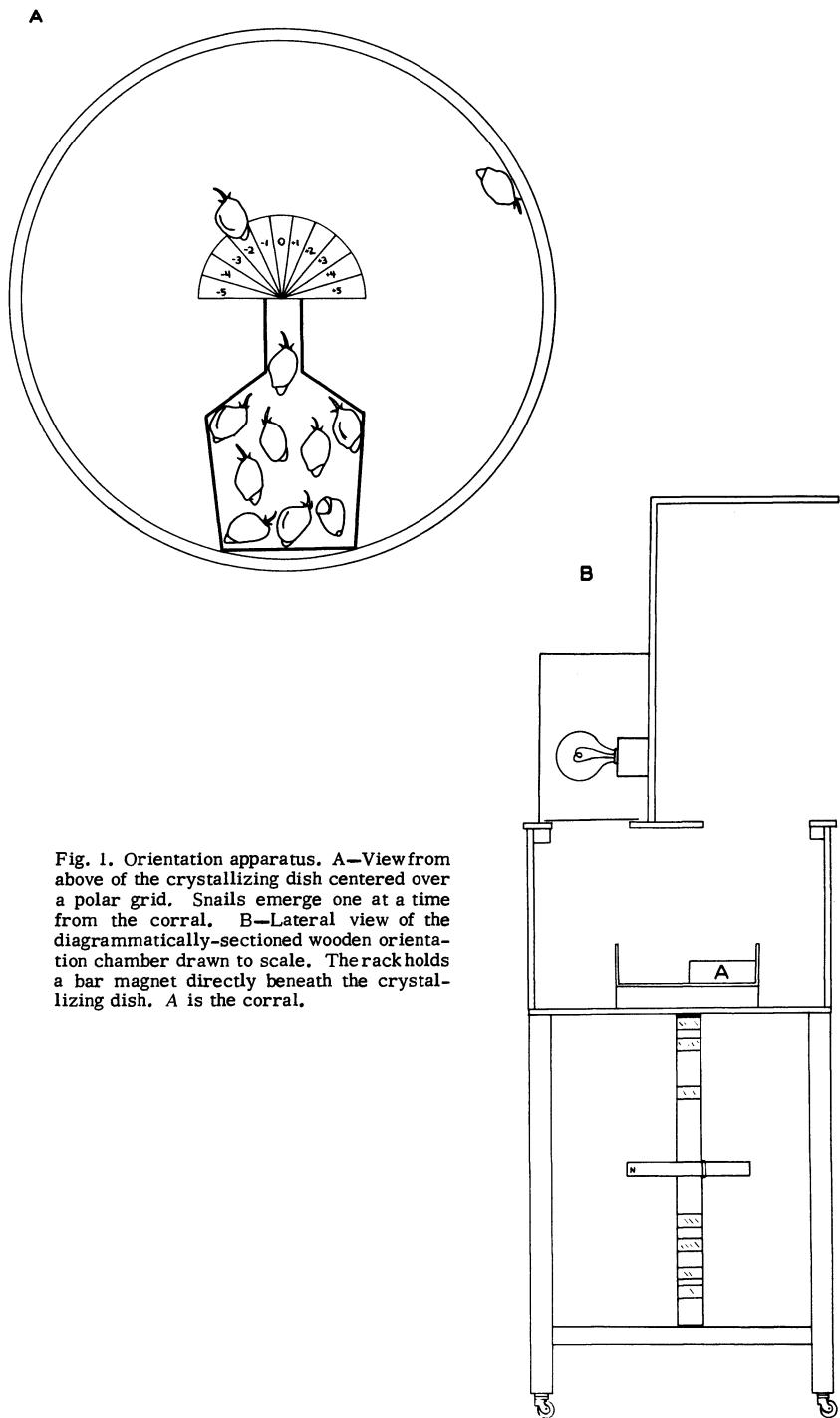


Fig. 1. Orientation apparatus. A—View from above of the crystallizing dish centered over a polar grid. Snails emerge one at a time from the corral. B—Lateral view of the diagrammatically-sectioned wooden orientation chamber drawn to scale. The rack holds a bar magnet directly beneath the crystallizing dish. A is the corral.

animals may be observed and recorralled. Beneath the box and centered directly under the polar grid is a wooden rack, freely rotatable about its vertical axis and containing calibrated slots to receive the bar magnet. By placing an 18-cm alnico bar magnet horizontally in slots at prescribed distances from the dish, a range of field strengths from 0.04 to 10.0 Oe and any orientation of the horizontal component can be obtained. The horizontal component of the natural magnetic field may, therefore, be augmented, reversed, or otherwise modified. The field produced at the level of the dish is relatively uniform and homogeneous in the small space traversed by the organisms, especially for the lower field strengths. The entire ensemble is mounted on casters so that it can be rotated to face any compass direction.

At this point several conventions should be recalled. First, the pole of a magnetic compass which points to the north magnetic pole is a north pole. Second, the direction of a magnetic field is the direction in which a north pole would move along one of the lines of force of the field, or the direction in which the north pole of a small compass placed in the field would point. For a bar magnet the direction of the field is toward the south pole of the magnet; and so, in the following account, the direction of an experimental field is the same as the direction in which the south pole of the bar magnet producing it is pointed.

Lest confusion arise when these conventions are applied to the earth's magnetic field, it should be recalled also that the north magnetic pole, the pole in the northern hemisphere, is the south pole of the geomagnetic field; and the direction of the geomagnetic field is north.

## EXPERIMENTS AND RESULTS

### Initial Demonstration of the Magnetic Response

Mud Snails. During the summer of 1959 an intensive examination for magnetic responsiveness was made at the Marine Biological Laboratory in Woods Hole, Massachusetts. The mud snail Nassarius obsoletus was selected as the experimental animal simply because it could be collected in abundance at low tide from nearby mud flats and because it possesses convenient behavioral attributes for measuring orientational reactions.

A simple experimental design was used. Each experiment comprised six sets of ten snail emergences—two sets in the earth's magnetic field, whose horizontal component is about 0.17 Oe in Massachusetts; two sets in an augmented horizontal field of 1.5 Oe which was north-directed and parallel to the initial path of emergence; and two sets in a 1.5-Oe horizontal field which was east-directed and at right angles to the path of emergence. The orientation chamber was symmetrically illuminated and directed southward. The six sets were run in scrambled order each time and the observer was not informed of the absence or presence of the bar magnet until the conclusion of the experiment.

In view of innumerable demonstrations of diurnal and tidal variations in the response patterns of organisms, it seemed clearly possible that any snail orientational response to modifications in geomagnetism might also be of a variable nature rather than of an unvarying and direct one. Accordingly, experiments were conducted at all hours between 5 AM and 9 PM to explore the possibility of time-related alterations in response.

Some results of the 2-month study are presented in Figure 2. Summarized are 564 experiments involving more than 33,000 individual orientations. When the mean amount of turning is considered for each hour, a daily variation is readily apparent, for both the experimental and control groups. Snails tended to move straight ahead in the early morning, turning progressively to the left to a maximum over the noon hour and then turning less so until about 7 PM.

The lower half of the figure depicts the magnetic effect expressed as the hour-by-hour differences between the means of controls in the earth's field and the experimentals. It is of great interest that a diurnal variation is also present in the magnetic effect. Between 7 AM and 9 PM the probability is less than  $10^{-3}$  that the experimentals and controls belong to the same population. On the other hand, during the early morning the presence of the magnet appears to produce turning in the opposite direction. The significance of the structure of the daily variation in magnetic effect will be commented upon below.

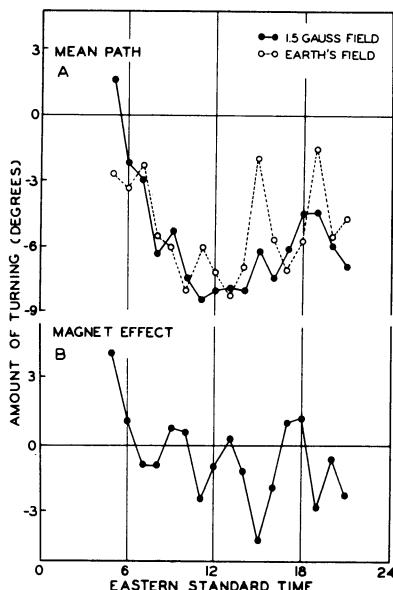


Fig. 2. A—Mean path of experimental and control snails as a function of hour of the solar day. The standard error for each point is about  $1\frac{1}{2}^{\circ}$ . B—Differences between the mean path of experimental and control snails for each hour. (1 Gauss = 1 Oe.)

For many organismic processes possessing diurnal variations, appropriate analyses have disclosed the simultaneous occurrence of lunar daily variations. These variations are a consequence of the 24 hr and 50 min period of the earth's rotation relative to the moon. These same snail data possessed a lunar daily variation. When differences of experimentals from controls were taken for each lunar hour, a magnet effect rather similar in gross trend to that of the solar daily magnetic effect was discovered. During the four hours prior to moonrise the magnet induced right-turning. Except for another peak of right-turning just prior to upper transit, the magnet throughout the rest of the lunar day induced increased left-turning until an hour before lower transit. Interestingly, the form of the lunar daily variation was practically superimposable upon the form of the lunar daily component in the spontaneous activity of white mice recorded under constant conditions during an overlapping period in Evanston, Illinois.

When both solar and lunar daily variations are present, they would be expected to produce by periodic interference longer-term variations whose periods are equal to, or submultiples of, the  $29\frac{1}{2}$ -day synodic month. Such an expectation was confirmed for the magnet response by the discovery of a semimonthly, or 15-day, variation in the amount and direction of induced turning. Experimental snails turned to the right of controls on the days just before each new and full moon and turned maximally to the left of the controls near the times of the moon's quarters.

Further analysis was directed to the question of whether the snails could distinguish between the N-S and E-W orientations of the experimental field. The answer was clearly in the affirmative, although, again, the nature of the response was one of systematic variation rather than of invariability. For example, in the early morning hours the E-W field was more effective in producing left-turning; this was followed by a gradual drift toward increasing relative effectiveness of the N-S field. Shortly before noon the N-S field displayed maximum relative effectiveness in producing comparable turning, whereafter there was a return to a greater effectiveness of the E-W field in the evening. In a similar fashion experimentals in the two fields were found to contribute quite different patterns to the mean semimonthly variation in response to the 1.5-Oe field, suggesting the simultaneous presence of a lunar daily variation in relative response to the two orientations. The E-W field produced relatively much greater left-turning about 2 to 4 days before the lunar quarters, whereupon a rapid reversal occurred, the N-S field producing comparably greater left-turning immediately after the lunar quarters.

In summary, this initial study demonstrated that at least one organism, the mud snail, can respond to a horizontal magnetic field only about nine times stronger than the earth's field. It indicated that the snail can distinguish parallel from right-angle horizontal orientations of this

field relative to its body axis. Clearly the character of the snail response to magnetism is a complex one, related to its physiological clock systems.

Planarians. To determine if the magnetic response was of wider biological distribution, the study was extended to include the common planarian Dugesia dorotocephala, a nonmarine organism which is phylogenetically rather far removed from the snail.

For flatworms the orientation apparatus was a glass petri dish centered over a polar coordinate grid within a black-lined chamber. The interior of this chamber was illuminated through a small circular opening in the top directly over the origin of the grid. The only other light in the system was a weak source placed horizontally, parallel to the zero axis of the grid to the rear of the dish. When a photonegative planarian was brushed into the horizontal light just short of the origin of the grid, it moved away from the light source, i.e., through the origin of the grid parallel to the zero axis. When the worm reached the origin the horizontal light was extinguished. The deviation in worm path from the initial direction was then recorded as the point, to the nearest 5°, where the worm crossed the circular arc 1 in. from the origin.

In this initial study the experiment comprised 45 to 140 orientations each in the earth's field, an east-directed 4-Oe horizontal field, and also, in most cases, a north-directed 4-Oe horizontal field. During the three months from November 1960 through January 1961, 53 experiments were conducted, always between 9 AM and 3 PM to minimize the effect of any daily variation. The apparatus was directed toward magnetic north.

Results of this series of experiments are presented in Figure 3. Quite conspicuously, worms in the earth's field displayed a monthly rhythm in direction and amount of turning. At the time of new moon the worms on the average turned about 10° to the left of the zero axis; at the time of full moon they turned about 10° to the right. This monthly rhythm was subsequently found to undergo an annual variation in which the form of the monthly cycle illustrated was repeated during the late fall and winter months of 1961-2.

Response to the experimental fields manifested itself in substantial alterations of the synodic monthly cycle. When the same worms evincing the monthly cycle in the earth's field were made to orient in the E-W field, the amplitude of the cycle was reduced; in the N-S field the cycle was abolished. Again it is clear that the apparent effect of the experimental magnetic field depends upon the rhythmic behavior of the organism. For example, the effect of the east-directed 4-Oe field at the time of full moon was to produce left-turning relative to controls; at the time of new moon, right-turning. Also evident is the fact that the planarian, too, can distinguish between horizontal fields parallel and perpendicular to its body axis, and, as with snails, the influence of a parallel field is greater than that of a right-angle field during the noontime hours.

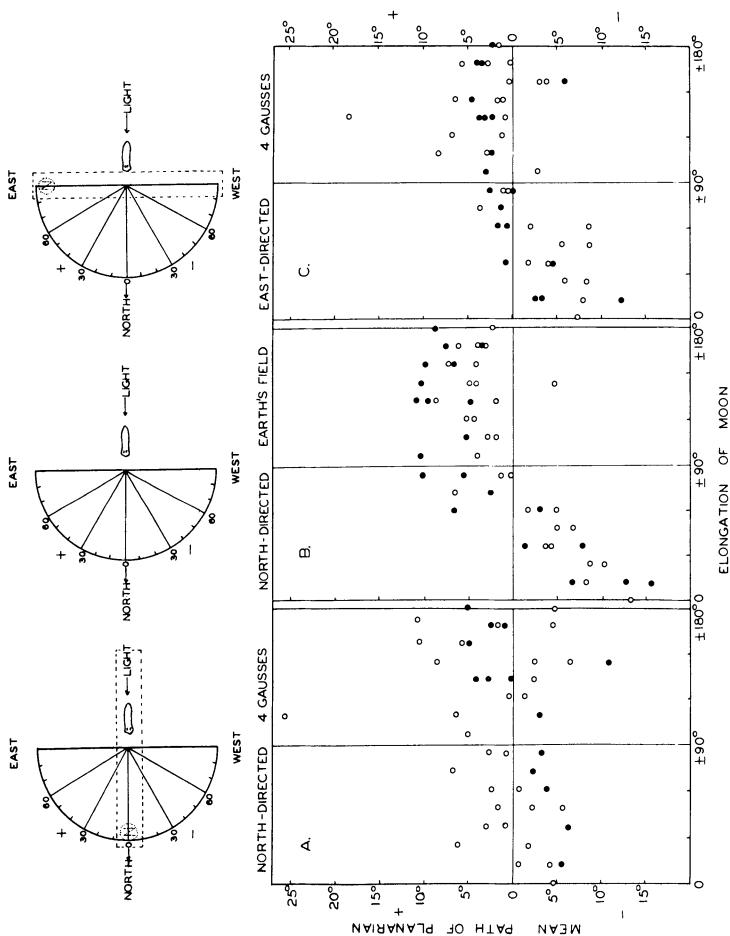


Fig. 3. The mean angular path of initially north-directed planarians as a function of elongation of the moon ( $0^\circ$  = new moon;  $180^\circ$  = full moon); with (A) a north-directed experimental magnetic field of 4 Oe, (B) the earth's field alone, and (C) an east-directed magnetic field of 4 Oe. Solid circles are the first experimental series (November and December); open circles, repetition of the experiment (January). (Reprinted from Biological Bulletin, October, 1962).

Synodic monthly rhythms have been demonstrated in both snails and planarians. They are components of the orientation reactions in "control" animals, and they are conspicuous in the magnetic response, where they may be of large amplitude and involve even reversals in the sign of response. For these reasons, monthly rhythms may be of considerable importance in the characterization of any response to weak experimental or natural magnetic fields. To randomize for these elements requires uniform distribution of data over a period of a synodic month or multiples of a month. This is the procedure which has been used in all the experiments to be described.

#### Response in Asymmetrical Light Fields

In further experiments the orientational apparatus has been either left symmetrically illuminated or rendered asymmetrical by introducing a light bias. For snails, half of the white orientation chamber was lined with matt black cardboard; for planarians, the chamber was converted to a two-light system by introducing a second light source 90° to the right of the central axis. The effect of a light bias was to reduce the variance in amount of turning by focusing more sharply the photic response.

In a set of 36 experiments the responses of snails orienting in symmetrical and asymmetrical light fields have been compared. When one side of the orientational chamber is lined with black the photopositive snails tend to turn more or less strongly to the opposite, white, side. The degrees of turning to the white side was found to be correlated with the direction and amount of turning shown by the same snails in a symmetrical field. For example, in a field with black to the left the snails which exhibited strongest right-turning in apparent response to the asymmetry were ones which had already been found to turn right in a symmetrical field; and snails turning least strongly to the right, or white side, were ones which were left-turning in the symmetrical field. In other words, the response to asymmetrical lighting was superimposed upon the turning response of the snail in symmetrical light. The coefficient of correlation between amount of turning under the two conditions of symmetrical and asymmetrical lighting with black on the left was + 0.70.

Some of the complexity of animal orientation reactions is demonstrated by the finding that the snail itself may possess a physiological asymmetry. The coefficient of correlation between turning in symmetrical and asymmetrical light fields for series when black was on the right was found to be only + 0.47. It was possible to abolish this asymmetry in snails by increasing the horizontal magnetic field to 5 Oe when snails were turning in the light field with black on the right. Then the correlation between amount of turning with black on the right and of turning in the symmetrical field rose statistically significantly to

+0.74. These results suggest that in some manner magnetoreception may be related to photoreception.

#### Relationship between Body Axis and Orientation of the Horizontal Component

Mud Snails. In 1959 it was shown that snails can differentiate between horizontal fields which are parallel and perpendicular to their body axis. Since then this capacity has been subjected to further study in the following manner.

In each experiment, groups of ten snails initially north-directed in an asymmetrical field were caused to emerge in 5-Oe horizontal fields directed north, east, south, and west. For each experimental group a control group was run in the earth's field alone. The ten paths in the experimental condition were then reduced to a mean value and compared with the mean of the control group. Over a 2-month period, 104 experiments involving 8320 orientations were recorded. The mean difference between experimentals and controls for each of the four field orientations is shown in Figure 4A. The snails appear to distinguish the north-directed field from the south- and the perpendicular east- and west-directed fields.

A further experiment has shown that snails can distinguish compass direction when they orient in the earth's field alone. By pointing the

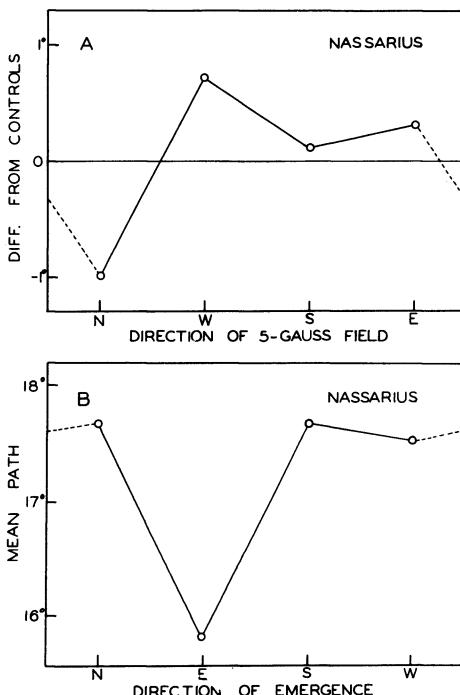


Fig. 4. A—Differences between mean paths of snails initially north-directed in the earth's field and comparable paths in experimental 5-Oe horizontal fields in each of four compass directions. Path angle is expressed as difference from the interpolated controls in the earth's field alone. B—Mean path of snails emerging into a constant asymmetrical light field when the orientation apparatus is oriented to each of the four compass directions in the earth's magnetic field alone.

apparatus in the desired direction, snails were made to emerge north, east, south, and west. The results of 68 experiments involving 2720 individual orientations are shown in Figure 4B. Snails made to emerge to compass east turn significantly to the left of the mean paths which they take when emerging in other compass directions.

It should be recalled that a snail moving east in the earth's field bears the same relationship of body axis to field orientation as a snail moving north in a west-directed experimental field. When the response patterns for the two experiments presented in Figure 4 are compared in terms of field orientation relative to body axis, they are found to be mirror image. This suggests that magnetism may be involved in the compass-direction effect for snails.

Planarians. Initially north-directed in a two-light system, planarians were made to orient in a series of 10-Oe horizontal fields directed north, east, south, and west. Control runs in the earth's field also accompanied each experimental group. The results of 20 experiments are summarized in Figure 5A. Each point in the figure represents the mean for all experiments of the difference between the average path of 15 experimentals and 15 concurrent controls within each experiment. The worms clearly differentiate between parallel and perpendicular fields and between north and south fields in a manner similar to, but more pronounced than, that in snails.

A compass-direction effect has been found in planarians, also. The results of 59 experiments involving a total of 3540 orientations in the

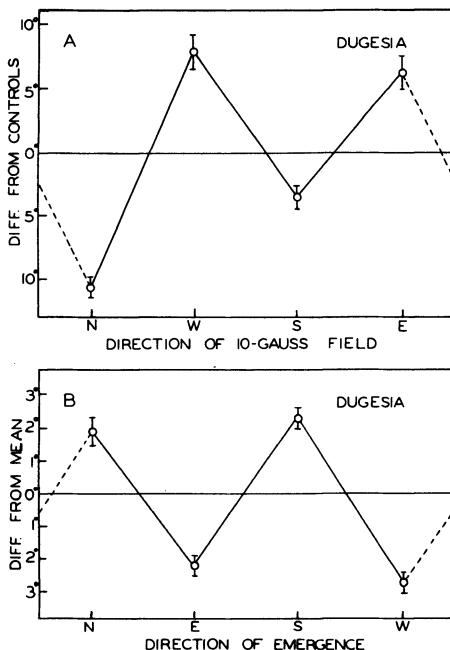


Fig. 5. A—Differences between the mean paths of *Dugesia* initially directed northward in the earth's field and comparable paths when experimental horizontal 10-Oe fields, directed in each of four compass directions, are superimposed. Path angle is expressed as difference from interpolated controls in the earth's magnetic field alone. B—Difference between mean path of planarians for each of four compass directions and the mean path in the same series for all four directions taken together, when the orientation apparatus is oriented to each of the four compass directions in the earth's magnetic field alone.

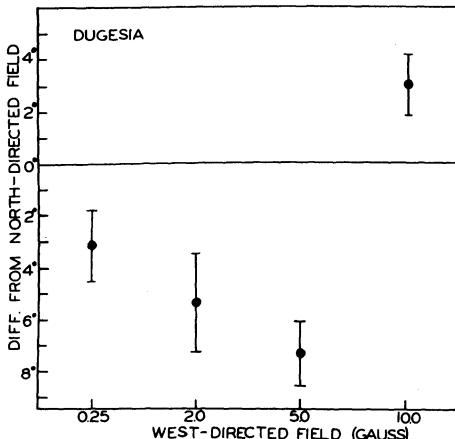


Fig. 6. Difference between mean paths of *Dugesia* initially north-directed in experimental north-directed magnetic fields which supplement the earth's field to yield the horizontal intensities indicated and the mean path resulting from rotation of the supplementing magnet 90° in a counter-clockwise direction.

earth's field alone are shown in Figure 5B. Again the pattern resembles, in general, that for the compass-direction effect in snails. As with the snails, the compass-direction effect is the mirror image of the response to orientations in the experimental magnetic field. However, in the case of planarians, an explanation for this inverse relationship in sign of response has been provided by the following experiment.

#### Relation of Horizontal Field Intensity to Orientation

Planarians were made to orient in asymmetrical lighting in a series of experimental horizontal field intensities—0.25, 2.0, 5.0, and 10.0 Oe—directed to north and west. The order of the conditions was always shuffled within each experiment. Differences between turning in north and west fields for each horizontal intensity are presented in Figure 6 for 24 experiments performed at the same time of day and distributed over a 2-month period. In going from 0.25 to 5.0 Oe the N-directed field clearly induced increasing left-turning. Between 5 and 10 Oe the direction of induced turning is reversed. It will be recalled from the preceding experiments that worms emerging east and west in the earth's field turned left relative to those emerging north; ones emerging in 10-Oe east- or west-directed fields turned right relative to those in the north-directed field.

#### Resolution of Magnetic Field Direction

Another experiment was designed to explore in more detail the differentiation of horizontal-field direction which the planarian had already demonstrated for north- and west-directed fields. In this experiment the amount of turning of the planarians in response to a 5-Oe horizontal field oriented at each of the seven 15° intervals from north to west was determined. In every experiment, 11 sets of 15 orientations each were

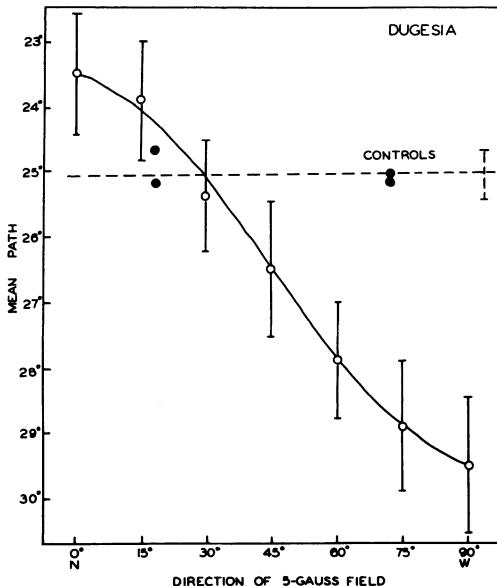


Fig. 7. Open circles illustrate the relationship of mean path to magnetic orientation for initially north-directed *Dugesia* in an asymmetrical light field. Worms are subjected to an experimental 5-Oe field with the south pole of the magnet changed by 15° intervals from north to west. The means for each of four successive controls in the earth's magnetic field alone, which were interpolated in random order in each experimental series, are indicated by the solid circles. Standard errors of the mean are shown. (Reprinted from Biological Bulletin, October, 1962).

observed in north-directed worms. The 11 sets were always run in shuffled order and consisted of the seven different orientations of the bar magnet and four control sets in the earth's field alone. Results of 42 experiments conducted over a 2-month period are presented in Figure 7. Clearly, the worms can resolve horizontal field direction with a remarkable mean precision.

#### Magnetic Response and Metabolic Rate

An investigation during the summer of 1958 showed that the metabolic rate of mud snails kept under constant conditions in the laboratory undergoes solar and lunar daily variations. During the study of snail magnetic responses in the summer of 1959, it was possible to record continuously and automatically the oxygen consumption of snails from the same population being used for the orientational studies. When the data on magnetic response and metabolic rate were compared, a possible relationship, which is presented in Figure 8, was discovered. The daily variation in rate of oxygen consumption is the mean of 29 days recorded

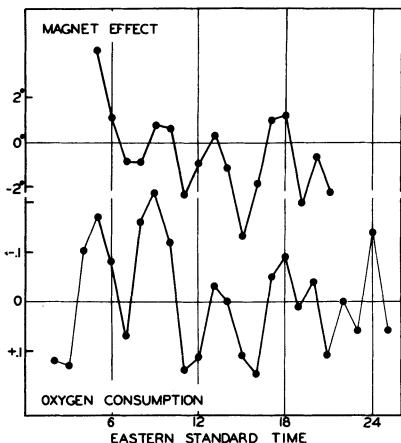


Fig. 8. Solar daily variations in Nassarius of the orientational response to a 1.5-Oe horizontal field and of metabolic rate under constant conditions in the laboratory during an overlapping period. The ordinate for oxygen consumption is in arbitrary units and is inverted to facilitate comparison.

from June 19 to July 17, 1959. Before averaging, the hourly readings for each day were converted to deviations from the daily mean for that day. The range of the variation is 4% of the mean rate. The daily variation in magnet effect is the same as in Figure 2. It is suggested by the figure that the amount of left-turning in response to the 1.5-Oe field is inversely related to metabolic rate.

Further analysis of these metabolic data has suggested that the substantial day-to-day changes in the mean daily rate were related to the day-to-day changes in world-wide geomagnetic data, as measured by the international magnetic character figure. The relationship of metabolic rate to geomagnetic activity during this period appeared to be one of organismic anticipation, because the highest coefficients of correlation were found when metabolic rate was treated with a 1-day lead ( $r = -0.55$ ) or a 2-day lead ( $r = -0.54$ ) on magnetic activity. The relationship is shown in Figure 9.

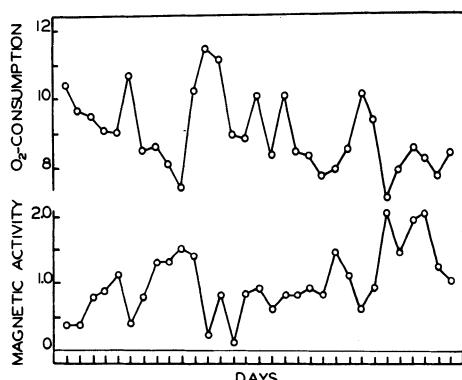


Fig. 9. Mean daily metabolic rate in Nassarius and the international magnetic character figures for June 19 to July 19, 1959. Values for oxygen consumption have been displaced one day to the right. Units for oxygen consumption are the same as in Figure 8. Character figures are from the Journal of Geophysical Research 65:790, 792, 1960.

## CONCLUSION AND SUMMARY

There remains no reasonable doubt that living systems are extraordinarily sensitive to magnetic fields. By extremely simple experiments it is possible to prove that highly diverse types of animals and plants may have their orientation modified by artificial fields of the order of strength of the geomagnetic field. This has already been established in our laboratory not only for the snails and flatworms, but also for the fruit fly *Drosophila* and the unicellular *Paramecium*. The colonial flagellate *Volvox* also is sensitive to very weak magnetic fields (personal communication from Dr. John D. Palmer). The nature of the organismic response is, however, far from simple. The systematic and periodic alterations in the strength and character of biological response suggest a highly differentiated response mechanism within the organism and belie any conclusion that the responsiveness is adventitious. To the contrary, the nature of the response properties suggest that the organism is normally integrated with its geomagnetic environment to a striking degree.

The perceptive system for magnetism is clearly functionally related to the three-dimensional organization of the organism as a whole. The organism behaves as if its magnetic-field responder were altering its orientation relative to fixed bodily axes in space in a highly regulated manner, with the predominant periods of the geophysically correlated biological clocks. Clearly, the perceptive mechanism for weak magnetic fields is not an independent system functionally isolated from the remainder of the living organism. To the contrary, the perceptive system is continuously under the influence of the living system.

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## *Chapter 2*

# Actions of a Very Weak Magnetic Gradient: The Reflex of the Dowser

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The way in which the dowser holds his divining rod (Figure 1) is common knowledge, although few people have tried it themselves. Whether the dowser really gets signals coincident with the presence of water is a very controversial question. In all events it appears undeniable that above-ground dowsers obtain, when certain physical conditions are present, a signal which is the involuntary upward or downward movement of the firmly held rod. It is independent of their will if they are employed solely in maintaining their grip. The interpretations of this signal in terms of water are very doubtful. Nevertheless, a correlation with the presence of water is manifested quite often.

If we let ten dowsers pass along a certain route, for example a quarter mile of road through a forest, and leave a mark wherever they get a signal, then experience shows that the marks of all the dowsers will coincide within 1 or 2 yards of the most frequent mark. Such verifications, made in a large number of cases,\* lead one to believe that there exists a physical agent causing the reflex of the dowser above the ground.

### THE MAGNETIC DOWSER

Through experiments described below and published in a recent book<sup>1</sup> we have established that:

1. The dowser, walking with uniform speed at his normal rate and with his rod in position, has his reflex started when he moves

\*The controversy of the dowsers versus the scientific world is such that accounts of such experiences are practically excluded from scientific literature. To find them, one must have the courage to explore the so-called literature of the dowsers and attempt to read it with a critical mind. We cite in this regard: Sir William Barrett and Th. Besterman, "The Divining Rod," 1926; J. Cecil Maby and T. Bedford Franklin, "The Physics of the Divining Rod," 1939; Dr. Jules Regnault, "Baguettes et Pendules," 1948; Dr. Joseph Wust, cited by E. Hartmann, "Vorstoß in Biologisches Neuland," 1964; S. J. Tromp, "Psychical Physics."



Fig. 1. 1693 engraving showing how the divining rod is held.

through a region where the earth's magnetic field is not entirely uniform and an anomaly is present. If we characterize this anomaly by a "gradient" giving the variation of the field with respect to distance, we arrive at the following facts:

- a. A gradient of 0.3 to 0.5 mOe/m can be detected, but with a time lag of the order of 1 sec.
- b. If the gradient increases to 2 or 3 mOe/m, the detection is more accurate.

- c. If the gradient falls below 0.1 mOe/m, the detection is wholly inaccurate. There is apparently a threshold below which the gradient cannot be accurately detected.
2. If many small anomalies are repeated in this way within a few meters, detection is improved in the sense that the signal becomes irresistible, i.e., no supplementary muscular contraction succeeds in restraining the rod. There is a slow integration (within seconds) of the causes contributing to the "signal."
3. If upon arriving at a gradient, one finds himself in a field which keeps increasing (within the interval), one witnesses a saturation: the human body adapts itself to the new value of the field; 20 to 50 mOe is large enough to cause this saturation. If one wishes to detect a signal despite this saturation, it is necessary to walk faster.
4. In a car or a plane one can detect a gradient  $\Delta H/\Delta x$  weaker than that detectable while on foot, provided that the higher velocity  $\Delta x/\Delta t$  of these vehicles produces a time variation of the field  $\Delta H/\Delta t$  of the order necessary for detection when moving by foot, i.e., more than 0.3 mOe/sec.

### THE ART OF THE DOWSER

We are not the first to have reported the sensitivity of a dowser to a magnetic field. According to a very old book,<sup>2</sup> when the Abbot of Vallemont—in reality the Reverend Father Le Lorrain, S. J., Professor of Physics at College Louis le Grand—places a lodestone before a well-known dowser, "the rod moves." S. J. Tromp ("Psychical Physics") causes the dowser to operate with artificial magnetic fields. Joseph Wust finds magnetic anomalies on the ground where the dowsers react. However, none of these authors connects these effects with the detection of water.

It appears to us that one may provisionally conclude that the dowser does not detect still water in a pond or running water in a river, but he can detect

- a. water filtering through porous media, and
- b. water in permeable layers adjacent to beds of clay,

since in these two cases water produces electric currents through electrofiltration potential and concentration batteries. If the medium is sufficiently conducting, and the current in the soil is sufficiently high, then there exists at the surface of the soil a small magnetic anomaly. The effects of electrofiltration and of clay potential are well known to geophysicists and can be calculated. We came to the conclusion that 1 mOe/m is very difficult to obtain, but one can obtain values of 0.1 mOe/m. On the ground, the magnetic dowser as we have described him is almost at the limit of his sensitivity when he attempts

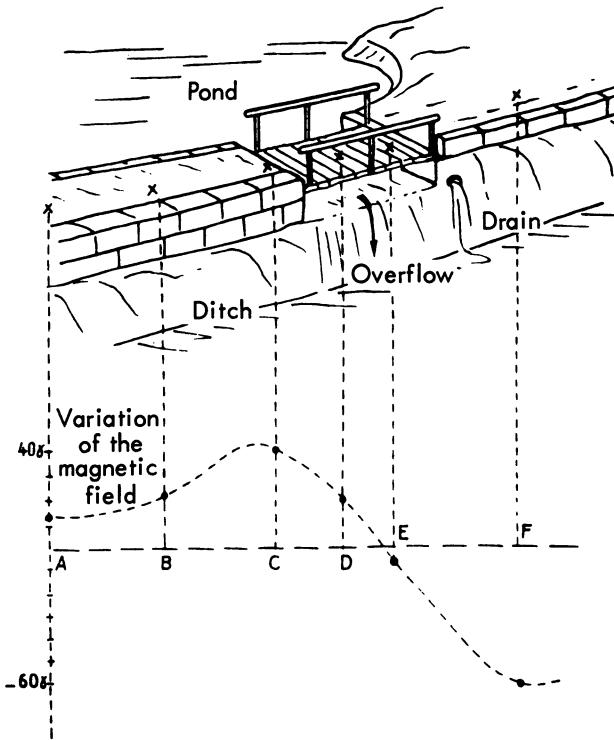


Fig. 2. Magnetic anomaly along an embankment bordering a pond which has various drains. The variations in the magnetic field are expressed in  $\gamma = 10^{-5}$  Oe.

to detect water. In our opinion he detects it only if he experiences signals which change with time; considerable flood or drought causes disappearance of the signals, either by suppressing the stream of current or by distributing it uniformly. At the same time, these conditions limit considerably the chances of the dowser to find water close to the surface of the soil.

Figure 2 represents a site (a bank separating a pond from a ditch below) where a dowser will obtain strong signals, corresponding to a magnetic anomaly, as we have proven.

#### MAGNETIC TRAPS

The dowser can be made to err in cases where the magnetism in the ground is not due to water:

1. Objects encased in iron, unexploded shells, etc., give signals. One often finds that they cause a magnetic anomaly too large (2 to 3 mOe/m) to be attributed to water.

2. Certain rocks, basalt among others, become magnetized after being struck by lightning.

3. Ordinary humus contains a nonmagnetic iron oxide. But if it has been reduced in certain spots by organic decomposition or fire, then we have a magnetic variation: a camp fire in a forest often gives the dowser a signal. One often can detect tombs, either because of this mechanism or because of the deformation of a system of electric currents.

### EXPERIMENTS WITHOUT AUTOSUGGESTION

In most scientific media, anything which is considered as not having a basis in physics is attributed to autosuggestion. Nevertheless, the fact that the dowser is sensitive to a magnetic field can be verified in practice by asking the dowser to indicate the presence or absence of a field controlled without his knowledge. This is possible if one employs the magnetic field of coils and the current is alternately turned on and off, with every precaution taken to prevent the dowser from knowing if the experimenter has turned the current on or off. Figure 3 indicates the field which is established around such a frame. We have described such experiments<sup>3</sup> and shall summarize them briefly:

1. It is necessary to find a site 12 to 15 m in length which does not give a magnetic signal.

2. Along the length of the rectilinear path which the operator follows, a frame is placed on an easel. The frame is about 50 cm by 100 cm, is wound about 100 times with fine wire, and is fed by a current of a few milliamperes. It produces a few millioersteds in its center and a few tenths of a millioersted at a distance of 1 m in front of it, the approximate distance at which the operator passes.

3. In following his path, the operator, walking at normal speed (1 to 1.20 m/sec), passes through an anomaly of a few tenths of a millioersted in  $1\frac{1}{2}$  to 2 sec.

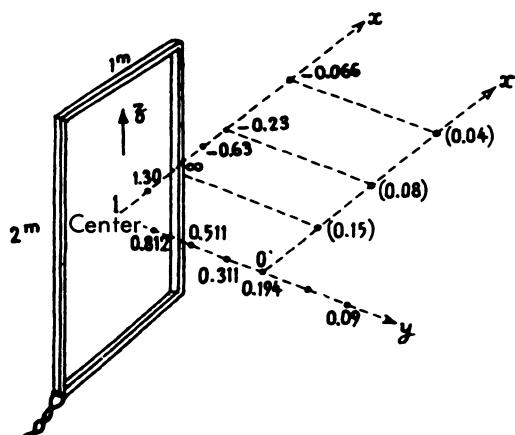


Fig. 3. Magnetic field of a  $1 \times 2$  m rectangular coil. The field in the center is taken as unity.

4. After he is trained to hold his rod for 15 or 20 sec, he is made to pass back and forth and told that the current is now on. He shows that he detects it, and thus becomes accustomed to the field. A second run is made, and he is told this time that the current is off. He indicates that he does not sense anything. He is then made to go back and forth without knowing whether the current is on or off.

Under these conditions a good operator is never wrong, if he is not overworked. A series of 5 or 6 experiments comprising 30 to 36 passages is acceptable. However, in these experiments one never observes the irresistible signal often obtained on the ground. This irresistible effect is obtained if we place two frames in series about 3 m apart and allow the operator to pass before them in succession. This shows that the duration of the influence (1 or 2 sec) of a single frame is too short and that the effect is greatly increased by letting the forces integrate over a period of time.

#### CONSEQUENCES FOR BIOMAGNETISM

We consider it established that the dowser while walking reacts to a variation of 0.3 mOe/sec. We also carried out experiments in which the field was varied with time and the dowser remained immobile, but we do not wish to discuss them here. They are not absolutely equivalent.

One important point is that most persons are sensitive. It is necessary only that the person should be willing to learn how to hold the divining rod. Furthermore, when they are initiated, their first sensation must come from a good signal, with no possibility of auto-suggestion. We found:

1. A good dowser is not sensitive to a much weaker gradient than a poor dowser, but he has a more rapid and accurate reflex.
2. One observes between the palms of the good dowser's hands an electrical resistance  $\frac{1}{3}$  or  $\frac{1}{4}$  that of the poor dowser. This is lessened if his hands are moist.
3. Certain very talented subjects can detect while standing still, observing a trembling of the divining rod.
4. These very talented subjects may perceive a small electric tingling in their palms during the signal.
5. It is much more difficult to determine the reality of the signal with a pendulum than with a rod. However, we think that the pendulum dowser detects approximately the same gradient as the rod dowser.

It appears that physiology has not yet attained a level at which explanation can go very far. However, we shall risk a few remarks from the point of view of physics.

- a. Let  $\mu$  be the magnetic moment of a molecule in the human body, and let  $H$  be the strength of the geomagnetic field (0.47 Oe). The energy

$\mu H$  is very small compared to  $kT$  ( $k=1.38 \times 10^{-16}$ ), the energy per degree of freedom in a molecule. It is impossible that sensitivity to a magnetic field would be caused by elemental effects. For example, a photon  $h\nu$  causes a detectable photoelectric effect, with an energy much greater than  $kT$ . With regard to the sense of hearing, the kinetic or potential energy which can be detected by the tympan at the threshold of audibility is also considerably greater than  $kT$ .

b. The chest and arms of the dowser advancing in the magnetic anomaly will present living electrical circuits, which are the seat of an induced emf. One can calculate the emf; it is less than a microvolt. Moreover, this induction effect has a vector character. If the dowser were sensitive to it, he could act like a compass and find the direction of the field. This is absolutely not the case. The sensitivity of dowsers can therefore not depend on an induced emf.

c. On the other hand, nuclear magnetic resonance is an effect which would appear to have possibilities.

The nuclei of atoms are magnets on the one hand (magnetic moment  $\mu$ ) and gyroscopes on the other. The earth's magnetic field gives rise to precession of the nuclei; the precession rate is characteristic of the field, for example, 2000 rps for a proton and 0.48 Oe. Should the dowser be in a nonuniform field, some of the protons in his body will have a speed of 2000 rps, others 2001, corresponding to a variation of 0.25 mOe. These protons are fixed in the bones and muscles and mobile in the blood. They have, on the other hand, a relaxation time sometimes short, sometimes long (many seconds), depending on the molecules to which they belong. The circulation of the blood causes waves of mobile protons to come in contact with the fixed protons or other systems of molecules, beating at a frequency of  $2001 - 2000 = 1$  cps. No matter how weak this is from one proton to another, it becomes evident when the volume is considerable.

If these vibrations (beats) play any role in living matter, for example in reducing muscle tone, this would suffice to cause a movement of the divining rod if it were in an initially unstable position.

The noticeably long hysteresis of the dowser's reflex could well be explained. Too strong a magnetic gradient could not be detected because it would produce too rapid a beat. It can also explain the fact that the movement of the dowser produces significant effects in a gradient. Even if the dowser is standing still in a magnetic gradient, the blood circulation may produce a beat which he can detect if he is very sensitive. On the other hand, the increased effect of a prolonged or repeated stimulus is explained equally well.

The consideration of a possible intervention of nuclear magnetic resonance is very attractive. In addition, it is not a vector effect, the speed of the nuclei depending on the absolute value of the magnetic field and not on its orientation. This agrees again with what we observe with the dowser.

Nevertheless, it is still not known how the physiological functions of the complex molecules of living matter, muscle contraction for example, are disturbed by a phase disorder in the rotation of the component nuclei under the action of a magnetic field.

Whatever it is, the curious phenomenon of the dowser's reflex, inasmuch as it is caused by a small magnetic variation, obliges us to consider wholly new possibilities for magnetic action on living matter.

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## *Chapter 3*

# Proposed Mechanisms for the Navigation of Migrating Birds

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The question of how migrating birds find their way when flying at night at great altitudes or in clouds, when no geographical landmarks can be seen, is an old puzzle. The hope of gaining new ideas for the guidance of intercontinental missiles has prompted the armed services to expend considerable research efforts on this quest. The number of papers dealing with this subject is quite impressive, but no final answer has yet been found.<sup>1</sup> In the following, two hypotheses are proposed. One of them is a new mechanism, the other a physical phenomenon so far not considered in relation to bird migration.

### WING FLAPPING AS d-c TO a-c CONVERTER

In a conductor which moves in a magnetic field, electromotive forces are generated which are perpendicular to the direction of motion and to the field direction. Their strength in volts is given by  $\text{emf} = Hv \cos \alpha / 10^8$ , where  $H$  is the field strength in oersteds,  $v$  the velocity of the conductor in centimeters per second, and  $\alpha$  the angle between the velocity and field vector. When a conductor of finite size is moving at constant speed in a homogeneous field whose direction does not change (that is, in a field with zero gradient and zero curl), as is the case for the geomagnetic field at a great height above the ground, these electromotive forces are compensated through the polarization of the conductor. A polarization current will flow whenever the emf changes due to a change in the direction of the velocity of the conductor relative to the magnetic vector. Intensity and duration of these polarization currents depend on the time derivative of the emf and on the electric resistance and capacitance of the conductor.

Living matter can be considered as a second-class conductor. The capacitance of a bird 5 in. long and 2 in. in diameter is about  $10 \mu\text{F}$ , and with a wingspan of 1 ft the capacitance of the wing ends could be

$5\mu\mu F$ ; the resistance of the fleshy part of the body and wings is of the order of  $1000\Omega$ . The circuit in which the polarization currents will flow has a time constant of the order of  $10\text{ nsec}$ . But even if we consider the resistance of the horny parts of the wings to be  $100\text{ M}\Omega$  the time constant would be of the order of milliseconds and certainly negligible compared to the time during which a flight-direction change occurs, or compared to the wing-flapping cycle. The impedance of the circuit can therefore be considered as purely capacitive and consequently the polarization current will be in phase with the time derivative of the emf generated by the geomagnetic field during flight.

Let us assume that in the temperate climates, over which the flyways of most migrating birds lead, the horizontal component of the geomagnetic field is  $H_h = 0.21\text{ Oe}$ , pointing northward, the vertical component  $H_v = 0.50\text{ Oe}$ , and the inclination  $I = 67.5^\circ$ . Furthermore, assume our bird travels with a speed of, say,  $100\text{ m/sec}$  in the north-south direction and would err in the direction by  $1\text{ deg/min}$ . In this case the change of the emf per centimeter of conductive tissue would be  $0.006\mu\text{V/sec}$ . It would produce, according to the above data on capacity, a polarization current of the order of  $i = 10^{-19}\text{ A}$ .

To measure such extremely small currents, even with our highly developed electrotechnique we have to resort to an artifice: converting the d-c current into an a-c current with the help of a vibrating-reed condenser. My hypothesis is that a bird is capable of doing the same by means of its wing flapping.

When the bird flies southward, an emf of  $50\mu\text{V}$  will be generated in each centimeter of its wingspan while the wings are in the horizontal position. But in the uppermost and lowest position of the wings (say  $45^\circ$  above and below horizontal), the emf will be only  $35\mu\text{V/cm}$ . The difference of  $15\mu\text{V/cm}$  will cause a flow of a polarization current from one wingtip through the body toward the other wingtip. The direction of this current will change four times during each complete flapping cycle.

Should our bird now turn toward the east, then in the uppermost position of the wings the emf in the left wing will be  $50\mu\text{V/cm}$ , as in the horizontal position; but in the right wing it will be merely  $20\mu\text{V/cm}$ . In the lowest position of the wings, the situation is the same, but with right and left reversed. Consequently, while the wings are above horizontal the current in the right wing will be about three times stronger than in the left wing; and during the time the wings are below horizontal, the current in the left wing is the stronger. Considering only the magnitude of the current flowing between wingtip and body, the currents are symmetrical while flying south or north, but asymmetrical when flying east or west.

In general, the emf generated per centimeter of wing length is

$$V = v \cdot 10^{-8} [H_h \cos \alpha \sin(\phi \sin \beta) + H_v \cos(\phi \sin \beta)]$$

where  $\alpha$  is the angle between the wing in the horizontal position and the

direction of the horizontal component ( $H_h$ ) of the geomagnetic field;  $\beta = 2\pi\nu t$ , where we assume that the angular velocity of the wing movement follows a sine function of time with frequency  $\nu$ ;  $\phi$  is the maximum angle between the horizontal and uppermost or horizontal and lowest wing position. The current flowing from the right wingtip toward the body and from the body toward the left wingtip is

$$i = lCv \cdot 2\pi\nu \cdot 10^{-8} \phi \cos \beta [H_h \cos \alpha \cos (\phi \sin \beta) - H_v \sin (\phi \sin \beta)]$$

When the bird flies southward along the magnetic meridian,  $\alpha = +90^\circ$  for the right and  $-90^\circ$  for the left wing; therefore, the first term in the bracket is zero. The second term has the same sign for both wings, but changes its sign each time the wings cross the horizontal position. As a consequence, the current through the body changes its direction twice during each flapping cycle. Our bird would observe an alternating current with a frequency twice the wing-flapping frequency, reaching a peak value of  $10^{-14}$  A halfway between the horizontal and uppermost and between the horizontal and lowest position of the wings. This current is five orders of magnitude larger than the current we obtained from a flight-direction change alone.

Should our bird now turn eastward, the first term in the bracket will become different from zero. This involves an increase in the peak value of the current by about 80% in the left wing when it is above the horizontal and in the right wing when it is below the horizontal; it reduces the current 90% in the left wing when it is below the horizontal and in the right wing when it is above the horizontal. Consequently, the frequency of the alternating current will drop to practically one half its previous value and the currents will no longer be synchronous in the two wings.

TABLE I  
Beep Signal Pattern

Each flapping cycle is, for the sake of simplicity, divided into eight equal time intervals.

Direction	Wing	1	2	3	4	5	6	7	8	1	2	3	4	5	6
wing up															
Southward	right	+	-			+	-			+	-			+	-
	left	-	+			-	+			-	+			-	+
wing down															
Eastward	right	#				=				#					=
	left	#			=					#					=
wing up															
Westward	right		=			#				=			#		#
	left	=				#				=			#		#
wing															
Northward	right	+	-			+	-			+	-			+	-
	left	-	+			-	+			-	+			-	+

Should a bird have a sensor for these small polarization currents, it could "hear" beep sounds from each wing. Table I lists the beep pattern for the four main geomagnetic flight directions. The + sign indicates that the current is flowing toward the body, the - sign that it is flowing away from the body. The = and # signs stand for 80% higher peak values. This guiding pattern is similar to signals transmitted to pilots to guide an airplane along the gliding path when it approaches the landing strip.

With respect to the question of whether birds could at all detect potential differences of a few microvolts, or currents caused by such small potential differences, we may note that experiments made by Lissman and Machin<sup>2</sup> suggest that the fish Gymnarchus niloticus can detect currents caused by a potential difference of 1  $\mu$ V between head and tail.

### THE RELATIVISTIC GEOELECTRIC FIELD

An entirely different phenomenon, which could be used by birds for navigation purposes, is a consequence of the special theory of relativity.

Electrodynamics of ponderable matter can be treated in a four-dimensional language by introducing two antisymmetric electromagnetic tensors  $F^{\mu\nu}$  and  $H^{\mu\nu}$ , the components of which in proper coordinates are given in terms of quantities appearing in the Maxwell field equations:

$$F^{\mu\nu} = \begin{vmatrix} 0 & B_z^0 & -B_y^0 & -E_x^0 \\ -B_z^0 & 0 & B_x^0 & -E_y^0 \\ B_y^0 & -B_x^0 & 0 & -E_z^0 \\ E_x^0 & E_y^0 & E_z^0 & 0 \end{vmatrix} \quad H^{\mu\nu} = \begin{vmatrix} 0 & H_z^0 & -H_y^0 & -D_x^0 \\ -H_z^0 & 0 & H_x^0 & -D_y^0 \\ H_y^0 & -H_x^0 & 0 & -D_z^0 \\ D_x^0 & D_y^0 & D_z^0 & 0 \end{vmatrix}$$

(Here  $\mu$  refers to columns and  $\nu$  to rows.) Maxwell's equations will be represented now by the tensor equations

$$\frac{\partial F_{\mu\nu}}{\partial x^\sigma} + \frac{\partial F_{\nu\sigma}}{\partial x^\mu} + \frac{\partial F_{\sigma\mu}}{\partial x^\nu} = 0$$

and

$$\frac{\partial H^{\mu\nu}}{\partial x^\nu} = J^\mu$$

These equations are true in all sets of coordinates if true in one. In particular, we can relate the values of  $E$ ,  $H$ ,  $D$ ,  $B$ , and  $J$  in any desired system of coordinates with the values of the corresponding quantities

as directly measured by a local observer, using proper coordinates in which the material is at rest. Of special interest for us will be the transformation of the total current  $J$ . One can separate  $J$  into conduction current  $C$  and convection current  $\rho v$ . Making this separation, we obtain the following expression for the charge density in matter moving parallel to the  $x$  axis with the velocity  $v_x$ :

$$\rho = \rho_0 / \sqrt{1 - v_x^2/c^2} + (C_x^0 v_x/c^2) / \sqrt{1 - v_x^2/c^2}$$

This equation shows that, even when the charge density is zero in proper coordinates, charge density and convection current can appear in other coordinates, provided the conduction current is not zero in proper coordinates. This possibility, that different observers may disagree as to the relative number of positive and negative charge carriers in a given volume element of the material, arises when there is relative motion of the two kinds of charge carriers and is a consequence of the concept of simultaneity as provided by the theory of relativity.

As Laue<sup>3</sup> has shown, this relativistic phenomenon can be readily understood if we draw the world lines of the resting protons and moving conduction electrons of the conductor in a Minkowski diagram (see Figure 1). In the coordinate system at rest relative to the conductor ( $x^0, ct^0$ ), due to the electric neutrality of the conductor an equal number of world lines of protons and electrons will start from any given length of the conductor. But, seen from the moving coordinate system ( $x, ct$ ), we can see that for any given length of the conductor the number of proton world lines will be greater than the number of electron world lines. (In Figure 1 we have 11 proton lines for 10 electron lines in the moving coordinate system.) Hence the conductor will appear to have an excess positive charge. In a current-carrying ring which moves with a

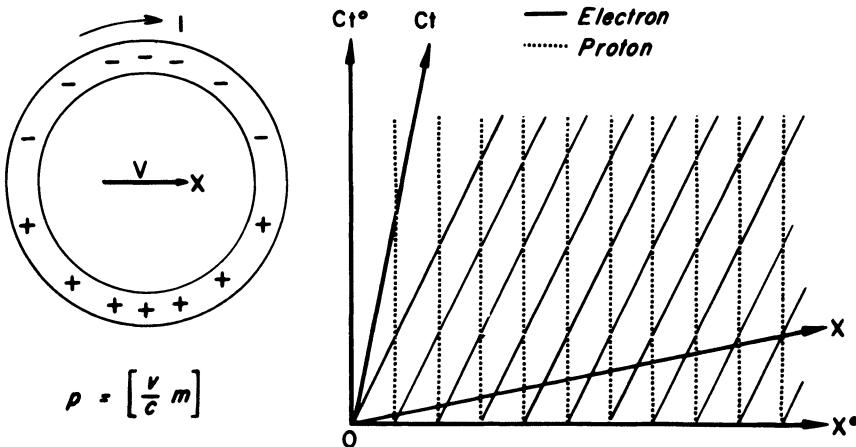


Fig. 1

velocity  $\vec{v}$  relative to an observer (see left side of Figure 1), it will appear as if the upper part of the ring carries an excess negative, the lower part an excess positive charge, although the total charge will be zero in both coordinate systems. A consequence of this relativistic effect is that a ring current producing a magnetic moment  $\vec{m}$  will seem to have an electric dipole moment  $\vec{P}$  for a moving observer.

A magnetic moment is always the consequence of a ring current. The earth has a magnetic dipole moment; it is irrelevant how we explain its origin. For every observer moving in a direction which has a component perpendicular to the direction of the magnetic dipole axis of the earth, hence for the flying bird, the earth will represent an electric dipole. The vector of this electric dipole will be perpendicular to the vector of the earth's magnetic moment as well as to the component of the bird's velocity vector, which is perpendicular to the magnetic moment vector.

As a straightforward calculation shows, a bird flying at a magnetic latitude  $\phi$  will observe a vertical potential difference

$$V_v = H_e \cdot 10^{-8} v (\sin^2 \alpha + \cos^2 \alpha \sin^2 \phi)^{1/2} \cdot 2 \sin \alpha \cos \phi$$

where  $H_e = 0.35$  Oe is the field strength on the magnetic equator and  $\alpha$  is the angle between the flight direction and the magnetic meridian. At the same time, it will sense a horizontal potential difference in a direction perpendicular to its flight direction:

$$V_h = H_e \cdot 10^{-8} v (\sin^2 \alpha + \cos^2 \alpha \sin^2 \phi)^{1/2} (1 - \sin^2 \alpha \cos^2 \phi)^{1/2}$$

For example, our bird with a wingspan of 1 ft and flying with a speed of 100 m/sec over the U.S. in the north or south direction should experience a potential difference of 0.6 mV between wingtips when the wings are in horizontal position and 0.42 mV when they are in the uppermost or lowest positions. If the bird turns east, a vertical potential difference of 0.9 mV will appear between their uppermost and lowest positions. If the bird turns west, the vertical potential difference will reverse its polarity. As a consequence of wing flapping, polarization currents are generated as in the case first discussed.

A satellite moving with a speed of 10 km/sec could build up potential differences of several volts between its different parts. Of course, without a surrounding conducting medium at rest relative to the earth, supplying a reference system to which this constant potential difference could be related, it cannot be observed with instruments moving with the satellite. To detect this potential difference, an antenna which, like the wingtips of the bird, would change its position periodically relative to the coordinate system of the earth would be necessary. Since satellites usually rotate, a single rod with a sensitive a-c current ammeter would be sufficient.

## CHAPTER 3

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*Part V*

## BIBLIOGRAPHY AND INDEXES

# Bibliography of the Biological Effects of Static Magnetic Fields

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## SCOPE OF THIS BIBLIOGRAPHY

Interest in the biological effects of magnetic fields has increased in recent years as evidenced by the growing number of investigators reporting in this area. Recently a bibliography on this subject has appeared:

Davis, L. D., K. Pappajohn, and I. M. Plavnieks. 1962. "Bibliography of the Biological Effects of Magnetic Fields." Fed. Proc. 21:1-38.

The above reference contains items which are not pertinent to the subject. It also includes reports on the effects of alternating magnetic fields. The effects of electric currents induced by alternating magnetic fields (magnetic induction) are different in nature and magnitude from the interaction of materials with static magnetic fields. For this reason, the list of articles presented below contains only reports where static magnetic fields were used. In order to guide the investigator into the literature with greater efficiency, the bibliography is organized as follows:

1. Biological Effects of Static Magnetic Fields (Biomagnetic Effects)
2. Biomagnetic Effects at Geomagnetic Field Strengths (Biogeomagnetic Effects)
3. Interactions of Magnetic Fields with Living Tissues
4. Clinical Applications of Static Magnetic Fields

Other areas of investigation into effects of magnetic fields not covered in this bibliography are reports on the magnetic properties of living organisms, of tissues, and of derivatives from tissues. Similarly, this bibliography does not include reports on the effect of magnetic fields on chemical reactions *in vitro* such as that presented in Part II, Ch. 7 of this volume.

Brief reviews of some phases of biomagnetism may be found in:

Supported in part by research grant C4561, National Institutes of Health.

- Alexander, H. S. 1962. "Biomagnetics." Am. J. Med. Electron. 1:181-187.
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### 1. BIOLOGICAL EFFECTS OF STATIC MAGNETIC FIELDS

Investigations utilizing static magnetic fields of more than 100 times that of the field of the earth (approximately 0.5 Oe) are considered studies of biomagnetism for purposes of this bibliography.

Investigations in which the experimenter utilizes differences in the static magnetic properties, i.e., the use of magnetic susceptibilities to uncover differences in tissue properties, do not reveal the existence of a biomagnetic effect and consequently such reports are not included. An example of such a report is the recent paper

- Senftle, F. E., and N. Thorpe. 1961. "Magnetic Susceptibility of Normal Liver and Transplantable Hepatoma Tissue." Nature 190:410-413.

The references listed are presumably complete. If any have been omitted, the author would appreciate the information for future correction and addition.

Whenever an article is not a scientific report, the nature of the printed reference is indicated in parentheses as "abstr." for abstract of a presented paper or "Remarks" for comments upon or summary of discussion.

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## 2. BIOMAGNETIC EFFECTS AT GEOMAGNETIC FIELD STRENGTHS (BIOGEOMAGNETIC EFFECTS)

The reports in this section are of investigations of the effect of the earth's magnetic field on living tissues or organisms, and of the ef-

fects of externally applied magnetic fields up to 100 times that of the earth's field. The term applied to biological phenomena in this range of magnetic field intensity is "biogeomagnetic effects."

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### 3. INTERACTIONS OF MAGNETIC FIELDS WITH LIVING TISSUES

Reports have appeared of prompt effects of static magnetic fields on living tissues in response to applied external magnetic fields. It is in this sense that "interactions" is used in the heading for this group of publications. Note also that these reports refer to effects on tissues as apart from effects on the entire organism.

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#### 4. CLINICAL APPLICATIONS OF STATIC MAGNETIC FIELDS

Speculation as to the curative powers of magnetic fields has been indulged in since magnetic phenomena were first discovered. The record of such work is summed up in reviews by:

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Quinan, J. R. 1886. "The Use of the Magnet in Medicine: A Historical Study." *Maryland Med. J.* 14:460-465.

The references below are in the main for static magnetic fields. Curative powers have been claimed for alternating magnetic fields applied in various ways. Where such references imply or suggest that static magnetic fields may be used, they are listed, though it is possible that this listing may not be exhaustive.

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Hansen, K. M. 1938. "Some Observations with a View to Possible Influence of Magnetism upon the Human Organism" *Acta Medica Scandinavica* 97:339-64.  
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ments with Magnets at the Edison Laboratory." New York Med.  
J. 56:729-32.

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BARNOTHY, JENO M. President of Biomagnetic Research Foundation, Evanston, Illinois; owner and technical director of Forro Scientific Co. Born 1904, Kassa, Hungary. Ph. D. 1933 in physics, Royal Pázmány University, Budapest, Hungary. Dozent in Cosmic Radiation; Professor of Physics, University of Budapest until 1948; president of physical section of Hungarian Association of Science. Organized symposium of Medical Physics in 1947. Eötvös Medal of Hungarian Academy of Science for cosmic radiation research. Professor of Physics, Barat College of the Sacred Heart. 73 publications on cosmic radiation, nuclear physics, astrophysics; patents in nuclear instrumentation; 28 publications in biomagnetism.

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CAMPBELL, WILLIAM F. Plant Physiologist, U.S. Dept. of Agriculture. Born 1928, Mont Vernon, Illinois. Ph.D. 1964 in botany, Michigan State University. Eight publications in Agronomy and Radiation Botany; five publications in biomagnetism.

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