

Biological Effects of Magnetic Fields

Madeleine F. Barnothy

2

**BIOLOGICAL EFFECTS
OF
MAGNETIC FIELDS**

Volume 2

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Edited by

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PREFACE

We define biomagnetism broadly as the science of processes and functions in living organisms induced by static magnetic fields. Biomagnetic research has greatly increased since the publication of the first volume of this series. While much of this work has been reported in various international journals, there exists a need for this work to be collected together in one place. This book covers a rather wide area of research, both with respect to the strength of the field, ranging from "zero" to 150,000 oersteds, and with respect to the various specimens and their biological functions. It is designed primarily to help clarify the action of magnetic fields on biological systems with the hope of achieving a better understanding of the fundamental physiological processes occurring in them. In some chapters it is suggested that magnetic fields could ultimately be used in the treatment of disease.

I would like to take this occasion to thank the contributors for their generous interest and willing cooperation.

M. F. B.

June 1969

Chicago, Illinois

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INTRODUCTION

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Biological processes are mainly complex chemical reactions. Chemical properties result from the arrangement and motion of electrons and nuclei in molecules, as determined by the interactions of the magnetic and electric fields of these particles making up the molecules. The principles of chemistry and biology are therefore understood in terms of electrodynamics and quantum mechanics. These two theoretical tools enable us to analyze and interpret chemical reactions. Consequently, electric and magnetic fields would seem to be the natural experimental devices to be used to obtain information regarding the basic phenomena themselves.

The biological medium in which these chemical reactions occur is a weakly conducting electrolyte; this makes it difficult to establish a strong electric field. External electric fields, therefore, seem to be restricted to the investigation of phenomena in which the current conduction is of semiconducting nature. This restriction does not apply to external magnetic fields because the medium is essentially nonmagnetizable. The applied magnetic field, therefore, is not affected by the continuum encountered in biological systems, and thus seems to be ideally suited to be used as a probe.

In view of the very small energy which a magnetic field can impart to a biological system compared to the energy of thermal motion at room temperature, it is not surprising that most of the established biomagnetic effects are observed *in vivo*, where a sort of built-in amplification mechanism in the living biological system is utilized. This mechanism, interposed between basic phenomena and the observed biomagnetic effect, complicates a clear interpretation of the fundamental process itself. Nevertheless, there is hope that when we have penetrated this new territory further, we will develop techniques and instruments which will enable us to observe the effects of magnetic fields on simpler systems.

The most frequently considered mechanisms are:

1. The existence of transient free radicals which interact with the magnetic field; 2. A change in the rate or mechanism of diffusion across a membrane; 3. Semiconductor effects which would be influenced by the applied field; 4. A change in the rate of production of hormone secretion, for example, oxidative processes may be altered, influencing the oxidation rate of unsaturated steroids; 5. A distortion of the bond angles via paramagnetic molecules which change the fit between enzyme and substrate; 6. A change in rotational polarization of molecules with specific reactive sites; and 7. A change in the rate of proton tunneling in DNA molecules caused by the applied magnetic field.

The first chapter of this volume is devoted to a discussion of various types of commercially available magnets. Advantages of the different types of magnet assemblies from the standpoint of biomagnetic experimentation are presented. The second chapter contains an up-to-date bibliography with a short résumé of all investigations performed in low magnetic fields. The last chapter discusses the magnetic properties of biological materials on the basis of a carefully screened bibliography. These data should prove particularly helpful in devising new experiments, or in interpreting already established biomagnetic effects.

In reviewing the experimental material collected in 17 chapters of this volume, some common aspects of biomagnetic effects emerge:

1. The existence of a threshold field strength, below which the field is ineffective. This is indicated by investigations on respiration rates, alkali ion excretion, and on the mitosis rate of liver tissues. However, the value of the limiting field strength seems to change with the parameter under investigation. Moreover, above this threshold value the effect is not proportionally enhanced in stronger fields.
2. Many effects have been found to persist for long periods after the termination of magnetic exposure. For instance, abnormalities were found in organs of mice 196 days later, the number of astrocytes in the cerebral cortex was still elevated 20 days later, and arrhythmic contractions of vagal heart preparations were seen 4 days after exposure.
3. Several observations support the view that magnetic fields produce organismic responses characteristic of regenerative processes mobilized by this environmental stimulus.
4. The fact that oxidative processes may be altered by the field is

indicated by many findings; for example, neuroglia and neurons show hypoxic lesions, the rate of oxygen consumption is reduced in some, enhanced in other tissues.

5. Observations on the central nervous system support the view that current conduction in it is of semiconducting nature.

6. A change in the activity of enzyme-substrate system was found at high fields and also in "zero" fields.

7. Embryonic development is particularly susceptible to the action of the magnetic field; for example, the development of frog embryos stopped soon after gastrulation.

Whether the observed differences from the normal functioning of the organism under the influence of the magnetic field should be considered as harmful or beneficial can not yet be settled. The observation that mice look and behave younger after having been reared in magnetic fields would imply that the field has a salutary effect. But, in any case, the accumulated information demonstrates that biomagnetism is increasingly fulfilling an important role as a new avenue through which we can achieve a better understanding of fundamental physiological processes. Experiments indicating the arrest of spontaneous tumors raise the hope that magnetic fields could be used in medical therapy even before we have clarified all of its aspects, just as X rays are now used even though the precise mechanism remains to be elucidated. The fact that ECG patterns taken in a magnetic field present simultaneously information on blood flow, offers a new, noninvasive method of studying cardiac performance. In man's endeavor to widen his horizon by wandering beyond the space confinement of the earth, it is imperative to know what effects the explorer would be subjected to during his voyage through near-zero magnetic fields of interplanetary or interstellar space, or if, for example, he were exposed to very strong magnetic fields in spaceships driven by ion engines. However, apart from such practical aims, it is part of human nature to want to understand the laws of nature without being too concerned with the application. As history has shown, this drive, this instinct for basic research, has always been the mainspring for new fundamental insights into ourselves and our environment.

It is our hope that this second volume of *The Biological Effects of Magnetic Fields* will stimulate both those already in this research and those about to enter it.

MAGNETS IN BIOLOGICAL RESEARCH

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TERMS AND UNITS

The starting point of any discussion of magnetic fields is generally a description and rationalization of the terms used in the discussion. This approach seems appropriate in view of the many sets of units in use—cgs-emu, cgs-esu, mksa—and the different ways in which magnetic effects have been defined historically. Complicating the matter of units is the fact that measurable magnetic fields range from the geomagnetic field (earth's field, approximately 0.5 G) to the 10^6 G produced by the spectacular implosion technique.

The earliest concept of a magnetic field was described by Faraday in terms of magnetic flux lines emanating from magnetic poles. It was then appropriate to define a *unit field* as one in which the flux density, or flux per unit area, is 1 line/cm². In the cgs-emu system this unit field is called the *gauss*, the term most commonly used today. However, the cgs system will ultimately be replaced by the internationally accepted mksa system. Just as the cycle/sec unit has been replaced by the hertz, so will the gauss be replaced by the *tesla*. Table I lists some of the more common terms and their units.

TABLE I
Units of Magnetism

Term	cgs unit	mksa unit	Relationship
Flux ϕ	line	weber	$1 \text{ Wb} = 10^8 \text{ lines}$
Flux density B	gauss*	tesla	$1 \text{ T} = 1 \text{ Wb/m}^2 = 10^4 \text{ G}$
Field intensity H	oersted	ampere-turn/meter	$1 \text{ Oe} = 79.58 \text{ At/m}$

* Geomagnetic fields are generally described in gammas (1 G = 10^5 gammas).

Note that field intensity H and flux density B are not the same. When used in its rigorous sense, H means a *magnetizing field*, or magnetizing force, and B denotes the flux density, i.e., the magnetic field induced in any region by the magnetizing field. The flux density B resulting from an inducing magnetizing field H depends on the permeability of the material μ in which the field is induced.

Stated simply:

$$B = \mu H$$

In the cgs-emu system, $\mu = 1$ (in a vacuum) and $B = H$; thus the confusion that the terms for which the symbols stand are assumed to be the same, rather than equal in a vacuum.

We may note that the permeability of air (at 20°C and atmospheric pressure) is 1.000024; thus field intensity H and flux density B in the gap or volume (without the specimen) are for all practical purposes numerically the same.

When using biological samples where the permeability of the material is unknown and might even change considerably within the sample, it is more appropriate to describe the magnetic field by indicating field intensity.

HISTORY

Reviewing the history of electromagnets, one finds that, despite Oersted's 1820 discovery of the effect of an electric current on a magnetic needle, and the subsequent application and expansion of this principle by Ampere, Arago, Weber, Henry, Faraday, and others, it was over half a century later that Pierre Weiss built the first true laboratory electromagnet. The electromagnets commonly found in research laboratories today are direct descendants of the type built by Weiss in the late 1800's. In the 1930's the design of electromagnets was reviewed, with the objective of optimizing the design for higher field strengths. Designs of two large magnets, one by Dreyfus in Sweden and the other by Bitter at MIT, made at about the same time, have provided the design criteria for most present-day magnets. The basic yoke, pole-piece, and coil designs of laboratory electromagnets have not greatly increased the available field intensities in the past 25 years. Actual field performance of iron-core electromagnets does not vary significantly from the empirical calculated performance; therefore designs have become quite standardized around the H-frame or closed-yoke design

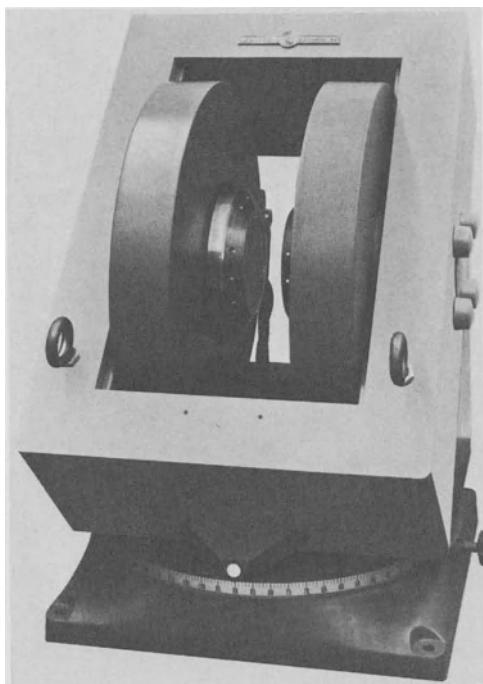


Fig. 1. Precision 9-in. electromagnet. Courtesy of Varian.

(Fig. 1). For some experiments, where maximum access to the air gap of the magnet is critical, a modification of the H-frame yoke design, called a C-frame or open-yoke magnet, can be useful (Fig. 2).

UNIFORM FIELDS

With the increasing use of magnetic fields in basic research, techniques were sought to improve magnetic field uniformity. Great impetus was given to the search by the discovery of nuclear magnetic resonance (NMR) and electron paramagnetic resonance (EPR). The homogeneity requirements of these two spectroscopic techniques were responsible for the dramatic improvements made in the technical areas of magnetic field homogenization and stabilization within the past 15 years. Specially designed electromagnets and sophisticated magnet current control systems can now produce magnetic fields which are homogeneous to one part in a million, and stable to one

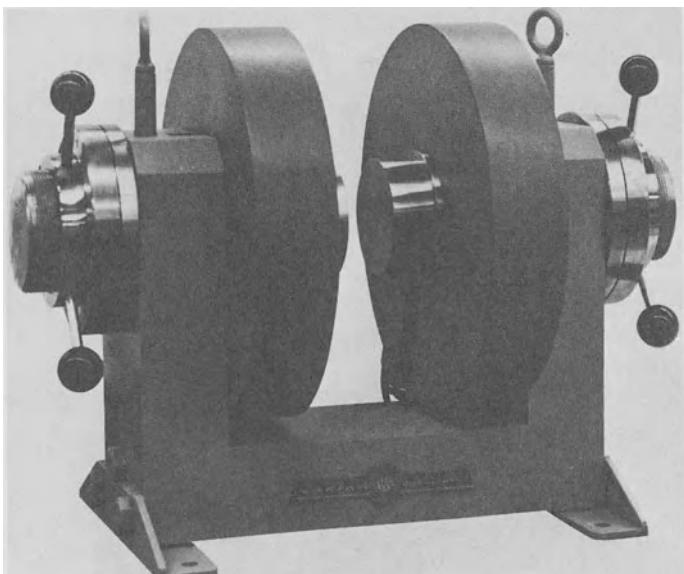


Fig. 2. Four-inch open-frame electromagnet with adjustable air gap.
Courtesy of Varian.

part in 100 million. This rapid progress was made possible by significant technological advances in the field of metallurgy, in finish-machining and heat-treating processes, and in field-sensing control circuits. The most important factors in the generation of uniform magnetic fields are the shape of the pole cap, its metallurgical history, and the processing it receives during manufacture. These same factors are also important in the shaping of nonuniform fields such as constant-gradient and constant-force fields. Care must also be taken in the design of the remainder of a magnet in order to achieve homogeneous or precisely shaped fields. For example, a magnet with a nonsymmetrical yoke, such as a C-frame, produces a field distribution which changes significantly as the field intensity is varied, due to forces on the nonsymmetrical yoke, which in turn create nonparallelism of the pole faces.

Large homogeneous fields for use on large biological samples are normally difficult to achieve except in large magnets such as 12-in. or 15-in. pole magnets, due to field fall-off at the pole-cap edges. However, special (ring-shim) pole caps based on the design concepts of Rose⁽¹⁾ are now available to enable the smaller magnets to achieve large homogeneous fields. Typically, a 9-in. magnet with specially designed pole caps can produce a field of nearly 8000 G in a 3-in. air gap, homogeneous within several parts

in 100,000 over a 2-in. diameter in the median plane, and 99% uniform over a 6-in. diameter. For very large samples, magnets with 12-in. or 15-in. pole diameters produce fields of 8 to 10 kG in 6-in. air gaps with slightly less uniformity than that described above.

Most commercial magnets are mounted to produce horizontal fields (i.e., the pole axis is horizontal), but generally only minor mounting modification is required to orient the field vertically, an orientation found very useful in many biological applications (see Fig. 3). The extra cost for such a modification is usually largely offset by elimination of the more expensive, conventional, multipurpose mount.

By and large, biomagnetic experiments indicate that the biological effect of the magnetic field is not instantaneous, but requires a continuous exposure to the magnetic field for a certain length of time for the effect to be observed. The reason for this could be either that the minute biological changes have to be accumulated, or that the physical precursor causing the biological change has to act for a certain time in the same direction before a significant biological change takes place.

The overwhelming majority of the cumulative physical effects which

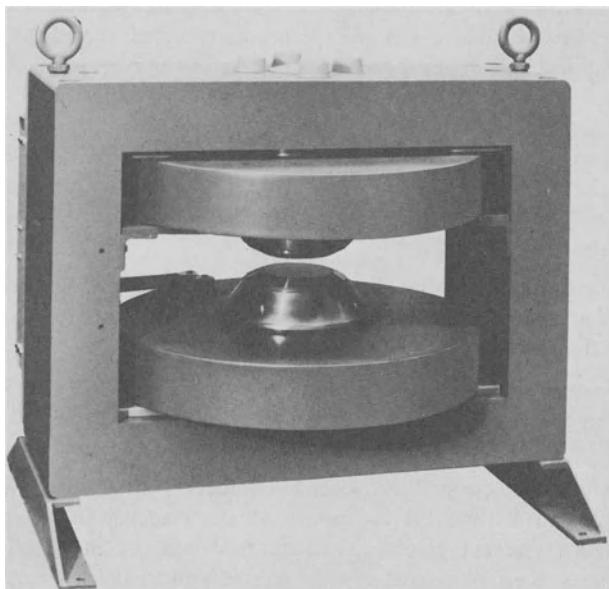


Fig. 3. Nine-inch electromagnet mounted for vertical-field experiments.
Courtesy of Varian.

could be considered as precursors of biomagnetic effects is reversible, meaning that a change in the direction of field and/or gradient vectors relative to the coordinate system of the specimen (organ, cell, molecule) will entail a change in the direction or sign of the physical effect. 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 requirement can be fulfilled only if the specimen remains in an unaltered position relative to the field or gradient vectors. 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 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. It is, therefore, sufficient that unrestrained animals be placed in a magnetic field with vertical field and gradient vectors in order to ensure that the animal remains in constant position relative to the field and gradient vectors. But one should expect a difference between the results of experiments executed in vertical and in horizontal magnetic fields. A more detailed discussion of this question was given by J. M. Barnothy in the first volume of this series.⁽²⁾

For a given magnet system, magnetic field intensity, field uniformity, and air-gap width are closely related and strongly influenced by the choice of pole caps. The following general rules apply within reasonable limits, when specifying an electromagnet with pole caps of cylindrical or conically tapered design.

1. Field intensity varies directly with the pole-piece diameter of the magnet and the power input, and inversely as the air-gap width and pole-cap-face diameter.
2. Field uniformity varies directly with pole-cap-face diameter (and therefore with the pole diameter of the magnet), and inversely as the field intensity and air-gap width.

One can find specific conditions which contradict one or more of the above general statements, but they do furnish a basic guideline in the selection of a magnet system. Figure 4 depicts the field intensity characteristics of various sizes of electromagnet systems plotted against air-gap widths. The diameter of the iron core in inches and the power consumption of the electromagnet in kilowatts are also indicated.

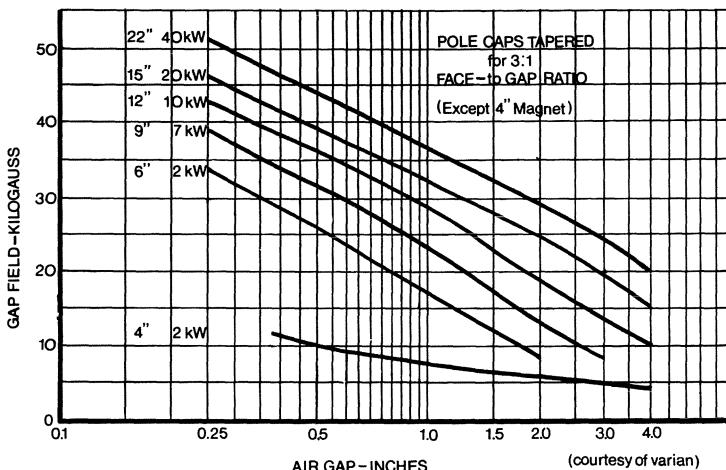


Fig. 4. Field intensity characteristics of various sizes of electromagnet systems plotted against air-gap widths. Courtesy of Varian.

CONTROL OF MAGNETIC FIELDS

A very important advantage of electromagnets is the degree of control which can be exercised over the magnetic field. The field can be continuously adjusted to the desired value by controlling the amount of current delivered to the magnet coils. Generally, the controls are an integral part of a power supply furnished as part of a magnet system. Storage batteries or DC generators were commonly used as power sources for early magnet systems because of the stability of their current output and their adaptability to different load impedances. Gradually, batteries gave way to current-regulated, vacuum-tube power supplies and finally to field-regulated, transistorized supplies.

The development in recent years of stable magnetic field sensing and feedback control circuits has greatly increased the flexibility and utility of iron-core electromagnets, as well as the simplicity of their operation. Power supplies which allow the user to dial the desired air-gap field to an accuracy of better than 1%, linearly scan a pre-set field range, and automatically reverse the field polarity are now in common use. Field-regulated power supplies essentially eliminate the undesirable effects of magnet iron hysteresis, extraneous magnetic field disturbances, and field variations due to temperature changes. Field stabilities better than one part in 100,000 are achieved.

A power supply is generally chosen which provides a DC output that maximizes the field-producing capabilities of the electromagnet at moderate cost. Typically, 4-in. and 6-in. magnets use 1 to 2 kW, 9-in. and 12-in. magnets need 5 to 15 kW, and 15-in. or larger magnets require 20 kW or more. Solid-state power supplies for electromagnets are generally water-cooled to eliminate heat dissipation to the laboratory work area, prolong component life, and reduce power-supply size. Since the major portion of the power is converted to heat in the magnet coils, these too require water-cooling. Good magnet and power-supply design must incorporate over-temperature-sensing devices and feedback protective circuits in both parts of the magnet system to guard the equipment against possible cooling-water failure. If over-temperature protection is not built into the system, some form of safety circuitry may be added during installation. In such cases the cooling-water inlet should contain full-time monitors of water temperature, pressure, and flow. These may be electrically connected into a safety servo system which shuts off system power when one of the pre-set limits is exceeded.

Some studies require the maintenance of constant sample temperature over relatively long periods of time. A magnet system with well-designed, efficient cooling circuits facilitates sample temperature control, since about 95% of the input power is discharged as heat through the water, and a modest room air conditioner can easily handle the rest. In less efficiently cooled magnets, however, magnet poles absorb coil heat at different rates, as magnet power and cooling-water temperature vary. Special water-cooled copper or aluminum discs may be placed against the pole faces to isolate the sample from these temperature variations. A system of this type can generally maintain pole-cap temperatures within a few degrees, and can be constructed or purchased for several hundred dollars. If air-gap space does not allow the placement of such cooling plates, the use of a closed-loop heat exchanger in the magnet cooling circuit may be necessary. Closed-loop cooling systems generally consist of a water-to-water or water-to-air heat-exchange mechanism in which the internal water-cooling loop is closed through the magnet system. A temperature sensor in this loop controls the flow rate of the cooling medium in the external loop of the heat exchanger, which in turn controls the internal loop heat-removing capacity.

PERMANENT MAGNETS

Although industrial usage of permanent magnets has increased steadily for the past thirty years, their utilization in research applications was

hampered by problems of field nonuniformity, low field intensity, and the difficulty of field shaping and control. The development of the more efficient anisotropic Alnico alloys in recent years has minimized or eliminated most of these difficulties. The major remaining disadvantage of permanent magnets is that of regulating or varying the field. Solutions to this problem normally entail the use of auxiliary field coils to give the desired net field effect.

The argument for using permanent magnets in biological studies is enhanced by the need for long, uninterrupted periods of steady field, by physical size restrictions, and by the relatively low field requirements of most biological experiments. The selection of the proper material and the design of the magnet to provide a specific field intensity and the desired flux distribution throughout the working volume are simply-stated requirements but represent a challenge even to the experienced magnet designer. Fortunately, the permanent magnet industry is large enough that a fair number of suppliers may be found who can furnish not only the desired end product, but the initial design capability as well. A comprehensive treatise on the subject of permanent magnets is listed in the references.⁽³⁾ Table II depicts the relative properties of some of the more commonly used permanent magnet materials.

Commercial sales of permanent magnets are divided almost evenly between Alnico and ceramic materials, with Alnico specified for most applications requiring fields near 10,000 G, and ceramic magnets preferred for lower-field applications.

TABLE II
Some Properties of Permanent Magnet Materials

Name	Coercive force H_c , Oe, nominal	Retentivity (residual induction), G, nominal	Energy product, $(BH)_m \times 10^{-6}$
Cobalt steels	200	10,000	< 1.0
Alnico 3	480	7,000	1.4
Alnico 5	640	12,600	5.5
Alnico 7	1050	7,700	2.9
Alnico 8	1650	8,200	5.3
Sintered Alnico 5	620	10,900	4.0
Ceramic types (Barium and strontium ferrites)	500–1500	7,000–11,000	1.5–4.0

Most permanent magnet materials are not machinable except by grinding. They are usually cast in the shape desired and finished by grinding. Depending upon the material, heat treatment is very important in obtaining the desired magnetic properties, and is usually performed by the manufacturer before finishing operations. The magnet is then generally subjected to heating and cooling and to a weak field of opposite direction to induce small changes of the material's magnetic state. These operations stabilize the finished magnet against the effect of stray fields, mechanical shocks, or temperature changes.

Field uniformity on the face of permanent magnet materials is poor. Where uniformity is a requirement iron pole caps are attached to the pole faces of the finished permanent magnet. With proper techniques it is possible to obtain the same uniformity as that of an electromagnet. The uniformity of the field is determined by the ratio of the pole-face diameter to the air-gap width, and by the parallelism of the pole faces. It is also possible to shape the pole faces to produce fields with quite high gradients $\delta H/\delta x$ along an axis of the air gap. Depending on magnetic field intensity, gradients ranging from 1 G/cm to more than 1000 G/cm can be created.

GRADIENT FIELDS

A number of biological studies require the use of magnetic fields possessing a gradient across the sample volume. For example, most methods of magnetic-susceptibility measurements require either a constant field gradient $\delta H/\delta x$ or a constant force $H(\delta H/\delta x)$ across the sample and for some additional distance on the axis along which the sample is moved. Because of their importance in biological studies, pole caps which produce these types of fields will be discussed in slightly greater detail.

Within the past ten years, constant force pole-cap designs which achieve fields with high $H(\delta H/\delta x)$ products have generally replaced other types in magnetic-susceptibility experiments. In a constant force field, two samples of equal mass experience a force proportional to their susceptibilities. In a constant gradient field, however, the two samples experience this force in direct proportion to their susceptibilities only if the samples are located in exactly the same position. The unit of the constant force field is the kG²/cm. Products of the order of 12 kG²/cm can be achieved in a 2-in. air gap of a 12-in. electromagnet, or in the 1-in. gap of a 4-in. magnet. A given design of a constant force pole cap is always optimized for a specific air gap. Varying the width of the air gap away from the optimum results in

serious degradation of the constancy of $H(\delta H/\delta x)$. Although a constant force pole-cap pair should not be used for more than one gap, it can be used for different values of magnetic field. The accuracy of a constant force field measurement is important in many studies. Though the value of H can be determined to a reasonably high degree of accuracy, the value of $\delta H/\delta x$ cannot. There are two reasons for this: the difficulty in accurately positioning the field-measuring probe and the averaging of the value of H over the probe dimension. Therefore, at present, the product $H(\delta H/\delta x)$ can be measured to little better than 1%.

The use of magnetic fields with a gradient across the biological sample is mentioned frequently in the literature. It is not difficult to locate an area in the gap of a magnet where fields with a gradient of 1000 G/cm exist. Generally, however, a fairly accurate special pole-cap design is needed to provide gradient fields over larger volumes, known to within 1%. Pole-cap designs vary considerably depending on the nature and direction of the field gradient. If high gradients are desired along the magnet pole axis, a conically tapered pole cap opposed by a tapered pole cap with a flat or slightly concave face of small diameter will suffice to produce gradients of the order of several thousand gauss/cm over small samples (Fig. 5). Fields with

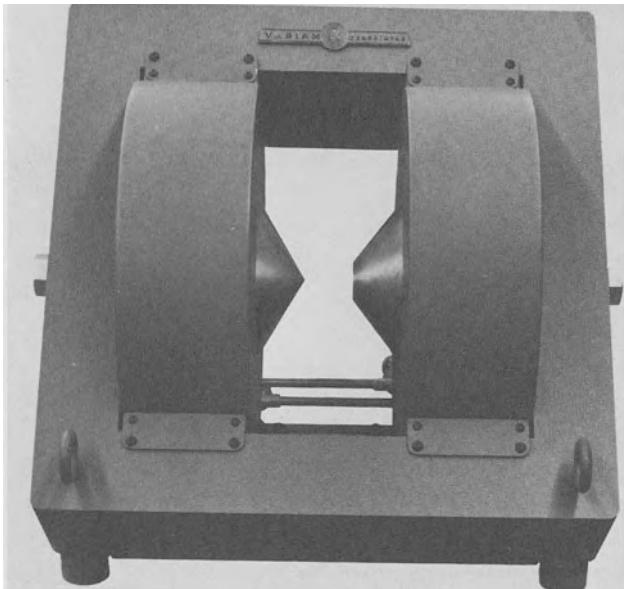


Fig. 5. Six-inch electromagnet with special pole faces for high magnetic field gradients. Courtesy of Varian.

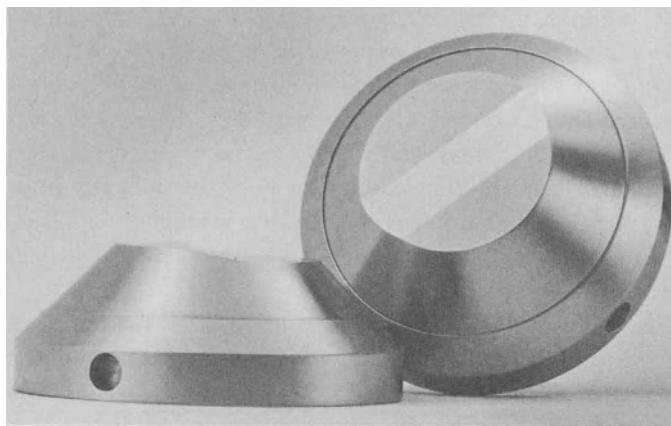


Fig. 6. Specially shaped pole caps for constant gradient field. Courtesy of Varian.

gradients of several hundred gauss, constant to 1% (usually along one axis), are produced by specially shaped pole caps such as illustrated in Fig. 6. Performance of a 4-in. electromagnet with constant force and constant gradient pole caps is depicted in the graphs in Fig. 7 and Fig. 8, respectively. Special pole caps which produce a constant force or constant gradient add only a few hundred dollars to the price of a small electromagnet.

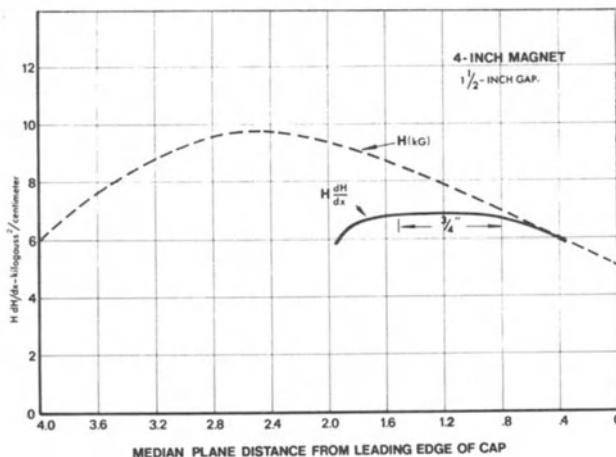


Fig. 7. Performance of a 4-in. electromagnet with constant force pole caps.

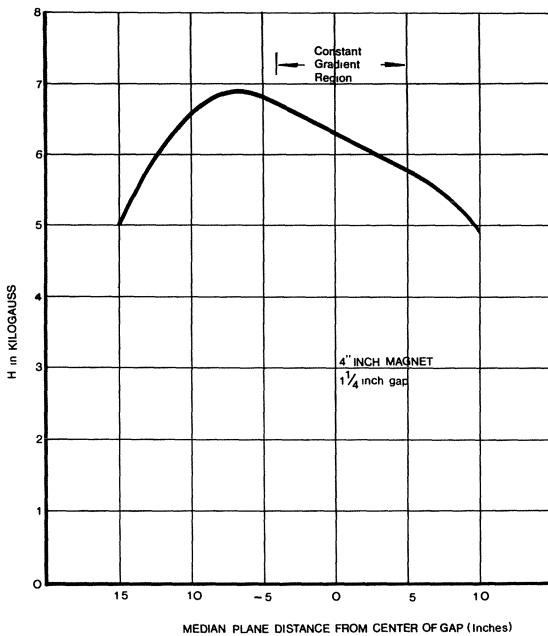


Fig. 8. Performance of a 4-in. electromagnet with constant gradient pole caps.

INTENSE MAGNETIC FIELDS

The term high magnetic fields generally refers to field intensities in excess of 50 kG, fields above those attainable in conventional, commercial iron-core electromagnets. The techniques of high-field generation can be divided into three groups, though all are similar in that the fields are produced by solenoids powered by large electric currents. The techniques are:

1. Use of normal conductors, cooled with water;
2. Use of normal conductors, cooled with cryogenic liquid such as hydrogen or nitrogen; and
3. Use of superconductors operating at liquid-helium temperatures.

With these choices available, one might presume that high magnetic fields are in extensive use in fundamental studies. However, the situation is not as favorable as one might think, especially when fields over 100 kG are sought. Regardless of the techniques used, severe technical difficulties arise

and fundamental limitations are encountered such as the strength of materials subjected to tremendous magnetic field forces, as well as other physical limitations of the systems.

The need for intense magnetic fields (above 100 kG) in research is shared by almost all of the major divisions of physics from high-energy physics to biophysics. The first devices to reach these levels were large copper coils which were designed to accept extremely high current for periods of less than 1 sec. As early as 1923, interest in intense magnetic fields had stimulated efforts to create fields above 100 kG over useful working volumes.⁽⁴⁾ In 1924, a Russian physicist, Kapitza, succeeded in generating pulsed fields to 500,000 G for several milliseconds by discharging a large AC generator across a small jelly-roll-shaped coil. He was able to perform many physical measurements during this short time. In 1936-7 Bitter at MIT developed a water-cooled electromagnet capable of generating and maintaining fields of 100 kG within a 1-in. air core.⁽⁵⁾ His solenoid design incorporated thick, insulated copper plates perforated with holes through which water was forced under very high pressure to dissipate the heat generated by the megawatts of power consumed. Most high-field copper solenoids in use today are built around the basic design principles of the early Bitter coils. Although pulsed fields will never eliminate the need for continuous intense fields, they have recently been pushed to new levels of intensity. Kolm and Foner have generated fields of 750,000 G at the Lincoln Laboratory of MIT for periods of 120 millionths of a second.

Pulsed fields are rarely suitable for biological research. The extreme fast rise and collapse of the magnetic field generates strong induced currents in the biological specimen. The biological effect of these eddy currents may be much stronger than that of the magnetic field and could overshadow the latter.

In recent years the Air Force has set up a sizable National Magnet Laboratory at Cambridge, Massachusetts, operated by MIT, which houses many such high-field solenoids.⁽⁶⁾ The highest continuous field produced by any solenoid to date is the 250,000 G generated in a magnet consisting of three coaxial solenoids having an inside diameter of slightly over 2 in. At full power it consumes 16 MW and uses 2000 gals of water per minute. Table III lists a few of the solenoids housed in the laboratory.

The National Magnet Laboratory facilities are made available to qualified research personnel from outside organizations. Application may be made by addressing the Assistant Director of the Laboratory. Several biomagnetic experiments have already been performed in the Laboratory.⁽⁷⁾ The most recent was a study of the behavior of squirrel monkeys in fields of

TABLE III
Magnets at National Magnet Laboratory

Magnet type	Bore size, in.	Maximum field range, kG	Number available
Standard solenoid	1 $\frac{1}{4}$	172-225	3
Standard solenoid	2 $\frac{1}{8}$	100-200	14
Solenoid with 1 $\frac{1}{8}$ in. dia. radial access	2 $\frac{1}{8}$	97	1
Standard solenoid	4	65-103	5
Standard solenoid	6	92-125	2
Standard solenoid	13-14	40-50	2

95 kG, conducted by Dr. D. E. Beischer and co-workers from the Naval Aerospace Medical Institute.

Magnets of the type found at the National Magnet Laboratory obviously require large investments in both primary equipment and support facilities. With power and water consumption rates measured in megawatts and gallons per second, respectively, these magnets are far beyond the budgets of all but the largest institutions. A typical copper solenoid designed for continuous fields of high intensity (100 kG) in a 2-cm working space requires a power of about 2 MW and over 500 gal/min of cooling water. A fairly up-to-date list of the major institutions where magnetic fields in excess of 100 kG have been generated and used is given in the references.⁽⁸⁾ It is worthwhile noting that the total number of such institutions in the world by 1967 was less than ten! Of these, all but three are government-sponsored.

The field intensity in a typical air-core solenoid is highest at the geometric center of the coil, on the axis of the bore, falling off rapidly toward the ends. Extremely high field gradients exist near the ends of the solenoid and this fact makes such devices of interest for biomagnetic studies. Though pulsed magnets represent one of the least expensive techniques for the production of very high fields, the field is a transient one, constantly varying with time. Therefore, they are impractical in most biological studies where experiments must be done over longer times in constant fields. For such work the use of supercooled or superconducting coils should be considered.

Supercooled solenoids circumvent the heavy power consumption through decreasing the electrical resistance of the conductor. However,

energy must be expended to produce the cryogenic liquid, and, if the magnet is to be used continuously, the refrigeration power can be greater than the electrical power used in a conventional high-field solenoid. A 2-in.-ID copper coil which operates continuously for about an hour until the liquid-nitrogen coolant is exhausted was constructed by Adair *et al.*⁽⁹⁾ The solenoid achieves a field of 36 kG with a power input of 62 kW. This coil represents a minimum capital investment for a steady DC field research coil. Higher fields are achieved by increasing power input and reducing the operating time to pulsed operation.

In most cases, steady magnetic fields between 50 kG and 100 kG are most economically produced in superconducting solenoids. These solenoids consist of coils wound with a special wire or ribbon conductor which becomes superconducting (i.e., has zero resistance) when maintained at a temperature near absolute zero. The availability of these conductors, particularly niobium-zirconium, niobium-titanium, and niobium-tin, has made possible the commercial development of solenoids with 1- to 2-in. working bores (at room temperature) and with fields to 100,000 G. The maximum field strength which can be produced with superconducting solenoids is limited by the fact that superconducting materials lose their superconductivity in high magnetic fields. Larger working volumes are possible at some sacrifice of field intensity and homogeneity. Typical research systems, including solenoid, power supply, and all necessary cryogenic hardware are priced in the range of \$ 10,000 to \$ 40,000 depending on field intensity, field uniformity, and field volume required. The major disadvantages of

TABLE IV
Types and Field Ranges of Magnetic-Field-Producing Devices

Type of magnetic-field-producing device	Upper operating range, kG	Most practical application range, kG
Permanent magnet	20	0-8
Laboratory electromagnet	40	0-30
Supercooled solenoid	100	25-60
Superconducting solenoid	140	30-100
Solenoid, water-cooled (intermittent operation)	250	75-150
Solenoid, liquid-helium-cooled (pulsed operation)	750	0-400
Implosion device	10,000	500 and up

superconducting solenoid fields in biological experiments are the relatively high operational expense (liquid-helium costs), the limited working space in the magnetic field, and the difficulty in maintaining a specific temperature over the sample. On an experimental basis magnetic fields to 140,000 G have been generated. A cryogenic solenoid of sophisticated design is being built at the new magnet laboratory on the St. Lawrence River in Montreal.⁽¹⁰⁾ It is expected to achieve a maximum field of 250,000 G for continuous-duty cycles of 10 to 15 min, using a combination of a supercooled aluminum core surrounded by a superconducting solenoid. This design by Dr. R. Stevenson circumvents the exposure of the superconductive material of the outer coil to fields higher than 80,000 G and raises the magnetic field to 250,000 G through the inner supercooled aluminum coil. The intense fields will be used primarily in studies of optical and magnetic properties of paramagnetic and antiferromagnetic solids.

MAGNETIC-FIELD-MEASURING INSTRUMENTS

Magnetic field measurements may be made using any one of over a dozen different principles, each of which has been the basis of a commercial instrument. A representative list of these instruments is given in Table V. Several of the instruments are particularly adaptable to the measurement of fields in biological studies and will be discussed here in some detail. The choice of the field-measuring instrument is generally determined by the type of field measurement to be made, namely, absolute measurements of field intensity (flux density per unit area), or relative measurements of the difference in field intensity between two points. Field measurements made with instruments having probes with small active elements are generally more precise (relative measurements) than those made with larger probes, though not necessarily more accurate (absolute).

The NMR (nuclear magnetic resonance) fluxmeter (Fig. 9) is the most accurate instrument (1 part in 10^6) for absolute measurements of flux density in the range of 1 kG to 50 kG. It is based on the principle of the energy absorption of protons when resonance occurs in a sample placed in a DC magnetic field and a weak perpendicular rf field of the proper (resonant) frequency. The NMR fluxmeter's primary limitations are the degree of field uniformity required over the sample and the probe size. If a magnetic field gradient of more than 1 G exists over the probe's active sample volume, the conditions of NMR resonance will not be met and no signal will be observed. The active sample volume is typically 1 cc or so, though the probe may be several cm thick to accommodate rf and receiver coils. NMR flux-

TABLE V
Magnetic-Field-Measuring Instruments

Instrument	Principle of operation	Features
Compass magnetometer	Movement of compass needle from N-S	Economy, 5-10% accuracy, used only with fields comparable to that of earth
Fluxmeter	Search coil and galvanometer with zero restoring torque on coil	Relative motion between coil and magnetic field, 1% accuracy
Rotating-coil gaussmeter	Coil rotated with constant velocity in field	Potentially accurate to 0.01%, wide measuring range
Hall-effect gaussmeter	Applies current to Hall-effect sensor and amplifies voltage output, which is proportional to magnetic flux density	Economy, small probe, accurate to 0.1%, wide measuring range
NMR fluxmeter	Number of protons in nucleus determines resonant frequency in magnetic field	Highest accuracy, wide measuring range
Magnetoresistance meter	Resistance change due to magnetic field	Simplicity, nonlinear at low fields
Fluxgate magnetometer	Core saturated equally in both directions; signal generated when unknown field unbalances core	High sensitivity at low fields, 3-5% accuracy

meter prices range from \$ 500 for the simplest devices to \$ 2000 for more sophisticated units.

A useful low-field-measuring instrument is the fluxgate magnetometer. It is useful in applications where determination must be made of the direction or magnitude of a low magnetic field. It consists of a magnetometer probe connected to a DC meter, and it has a measuring range of 1 mG to approximately 10 G, with a typical accuracy of several percent. The advantages of the instrument are its relatively modest cost, ease of operation, sensitivity, and portability, though it has limitations in its measuring range. Prices for magnetometers of this type range from \$ 500 to \$ 800.

The rotating-coil gaussmeter is less accurate (0.1 to 0.01%) than an

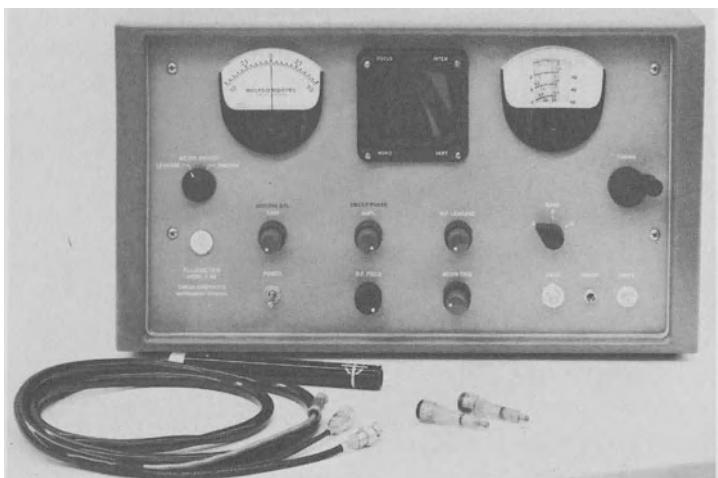


Fig. 9. NMR fluxmeter. Courtesy of Varian.

NMR instrument, costs about the same, but utilizes a smaller probe, and does not require a uniform field. It senses the magnetic field through the rotation of a wire coil in the field. The coil is rotated at a speed of several thousand rpm. This improves its sensitivity over that of the flip-coil. In comparison with other types of gaussmeters, the rotating-coil gaussmeter has some disadvantages, such as mechanical wear, electrical noise, and size of the mechanical unit.

Probably the most widely used field-measuring instrument in biomagnetic research is the Hall-effect gaussmeter. Hall-effect gaussmeters utilize

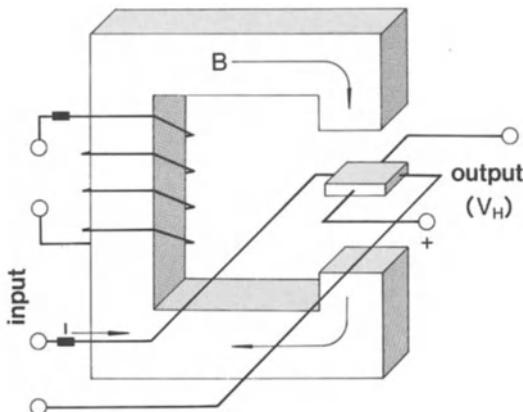


Fig. 10. Simplified Hall-effect circuit diagram.

the principle of charged-particle deflection in a magnetic field. A small crystal, generally a semiconductor, is placed in the magnetic field and a current is applied to it at right angles to the field (Fig. 10). As the charged particles move across the crystal they are deflected to the side, in direct proportion to the current density through the Hall element and the magnetic field intensity. A measurable electric potential called the Hall voltage is created across the sides of the crystal. The Hall-effect equation is

$$\mathcal{V}_H = wR_H(jB)$$

where \mathcal{V}_H is the Hall output voltage, w is the Hall-element width, R_H is the Hall coefficient, j is the current density through the Hall element, and B is the magnetic field strength. For any given conductor the voltage can be related directly to field intensity on a calibrated meter.

An advantage of the Hall-effect gaussmeter is the small size of the sensing element, a few mm^2 , which allows the mapping of the magnetic field over the area or volume of small biological samples. Some instruments are equipped with twin sensing elements and can be used to measure incremental fields or gradients. Accuracies of the order of 0.01% (relative) and 1% (absolute) can be achieved. A disadvantage is the fragility and relatively high temperature coefficient of the sensing element. Hall-effect gaussmeters are priced according to instrument accuracy and versatility, ranging from \$ 300 to \$ 2500.

ZERO FIELDS

Many biomagnetic studies require the use of control specimens around which the geomagnetic fields have been reduced to a fraction of their normal value. Though it is possible to create a working area with essentially zero-field, the maintenance of this condition in the presence of variations of direction and magnitude of the environmental magnetic fields may require fairly sophisticated servo-control systems. The magnetic field in a normal laboratory is generally a combination of the geomagnetic field and the stray fields produced by nearby AC or DC electric currents, rotating machinery, or magnetic devices. Field intensities ranging from $\frac{1}{2}$ G to 100 G or more are often measured at different locations in a typical laboratory. Both magnitude and direction of the field can be significantly affected by the movement of large metal objects in the room, switching of electric power, and other normal laboratory activities.

One technique for creating a field-free working environment of only a

few gammas ($1 \text{ gamma} = 10^{-5} \text{ G}$) is to compensate the components of the magnetic field using small permanent magnets. Permanent magnets, discussed elsewhere in this chapter, can be utilized quite economically (less than \$100) but may require a considerable investment in time to ascertain precise location for maximum nullification of the field, and they do not compensate for the daily variations of the geomagnetic field.

A better technique of field compensation is through the use of orthogonally placed electric-coil pairs known as Helmholtz coils. A typical installation consists of three pairs of coils, each pair placed at right angles to the other two pairs. The coils are wound with several hundred turns of small-diameter copper wire, which are energized by a stable DC source of several amps. Magnetic field variations within the control area can be detected and corrected by servo systems coupled to appropriately placed field sensors, if desired. Very precise control of the geomagnetic field can thus be achieved, the cost being directly proportional to coil size and accuracy of control. Simple, nonservo systems to reduce the magnetic field over a control volume of about 100 cc to less than ± 50 gammas could be constructed for about \$200. Servo-controlled coil systems could increase the cost by an order of magnitude. In biological studies where visual observation, temperature control, and limited movement of the specimen are desirable, the use of Helmholtz compensating coils has distinct advantages over magnetic shielding.

A magnetic shield diverts the magnetic field around the shielded object, presenting a low-reluctance path for the magnetic lines of force to follow (Fig. 11). The shield does not stop or absorb the lines of force. Thus, the effectiveness of a magnetic shield is directly proportional to the thickness of the shield. Thinner materials saturate at relatively low field levels, thereby limiting the level of attenuation that is achieved. The factors which must be considered in the design of a shield are the type of magnetic field, the magnetic field intensity (both AC and DC levels), and the degree of attenuation desired.

The choice of the magnetic alloy is a most important consideration in shield design. Generally, very soft magnetic alloys containing approximately 79% Ni, such as Superalloy, Permalloy, and Mu-metal, are best for shielding the earth's field and lower fields. These materials have very high permeabilities at low fields but tend to saturate at modest field intensities. Magnetic shielding materials are sensitive to shock and cold-working stresses; however, their shielding characteristics can be restored by annealing the finished product. Magnetic continuity between the sections of any single shield layer is essential, and close-fitting joints with generous overlaps

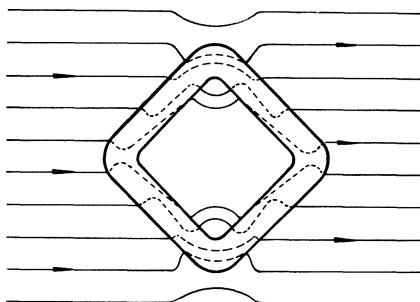


Fig. 11. Effect of magnetic shielding.

are necessary. Shields fabricated with multiple layers must incorporate non-magnetic spacers between layers for most effective shielding. Several areas of geophysical research, as well as space-simulation programs, require a large space having a controlled low-level field. One such magnetic shielded room, described by Patton and Fitch,⁽¹¹⁾ was constructed for approximately \$ 10,000. A small (4 in. in diameter, 15 in. long) Mu-metal shield for smaller control setups can be purchased for around \$ 600. The U. S. Naval Ordnance Laboratory, Maryland, has a room with 1000 ft³ of working area in which a zero field with 50-gamma deviation can be maintained to perform experiments on humans. The Kettering Foundation, Oakland, Michigan, can maintain a field of zero intensity with a deviation of only ± 0.1 gamma in a volume of a few cubic feet.

In summary, there are two ways of creating a zero-field space; compensating the force lines by creating a magnetic field of equal and opposite intensity, and shielding the force lines away from the space. Of these, field compensation generally creates fewer problems for experimenters in biology. A further discussion of zero-field installations can be found in the next paper (see page 29).

EPR AND NMR

In the search for new research tools to investigate biological systems, two magnetic resonance spectroscopic techniques have gained rapidly in prominence. These techniques are electron paramagnetic resonance (EPR) and wide-line nuclear magnetic resonance (NMR). In principle EPR and NMR resemble each other closely.⁽¹²⁾ In both techniques one uses a strong DC magnetic field and a weak rf field to excite a resonance in a sample containing particles that have a magnetic moment. At resonance the sample

absorbs rf energy and this absorption is observed as a spectral line in the output of a graphic recorder. Of these two spectroscopic tools, EPR has been most widely used in biological applications. A brief fundamental description of EPR and its application to biological problems follows.⁽¹³⁾

"To describe briefly the basic principles of electron paramagnetic resonance, it is necessary to concentrate first on a single electron. We 'see' the electron only by its interaction with other matter, and this interaction can be described by attributing to the electron certain 'physically measurable variables,' such as charge, mass, intrinsic angular momentum (spin), orbital angular momentum about some point in space, and magnetic moment.

If the single electron is placed in an applied magnetic field, the property of interest is its magnetic moment, whereby a torque is exerted on the electron tending to align its moment with the direction of the applied field like a compass needle. However, the electron also has intrinsic angular momentum that causes it to behave like a toy gyroscope or top and precess about the field direction instead of aligning with it.

In EPR spectroscopy the frequency of absorbed radiation depends directly on the strength of the applied magnetic field. To see this absorption one may either adjust the frequency to correspond to resonant absorption or adjust the strength of the magnetic field (and hence energy levels) until the energy separation corresponds to the quantum energy of the radiation. Both methods of scanning a spectrum are used in practice, with the latter procedure of sweeping the applied magnetic field the most favored. Frequencies in the range of 1,000 to 35,000 megacycles per second are most commonly employed with concomitant applied fields from 0 to 15,000 gauss.

Principal components of an EPR spectrometer operating at 10,000 megacycles per second include the following:

- An electromagnet whose homogeneous gap field can be swept continuously from zero to over 5,000 gauss.
- A sweep generator for producing a small audio modulation of the main field at the center of the air gap.
- A stable microwave oscillator (for example, a klystron) supplying rf energy at a frequency of 10,000 megacycles per second.
- A sample cavity resonating at the klystron frequency.

- A bolometer or crystal detector for demodulating the microwave power reflected from the sample cavity.
- An audio amplifier, and a phase-sensitive detector for conversion of the detected audio signals into low-noise dc signals for display.

The sensing element of an EPR spectrometer is a rectangular microwave cavity placed in a suitably stable and uniform magnetic field. Provision is made for sweeping the applied field in a linear fashion through the resonant condition with varying rates and sweep magnitudes for optimum display.

The sample is centered in the cavity at a position of minimum microwave electric field to reduce dielectric loss and is exposed to an audio frequency field produced by modulating coils fixed on the outside of the cavity as well as to the microwave and external magnetic fields.

The value of EPR as a spectroscopic tool results from the fact that an electron confined to an atom or molecule interacts with its surroundings and affects its magnetic energy levels. The resulting absorption spectrum reflects the change in energy levels and reveals an interaction that can be used to determine chemical structure as well as serve as a 'fingerprint' of a particular molecule.

Not all atoms and molecules are susceptible to study by this technique. A necessary requirement is that there be a net unpaired electronic magnetic moment associated with the atom or molecule under investigation. EPR can be observed for odd molecules and free radicals, biradicals, triplet electronic states, transition element ions, conduction electrons in metals and semiconductors, impurities in semiconductors, and radiation damage sites.

Samples usually are placed in quartz tubes or flat cells to minimize extraneous microwave loss, although a great variety of arrangements are permitted. With auxiliary apparatus, samples can be studied at pressures varying from vacuum to 100,000 atmospheres; temperature from 0.5° to 1000°K; and under simultaneous bombardment by electron, X-ray, ultra-violet, or visible radiation.

Up until a few years ago EPR was used almost exclusively by physicists to study paramagnetic ions of the transition elements in single crystals at very low temperatures. In recent years the physical-organic chemist has applied this technique to elucidate mechanisms of various chemical reactions.

It took only a few years after the discovery of EPR before this tool was applied to biological problems. For the past quarter century, the idea has been current that many of the electron-transport processes in biological systems may go by one-electron steps. Evidence for these led Michaelis to make his famous proposal that, 'It will now be shown that all oxidations of organic molecules, although they are bivalent, proceed into two successive univalent steps, the intermediate being a free radical...' Although Michaelis was convinced of the above, others required more direct proof than could be provided at the time. Because EPR is applicable directly to the detection and identification of free radicals, this tool made a tremendous contribution to the state of knowledge in biochemical reactions.

A large body of work also has been done in the study of biological oxidation-reduction reactions. Examples are studies of enzyme-substrate reactions where both substrate and enzyme radicals have been detected and valence state changes of metalloflavoprotein paramagnetic ions.

Photochemical studies have been performed with EPR on living cells, such as chlorella and chlamydomonas, with the direct determination of action spectra, and certain psychotropic drugs such as chlorpromazine have been studied using EPR together with UV photolysis.

Considerable interest has been generated in possible clinical uses of this type of spectrometry. The fact that free radical reactions have been shown to occur in biological systems raises the question of whether deleterious changes in body chemistry might not demonstrate their presence by altered free radical concentrations. The study of radiation damage effects and carcinogenesis are two cases in point.

Though it is possible to build one's own working EPR system, one generally sacrifices sensitivity, and many paramagnetic biological samples require the highest possible instrument sensitivity for signal detection. The recent development of small, complete EPR spectrometers costing less than \$ 30,000, with simplified operational characteristics and possessing extremely high sensitivity has dramatically increased the utilization of EPR techniques in biological studies."

Though the body of published work on biological applications of wide-line NMR is somewhat less imposing than that of EPR, it has nevertheless

become of significant interest.⁽¹⁴⁾ There are over 100 nuclear species that possess a magnetic moment. The nuclei most commonly studied by wide-line NMR are H¹, F¹⁹, Na²³, Al²⁷, H², P³¹, N¹⁴, and Cl³⁵. Of these, studies of the H¹ nuclei far outnumber all others, because of its relative abundance in nature. Wide-line NMR is used to study chemical bonding, crystal structure, and relaxation times in substances where the lines are too broad to be studied by high-resolution NMR. Substances with short relaxation times (such as solids and viscous liquids) and substances with large quadrupole moments have broad lines and cannot be investigated using high-resolution NMR techniques. Biological samples typically fall in this category.

The instrumentation for wide-line NMR studies consists of an electromagnet system, a probe, an audio modulation system, rf unit, power supplies, and some form of signal display. A block diagram of a wide-line NMR system is shown in Fig. 12.

Much of the instrumentation used in EPR spectrometers is compatible with NMR instrumentation requirements, and combination systems may be purchased. Construction of most elements of the NMR system being fairly well known, one may purchase the more sophisticated components,

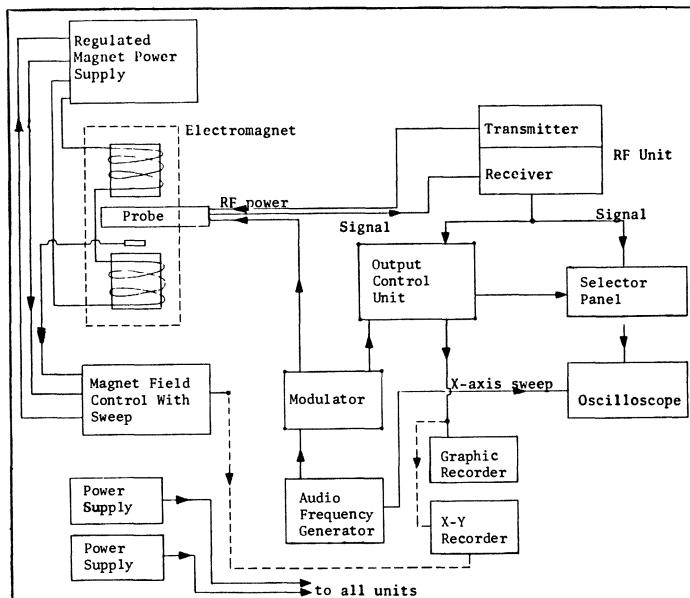


Fig. 12. Block diagram of wide-line NMR spectrometer system.

such as the magnet system, the rf unit, and probes, and build one's own NMR system. A useful system can be constructed from purchased parts for approximately \$ 10,000 excluding magnet system. Commercial systems are priced from \$ 20,000 to \$ 30,000.

As biology researchers turn more and more toward investigation of smaller systems, EPR and wide-line NMR will become more widely recognized as extremely useful, sophisticated experimental techniques. There is good reason to believe that when EPR and NMR techniques are used to gain additional information in biomagnetic studies, they will help to clarify the yet-eluding mechanism by which magnetic fields produce biological effects.

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EFFECTS OF NEAR-ZERO MAGNETIC FIELDS UPON BIOLOGICAL SYSTEMS

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INTRODUCTION

Man's exploration of space is resulting in his prolonged separation from the terrestrial magnetic field. In Fig. 1, a typical distribution of the geomagnetic field at the earth's surface is shown on a Mercator projection. Spacecraft, at the altitudes and latitudes of the usual near-earth orbits,

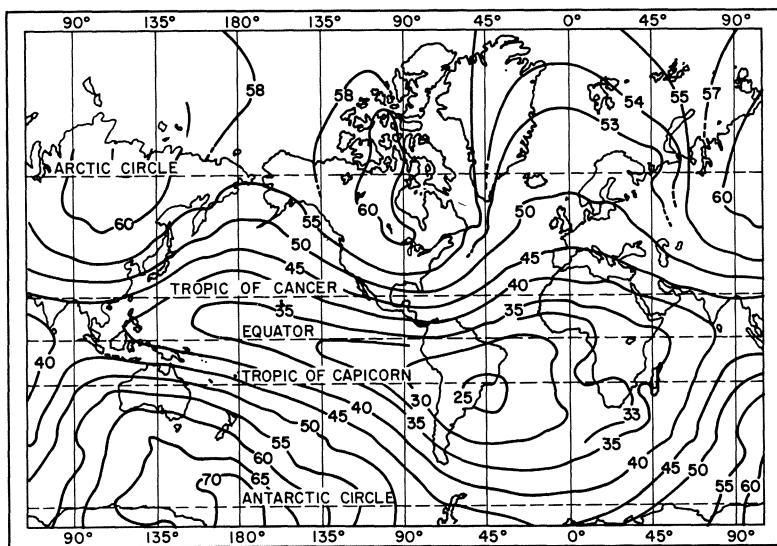


Fig. 1. The total intensity of the earth's magnetic field. Expressed in kgammas (0.01 Oe).
Source: U. S. Geological Survey No. 1703.

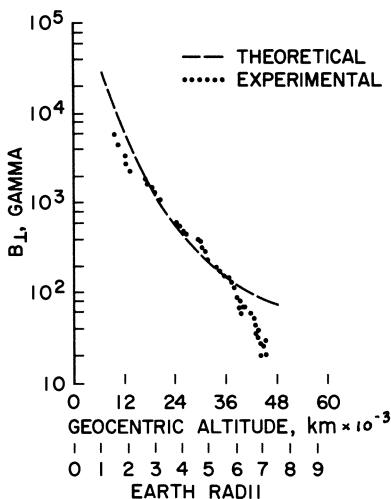


Fig. 2. Field magnitude measurements during a magnetic storm (Explorer VI). After Smith *et al.* (1963).

will be exposed for the most part to magnetic fields no lower than those around Rio de Janeiro at sea level. But in spaceflights carrying him more than 10 earth radii (about 1/6 the distance to the moon) away from the earth's center, man finds the intensity of his magnetic environment to be near zero (Fig. 2). Interplanetary probes [Ness and Wilcox (1965); Schardt and Opp (1967)] have revealed extremely low magnetic fields, i.e., in the range of a few gammas (10^{-5} Oe)* of intensity, and planetary probes [Koenig *et al.* (1967); Michaux (1967); Hess (1967), and Colburn (1968)] show fields of considerably less than 100 gammas around all those bodies which are man's first scheduled extraterrestrial landing sites (Table I). In addition, the makeup of the present generation of manned spacecraft has been estimated to cause only minimal modification of these ambient, null magnetic fields within the vicinity of the crew [Modisette (1966)].

Beischer (1967) has recently expressed most succinctly the basis for anticipating biological problems related to exposure to the nearly null magnetic field of outer space as follows: "It is hypothesized that the presence of a magnetic field during the major part of the development of life on earth has played a certain role in development and that living beings probably cannot be removed from the geomagnetic environment without

* See *Definitions*, p. 51.

TABLE I
**A Comparison of Representative Magnetic Field Intensities Adapted from Beischer
(1963)**

Alnico magnet surface	~ 1000 Oe	~ 10 ⁸ gammas
Solar flares at sun's surface	~ 100	~ 10 ⁷
Sun surface during quiescence	~ 1	~ 10 ⁵
Earth surface, polar	~ 1	~ 7 × 10 ⁴
Interplanetary during magnetic storms	~ 10 ⁻³	~ 100
Moon, Mars, Venus	< 10 ⁻³	< 10 (Moon)
Interplanetary, normal	~ 10 ⁻⁵	~ 5

penalty." In this respect, the findings of Harrison and Funnel (1964), recently confirmed by Watkins and Goodell (1967), of a correlation between the time of extinction of certain living species and the occurrence of geomagnetic polarity reversals, provide suggestive evidence for a significant influence of geomagnetism upon terrestrial life.

TECHNIQUES FOR PRODUCING LOW AND VERY LOW MAGNETIC FIELDS

We are concerned here with the experimental alteration or reduction of the ambient magnetic field and shall expand on Barnothy's list (1964) of four major categories of techniques for accomplishing this.

1. *Superimposition* of fields, a few oersteds in intensity, is done by using bar magnets, usually in order to change the direction of the geomagnetic field vector, a technique described in detail by Brown *et al.* (1960).

2. *Astatization* is the term for nullifying the local components of the earth's field with appropriately positioned permanent magnets.

3. *Shielding* of the subject from the geomagnetic field can be accomplished by surrounding the experimental region completely with metal sheets of very high magnetic permeability, so-called Mu-metal. Such material is thought to deflect the force field by concentrating it within the metal substance. Concentric layers of Mu-metal can bring the field contained in the experimental volume down to a few gammas or even lower. Such equipment usually imposes strict size limitations on the working volume. But there are exceptions, such as the metal room of dimensions adequate to accommodate human subjects which is in use by University of Illinois in Chicago, and was described recently in a paper presented by Cohen (1967).

4. *Compensation:* Large near-zero magnetic field working volumes can be obtained probably most cheaply by the use of a system of compensating coils of the Helmholtz type. Three coils, oriented in the planes of the three natural dimensions, can be activated so as to nullify all three vectorial components of the earth's field. With appropriate circuitry, the currents in the coils can be modulated to follow and compensate the natural daily changes in the earth's field. These changes are generally on the order of ± 100 gammas/24 hr. But smaller fluctuations occur at much higher frequencies within the day. These fast components have, in fact, been the subject of some interesting correlations with encephalographic activity of 8–16 Hz frequency [Lebedev (1966)].

Such an arrangement of coils with a compensating electronic servo system has been constructed at the NASA-Ames Research Center to provide

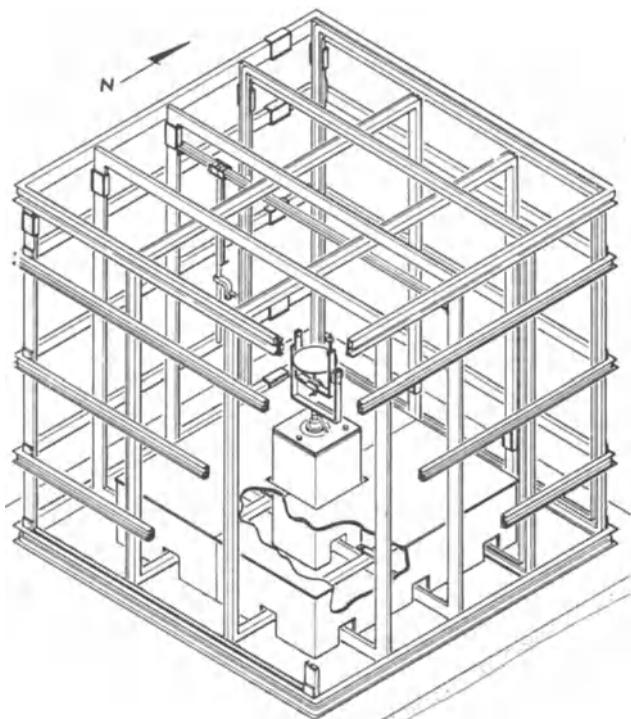


Fig. 3. Hypercubic coil assembly of space magnetic environment simulation Laboratory at the NASA-Ames Research Center. From Iufer and Droll (1967).

a usable volume of some three cubic feet within which the magnetic field intensity can be kept in the range of a few gammas or less, comparable to interplanetary levels. This is illustrated in Fig. 3 and is described in detail in the report by Iufer and Droll (1967). By virtue of its open construction, this arrangement has the important advantage for biological experimentation that control subjects can be placed nearby the test subjects and be exposed for prolonged periods to the same environmental conditions with the exception only of the magnetic field.

Other facilities are located in Oakland, Michigan, at the Kettering Foundation, in Silver Springs, Maryland, at the U. S. Naval Ordnance Laboratory, and at the NASA-Goddard Space Flight Center, Greenbelt, Maryland. More compact versions are available commercially.

5. *Combinations* of Mu-metal shielding and active electrical compensation are in use, e.g., the facility at the Institute of Geophysics and Planetary Physics of the University of California at Los Angeles [Barry *et al.* (1967)]. Combinations are also offered in equipment made commercially. Figure 4 shows in use, in our laboratory, the bottom half of such a flux tank with its concentric metal walls enclosing a set of coils surrounding a typical small rodent cage constructed of nonmagnetic and demagnetized materials.

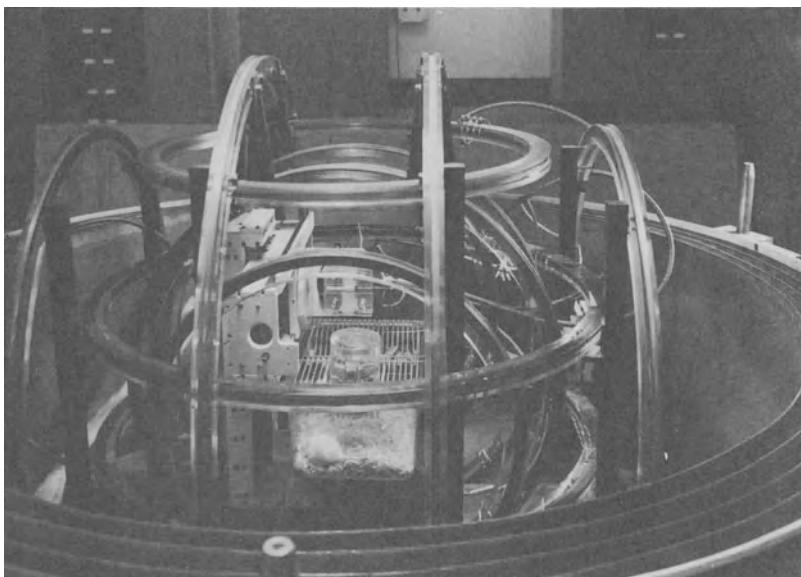


Fig. 4. Magnetic shielding tank composed of concentric cylinders of Mu-metal, opened to show the three-axis compensating coil assembly surrounding an experimental subject.

As the papers of Iufer and Droll (1967) and of Cohen (1967) have indicated, a primary purpose of near-zero magnetic field facilities is to permit the measurement, by sensitive magnetometers, of minute magnetic fields in a variety of equipment and in living systems. The importance of this aspect of very low field studies involving magnetic fields produced by living systems themselves cannot be overestimated [Beischer (1966)].

But the most fascinating application of near-zero fields should be in studying the effects of eliminating, as completely as possible, the geomagnetic force to which all living systems are subjected in nature on the earth. Certainly, this is the obvious and ideal approach for ground-based simulation of the near-zero magnetic field of certain planets and of outer space. It is only regrettable that for research purposes in other aspects of space biology, it is not possible to achieve analogous, ground-based simulation of the near-zero gravitational field of outer space.

LOW AND NEAR-ZERO MAGNETIC FIELD STUDIES

Table II presents a summary of selected reports of biological studies of low or very low magnetic fields. Most of the studies tabulated concern the effect of a marked reduction of the steady-state magnetic field intensity on the biological subject; therefore, the criteria of field gradient and fluctuations with time are omitted. Also, field direction is considered only where it is the independent variable, as in certain migration and growth studies.

Most of the work cited is quite recent, probably the result of interest in current space exploration and of the development of modern low-field-producing equipment. Almost all the reports selected were based on experiments with statistical analyses of the results. When experiments upon similar species under similar magnetic field conditions are compared, there appear to be fewer contradictions than might be suggested by a cursory scanning of the unorganized biomagnetic literature.

While there has been a great deal of emphasis on general growth and behavioral functions of whole organisms in low or null magnetic fields, neither the anatomical subcellular or organ level of function has been studied. And it is at these simpler levels of biologic function that one must seek the answers to the theoretical problems posed by the findings on the more complex levels.

Of the four major taxonomic groups not represented by work reviewed here (the Fungi, Echinoderms, Amphibians, and subhuman Primates), the first three lend themselves well to prolonged exposure within the type of equipment required to produce very low fields, and all four have been well

TABLE II
Summary of Selected Reports of Biological Studies of Low or Very Low Magnetic Field Effects

Biological classification	Specific common name	Level of organization	Function observed	Specific parameter measured	Magnetic field intensity*	Exposure duration	Effects observed	Author and year
Simple Algae	<i>Euglena</i> , <i>Chlorella</i>	Cellular	Reproduction rate	Reproduction rate	<1 mOe to 10^8 mOe	1-3 weeks	Reproduction accelerated in very low fields, inhibited in high fields	Halpern (1966)
	<i>Volvox</i>	Cellular	Locomotion	Turning from migration course by coenobia	5000 mOe	Approx. 1 min	Increased turning in augmented and redirected fields ($p < 0.01$ to $p < 10^{-6}$)	Palmer (1953)
Schizo-mycophytes	Bacterium <i>Staph. albus</i>	Cellular	Reproduction	Size and number of colonies	51 mOe	72 hr	Reduction in size and number of colonies: 15-fold	Becker (1963)
Angiosperms	White Clover (seeds)	Whole organism	Growth success	Germination success	< 0.7 mOe	48 hr	10-20% increase in germination after 31 hr ~ 1000 seeds/batch	Halpern and Dyke (1966)
	Winter Wheat (seedlings)	Whole organism	Growth	Root orientation	Hor. Geo.-vector, equivalent	Growth period	Orientation parallel to Geo., or applied field, all cases (~ 200 mOe)	Pittman (1964)

TABLE II (Continued)

Biological classification	Specific common name	Level of organization	Function observed	Specific parameter measured	Magnetic field intensity*	Exposure duration	Effects observed	Author and year
Protozoans	Ciliate (<i>Paramecium</i>)	Cellular (= whole organism)	Growth (reproduction)	Rate of reproduction	<1 mOe to 10° Oe	3 weeks	Growth accelerated under very low fields, inhibited under high fields	Halpern (1966)
	Ciliate (<i>Paramecium</i>)	Cellular (= whole organism)	Locomotion	Direction of migration path and amount of turning (klinokinesis)	1300 mOe	several seconds	Path distribution altered by applied magnetic field: S.D. increased by 6° ($p < .001$)	Brown (1962)
Flatworms	Planarian (<i>Dugesia</i>)	Whole organism	Locomotion	Direction of migration and klinokinesis (turning)	170 mOe (Geo.) to 10 Oe	Approx. 16 sec	Orientation modified ca. 10° from mean path by altered Geo. field ($p < .05$ to $< .005$)	Brown (1962)
Arthropods	Fly (<i>Drosophila</i>)	Whole organism	Locomotion	Orientation in phototaxis and activity	2000 mOe	1 min	Applied field influenced degree of turning ($p < .001$, all field configurations)	Picton (1966)
	Beetle (Cockchafer)	Whole organism	Locomotion	Ultraoptic orientation, migration	10^4 mOe	Several hr	Avoidance, or 50° shift of preferred sectors relative to field vector ($p < .001$)	Schneider (1963)

TABLE II (Continued)

Biological classification	Specific common name	Level of organization	Function observed	Specific parameter measured	Magnetic field intensity*	Exposure duration	Effects observed	Author and year
Mollusks	Snail (<i>Nassarius</i>)	Whole organism	Locomotion	Orientation during phototaxis	170 mOe to 1500 mOe	12-60 sec	Approx. 15° leftward shift in mean paths in increased or altered fields ($p < .005$)	Brown and co-workers (1960)
	Snail (<i>Nassarius</i>)	Whole organism	Locomotion	Orientation during phototaxis	40 mOe to 10 Oe	Approx. 1 min	Approx. 2° shift of mean path, max. at superimposed fields nearest Geo. levels ($p \text{ to } < .001$)	Brown and co-workers (1964)
Birds	Chicken (embryo)	Tissue <i>in vitro</i>	Growth	Blastoderm diameter, Axial orientation, Fe composition, anomaly formation	1100 mOe to 30 Oe	2 days	No effect at low fields; 30% reduced diam. and 33% increase in N-S orientation in higher fields; appearance of anomalies, decr. Fe	Veneziano (1965)
	Chicken (embryo)	Tissue <i>in vitro</i>	Growth	Embryo size and development	Approx. 0.05 mOe	4 days	Growth unaffected by very low field	Greene and Halpern (1966)
Sparrow	Whole organism	Behavior	Amplitude and character of motor activity	600 to 1700 mOe	2-9 hr	Increase and change in motor activity, 2- to 4-fold in 87% of cases	El'darov and Kholidov (1964)	

TABLE II (*Continued*)

Biological classification	Specific common name	Level of organization	Function observed	Specific parameter measured	Magnetic field intensity*	Exposure duration	Effects observed	Author and year
Rodents	Mouse	Cellular, tissue <i>in vivo</i>	Cytologic: (lysosomal enzyme activity)	Acid phosphatase content of peritoneal macrophages	< 0.8 mOe	18 hr	31% reduction in enzyme activity from very low field animals ($p < .001$)	Conley and co-workers (1966)
Hamster		Tissue <i>in vitro</i>	Growth	Tissue culture size	Approx. 0.5 mOe	Few days	No effect of very low field on tissue culture growth	Greene and Halpern (1966)
Mouse	Whole organism	Aging, growth, reproduction and behavior	Life span, litter size, activity, positioning and pathology	1.0 \pm 0.5 mOe	1 year	Shortened life span (6 months), diffuse tissue hyperplasia, infertility (F_4), cannibalism and supine positioning	van Dyke and Halpern (1965)	
Primates	Man	Subcellular (chemical)	Human enzyme reactivity	Quantity of specific substrate converted	< 0.5 mOe to 5 kOe	Several minutes	No significant alteration of enzyme reactivity in high or very low fields	Conley and co-workers (1967)
Man		Tissue <i>in vitro</i>	Growth	Tissue culture size	Approx. 0.5 mOe	Few days	No effect on growth of three tissue culture types	Greene and Halpern (1966)

TABLE II (*continued*)

Biological classification	Specific common name	Level of organization	Function observed	Specific parameter measured	Magnetic field intensity*	Exposure duration	Effects observed	Author and year
Man	Whole organism	Death, behavior, nerve disorders	Vital statistics (metropolitan)	Geo. (fluctuations)	5 years	Striking correlation of illness, deaths, etc., with 67 instances of sharp Geomag. disturbances	Düll and Düll (1935)	
Man	Whole organism	Cardio-vascular, cytologic	Blood pressure, leukocyte count	Geo. (fluctuations)	1 year	Time correlation between biol. parameters in 43 subjects and Geomag. fluctuations	Alvarez (1955)	
Man	Whole organism	Behavior	Rate of psychiatric hospital admissions	Geo. (fluctuations)	1 month	Positive correlation between Geomag. intensity and admission rate ($p < .001$)	Becker and co-workers (1961)	
Man	Whole organism	Behavior, nervous, cardio, resp., alimen-tary, endocrine, cytol.	Psych, tests, CFF, EEG, EKG, BP, resp. temp., weight, PBI, leukocytes	Approx. 0.5 mOe	10 days	Depression of CFF (flicker fusion) threshold, otherwise no significant changes	Beischer and Miller (1964)	
Man	Whole organism	Behavior, ner-vous function, misc. functions	Psych. tests, CFF, motor tests	< 0.5 mOe	10 days	Decreased CFF threshold ($p < .001$)	Beischer and co-workers (1967)	

* Geo. or Geomag. refer to intensities \cong 500 mOe.

studied in laboratories over the years. The genetics of the fungus *Neurospora*, cell replication in the sea urchin egg, and the embryonic development of the frog ought to be fruitful systems for biomagnetic research, for example.

ORIGINAL STUDIES *

As an example of biological studies in very low fields, the description of a two-part effort from our own laboratories is presented here.

Part One: *In Vivo* Studies

This section describes our observations on the influence of a very low magnetic field upon a selected reaction in animals to the introduction of a foreign biopolymer. Control animals were kept in the geomagnetic field, but were in otherwise identical surroundings.

The very low magnetic field area, described in greater detail by Iufer and Droll (1967), was located at the center of a 12-ft cube surrounded by open rectangular magnetic compensating coils (Fig. 3), working in a manner analogous to the Helmholtz principle and balanced to maintain a total field of less than 80 gammas (0.8 mOe) during the incubation period. The coil system occupied one half of an isolated building of 15 by 30 ft, so that control animals, located within this building but outside the coils, shared the same conditions of caging, ventilation, light, heat, and humidity as the experimental animals. Temperatures were recorded by calibrated hygro-thermographs at each location.

Materials and Methods

All animals were young, male, C₃H mice; controls and experimental animals were housed and handled alike. Eight animals were studied in each experimental run, four in the low-field area and four in the control area. They were in place 18 hr starting immediately after injection.

Since the acid phosphatase activity of serosal macrophages is correlated with their phagocytic activity, we used this measurement as an index of the reaction to the intraperitoneal injection of the foreign substance. Initially, we injected chicken egg albumin producing the qualitative reaction illustrated in Fig. 5. Later, using the lipopolysaccharide of *Escherichia coli*, we found [Conley *et al.* (1965)] that among selected strains the C₃H mouse

* These studies were made in association with Mr. Willie J. Mills and Mrs. Patricia A. Corsaut.

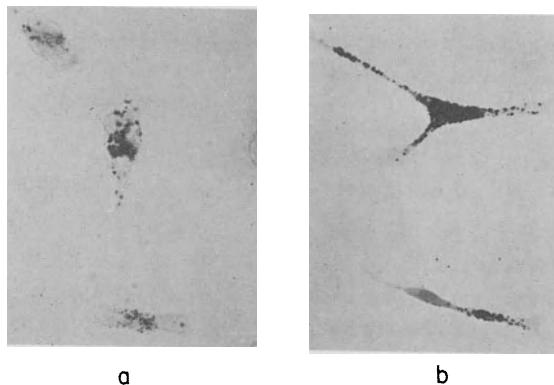


Fig. 5. Photomicrographs of serosal macrophages stained for acid phosphatase after harvesting by peritoneal lavage from (A) untreated control mouse, and (B) mouse injected 24 hr previously with a foreign protein, showing increased enzyme in cytoplasm. Gomori acid phosphatase stain, counterstained with nuclear red, 1000 \times .

exhibited the most distinctive quantitative peak in this reaction, and that it occurred after approximately 18 hr of incubation (Fig. 6). Peritoneal macrophages were obtained by saline lavage from all mice within 30 min after removal from the test chamber; a blind selection technique was used. Individual cell suspensions were assayed for total acid phosphatase activity

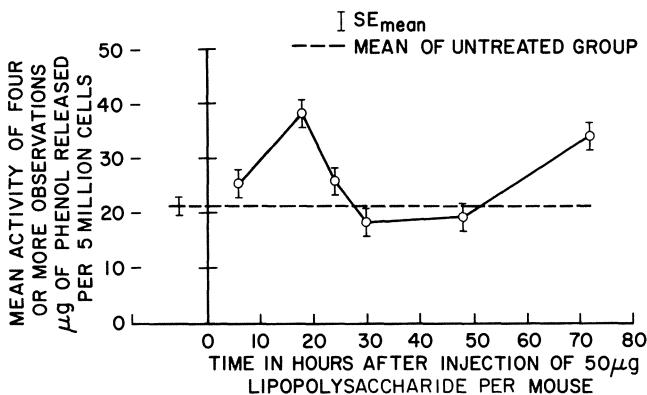


Fig. 6. Graph of acid phosphatase activity in C₃H mouse macrophage suspensions following injection of foreign material; note peak activity after 18 hr of incubation.

by a modified Lowry spectrophotometric method [Bessey *et al.* (1946)]. The activity was calculated in relation to the numbers of cells in the fluid as determined by counts made on a standard hemacytometer, and was expressed as micrograms of phenol released by the enzyme action per five million cells.

Results

We found consistently lower acid phosphatase activity in cell suspensions from animals which had been kept at the very low field intensities during incubation, as compared with the activity found in the controls.

The data from one series of experiments (Table III) were subject to an analysis of variance based on a randomized, complete block design utilizing treatment (null-field exposure vs. control) as blocks with two levels, and the times of the experiments (six different dates) as blocks with six levels. The average treatment means were highly significantly different ($p < 0.001$). The average response for the control group was 19.3, and for the magnetic compensating coil (null-field) group, 13.6 micrograms of phenol released per five million cells, representing a 31% reduction in activity. Since it was thought that environmental temperature could possibly have played a role in the difference between the responses of the null-field and the control animals, an analysis of covariance was performed, utilizing the average temperature ambient in the coil or on the control bench as a

TABLE III

Enzyme Activity *in Vivo*: Effect of Exposure to Nullled Magnetic Field, Showing Mean Acid Phosphatase Activity in Mouse Peritoneal Macrophage Suspensions, Expressed as Micrograms of Phenol Released per 5 Million Cells

Experiment number	1	2	3	4	5	6	Means
Treatment group:							
null-field chamber (< 0.8 mOe)	13.65	22.58	16.92	13.50	5.32	9.88	13.64*
Control (geomagnetic field: ~ 500 mOe)	21.05	25.58	25.58	18.15	10.75	15.12	19.32*

* Difference between treatment means is significant at $p < 0.001$, by analysis of variance, randomized complete block design.

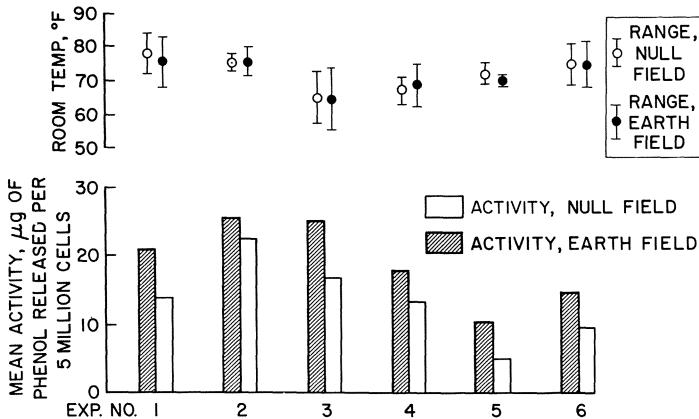


Fig. 7. Graphs showing the effect of a null magnetic field and the lack of an effect of room temperature upon the acid phosphatase activity of stimulated mouse peritoneal macrophages.

concomitant variable for the particular day of experiment. The ambient temperature was found to be nonsignificant as a factor in response to treatment (Fig. 7).*

In addition, continuous records of the local geomagnetic field [Breiner and Langan (1965)] taken during the periods of our experiments, were examined. The data were reduced and an analysis revealed no correlations between fluctuations in the ambient natural field and the pattern of our biological findings.

In another series, we achieved a macrophage priming effect by the intraperitoneal injection of sterile, complete Freund's adjuvant a few weeks prior to the injection of the lipopolysaccharide. This yielded a greater number of macrophages per animal with generally higher acid phosphatase activity in both the null-field and control groups than was seen in the first series. Nonetheless, the experimental results were the same as in the first series, with significantly ($p < 0.002$) lower enzyme activity from all null-field groups as compared with controls (Fig. 8).

In two sham experiments, four groups of mice were treated as in the first series, except that the compensating coils were not activated. No significant differences between the groups were observed.

* We are indebted to Mr. Eugene Averkin, statistician, for his help in the analysis of our data.

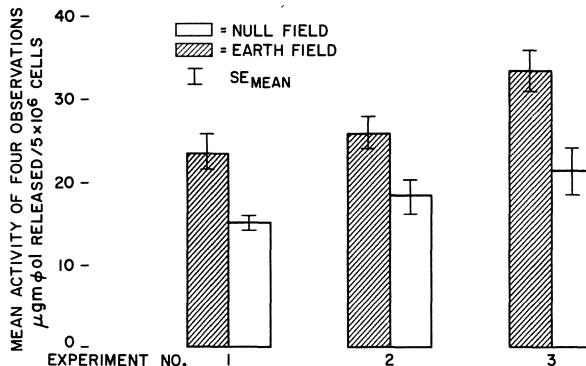


Fig. 8. Macrophage acid phosphatase activity: effect of nulled magnetic fields after preparation of mice with Freund's adjuvant.

Conclusions

While it appears that between-day factors produced differences in activity at least as great as those seemingly related to magnetic field differences, no correlations with day-to-day temperature or the concurrent small geomagnetic fluctuations were found. The consistently lower activity levels in cells from the chamber animals implies a possible inverse relationship between this particular aspect of cell function *in vivo* and the ambient magnetic field strength.

Part Two: *In Vitro* Studies

The following possibilities, singly or in combination, are suggested by the findings described in Part One:

1. The rate of production of enzyme by the macrophages was reduced.
2. A defective enzyme was produced.
3. The enzyme produced underwent accelerated denaturation in the reduced magnetic field.

The presence of paramagnetic transition metals in hydrolytic enzymes of the type assayed in the above study suggests that magnetic field effects on enzymes might derive from direct action on such metals. Smith and Cook (1963), Akoyunoglou (1964), and Wiley *et al.* (1964) have all shown enhancement of enzyme activity following exposure to high magnetic fields. But Maling *et al.* (1965) found no effect when the enzyme-substrate systems they used were allowed to react in a 100-kOe field. Since none of these

studies involved acid phosphatase or the null-field environment, we made observations of both nulled and high (kOe) magnetic field effects on the activity of this enzyme, as well as two others, *in vitro*.

Materials and Methods

The nulled-field (< 0.5 mOe) observations were made in the Ames space magnetic environment simulation facility (Fig. 3). Temperature regulation of experimental and control incubation vessels was achieved with a Precision closed-circuit, constant-temperature water bath. The high-field observations were made using a Varian Model V-4007 6-in. water-cooled electromagnet having V-4037 tapered pole caps with 3-in. faces providing a uniform field of 5.7 kOe with 95% homogeneity over a 2½-in. diameter.

A closed plastic water bath held the experimental test tubes in groups of three within the uniform-field area. This was circulated in parallel with the control incubation vessel by means of a Haake Model F constant temperature, circulating water pump.

The enzyme-substrate reaction systems studied consisted of the quality-control standards for alkaline and acid phosphatase,* with reactivity assayed by the Lowry-Brock method, and the standards for the glutamic-oxalacetic transaminase reaction,[†] using the Reitman-Frankel assay method. Thirty- and 60-min incubation times were used, respectively [Bessey *et al.* (1946); Reiman and Frankel (1957)].

Results

The results listed in Table IV show the lack of any significant effect of either high or low field upon the *in vitro* activities of the three enzymes.[‡] Our results are consonant with those of Maling and co-workers. But neither their work nor ours should be construed to contradict the findings of the three other groups, since both Maling and we used fresh, untreated enzyme which was already in contact with the substrate by the time the altered magnetic field was applied, and this application was only for the duration of the reaction period. The earlier workers had all pretreated their enzymes by exposure to high fields for at least an hour, and Wiley's group even showed a correlation between length of exposure and percent enzyme reactivation.

* From Dade Reagents Inc., Miami, Florida.

† From Sigma Chemical Co., St. Louis, Missouri.

‡ We thank Mrs. Margaret N. O'Neill for her advice on the statistical treatment of these data.

TABLE IV

The Lack of Significant Effect of Either High or Low Magnetic Fields upon the *in Vitro* Activities of Three Common Mammalian Enzymes Is Shown

Enzyme	Activity level*			Significance ^{II}
	Null (< 0.5 mOe)	Geomagnetic (control: 0.5 Oe)	High (5 × 10 ³ Oe)	
Experiment number				t-values (df ≤ 18)
1 Acid phosphatase [†]	1.21 (± 0.01)	1.22 (± 0.02)		0.500
2 Acid phosphatase [†]		1.34 (± 0.03)	1.36 (± 0.04)	0.444
3 Alkaline phosphatase [†]	6.02 (± 0.18)	5.83 (± 0.27)		0.189
4 Alkaline phosphatase [†]		5.33 (± 0.41)	4.90 (± 0.18)	0.962
5 Transaminase [‡] (glutamicoxalacetic)	92.3 (± 1.19)	93.2 (± 0.99)		0.600
6 Transaminase [‡] (glutamicoxalacetic)		93.5 (± 0.74)	94.6 (± 0.88)	0.960

* Means (± S.E.).

[†] Lowry-Brock units.

[‡] Reitman-Frankel units.

^{II} t-values < 1.0 with df = 18 indicate a lack of significance at the 70% level ($p > 0.30$).

Conclusions

In our *in vivo* experiments [Conley *et al.* (1966)] the enzyme was released by sonic disruption of the macrophages within a half hour after removal of the mice from the nulled field, and it is possible that a qualitative change in the enzyme could have been produced by the field and could have caused the results we got. So the *in vitro* studies presented in this section were done with the idea that if altered magnetic fields do, in fact, cause some such direct molecular changes in the enzymes resulting in denaturation, then the application of such fields could be expected to produce altered reaction rates when the enzyme-substrate system was exposed during the reaction period. Our finding of the lack of such an effect on fresh enzymes does not completely exclude the possibilities that malformation or denaturation were operative mechanisms in the *in vivo* experiments. But the results do seem to favor instead, a gradual, cumulative effect of the markedly reduced magnetic field. This effect is visualized as equivalent to the *loss of a favorable bias* on a complex sequence of cellular events thereby resulting in a quantitative change, and in this case, suggesting the first of the three

possible explanations cited in the introduction to this part, i.e., a reduced enzyme-production rate.

Discussion

In reconciling our findings, we must seek an explanation for an effect which appears to require more of the physiological milieu than a simple enzyme-substrate system, and which may be related more to the rate of production of a specialized enzyme protein than to the quality of that product. A possible inference is, that to be biologically effective, a magnetic field may have to act across a rather extensive conduction system, on the molecular scale, and for a fairly long time, in proportion to traditional electrical phenomena. Both conditions imply a cumulative action of the force field. A somewhat parallel inference was drawn by Solov'ev (1963), who felt his own experimental findings indicated that those biological media which appeared sensitive to an external, applied magnetic field could be characterized by their relatively slow processes.

In summary, the absence in the second set of our own experiments, of an effect of either high or low magnetic fields upon *in vitro* enzyme-substrate reactions, in the presence of our earlier positive findings of an effect from null-field exposure of intact animals together with the positive findings of others using enzyme pretreatment systems, suggests that any biological influence which magnetic fields do exert may be detectable only in cases of fairly prolonged exposure of complex sequences of cellular or biochemical events rather than in systems involving the instantaneous application of a direct magnetic force upon a single, specific chemical reaction.

COMMENTS

The complexity of the problems in the search for and exploration of biological effects of magnetic fields is almost overwhelming. It would appear to be the obligation of conscientious workers in this field to avail themselves of the insights of both of the major disciplines, chemical physics and integrative biology, applicable here. Not many researchers are equipped to do this alone, but all workers can encourage the collaboration of scientists with complementary training.

Also, the general advice of Longuet-Higgins (1962) as to how to perform biological experiments would seem to apply here; he writes that it is usually more fruitful to look for simple physical analogies than to engage in purely quantum mechanical discussions. But it is important in biomag-

netic theorizing to keep in mind that analogies to radiobiological phenomena should be applied with caution, since in radiation experiments the environmental variable is a form of *energy*, while with magnetic field studies, it is a form of *force*, two entities with quite different physical dimensions.

While experiments with intact organisms are very hard to interpret, they serve two important purposes: first, they provide manageable units for initial screening studies; and, second, they provide ready evidence for the practical significance of any demonstrable magnetic field effects.

But most importantly now are needed *in vitro* studies of any implicated biological systems, and experiments upon *in vivo* systems such as the previously uninvestigated Fungi, Echinoderms and Amphibians, made as simple as their integrity will allow, so as to permit more explicit theoretical interpretation.

In addition, there is a need for following such simple systems through a broad range of magnetic field intensities, so that if and when biological alterations are elicited, a quantitative correlation with field strengths might be made.

Lastly, it seems that much of the research for significant interactions between biological systems and the magnetic environment has suffered from a lack of persistence. The pursuit of both specific positive and negative findings could have been extended by simple repetition, i.e., the acquisition of more data, with great profit in many instances.

ACKNOWLEDGMENTS

Special thanks are due Messrs. Ernest J. Iufer and Paul W. Droll, physicists, of the Vehicle Environment Division, NASA-Ames Research Center. Dr. Torquato Gualtierotti and Mr. David Alltucker, recently of the Environmental Biology Division at Ames, are also to be thanked for their advice and the use of their high-field equipment.

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DEFINITIONS

In the cgs system of physical units, the gamma may represent either 10^{-5} gauss or 10^{-5} oersted because in a vacuum or in air, the permeability constant, μ , equals unity. There is, therefore, a 1:1 relationship between the induced magnetic flux density, B , in gauss, and the applied magnetic field intensity, H , in oersteds, since $B = \mu H$. The studies tabulated here were reported primarily in terms of applied or observed magnetic field intensities measured in the air ambient to certain biological systems. To facilitate comparison, we have converted the reported intensities to oersteds (Oe) or millioersteds (mOe).

In this review, the term *low fields* refers to intensities in the geomagnetic range of a few hundred millioersteds, up to intensities of a few to several oersteds. The terms *very low*, *near-zero*, *null*, or *nulled fields* all refer to intensities less than 1 mOe.

THE EFFECTS OF A MAGNETIC FIELD ON DNA SYNTHESIS BY ASCITES SARCOMA 37 CELLS

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Barnothy has reported that exposure to a magnetic field increased the rejection of tumor transplants in mice and increased the life-span.⁽¹⁾ Gross found that magnetic pretreatment of tumor-bearing mice increased the average life-span, but the increase was not statistically significant.⁽²⁾ Since any *in vivo* effects may be indirect, *in vitro* findings are of interest. Mulay and Mulay found increased degeneration of ascites Sarcoma 37 cells (but not of the solid tumor) exposed to higher intensity magnetic fields (4400 to 8000 Oe).^(3,4) Butler and Dean reported that the growth of KB cells in tissue culture was inhibited by a 4000-Oe field.⁽⁵⁾ On the other hand, Hall *et al.*⁽⁶⁾ found no lethal effects of 5000-Oe or 77,000-Oe fields on HeLa cells, in agreement with negative results of a 1200-Oe field on the same cells reported by Halpern and Greene.⁽⁷⁾ The same authors reported further negative results of fields from 0.0005 to 1200 Oe on the growth of HeLa, KB, WI-38 as well as Chinese hamster and chick embryo cells.⁽⁸⁾

In the case of microorganisms, Gerencser *et al.*⁽⁹⁾ found inhibition of *Serratia marcescens* and *Staphylococcus aureus* by inhomogeneous fields as did Hedrick for *S. aureus* in a homogeneous field.⁽¹⁰⁾ Jennison found no inhibition of bacteria or yeast⁽¹¹⁾ but Hedrick *et al.* did find a significant reduction in cell population of *Saccharomyces cerevisiae* exposed to a 4600-Oe field.⁽¹²⁾ Of some interest, too, is the report that tumor production by *Bacterium tumefaciens* on *Pelargonium zonale* was inhibited by a magnetic field but there was no effect on the growth or morphology of the organism.⁽¹³⁾

Assuming that magnetic fields do inhibit growth, theoretical treatments have been given, among others, by Valentinuzzi,^(14,15) Mulay and Mulay,⁽⁴⁾ Dorfman,⁽¹⁶⁾ and Winterberg.⁽¹⁷⁾

In our laboratories, aside from some of the early *in vitro* observations of I. L. Mulay,⁽³⁾ we have concentrated on the effects of magnetic fields on the respiration of cells and tissues, and have observed inhibition of the respiration of tumors and embryonic normal tissues.⁽¹⁸⁻²²⁾ In the course of this work we had the opportunity for a brief study of the effects of a magnetic field on DNA synthesis by ascites Sarcoma 37 cells with the autoradiographic technique and these results are reported in this paper. Since these experiments indicate that a magnetic field can inhibit DNA synthesis they would seem to be consonant with the positive findings of others on growth inhibition. The diversity of biological effects attributed to magnetic fields—such as those on growth, morphological changes, and respiration illustrated in the foregoing paragraphs as well as other effects such as those on the vertebrate nervous system⁽²³⁾—make a theoretical treatment difficult. If, however, one could relate these changes to such fundamental processes of growth as the rate of synthesis of macromolecules, or bioenergetics, a theoretical analysis might be more readily possible.

MATERIALS

Ascites Sarcoma 37 (S 37) cells were obtained from 5- to 7-week-old female Boontucky (BT) mice 5 days after intraperitoneal transplantation. The tumor suspension in ascites fluid was diluted with Hanks Basal Salt Solution (BSS) in a ratio of 1:3 by volume.

Tritiated thymidine ($^3\text{HTdR}$) was purchased from Schwarz Bio-Research, Inc. The aqueous solution containing 0.5 mc/ml was diluted in BSS to a concentration of 10 $\mu\text{c}/\text{ml}$. The solution was kept refrigerated in sterile bottles.

The photographic emulsion (Kodak Nuclear Track Emulsion NTB₃) was obtained from Eastman Kodak Co. It was kept in the dark in the refrigerator. Kodak Developer D19 and Kodak Acid Fixative were used.

The cells were stained with a 1% solution of orcein in 45% acetic acid.⁽²⁴⁾

PROCEDURE

Aliquots of 0.5 ml of the freshly prepared tumor cell suspension were pipetted into sterile test tubes and stoppered with sterile cotton plugs. The experimental sample was exposed to a magnetic field of 7300 Oe

obtained with a permanent Alnico magnet with a pole gap of 1.5 cm. The field strength was measured with two instruments: a Model A-2 gaussmeter fitted with probe FA-21 from G.R.H. Halstell Co. and a Model 150 gaussmeter with probe T-1501 from F. W. Bell, Inc. The field strength was measured in a plane equidistant from the pole caps at several positions on the *X* and *Y* axes: no measurable gradient was observed. The exposures were made at $37 \pm 0.1^\circ\text{C}$ in a water bath, and the control sample was maintained in the same water bath at such a distance from the magnet that the field strength at the center of the control sample measured 0 Oe. The setup was identical with that used in the previously reported respiration measurements.^(18,19)

In preliminary experiments the times of exposure to the magnetic field and the length of exposure to the $^3\text{HTdR}$ were varied. In some experiments the $^3\text{HTdR}$ was added while the cells were exposed to the magnet, and in others it was added after removal of the experimental sample from the magnet to a control position, the period of labeling varying from 30 to 60 min. All procedures gave similar results: decreased DNA synthesis in the cells exposed to the field and a somewhat wider scatter of rates of synthesis in the nonexposed cells.

For the experiments reported in this paper the following procedure was adopted. Thirty minutes prior to the termination of the experiment 0.5 ml of $^3\text{HTdR}$ solution was added to the exposed sample in the magnet and to the nonexposed sample (final dilutions, 5 $\mu\text{c}/\text{ml}$). Thus, regardless of the total magnetic exposure time, the labeling took place during the last 30 min of the exposure. At the end of the desired exposure period (1, 2, or 3 hr) all metabolic processes were terminated by addition of acetic acid-alcohol mixture (1:3, v/v) and the samples were kept at room temperature for 20 min. The cells were separated by centrifugation at 8000 rpm for 20 min and the cells were washed with the fixing solution to remove all unincorporated isotope.

The cells were stained by adding 1% acetic-orcein stain (3 times the packed cell volume). After staining for 10 min the cells were uniformly spread on microscopic slides by gently smearing one drop from a Pasteur pipette to give a layer about one cell thick. The slides were protected from dust and allowed to dry in the air at room temperature.

Kodak Nuclear Track Emulsion NTB₃ was transferred in the dark-room to a black-coated cell* measuring 4 by 1.25 by 0.25 in. and maintained at 45°C in a waterbath for about 30 min. The slides, warmed on

* The authors thank Mr. J. D. Forney for preparing the cell.

the waterbath cover, were dipped into the emulsion for 10 sec, removed and held at room temperature in a vertical position until the emulsion had gelled. The technique was similar to that of Messier and Leblond.⁽²⁵⁾ It was advantageous to wipe the emulsion from the blank surface of the slides for better definition later. The coated slides were dried in a stream of cold air for about 30 min and were then placed in plastic slide boxes, sealed with plastic electrical tape, and stored in desiccators containing silica gel. Low humidity is essential for good results.⁽²⁵⁾ The emulsion was exposed to the isotope for 72 hr at about 4°C. The slides were then removed to the dark-room, developed for 5 min with Kodak D19 developer, washed for 15 sec in running tap water, and fixed with Kodak acid Fixative for 5 min. After washing for 30 min the slides were dried and observed with a microscope under oil immersion. The DNA synthesis is measured by the amount of tritiated thymidine incorporated into the cell. This, in turn, is determined by the grain counts per cell obtained with the autoradiographic technique. For each determination grain counts were made from 20 cells per slide and the average count per cell was recorded.

RESULTS AND DISCUSSION

The grain counts per cell (each figure is the average of 20 cells) for S 37 cells exposed to the magnetic field of 7300 Oe for periods of 1, 2, and 3 hr, together with the counts of the unexposed cells, are given in Table I. It can be seen that the exposures for 1, 2, and 3 hr (28, 23, and 30 experiments, respectively) produce significantly lower grain counts, representing depressions of DNA synthesis of 17.8%, 23.7%, and 21.7%, respectively. The mean counts are plotted in Fig. 1. From the data available it cannot be said that the longer exposures were more effective in diminishing the DNA synthesis under the conditions of the experiments. Similar conclusions would have to be drawn regarding the effects of length of exposure to a magnetic field on the depression of respiration of S 37 and mouse embryo tissues.^(18,19,21,22) Both DNA synthesis and respiration of S 37 are depressed by magnetic fields and, on the whole, the order of depression is similar: 20–30%.

CONCLUSIONS

Exposure of ascites Sarcoma 37 cells to a 7300-Oe magnetic field for periods of 1 to 3 hr produces a decrease of DNA synthesis of about 18 to 24% as determined by the tritiated thymidine uptake measured by auto-

TABLE I

Grain Counts per Cell of Ascites Sarcoma 37 After Uptake of Tritiated Thymidine
(Each Figure Is Average of 20 Cells)

Exposure time to 7300-Oe field, hr	1		2		3	
	Unexposed	Exposed	Unexposed	Exposed	Unexposed	Exposed
69.7	40.8	65.1	53.45	40.65	28.1	
49.5	47.7	65.0	48.75	32.5	28.4	
58.5	49.75	71.0	48.25	35.7	32.15	
52.95	49.25	73.35	40.95	45.15	36.85	
51.6	39.5	75.95	54.45	38.65	34.4	
48.65	44.2	77.2	38.6	42.9	27.75	
66.2	58.3	66.7	44.95	84.9	53.35	
88.3	52.0	41.65	38.8	81.1	58.75	
78.4	61.8	56.3	42.4	77.45	56.9	
71.5	58.05	52.9	38.35	76.3	61.65	
66.05	48.55	43.25	35.95	67.95	58.0	
78.6	54.95	71.35	54.2	64.05	55.55	
77.8	55.15	68.15	50.95	73.05	52.9	
75.8	48.15	73.15	68.85	68.4	52.8	
80.75	48.2	81.5	63.7	73.2	61.9	
73.0	58.55	84.75	61.05	63.7	57.8	
67.4	62.45	84.15	66.0	76.75	60.55	
73.7	62.85	83.15	57.05	71.45	58.3	
68.45	56.85	68.15	62.95	42.9	33.75	
73.75	55.45	68.3	61.15	30.3	38.6	
65.15	62.45	77.15	64.0	32.95	35.55	
50.45	52.95	69.65	67.15	49.6	29.25	
61.45	61.45	77.2	55.1	53.25	26.1	
52.75	53.05			42.9	33.6	
51.2	50.3			57.25	40.15	
61.2	56.75			53.45	42.4	
55.8	53.8			49.15	38.45	
57.2	53.15			56.7	41.15	
				55.95	45.8	
				50.65	42.4	
Mean	65.03 ± 2.13*	53.44 ± 1.20	69.35 ± 2.43	52.92 ± 2.17	56.30 ± 2.93	44.11 ± 2.20
	<i>p</i> < 0.001		<i>p</i> < 0.001		<i>p</i> < 0.01	

* S.E.

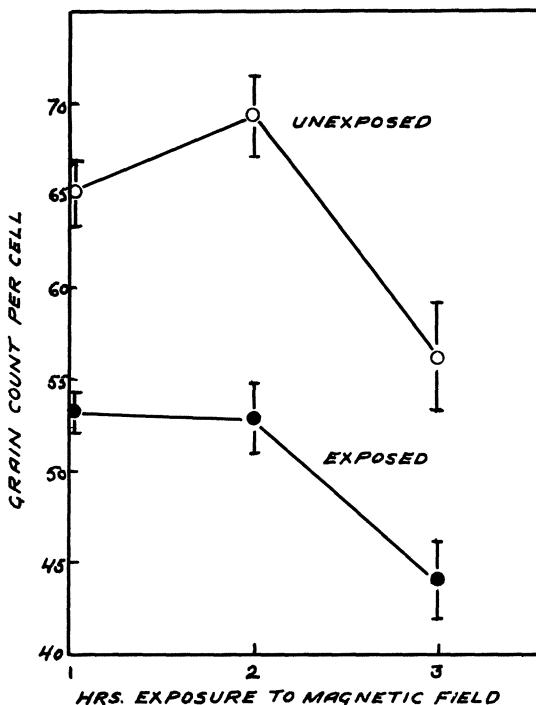


Fig. 1. Effects of exposure of ascites Sarcoma 37 cells to a 7300-Oe magnetic field on DNA synthesis. Bars represent standard error.

radiography. The depression of respiration by the magnetic field is of the same general magnitude.

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THE EFFECT OF STRONG INHOMOGENEOUS MAGNETIC FIELDS ON SERUM-FREE CELL CULTURES

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An investigation of the effect of strong magnetic fields on serum-free cell cultures of rabbit myocardium and of mouse lung fibroblast isolate was carried out. It was found that in each case there was a significant increase in the growth rate of cells when exposed continuously for two to seven days to a static magnetic field of 14,600 Oe, with a 5000-Oe/cm gradient.

In the *first experiment* Sykes-Moore culture chambers were used in permanent magnets (8 kOe, 1.0 kOe/cm) and in an electromagnet (29 kOe, 4.0 kOe/cm). The rabbit myocardium cell line in permanent culture had been adapted to rapid growth in a heat-stable serumless medium. The fluid volume in the chambers was 0.7 ml and contained approximately 1.5×10^5 cells. Following three days of exposure to the field, the cells were stained with Giemsa stain and compared with controls kept in dummy magnets. The three permanent magnets and the three dummy magnets were kept in an incubator. When the electromagnet was in use, the culture chambers were in a water bath kept at constant temperature of 37°C by a thermo-circulator. Each experiment was repeated several times.

There was no gross morphological difference between magnet and control cell preparations. However, a frank difference in the staining intensity could be observed: the cells in the magnetic field stained much

more intensely than the controls and the cell layers seemed to be more homogeneous. No attempt was made to determine the effect on the cell growth.

In the *second experiment* the basic cell techniques were altered so that an accurate determination of the effect of the magnetic field on cell growth could be obtained.

Disposable plastic petri dishes with an inner diameter of 50 mm were hermetically enclosed in thermostats turned from solid aluminum blocks. Through the circular water channels in these blocks a thermocirculator pumped water ($37.5 \pm 0.1^\circ\text{C}$) at a rate of 1.5 liter/min. In each experiment five identical thermostats were used, one between the magnet poles, the others serving as controls.

The magnet poles were kept at a temperature of $20 \pm 2^\circ\text{C}$ by water-cooling. The thermostat in the magnet was enclosed in a second copper thermostat, heat-insulated from the pole caps as well as from the inner thermostat, and kept at a 0.15°C lower temperature than the inner thermostat, by the recycling water. The temperature of each successive thermostat was lower by 0.05°C than that of the preceding one, following in the direction of the water flow; the thermostat in the magnet was always in the middle position, with respect to the water-flow sequence.

The control cultures and thermostats (shielded from the stray magnetic field of the electromagnet) were in a geomagnetic environment, heat-insulated by 2-in. of styrofoam; a 1-in.-thick brass disk simulated the upper pole cap of the thermostat in the magnet.

The 4-in. Varian electromagnet was used with a flat upper and conical lower pole cap, producing a field of 19,600 Oe in the center of the culture dish, at the level of the culture, with a vertical gradient of 11,100 Oe/cm. The average field strength was 14,600 Oe over the area of the culture dish and had an average gradient of 5000 Oe/cm. The AC ripple amplitude was 2 Oe. In some experiments a lower field strength was used and sometimes additional dishes of the control culture were placed in an incubator kept at 37.5°C .

Two cell lines were used. The rabbit myocardium cell line had undergone over 300 *in vitro* passages and was propagated on a medium of 99.4% Eagle's minimum essential medium and 0.6% peptone dialysate. The mouse lung line had undergone over 500 *in vitro* passages and was propagated on a medium consisting of 99% medium No. 199 and 1% full bactopeptone. Both lines routinely reached maximum growth in 4 to 5 days at which time about 1.5×10^6 cells/ml of medium are present in the rabbit myocardium isolate, and about 1.0×10^6 cells/ml, in the mouse lung fibroblast culture.

In some experiments, run at a temperature lower than 37.5°C, the maximum growth was reached later.

All petri dishes to be used in one experiment were planted from the same cell suspension in fresh complete media. The cells to be tested were harvested after 2 to 7 days of growth in complete media by scraping with nicrome wire.

The rabbit heart cells were harvested from the petri dishes by adding 0.1 ml of 5% trypsin and 0.1 ml calf serum to each dish. After standing for 5 min the cells were agitated by pipetting and placed in screw-capped plastic tubes. The calf serum was added to prevent clumping of cells which occurs following trypsinization in serum-free cultures. The mouse lung fibroblast cells grow in the supernatant and freeing of the cells is not necessary; only 0.5% trypsin was added to keep cells from sticking. The medium contained 50 units of penicillin and 50 µg of streptomycin per ml of medium to inhibit bacterial contamination. The tubes containing the harvested culture were sometimes cooled to 5°C until counting, when counting was delayed.

From the total cell medium harvest (about 5 ml), $\frac{1}{2}$ ml was pipetted in 20 ml saline and the cell number counted with a Coulter particle counter using a $100-\mu\text{-diameter}$ aperture. During counting the diluent was agitated by a magnetic stirrer. When the sample was counted at 11 different sensitivity levels (obtained through variation of the threshold setting and aperture current) a ten-channel, particle-volume spectrum was obtained, in which the cell volume increased by a factor of two for each successive channel. In each of the two cultures two well-defined particle sizes could be observed, differing in volume by a factor of 60 to 130 (or by a factor 4 to 5 in diameter). The larger size corresponded to the cells, the smaller size to cellular debris. Later, only the cell number of the first six channels was counted to find the location of the minimum of the differential counts, at which latter setting the actual counting was made. The peak of the cell-size distribution had a half-height width which corresponded to a deviation of about $\pm 15\%$ in cell diameter from the mean for both cell cultures.

Under the prevailing experimental conditions, the inhomogeneous magnetic field enhanced the growth of the serum-free cell cultures of rabbit myocardium isolate and mouse lung fibroblast. Figure 1 shows the relative difference (in percent) in cell number between magnet and control cultures as a function of the cell density at the end of the magnetic exposure. The standard deviation of the cell count among the control dishes averaged $\pm 3\%$. Since each cell culture was investigated more than six times, the existence of an enhancement of serum-free cell cultures through strong inhomogeneous magnetic fields is established on a probability level better

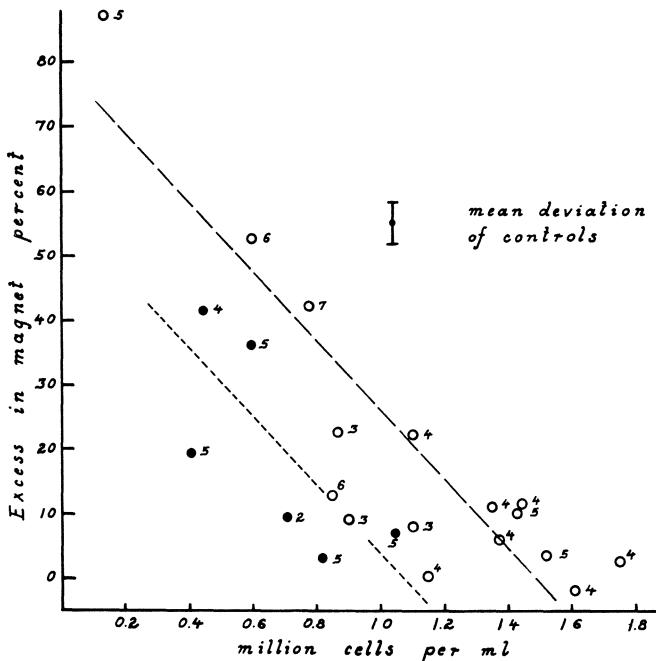


Fig. 1. Growth of serum-free cell cultures of rabbit myocardium cells (open circles) and mouse lung fibroblast cells (dots) in a magnetic field. The numbers beside the observational points indicate the exposure time in days. Average field strength was 14,500 Oe, the gradient, 5000 Oe/cm.

than 1:10⁵. There is some indication that at temperatures lower than 37.5°C the effect is larger.

From Fig. 1 we see that the difference between magnet and dummy cultures decreases with increasing final cell count; we infer from this that the observed phenomenon is akin to that observed in bacterial cultures,⁽¹⁾ namely, it is observed during the logarithmic growth phase, and vanishes after the biological space becomes saturated, suggestive of a factor acting during rapid cell division. However, while bacterial cells show an inhibition in strongly inhomogeneous magnetic fields, tissue cell cultures show an enhanced growth.

Two exposures corresponding to a field strength of 7000 Oe at the center of the culture dish decreased the cell growth of the magnet culture. This may indicate an inhibition at lower field strength, contrary to the enhancement at high field strength, and could explain the negative results

of earlier investigators. Pilot experiments with other cell lines showed that the sensitivity to a magnetic field varies greatly with cell type and even with the age of the serial culture.

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EFFECTS OF MAGNETIC FIELDS ON CELLULAR RESPIRATION

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There are several reasons for our interest in the possible effects of magnetic fields on cellular respiration. First, the literature contains many reports on the effects of magnetic fields on cellular proliferation but very few on metabolism, which furnishes the energy for proliferation. Second, some of the observations in the literature on biological effects of magnetic fields are relatively qualitative. Respiration can be measured with considerable accuracy and a quantitative technique has greater significance than more qualitative ones. Third, if a consistent pattern of effects can be obtained it may lead to helpful investigations of mechanism. Finally, members of our laboratory have had considerable experience in cellular respiration measurements.

Our studies have employed a constant-pressure differential type of respirometer^(1,2) designed to use small (1-4 mg) amounts of tissue or cell suspension. The latest design of the instrument and its use will be detailed later in this paper. In earlier experiments a tissue sample in the respirometer chamber was exposed to the 7300-Oe field of a permanent Alnico magnet in a pole gap of 1.5 cm. The respiration of a second control sample was measured in another respirometer placed in the same constant temperature bath as the experimental one and shielded from the magnet. Under these conditions field-gradient considerations were minimal owing to the small cross-sectional area of the tissue specimens; no gradient was measurable over this area. With this procedure the following results were obtained^(3,4):

1. The respiration of 5-7-day ascites Sarcoma 37 (S 37) was depressed in the field (Table I);
2. In a smaller number of experiments the respiration of ascites Ehrlich adenocarcinoma was also depressed;

TABLE I
Effect of a 7300-Oe Magnetic Field on the Q_{O_2} of Sarcoma 37 at 37°C
(10 experiments)

	Q_{O_2} at exposure time, min				Mean \pm S.E.*
	60	120	180	240	
Control	3.60	2.95	2.54	2.36	2.87 ± 0.14
Experimental	2.22	2.16	1.89	1.83	2.03 ± 0.11
% Depression	38.3	26.7	21.7	22.4	$0.84 \pm 0.18^*$

* $N = 37$, $t = 4.67$, $p < 0.001$.

3. The respiration of embryo mouse kidney was depressed, the younger the kidney (as determined by the curved-crown-rump lengths of the embryos⁽⁵⁾), the greater the depression (Table II);

4. The respiration of adult mouse kidney was not significantly affected (Table III): in individual experiments sometimes slight stimulation and

TABLE II
Percent Depression of Q_{O_2} of Embryo Mouse Kidney in a 7300-Oe Magnetic Field
(12 experiments)

Embryo length, mm	Percent depression at exposure time, min			
	60	120	180	240
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.

TABLE III
Effect of a 7300-Oe Magnetic Field on the Q_{O_2} of Adult Mouse Kidney
(Age range, 1 day to 19 weeks; nine experiments)

	Exposure time, min				Mean \pm S.E.
	60	120	180	240	
Control	2.5	2.7	2.6	2.7	2.63 ± 0.14
Experimental	2.6	2.6	2.5	2.7	2.60 ± 0.16
				0.03 ± 0.21	

sometimes slight depression were observed and there was no apparent correlation with the age of the mice;

- 5. Yeast respiration was invariably stimulated in the field; and
- 6. In an attempt to observe the effects of field strength the respirometer containing S 37 was moved different distances from the center of the pole faces and limited data suggested that the degree of depression of respiration varied with the field strength.

As pointed out in an earlier paper⁽⁴⁾ experiments with a permanent magnet and two respirometers are tedious, and working with a separate control in a second very sensitive respirometer adds to the experimental hazards. It would be desirable to make control and experimental observations on the same tissue or cell sample. Moreover, despite the attempt mentioned in the preceding paragraph, it is not readily possible to study the effects of variation of the field strength with a single permanent magnet. In addition, the use of intermittent fields on the same sample—alternate measurements of respiration in and out of the field—would show whether the effects were reversible. Finally, the use of intermittent fields could provide greater total energy because of the repeated establishment and collapse of the fields and thus might increase the effects.

The first attempt in our laboratory to study the effects of an intermittent field were made by mounting a quadruple permanent magnet (6000 Oe) on a dolly so that the magnet could be brought by hand to and from the specimen in a respirometer. The results of such experiments⁽⁶⁾ confirmed that respiration of S 37 and of mouse embryo tissues was depressed by the field while that of yeast was stimulated. It also was confirmed that the respiration of adult mouse kidney was unaffected by a constant field but it appeared

that the intermittent field stimulated the respiration of this tissue. This technique was awkward at best and, furthermore, it did not allow the study of varying field strengths. The acquisition of an electromagnet as a result of a grant from the National Cancer Institute provided an opportunity for an improved study of the effects of magnetic fields on respiration.

PRESENT RESPIRATION TECHNIQUES

The instrument (designed by J. C. F.) is based on those described earlier^(1,2) but adapted for use with the electromagnet. It is shown schematically in Fig. 1. It is a closed system of two gas chambers connected by a precision bore capillary U-tube 1 mm in diameter. In Fig. 1, *E* is the control vessel and *O* is the experimental vessel. (Flask *J* is an experimental chamber for general use, while *O* is designed for use in the magnet.) Chamber *O* is 13 in. in length so that it can reach into the center of the pole gap of the magnet. The tissue to be studied is placed in a small glass boat *Q* attached to a glass rod *P*, so that the boat can be positioned at the distal end of the chamber *O*. (*M* and *N* are side and top views of tissue boats for use with *J* and not employed in the present work.) The tissue (or cell suspension) is bathed in a physiological saline solution. Around the rod *P* is wound a strip of filter paper moistened with a few drops of 20% potassium hydroxide to absorb the carbon dioxide expired by the tissue. The setup of the experimental flask in its constant temperature jacket is shown in more detail in Fig. 2. Returning to Fig. 1, the experimental chamber *O* is attached by joint *F* to the capillary U-tube *G* which contains an index drop of odorless kerosene colored with Sudan III. The control chamber *E* is attached to *D*. A stopcock *C* closes the system to outside air. As long as the system is closed, both chambers of the respirometer are similarly affected by environmental barometric pressure and temperature. Silicone grease is used to lubricate all joints; obviously, prevention of all leakage is essential.

As compared with the Warburg respirometer, very small amounts of tissue (1–4 mg) may be used. The apparatus is not shaken so optimum gas exchange is secured by using only enough physiological saline to thoroughly moisten the tissue. It has been noted that the rate of respiration decreases with the depth of the medium above the tissue.⁽⁷⁾

Equilibrium of the air and water vapor in the two sides of the respirometer is approximately obtained by the empirical adjustment of the amount of distilled water in the control flask *E* and by depositing a few drops of distilled water at the proximal end of the experimental flask before final assembly of the respirometer. Before oxygen consumption measurements are

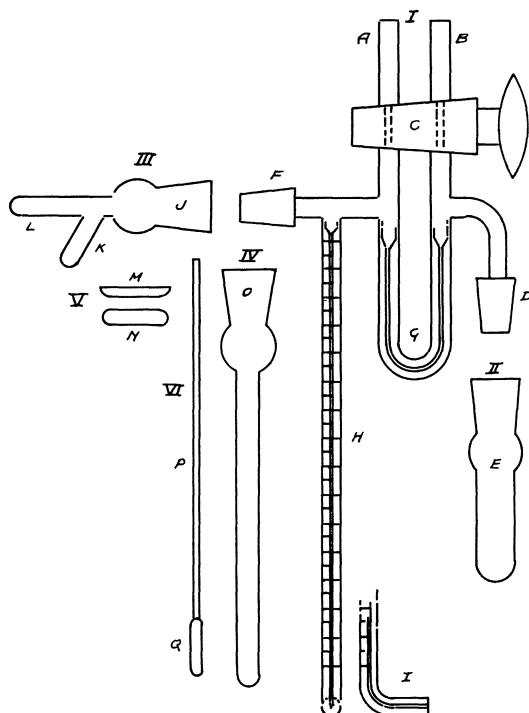


Fig. 1. Constant pressure microrespirometer: *A*, *B*, air inlet and outlet arms; *C*, double bore stopcock; *D*, joint to connect control vessel; *E*, control vessel; *F*, joint to connect experimental vessel; *G*, capillary U-tube; *H*, calibrated pipette; *I*, elbow from *H* to mercury reservoir; *J*, experimental vessel for general use; *K*, arm for KOH; *L*, tissue boat arm; *M*, tissue boat (side); *N*, tissue boat (top); *O*, experimental vessel for use with magnet; *P*, glass rod, sealed to *Q*, tissue boat.

begun, a period of equilibration is allowed of approximately 40 min, depending upon the amounts of tissue and medium used. Subsequently the mercury column in the calibrated capillary pipette *H* is adjusted to a predetermined level by means of a leveling screw attached to a mercury reservoir at *I*. The hair line of a reading telescope on the outside of the water bath is made to coincide with the meniscus of the index drop in *G*. This position remains the reference point throughout an experiment.

After equilibration the stopcock *C* is closed and readings are made as

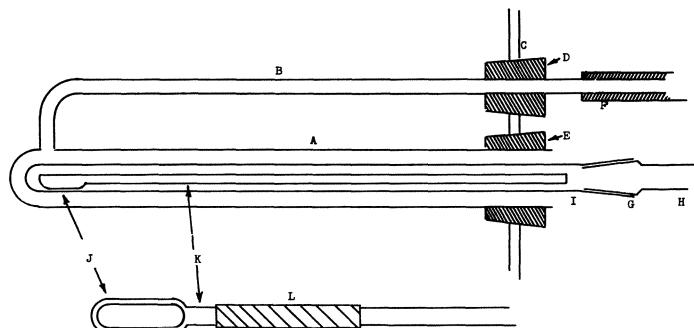


Fig. 2. Microrespirometer (experimental vessel): *A*, water jacket for respirometer vessel; *B*, water intake tube; *C*, wall of water bath; *D*, rubber stopper for inlet tube; *E*, rubber stopper for water jacket; *F*, rubber tube connection to circulating pump; *G*, ground joint to connect vessel and respirometer; *H*, experimental arm of respirometer; *I*, experimental respirometer vessel; *J*, tissue boat; *K*, glass rod attached to boat; *L*, wrapped strip of filter paper saturated with KOH solution.

described below at intervals of not less than 10 min. This is the shortest period in which sufficient oxygen is absorbed to permit reliable measurement. The first two readings are usually discarded and are observed only to be sure that the tissue is respiring properly. As the respiring tissue uses oxygen, the index drop in *G* moves toward the experimental flask. To make a reading the index drop is returned to its original position by moving the leveling screw attached to the mercury reservoir. The differences between successive mercury level readings in *H* represent the volume of oxygen absorbed by the tissue during the respiration period. Changes of ± 0.1 mm of mercury are measurable so that differences of the order of 1 mm between control and experimental readings (as in Fig. 3) are significant and reproducible.

To study the effects of intermittent fields the respiration was usually measured for 10 min with the field activated as the experimental period and 10 min with the field inactivated for a total of 2 hr. Of course, other periods of exposure may be used. Figure 3 represents the results of a single experiment on the respiration of S 37. Successive readings in mm of mercury are plotted with the magnet (80 Oe) alternately on and off for 10-min periods. The reproducibility and reversibility of the effects is illustrated although the total respiration of the cells declines somewhat toward the end of the experiment.

In these experiments, where the same tissue sample serves as both control and subject in a single respirometer, it is not necessary to know

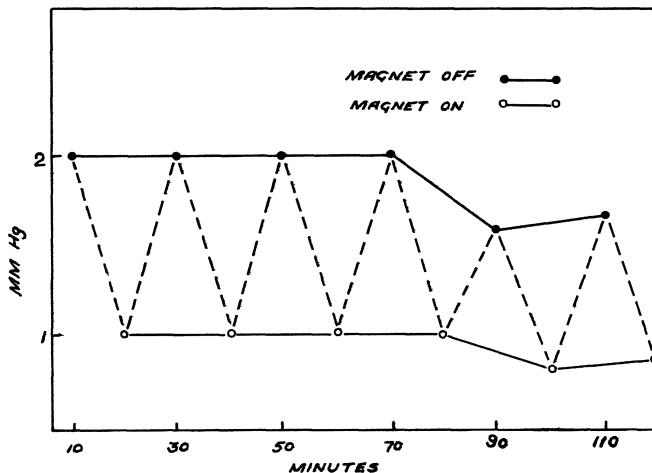


Fig. 3. Effects of an intermittent magnetic field of 80 Oe on respiration of ascites S 37 sarcoma cells. The dotted line connects 10-min respiration values and shows reversibility of effect.

the Q_{O_2} (μl of oxygen absorbed per mg dry weight of tissue per hr) in order to determine the influence of the magnetic field. Under these circumstances each experiment stands alone, but with an experienced operator results of separate experiments are generally in reasonably good agreement in terms of percent stimulation or depression, as will be seen later. Dispensing with calculation of the Q_{O_2} also eliminates a source of error in the tissue weighing.

If it is desired to determine the Q_{O_2} , as is necessary when separate control and experimental respirometers are used, one must, of course, determine the dry weight of the tissue samples. At the conclusion of the respiration determinations the tissue is placed in a weighed glass crucible and dried overnight at 80°C or until constant weight. After cooling to room temperature in a desiccator the crucible is weighed on a microbalance. Q_{O_2} is given by

$$Q_{O_2} = \frac{RkF}{WT}$$

where R is the difference between two successive capillary readings, k is the respirometer constant, F is the conversion factor to NTP (760 mm and 0°C), which may be obtained, for example, from Dixon,⁽⁸⁾ W is the dry weight of the tissue in mg, and T is the time in hr. The respirometer constant

k must be determined for each respirometer from the formula

$$k = \frac{W}{DL}$$

where W is the weight of a mercury column in g in the reading capillary of length L in mm and D is the density of mercury at the environmental temperature (tables of D may be found in Dixon⁽⁸⁾ or Umbreit *et al.*⁽⁹⁾). In the respirometers we used k has varied from 0.7 to 1.53.

The respirometer is immersed in a constant temperature bath which in most of our experiments was kept at $37 \pm 0.01^\circ\text{C}$. The main bath is a glass aquarium but the experimental chamber is enclosed in its own elongated bath attached to the side of the main bath as diagrammed in Fig. 2. Because of the great sensitivity of the respirometer the temperature must not deviate more than $\pm 0.01^\circ\text{C}$ as determined by a Beckmann thermometer. This is accomplished by using a 250-W infrared lamp mounted external to and nearly against the bath wall and which is activated by a mercury thermoregulator and vacuum-tube relay. In order to keep the temperature of the whole system as uniform as possible a pump circulates the water from the neighborhood of the thermoregulator in the bath proper to the distal end of the water jacket for the experimental flask as indicated by B of Fig. 2. To minimize heat loss the glass walls of the bath are covered with aluminum foil, shiny side toward the water, and the bath is further encased in sheets of urethane foam. The entire bath is mounted on a jack which may be used to vary the height, and this in turn is mounted on tracked rollers so that the experimental chamber may be moved in and out of the pole gap of the magnet.

The magnet employed in this work was a $9\frac{1}{2}$ -in. water-cooled Harvey Wells electromagnet with a pole gap of 2 in. to accommodate the water jacket of the experimental respirometer chamber. In the experiments to be described the field strength was varied from a residual 40 Oe, which remains during the period after the power supply has been shut off, to 10,000 Oe. The field over the experimental area was homogenous within the limits of our measurements.

EXPERIMENTAL METHODS AND MATERIALS

The experiments reported here⁽¹⁰⁾ are restricted to studies with intermittent fields in which the respiration of cells or tissues was measured for alternate 10-min periods with the magnet off (control) and on (experi-

mental) for a total of 2 hr giving six control and six experimental readings. However, the effects were verified using longer control and experimental periods (20, 30, and 60 min), with correspondingly larger volumes of oxygen consumption and reduced error in measurement; entirely similar results were obtained. Results are reported in mm of Hg rather than in Q_{O_2} as explained earlier.

Female Boontucky (BT) mice (a random bred strain which originated from the Rockland All-Purpose strain and which has been maintained in our laboratory for many years) were the source of animal tissues. Ascites Sarcoma 37 (S 37) cells were used 3 to 9 days following serial intraperitoneal transplantation. Two drops of tumor suspension in homologous ascites fluid were used for each experiment. Slices of embryo kidney and liver, adult kidney and liver of 14- to 21-week-old mice, and neonatal liver (of mice 2-3, 3-4, 6-7, and 10-11 days of age of the same litter) were suspended in Hanks Basal Salt Solution (BSS). Baker's yeast (*Saccharomyces cerevisiae*) from commercial Fleischmann's cake yeast were also suspended in BSS. Intermittent magnetic fields of from 40 to 10,000 Oe were used as shown in Table IV. Not included in the table are four experiments on HeLa cells in Eagle's medium.

RESULTS AND DISCUSSION

Field strengths of 80 Oe or higher lowered the respiration of S 37. Lower field strengths were without effect and higher field strengths up to 10,000 Oe produced no significantly greater effect. It would appear that a critical field strength of 75 to 80 Oe is necessary to obtain the effects. The average depression of respiration in 25 experiments at 80 to 10,000 Oe was $28.3 \pm 2.6\%$ ($p = 0.02$).

A similar picture emerges from the data on embryo mouse kidney and liver. In seven experiments with embryo kidney, field strengths of 85 through 5000 Oe produced an average respiratory depression of $29.3 \pm 3.5\%$ ($p < 0.05$). Lower field strengths were without effect and the critical value appears to lie between 80 and 85 Oe. Six experiments on mouse embryo liver showed depression of respiration by field strengths of 80 to 10,000 Oe but not at 70 and 75 Oe. Likewise, in three experiments at 100 Oe, the respiration of young neonatal mouse liver (ages 2-3, 3-4, and 6-7 days) was depressed to a similar extent. In Table IV the respiration data for embryo and young neonatal liver have been combined, giving an average depression of $20.6 \pm 1.0\%$ ($p = 0.01$) in the nine experiments. On the other hand, the respiration of adult mouse liver (field strengths of 100, 5000, and

TABLE IV

Effect of Intermittent Magnetic Fields on Respiration of Mouse Tissues and Yeast
Percent Change (D, Depression; S, Stimulation)

Field strength, Oe	Ascites sarcoma 37	Embryo kidney	Adult kidney	Embryo and neonatal (2-7 days) liver	Adult and neonatal (8-11 days) liver	Yeast
40	0 0					
50-60	0					
70	0 0	0		0		
75	0			0		0
80	D31.5* D33.3	0 D12.0		D19.8 D24.0		S10.2 D 6.8 S 9.3
85		D34.6*				S30.4* S42.1
90		D29.3 D29.3				S37.5 S27.7
100	D16.2 D31.5 D42.9 D30.0	D20.7 D45.4 D20.7	0	D14.8 D20.7 D23.7	0 0	S36.0
200	D18.7					
500	D53.2 D21.5 D29.9 D31.5					
3000	D34.4 D29.5					
5000	D11.1 D24.9	D25.1	0	D19.2	0	S63.2 S55.7
7300	D40.0 D33.3 D27.2 D 5.4 S 5.0					
10000	D53.2 D22.5 D33.3 D25.7 D32.1		0	D21.8	0	S27.3
Mean change at and above critical field strength [†]						
	D28.3±2.6 (p = 0.02)	D29.3±5.3 (p < 0.05)	0	D20.6±1.0 (p = 0.01)	0	S40.0±5.0 (p = 0.03)

* Approximate critical field strength.

[†] ± S.E.

10,000 Oe) or of the livers of mice 8–9 and 10–11 days of age (field strengths of 100 Oe) was unaffected.

Yeast respiration was stimulated by field strengths of 85 Oe or higher. In eight experiments the average stimulation was $40.0 \pm 5.0\%$ ($p = 0.03$). The critical field strength appears to lie between 80 and 85 Oe: 80 Oe gave marginal and insignificant effects and 75 Oe was ineffective.

Only four experiments were performed with HeLa cells. Field strengths of 70, 80, 100, and 1000 Oe produced no effect, 44.7% depression, 40.1% depression, and 42.9% depression, respectively.

It will be seen that the effects of the intermittent magnetic fields generally paralleled those previously observed with permanent magnets^(3,4): the respiration of tumors and embryo tissue was depressed; that of adult kidney was not significantly affected; and yeast respiration was stimulated. They also paralleled the limited results with intermittent fields obtained by moving tumor, embryo tissue, and yeast in and out of the field of a permanent magnet.⁽⁶⁾ However, the stimulation of adult mouse kidney respiration in the intermittent field (as contrasted with the lack of effect in a constant field) was not confirmed in these experiments.

The early results with the permanent magnet⁽⁴⁾ suggested that the degree of depression of respiration of S 37 was dependent on the field strength. This was not confirmed when a series of field strengths were employed in the electromagnet. On the contrary, with all cells or tissues which responded to the magnetic field a relatively low critical field strength was found below which there was no response and above which the response was quantitatively constant within experimental error regardless of field strength.

It seems apparent that the more actively proliferating cells are most responsive to the fields: all tumor cells investigated (S 37, Ehrlich adenocarcinoma, and HeLa cells) and embryo and young neonatal tissues displayed a reduced respiration in effective fields whereas adult and older neonatal tissues did not. Yeast cells also responded to the field, but with stimulation rather than depression of respiration. The reason for this difference in response between yeast and proliferating animal tumor cells is not known. Possibly investigation of cells at different stages of the proliferation cycle may provide a clue.

Finally, the effects of a magnetic field on the respiration of responsive tissues seems to be fully reversible under the conditions studied. At this time we do not attempt to advance a theoretical explanation for the observed behavior.

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MAGNETIC FIELD AND *IN SITU* ACETYLCHOLINESTERASE IN THE VAGAL HEART SYSTEM

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INTRODUCTION

It seems that man has long been fascinated by the mysterious power 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 19th century. A survey of biomagnetics publications^(1,2) indicates a recent intensification of interest in this field (Table I). This survey reveals a sharply rising interest in the biomagnetics field over the last decade or so. Such borderline disciplines, as biomagnetics arise naturally as sciences branch out, and, also naturally

TABLE I
Biomagnetics Papers Published in Years 1880-1964

Year	Biomagnetics papers
1880-1900	15
1901-1930	17
1931-1940	23
1941-1950	25
1951-1960	41
1961-1964	86

the interest in biomagnetics has increased as collateral research has progressed.

In biomagnetics, a problem has arisen that is frequently seen in such rapidly developing fields: the difficulty of finding suitable systems for direct study of the phenomena. The magnetic effect on biological material is almost certain to be very small if the biological system is so complicated as to mask the effect looked for. On the other hand, if the system is too simple, the sensitivity may be lost. If Sir Charles Sherrington had used another organism than the cat to discern the intricate communication system in the brain, our knowledge of neurophysiology would have been a blank, at least in certain areas. Had Morgan and Muller used other biological material than *D. melanogaster* in their genetic studies, they might well have failed to reach their conclusion of decades ago. Similarly, molecular biology could not have revealed the genetic code without the aid of *E. coli*. Thus, at this stage of development in biomagnetics, it is a matter of considerable importance that appropriate biological material be selected for some strategic experiments, in order to reveal the basic mechanisms of magnetic interaction with biological matter.

Free radicals have been found much more widely distributed in chemical and biological systems than was previously realized. Thus it appears that the importance of magnetic interactions with biological matter may not be realized yet. The high magnetic permeability of biological fluid and biological matter may contribute immensely to the biomagnetic effect.

Altered catalytic enzyme activity under the influence of an external magnetic field was demonstrated for carboxydismutase,⁽³⁾ for glutamate dehydrogenase,⁽⁴⁾ and for trypsin.⁽⁵⁾ Failures to demonstrate such changes in some of the *in vitro* studies⁽⁶⁾ are largely due to improper handling of biological systems and failure to expose the material to the magnetic field for a sufficient time to permit perturbation of biological material. Furthermore, random distribution of the test substance (enzyme) with respect to the substrate in the *in vitro* system makes it difficult to demonstrate a change in conformation. On the other hand, in *in situ* biological material, which is highly organized and sensitive to environmental changes, the magnetic effect is rather easily demonstrated, at least in certain isolated biological systems.⁽⁷⁾ The problem, then, is one of properly isolating the *in situ* system so as to remove masking interferences without losing the sensitivity.

Out of the enormous number of biological systems, it seems evident that a lucid mechanism of the biomagnetic effect will be evolved in the near future. In the past decade we have successfully utilized a rather delicate biological system—namely, the vagal heart system—to study various phys-

ical parameters such as pressure,⁽⁸⁾ temperature, and frequency of stimulation. It seems to be an ideal system to study *in situ* the kinetics of the acetylcholinesterase (AChE) system.⁽⁹⁾ In the past few years we have made use of this system to develop some special approaches to magnetokinetics to probe the active site of the AChE, especially with respect to the conformation of this enzyme.

In the succeeding pages I describe in some detail this biological system in conjunction with the magnetic field, the effects obtained from the experiments, and the tentative conclusions drawn from the studies. It is hoped that this study will serve as a mirror to view the magnetic effect on the biological system from a different angle, to broaden the horizons of enzymology.

MATERIALS AND METHODS

Magnetic Equipment

Permanent Magnetic Field

The magnets used for this study were General Electric assembly magnets. The pole face is 4 in. in diameter and pole gap is 2.5 in. wide. The strength of such a magnet gives a field of about 2000 Oe. Through specially made pole pieces, the field strength can be increased up to 10,000 Oe without much difficulty.

The field strength produced in the pole gap was monitored by a Gaussmeter Model 90 (Empire Scientific Corp.). The exact strength of the field and the frog cardiac contractions were recorded simultaneously on a two-channel Massa recorder during the experiment. The experimental setup is shown in Fig. 1.

Inhomogeneous Magnetic Field

The DC electromagnet for the nonhomogeneous field was constructed at Lawrence Radiation Laboratory with a special pole design to produce a field strength up to approximately 17,000 Oe (midfield) with optional choice of polarity. Thus the experiment can be conducted at will with a swift change of polarity. A magnetic probe at the convex pole (see Fig. 2) showed 21,000 Oe, and a probe at the concave pole showed 16,000 Oe. Since the distance between pole pieces is 1.08 cm, the average gradient would be calculated at 4900 Oe/cm.

THE BIOLOGICAL SYSTEM

Vagal Heart Preparation

The frog *Rana pipiens* was the source of the vagal heart preparation used for this experiment. The frog was pithed (i.e., the brain and spinal cord were destroyed with a probe) to obviate the use of anesthetics, which undoubtedly affect the test. The operation procedure included three major steps.

First step—isolation of the heart from the general circulation. The heart was exposed by a midline incision in the abdomen and chest. The superior vena cavae were ligated first (Fig. 3A) then the pulmonary arteries and veins. The posterior vena cava was isolated and cannulated (Fig. 3B). This cannulation serves two purposes. The first is to rinse the cardiac cavities

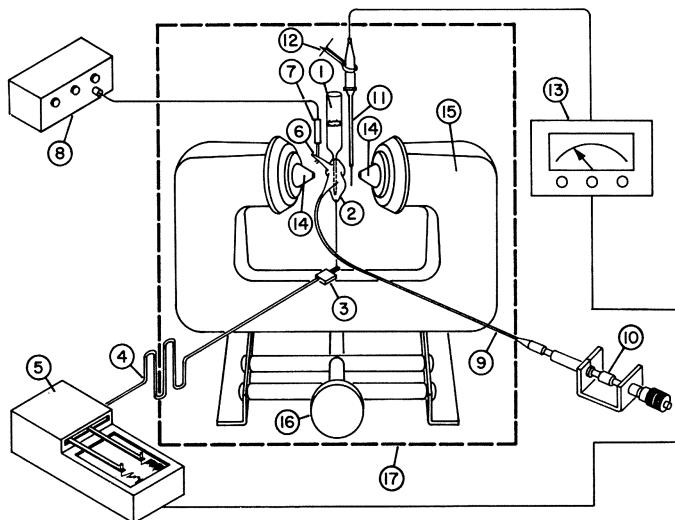


Fig. 1. Diagram of the permanent magnetic field on the vagal heart system. A cannula (1) perfuses the frog heart (2), which is connected to a Statham transducer (3) leading through a cable (4) to a recorder of cardiac contractions (5). The vagal nerve (6) is stimulated through a pair of platinum electrodes (7) by a stimulator (8). Polyethylene tubing (9) and a micro-injector (10) provide for injection of chemicals. The magnetic probe (11) held in place by a clamp (12) is connected through a gaussmeter (13) to the recorder (5). Also shown are the pole pieces (14) of the magnet (15) and the motor (16) for moving the magnet in and out of position. The incubator (17) maintains a temperature range from 0 to 55°C.

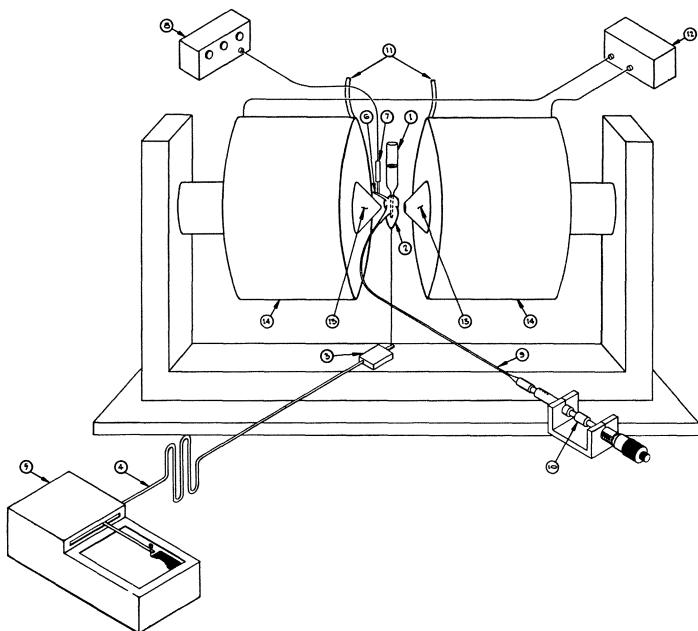


Fig. 2. Diagram of the inhomogeneous electromagnetic field experiment on the vagal heart system. Nos. 1 through 10 are the same as in Fig. 1; (11) water-cooling coil; (12) power supply; (13) pole pieces; (14) coils; (15) moisture chamber.

free of blood to ensure that pseudo-acetylcholinesterase cannot interfere with the subsequent test for true AChE activity. The second is to provide nutrition for cardiac muscle on the one hand and to allow the injection of test chemicals on the other.

Second step—isolation of vagus nerve. Once the heart is perfused with Ringer solution, we proceed to the next step, the isolation of the nerve. As a rule, the nerve should be handled as little as possible. When handling is necessary, it should be done only with a smooth-surfaced object, for example, a glass needle. The relative positions of vagus nerve and sympathetic branch are shown in Fig. 4A, as indicated by the dotted lines. Fig. 4B shows the positions of the two nerves exposed.

Third step—cardiac cannulation. The heart was cannulated through the aortic bulbus with a specially made cannula (Fig. 5). (The dimensions described here are for adult *Rana pipiens* only.) After cannulation the heart was isolated from the body. The whole operation usually takes about 35–

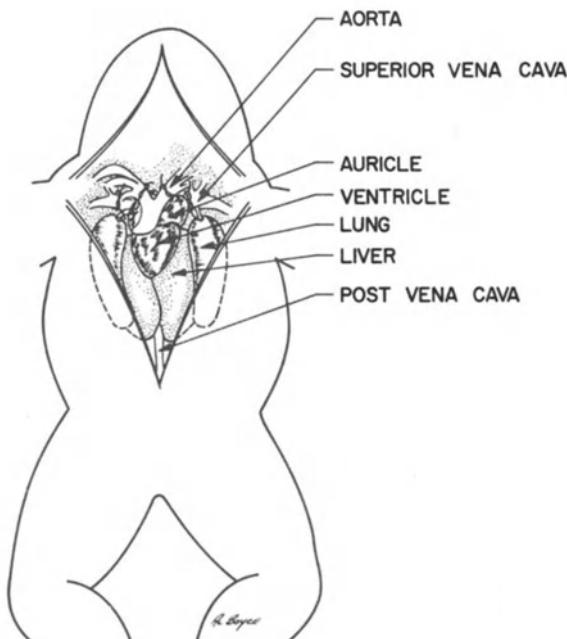


Fig. 3A. The heart of the frog, *Rana pipiens*, showing the exposed superior vena cava, auricle, ventricle, and aortic bulbus.

45 min. In our hands such a preparation lasts for several days without difficulty. This long life makes it a valuable biological tool for studies such as magnetokinetics and radiation experiments to investigate enzyme kinetics *in situ*.

Measurement of Acetylcholinesterase Activity

Another special advantage of the vagal heart preparation for the study of enzyme kinetics *in situ* is that the activity *in situ* acetylcholinesterase can be measured accurately, and continuously. This is done indirectly by the measurement of the magnitude of contraction of the heart, which can easily be shown to be proportional to the acetylcholinesterase activity.

Theory of Acetylcholinesterase Kinetics In Situ

The equation of Michaelis and Menten⁽¹⁰⁾ for determining the dissociation constant of the enzyme-substrate complex ES has been applied to numerous enzymes,^(11,12) and to the reaction with partially purified extracts

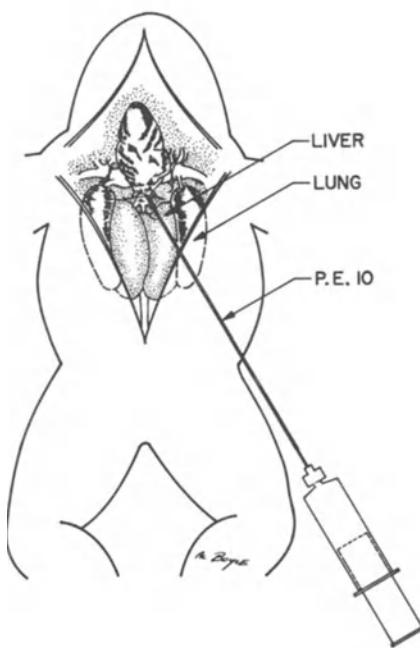
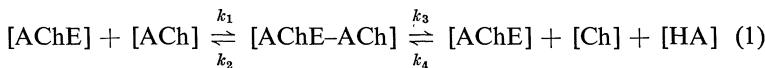


Fig. 3B. The heart is flipped over cephalically to expose the sinus for cannulation.

of *Cypridina*.⁽¹³⁾ We have successfully employed this treatment to *in situ* acetylcholinesterase⁽⁹⁾



where $[\text{AChE}]$ is the concentration of acetylcholinesterase, $[\text{ACh}]$ is the concentration of the substrate acetylcholine, $[\text{Ch}]$ is the concentration of choline, and $[\text{HA}]$ is the concentration of acetic acid. For simplicity, let $[\text{C}] = [\text{AChE-ACh complex}]$ and $[\text{p}] = [\text{Ch}] + [\text{HA}] = [\text{product}]$. Then

$$k_1[\text{AChE}][\text{ACh}] - k_2[\text{C}] = k_3[\text{C}] - k_4[\text{AChE}][\text{p}] \quad (2)$$

Since during the initial stage of the reaction $[\text{p}]$ is infinitesimally small, the whole term containing this quantity can be eliminated. And thus Eq. (2) can be simplified by transposing and grouping, yielding Eq. (3)

$$\frac{[\text{AChE}]}{[\text{C}]} = \frac{k_2 + k_3}{k_1[\text{ACh}]} \quad (3)$$

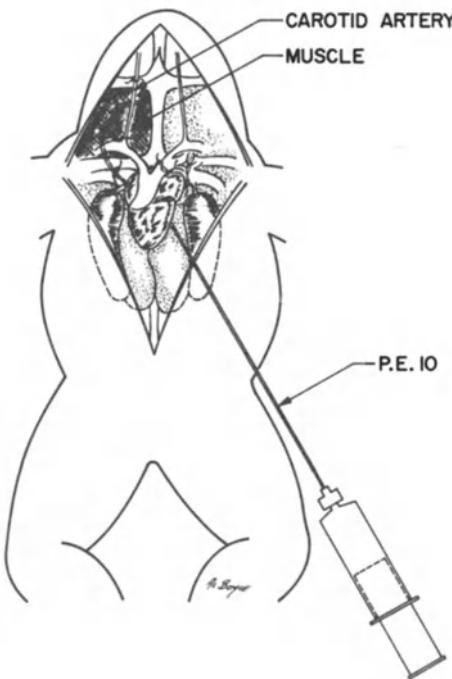


Fig. 4A. Exposed view of palate to show the relative positions of muscle, carotid artery, sphenoid bone, and the right vagus nerve in dotted lines.

Letting $[AChE]^* = [AChE]_t - [C]$, where $[AChE]_t$ is the total molecule in the system, and substituting into Eq. (3)

$$\frac{[AChE]^*}{[C]} = \frac{[AChE]_t - [C]}{[C]} = \frac{[AChE]_t}{[C]} - 1 = \frac{k_2 + k_3}{k_1[ACh]} \quad (4)$$

$$\frac{[AChE]_t}{[C]} = \frac{k_2 + k_3}{k_1[ACh]} + 1$$

The maximal velocity V is proportional to $[AChE]_t$. Any actual velocity ν is proportional to the active enzyme or $[C]$.

Let

$$k_m = \frac{k_2 + k_3}{k_1}, \text{ the Michaelis constant}$$

then

$$\frac{[AChE]_t}{[C]} = \frac{V}{\nu} = \frac{k_2 + k_3}{k_1[ACh]} + 1 \quad (5)$$

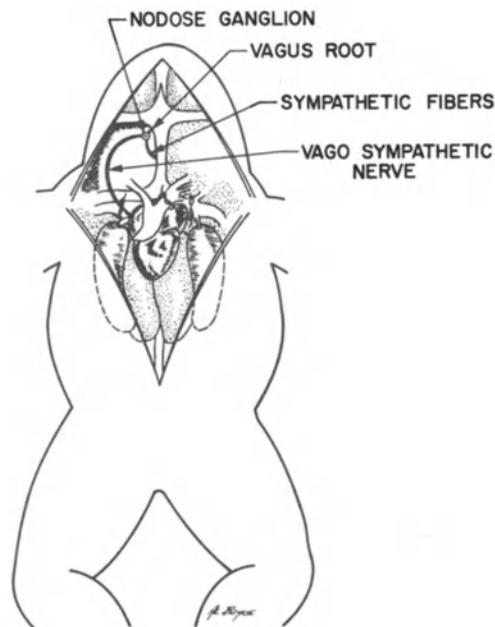


Fig. 4B. Same view as in Fig. 4A, except the muscle was removed, and thus vagus root, superior sympathetic branch, nodose ganglion, and vagosympathetic trunk are in view.

STRAUB'S CANNULA

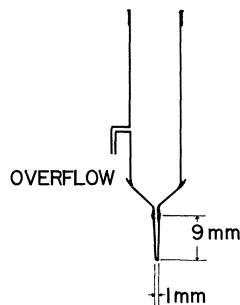


Fig. 5. Dimensions of the Straub's cannula for adult *Rana pipiens* heart. These dimensions are crucial for a successful cannulation. The rate of flow by gravity for maintaining a hydrostatic pressure is about 64 drops/min.

Multiplying each side by [ACh] and rearranging yields the familiar Michaelis-Menten equation

$$v = \frac{V[ACh]}{k_m + [ACh]} = \frac{V}{(k_m/[ACh]) + 1}$$

or

$$\frac{1}{v} = \frac{(k_m/[ACh]) + 1}{V} = \frac{k_m}{V[ACh]} + \frac{1}{V}$$

This is equivalent to the straight-line expression where k_m/V is the slope and $1/v$ is the intercept.

When [ACh] is very high, we have a situation approximating that at the instant at which electrical shocks are applied to the nerve. The first term on the right side approaches zero, and $1/v = 1/V$ (the intercept). The value V , maximal velocity, can be evaluated. The slope k_m/V is evaluated readily to yield the k_m value.

When a reversible or competitive modifier [I] is present, the Michaelis-Menten equation has the form

$$\frac{1}{k_3[ES]} = \frac{k_m 1}{k_3[E][S]} \left(1 + \frac{[I]}{k_1} \right) + \frac{1}{k_3[E]}$$

This can be modified to show that $[I]k_m/k_1k_3[E]$ represents the effect of the modifier in removing enzyme from the reaction. In this case, [I] may have either a positive or a negative sign, depending on whether the modifier is an inhibitor or an accelerator. The term [IE] (modifier-enzyme complex) represents the concentration of enzyme molecules diverted toward the main reaction.

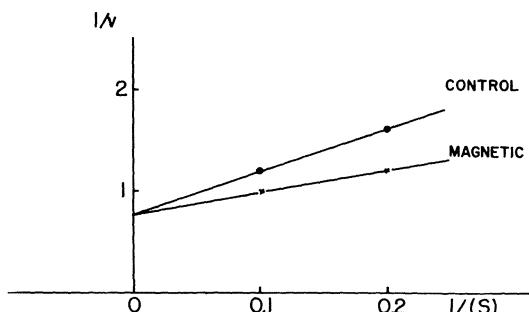


Fig. 6. Magnetokinetic of AChE *in situ*, the typical double reciprocal plot.

TABLE II

	2	5	7	10	15	20
Time of stimulation, sec	2	5	7	10	15	20
Substrate introduced by vagal stimulation (units of 1×10^{-7} moles/liter)	2.76	6.89	9.65	13.8	20.7	27.6
Time required for complete hydrolysis, sec	30	42	54	66	90	114
Relative velocities	0.092	0.16	0.18	0.21	0.23	0.25
$1/[S]$	0.362	0.145	0.103	0.072	0.048	0.036
$1/\nu$	10.8	6.25	5.5	4.7	4.3	4.0
Plot $1/[S]$ vs. $1/\nu$; the intercept $1/\nu$ is 3.2, so $V = 0.31 \times 10^{-7}$ moles/liter/sec.						
The slope $k_m/V = (10.8 - 4.0)/(0.362 - 0.036) = 21.25$						
hence						
k_m	$= 21.25 \times 0.31 \times 10^{-7}$ moles/liter					
	$= 6.3 \times 10^{-7}$ moles/liter					

In the AChE system *in situ* the magnetic field proved to be an activator at low field strength. A typical double reciprocal plot ($1/\nu$ vs. $1/[S]$) is given in Fig. 6. What this plot shows is that the magnetic field increases the AChE activity; the common intercept of the two lines shows that the magnetic field is acting at the same site on the enzyme molecule as the substrate. The initial velocity of ACh hydrolysis and k_m were calculated by a standard method. A practical calculating procedure is illustrated in Table II.

RESULTS

Effects of a Permanent Magnetic Field on the Rate of Hydrolysis of Acetylcholine

When the vagus nerve is stimulated, the immediate effect is cardiac arrest of duration proportional to the amount of stimulation. The effect of a low magnetic field on the duration of this arrest (i.e., on the rate of hydrolysis of ACh) was determined in a series of experiments, each performed upon a different vagal heart preparation before, during, and after the application of a magnetic field of 2700 Oe field strength. The average gradient for this magnet is about 500 Oe on the edge. But if the preparation is placed in the middle of the field, the gradient is very small indeed; therefore this field can be considered to be relatively homogeneous. The relative velocities of the hydrolysis (ν) are shown in Table III. A rep-

TABLE III

Effect of Homogenous Low Magnetic Field on the Hydrolysis of Acetylcholine in the Vagal Heart System

S	V_0 , Pre	H	t, min	V_m , During	V, Post	Reversal
Vagal st.	0.69	2700	25	0.77	6.6	—
Vagal st.	0.78	2700	50	5	7.5	—
Vagal st.	0.78	2700	40	0.78	1.67	Taurine

V_0 , velocity of hydrolysis before the application of magnetic field.

V_m , velocity of hydrolysis after the application of magnetic field.

H, magnetic field strength, Oe.

t, time interval during which magnetic field was applied.

S, substrate, was introduced by vagal stimulation.

representative experiment is illustrated in Fig. 7A. In every case, the acetylcholine during application of the magnetic field was hydrolyzed more rapidly than was an equivalent vagal stimulation before the application of the magnetic field.

The control values for absolute velocity showed remarkable constancy, all in the neighborhood of $(6.7 \pm 0.7) \times 10^{-10}$ moles/sec. The application of a magnetic field produced increases in the rate of acetylcholine hydrolysis ranging from 50% up to several times the original value. In some preparations the rate did not return to normal immediately after cessation of the field; however, it did so eventually, sometimes as long as 18 hr after cessation of the field. (This is in contrast to *in vitro* experiments, in which the effect usually is irreversible.)

The rate of hydrolysis did not seem to increase proportionally as the field was increased to 10,000 Oe (Table IV). There were strong accelerations

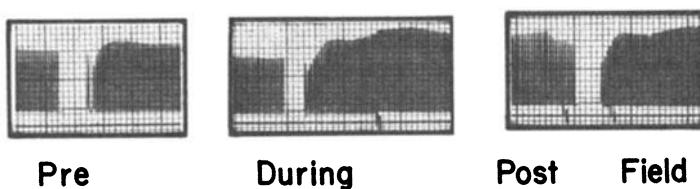


Fig. 7A. Effect of a magnetic field on vagal stimulation.

TABLE IV

Effect of High Permanent Magnetic Field on the Hydrolysis of Acetylcholine in the Vagal Heart System

Substrate*	V_0 , Pre	H in Ce	t , min	V_m , During	V , Post	Reversal
Vagal st.	0.67	10.000	5	0.67	0.67	—
Vagal st.	0.68	10.000	25	5	6	Taurine 15 $\mu\text{g}/\text{ml}$
Vagal st.	0.68	10.000	35	5	6	18 hr 0.78
Vagal st.	0.77	10.000	40	—	1.2	—
Vagal st.	0.67	10.000	50	2.0	5.0	—
Vagal st.	0.68	10.000	50	2.0	—	—
Vagal st.	0.68	10.000	60	1.9	5.8	No reversal

* Substrate was introduced by vagal stimulation.

V_0 , velocity of hydrolysis before the application of magnetic field.

V_m , velocity of hydrolysis after the application of magnetic field.

H , magnetic field strength, Oe.

t , time interval during which magnetic field was applied.

after application of the field for more than 25 min. There were no effects for a short brief period of application of the magnetic field.

Taurine, a normal constituent of tissue, seems to reverse the magnetic effect. Some metallic ions enhance the magnetic effects, while others decrease it. This finding is important because it may relate to the basic underlying mechanism through which the magnetic field acts on the biological system. Studies along this line are actively in progress.

Effects of a Inhomogeneous Magnetic Field on the Vagal Heart System

Low Field Strength (4000 Oe)

The effect of a low magnetic field strength on the time of hydrolysis of introduced ACh (injected into the perfusing medium) was determined in a series of experiments. Each was performed upon a different vagal heart preparation before and during the application of a magnetic field of 4000 Oe field strength and average gradient of 830 Oe/cm. The results of all the individual experiments are presented in Table V, and a representative experiment is demonstrated in Fig. 7B.

The control values for absolute velocity of hydrolysis showed appreciable individual variability. However, in every case the ACh introduced during application of the magnetic field was hydrolyzed more rapidly than

TABLE V

Effect of a 4000-Oe Magnetic Field on ACh Hydrolysis Rate (Magnetic Field Gradient 830 Oe/cm)

Substrate	V_0 , 10^{10} moles/liter/sec	V_m , 10^{10} moles/liter/sec	V_m/V_0
ACh	7.9	10.1	1.28
	9.6	11.6	1.22
	6.5	11.1	1.71
	4.6	5.8	1.27
	17.0	24.5	1.44
	33.2	39.6	1.18
	10.0	12.3	1.23
	19.7	30.0	1.52
	10.1	15.8	1.57
Average	13.1	17.8	1.38

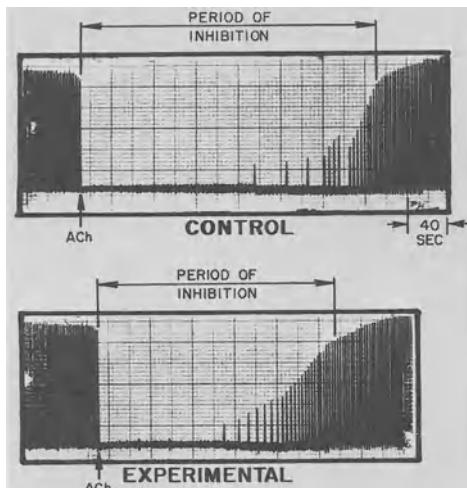


Fig. 7B. Effect of a magnetic field on the activity of AChE. Upper tracing, control; lower tracing, magnetic field of 4000 Oe. Arrows mark the introduction of 1×10^{-7} g ACh into the sinus. The magnetic field was initiated 30 min before the introduction of acetylcholine.

was an equivalent dose introduced before the application of the field, and the increase was independent of the control rate. The mean increase was 40% (range 18 to 71%).

The effect of a low magnetic field was also shown to be reversible by several simple organic compounds such as dimethylsulfone oxide and taurine as well as cholinesterase inhibitors (physostigmine, TEPP, and DFP). A low magnetic field seems to act on the active site directly and specifically. In a magnetic field, especially when the field is inhomogeneous, several side effects begin to emerge, such as arrhythmia and decrease of contractility as well as inactivation of the enzyme.

High Field Strength

A similar series of experiments on ten different vagal heart preparations was performed utilizing a much higher magnetic field strength, 17,000 Oe (average gradient of 4900 Oe/cm). The data for individual experiments are

TABLE VI

Effect of 15,000-Oe Magnetic Field on ACh Hydrolysis Rate (Field Gradient = 3700 Oe/cm); All Conditions Same as in Table V. ACh 4.42×10^{-7} moles-liter

ACh	V_0	Field strength, Oe	Time of exposure, min	V_m	V_m/V_0
*4.42	10.0	15,000	30	92.0	9.2
4.42	7.6	15,000	30	73.9	9.7
4.42	11.0	15,000	30	15.8	1.44
4.42	18.5	15,000	30	34.5	1.88
4.42	30.3	15,000	30		3.17
4.42	9.5	15,000	30	38.1	4.03
4.42	10.9	15,000	30	15.1	1.39
4.42	5.5	15,000	30	17.9	3.24
4.42	7.4	15,000	30	25.2	3.41
4.42	5.4	15,000	30	15.4	2.88
4.42	5.4	15,000	30	15.4	2.88
<hr/>					
$\dagger 7.5 \times 10^{-9}$	6.7	17,000	25	12.5	1.88
7.5	6.8	17,000	50	17.1	2.52
7.5	6.8	17,000	60	15.0	2.20
7.5	6.8	17,000	60	16.3	2.39

* Acetylcholine was introduced by microinjection into the sinus.

† Acetylcholine was introduced by vagal stimulation.

V_0 , the initial velocity of hydrolysis without magnetic field.

V_m , the velocity of hydrolysis in the magnetic field.

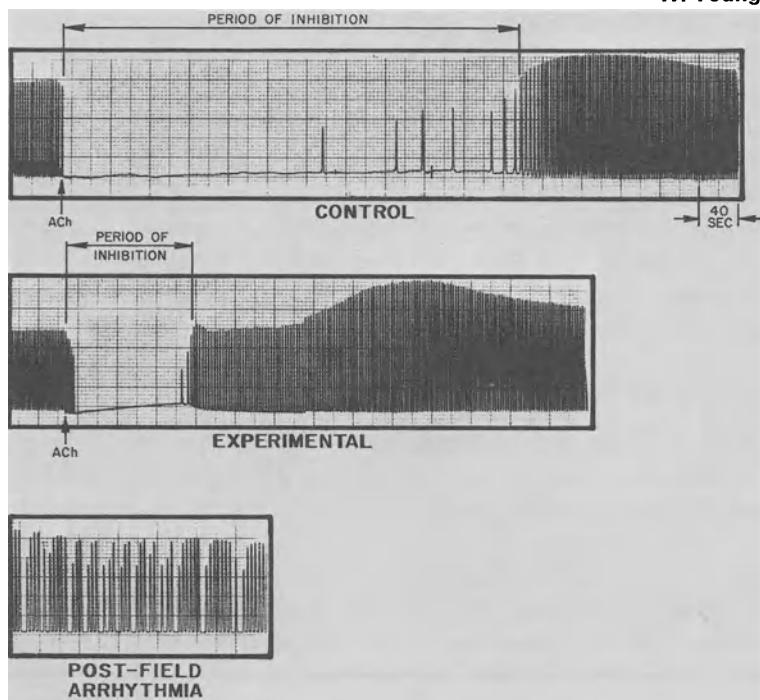


Fig. 8. Same as Fig. 7B, except that the magnetic field is 15,000 Oe in the middle tracing. The arrhythmic contractions frequently occur after magnetic application, usually 20 or more min after cessation of the field is shown at the bottom.

presented in Table VI, and a representative experiment is shown in Fig. 8. While the control rate of ACh hydrolysis showed considerable individual variation, the application of the 17,000-Oe magnetic field invariably resulted in a marked increase and again the increase was largely independent of the control rates. The mean was 250% (ranges from 44 to 870%). For comparability, all the measurements of the effect of magnetic fields upon acetylcholine hydrolysis rate, both at 4000 and 17,000 Oe, were made at 30 min after initiation of the magnetic field.

Arrhythmic Contraction in Relation to Denaturation of Acetyl-cholinesterase

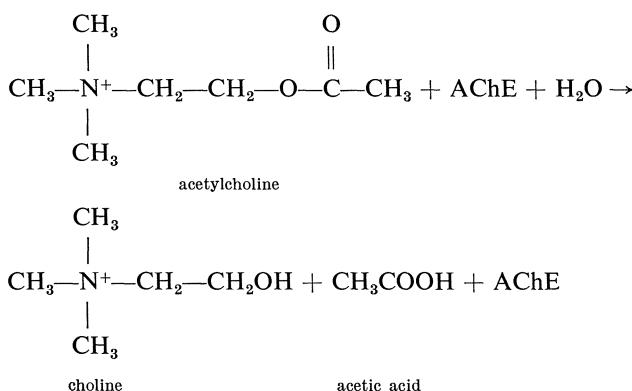
In the case of the 4000-Oe field, there was no evidence of a delayed magnetic field effect on cardiac rhythm. A slight hemodynamic effect was observed. In the case of the 17,000-Oe field, however, arrhythmic contractions were commonly observed to occur 20 min after the initiation of the

field, or later, after cessation of the field. Such effects suggest that the high-intensity field may have produced some damage to the pacemaker as well as to the conducting mechanism. In some preparations normal cardiac rhythm was reestablished spontaneously within 1 to 3 days; in others, irregularity was observed to persist for as long as 4 days after the cessation of the field; thus it appears that some permanent damage was inflicted on the heart.

The mechanism of the magnetic arrhythmia was examined in some detail. Several organic compounds which are effective in treating certain types of arrhythmia were tested against magnetic arrhythmia and found to be ineffective. These were glucose, which is very effective against phosphate-induced arrhythmia; digitalis, which effectively corrects conduction-fault arrhythmia; and oxygen, which is effective against ischemic arrhythmia.

Magnetic arrhythmia is, however, corrected by the injection of micro-quantities of AChE. When the AChE is washed out of the heart, the irregularities reappear, and these can again be corrected by further injections of AChE. The results suggest that the damage inflicted by the magnetic field seems to reside in the enzyme. It is therefore tentatively concluded that the arrhythmic contraction induced by a high-intensity magnetic field is due to the spotty destruction of AChE in certain exposed postsynaptic areas. Thus the normal rate of hydrolysis, corresponding to the presynaptic release of acetylcholine, cannot be carried out. The net result is the development of arrhythmia.

Since acetylcholinesterase activity involves the hydrolysis of acetylcholine



it is pertinent to investigate deuterium oxide and deuterated substrates injected into the cardiac sinus, to discern whether magnetic fields act on

TABLE VII

Effect of 17,000-Oe Magnetic Field on the Deuterated Acetylcholine Hydrolysis Rate. Test Dose of Deuterated Acetylcholine 4.13×10^{-8} moles/liter

Substrate ACh- <i>d</i> ₁₆	Time of exposure, min	<i>V</i> ₀	<i>V</i> _m	<i>V</i> _m / <i>V</i> ₀
4.13×10^{-8}	30	2.9	6.1	2.12
4.13	30	1.20	2.3	1.93
4.13	30	0.95	2.3	2.46
4.13	30	2.5	5.5	2.20
Average		1.8	4.1	2.18

*V*₀, velocity of hydrolysis without magnetic field.

*V*_m, velocity of hydrolysis with magnetic field.

*V*_m/*V*₀, the ratio of the velocity of hydrolysis with and without magnetic field.

Substrate, by injection.

the hydrogen bond.^(14,15) The effect of a magnetic field on the hydrolysis rate of deuterated acetylcholine is shown in Table VII. The average rate of hydrolysis of the deuterated acetylcholine (ACh-*d*₁₆) is much lower than that of protiated acetylcholine either with or without the magnetic field.

A possible interaction of the magnetic field with acetylcholinesterase is revealed by Table VIII. The rate of hydrolysis is much higher for protiated ACh than for deuterated ACh either with or without the magnetic field. Also, the magnetic field increases the rate of hydrolysis in both protiated and deuterated ACh. It is conceivable that the magnetic field effect is super-

TABLE VIII

The Effect of Magnetic Field on the Velocity of Hydrolysis of ACh and ACh-*d*₁₆ in the Vagal Heart System

Magnetic field, Oe	<i>V</i> _H	<i>V</i> _D	<i>V</i> _H / <i>V</i> _D
0	12.6 (10)*	1.9 (4)	6.3
17,000	42.4 (10)	4.1 (4)	10.3
<i>V</i> _m / <i>V</i> ₀	3.36	2.16	

*V*_H, the rate of hydrolysis of protiated acetylcholine.

*V*_D, the rate of hydrolysis of deuterated acetylcholine.

*V*₀, *V*_m, same as Table III.

* The numbers in parentheses refer to the number of experiments.

imposed on a deuterium-isotope rate-effect. This is not borne out, however, by the V_m/V_0 ratios = 3.36 for the protiated and 2.16 for the deuterated acetylcholine. Thus it seems possible that the substrate molecules *per se* are affected by the magnetic field. This leads to consideration of interaction of ligand fields of the substrate molecule and the active site of the enzyme molecule,⁽¹⁶⁾ which play a paramount role in the biological system with respect to the magnetic field, but which are beyond the scope of this discussion.

Magnetokinetics of AChE *In Situ*

Cholinesterase Inhibitor Experiments

Physostigmine, neostigmine, tetraethylpyrophosphate (TEPP), and diisopropyl fluorophosphate (DFP) are well-known specific inhibitors of acetylcholinesterase activity. It is logical to determine whether this class of inhibitors can prevent the magnetic effect observed above. Figure 9 shows the velocity of ACh hydrolysis in the untreated control preparations, in preparations under the influence of the magnetic field and in preparations with physostigmine added to the medium. The rate of hydrolysis was markedly decreased by physostigmine and increased by magnetic field in comparison with the normal controls.

A double reciprocal plot indicates clearly that all three curves—control, magnetic field, and AChE inhibitor—intercept the $1/v$ axis at the same point. This is commonly accepted as evidence for competitive activity, and suggests

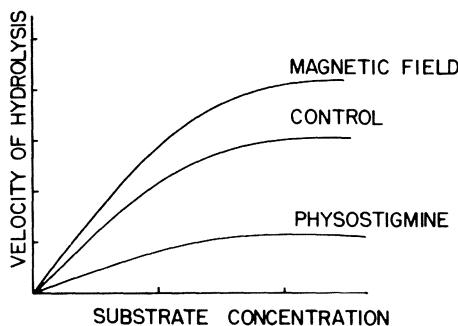
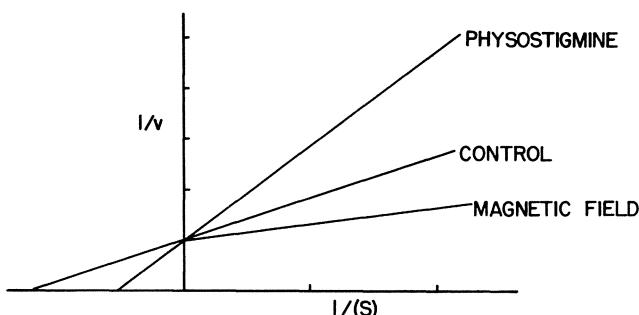


Fig. 9. Comparison of physostigmine (an AChE inhibitor) and magnetic field (acts as AChE activator in low field) on the *in situ* AChE. As the concentration of substrate increased the velocity of hydrolysis leveled off.

Fig. 10. Double reciprocal plot, $1/v$ vs. $1/[S]$.

strongly that magnetic field acts as a competitive activator (Fig. 10). This competitive activation almost always occurs when a low field is applied.

Alkali Metal Ions in the Medium

In earlier studies we have compared the alkali metal ions with respect to their effects upon ACh hydrolysis in the vagal heart preparation. We observed that partial replacement of potassium ions by cesium ions results in a marked increase in hydrolysis rate. It is pertinent here with respect to the mechanisms to determine whether the increased rate induced by cesium ions is altered by a magnetic field. The data for such a study are presented in Table IX. In that experiment, replacement of one-fortieth of the potassium ions of the perfusing medium (on a molar basis) by cesium ions,

TABLE IX
Combined Effect of Alkali Metal Ion Alteration and Magnetic Field

Medium	ACh substrate, $\times 10^7$ moles/liter	Magnetic field, Oe	Time, min	ACh hydrolysis, moles/liter/sec
1.* Cs ions absent	4.42	0	—	12.3×10^{-10}
2. [†] Cs ions present	4.42	0	—	32.5
	4.42	5000	30	33.5
	4.42	15000	30	111.0

* Medium 1 is a modified Ringer's solution, previously determined to produce ACh hydrolysis rates in the range suitable for these studies. Composition: NaCl, 112.3 mM; KCl, 1.62 mM; CaCl₂, 2.02 mM; NaHCO₃, 1.78 mM; NaH₂PO₄, 0.004 mM.

[†] Medium 2 is identical with medium 1, with the sole exception that 1/40 of the K⁺ ions are replaced by Cs⁺ ions (on a molar basis).

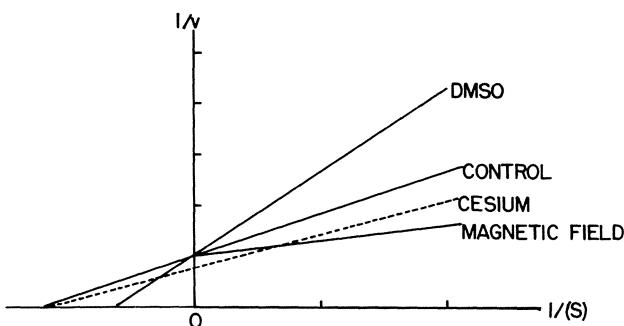


Fig. 11. Double reciprocal plot shows the kinetics that DMSO acts as a competitive inhibitor and magnetic field (low field) acts as an activator. This is in contrast to cesium ions which act as a noncompetitive activator.

resulted in a 2.6-fold increase in the rate of hydrolysis. The rate increased again slightly, but not significantly, at 30 min after initiation of a 5000-Oe magnetic field. However, when the field strength was raised to 15,000 Oe and maintained there for 30 min, subsequent measurements showed a more than threefold increase over the already elevated rate induced by cesium ions. The total increase over the control rate (K^+ ions, no magnetic field) was therefore ninefold.

This increase was not simply a synergistic effect. The mode of acceleration of Cs is different from that of the magnetic field. Cesium is a non-competitive activator, whereas the magnetic field is a competitive activator in the case of a low field strength and in the early phase of response to a high field strength (Fig. 11). The displacement of the $1/v$ intercept for alkali metal ions and their meeting at the same point on the $1/[S]$ axis indicates that the mechanism of acceleration is not the same as that of the magnetic interaction.

I have demonstrated that urea and guanidine, typical hydrogen-bond-breaking agents, act almost like the magnetic field in the vagal heart system (unpublished). This suggests that a magnetic field may act at the hydrogen-bonding energy level.

DISCUSSION

The observations described here would appear to establish that the application of magnetic fields ranging from 2700 to 17,000 Oe produces several effects, including a consistent decrease in the duration of vagal

inhibition, a decrease in cardiac contractility, and a high frequency of cardiac irregularity in high fields.

Applied magnetic fields below 4000 Oe and relatively homogeneous seem to accelerate the rate of hydrolysis. Strong inhomogeneous fields accelerate the enzymic activity in the early phase, but elicit arrhythmic contraction in the later phase. The arrhythmic contraction can be temporarily abolished by the injection of acetylcholinesterase. Thus the strong field in the later phase (60 min or more exposure to the field) seems to inactivate the enzyme. This is in line with the observation of Haberditzl and Muller⁽³⁾ with *L*-glutamic dehydrogenase.

The well-established domain concept is indeed corroborated with membrane systems in the living cell. A vast array of experimental evidence shows that the process of magnetization generally consists of a rearrangement of molecular structures, first as an increase in the volume of those domains magnetized in or near the field direction, and finally by rotations of domains (magnetization) toward the field direction. The analogy of the domain pattern of cobalt film⁽¹⁶⁾ with the synaptic membrane of the synaptic junction is striking; the dimensions of the cobalt domain and those of the folding in the postsynaptic membrane are in the same general order of magnitude.

The most likely site of magnetic interaction with the vagal heart system is upon the AChE of the myoneural junction. If this be a site of action of the magnetic field, the observed decrease in vagal inhibition period would have to be interpreted as an increase in AChE activity. Recent *in vitro* studies indicate that for four different enzymes, trypsin,⁽⁵⁾ carboxydismutase,⁽³⁾ glutamate dehydrogenase,⁽⁴⁾ and catalase,⁽³⁾ the application of a magnetic field has also resulted in increased enzyme activity. Similarly, in our *in situ* studies we reported that a magnetic field modifies AChE activity in the vagal heart system,⁽⁷⁾ but more strongly than in those *in vitro* studies. It is mostly likely that the field gradient used in our experiment is much higher than those used by the other workers.

In a low magnetic field enzyme activity is increased, and the AChE inhibitors, such as TEPP, physostigmine, and DFP, can reverse the magnetic effect. In a high inhomogeneous field when the enzyme was inactivated, administration of AChE temporarily relieved the effect exerted by the magnetic field on the vagal heart preparation. Thus it is clear that a magnetic field acts almost exclusively on the enzyme molecules, perhaps to produce conformational change at low field strength and destruction at high inhomogeneous field strength.

Double-reciprocal plots indicate that there is a competitive modifi-

cation. The application of taurine and dimethylsulfone oxide modifies this curve, indicating magnetic interaction with the anionic site of the acetylcholinesterase.

Because the substrate ACh possesses a quaternary nitrogen, it appears likely that ACh molecule *per se* is affected by the magnetic field. This is in line with the results of the protiated and deuterated acetylcholine studies. The slower rate of hydrolysis of deuterated acetylcholine is probably due to increased strength of the hydrogen bond between the esteric site and the carboxyl carbon of the acetylcholine molecule.⁽¹⁵⁾

Theoretically the magnetic properties of AChE are due to the π electron in the acid group of the esteric site and to the unpaired electron in the histidine when the substrate begins to react with the enzyme. During the reaction of enzyme hydrolysis the ACh molecule acts as a small magnet with magnetic dipole lining up parallel to the magnetic field. At the same time the enzyme molecules are readjusting themselves by conformational changes. Under the influence of a high magnetic field, the enzymes spread out on account of the breakage of the hydrogen bonds, as they do under the influence of urea or guanidine.

On the other hand, when no substrate is present the π electrons behave differently. When the molecule is in its normal conformation, the high magnetic field tends to disrupt or denature it.

Bridge Formation

The magnetic field exerts force on the electron clouds at the active site and the resulting distortion of the orbital electron could facilitate some transition of the hydrogen bond. A sulphydryl group tends to repulse the orbital electron and thus slows down the reaction. Sulphydryl-containing compounds such as glycine and reduced glutathione have been shown to reverse the effect of a low magnetic field (unpublished observation). On the other hand, cesium ion forms a bridge; therefore the forbidden path or gap is open and the electron can take this short cut. Consequently, the rate of hydrolysis is increased. A magnetic field could interfere with or facilitate the transfer of the electron in a similar fashion. Once the electron has been so transferred, the deformed orbit seems to have temporary memory, just as does the domain pattern after the application of a magnetic field.

The similarity of the effects of the ions, urea, guanidine, and the magnetic field on AChE activity in the vagal heart system implicates a bonding effect.

SUMMARY

1. The vagal heart preparation has been described. This biological system permitted us to study acetylcholinesterase activity *in situ* in relation on various physical factors on a native enzyme.
2. The activity of the enzyme can be traced by introducing substrate either by injection of microquantities of acetylcholine or by vagal stimulation to perturb the biological equilibrium.
3. It was found that a magnetic field invariably increased AChE activity at low field strength. The increased activity can be reversed by application of AChE inhibitors or some small organic molecules such as dimethylsulfone oxide and taurine.
4. A nonuniform magnetic field consistently increases the rate of hydrolysis of acetylcholine introduced either chemically into the isolated frog vagal heart preparation or by vagal stimulation in the early phase of the applied field. Prolonged exposure of the field produces arrhythmia.
5. The magnetic effects seem to be reversible, especially in the low field.
6. Magnetokinetics are discussed in conjunction with bridge formation and conformational changes of the enzyme molecule.

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EFFECTS OF THE MAGNETIC FIELD ON INTERNAL ORGANS AND THE ENDOCRINE SYSTEM OF MICE

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During the last few years it has been established that homogeneous magnetic fields produce morphological, functional, and behavioral changes in living organisms.⁽¹⁾ There have been various hypotheses proposed⁽²⁻⁶⁾ to explain these observations.

Modern pathology is increasingly concerned with the etiology of physical irritants such as heat, cold, ultraviolet radiation, electromagnetic waves of various frequencies, and the like. The reaction repertoire of the various organs is very limited; hence, stimuli of so widely different nature evoke often, though not always, similar pathological changes in them. Selye⁽⁷⁾ developed a working hypothesis assuming that most of the above enumerated stimuli influence the central homeostatic role of the pituitary-adrenocortical axis. An increased adrenocorticotropic hormone (ACTH) secretion stimulates adrenal activity and the increased titer of corticoids provides protection against the effect of these noxious stimuli. He has introduced the term "general adaptation syndrome" which is considered to be the sum of all nonspecific systematic reactions of the body which ensue upon long-continued exposure to a stressor stimulus. The general adaptation syndrome has three stages (Fig. 1A): the first stage, the alarm reaction is biphasic: shock and countershock. During the shock phase

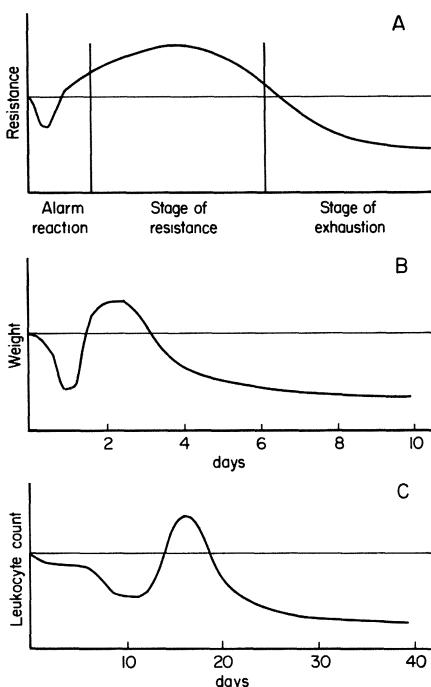


Fig. 1. (A) Changes in resistance to a specific stressor in different phases of the general adaptation syndrome, according to Selye; (B) changes in body weight of mice exposed to a homogeneous magnetic field of 9000 Oe; and (C) changes in the number of leukocytes in the blood of mice exposed to a homogeneous magnetic field of 4200 Oe.

symptoms of nervous and vascular depression occur (blood pressure falls, body temperature drops, etc.). If the shock is not fatal, a countershock phase appears, characterized by an increased release of ACTH with subsequent corticoid release, thereby increasing the resistance. The second stage is the stage of resistance, in which adjustment initiated already during the countershock phase gains supremacy: adaptation has taken place. A repletion of the secretary granules of the adrenal cortex takes place during this stage. The third stage of the syndrome is the stage of exhaustion in which the acquired adaptation can no longer be maintained, and the symptoms of shock reappear. During this stage there might occur an absolute excess or imbalance of the hormones whose secretion is evoked in the process

of adaptation and this may result in a pathological condition, termed "disease of adaptation."

The sudden weight drop of mice during the first two days of exposure to strong (3,000–10,000 Oe) magnetic fields⁽⁸⁾ (Fig. 1B), similarly the decrease in leukocyte count of mice when exposed to magnetic fields⁽⁹⁾ (Fig. 1C) seem to have the usual characteristics of the general adaptation syndrome: alarm reaction, stage of resistance, and stage of exhaustion.

We pose the question to what extent can, in general, a similarity between effects of stress and those observed in animals exposed to magnetic fields be substantiated, and whether the magnetic field could not be looked upon as a rather weak nonspecific stressor. We report on three experiments in which the magnetic field evoked significant changes in the organs of mice.

In all three experiments the magnetic field vector was vertical. This precaution provided that, despite the movement of the mice in the cages, the direction of the field vector would not reverse its direction relative to the biological coordinate system of the animal.⁽¹⁰⁾

Prior to exposure to the magnetic field, the mice in all experiments were conditioned for one week to the confinement of the experimental cages.

Experiment I. Twenty random-bred Swiss female mice 10 weeks old were exposed for 35 days to a field of 4200 Oe; 20 female mice of the same age, strain, and average weight were kept during these 35 days in dummy magnet cages (controls), under conditions which were, with the exception of the field, identical in every respect (including the pole caps) to those in the magnet.

The magnetic field was produced by ten Alnico permanent horseshoe magnets weighing 275 lb each. The cages (in the magnet and dummy magnets) had an area of 80 cm² and were 3.8 cm high; two mice were placed in each cage. A thermocirculator kept the bottom of the magnet and dummy cages at 25°C; small electric fans provided adequate ventilation through the cage volumes. The experimental conditions of this experiment are described in detail in Volume 1 of this series, pages 110–112.

After 35 days of uninterrupted exposure the mice were removed from the magnet cages and from the dummy magnet cages and placed for 196 days in standard plastic mice cages, 5 mice per cage; then all mice were killed (all between 11 a.m. and 1 p.m.), magnet and control mice alternately. The organs were removed, placed in 10% formalin solution, then dehydrated and embedded in paraffin (the bone samples were decalcified before processing). Tissue sections, 5 to 6 μ thick were stained with hematoxylin and eosin and microscopically examined. In addition to the above stains, the liver and spleen sections were also stained with acridin orange for

fluorescence microscopic inspection and with methylgreen-pyronin with the aim of detecting the presence of degenerated cells, or a change in the pyroninophil granules in the megakaryocytes of the spleen. To reach further conclusions regarding glycogen and acid mucopolysaccharide distribution, the periodic acid Schiff reaction according to McManus was used. The "Azan" staining in Ladewig's modification was also tried. The examination of the sections was made by the pathologist of our team (I.S.) in a blind experiment.

The most striking abnormality found in the *adrenals* of the magnet mice was a narrowed zona fasciculata with normal zona glomerulosa and normal or slightly wider zona reticularis. The cells of the zona fasciculata were more or less disorganized, the usual cordlike arrangement of the cells was disturbed, and the clear demarcation of the zones was absent. To characterize the severity of the lesion in the adrenals a number system was adopted: 0 means a normal adrenal, + one where less than one half of the zona fasciculata showed the mentioned abnormalities, ++ when the lesion extended to more than half of the zona fasciculata and +++ when no characteristic structure of the zona fasciculata was visible, the cells of the zona reticularis seemingly replacing the cells of the zona fasciculata.

Of the 18 adrenals inspected in the magnet group three had normal adrenals. However, even in these seemingly normal fasciculatae from the vacuolization seen in the cytoplasm one could infer that the lipid content was less than in the adrenals of the controls. The histological preparation

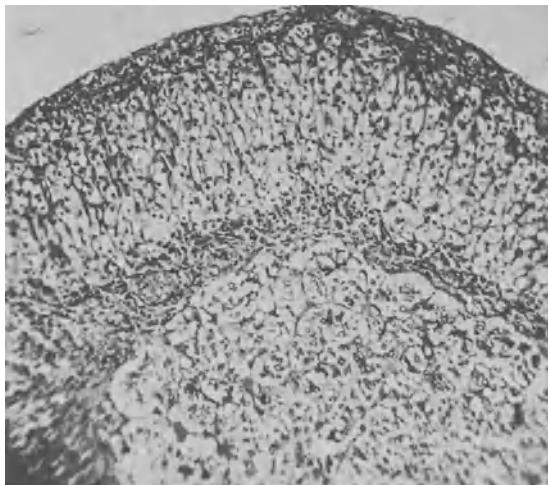


Fig. 2A. Normal adrenal. The cells of the zona fasciculata are faintly colored, rich in lipids, and show the usual cordlike arrangement. (Haem. eos. $\times 320$).

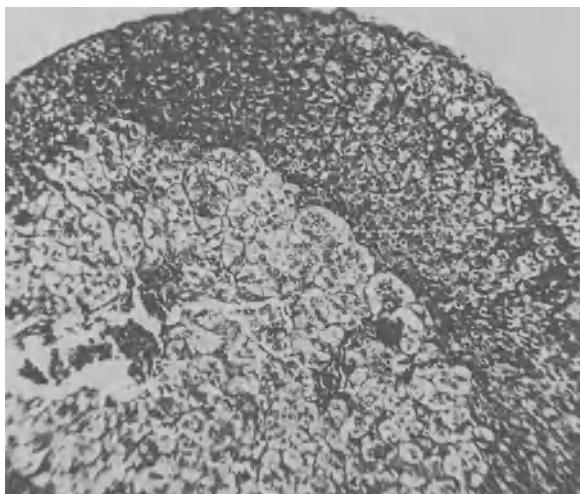


Fig. 2B. Abnormal adrenal after 4 days of exposure to 9000 Oe. The zona fasciculata is narrowed, the cells are darker, poor in lipids, and the cordlike arrangement is blurred. (Haem. eos. $\times 320$).

techniques used remove all three kinds of lipids (cholesterol, neutral fat, and functionally active steroids). Hence, in the sections made from organs embedded in paraffin one can see only the negative aspect, that is, the places where lipids have been. Our findings of reduced lipid content can not be interpreted as proving that we have found a reduced amount of functionally active steroids in the adrenals of the magnet group mice. In one adrenal the severity of the lesion was rated +, in six it was rated ++, and in eight as being +++. This means that in 78% of the mice the severity of the lesion was equal or greater than ++.

From 15 adrenals inspected in the control group, 13 had normal adrenals and in two a lesion amounting to + was seen. None of the controls had lesions reaching a severity of ++.

The most pronounced peculiarity seen in the *bone marrows* of the magnet mice was the decrease in the number of megakaryocytes (Mkc). In each marrow at least ten microscope fields ($\times 350$ magnification) were counted, the fields being selected so that there was a minimum number of bone trabeculae in them. All 17 inspected bone marrows of the magnet group had a smaller number of Mkcs per field than the average of the control group. The number of Mkcs per microscope field ranged in the magnet group from 4.0 to 7.7, compared to a range of 6.2 to 13.5 Mkcs per field in the mice of the control group.

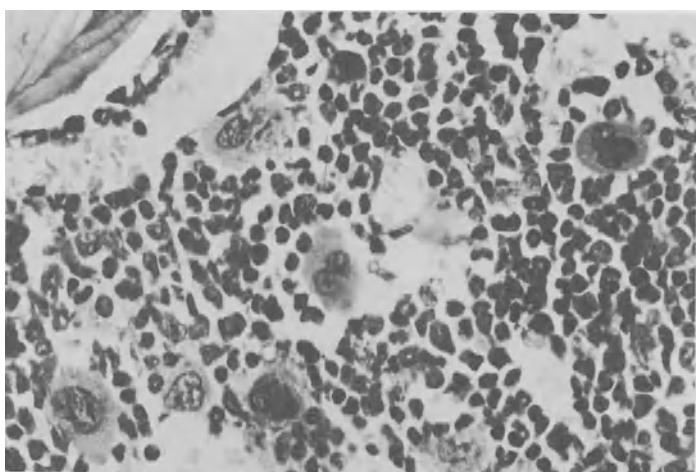


Fig. 3A. Normal bone marrow; seven megakaryocytes are seen. (Haem. eos. $\times 380$.)

It is known that in the *spleen* of rodents one can always find a certain number of multinucleated giant cells, which morphologically are difficult to differentiate from the typical megakaryocytes found in the bone marrows. In the spleens of the control mice we have always found such Mkcs in

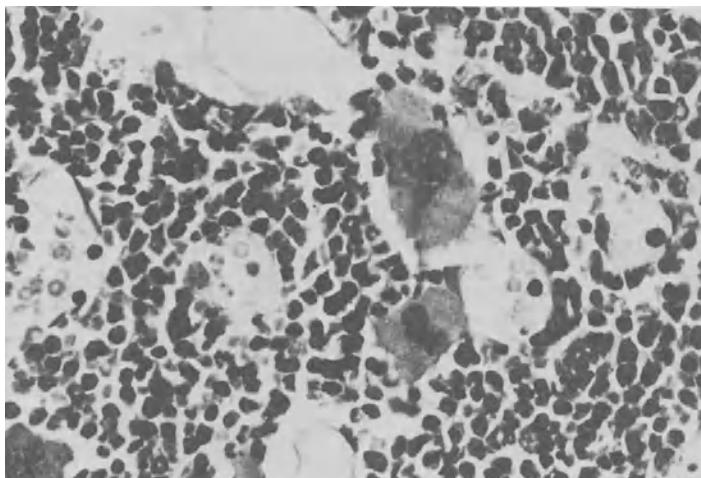


Fig. 3B. Abnormal bone marrow after 4 days of exposure to 9000 Oe. Only three megakaryocytes are seen over the entire microscope field. (Haem. eos. $\times 400$.)

smaller groups, scattered in the pulp of the spleen. Between such small groups there was always a larger area with no Mkcs in them. Megakaryocytes were never seen in the follicles.

The number of Mkcs in the spleens was counted in a similar manner as in the bone marrows. The number of Mkcs field in the spleens of the magnet group was higher than the average seen in the spleens of the controls, 6.3 to 15.0 Mkcs per field in the magnet mice, compared to a range of 3.8 to 9.7 Mkcs per field in the control mice.

The Mkcs in the pulp of the spleens of the magnet mice were generally either diffusely scattered, or in small groups, sometimes the groups being very close to each other. The follicles are usually resistant to the invasion of giant cells, but in several instances we have seen more than one Mkc at the periphery of the follicles. Degenerative symptoms, such as decrease in nuclear staining were observed. With eosin the cytoplasm of these cells stained to a stronger red color, but sometimes only a faint homogeneous cell ghost was observable. With PAS stain the Mkcs stained redder than the other cells and often very red small cells were seen in the spleens with direct transitions to formations which would correspond to Russel bodies. In these instances a slight increase in the number of plasma cells was also noted. Another abnormality of the Mkcs of the spleen in the magnet mice was that they did not have sharp boundaries, but were connected through extended processes to the surrounding proliferating reticular cells, the number of the latter being definitely increased. One got the impression that the Mkcs resulted from a fusion of the reticular cells. In some of the mice, where the Mkc increase was only slight or nil, nevertheless the number of reticular cells was abnormally high. The effect of the magnetic field could probably be best described as producing a reactive reticulosism.

In the *liver* sections of the magnet mice an increase in the number of mitoses, a large number of cells with large nuclei, and multinucleated cells were seen. Pycnotic cells, necrobiotic changes, and cells with poor nuclear staining were sometimes observed. The mitotic index was used as the parameter to characterize the liver lesion. The mitotic index was taken as the number of mitoses per 400 liver cells. Four thousand liver cells per mouse were counted. In 19 out of 20 liver sections of magnet mice the mitotic index was higher than in the control mice. The mitotic index ranges are: magnet group 5.4 to 10.3; control group 2.7 to 6.0.

Table I summarizes the findings in Experiment I for the adrenals, bone marrows, spleens, and liver sections of magnet and control mice. The last column lists the difference in magnet and control averages in percent of the control values and also the probability levels p that the observed effect

TABLE I

	Magnet	Control	Diff. in %
Percent of mice with lesions in the z.fasc. exceeding ⁺⁺	78	none	78 <i>p</i> < 0.00003
Number of Mkcs per field in bone marrow	5.60 ± 0.27	8.17 ± 0.48	-31.5 ± 6.7 <i>p</i> < 0.00004
Number of Mkcs per field in spleen	9.50 ± 0.60	6.49 ± 0.39	+46.5 ± 10.5 <i>p</i> < 0.0001
Mitotic index in the liver (per 400 liver cells)	7.32 ± 0.36	4.28 ± 0.26	+71.5 ± 10.3 <i>p</i> < 0.0000001

would be due to chance alone. The probability levels were computed by using Student's *t*-test, with the exception of the lesion in the adrenal cortex, where a 2×2 contingency table was employed. The indicated errors are standard errors.

The abnormalities to be noted in Table I are all of very high statistical significance. They were found in mice which were sacrificed 196 days after termination of magnetic exposure. Should the effects be the manifestation of a general adaptation syndrome evoked by the stress of the magnetic field, it would be reasonable to expect that the magnitude of the observed effects would increase if the mice were killed immediately after termination of the magnetic exposure.

Experiment II. Forty-seven-day-old, random-bred, male Swiss mice weighing 30 g were kept in a vertical homogeneous magnetic field of 9000-Oe strength with less than 200 Oe/cm gradient. The field was produced by a 7-in. electromagnet. The current feeding the electromagnet was rectified from 3-phase 60 cycle and was very well filtered with chokes, condensers, and the large inductance of the magnet coils. The upper pole of the magnet was the north pole. The cage area was 310 cm² the height 7 cm (2170 cm³ volume); thus the living area was 70% of that available in standard plastic mice cages. Water-cooling of the magnet coils maintained the temperature of the magnet within $\pm 3^{\circ}\text{C}$ of room temperature. A dummy magnet cage identical in every respect was used, with an iron disk simulating the pole cap of the magnet. Both cages (together with the pole cap) were enclosed

in larger copper boxes, which were open on two opposite sides and were thermally insulated from the electromagnet. Small electric fans maintained a light breeze through the copper boxes and the cages, keeping the temperature in the magnet and dummy magnet cages similar to within $\pm 0.5^{\circ}\text{C}$. The room was ventilated by filtered and temperature-controlled air. Both magnet mice and control mice in the dummy magnet had free access to tap water and Purina Mouse Chow.

Rodents are known to be sensitive to high-frequency sound with stressful effects. We have, therefore, investigated with a sensitive audiometer the noise level between 31.5 to 31,500 Hz in the magnet and dummy magnet cage areas. The humming tone of the power supply of the magnet produced an increase of 4.5 db in both cages at 250 Hz, but for frequencies above 2000 Hz no difference was observed with the power supply on or off, nor was any measurable loudness difference in the entire frequency range from 31.5 to 31,500 Hz observed when the magnet was energized, or when the water-cooling was turned on. Due to the rather large weight (about 2000 lb) of the magnet yoke and coils, it is not surprising that no high frequency vibrations were generated.

Ten mice of the experimental group were kept in the magnetic field for 13 days (subgroup *a*) and killed immediately after removal; six mice of the same batch were kept for 4 days in the field and killed immediately after removal (subgroup *b*). Both mouse groups and their controls were conditioned for one week to the conditions of the experimental circumstances, and magnet and control mice were killed within a few hours.

The experimental circumstances in this experiment, compared with those of Experiment I, differed on the following points: (a) the field strength was 9000 Oe instead of 4200 Oe; (b) the mice were killed immediately after termination of exposure and not 196 days later; (c) the treatment time was 13 and 4 days, respectively, compared to 35 days in Experiment I; (d) male mice were used whereas females were used in Experiment I; and (e) ten and six mice, respectively, were kept in cages 7 times larger in volume, compared to two mice per cage in Experiment I. In every other respect the two experiments were conducted in exactly the same way; removal of the organs, staining, and inspection of the tissues were done described in connection with Experiment I.

For each of the four investigated parameters—disorganization of the zona fasciculata of the adrenal cortex, number of Mkcs in the bone marrow number of Mkcs in the spleen and mitotic index in the liver tissues—we also investigated whether the difference in exposure time (4 and 13 days, respectively) was of relevance.

In subgroup *a* seven adrenals were examined; in one the zona fasciculata was normal, in two they were classified as +, and in four as ++. In subgroup *b* three adrenals were inspected; all three showed lesions amounting to ++. All the adrenal sections of the controls were normal. It should be mentioned that none of the adrenal sections of the exposed mice showed total disorganization of the zona fasciculata, that is, lesions of +++ severity. In subgroup *a* 57% of the mice and in subgroup *b* 100% of the mice had lesions amounting to ++. At this juncture we do not want to attach significance to this difference between the two subgroups, but we plan to investigate this question later. We conclude, thus, that under the experimental conditions of this experiment on the average 70% of the exposed mice had lesions amounting to more than one half of the zona fasciculata being disorganized. Figure 2A shows the adrenal section of a control mouse; Fig. 2B shows that of one of the mouse of subgroup *b*.

The number of megakaryocytes in the *bone marrow* of subgroup *a* was in all ten mice less than the number of Mkcs in the marrows of the controls. In subgroup *b* two had equal number of Mkcs per field as the control mice. The respective ranges are: subgroup *a*, 4.9 to 6.8; subgroup *b*, 6.1 to 8.0; and controls, 7.3 to 10.0 Mkcs per field.

We conclude that a 9000-Oe magnetic field produces after 4 days of exposure a 16.3% decrease in the number of Mkcs, while 13 days of exposure produces a 27.0% decrease; however the difference between the average number of Mkcs per field in the two subgroups is 0.87 ± 0.42 which is significant only at the 5% level.

The number of Mkcs in the *spleens* in both magnet subgroups was larger than the control values. The Mkcs per field ranges are: subgroup *a*, 7.0 to 11.0; subgroup *b*, 8.0 to 13.2; and control group, 5.7 to 7.0, with one mouse spleen having 9.1 Mkcs per field. The difference in the number of Mkcs per field between subgroup *a* and subgroup *b* was not significant.

The mitotic index in the *liver* tissues of nine mice of subgroup *a* was larger by a factor of 2 to 3 than that in the control liver sections; one magnet mouse had an excess of merely 40%. In subgroup *b* again all liver sections have larger mitotic indexes than either of the control livers. The ranges are as follows: subgroup *a*, 7.2 to 15.1; subgroup *b* 7.2 to 11.9; and control group, 4.5 to 6.7.

Four days of exposure to a 9000-Oe field produced a 74% increase in the mitotic index above the average value found in the livers of the controls, while 13 days of exposure increased the mitotic index by 129%. The difference between the mitotic index of the two subgroups is 2.84 ± 1.11 which

is significant on a probability level of 2%. We conclude that the increase in the number of mitoses does depend on the length of exposure.

The lesions in the liver tissues were characterized by the appearance of some edema, poor nuclear staining due to chromatolysis, and pyknosis. Sometimes fibrinoid degeneration (eosinophil necrobiosis) of the cells was seen; the reticuloendothelial system showed symptoms of irritation. The extremely high mitotic index, 2.3-times higher than in the controls, can be considered as being a sign of rapid regenerations taking place immediately after termination of magnetic exposure. Figures 4, 5, and 6 illustrate our findings in the livers of magnet group mice.

Table II summarizes the results of Experiment II.

In experiments we conducted over many years on thousands of mice we have found that young animals are always somewhat more susceptible to the effect of magnetic fields.⁽¹⁾ Cook *et al.*⁽¹¹⁾ found that actively proliferating cells, such as embryo and neonatal tissues, display a reduced respiration in magnetic fields, while no effect of the field was found on adult tissues. Several investigators have also demonstrated that the responses to general stress are more manifest in young animals. For example, Shapiro⁽¹²⁾ found that administration of cortisone to 2- to 5-day-old and to 30- to 33-day-old rats causes a greater adrenal atrophy in the newborn, suggesting

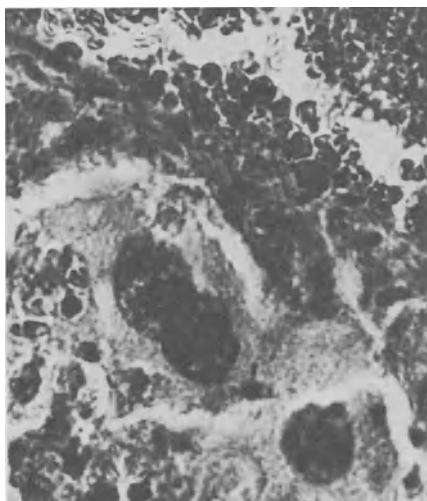


Fig. 4. Abnormal liver after 15 days of exposure to 4200 Oe. Vena hepatica is wide and filled with blood. One cell in the process of nuclear mitosis is seen. (Haem. eos. $\times 350$.)

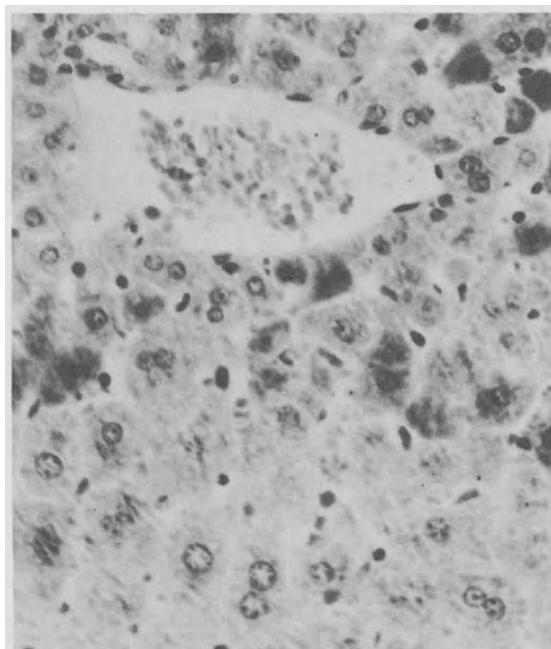


Fig. 5. Abnormal liver after 15 days of exposure to 4200 Oe. Vena centralis is wide. One cell undergoing mitosis and near to it another cell with large nucleus can be seen. (Haem. eos. $\times 150$.)

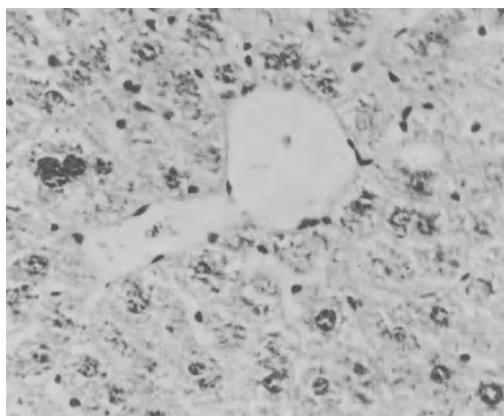


Fig. 6. Abnormal liver after 4 days of exposure to 9000 Oe. Vena centralis is very wide; it is surrounded by liver cells with poor nuclear staining. (Haem. eos. $\times 150$.)

TABLE II

	Magnet	Control	Diff. in %
Percent of mice with lesions in the z.fasc. exceeding ++ *	70	none	70 <i>p</i> < 0.07
Number of Mkcs per field in bone marrow	group <i>a</i> : 5.91 ± 0.21	8.09 ± 0.63	-27.0 ± 8.2 <i>p</i> < 0.005
	group <i>b</i> : 6.78 ± 0.37		-16.3 ± 9.1 n.s.
Number of Mkcs per field in spleen*	9.91 ± 0.44	6.98 ± 0.45	$+41.9 \pm 9.1$ <i>p</i> < 0.0003
Mitotic index in the liver (per 400 liver cells)	group <i>a</i> : 11.84 ± 0.80	5.17 ± 0.35	$+129.0 \pm 16.0$ <i>p</i> < 0.000002
	group <i>b</i> : 9.00 ± 0.81		$+74.0 \pm 17.0$ <i>p</i> < 0.0007

* We list the average of the two subgroups whenever the difference between them was not significant (N.S.).

a greater sensitivity of the steroid feedback mechanism to cortical hormones during early postnatal period. Based on these considerations we devised as our next experiment the following investigation.

Experiment III. C₃H inbred mice were obtained from Jackson Memorial Laboratory (4 males and 4 females). Two male-female pairs of this original stock were mated in magnetic fields and the other two male-female pairs in were mated dummy magnet cages. The ten magnets used in this experiment were the same permanent magnets we used in Experiment I. However, to provide greater space for mating and later for the lactation of the newborn, the height of the cages was increased to 6 cm and the cage area to 150 cm² (900-cm³ cage volume); the field strength was thereby reduced to 2200 Oe. The dummy magnet cages were again of the same dimensions as the magnet cages. One male and one female mouse were put in a cage. The males were kept in the field until pregnancy was well in progress (about 15 days); the females remained there during the entire period of pregnancy and lactation of the offsprings, thus for more than 40 days. Twenty-day-old offspring were weaned and placed (males and females separately) in

standard plastic mouse cages where they were kept until maturation. These *first-generation* magnet-born mice were thereafter brother-sister mated again in the magnetic field (and dummy-magnet-born offspring in dummy magnets) and the same procedure was followed as before. The organs of 20 *first-generation* mice and 8 *second magnet-born generation* mice were examined in the same manner as described earlier.

The experimental circumstances in this experiment differed from the two previous ones in that respect that (a) the field strength was only 2200 Oe; (b) the treatment time was 40 days of which 20 days were prenatal exposures; (c) inbred C₅₇H-strain mice were used; (d) male and female mice were used; and (e) the cage volume was intermediate between the volumes used in Experiments I and II.

Of the 10 mice (3 males, 7 females) of the *first magnet generation* one had a normal adrenal cortex, in one the lesion of the zona fasciculata was classified as +, in six it was classified as ++ and in two as +++. Of the eight mice (4 males, 4 females) of the *second magnet generation* three had normal adrenals, one had a lesion amounting to +, one amounting to ++ and three were classified as +++. Thus in the first generation 80%, while in the second generation 50% of the mice had lesions ++ or greater in severity. In evaluating this difference by using a 2×2 contingency table it was found to be significant only at the 10% level. The severity of the lesion in the adrenal cortex of the females was slightly, though not significantly, higher than in the males. Of the dummy-magnet-born mice one mouse had a disorganization of the zona fasciculata amounting to +, all the others had normal adrenals.

The average number of Mkcs per field in the *bone marrows* of the first generation was 6.72, while it was 6.16 in the second magnet generation, the difference not being significant. Nor was the difference between males and females significant. Compared to the number of Mkcs per field in the mice born in dummy magnets a decrease of 14.5% was observed. The ranges were: magnet-born mice, 4.5 to 8.6; controls, 6.3 to 9.8 Mkcs per field.

The average number of Mkcs per field in the *spleens* of the first magnet generation was 6.18 ± 0.90 and 4.85 ± 0.33 in the second magnet generation. The number of Mkcs per field in the dummy magnet mice was 4.24 ± 0.59 ; that is, the second magnet-born generation had within the error limits the same number of Mkcs per field as their controls. Whether this indicates that in the second generation an adaptation to the magnetic field had taken place, or other unknown factors played a role, cannot be decided. Males and females did not show significantly different increases in the

number of Mkcs compared to the values found in the corresponding control males and females. The ranges were: first generation, 3.4 to 11.6; second generation, 2.7 to 5.7; and controls, 2.1 to 6.2 Mkcs per field.

The mitotic indexes of the *liver* tissues of the first and second magnet generation were 6.73 and 6.90, respectively, that is, within the error limits identical. Compared to dummy-magnet-born mice the mitotic index increased by 39%. Females showed a slightly but not significantly higher mitotic index than the males. The ranges were: magnet-born mice, 4.1 to 9.2; controls, 1.7 to 7.2. Table III summarizes the results.

Although in this experiment we again found a disorganization of the zona fasciculata of the adrenal cortex, a decrease in the number of Mkcs in the bone marrow, an increase in the number of Mkcs in the spleens, and an increased number of mitoses in the liver tissues, only the percentage lesion of the adrenal cortex and the decrease in the number of Mkcs in the bone marrow deviated significantly from the values found in dummy-magnet-born mice. To get an indication of whether these much smaller effects may have been caused by the reduction of the field intensity or by an adaptation of the mice to the magnetic environment during the prenatal exposure, we have compared the investigated parameters with those found in the four mice of the original stock, which were exposed to the same magnetic field as adult mice. From the original stock magnet mice one had a lesion in the zona fasciculata classified as ++, the other three had normal adrenals, that is, only 25% of the mice showed a lesion amounting in severity

TABLE III

	Magnet	Control	Diff. in %
Percent of mice with lesions in the z.fasc. exceeding ++	66.6	none	66.6 <i>p</i> < 0.003
Number of Mkcs per field in bone marrow	6.50 ± 0.31	7.60 ± 0.48	-14.5 ± 7.5 <i>p</i> < 0.07
Number of Mkcs per field in spleen*	6.18 ± 0.90	4.24 ± 0.59	$+46.0 \pm 25.4$ <i>p</i> < 0.10
Mitotic index in the liver (per 400 liver cells)	6.80 ± 0.35	4.89 ± 0.92	$+39.1 \pm 21.8$ <i>p</i> < 0.10

* The listed value is that found in the first magnet-born generation.

to ++. The number of Mkcs per field in the bone marrow of the original stock was essentially the same as in the controls.

We conclude that under the experimental conditions of Experiment III no significant effect was manifested in the spleens and in the liver sections of the exposed mice. Moreover a 2200-Oe field is not strong enough to produce changes in the organs if the animals are exposed as adults to the field. Thus, tentatively we may say that the lesion in the zona fasciculata of the adrenal cortex and the reduction in the number of Mkcs in the bone marrow has been intensified by the pre- and postnatal exposure of the animals to the field.

DISCUSSION

Table IV summarizes the results of the three experiments. Listed are differences relative to control values in percent.

The Adrenal Cortex

The adrenals of 46 mice exposed to magnetic fields were inspected in the three experiments: 17% of these had normal adrenals as far as the structure of the zona fasciculata is concerned, but even these had seemingly a reduced lipid content; 11% had lesions amounting in severity to +; 46% had lesions classified as ++ and 26% lesions classified as +++. All together in 72% of the exposed mice the severity of the lesion exceeded ++. From a total of 25 inspected adrenals of control mice 88% had normal adrenals, 12% had disorganization of the zona fasciculata amounting to +, and none showed lesions classified as ++ or +++. These findings establish the existence of an effect of strong magnetic fields on the adrenal cortex.

TABLE IV

Experiment	Percent of mice with lesions exceeding ++ in the z.fasc.	Decrease in Mkcs per field in bone marrow	Increase in Mkcs per field in spleen	Mitotic index increase
I (4200 Oe)	78	31.5	46.5	71.5
II (9000 Oe)	70	27.1	41.9	129.0
III (2200 Oe)	67	14.5	N.S.*	N.S.

* N.S. means not significant.

Many investigations have revealed that the adrenal cortex is an organ which participates in the physiological and pathological processes of most organs of the body. The abnormalities we observed in the zona fasciculata of the adrenal cortex when mice were exposed to magnetic fields is probably one of the most significant results of our investigations.

A 4200-Oe field produced in 78% of the mice a disorganization of the zona fasciculata amounting in severity to ++ or more (that is, a lesion in which more than one half of this zone was disorganized), and this effect was still observable 196 days after termination of magnetic exposure; of the mice killed immediately after termination of exposure to a 9000-Oe field 70% showed lesions of the same severity. This seems to indicate the following:

1. The severity of the lesion is not dependent on the field strength within this field-intensity range; and
2. The magnitude of the lesion does not change considerably with the time elapsed after termination of exposure, at least not up to $6\frac{1}{2}$ months.

The disappearance of clear demarcations between the zones of the adrenal cortex occurs whenever the normal synthesis and secretion of the adrenocortical hormones are disturbed. The stress-induced alterations of the rate of ACTH secretion are the result of the interplay of various factors, some of which are yet not fully established or understood. Superimposed upon the basal secretion of ACTH by the pituitary gland, there seems to be a hypothalamic neurohumoral stimulating ACTH release, which, in turn, increases corticoid secretion. Neural stresses, such as noise, or strange environment release ACTH via action on the posterior lobe of the pituitary, while systematic stresses, such as hemorrhage or drugs require the presence of the anterior lobe of the pituitary gland.⁽¹³⁾ The cells of the zona fasciculata being most strongly under the influence of the adrenal-stimulating pituitary hormones are the most sensitive cells of the adrenal cortex. Thus, it is not surprising that under the influence of a static magnetic field a disorganization and narrowing of this zone was observed, while the zona glomerulosa remained unchanged, and the zona reticularis was either unchanged or slightly widened. However, under ordinary conditions of stress the zona fasciculata is widened, not narrowed. On the other hand, Akabana⁽¹⁴⁾ found that administration of alcohol widened, while that of acetaldehyde narrowed, the zona fasciculata. Saito⁽¹⁵⁾ found that in rats ascites tumor implants produce first a widening, later a narrowing, of the zona fasciculata, accompanied with simultaneous widening of the zona reticularis. The fact that we observed a narrowing of the zona fasciculata and particularly that this

narrowing persisted for such a long time after termination of magnetic exposure seems to rule out the possibility that we have here a manifestation of the usual general adaptation syndrome.

In the hope of gaining a deeper insight into the causes of the abnormalities found in the adrenals, a pilot experiment was made in cooperation with Dr. S. Marotta and Miss Ch. Lav of the Physiology Department of the University of Illinois.

We exposed a total of 28 Sprague-Dawley male 25-day-old rats to 9000-Oe fields for periods ranging from 3 to 14 days. Twenty-six rats were placed in dummy magnet cages during the same periods. The experimental circumstances were the same as in Experiment II. In each experiment the dummy-magnet-group rats were of the same age and same weight as the magnet-group rats. In each instance an equal number of rats from the exposed group and from the control group were anesthetized with sodium pentobarbital (4.5 mg/100 g) intraperitoneally, always at 10 a.m., and blood was obtained from the abdominal aorta. Adrenal weight and corticosterone content of the gland and of the plasma were measured. The steroid concentration was determined by the method of Guillemin *et al.*⁽¹⁶⁾ On the average, the adrenal weight of the magnet-group rats was 9% smaller than the average adrenal weight of the control rats; however, the difference was significant only on a 3% probability level. No differences exceeding the error limits were found in the corticosterone concentration of either the adrenal gland or of the blood plasma.

The Bone Marrow

In all three experiments the number of megakaryocytes per microscope field in the bone marrow of the magnet-group mice decreased significantly compared to their controls. Since the decrease was slightly smaller when the field strength was increased from 4200 to 9000 Oe, the effect must be considered as to be field insensitive in this field-strength range. Comparing the results with respect to the length of exposure to the field, we find that 4, 13, and 35 days of exposures, respectively, decreased the number of Mkcs per field by 16.3%, 27.1%, and 31.5%, respectively, indicating that at least up to 35 days longer exposures to the field intensifies the abnormality. Experiment III may indicate that between 2000 and 3000 Oe a saturation has occurred, but that below this value the effect is field sensitive.

Lorber⁽¹⁷⁾ found after the application of the stress of a variety of surgical procedures within a few days an increase in the number of Mkcs in the bone marrow.

On the basis of morphological, immunological, and chemical studies it is generally accepted that thrombocytes are formed when the cytoplasm of the older Mkcs become fragmented. In some diseases (acute purpura hemorrhagica) a great increase in the Mkcs is observed, but less than 20% of these show evidence of platelet production. Diseases destroying the bone marrow, such as leukemia, reduce the Mkc count without changing the morphology of the Mkcs.⁽¹⁸⁾ Ebbe *et al.*⁽¹⁹⁾ found that transfusion-induced thrombocytosis did not affect either the rate of maturation of the Mkcs, or the influx of cells into the megakaryocytes' compartment from precursor compartments. Likhachev⁽²⁰⁾ reports on a 30% drop in the number of platelets in human blood during a 15-min exposure of the subject's head to 5000-Oe field.

The Spleen

The number of megakaryocytes per microscope field increased in the spleens of the magnet-group mice significantly, both in Experiment I and II, while no significant increase was found in Experiment III. Nor was a significant difference observed between subgroups *a* and *b* of Experiment II with 13-day and 4-day exposure times, respectively. We conclude that there exists a lower limit of field strength, but that in the range from 4200 to 9000 Oe the increase in the number of Mkcs in the spleen is field-independent and independent of the length of exposure between 4 and 35 days: moreover this effect of the magnetic field persists up to at least 6½ months after termination of magnetic exposure.

A large increase in the number of Mkcs in the spleens of animals stressed with Walker tumor transplants was found by Selye.⁽²¹⁾ Such increases, however, are usually associated with erythropoiesis and myelopoiesis in the red pulp of the spleen, symptoms not seen in our magnet-group mice. Megakaryocyte proliferation is characteristic of the reaction to neoplastic tissues and to treatment with certain tissue extracts, but not seen with ordinary, nonspecific stress, such as restraint, cold, traumatic injuries and the like.

Megakaryocytes, may occur in the spleen in extramedullar blood forming foci. However, in the spleens of the magnet-group mice we have only occasionally seen cells somewhat resembling myelocytes in the vicinity of the Mkcs groups.

Recent observations of Shanoff and Kim⁽²²⁾ and of Aschoff *et al.*⁽²³⁾ revealed the presence of Mkcs in the lung tissues of humans and animals

under normal conditions and also in various pathological states. After the administration of adrenaline the number of Mkcs in the lung decreased with a simultaneous increase in the number of thrombocytes. Kaufman *et al.*⁽²⁴⁾ inferred from their investigations on blood withdrawn with an intercardiac catheter that Mkcs are formed in the bone marrow and about 20 to 50% of these enter the blood stream and migrate through the capillaries to the lung, where they take part in thrombocyte production. They estimate that about 7 to 17% of all thrombocytes originate in this manner. It should be mentioned that in all lung tissues of the exposed mice inspected we have found a certain, though rather small, number of Mkcs. Whenever the need for an increase in the thrombocyte number arises, more Mkcs must enter the pulmonary circulation, occasionally a not negligible number of these might be immature Mkcs. If this picture of thrombocyte formation is correct, then it seems plausible that under the influence of a magnetic exposure a greater need for thrombocyte production may arise and accordingly both mature and immature Mkcs would be forced to leave the bone marrow in increased number, explaining the decrease found in the number of Mkcs in the marrow. Most Mkcs would remain in the capillaries of the lung but some might enter the systemic circulation and the side branches of the celiac artery, from where they could reach the spleen and occasionally the liver. In some instances we have seen Mkcs in the liver tissues of the magnet-group mice. One can not exclude the possibility that the smaller immature Mkcs may readily traverse through the lung.

According to another theory Mkcs are locally formed whenever endomitosis of the reticular cells is not followed by cytokinesis and many large cells with many nuclei are formed.⁽²⁵⁾ Megakaryocyte formation would hence correspond to a reactive reticulosclerosis. The results obtained from the experiments described herein seem to lend more support to the latter interpretation, because in most tissue sections we did see relationships between the Mkcs and the surrounding reticulum. One might speculate that under the influence of the magnetic field the resistance of the Mkcs decreases, and they may easily undergo necrobiotic processes. In this sense, the increase in the number of Mkcs in the spleens would be a manifestation of a general reaction of the organism, for instance, to a stimulation of the reticuloendothelial system. Such reactions are observed in acute hepatitis, or after subcutaneous injection of caseine, and are always linked with an increase in the number of Mkcs in the spleen.⁽²⁶⁾ The stimulation of the reticuloendothelial system is part of the general defense mechanism of the organism, and as such might also affect the bone marrow and explain our findings of a reduced number of Mkcs in them.

The Liver Tissues

A statistically very significant number of cells undergoing mitosis were found in Experiment I and II, while no significant increase in mitosis was observed at the lower field strength of Experiment III. The difference between the 71.5% increase in mitosis above control value in Experiment I and 129% increase in Experiment II has to arise from the differences in the experimental circumstances of the two investigations. It can be interpreted either as meaning that this effect is strongly field-strength-dependent, or, that in Experiment I the smaller increase in the mitotic index was a consequence of the long time interval between termination of exposure and killing of the mice. Some data, not reported here because they were obtained using too small a sample of animals, tend to favor this second interpretation.

The liver is a very complex and sensitive organ, which reacts to comparatively mild lesions with observable morphological changes. The effect of noxious influences is primarily seen in the cells of the parenchyma and in the reticuloendothelial system. The gross morphological picture is always rather similar, probably varying in intensity, independently of whether it is a consequence of a disease of the liver or provoked through different experimental stimulations.

The microscopic examination of the liver tissues in the mice group killed immediately after termination of exposure revealed some edematous conditions, seen on the periphery and the center of the lobules. The capillaries around the portal vein were widened and the Disse's spaces were much wide than usual. The cells in the center of the lobules stained much more poorly, or, conversely, the cells became smaller with small nuclei and stained darker than usual, that is, they became pyknotic. In some sections the cytoplasm of the cells stained with eosin to a darker red than usual and in these same cells the nuclear staining was the poorest. No glycogen was seen in the central portion of the liver. The cells in the peripheral and midzones were generally larger than normal; their cytoplasm was vacuolated and contained some glycogen. Fatty deposits were rarely seen. The absence of vacuolization in the central zone is surprising since it is usually found whenever a lesion occurs in that zone. Many of the larger than normal cells of the peripheral and midzones underwent mitosis which one could explain as a reaction to the lesion, the liver replacing in this manner the destroyed and/or necrobiotic cells. The number of dividing cells is hence a measure of the lesion suffered and the mitotic index is used by many to assess the degree of regeneration. In addition to the injury to the parenchyma, a certain irritation of the reticuloendothelial system was also

seen, manifested in the slight increase in the number of Kupffer cells and in their increased glycogen phagocytic action. No proliferation of the connective tissues was seen.

When the mice were killed a long time after magnetic exposure (Experiment I), the injury to the central parenchyma was much less pronounced and at most a few pyknotic liver cells were seen; nevertheless, the number of large liver cells and the number of cells undergoing mitosis was still above normal.

Ladewig stain and fluorescence microscopic examination of the liver sections did not add further insight to our findings; we did not see the red or purple staining of the cells usually characteristic of dead cells when using Ladewig stain, nor did we find pyroninophil granules.

The abnormalities described here differ only quantitatively from those occurring after injuries of different nature. For instance, Selye⁽²⁷⁾ reports of cloudy swelling, nuclear pyknosis, distention of Disse's spaces, and the appearance of edemas in the liver of stressed animals. Fibrinoid necrosis can also occur with liverstasis as a result of heart failure. After prolonged chloroform or tetrachlorethylene inhalation (Kylin *et al.*⁽²⁸⁾) and after injection of chloroform or phosgene (Sümegi *et al.*⁽²⁹⁾) abnormalities were manifested as circulatory disturbances and fibrinoid necroses. Szende *et al.*⁽³⁰⁾ observed glycogen reduction, differences in the size of the cell nuclei, together with the appearance of large nucleoli and tiny focal liver cell necroses after administering tannic acid or isoniazid.

Finally we wish to mention still another long-lasting change we observed on many mice exposed to magnetic fields, which might be related to the effects described herein, namely that after exposure to a magnetic field of 4200 Oe the body temperature (measured in the vagina, or in the rectum) dropped by 0.7°C and the temperature returned to normal values only 3 months later.

CONCLUSION

Exposure of mice to homogeneous static magnetic fields in excess of 4200 Oe, and exposure times longer than 4 days produce a disorganization and narrowing of the zona fasciculata of the adrenal cortex, a decrease in the number of megakaryocytes in the bone marrow, an increase in the number of megakaryocytes in the spleen, and an increase in the number of mitoses in liver tissues. The high statistical significance found for each of these four parameters establishes the existence of these changes in organs of mice exposed to strong magnetic fields.

While some of the observed abnormalities are seen when an organism is subjected to a variety of stressors, some differences should be pointed out. Under ordinary conditions of stress the fasciculata is widened not narrowed; the number of megakaryocytes usually increases in the bone marrow and does not decrease; and, finally, while megakaryocyte proliferation with ectopic myelopoiesis in the spleen is characteristic of the reaction to neoplastic tissues and treatment with certain tissue extracts, it is not seen with ordinary non-specific stress. Furthermore, should the magnetic field behave as a non-specific stressor, we would have to expect a decrease in the intensity of the effects as the time elapsing after termination of magnetic exposure is increased; such decrease in severity of the effects was observed only in the mitotic index of liver tissues.

The circumstance that a change was evident in the zona fasciculata of the adrenal cortex suggests that during the exposure to a magnetic field some hormone imbalance was created. The abnormalities observed in the spleens and livers of the exposed mice could best be described as resulting from a general stimulation of the reticuloendothelial system, which, in turn, might be a manifestation of the defense mechanism of the organism against the stimulus of the magnetic environment.

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CHANGES IN SODIUM AND POTASSIUM CONTENT OF URINE FROM MICE SUBJECTED TO INTENSE MAGNETIC FIELDS

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INTRODUCTION

Man is being exposed to increasingly higher magnetic fields on earth. Such exposures occur in plasma and solid state physics laboratories, industrial factories using magnetic forming devices, and other engineering disciplines. Betatrons and cyclotrons with magnetic fields ranging to 20 kOe are used for accelerating charged particles. It is reasonable to expect that in the near future man will be exposed to an increasing number of sources of even higher intensities. Magnetic fields to shield men against damaging radiation during extended space flights are contemplated.

We realize how important it is for man to function properly in whatever task he is performing, especially when conducting technically sophisticated research, or when performing potentially hazardous tasks. Whether magnetic fields of high intensity produce harmful effects on man himself is not known. We do know that man has always lived in a weak geomagnetic field of around 0.5 Oe without knowledge of any harmful effects. Although there have been some reports of man noticing effects when being exposed, there have been no controlled physiological studies to obtain data on human tolerance to high magnetic fields. It has been reported⁽²⁾ that one person experienced a slight sensation of pain from teeth containing fillings when working around magnetic fields. Becker *et al.*⁽¹⁾ reported on an increase of admissions to mental hospitals following violent magnetic storms. Beis-

cher⁽⁸⁾ mentioned that persons working around a magnetic forming machine had complained of abdominal pain while the machine was operating.

There is a strong possibility that magnetic fields of high intensity influence the electrical processes of living tissue. When this occurs, there may be a change in the gradient of electrical potential or the chemical concentration across the cell membrane. When Gualierotti and Capraro⁽⁵⁾ demonstrated that a magnetic field of 500–600 Oe decreased the inward flux of sodium ions across a frog's skin, and this decrease in sodium transport was given as the reason for sudden depolarization in frog skins exposed to a field of 10 kOe,⁽⁴⁾ the question arose whether cations in the body fluids will be affected by exposure to high magnetic fields?

MATERIALS AND METHODS

To study the response of urinary cations, we began by exposing mice to a magnetic field of 14 kOe for 24 hr,⁽⁷⁾ and then later exposed mice to a 7200-Oe field for 96 hr.⁽⁹⁾

STUDY I

In the first study a water-cooled Harvey-Wells L-128, 12-in. electromagnet with 6-in. pole faces (Fig. 1) was used. The poles of the magnet were positioned vertically with the field lines oriented horizontally.



Fig. 1. Twelve-inch electromagnet with 6-in. pole faces and power supply.

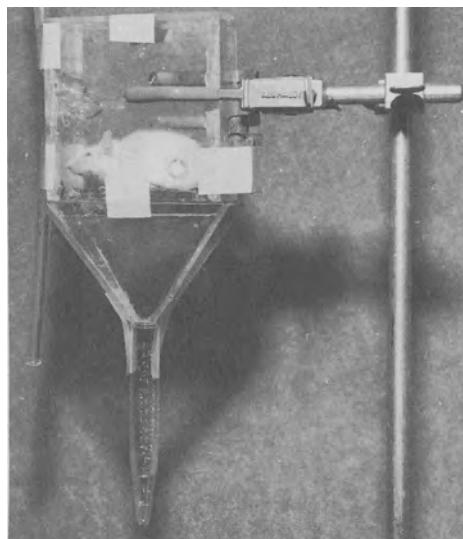


Fig. 2. Side view of metabolic cage used for collecting urine.

Female albino Swiss mice of the Webster-Fairfield strain, approximately 22 weeks old and weighing an average of 24.3 g were used. Thirteen mice, six experimental and seven controls were housed individually in plexiglas metabolic cages (Fig. 2) as described by Hanneman *et al.*⁽⁷⁾ The metabolic cage was positioned between the poles of the magnet. Food (Purina Laboratory Chow) and water were always available for the animals. At the beginning of each experiment, the mice (in their cages) were placed in the magnet and dummy magnet for a 24-hr period to permit the mice to adjust to the confined environment before urine was collected for analysis. The sampling periods consisted of the 2 days prior to exposure (preexposure period), a 24-hr exposure period, and the 2 days immediately following exposure (postexposure period). The exposed and control mice were handled and housed identically. The urine samples were analyzed for Na and K concentration with a Beckman Model B flame spectrophotometer.

RESULTS

The results in Table I are expressed as milligrams per milliliter of urine. The Na concentration of the urine from the exposed mice increased from 2.34 to 4.29 mg/ml following exposure. This was an increase of 1.95 mg,

TABLE I
Average Daily Na and K Concentration in Urine per Mouse

	Na, mg/ml		K, mg/ml	
	Exposed	Control	Exposed	Control
Preexposure	2.34	3.01	9.14	9.01
Postexposure	4.29	3.69	14.59	9.99
Difference	+1.95	+0.68	+5.45	+0.98
D. F.	10	12	10	12
t value	2.457	0.646	2.671	0.646
Probability level, %	3	N.S.*	2.5	N.S.

* N.S., not significant.

which was significant at the 3% probability level. The difference in the Na content in the urine from the controls for the same periods was 0.68 mg, which was not significant. The K concentration increased in urine from the exposed mice from 9.14 to 14.59 mg/ml following exposure. This increase of 5.45 mg/ml was significant at the 2.5% level of probability.

STUDY II

In the second study a water-cooled, Varian Associates 4-in. electromagnet, Model V-4004 (Fig. 3) was used. The field strength ranged from 7200 Oe at the center of the field to 5200 Oe at the periphery of the poles.

As with the first study, female albino Swiss mice of the Webster-Fairfield strain were used. However, these mice were approximately 9 months of age and weighed an average of 32.5 g. The metabolism units and method of urine collection were the same as in the first study, but in this study data were collected from ten mice for 8 days in a control environment (no exposure). Two weeks later data were collected from these same ten mice in a test environment, in which the mice were exposed for 96 hr in a magnetic field. The first 2 days of the study (for both environments) were termed preexposure and the last 2 days were termed postexposure. The terms pre and post were used for making comparisons between the control and test environments. The mice were placed between the poles of an aluminum dummy magnet (Fig. 4) except for the 96-hr exposure in the test environment when they were placed between the poles of the electromagnet. To regulate better the daily urine output the same quantity of water

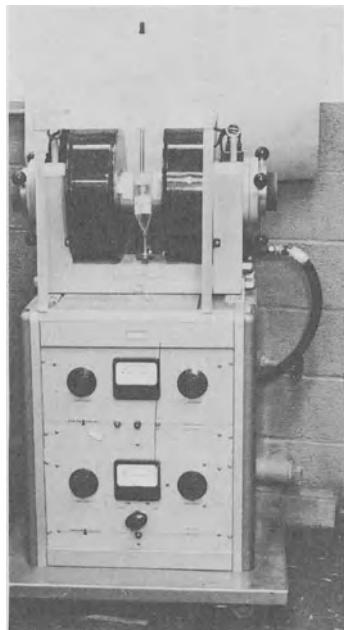


Fig. 3. Four-inch electromagnet and power supply with metabolic case positioned between poles.

was given to each mouse each day. These mice were also given a 24-hr adjustment period before any urine was collected. Toluene was used as the preservative and to eliminate evaporation during collection. Daily urine samples were collected from each animal during the days for both environments. A Beckman Model B flame spectrophotometer was used for the Na and K analyses.

RESULTS

Because each mouse was used for obtaining data both in the control and test environment, several statistical methods were suitable for evaluating the results. First, a comparison was made between the preexposure and postexposure data for both control and exposed conditions. Second, a comparison was made between the control and exposed groups during, first the preexposure period and then the postexposure period. The Na and K excretion in this study is expressed as average daily output per mouse.



Fig. 4. Dummy magnet setup used in obtaining control data.

Table II shows the urinary Na excretion from the exposed mice increased from 3.26 mg before exposure to 6.25 mg following exposure. This 2.99 mg increase was found to be significant at a 1% probability level. The difference between the same periods for the control data was not significant. The K excretion of the exposed group increased from 7.81 to 14.44 mg per day. This was significant at the 1% probability level. Although the K excretion in the control phase changed from 7.17 to 10.16 mg this difference was found not to be statistically significant.

Table III showed the average daily Na excretion during the preexposure period was 3.53 mg for the control and 3.26 mg for the exposed. This difference of 0.27 mg was not significant. However, the difference during the postexposure period between the control and exposed mice was 4.45 mg and 6.25 mg, respectively. This difference was statistically significant at the 3% probability level. In comparing the K output during the preexposure period the control animals excreted 7.17 mg per day while the exposed excreted 7.81 mg; however, this difference was not significant. In comparing the postexposure periods, the K excretion for the exposed averaged 14.44 mg

TABLE II
Comparison of Pre- and Postexposure Data

	Average daily output of Na and K per mouse			
	Na total, mg		K total, mg	
	Exposed	Control	Exposed	Control
Preexposure	3.26	3.53	7.81	7.17
Postexposure	6.25	4.45	14.44	10.16
Difference	+2.99	+0.92	+6.63	+2.99
D. F.	18	18	18	18
<i>t</i> value	4.545	1.371	4.774	2.033
Probability level, %	1	N.S.	1	N.S.

and the controls averaged 10.16 mg per day. This difference of 4.28 mg was statistically significant at the 2% probability level.

DISCUSSION

In both studies the excretion of urinary Na and K from female mice was found to be influenced by exposure to a magnetic field. The results obtained from these studies show a statistically significant increase in Na and K following both a 24-hr exposure at 14,000 Oe and a 96-hr exposure with the field intensity reduced by one-half.

TABLE III
Comparison of Control and Exposure Data

	Average daily output of Na and K per mouse			
	Na total, mg		K total, mg	
	Preexposure	Postexposure	Preexposure	Postexposure
Exposed	3.26	6.25	7.81	14.44
Control	3.53	4.45	7.17	10.16
Difference	-0.27	+1.80	+0.64	+4.28
D. F.	18	18	18	18
<i>t</i> value	0.464	2.412	0.525	2.696
Probability level, %	N.S.	3	N.S.	2

The temperature around the metabolism cage and mice was maintained at $78 \pm 2^{\circ}\text{F}$ and the relative humidity ranged from 45 to 65%. During the studies all animals were under continuous fluorescent lighting. There was no significant change in food or water consumption for any of the mice during the study. Moreover, there was no appreciable change in the quantity of urine excreted.

More recently, a study was conducted in which female mice about 24 weeks of age and weighing an average of 31.7 g were exposed to a magnetic field of 1000 Oe for 24 hr. They were studied under the same environmental conditions as in the 96-hr study. There was an increase in excretion of Na and K in the urine following exposure; however, the increase was found not to be significant. This suggests that the effects produced are influenced by both the magnitude of the field and the duration of the exposure.

As more biomagnetics research is being conducted, the more it becomes apparent that the presence of a magnetic field imposes a stress either on the body as a whole or on certain body systems. It is thought that changes which do occur during exposure appear to do so at the cellular level and more than likely involve ion changes at the cellular level. This may be a change in the gradient of electrical potential or the chemical concentration across the cell wall or within the cellular substance itself.

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CHANGES IN THE ERYTHROCYTE SEDIMENTATION RATE OF RABBITS DUE TO EXPOSURE OF THE CENTRAL NERVOUS SYSTEM TO A CONSTANT MAGNETIC FIELD

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Several investigators have studied the effect of a constant magnetic field (CMF) on the blood picture,⁽¹⁻⁶⁾ and others have examined its effect on the erythrocyte sedimentation rate (ESR) *in vivo* and *in vitro*.⁽⁷⁻⁹⁾

However, these workers did not always find themselves in full agreement, not only as regards their interpretation of their findings, but also as regards the results themselves, for they were contradictory.⁽⁸⁻¹⁰⁾

In undertaking the study of the action of a CMF on the ESR our basic position was as follows: in the diagnosis of diseases the ESR plays a fundamental role in the general clinical analysis of the blood and it could be found that the change in ESR brought about by the CMF is strictly constant for diseases of a given type. In that case determination of the ESR in such a situation would increase the probability of a correct diagnosis. Another purpose of our investigation was to elucidate the mechanism of interaction between the CMF and blood flowing through the vessels and to examine the role of the central nervous system (CNS) in the processes leading to a change in the ESR. Such an investigation could yield fundamental results which could serve as the basis for examination of problems allied to that of the ESR.

I shall not dwell further on the published data concerning the effect of the CMF on the blood picture, which has been adequately dealt with in the last decade, except to say that none of the experimental investigations so far reported include a strictly theoretical account of interaction between

the CMF and blood flowing along vessels, despite no lack of suggestions that such a possibility exists.

Let us turn now to the possible theoretical interpretation of interaction between the CMF and blood flowing along the vessels.

THEORETICAL CONSIDERATIONS

In our examination of interaction between the CMF and flowing blood we shall assume that the regulatory functions of the CNS are so trivial that as a zero-order approximation they can be disregarded, although subsequently we shall introduce a coefficient (determined experimentally for rabbits) allowing for effects of the CMF on the ESR mediated via the CNS. To examine the interaction between the CMF and blood flowing along the vessels we use a model which would not disturb the physiological nature of the blood or the functions which it performs in the living organism. These conditions are satisfied by the following case: erythrocytes moving in the blood stream as in Chizhevskii's arrangement,⁽¹¹⁾ on the one hand, and plasma bathing of the erythrocytes, on the other. In this latter case the term plasma applies to the plasma itself and to the salts which it contains, together with all the blood cells except the erythrocytes. By treating all the blood cells as plasma we can operate with the plasma as a homogeneous entity, and we can assess all the physiological changes in structure or number of cells from changes in the hydrodynamic and physicochemical indexes of the plasma as a whole, without considering them separately for each particle. In this way, while preserving physiological properties and their possible changes in a magnetic field, we considerably simplify the problem of the theoretical examination of our adopted system of plasma-erythrocytes with constant magnetic field. In other words, we change the interaction of a multiphasic and multicomponent system with a magnetic field to an interaction of a two-component system, fulfilling the same physiological functions. If the experimental findings confirm our theoretical calculations, it means that the model we have chosen is valid, while any discrepancies between their values can be used to assess the degree of validity of the chosen model.

At this stage certain physicochemical properties of erythrocytes which will be necessary later may be mentioned.

The erythrocyte consists of the shadow and the endoglobular contents. The most interesting of the endoglobular contents is hemoglobin, the chief functional unit of the erythrocyte. From the structural formula of this chemical compound and its three-dimensional crystal lattice, which are

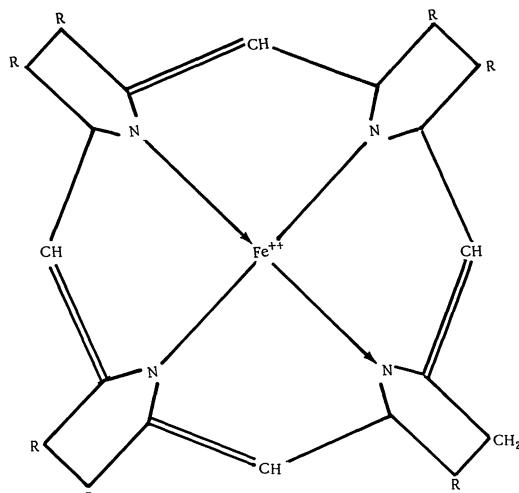


Fig. 1. Structural formula of hemoglobin.

shown in Figs. 1 and 2, it can be deduced that, as one of the class of complex compounds known as ligands, it will possess a dipole moment and will thus be able to interact with the surrounding aqueous medium.

Various workers⁽¹²⁻¹⁴⁾ have shown that the hemoglobin of the erythrocyte can react with surrounding water molecules to give charged complexes or complexes with free radicals. I have determined the sign and magnitude of the charge carried by the erythrocyte.⁽¹⁵⁾ Hence, when considering interaction between a constant magnetic field and blood flowing along the vessels,

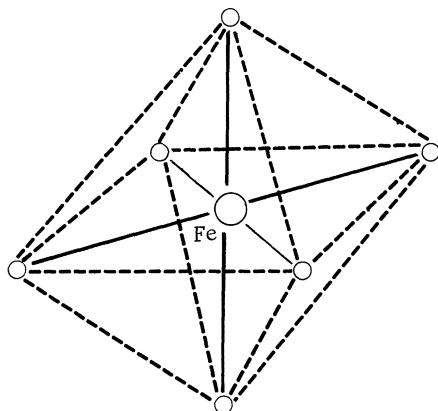


Fig. 2. Three-dimensional crystal lattice of hemoglobin.

we are inevitably compelled to examine interaction between the magnetic field and an electrolyte, containing charged particles, flowing along the vessels. I should mention here that the blood plasma is by nature an incompressible Newtonian fluid, because $\eta\rho = \text{const}$, and if we examine the flow established in segments of blood vessels which are cylindrical in shape and from 1 to 18 mm in diameter we can conclude that in this case it is laminar in character, and we are justified in using the formulas of hydrodynamics to describe the distribution of velocities over the cross section of a blood vessel.⁽¹⁴⁻¹⁷⁾

In our calculations we further assumed that the vector of the magnetic field H is directed perpendicularly to the vector of forward velocity of the plasma flow. By combining solutions of hydrodynamics and Maxwell's equations for an electrically conducting fluid, we obtain a connection between the velocity of flow of this fluid and the intensity of the magnetic field.^(18,19)

The decrease in the velocity of blood flow along the vessels in a magnetic field can be associated with an increase in its viscosity, because viscosity is inversely proportional to velocity. In a field of 5000 Oe, for example, the viscosity is increased, and, consequently, the velocity is decreased by a factor of 2.3. Assuming that the magnetization of the erythrocytes persists for a time long enough to allow the measurement of ESR one would expect that the ESR as a function of viscosity will decrease.

Limitations of space do not permit the derivation of the formulas for the ESR during exposure to a magnetic field to be presented fully; we might write the end result of these computations

$$U_{\text{ESR}} = \frac{\bar{v}(\rho_e - \rho_p)g}{2.5\pi\eta^*\bar{r}_e} \cdot \frac{\bar{n}_0}{n} \cdot B + \delta(H)$$

In this equation, \bar{v} represents the volume (mean) of the erythrocyte; ρ_e the density of the erythrocyte; ρ_p , the density of plasma; η^* the viscosity of plasma in a magnetic field; \bar{r}_e the mean radius of the erythrocyte; \bar{n}_0 and n the mean number of erythrocytes and the number of erythrocytes in the particular animal concerned, respectively; and $B = 12F + 2.5G - 2.5A + 12$ is a term expressing the quantitative proportions of protein fractions in the blood plasma (F stands for fibrinogene, G for globulin, A for albumin). Since we consider that all changes taking place in all cells other than the erythrocytes belong to the plasma, this term will also characterize the change in the microscopic physicochemical properties of the plasma. The term $\delta(H)$ is determined experimentally in encéphale isolé preparations and it

describes the quantitative contribution made by the CNS as a regulatory organ reacting in one way or another to the action of the magnetic field.

EXPERIMENTAL PROCEDURE

Thirty-five rabbits were divided into five groups with seven animals in each group.

Group 1 consisted of rabbits injected with turpentine. Each rabbit of this group was placed in a magnetic field for 15 min daily.

Group 2 consisted of rabbits also receiving turpentine. Each rabbit was placed for 15 min in a magnetic field only on one of the six days devoted to the experiment.

The rabbits of group 3 also were injected with turpentine. Each rabbit was placed in a magnetic field for 15 min daily, and the *encéphale isolé* operation was performed on one of the animals.

The rabbits of group 4 were also injected with turpentine. Each rabbit was placed only once in the magnetic field for 15 min during the six days of the experiment and the *encéphale isolé* operation was performed on one of the animals.

The rabbits of group 5 also were injected with turpentine; these rabbits were not placed in a magnetic field during any stage of the investigations.

The dose of turpentine injected was 0.5 ml/kg body weight. Turpentine was injected into the lateral surface of the abdomen to produce inflammation. The *encéphale isolé* operation was performed as described,^(20,21) the spinal cord being divided at the level of the first cervical vertebra. The animal's normal respiration was maintained by means of the AID-1 artificial respiration apparatus.

Blood was taken from all the animals before and after exposure to the magnetic field for measurement of the ESR, the hemoglobin concentration, and the leukocyte count.

The magnetic field was generated by a highly stabilized electromagnet whose intensity could be varied from zero to 7000 Oe. The area of the pole pieces was 100 cm², and this, together with the use of Rose's bars and shorting plates, ensured high uniformity of the field. The intensity of the magnetic field was checked by means of a type E-11-2 apparatus, and during the experiment it was 5000 Oe. The seventh rabbit in each group was used as a control.

Mechanical action was excluded during the experiment. The external environmental temperature was maintained at 22°C on every day of the experiment.

The rabbits were placed in the magnetic field for 15 min. The control animals also were placed between the pole pieces but the current was not switched on. The rabbits were introduced into the magnetic field so slowly that there was no possibility of generation of an induced electromotive force because of a change in $\partial H/\partial t$.

The experimental results were subjected to statistical analysis, although the need for this was minimal because of the high reproducibility of the values obtained.

EXPERIMENTAL RESULTS AND DISCUSSION

The results of experiments to study changes in the blood picture during the action of the CMF on the CNS of the rabbits are given in Table I. Let us briefly analyze the change in each parameter presented therein. Comparison of the changes in ESR in the rabbits of group 1 during the six days of the experiment shows that its value decreased under the influence of the CMF, its value being smaller on each successive day. Comparison between these changes and the ESR values in group 5 (control) reveals that, whereas the ESR was still very high on the 6th day in rabbits not exposed to the CMF, it had returned to normal in the rabbits of group 1. We thus find a dual effect: after exposure to the CMF (in the presence of intensive inflammatory processes, when the ESR is high) the ESR falls, while on the other hand the action of the CMF on the CNS leads to very rapid normalization of the ESR. To elucidate the role of the CNS in quantitative processes leading to a decrease in the ESR, experiments were carried out in which the spinal cord was divided at the level of C1 to exclude the CNS. Their results are given in Table I (group 3), and they show that the contribution of the CNS to the decrease in ESR was that inhibition, developing in the CNS under the influence of the CMF,^(22,23) spreads to regions responsible for the circulatory system, especially to the medulla and diencephalon. These arguments are confirmed by data for the leukocyte count in the blood before and after exposure to the magnetic field and also for *encéphale isolé* rabbit preparations.

Whereas the leukocyte count of the rabbits of group 5 rose steadily as a result of the inflammation, in the rabbits exposed to the action of the CMF on their CNS it rose extremely rapidly. However, the rise continued in the presence of CNS regulation. In the *encéphale isolé* preparations placed once only in the magnetic field (group 4) it rose just as in the controls, and the magnetic field had no action on it. The increase in the leukocyte count in the blood of the rabbits of group 1 was evidently due to positive

TABLE I
Changes in Blood Picture of Rabbits Following Action of Constant Magnetic Field on the CNS

Group of rabbits	1st day			2nd day			3rd day			4th day			5th day			6th day		
	ESR, mm/h	leuko- cytes	Hb	ESR, mm/h	leuko- cytes	Hb	ESR, mm/h	leuko- cytes	Hb	ESR, mm/h	leuko- cytes	Hb	ESR, mm/h	leuko- cytes	Hb	ESR, mm/h	leuko- cytes	Hb
1	8 — 8	7000 10200	65 64	57 45	9600 11400	66 55	43 33	9600 18000	57 50	30 16	14000 19000	48 44	12 6	15000 27000	44 43	8 4	14000 21000	50 46
2	6 — 5	6000 10000	67 64	43 23	6800 11000	65 60	48 28	8000 14000	62 58	56 30	9000 18000	58 54	61 31	9000 18000	56 52	62 30	10000 20000	56 50
3	10 — 11	5000 6000	66 64	55 22	6500 6800	62 59	42 28	9500 10000	58 53	35 18	11000 10500	54 50	22 12	14000 14500	50 48	14 8	16000 15500	52 48
4	11 — 10	6000 6200	64 60	45 25	6800 6500	60 54	54 26	7000 7500	56 50	49 26	7800 8000	52 48	58 29	8500 8500	50 48	56 26	9500 9500	52 48
5 (control)	7	3800	65	45	6800	66	56	7500	63	60	8000	60	65	8500	60	65	9500	58

Numerator denotes results before exposure, denominator after exposure to the magnetic field.

induction of the subcortex by the inhibited cortex, and removal of this inhibition by transection at the C1 level naturally resulted in no change in the leukocyte count as a consequence of exposure to the CMF. These conclusions are in full agreement with published data^(24,25) on nervous regulation of the blood system. The available data^(22,23) on inhibition of cortical activity in a magnetic field provide added confirmation. The increase in the leukocyte count in the blood under the influence of the CMF is evidently a positive factor at a certain stage of action of the magnetic field. This follows from the extent to which the inflammatory process subsided more rapidly in the presence of the magnetic field than in its absence. However, the sudden increase in the blood leukocyte count may have a lethal outcome. This factor must obviously be taken into consideration when working with strong magnetic fields.

The changes taking place in the blood picture were independent of the mode of action (with a single exposure), while in the case of repeated exposures of a living organism in which inflammatory changes are taking place a certain therapeutic effect was manifested. After a more detailed study, it is possible that this effect could be utilized in clinical practice.

The study of changes in the blood hemoglobin concentration under the influence of the action of the CMF on the CNS in rabbits revealed, on the one hand, a clear tendency for the hemoglobin level to fall under these conditions. However, a comparison of all the results obtained on this problem showed, on the other hand, that this tendency is not so clear and, in particular, that there is no hint of an effect of the CNS on the magnitude of the fall. This question will have to be left open; we can state only that either the decrease in hemoglobin concentration is a process with a long latent period, or that the hemoglobin concentration in the rabbit's blood falls entirely on account of magnetic field forces, acting destructively on the thin membrane of the oldest erythrocytes, leading to hemolysis and subsequent breakdown of the hemoglobin in the plasma.

To verify this hypothesis, the platelet count was determined in the blood before and after exposure of the CNS to the action of the magnetic field. Exposure to the CMF was found to cause a marked decrease in the platelet count. The number of platelets in human blood fell from 282,000/mm³ to 196,000/mm³ during exposure of the subject's head for 15 min to a magnetic field with intensity of 5000 Oe. It would be too rash to draw final conclusions regarding the decrease in hemoglobin concentration in the blood on the basis of a decrease in the platelet count, but as a working hypothesis it may be acceptable.

Results similar to those described above indicating changes in the blood

picture in a magnetic field were also obtained in our experiments on rabbits when the dose of turpentine injected was doubled and halved. When the dose was doubled, the results indicated not merely intensification of the inflammation, but also that the higher the ESR of an animal placed in a magnetic field the greater the relative changes leading to slowing of the ESR. Again, the more acute the course of the inflammation (the larger the dose of turpentine injected), the smaller the contribution made by the CNS to the slowing of the ESR and vice versa.

Two ways in which the action of a CMF may be used for clinical purposes can thus be discerned. The first is the use of powerful magnetic fields in the presence of relatively weak inflammatory processes, the second the use of weak magnetic fields in the presence of intensive inflammatory processes. In any event, we can presume that changes in the blood picture under the influence of the CMF can be used for diagnostic purposes. The role of theory in this matter is very important. The values obtained for slowing of the ESR in a magnetic field agree closely with those obtained by calculation using the model suggested above. The model, of course, is imperfect and needs improvement, yet the results of calculations made on its basis have led to conclusions which are already applicable in practice.

To sum up, we can say that by means of a full and comprehensive study of changes in the blood picture produced by exposure of the intact organism to a constant magnetic field, data can be obtained which may prove useful to the physician by aiding him in the diagnosis of diseases not only of the vascular system, but also of other systems not directly connected with it.

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SENSITIVITY OF SOME PLANT MATERIAL TO MAGNETIC FIELDS

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INTRODUCTION

The effects of magnetic fields on the growth and development of organisms have been studied for over a century. A variety of plant material has been used in such experiments, including both vascular and nonvascular plants. These studies have been largely concerned with growth responses, rather than anatomical effects. The investigations described in this chapter include not only growth responses of a filamentous alga and the adventitious roots of several angiosperms, but also the effects of heterogeneous magnetic fields of 500–4500 Oe on the activity and anatomy of root meristems and the morphogenesis of the derivative cells and tissues of these meristems.

PART. I. ANOMALOUS DEVELOPMENT OF FILAMENTS OF *PITHOPHORA* SP.

Pithophora sp., a common freshwater alga, was grown in a heterogeneous magnetic field of ca 800 Oe generated by permanent magnets obtained from magnetron tube assemblies (Fig. 1A). The alga was cultured in dechlorinated water in microaquaria with a diameter of 9.5 cm and a depth of 9.0 cm. Each experimental microaquarium was placed within the interior of a magnet, leaving about 1 cm of space between the magnet and the microaquarium. Each experimental microaquarium had a matching control. Other experimental and control cultures were established in 30-ml plastic tissue culture flasks and placed in heterogeneous magnetic fields of 800–1500 Oe (Fig. 1B). Periods of exposure varied from 15–60 days.

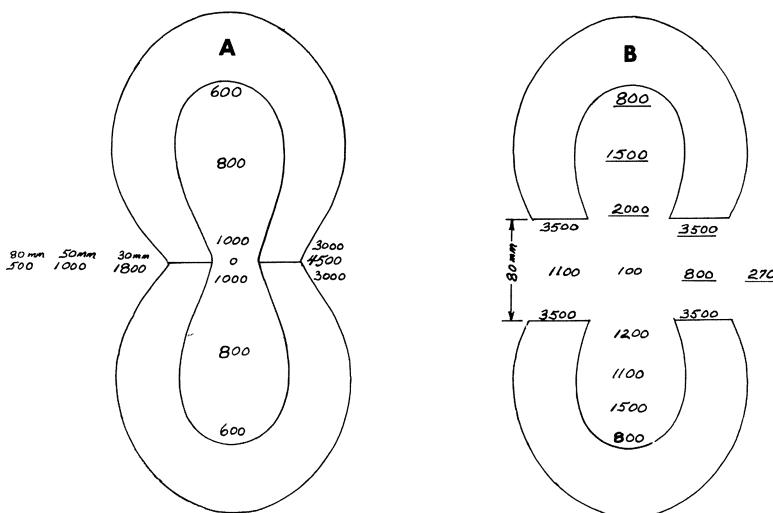


Fig. 1. Hall-effect gaussmeter readings of typical magnets used. (A) Readings for pair of horseshoe magnets in attracting position with poles joined; (B) readings for attracting (not underlined) and repelling (underlined) positions of horseshoe magnets with poles held 80 mm apart by brackets. Experimental algal cultures, bulbs, and cuttings were placed inside the magnets at each end (A, B), between the poles (B), and inside and outside the junction of the poles (A).

Control filaments of *Pithophora* were cylindrical in shape with paraboloid tips and maintained a uniform cell diameter of ca 50 μ (Fig. 2A). Filaments exposed to the magnetic field exhibited anomalous development after about 14 days. Growth rate decreased and filaments became irregularly sinuous (Figs. 2B-D); the diameter of the filaments increased to ca 80 μ , except near the tips, which became constricted to 30–40 μ . Some filaments in control cultures eventually developed similarly malformed tips; however, these were fewer in number and formed later than those of comparable filaments exposed to the magnetic field. Control cultures did not contain more than 10–20% abnormal filaments after 60 days, while cultures exposed to the magnetic field had developed 50–100% abnormal filaments after 15–20 days.

The magnetic field also affected the rate and amount of akinete production. Production of akinetes (vegetative cells modified as thick-walled resting spores) is a normal phenomenon in *Pithophora*: however, cultures exposed to magnetic fields commenced production of akinetes 2–3 weeks sooner and produced 50–75% more akinetes than did controls.

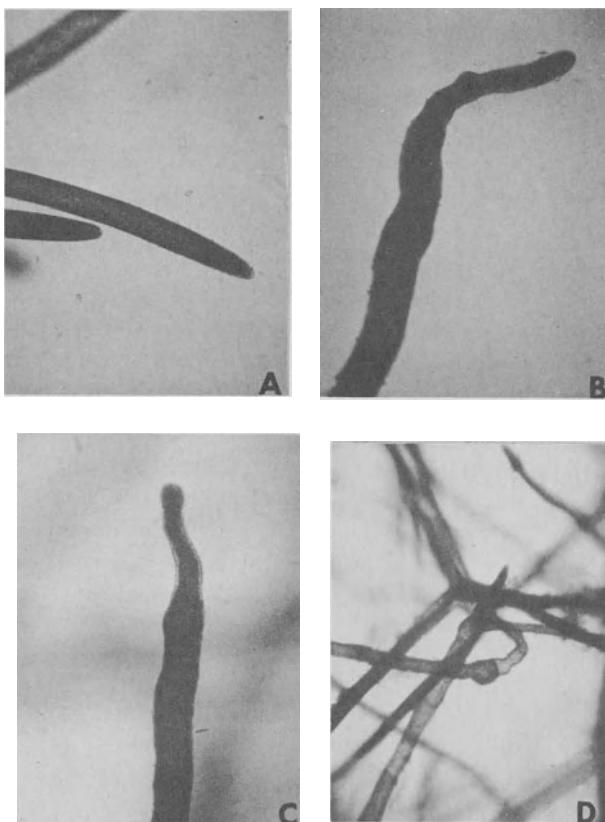


Fig. 2. *Pithophora* sp. (A) Normal filament tips; (B-D) typical changes in growth pattern after 15-30 days exposure to 800-1500 Oe. (A-C) 100 \times ; (D) 40 \times .

PART II. ANOMALOUS DEVELOPMENT OF ADVENTITIOUS ROOTS FROM BULBS OF *ALLIUM* AND *NARCISSUS*

Bulbs of *Allium cepa* and *Narcissus tazetta* were grown in glass jars of such a size that the bulbs rested in the necks with their lower portions in contact with tap water. Some root systems were shielded from light with aluminum foil wrapping; this did not appear to affect the results. Magnetic fields of 500-4500 Oe were used (Fig. 1); these fields were generated by permanent magnets obtained from magnetron tube assemblies. Experi-

mental bulb roots were exposed to magnetic fields for periods of 2 weeks to 2 months. Control bulb roots were grown under comparable conditions; no dummy magnets were used. Periodically, sample roots were removed from experimental and control bulbs and processed by standard cytological techniques. Over 20,000 sections of some 250 root tips were studied: these preparations revealed a number of abnormalities which we attribute to magnetic field exposure.

Roots exposed to magnetic fields elongated more slowly than control roots. This decrease in rate of growth and total length became more apparent as oersted-hours of exposure accumulated. Eventually elongation ceased, concurrent with enlargement of the root tips (Fig. 3M-1). This swelling and cessation of elongation was frequently followed by the appearance of a number of branch roots near the apex (Fig. 3M-2), the origin of a smaller root directly from the apical region (Fig. 3M-3), or sometimes a dichotomy of the apex to form two new root primordia (Fig. 3M-4). The branch roots or smaller roots also grew slowly, swelled at the apex, and occasionally bifurcated (Fig. 3M-2). These abnormalities were not infrequent, but appeared in the majority of roots produced by a bulb: on nearly every bulb exposed to the magnetic field the number of abnormal roots was between

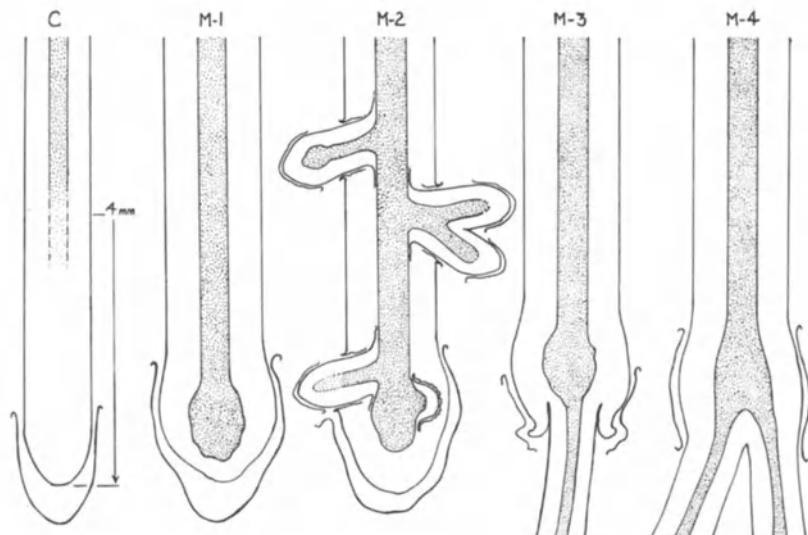


Fig. 3. Diagram of *Allium* root abnormalities. Stippled area represents mature vascular tissues. (C) Normal control root tip; (M-1 through M-4) patterns of abnormal development in experimental root tips.

50–100%. Direction of growth seemed relatively unaffected by magnetic fields in these experiments: no obvious reorientation was observed.

Root apical meristems exposed to magnetic fields followed one of three types of development. The most outstanding of these, as well as the most common, was the complete cessation of cell reproduction concurrent with cell maturation throughout the apical region as well as proximal to it (Figs. 4B, 5B). No mitotic figures were visible in sectioned root tips of this type,

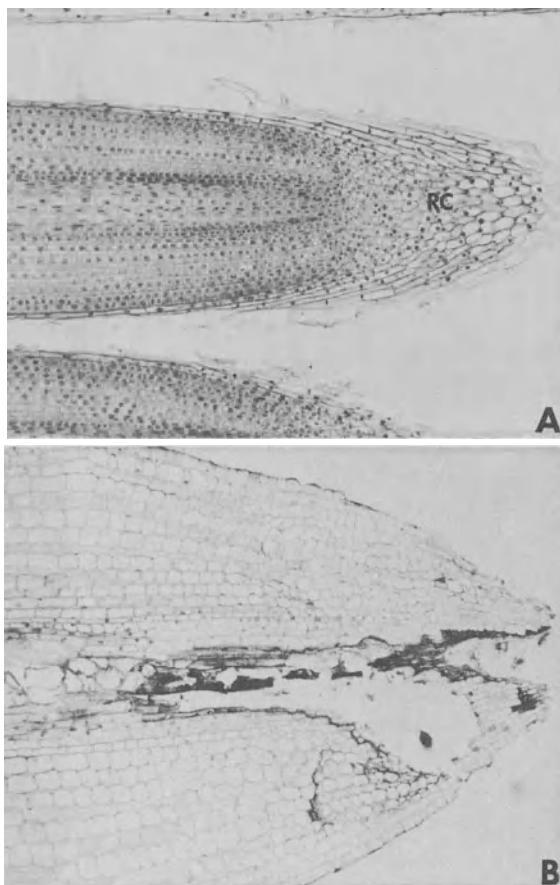


Fig. 4. *Allium* root tips. 80 \times . (A) Normal control root tip, showing apical embryonic region with typical meristematic cells, and well-developed root cap RC; (B) experimental root tip showing disintegration of embryonic region and root cap, development and distortion of stele, and enlargement of cortical cells.

and the cells were enlarged many times normal size. The root cap was no longer readily distinguishable and appeared partially or entirely disintegrated in many root tips (Fig. 4B). Staining reactions were abnormal: in some parts of the apex the nuclei were no longer chromatic, while others were excessively chromatic. The region of cell maturation finally extended into the former embryonic region; cell walls became excessively thickened in some areas, and the apex frequently disintegrated (Figs. 4B, 5B). Roots of

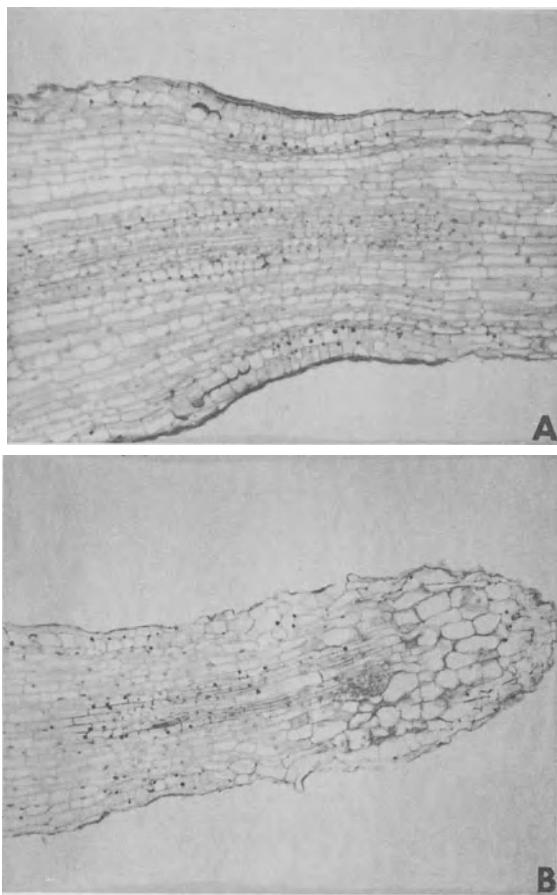


Fig. 5. Experimental *Allium* roots. 80 \times . (A) Branch root with tip disintegrated prior to emergence from parent root. Extreme enlargement and lignosuberization of adjacent cortical cells. (B) Root tip showing origin of branch roots from pericycle, development of stipe to the disintegrated root tip, enlargement of cortical cells, and lack of mitotic activity.

Allium with an apex which underwent this type of development frequently had numerous branch roots arising from the pericycle near the root apex; these branch roots in turn ceased meristematic activity and matured (Fig. 5A). Control roots of *Allium* produced fewer branch roots, and these usually arose several centimeters back of the root apex. Roots of *Narcissus* did not generally produce branch roots.

A second pattern of development was characterized by a constriction

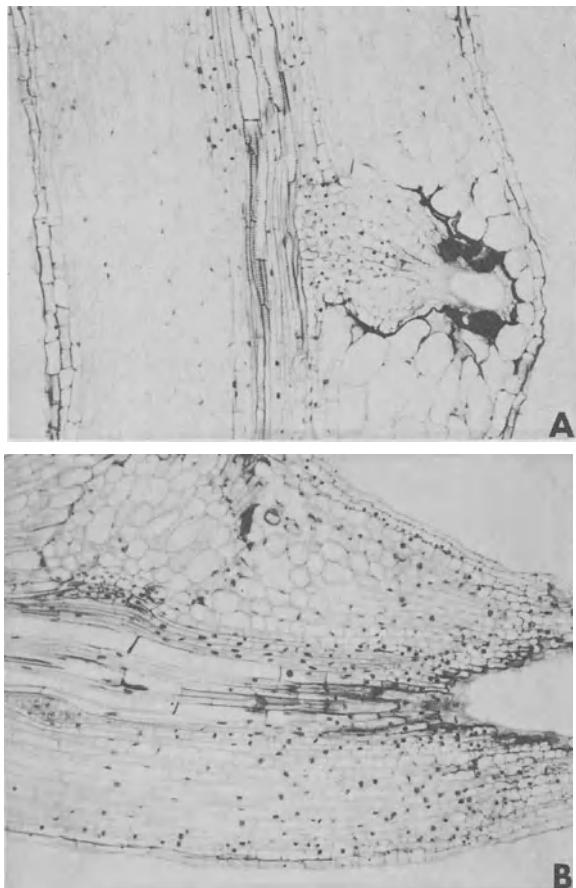


Fig. 6. Experimental *Allium* roots. 80 \times . (A) Constriction of root accompanied by modification of epidermal and hypodermal cells; (B) tip of same root, showing branch root origin from pericycle near apex, and enlargement of apical cells.

of the root to less than half its original diameter (Fig. 6A), followed by continued growth of the resulting smaller root. This smaller root, after a period of growth, also ceased cell reproduction, and the formerly meristematic cells enlarged considerably (Fig. 6B).

A third pattern of development was marked by regeneration of a new

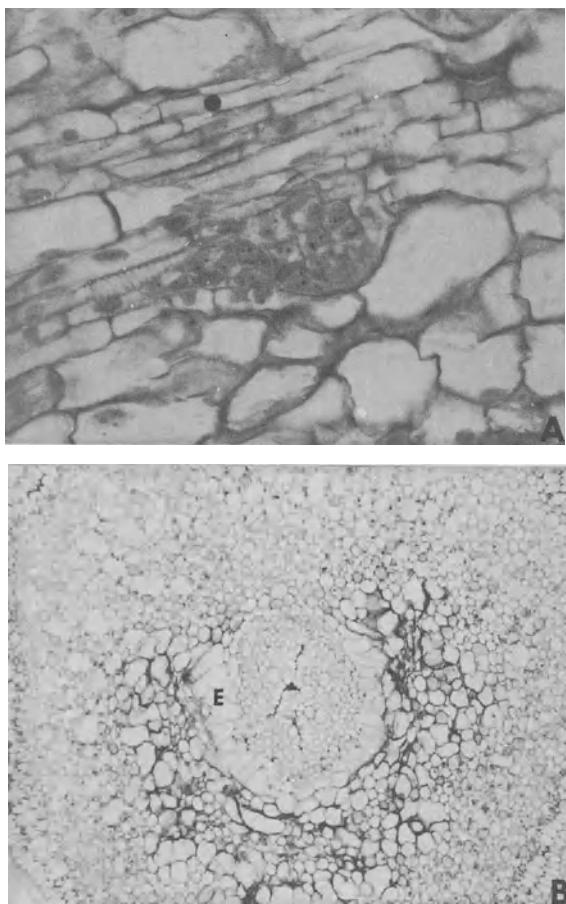


Fig. 7. (A) Branch root of *Allium* from Fig. 6B. Note mature xylem elements in adjacent stele of parent root apex, $333\times$; (B) Cross section of experimental *Narcissus* root ca $500\ \mu$ back of apex, showing lignin deposition in stele, enlargement of endodermal cells *E* around $\frac{3}{4}$ of circumference, and enlargement and lignosuberization of cortical cells around $\frac{3}{4}$ of circumference.

root or roots from the quiescent center (Fig. 8B). In cases of a double regeneration the root bifurcated. Branch roots also produced direct regenerations in the form of smaller roots that separated the tissues as they emerged from the apex of their quiescent parent.

Lignosuberization, a characteristic of dormant roots of many perennial monocots, was found in all experimental roots as they approached senes-

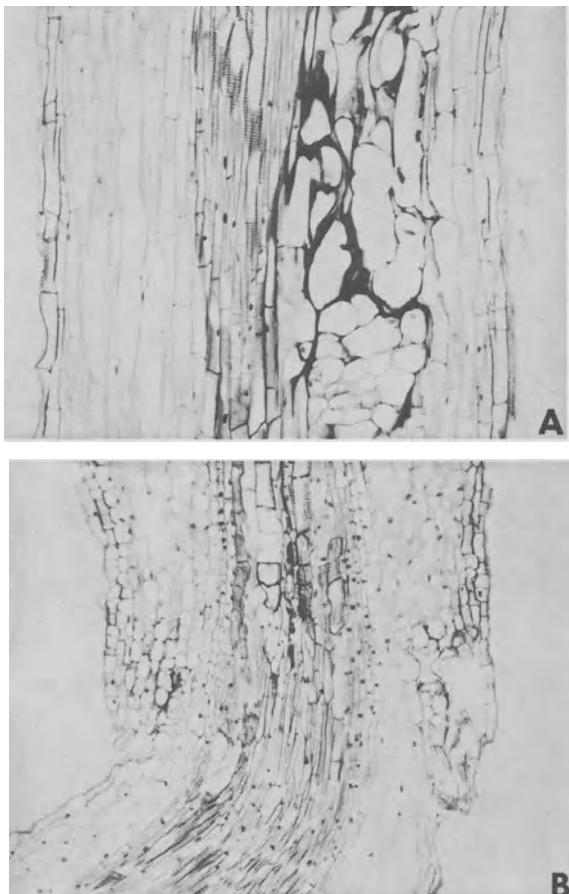


Fig. 8. Experimental *Allium* roots. 80 \times . (A) Longitudinal section showing enlargement and lignosuberization of cortical cells on one side and more nearly normal cortical cells on the other side of the stele; (B) longitudinal section showing result of regeneration of a new root from the quiescent center of the old root apex. Note smaller diameter of tracheary elements of new root.

cence after prolonged exposure to the magnetic field. This excessive deposition of wall material, accompanied by extreme cell enlargement, was seen in epidermal and hypodermal layers (Fig. 9A), generally or locally in cortical parenchyma (Figs. 7B, 8A), in the endodermis (Figs. 7B, 10B), and in cells distal to the former apical initials, so that the entire root tip

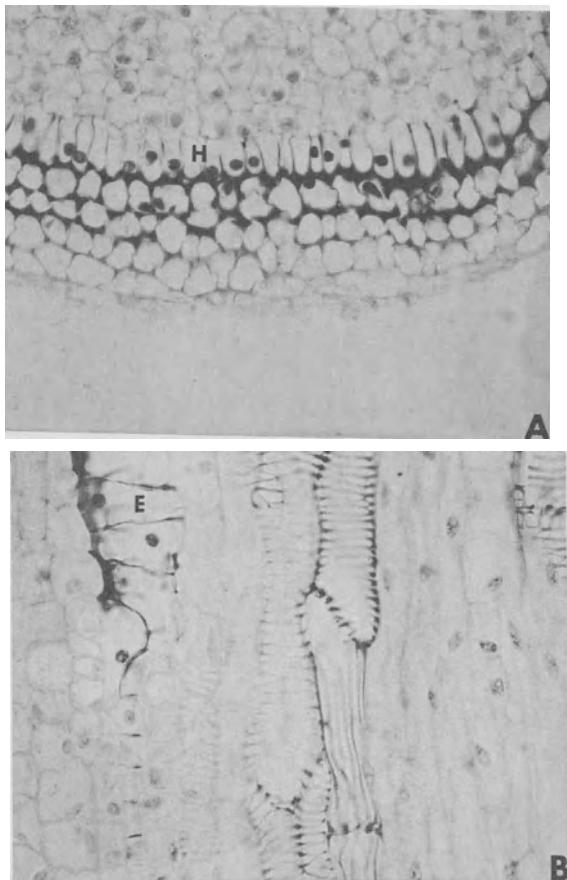


Fig. 9. Experimental *Narcissus* roots. (A) Cross section ca 500 μ back of apex, showing lignosuberized hypodermal H and epidermal cells: Outermost 3-4 cell layers are root cap, 80 \times ; (B) longitudinal section of apical region showing mature xylem, including two tracheary elements with longitudinal wall thickenings, and extreme enlargement of endodermal cells E. 333 \times .

was often sealed off from the outside. Lignosuberization was also found in older portions of the root and even around developing branch roots (Fig. 5A), some of which never emerged because cell reproduction had ceased in their embryonic region.

In control roots and in vigorously growing experimental roots of *Allium* and *Narcissus*, the first mature tissues were found several millimeters back of the root apex (Fig. 11A). However, as senescence of experimental roots

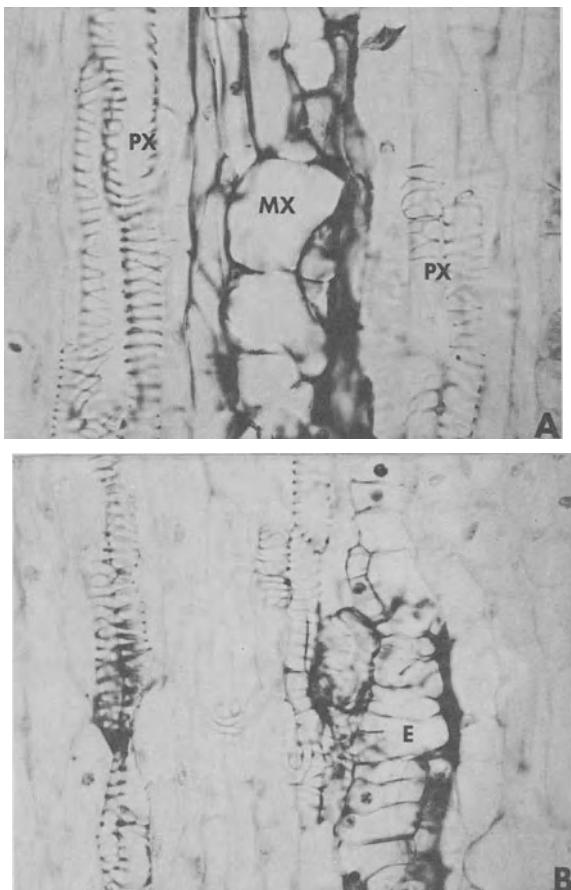


Fig. 10. Experimental *Narcissus* roots. 333 \times . (A) Enlargement, distortion, and excessive lignification of metaxylem MX elements; helical-reticulate wall thickenings in protoxylem PX; (B) excessive radial enlargement and lignosuberization of endodermal cells E.

progressed, the differentiation and maturation of tissues proceeded acropetally to the region of cell initiation (Figs. 4B, 5B, 6B, 7A), which had finally become inactive. Anomalies were most likely to be found in this region where mature tissues had replaced the normally meristematic cells. All tissues of the root showed one or more types of anomalous development. Deposition of wall materials was noticeably greater in tracheary elements of experimental roots. Protoxylem omitted the early types of wall sculpture and developed a scalariform or reticulate wall pattern (Figs. 12-14). Meta-

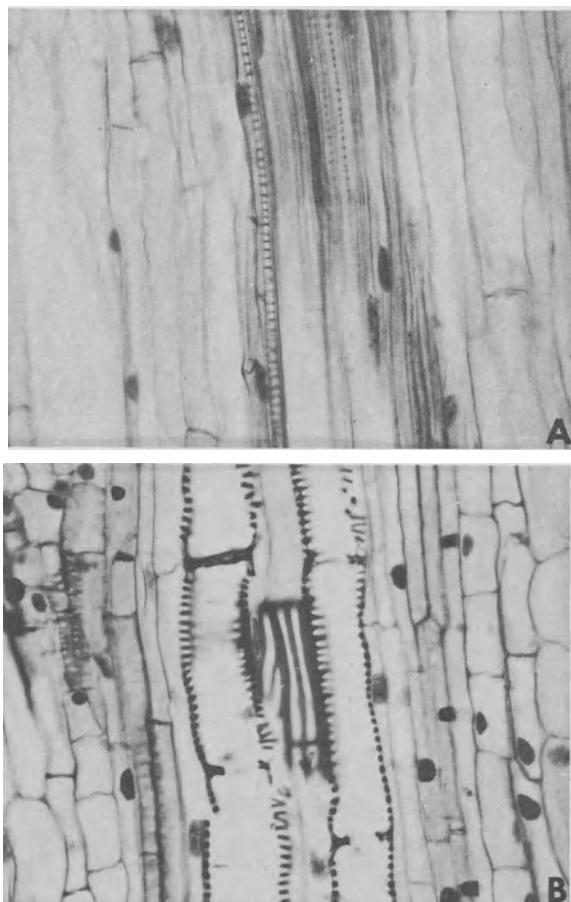


Fig. 11. *Allium* roots. 333 \times . (A) Control root showing first mature protoxylem with annular wall thickenings ca 4 mm back of root apex; (B) experimental root showing metaxylem tracheary element with longitudinal wall thickenings.

xylem frequently developed both scalariform and longitudinal wall thickenings or some combination of these types (Figs. 9B, 11B, 12A, 13, 14). Longitudinal wall thickenings were not found in tracheary elements of control roots.

Sieve elements also exhibited abnormal characteristics near the apex of roots exposed to magnetic fields. In this region the elements were unusually

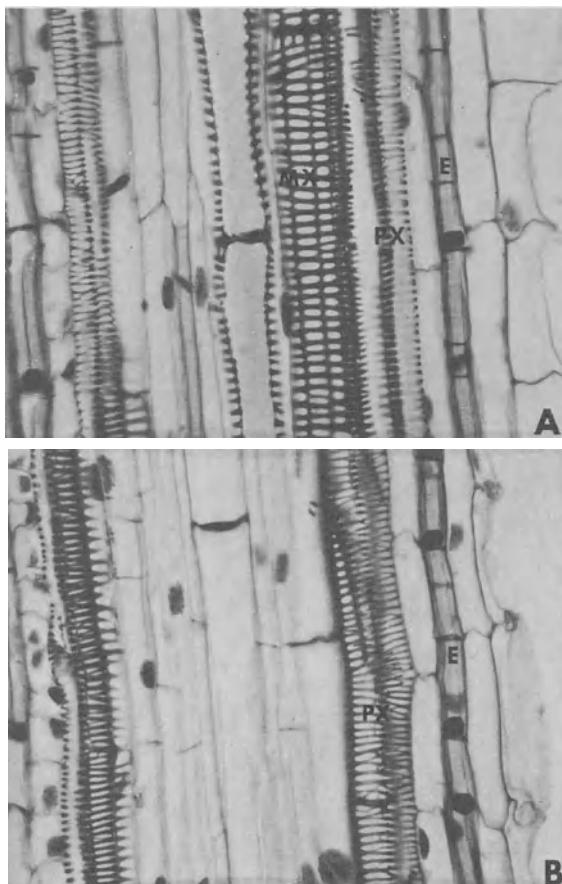


Fig. 12. Experimental *Allium* root. Longitudinal sections near apex showing protoxylem *PX* of larger diameter and more advanced wall characteristics (compare with Fig. 11A); wide, heavily lignified metaxylem elements *MX* with scalariform wall thickenings (Fig. 12A), and unusually wide and heavily lignosuberized caspary strips in endodermal cells *E*. 333 \times .

short and had either horizontal or oblique end walls; the oblique end walls often had compound sieve plates (Fig. 15A). Lateral walls of these elements had many sieve areas, as well as unusually heavy deposition of cellulose (Fig. 15B). In contrast, sieve elements of control roots possessed only horizontal end walls with simple sieve plates; these elements had thin lateral walls devoid of sieve areas.

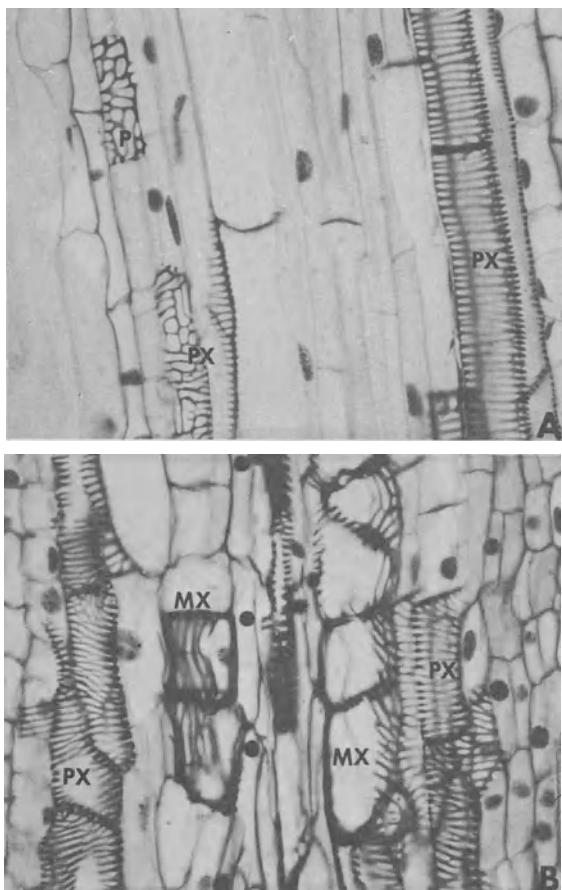


Fig. 13. Experimental *Allium* roots. 333 \times . (A) Reticulate pericyclic tracheid *P* top left, reticulate protoxylem tracheid *PX* lower left, and scalariform protoxylem *PX* at right; (B) Protoxylem *PX* with advanced wall characteristics of metaxylem, and metaxylem *MX* with reticulate and longitudinal wall thickenings.

The pericycle of experimental roots exhibited two types of abnormal development: an increase in cell size, especially cell length, and occasional differentiation of single cells or longitudinal series of cells into reticulate tracheary elements (Figs. 13A, 14).

The endodermis of experimental roots also showed an increase in cell size, which, in contrast to the pericycle, was largely radial and tangential (Figs. 7B, 10B). In addition, development of caspary strips was unusually extensive and frequently included the entire width of radial and transverse walls (Fig. 12). These caspary strips stained unevenly, showing numerous light bands and pitlike areas. In roots that had ceased elongation, the endodermis was frequently continuous down to and surrounding the formerly meristematic region of apical initials.

Cortical parenchyma of experimental roots exhibited cell enlargement comparable to that of other tissues, and, in addition, often showed varying degrees of lignosuberization (Figs. 7B, 8A). Occasional cortical cells exhibited peculiar patterns of this wall deposition (Fig. 15D). Other cortical cells, while not lignosuberized, showed heavy deposition of cellulose in a crossed lamellar pattern with numerous simple pits (Fig. 15C). Outer cortical cells often formed a pronounced hypodermal layer of lignosuberized cells (Fig. 9A); this hypodermis was usually most evident near the inhibited root apex.

Epidermal cells similarly showed lignosuberization (Figs. 5A, 9A) and

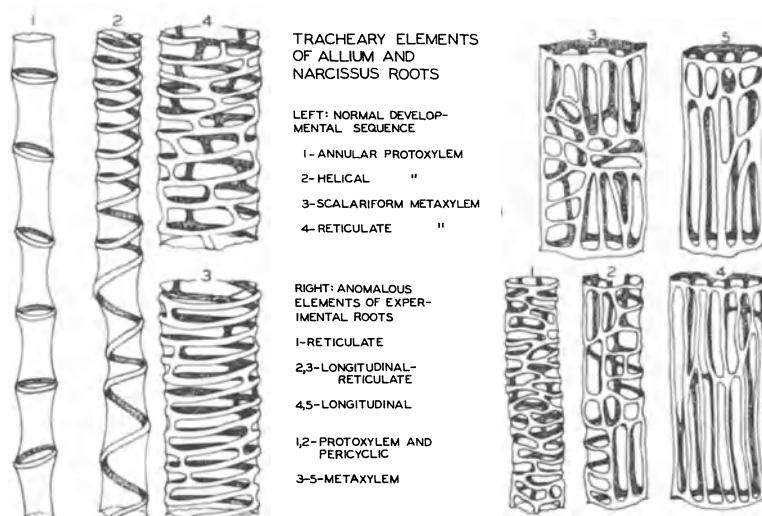


Fig. 14. Tracheary elements of *Allium* and *Narcissus* roots: normal and anomalous types.

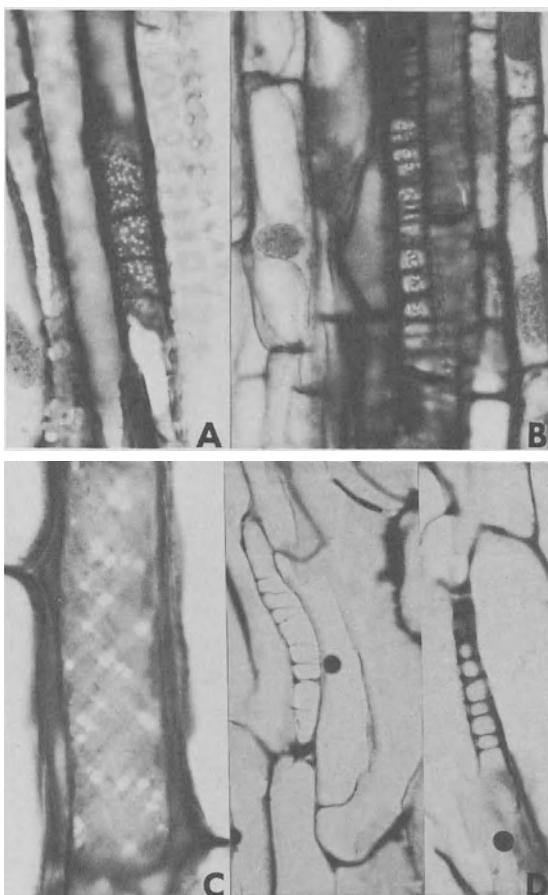


Fig. 15. Experimental *Allium* root. (A) Compound sieve plate in oblique common end wall between two thick-walled, sieve-tube elements; (B) numerous sieve areas in common longitudinal wall of adjacent sieve-tube elements; (C) cortical cells with unusually thick walls and pronounced crossed-lamellar deposition with numerous pits; (D) cortical cells showing anomalous thickenings and lignosuberization of cell walls. 832 \times ; (D) 333 \times .

an unusual decrease in length in regions of greatest inhibition of growth.

The anomalous behavior described here in adventitious roots of *Allium* and *Narcissus* exposed to magnetic fields did not occur in control roots. None of the preparations of control roots showed abnormal anatomical characteristics, and externally all of the control roots appeared normal.

PART III. ANOMALOUS DEVELOPMENT OF ADVENTITIOUS ROOTS FROM CUTTINGS OF COLEUS

Pairs of *Coleus blumei* cuttings were made from equal opposite axillary branches of vegetative plants. These cuttings were of similar length, and the members of each pair had essentially the same number of leaves and leaf

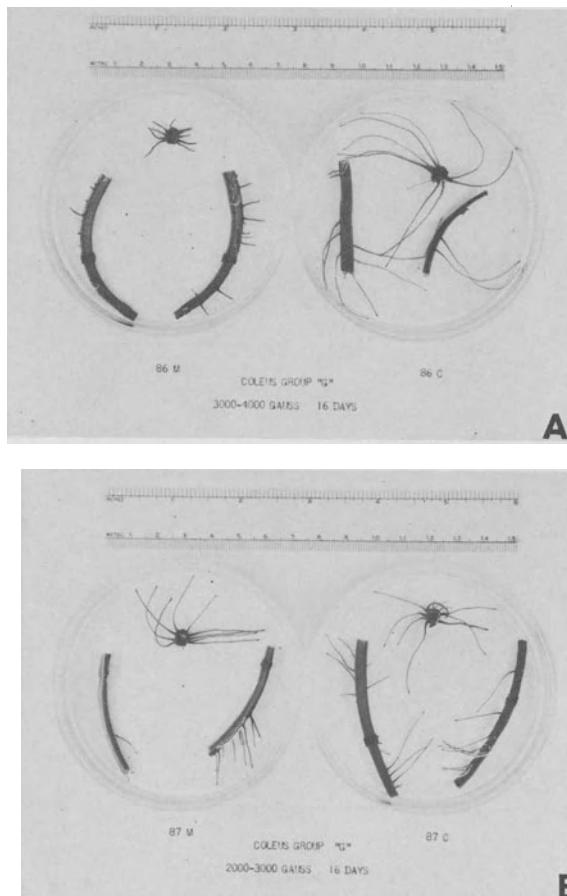
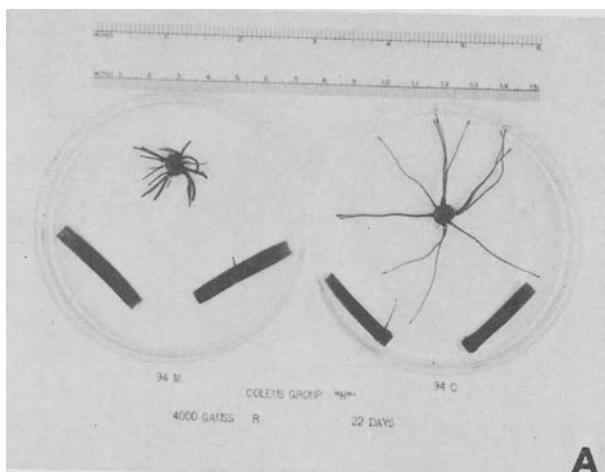
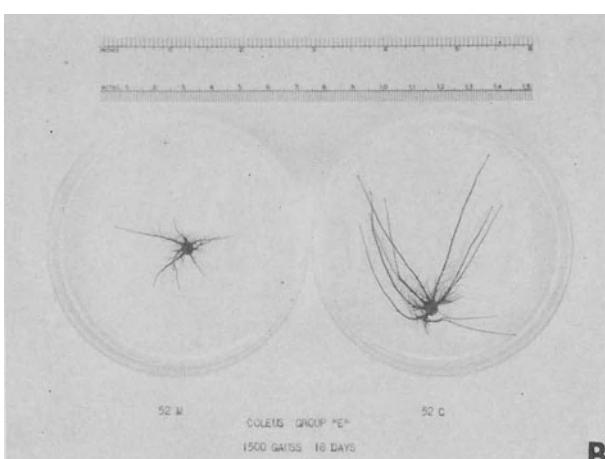


Fig. 16. *Coleus* cuttings with adventitious roots. 0.45 \times . Experimental cuttings at left, controls at right. Lowest node of each cutting seen in transection; the two internodes above split longitudinally. (A) Note shorter roots of experimental cutting; (B) note restriction of roots to lowest node and internode of experimental cutting.

surface area. Each cutting was set up floating in tap water in a small beaker and was supported by a cork disk with a hole in its center through which the stem of the cutting extended. One member of each pair of cuttings was exposed to a heterogeneous magnetic field of 2000–4000 Oe (Fig. 1); the corresponding member was used as a control. About 63 such pairs of matching cuttings were grown in a constant environment room at 20°C. During



A



B

Fig. 17. *Coleus* cuttings with adventitious roots. 0.45×. Experimental cuttings at left, controls at right. Note shorter roots of experimental cuttings, and lack of root formation above lowest node in both cuttings of Fig. 17A. Figure 17B shows only lowest node of cuttings.

TABLE I
**Number and Length of Roots of *Coleus* Cuttings Exposed to Magnetic Fields of
2000-3000 Oe Compared to Controls**

Day	Root number		Ave. root length, mm		Total root length, mm [†]	
	87M*	87C	87M	87C	87M	87C
8	0	0	0	0	0	0
9	0	1	0	0.5	0	0.5
10	6	6	0.5	1.0	3.0	6.0
11	8	12	2.0	3.0	16.0	36.0
12	11	18	3.5	3.5	38.5	63.0
13	17	25	7.0	7.5	119.0	187.5
14	20	25	10.0	10.0	200.0	250.0
15	22	33	12.0	13.0	264.0	429.0
16	22	33	12.5	16.5	275.0	544.5

* M, experimental cutting; C, control.

† Total root length is the number of roots times average root length.

the course of each experiment, the number, length, and distribution of roots developed by each cutting were recorded. At the close of each experiment, both stems and adventitious roots of the cuttings were fixed, transferred to 70% ethanol for photographing (Figs. 16, 17), and then processed by standard cytological techniques for microscopic examination.

TABLE II
**Number and Length of Roots of *Coleus* Cuttings Exposed to Magnetic Fields of
3000-4000 Oe Compared to Controls**

Day	Root number		Ave. root length, mm		Total root length, mm	
	86M	86C	86M	86C	86M	86C
8	0	1	0	0.25	0	0.25
9	0	6	0	0.5	0	3.0
10	4	12	0.25	2.0	1.0	24.0
11	14	16	0.5	5.0	7.0	80.0
12	19	18	1.5	7.5	28.5	135.0
13	23	18	3.5	15.0	80.5	270.0
14	23	21	3.5	17.5	80.5	367.5
15	24	22	5.0	20.0	120.0	440.0
16	29	24	5.5	25.0	159.0	600.0

Adventitious roots first emerged from the stem of a control cutting ca 8 days from the time the cutting was removed from the parent plant, and 1-2 days later from experimental cuttings. By 14-15 days the experimental cuttings had an average of 10% fewer roots than the control cuttings (Figs. 16A, 17; Table I). Although a few control cuttings developed fewer roots than their corresponding experimental cuttings (Fig. 16B; Table II), in all cases the total root length (sum of lengths of all roots of a cutting)

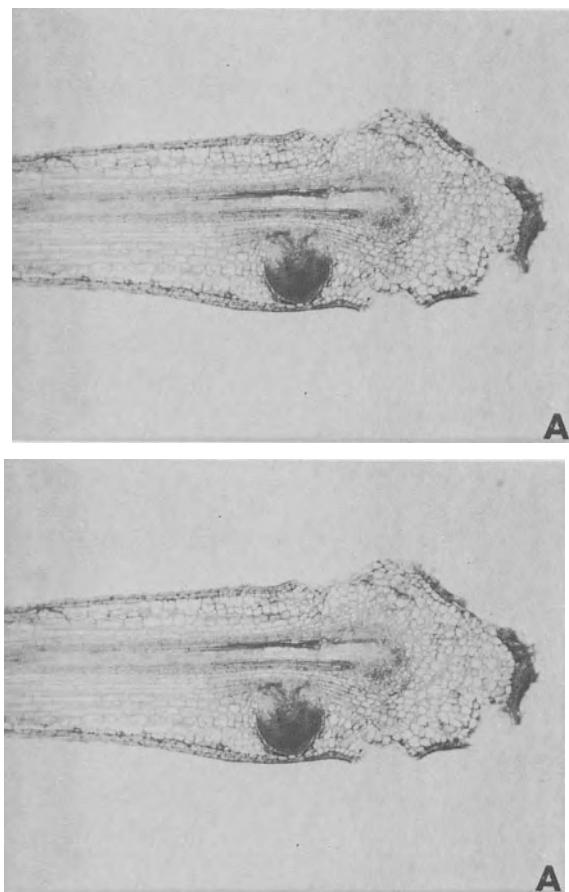


Fig. 18. Experimental *Coleus* root. Note lack of meristematic activity in root apex, accompanied by swelling of apex, disintegration of some portions, maturation of vascular tissues into apical region, and origin of branch root from pericycle close to apical region. (A) $90\times$; (B) $366\times$.

of experimental cuttings was less than that of corresponding control cuttings (Tables I, II): total root length of 63 cuttings exposed to magnetic fields averaged 42% less than total root length of 63 control cuttings at the termination of the series of experiments.

Roots of experimental *Coleus* cuttings exhibited anomalous anatomy comparable to that found in *Allium* and *Narcissus* roots exposed to magnetic fields. Root tips swelled in diameter, apical initials ceased mitotic activity, vascular tissues matured into the apical region, portions of the root tip disintegrated, and branch roots often arose within or close to the apical region (Fig. 18). Lignosuberization was evident in hypodermal cells. In control material which was studied, roots and root tips exhibited normal anatomy and mitotic activity.

In addition to anomalous root development, the lower nodes of the stems of the cuttings exposed to magnetic fields exhibited an unusually well-developed endodermis with caspary strips just exterior to the phloem fibers of the vascular bundles. In contrast, stems of control cuttings had a much less prominent endodermis with poorer development of caspary strips.

DISCUSSION

Exposure of plant material to magnetic fields well in excess of the earth's ca 0.5 G results in a variety of responses in different organisms, cells, and tissues. We believe that there is a consistent pattern whereby apparently various behavior is really a manifestation of the same basic responses of living material limited by the genome of the organism. We can expect no more than the capability resident within an organism, unless that capability be changed, as by mutation.

Alteration of the normal course of development results from alteration of the normal external and/or internal environment of the cell or organism. We feel that maturity is reached earlier in cells exposed to magnetic fields because protoplasts omit or telescope metabolic activities, and that this maturity may be expressed in unusual or bizarre forms of development. However, a cell will not deposit excessive lignin unless the capability for lignin deposition already exists in the genome of the cell.

The state of dormancy and senescence, decrease in elongation, and advanced type of wall sculpture seen in protoxylem are evidence of physiological aging and the attainment of an earlier maturity. Cell activity is affected at the molecular and submolecular level in a cumulative fashion over a period of days and weeks. Change is gradual and usually leads to

premature development, dormancy, senescence, and sometimes death. In roots of *Narcissus* and *Allium*, recovery may be effected by regeneration of a new root from the quiescent center (group of nonmeristematic cells surrounded by active meristematic cells and possessing capability for resuming meristematic activity) of the apical meristem in some instances, and by branch root formation from the pericycle in others. Similar regeneration from the quiescent center has been observed in X-irradiated roots of *Zea mays* and *Vicia faba* [Clowes (1963)], and it has been suggested that the quiescent center is less sensitive to X rays because its average rate of mitosis is much lower than that of the surrounding meristematic cells. The quiescent center and pericycle are probably less sensitive to magnetic fields for this same reason, since exposure to magnetic fields inhibits and gradually causes the cessation of cell reproduction in actively meristematic tissue.

Electric currents have also been shown to affect development of roots. *Allium* roots subjected to continuous applied electric currents of low intensities [Berry *et al.* (1947)] exhibited behavior similar to that of *Allium* roots exposed to magnetic fields: currents of relatively low densities promoted excessive formation of lateral roots, while currents of higher densities caused local swellings, twisting, and doubling, accompanied by disorganization of the vascular tissues, extreme vacuolization, and enlargement of cortical cells.

In *Coleus* cuttings exposed to magnetic fields of 2000–4000 Oe, we found a decrease in the number and length of adventitious roots, accompanied by anomalous anatomical development comparable to that of *Allium* and *Narcissus* roots: swelling of root tips, disintegration of some tissues, maturation of the stele to the region of root initials, and, frequently, a more profuse formation of branch roots, some of which arose near or in the apical region. As in *Allium* and *Narcissus*, this behavior suggests premature attainment of maturity and senescence.

Pithophora, a simple, filamentous, nonvascular plant, also exhibited evidence of early maturity when exposed to magnetic fields of 800–1500 Oe. Changes in growth pattern of experimental filaments included contortions, swellings, and constrictions, accompanied by early formation of an unusually large number of akinetes. Since akinetes are vegetative cells modified as thick-walled resting spores and are generally formed to enable the alga to survive a period when conditions are unfavorable for growth, the premature formation of large numbers of akinetes in magnetic fields suggests that the magnetic field generates an environment unfavorable for growth of *Pithophora* and thus hastens maturation and senescence in this alga.

While results of our experiments indicate that strong magnetic fields

inhibit growth and induce early maturation and senescence in plants, other investigations have produced apparently contradictory results. Mericle *et al.* (1964) exposed germinating grains and seedlings of barley to a magnetic field of 1200 Oe and found that the growth rate of both root and shoot was stimulated. However, all of these experiments were terminated after only 8 days, so it is difficult to compare the results with those of our experiments, in which effects of exposure to the magnetic field were not readily apparent until 14 days or later. Dycus and Shultz (1964) found a similar increase in root growth of *Coleus* cuttings exposed to magnetic fields of 2000 Oe, in contrast to our results with *Coleus* roots. However, they germinated seeds of 20 different species of plants in magnetic fields ranging from 0 to 6000 Oe and found that seeds in the strong magnetic field generally were slower to germinate and did not maintain the rate of growth shown by the plants in the geomagnetic and nulled fields.

Audus and Whish (1964) reported a definite magnetotropism (growth curvature of a plant organ down the magnetic gradient) in roots, coleoptiles, and hypocotyls of a number of vascular plants, and in sporangiophores of a fungus. In our experiments we noted no obvious orientation of roots or algal filaments in the magnetic field. However, we have not made comparable studies of the growth of individual roots and filaments.

SUMMARY AND CONCLUSIONS

The results of these studies demonstrate that prolonged exposure to heterogeneous magnetic fields of 500–4500 Oe may produce the following responses in adventitious roots of *Allium*, *Narcissus*, and *Coleus*: inhibition of cell reproduction followed by death of embryonic tissue and accompanied by senescence of all root tissues; and anomalous development of certain cells and tissues, including nuclear, cytoplasmic, and cell wall abnormalities, with omission of some normal developmental cell types.

Pithophora, a much less complex organism, necessarily showed much less complex effects of exposure to magnetic fields. However, prolonged exposure to heterogeneous magnetic fields of 800–1500 Oe may induce the following behavior in *Pithophora*: reduced and markedly abnormal growth of filaments, accompanied by premature and excessive formation of akinetes.

We conclude that magnetic fields of a strength well in excess of the geomagnetic field generated an environment unfavorable to growth and normal development, and promotive of early maturation and senescence of cells, tissues, and organisms.

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BIOMAGNETIC HYPOTHESES

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RULES OF THUMB FOR STANDARD MAGNETOACTIVE DIFFUSION

In the present theory⁽¹⁾ we assume that the growth process of cells is related to the diffusion mechanism of dissociated salts as, for instance, across the plasma membrane from the extracellular domain to and from the cytoplasm, or through the nuclear membrane between the nucleus and the cytoplasm.

Two simple rules of thumb appear to rule out the possibility that a magnetic field can influence the state of a cellular system through a mechanism interfering with the ionic diffusion process. Magnetic fields interact with charges in motion. The orbit of a charged particle in the presence of a steady magnetic field is helical. The radius of the circular part of the helix is called the Larmor radius R . For a singly ionized particle of mass 30 amu, at a temperature of 300°K

$$B \simeq 90/R \text{ (cgs)} \quad (1)$$

The first rule of thumb is that in order for a magnetic field to influence a system whose spatial extent is of order L cm, R must be of the order of L . For typical cells $L \simeq 10^{-4}$ cm, so that B must be of the order of 10^6 G to produce an effect.

Another rule of thumb is obtained in the following manner. Suppose a charged particle is moving in a viscous medium. Phenomenologically one may say that the particle is suffering ν collisions per second. If a magnetic field permeates the medium, then the charged particle moves in a helical orbit, with Larmor radius R —as discussed above—and with Larmor frequency Ω . In order for the magnetic field to produce an effect, the particle must execute at least one Larmor orbit before colliding, i.e., $\Omega > \nu$. For a particle of mass m and charge e , which is moving in a medium characterized

by a mobility coefficient μ [$\sim 10^{-3}$ cm/sec at 1 V/cm and $\nu \sim 10^{11}$ /sec for typical electrolytes⁽²⁾], and through which a magnetic field B permeates

$$\Omega/\nu = \mu B/c \text{ (cgs)} \quad (2)$$

where c is the speed of light. It follows that for B to produce an effect in such a medium

$$\Omega/\nu \simeq B \times 10^{-13} \gtrsim 1 \quad (3)$$

or B must be of the order of 10^{13} G.

In view of these severe criteria, one might well suppose that only the most gigantic of magnetic fields could influence the growth dynamics of a cell—via the mechanism of interfering with the ionic diffusion processes.

MAGNETOACTIVE DIFFUSION ACROSS THE CELL MEMBRANE

However, an encouraging turn of events occurs if one brings into play the effects of a potential difference which is known to exist across the cell membrane of many cells.⁽³⁾ To facilitate calculation we assume the cell to be in the shape of a cylinder (see Fig. 1). The electric field E , within the membrane, is given by

$$\mathbf{E} = 2\pi en(r - r_0)\hat{\mathbf{r}} \quad (4)$$

In this formula, the unit vector $\hat{\mathbf{r}} = \mathbf{r}/r$, and n is the density. Suppose one

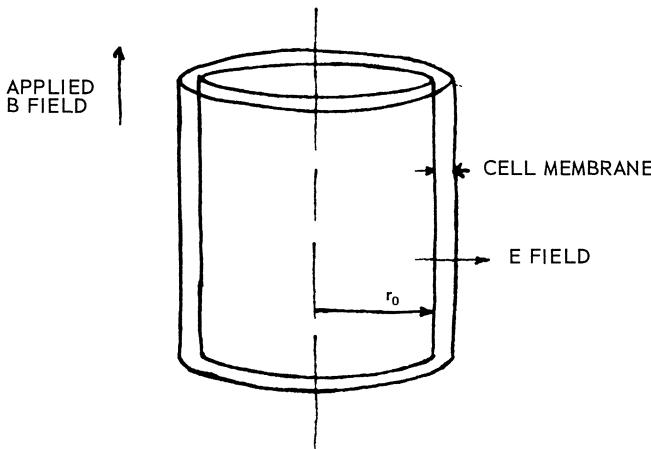


Fig. 1. Orientation of a cell in a magnetic field.

now superimposes a steady magnetic field \mathbf{B} which is parallel to the axis of symmetry of the cylinder (see Fig. 1), so that \mathbf{B} is normal to \mathbf{E} ($\mathbf{E} \cdot \mathbf{B} = 0$).

The problem we wish to consider now is the following: ions are diffusing across the cell membrane. The mean square (radial) displacement of an ion with the B field turned off is $\langle r^2 \rangle_E$. With the B field turned on it is $\langle r^2 \rangle_{EB}$. The effect of the magnetic field on the diffusion of ions across the membrane is then given by the ratio

$$\Delta = \langle r^2 \rangle_{EB} / \langle r^2 \rangle_E \quad (5)$$

For the above geometry, this expression becomes⁽⁴⁾

$$\Delta = [1 + (\Omega/\nu)^2] \exp\{-(\omega_p/\nu)^2(\Omega/\nu)^2\nu t/[1 + (\Omega/\nu)^2]\} \quad (6)$$

In this formula t is the time, and ω_p is the frequency

$$\omega_p^2 = 4\pi e^2 n/m \quad (7)$$

The charge-to-mass ratio e/m is that of the diffusing particle. The collision frequency ν is related to the mobility μ through

$$\mu\nu = e/m \quad (8)$$

Equation (3) indicates that $(\Omega/\nu)^2 \ll 1$, whence Eq. (6) reduces to

$$\Delta \simeq \exp\{-(\omega_p/\nu)^2(\Omega/\nu)^2\nu t\} \quad (9)$$

For a voltage difference of $\sim 10^{-4}$ stat-volt and $B = 10^5$ G, this equation [together with the aid of Eqs. (4), (6)–(8)] becomes

$$\Delta \simeq \exp\{-10^{-3}\nu t\} \quad (10)$$

We conclude that in the time $\nu t \simeq 10^3$ (i.e., after 10^3 collisions) $\langle r \rangle_E$ is reduced by the factor e^{-1} due to presence of a magnetic field of the order of 10^5 G.

The charge density necessary to produce the desired effect is obtained from the following expression for the potential V , which follows from Eq. (4)

$$V = \pi n e (r - r_0)^2 \quad (11)$$

This, together with the data that

$$V(r = r_0 + 10^{-6}) \simeq 10^{-4} \text{ stat-volt}$$

gives

$$n \simeq 10^{18} \text{ cm}^{-3}$$

which is slightly less than the density of free electrons of typical conducting media.

MAGNETIC FORCE ON A ROOT

Recent experimental evidence^(5,6) seems to indicate that, when placed in a uniform magnetic field, the roots of certain plants tend to reorient themselves with respect to the magnetic field. To explain this effect, we suppose the root carries a uniform solenoidal-like current I (see Fig. 2). Then, for a root of cross-sectional area A , a magnetic moment M is produced

$$M = IA \quad (12)$$

If the root is in the presence of a steady magnetic field, then a torque T will be exerted on the root which would tend to align it with the magnetic field. This torque is (to within a factor of the order of unity)

$$T \simeq MB = \frac{IAB}{10} \quad (13)$$

In this relation I is in amperes. Otherwise values are in cgs units. If we choose $T \simeq 10^{-5}$ dyne-cm as a typical value which might perturb the growth direction of a root, and $A \simeq 10^{-2}$, then Eq. (13) becomes $IB = 10^{-2}$. For a magnetic field of the order of 10^4 G, a surface current of 10^{-6} amp. ($1 \mu\text{A}$) would produce the desired effect—for the said model.

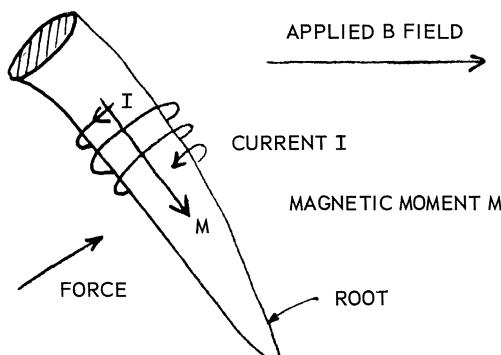


Fig. 2. Magnetic force on a root.

ACKNOWLEDGMENTS

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THE EFFECT OF HIGH-GRADIENT, HIGH-STRENGTH MAGNETIC FIELDS ON THE EARLY EMBRYONIC DEVELOPMENT OF FROGS

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INTRODUCTION

Data on the biological effect of static magnetic fields are presented in Volume 1 of the present series⁽¹⁾ and by further listings.⁽²⁻⁴⁾ They have failed to provide either specific explanations of particular results or any overall explanation of why magnetic fields in general or particular field strengths or field gradients should influence biological systems. Above all, no experimentally verified predictions of the effects of a given field on a given biochemical reaction, cellular process, or development of a given organism have been made. The most recent biophysics literature simply adds further contradictory evidence. For instance, Rabinovitch *et al.*⁽⁵⁾ show that even extremely high fields have no effect on certain basic enzymatic reactions while Levengood⁽⁶⁾ shows statistically significant results of much lower* fields than 20,000 G. Motivated by a review of this literature, we attempted to devise a critical experiment to test a hypothesis that would be applicable to some of the best of the reported experiments, but evidence for which would be enhanced by the choice of our conditions. We would like to report the results of this attempt, which on the experimental side proved to be rather striking and positive but in terms of the initial hypothesis left more questions than answers.

We postulated that there are cellular components in organisms which

* His claim that the effective field strength is higher, is in error. The end of an iron rod in a $\frac{1}{2}$ -W solenoid would not reach saturation. The measured field of 150 G probably more nearly represents the actual field.

are relatively large in volume and have a paramagnetic susceptibility larger than the rest of the tissue. Such cellular components would experience a force in a gradient magnetic field great enough to move them through the cell and to maintain a concentration gradient within the cell. This force would exist for as long as the cells continued to be exposed to a magnetic field. Such a cellular component might be a particle of ferritin. Ferritin, a protein complex of iron, containing as much as 20% by weight of the metal is relatively paramagnetic and plays an important function in plants and animals. Iron in both is needed for vital pigments and for respiratory or photosynthetic functions. In animals, the iron is used to make the hemoglobin incorporated in red cells and ferritin is the molecule used for iron storage in the animal. In order to test the effect of a magnetic field on ferritin, it seemed desirable to use a sensitive biological indicator. Early embryonic growth, during which ferritin is used to make the initial supply of hemoglobin of the animal, is such a sensitive indicator. It was therefore decided to check the early embryonic growth of the leopard frog (*Rana pipiens*) in a high magnetic field with a high gradient. Because any change in the embryonic development becomes more noticeable during the course of further differentiation, small effects on development and growth can thus easily be observed. At the same time we planned to check on the connection between any observed abnormalities and the postulated relative motion of ferritin within the embryo. The experiments therefore included a search for such particles and a check on the iron distribution in eggs and embryos. The experimental procedures and results will be described below. It should be pointed out at the start that even though the embryonic development was affected in a highly significant manner, the ferritin hypothesis has not been substantiated. In the light of these results an alternate hypothesis and further experiments are suggested to shed more light on these results. The high degree of their significance and the relative ease with which they could be repeated suggest the desirability of providing a firm theoretical basis for these observations.

EXPERIMENTAL PROCEDURES

The experiment was planned to allow the early embryonic development from fertilization to hatching of the eggs of the leopard frog (*Rana pipiens*) to proceed in the presence of a very high field with a very high gradient under extremely closely controlled conditions. These conditions are:

1. To keep temperature difference between the exposed samples and the controls to below 0.1°C;

2. To have a similar exposure to ambient light;
3. To maintain all embryos in the same nutrient fluid; and
4. To have all embryos genetically similar.

To provide a high magnetic field with a high gradient at reasonable cost can only be done by restricting the volume in which the field and the gradient are high. This necessitated the design of a holder which would place the developing eggs in this restricted portion of the magnetic field and which would furthermore physically fit into the magnet while allowing the necessary space for controlling the temperature. A holder made of inert material is also required. Stainless steel, in particular, proved to be quite unacceptable. Frogs' eggs in contact with stainless steel do not develop and we had to switch to a teflon holder. The resulting design of the whole system is shown in the first three figures. Figure 1 shows one of the pair of holders with 14 compartments, into 13 of which are placed the freshly fertilized frogs from one female. Samples and controls are put in their holder shortly after fertilization, as soon as the animal-vegetal axis has rotated into the vertical direction. One space is left unoccupied since the thermistor which monitors the temperature control is placed in that compartment. The fact that the egg assumes a particular orientation with respect to the gravitational field is made use of in the plan of the experiment. It allows the axis of the magnetic field and the direction or gradient of the magnetic field to be applied in a fixed relative orientation to the egg in all eggs. As even the hatched embryos spend most of their time with their

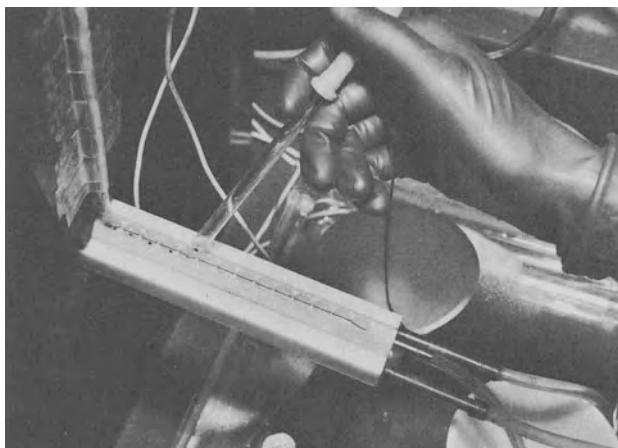


Fig. 1. Frog eggs being inserted into temperature controlled Teflon sample holder.

vertical axes vertical, our field has a relatively fixed orientation with respect to the animal throughout its exposure: that is, the direction of the magnetic field (vertical) and the direction of the gradient (pointing downward) remain during the entire experiment parallel to the biological axis. Thirteen eggs are placed in the sample holder and 13 in a similar control holder selected at random from a single fertilized batch at one time. The sample holder is then placed between the pole caps of the permanent magnet (Fig. 2) which was designed for this work and the control holder in an aluminum dummy. The permanent magnet Alnico V pole pieces and soft iron pole caps can be seen in Fig. 2.

The nutrient fluid, bottled spring water, is circulated through the compartments containing the eggs. The black string which can be seen in Fig. 1 acts as a wick to keep the fluid moving through the holder without a meniscus rising to the point where the fluid would overflow. This arrangement was essential to maintain a water-air interface for the development of these eggs as they usually would in fingerbowls. Without circulation the eggs did not develop because of the small volume of fluid around each egg.

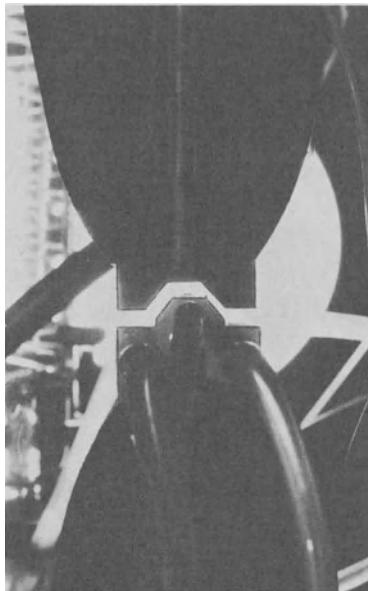


Fig. 2. Holder between shaped pole pieces.
Apparent asymmetry is due to camera angle.
Meniscus of water is just visible.

The smallness of the sample chambers was due to the restricted space at which a magnetic field of sufficient strength was available at a reasonable cost. More than 2000 cc of fluid was circulated by a two-tube peristaltic pump⁽⁷⁾ at a rate of about 500 cc/hr through each holder. The flow through both was regulated by a hose clamp to be equal, and the exit fluid from both was collected in the common reservoir from which it was recirculated through both holders. In that way the same nutrient fluid was passed through both sets of embryos (Fig. 3). The temperature regulation presented a challenge because of the poor thermal conductivity of the inert teflon in the holders. Three steps were found to be necessary to hold the temperature of the samples and the controls to within 0.1° of each other. The nutrient fluid first passed through a thermostatically controlled glass heat exchanger. Water from the heat exchanger was also passed through passages in both holders. Finally the room in which the experiments were performed was temperature controlled to $\pm 0.5^{\circ}$. The high precision ($\pm 0.1^{\circ}\text{C}$) thermistors to monitor both holders were repeatedly compared to ensure the validity of the temperature recordings.

The embryos were checked daily and any that had clearly stopped developing or had reached Shumway Stage 25, i.e., had hatched, were removed from their compartments. Initially it had been planned to put all eggs into the holder and not remove them from the magnetic field until hatching. However, when it was found that an appreciable number died, the problem of disintegrating eggs damaging the other eggs in the holder

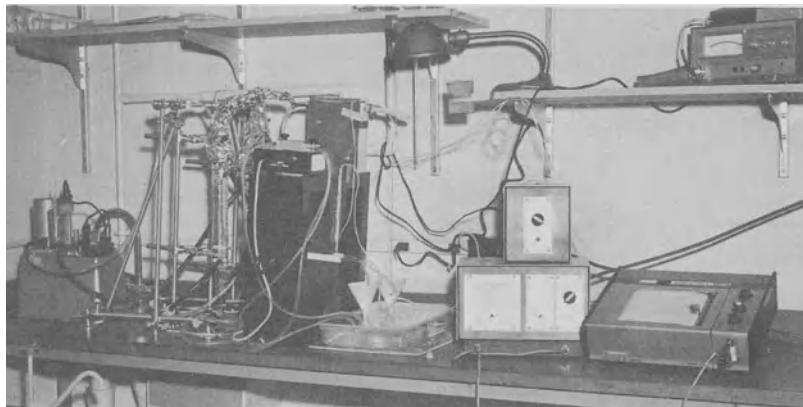


Fig. 3. Overall view of magnet with the sample holder between the pole, temperature control, and nutrient circulation system. On the top of the magnet frame we see in the foreground the peristaltic pump and in the back the aluminum block with the control sample holder.

was solved by removal. After the eggs had been removed they were either allowed to develop further in the finger bowls under continued observation or fixed with fixative or frozen in liquid nitrogen for later histological or chemical observation.

The magnetic field and its direction at the position of the embryo was measured with a Bell Hall-effect gaussmeter to confirm the manufacturer's specifications. The magnitude of the field at the approximate position of the embryo was approximately 10^4 Oe and its gradient in the vertical Z direction

$$\frac{dH}{dZ} = 8.35 \times 10^3 \text{ Oe/cm}$$

Therefore,

$$H \times \frac{dH}{dZ} = 8.35 \times 10^7 \text{ Oe}^2/\text{cm}$$

at the position of the developing embryo in the sample holder, compared to less than $1 \text{ Oe}^2/\text{cm}$ in the controls. This is caused by the earth's field of about 1 Oe and a field gradient of less than 1 Oe/cm. At a large distance from the magnet it would be expected that this product would be smaller by several more orders of magnitude, but, because of the temperature control and fluid circulation system, it was not considered worthwhile to remove the controls further. The product $(dH/dZ)H$ of the controls is nearly 10^8 times smaller than at the sample. Twenty-nine runs, each starting with 13 controls and 13 samples, were made during which control eggs hatched. Runs of this kind can only be made during the Spring of the year; even with profuse injection of pituitary *Rana pipiens* cannot be induced to ovulate with any reasonable chance of success during the Summer or Fall, unless colonies of frogs are kept under temperature-controlled conditions.

TABLE I

	Hatched	Died	Total
Samples	119	258	377
Controls	195	182	377
	314	440	754

$$\chi^2 = \frac{(119 - 157)^2}{157} + \frac{(195 - 157)^2}{157} + \frac{(258 - 220)^2}{220} + \frac{(182 - 220)^2}{220} = 31.4$$

with one degree of freedom. Therefore the significance of treatment exceeds the 0.1% level.

RESULTS

The twenty-nine successful runs contained 377 sample eggs and 377 control eggs. One hundred nineteen of the former and 195 of the latter hatched; most of those that did not, stopped developing after gastrulation. When these results are plotted in a 2×2 contingency table (Table I), χ^2 for these results is 31.4 with 1 degree of freedom. This is highly significant,

TABLE II

Run No.	Date	Number which hatched	
		Samples	Controls
1	01/21/67	1	4
2	01/27/67	3	6
3	03/08/67	2	7
4	03/17/67	10	9
5	03/21/67	4	11
6	03/31/67	6	6
7	04/03/67	3	8
8	04/07/67	1	7
9	04/10/67	0	5
10	04/14/67	10	8
11	04/24/67	9	9
12	04/27/67	2	6
13	05/01/67	4	2
14	05/09/67	3	13
15	05/12/67	12	12
16	05/16/67	0	3
17	05/19/67	0	7
18	05/23/67	4	12
19	12/30/67	3	9
20	01/19/68	0	2
21	01/26/68	5	8
22	02/09/68	2	4
23	02/13/68	3	6
24	02/19/68	12	11
25	02/21/68	7	6
26	03/04/68	0	2
27	03/13/68	7	5
28	03/18/68	6	7
29	03/25/68	0	2
Total		119	196

far exceeding the 0.1% level. It is therefore concluded that exposure to the magnetic field prevented the normal embryonic development of *Rana pipiens*. The detailed results of the twenty-nine runs are shown in Table II. They indicate that the effect may be more pronounced when a smaller number of eggs hatch.

INVESTIGATION OF THE FERRITIN HYPOTHESIS

As was pointed out in the introduction, the first hypothesis we had in mind when designing the experiment postulated the existence of ferritin aggregates. If such aggregates have a radius r and a magnetic susceptibility relative to the rest of the tissue, ΔK (cgs units), they will experience a force

$$F = H \left(\frac{dH}{dz} \right) \Delta K \text{ dynes/cm}^3$$

The concentration gradient for particles of volume V is given by

$$n/n_0 = \exp(FVz/kT)$$

The velocity v of a sphere in a fluid of viscosity η is given by

$$v = FV/6\pi\eta r$$

(see detailed discussion in Reference 8). We find that for

$$r^2 \Delta K > 10^{-15}$$

(r in centimeters) the velocity of the ferritin aggregate in our field

$$H \frac{dH}{dz} \sim 10^8 \text{ Oe}^2/\text{cm}$$

will be 300μ per day. The equilibrium concentration gradient will exceed 1000:1 across a 1-mm distance, even against thermal motion at 20°C . The product $r^2 \Delta K$ would, for instance, be larger than 10^{-15} if its properties correspond to the pairs of values given in Table III. The ferritin hypothesis then postulates that the motion of these ferritin particles will somehow interfere with the formation of hemoglobin in the developing embryo. The requirement of about $1 \mu\text{g}$ of iron is supplied in each egg in the form of ferritin and as shown by Brown and Caston⁽⁹⁾ converted to hemoglobin iron just before hatching. To prove our postulate it would be necessary to

TABLE III
Pairs of Values for Spherical Ferritin Aggregates for Which $r^2 \Delta K > 10^{-15}$

ΔK , cgs units	r , cm
10^{-4}	0.03×10^{-4}
10^{-5}	0.1×10^{-4}
10^{-6}	0.3×10^{-4}

know the magnetic susceptibility and size of the ferritin particles in the frog egg and to determine that they are displaced within the egg or within certain cells of the embryo and thus cause cessation of development. In the absence of direct measurement of the magnetic susceptibility of these particles any of the values given in Table III appear possible from a knowledge of the properties of ferritin suspensions. We therefore concentrated on attempts to (a) locate the ferritin exactly; (b) determine the size of the aggregates; and (c) determine any preferential distribution of iron before or after magnetic field exposure. These attempts have not been successful. We have not been able to locate particles with the light or electron microscope or with the electron microprobe, and we were not able to determine chemically any nonrandom distribution of iron in different quarters of the embryos. Our inability to make these observations does not prove the absence of such particles, but suggests that ferritin may occur as separately suspended or dissolved molecules. In that case, $r^2 \Delta K$ would be much smaller than 10^{-15} and even a much higher product of magnetic field strength and gradient would be insufficient to produce a significant displacement of such ferritin molecules.

DISCUSSION

The experimental results present clear-cut evidence for the existence of the inhibiting effects of the product of a high-strength magnetic field and gradient on the early development of frog embryos. The hypothesis that these results are due to forces on ferritin particles are unsupported by direct evidence which we sought. The experiments by J. M. Barnothy⁽¹⁰⁾ and M. F. Barnothy⁽¹¹⁾ on mice might however be taken as indirect supporting evidence. They could be interpreted as showing a response of the white cell formation to gradient magnetic fields. The formation of white cells and

the formation of red cells may possibly be observable as a response in the white blood count. The formation of the white cells is related to resistance to X-ray radiation exposure. On the other hand, effects at low fields or experiments such as those of Levengood cannot in our mind be related to ferritin displacement in a high gradient field.

We therefore feel that another hypothesis of a more general nature might be required. It is needed to stimulate further critical experiments. Perhaps a particle, be it a molecule or a cellular structure, exists which is common and has a magnetic moment. This might be the highly oriented mitotic spindle structure of a cell, components of it, or a macromolecule. Such a particle with a magnetic moment might be formed only momentarily at particular cellular events, and the interaction of this magnetic moment with a magnetic field would provide the energy or orientational difference producing the biomagnetic effect. These events would require some common orientation with respect to each other in the cell or the organism in order to have a sufficient net magnetic moment that is not randomized by the thermal motion of individual molecules. It would therefore have to be intimately related to the structure within the cell.

Postulating such a biological entity with a magnetic moment may seem rather far-fetched. Further investigation of the relatively simple and standard system of frog development may clarify the need for such radical hypotheses. Ferritin particles might be found by more powerful methods as they become available, such as high-resolution electron microprobe techniques or mass spectrometry methods. With those one should be able to detect picograms of iron in small sections of the frog embryo.

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MUTAGENIC EFFECTS OF MAGNETIC FIELDS ON *DROSOPHILA MELANOGASTER*

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There is evidence [Cox (1964); Harrison (1966); and Dalrymple (1968)] of several complete reversals of the earth's magnetic field. The most recent and best documented event is the Olduvai which occurred about 2.0 to 1.9×10^6 years ago. The change in polarity is recorded in lava flows from Olduvai Gorge, Tanzania, Victoria, Australia, and Cocos Island in the eastern Pacific Ocean. This suggests that organisms existing today have evolved under reversing magnetic field orientations. This reversing process should produce a null magnetic field for the earth for a period of time. Current evidence indicates that the earth has had its field reduced nearly 30% in the last 100 years. Due to the ever increasing possibility that man will be exposed to magnetic fields other than that of the earth, it becomes important to ascertain if those alterations may induce genetic changes. Obvious areas of differing exposures are associated with space travel. These areas may involve both null and high intensity fields. Radar, cyclotrons, etc., which have magnets as component parts or as shielding devices, are other hazard areas of our modern era.

A brief review of the literature reveals the following. Brown (1960, 1962, 1963, 1964, 1966) found that small changes in magnetic fields influence organisms. Audus (1960, 1964) demonstrated magnetotropism of plants. Considerable work has been done on enzymatic activity with various magnetic field strengths [Smith (1966); Conley (1966); Mohr (1967)].

Lavengood (1967) concluded that *Drosophila melanogaster* pupa subjected to magnetic fields exhibited morphogenetic anomalies which were transmitted for 30 generations. He suggested a cytoplasmic type of inheritance associated with an enzymatic inhibition effecting development. Baranthy (1956, 1964), Gross (1962, 1964), and Dunlop (1964, 1965) demon-

strated an apparent effect on the division of cells. Beischer (1964) and Mulay (1964) reported no genetic effect on organisms by alterations of a magnetic field, but Chevais (1942) stated that mutations occur after the exposure of *Drosophila melanogaster* eggs to magnetic fields. With this controversy relative to biomagnetic responses, it is imperative that investigations of the mutagenic effects of these fields be carried on.

Levengood also indicated a recovery or healing effect in his study of *Drosophila melanogaster* as influenced by magnetic fields. The transmission of the magnetic effect through 30 generations clearly indicates some change in the biotic material. He recovered no standard Mendelian ratios, but this could be due to varying degrees of DNA fibril change in the spermatocytes and the subsequent healing effect. The suggestion of cytoplasmic inheritance would not appear to be appropriate as this type of trait is transmitted primarily through the female not through treated males, as his work demonstrates. It is possible that a combination nuclear change (mutation) and altered cytoplasm could result in the morphological changes observed. Dilution and redistribution of the altered cytoplasm into individuals with the appropriate nuclear composition could result in a reduction in expression of the trait which would be demonstrated after many generations.

For my investigation, *Drosophila melanogaster* was selected as the test organism, as there is a vast quantity of valid information concerning its genetic composition. The primary information reported is on sex ratios and the occurrence and transmission of new mutants.

Two sources of *Drosophila melanogaster* (wild stock) were utilized: Turtox and Carolina Biological—CBS. A culture with the sex-linked traits, yellow body, white eyes, and miniature wings (CBS), was also included in the study.

The first study utilized subcultures of the CBS stock. Permanent U-shaped magnets were fastened together in an adjustable aluminum frame, allowing for changes in the distance between pole pieces and a variety of field intensities per location. These magnets were arranged as illustrated in Fig. 1.

The two North poles were opposing each other, with the two South poles arranged the same way. The field strength occupying the central space between like poles (S-S) (N-N) was 0 Oe (positions *A* and *F*). There was a gradient toward the center (positions *C* and *D*). Since a gradient also exists toward the sides of the tubes (those closest to the pole pieces), the field strengths indicated are for the center of the culture tubes. The maximum field strength (as determined by a Hall-effect gaussmeter) at this point (positions *C* and *D*) was approximately 500 Oe. Positions *B* and *E* had

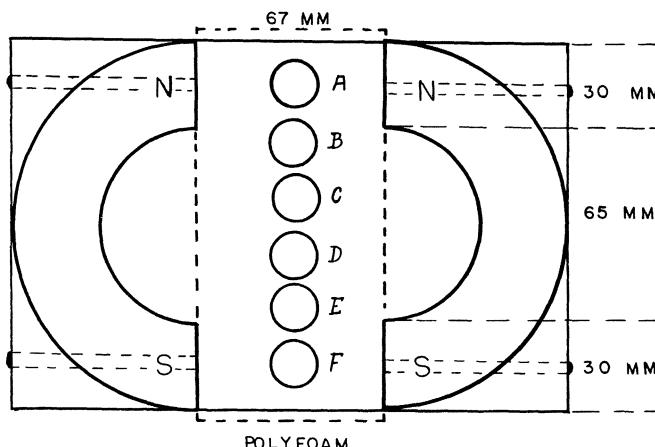


Fig. 1. Arrangement of magnets.

field strengths of 320 Oe. Within a restricted space, this magnetic setup produces both a moderate magnetic field [(520 Oe) of low gradient (*C* and *D*), 20 Oe/cm gradient in the direction of (*A* and *F*) and a lower field [(0 Oe) in the center with an average 100 Oe strength (*A* and *F*) with a high gradient of 300 Oe/cm to their respective poles].

A medium prepared with oatmeal agar, karo, and a mold inhibitor was used for all cultures. Five virgin female flies were placed in separate shell vials containing medium. The virgin male flies were treated identically. These vials were 12 mm in diameter and 45 mm long. The magnet pole diameter was 60 mm, allowing for complete tube exposure. All tubes were enclosed between two polyfoam strips and secured by rubber bands. These were placed in the magnetic field, and the tubes were arranged in selected positions. For the control flies, the enclosed tubes were placed in the same type of cubical, but without the magnets. Since all the culture tubes were completely surrounded by the polyfoam, no dummy magnets were needed for the controls. With this setup, any difference in the treatments and the controls (those at a normal geomagnetic background) must be attributed to the superimposed magnetic fields. Vials containing males or females were placed in position *A* (by flip of coin) with the opposite sex occupying the like position *F*. All positions within the magnetic field were treated in the same manner.

In this first reported study (Table I), all flies (CBS stock) were exposed to indicated fields for a period of 24 hr. Following this procedure, male and female flies from fields of like intensity were mated for 3 hr as individual

TABLE I

Comparison for Three Generations of Flies Subjected to Four Different Field Intensities for 24 hr Prior to Mating—Treatment A

Generation	$\Sigma \delta$	$\Sigma \varphi$	$\Sigma \Sigma \delta + \varphi$	$\Sigma \chi^2$	Pooled χ^2	Heterogeneity
0- to 100-Oe Field (high gradient)						
F ₁	81	79	160	1.3632	0.0250	N.S.
F ₂	1049	1013	2062	1.9974	0.6284	N.S.
F ₃	874	773	1647	11.0486	6.1936*	N.S.
Σ	2004	1865	3869		6.8470*	N.S.
$\text{Pooled } \chi^2 = 4.9936^*$						
320-Oe Field (low gradient)						
F ₁	78	86	164	3.6764	0.3902	N.S.
F ₂	1365 (917)	1338 (967)	2703 (1884)	9.0984* (1.8542)	0.2696 (1.3268)	* (N.S.)
F ₃	476	471	947	0.7374	0.0262	N.S.
Σ	1919	1895	3814		0.6860	N.S.
$\text{Pooled } \chi^2 = 0.1510$						
520-Oe Field (low gradient)						
F ₁	132	99	231	10.1038	4.7142*	N.S.
F ₂	1619	1544	3163	3.7488	1.7782	N.S.
F ₃	557	484	1041	7.7618	5.1190*	N.S.
Σ	2308	2127	4435		11.6114*	N.S.
$\text{Pooled } \chi^2 = 7.386^{\dagger}$						
Geomagnetic background						
F ₁	113	130	243	2.9420	1.1892	N.S.
F ₂	1238	1200	2438	9.6726	0.5922	N.S.
F ₃	222	217	439	4.6148	0.0568	N.S.
Σ	1573	1547	3120		1.8382	N.S.
$\text{Pooled } \chi^2 = 0.2166$						

* Significant, 5%.

† Highly significant, 1%.

TABLE II
**Comparison for Three Generations of Flies Subjected to Four Different Field
 Intensities for 72 hr Prior to Mating—Treatment B**

Generation	$\Sigma \delta$	$\Sigma \varphi$	$\Sigma \Sigma \delta + \varphi$	$\Sigma \chi^2$	Pooled χ^2	Heterogeneity
0- to 100-Oe field (high gradient)						
F ₁	351	291	642	4.5812	5.6074*	N.S.
F ₂	611	413	1024	67.3992†	38.2850†	†
	(361)	(307)	(668)	(9.1522)	(4.3652)*	(N.S.)
F ₃	436	396	832	13.0428†	1.9230	†
	(381)	(365)	(746)	(6.3452)	(0.3430)	(N.S.)
Σ	1398	1100	2498		45.8154†	†
					Pooled $\chi^2 = 17.7750^\dagger$	
(Σ)	(1093)	(963)	(2056)		(10.3158)*	
					Pooled $\chi^2 = 8.2198^\dagger$	
400-Oe field (low gradient)						
F ₁	230	241	471	5.644	0.2568	N.S.
F ₂	508	325	833	81.4976	40.2028†	†
	(337)	(278)	(615)	(10.9656)	(5.6600)*	(N.S.)
F ₃	623	523	1146	15.0386	8.7260†	N.S.
Σ	1361	1089	2450		49.1856†	†
					Pooled $\chi^2 = 30.1974^\dagger$	
(Σ)	(1190)	(1042)	(2232)		(14.6428)†	(N.S.)
					Pooled $\chi^2 = (9.8136)^\dagger$	
540-Oe field (low gradient)						
F ₁	87	70	157	1.8406	1.8406	N.S.
F ₂	109	100	209	1.9542	0.3874	N.S.
F ₃	241	203	444	5.6852	3.4802	N.S.
Σ	437	373	810		5.4802	N.S.
					Pooled $\chi^2 = 5.0566^*$	
Geomagnetic Background						
F ₁	62	67	129	1.7544	0.1936	N.S.
F ₂	191	170	361	3.3110	1.2216	N.S.
F ₃	100	74	174	8.7868*	3.8850*	*
	(57)	(54)	(111)	(1.0588)	(1.0588)	(N.S.)
Σ	353	311	664		5.3002	
					Pooled $\chi^2 = 2.6566$	
(Σ)	(310)	(291)	(601)		Pooled $\chi^2 = (0.6006)$	

* Significant, 5%.

† Highly significant, 1%.

TABLE III
Results of Null Magnetic Field (Less Than 0.05 Oe) on Transmission of Sex Ratio

	Total ♂	Total ♀	Total Flies	χ^2	χ^2	χ^2	$\Sigma \chi^2$	Pooled χ^2	Heterogeneity
F_1	1A	22	29	51			0.9606	0.9606	N.S.
	2B	20	21	41			0.0242	0.0242	N.S.
	Σ	42	50	92			0.9848		
F_2	1A	156	180	336	1.1904	4.6666*	0.3636	0.4000	7.5812
	2B	112	103	215	2.4614	4.0832*	0.2000	1.8836	8.6282
	Σ	268	283	551					0.3766
F_3	1A	96	66	162			5.554*	5.554*	*
									2.0908
									N.S.
Summary									
F_1	42	50	92				0.6956		
	F_2	268	283	551			0.4082		
	F_3	96	66	162			5.554*		
Σ	/	406	399	805			6.6592		
							Pooled $\chi^2 = 0.4082$		
Summary									
F_1	42	50	92				0.6956		
	F_2	268	283	551			0.4082		
	F_3	96	66	162			5.554*		
Σ	/	406	399	805			6.6592		
							Pooled $\chi^2 = 0.0608$		

* Significant, 5%.

pairs in separate tubes. Control flies were treated in exactly the same manner, but they were exposed to a normal geomagnetic field only, as has been previously mentioned.

An identical study (Table II) was carried on with a prolonged exposure time of 72 hr. After the mating period, all females were placed in individual tubes for 5 days to lay eggs. The female flies were then mated with a different male from the same group of treated flies. The second mating was a reciprocal mating and provided more detailed information on the genetic changes of individuals.

The third study (Table III) involved a set of Helmholtz coils ($45\frac{1}{2}$ -in. inside diameter, Fig. 2) large enough to produce in a volume of a cubic foot, less than one-tenth the normal field, henceforth called null field. The field measurement was obtained by using a special cathode ray tube with low voltage. The maximum deflection within the cubic foot utilized, was less than 1 mm on a scale of 12 mm, corresponding to < 0.05 G. The earth's magnetic field at the experimental location was approximately 0.55 G. Two treatments were utilized in this null field. In one, virgin flies were in the null field for 48 hr. They were then mated in the normal geomagnetic field for 2 days. The females were then placed in individual fresh culture tubes. The second set (Table IV) was treated as the first with the exception that the females were returned to the null field after 2 days of mating until the first offspring appeared, thus allowing oogenesis, as well as the stored sperm, to be subjected to the null condition.

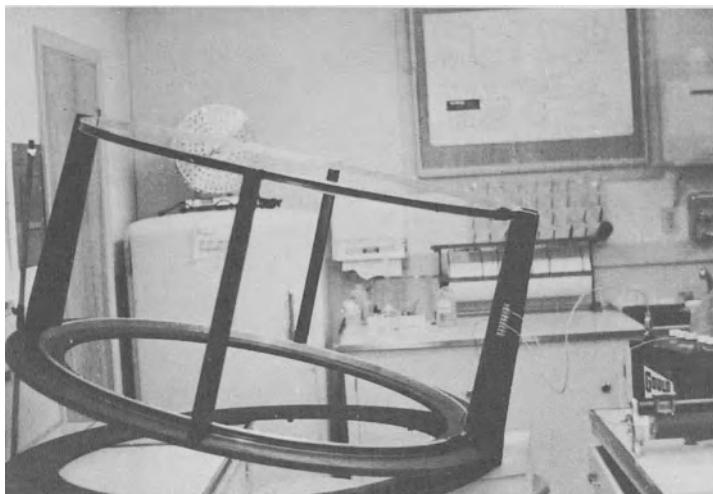


Fig. 2. Helmholtz coils.

TABLE IV

Results of Extended Residence in Null Magnetic Field (Less Then 0.05 Oe) on Transmission of Sex Ratio

Mating	Total ♂	Total ♀	Total Flies	$\Sigma \chi^2$	Pooled χ^2	Heterogeneity
F₁						
1-A	15	3	18	8.0000*	8.0000*	N.S.
2-B	14	20	34	1.0588	1.0588	N.S.
1-C	17	21	38	0.4216	0.4216	N.S.
Σ	46	44	90		9.4798*	
					Pooled $\chi^2 = 0.0444$	
F₂						
1-A	67	52	119	4.1730	1.8906	N.S.
2-B	84	67	151	2.2000	1.9138	N.S.
1-C	165	122	287	7.6366*	6.4424*	N.S.
Σ	316	241	557		10.2468*	
					Pooled $\chi^2 = 10.0986^\dagger$	
F₃						
1-A	105	96	201	2.1510	0.4028	N.S.
2-B	99	76	175	4.5074	3.0228	N.S.
1-C	161	121	282	7.0706	5.6736*	N.S.
Σ	365	293	658		9.0992*	N.S.
					Pooled $\chi^2 = 7.8784^\ddagger$	
Summary						
	Total ♂	Total ♀	Total Flies	Pooled χ^2		
F₁	46	44	90	0.0444		
F₂	316	241	557	10.0986 [†]		
F₃	365	293	658	7.8784 [†]		
Σ	727	578	1305	18.0214 [†]		
				Pooled $\chi^2 = 17.0122^\ddagger$		

* Significant, 5%.

† Highly significant, 1%.

The fourth study concerns the treatment of five pairs of flies (Turtox) placed in a test tube extending the length (90° change from previously mentioned culture tubes) of the magnetic field. These flies were mated in the tube and remained in the magnetic field of 0–520–0 Oe gradient until the appearance of the first offspring. A mutant fly with withered wings appeared among the F₁ flies and was removed (nonvirgin) for analysis of its offspring. Analysis of this mutation through five generations of sib-mating is detailed, as well as its transfer to a yellow, white, miniature stock (Tables V, VI). The appearance of anomalies along the pedigree is also indicated.

All experiments were conducted in an air-conditioned laboratory with an average temperature of 75 ± 3°F. Temperature was therefore considered to be uniform for all studies. The tubes were checked for moisture content, and a few drops of sterile distilled water were added periodically to keep the medium at a uniform consistency.

The results of each mating were tested for sex ratio by means of the χ^2 technique. A test of heterogeneity was also performed to determine the consistency of variation. This test is generally utilized to ascertain if data may be totaled. It should be pointed out that since this work would probably induce mutations which might alter the normal sex ratio in either direction, an excess of females or of males could be expected in a given mating. Considering this to be the case, a significant heterogeneity test could apparently best be interpreted as an indication that such a circumstance has actually taken place. Under normal conditions, one would hesitate to bulk significantly heterogeneous data; however, by recognizing that there have been significant deviations, though they may be in opposite directions, it is possible that a total evaluation of the mutagenic effect of magnetic fields would in actuality permit the totaling of the data into the various generations and would be of considerable value. Therefore, the combined summary (Tables I and II) indicates a comparison between generations, as well as between magnetic strengths, gradients, and duration of exposure. The same tests have been performed, removing the data that contributed to a significant heterogeneity, and are included in parentheses. In all cases, the data resulting in a high heterogeneity have come from one of the individual matings following magnetic treatment.

The procedure by which the total data for a treatment was analysed is presented in Table III. The individual χ^2 values per culture tube were recorded. These were then totaled and represented by the sum of the χ^2 . The number of degrees of freedom utilized here is the total of the degrees of freedom for each individual χ^2 (one). The pooled χ^2 is a new value for

TABLE V
Sex Frequency and Mutant Pedigree Data

χ^2 Values for Total Sex Ratio in the Mutant Pedigree (Table V)

Generation	Total	Total	Total	Total
F ₁	0.0400	0.0418	0.0400	0.0418
F ₂	1.2564	1.2564	2.0000	2.0000
F ₃	CF ₁	CF ₂	CF ₁	CF ₂
F ₄	CF ₃	CF ₄	CF ₃	CF ₄

Statistical values for each generation:

- F₁:** Progeny of treated parents. Total = 0.0400. $\chi^2 = 4.5000^*$, pooled $\chi^2 = 4.5000^*$.
- F₂:** Total = 1.2564. $\chi^2 = 12.6450^*$, pooled $\chi^2 = 12.6450^*$.
- F₃:** Total = 2.0000. $\chi^2 = 3.3504$, pooled $\chi^2 = 3.3504$.
- F₄:** Total = 2.0000. $\chi^2 = 3.3504$, pooled $\chi^2 = 3.3504$.

Other data points shown in the pedigree:

- F₁ not counted: Total = 0.0418.
- F₁ CF₁: Total = 0.0400.
- F₂ CF₁: Total = 2.0000.
- F₂ CF₂: Total = 1.2564.
- F₃ CF₁: Total = 0.0400.
- F₃ CF₂: Total = 0.0400.
- F₄ CF₁: Total = 0.0418.
- F₄ CF₂: Total = 0.0418.

* Significant, 5%.
† Highly significant, 1%.

the total data of a single mating generation. In order to obtain an overall total χ^2 test for all matings of a given generation, the pooled values were added and the degrees of freedom were computed as mentioned before. A new overall pooled value based on total data was then recorded for each generation. The interpretation of the heterogeneity test has already been discussed.

RESULTS

The pooled χ^2 values for data from control flies (subjected to a geomagnetic background) indicate no significant deviation from the normal 50:50 sex ratio (Tables I and II). There is one significant χ^2 value in the F_3 in Table II. However, this is due to a single tube of 63 flies. Since the significance disappears when the χ^2 is calculated for the 111 flies in the other tube, a deviation this large would be expected by chance alone approximately 5% of the time. Data from lab cultures of the same CBS stock for the same duration of time totaled 1181 males to 1181 females. These data indicate that no overall significant sex deviation was obtained from genetic background of the stocks utilized, and also substantiate that the exposure of flies to small vials had no effect on sex ratios.

Having established no culture-medium or small-vial effects on sex ratios, it is possible to relate deviation in sex ratios to the differing magnetic field strengths, gradients, and duration of exposure time. In Table I, it is seen from the overall pooled χ^2 at the end of three generations that the low field (high gradient) and field of 520 Oe (low gradient) were both significantly (5% level) effective in altering the sex ratio in favor of male offspring. In Table II, the effect of the low field (high gradient) is evident as a highly significant (1% level) deviation from the expected 50:50 sex ratio. The removed data are from matings with a highly excessive male count as may be seen from the results. The results at all field intensities given in Table II are significant. A field strength of 520 Oe (low gradient), appears to be less effective with longer exposure times (Table II compared to Table I). But it should be noted in Table II that of the original five matings, only one successfully produced offspring.

Table III includes the results of two matings of virgin flies which were cultured in the normal geomagnetic background for three generations following exposure to a magnetic field of one-tenth the geomagnetic background for 48 hr. No significant deviation of the sex ratio occurred in the F_1 . The F_2 data analysis indicates that in each mating, at least one culture tube had a significant χ^2 , i.e., a deviation from the expected 1:1 sex ratio. When I totaled the χ^2 for each mating, I found no significant deviation.

Table IV contains the statistical evaluation for three matings of flies which were exposed to one-tenth the normal geomagnetic field before mating, mated in normal geomagnetic background, and returned to the reduced field until the first offspring emerged. It should be noted that male fly No. 1 was mated to two virgins, A and C. Mating 1-A produced a significant shift in the sex ratio again toward an excess of males, though the total number of offspring was low. It should also be noted that the Yates correction factor was not utilized in these computations as they are to be totaled. Therefore, this factor is not appropriate, although only one degree of freedom is involved. The sum of the pooled χ^2 values for each generation is significant, evidence that a shift from the normal sex ratio has occurred in each generation.

The new or overall pooled χ^2 values for the data of each generation indicate no sex deviation in the F_1 , but highly significant deviation in the F_2 and F_3 . It should be noted that the 1-C mating in the F_2 and F_3 produced a significant total value which the 1-A mating did not, while the reverse was true for the F_1 . In all cases these were nonsignificant heterogeneity values indicating sex ratio variation in the same direction (excess of males) in all samples. The summary of the three generations has a highly significant total χ^2 as well as a highly significant overall pooled χ^2 . The pooled χ^2 , in turn, has a low level of heterogeneity.

Table V is a pedigree from a mutant fly with withered wings which appeared among the offspring of flies exposed to a field with a gradient of 0-520-0 Oe. The center of the chart shows that the parents produced 20 normal males, 13 normal females, no abnormal males and one abnormal female (withered wing). This sequence of listing normal male/female, then mutant male/female is followed throughout the table. The F_1 refers to the offspring of the mutant fly. The F_2 is the offspring of the F_1 individual and indicates a 3:1 ratio of normal to withered wing. The seven withered females were nonvirgins when found, and were placed together in one tube indicated as going to the right side of the table labeled F_3 . Their offspring were composed of 109 male and 106 female normal flies. These females were then mated (out cross) to a different strain (yellow, white-winged, miniature CBS stock) of flies, and the data are found on the left side of the table under CF_1 . The results of five of the flies are listed. Two of the flies died during the mating procedure. It should be noted that neither the CF_1 nor F_3 had mutant forms, as would be expected in the CF_1 , and based on chance matings, could be expected in the F_3 . The mutant reappeared in later generations in both stocks. It should also be noted that several lines in the CF_2 and F_4 categories (the same generation removed from mutant grand-

mothers) have significantly abnormal sex ratios, as indicated by Table IV. In the succeeding generation, additional types of mutants appeared and are expressed as a third grouping of numbers.

A χ^2 analysis of the sex ratio in the mutant pedigree (Table V) is presented in Table VI. The sex ratio does not deviate significantly until the offspring matings of the seven mutant (withered wing) female flies are studied. This deviation occurs in the F_4 and the CF_2 , both of which are the same generation removed from the original mutant. The significant χ^2 values in this table result from an increase in the female sex, a contrast to previous work in which there was generally an excess of male offspring. The χ^2 sums for the F_4 and the CF_2 , as well as the overall pooled χ^2 values, are highly significant. In the F_5 and the CF_3 , there are again significant deviations from the expected 1:1 sex ratio in both the χ^2 sums and the overall pooled values.

DISCUSSION

The works of Mulay, and of Close and Beischer, both indicated no mutagenic effect of magnetic fields on *Drosophila melanogaster*. In these works, "Muller's 5" strain of *Drosophila melanogaster* was used to rapidly indicate the presence of sex-linked lethal mutants. From the data presented, it seems that this procedure is not valid for such a procedure. If dealing with the DNA level, several generations of DNA replication would be necessary before the X chromosome would contain sufficient fibrils of mutated DNA for expression as a sex-linked lethal gene. To adequately test for a lethal gene of this type, "Muller's 5" should not be used until at least two or three generations of inbred matings have taken place, allowing time for the accumulation of mutated DNA fibrils within the chromosome. This procedure necessitates the maintenance of large quantities of flies for a longer period, reducing the effectiveness of "Muller's 5" as a time-saving device.

Tables V and VI may be explained on the basis of a sex-linked recessive lethal gene, resulting in a predominance of female offspring. This occurred four generations removed from the exposed parents. It was possible to maintain stocks perpetuating this condition, assuring the genetic basis of the trait, but the precise gene location has not been discovered. Likewise, the genetic basis of the withered wing (similar to Mulay's wrinkled wing) cannot be questioned, as it also was transmitted through several generations. This is, however, a recessive autosomal gene. It passed undetected through a mating and was recovered upon sib-mating. It has not been possible to

establish a pure line of this mutant, and there is an indication that penetrance, as well as an active healing effect or transforming effect, may act on this gene. Another mutant, Convergent Bands (an X pattern is formed middorsally by the first two segments of the abdomen), is also transmitted from generation to generation, but gradually disappears and cannot be maintained in pure stock. These characteristics are mutants in the true genetic sense since they may be passed from one generation to another.

In the third column of flies listed as F_5 and CF_3 in Table V, the term mutant does not necessarily infer being perpetuated from generation to generation. Several gross anomalies were included in this group, such as: three wings, missing leg parts, mosaic patterns, *etc.* However, these flies were incapable of breeding and may represent only developmental effects, not true mutants. These aberrant forms did not appear until the sixth generation removed from the treated parents, again suggesting that several generations are often necessary before the effect of the magnetic field on germinal tissue may be evaluated.

It appears from Tables I to IV that mutations (other than sex-linked) affecting sexual development may be induced by an increase in magnetic background or by a decrease in geomagnetic background. The duration of exposure to these backgrounds seems to influence the effectiveness of the treatment. These mutations appear to be autosomal, indicating that the X chromosome is not the only one susceptible to this type of treatment. In view of the various mutant types produced, the action of magnetism should be considered as a mutagen of the DNA in general. In this study, the major mutagenic effects of magnetic fields seem to occur in gonadal tissue. The exposed individuals did not appear to develop any somatic anomalies, as was anticipated since the germinal tissue is the more actively dividing. If the somatoplasm were effected, the production of tumors or other somatic anomalies would be expected. However, these would be restricted to relatively small areas, making observation difficult.

During DNA replication, the hydrogen bonds, those holding the purine and pyrimidine bases together, break. This allows for a complimentary chain to be formed, maintaining the genetic code. Bases are held by a C-N bond between the sugar and the base as part of the nucleotides making up the DNA strand. This bond is a dative (coordinate covalent, semipolar) bond which is partially ionic in character. The charge separation is said to constitute an electric dipole. The bond energy of the C-N bond is approximately 73 kcal/mole, a relatively low level for a covalent bond. It is possible that this semipolar bond accounts for the helical structure of the DNA

because of repulsion forces. These forces may be responsible for opening of the DNA helix upon breakage of the H bonds between bases.

In relating DNA to geomagnetic background, it appears that these electric dipole bonds are stable; however, a change in either direction of the magnetic background would change the strength of the forces exerted on these dipoles. It is conceivable that the repulsion forces of these bonds result in bond breakage, allowing for a mutation (change in sequence of bases upon the sugar phosphate chain). The proton tunneling effect of magnetism, as discussed by M. F. Barnothy (1964), may be involved in this breakage phenomena. Additional information is needed on the action between magnetic fields and these bonds.

Considering the geologic record of geomagnetic reversals, it seems that these changes could lead to greater genetic diversity and subsequent evolutionary development. There is some question concerning the effects resulting from the loss of the earth's magnetic field. The earth could receive an increased rate of high-energy radiation and reap the effects of greater numbers of genetic changes. However, it is possible that the atmosphere is sufficient to prevent the penetration of these particles. If so, the major effect on biotic material is the magnetic effect, not the radiation. This study indicates that a change in the intensity of the magnetic background has, in essence, an effect upon an organism's genetic composition.

CONCLUSIONS

Magnetic fields of lesser or greater strength than that of the geomagnetic background, whether with fields of large or small gradient, induce mutations affecting the sex ratios of *Drosophila melanogaster*. It was not ascertained whether these mutations are sex-linked or autosomal in nature.

The mutagenic effects of magnetic fields on genes controlling traits unrelated to sex also have been demonstrated. Mutations resulted in withered wing, convergent banding, and spotted abdomen, which were without sex-linked inheritance.

It appears that only a few DNA synthesis fibrils are affected by the magnetic field utilized, judging from the number of inbreeding generations before the appearance of anomalies.

There appears to be a healing effect of some of the mutations induced by magnetic treatment.

The concept that the reversal of the geomagnetic field alone, by virtue of passing through a zero and near-zero intensity, may be a contributing factor for stages in evolutionary development has been proposed.

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THE EFFECT OF MAGNETIC FIELDS UPON THE CENTRAL NERVOUS SYSTEM

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The possible effects of magnetic fields on neural functioning and human behavior have been much discussed since the time of Mesmer. Only within recent years, however, has this problem been subjected to appropriate experimentation, and at this writing there would appear to be little doubt that some interaction exists between central nervous system (CNS) function and external magnetic fields. This concept has in the past been viewed with considerable skepticism in scientific quarters since civilized man is exposed to a multitude of electromagnetic fields, all apparently with no effect whatsoever. The writer believes, however, that an expanding technology may well be productive of magnetic environments in the future, that could have significant effects upon the human population, that may or may not be undesirable. It therefore appears desirable to briefly review the present state of knowledge in this area and to attempt to categorize it in some fashion. Hopefully, this will serve to indicate the direction in which we should proceed to determine the basis for the interaction between neural structures and magnetic fields, for it is only with this knowledge that we can intelligently predict the possible undesirable effects of human exposures to new and different types of such force fields.

There have been several general reviews of the literature on biomagnetics published within the past few years,⁽¹⁻³⁾ and their convention of classifying effects according to the type of field exposure will be followed in this paper.

Certain alternating or modulated fields have a definite and indisputable effect upon CNS structures which appears to be limited chiefly to the production of magnetophosphenes⁽⁴⁾ through a direct action upon the retina.⁽⁵⁾

The possible mechanism of action appears to be quite unclear at this time and is the subject of some differences of opinion.^(6,7) It should be noted, however, that the majority of workers concerned with neural effects have been primarily interested in steady-state fields and the possibility exists for other neurological consequences of alternating fields outside of the frequency and field-strength ranges known to produce the magnetophosphenes.

Reports of the effects of steady-state magnetic fields have been considerably more varied and at times contradictory. Most workers have utilized moderate-to high-strength fields (800 to 91,000 Oe) and have reported alterations in the electroencephalogram most commonly consisting of increased overall amplitude and increase in the number of spindles.^(8,9) Other workers have noted a decrease in the frequency pattern with the appearance of delta wave forms,^(10,11) and alterations in the DC electrical activity of the CNS.⁽¹²⁾ More recently, Aleksandrovskaya and Kholodov have reported histological alterations consisting of increased gliosis in the rabbit brain on exposure to steady-state fields of 200 to 300 Oe.⁽¹³⁾ This report is of considerable significance since, if it is substantiated, it will place a serious restriction on any possible therapeutic use of this modality. It should, however, be noted that rabbit brains characteristically evidence histological lesions resulting from endemic diseases (encephalitozoönosis) rendering the interpretation of experimentally produced lesions difficult.⁽¹⁴⁾ No particular attempt has been made to detect any behavioral alterations resulting from exposure to any of the moderate- to high-strength fields. This is somewhat surprising in view of the fact that, whereas only a relatively few workers have utilized low-strength fields, the effects they report are entirely in the area of behavioral alteration.

Brown and his associates have reported a long series of experiments on exposure of lower animals to field strengths ranging from 1 to 10 G primarily aimed at detecting alterations in the biological cyclic activity.⁽¹⁵⁾ Most recently, Brown and Park have demonstrated alterations in the cyclic pattern of activity of planaria produced by altering the vectorial relationship between a photic stimulus and the natural magnetic field of the earth.⁽¹⁶⁾ One is forced to conclude that these organisms are capable of sensing some component of the earth's field and that its known circadian fluctuation may be the driving force for biological circadian rhythms. This would appear to substantiate the reports of Friedman *et al.* on studies relating behavioral alterations in human psychopathological population groups to geophysical parameters associated with naturally occurring variations in the earth's magnetic field.^(17,18) In this case, one may conclude that at least a segment of the human population reacts to these alterations in the earth's magnetic

field or to some other associated modality. This concept has been challenged in part by Pokorny and Mefferd in a similar recent study.⁽¹⁹⁾ In order to clarify this relationship further, Friedman and his associates looked for neurophysiological correlates of exposure to artificially generated magnetic fields in the human using low-strength fields applied to the head in a bi-temporal direction.⁽²⁰⁾ Simple reaction-time performance was examined in both schizophrenic and normal individuals so exposed. With steady-state fields of 5 and 17 G no changes could be detected. When fields of 5 to 11 G were modulated at rates of 0.1 and 0.2 cps, definite, statistically significant, temporary changes in reaction time were observed. A deliberate attempt was made with a small number of volunteers to determine any electrophysiological correlates of exposure to steady-state and similarly modulated fields up to 100 G in maximum intensity. No consistent alterations in either EEG patterns of frontooccipital DC voltages paralleling the alterations in reaction time were observed.⁽²¹⁾ The field strengths utilized were considerably lower than those reported to produce either EEG or DC changes in lower animals. In regard to the DC voltages particularly, it should be emphasized that the externally measured frontooccipital voltage is a crude determination and reflects only major alterations in the state of consciousness. It is possible that subtle alterations in both parameters occurred in these experiments and were not detected by the methods utilized. These experiments in humans have been suspended following Kholodov's communication to us of his observations of cerebral gliosis associated with field exposure. A study parallel to his is currently underway in an attempt to duplicate his findings.

If Brown's thesis relating the cyclic pattern of the geomagnetic field to biological cycles is correct, then exposure of an organism to an environment lacking this factor should produce detectable alterations in some physiological or psychological parameter. Beischer has reported the only full-scale study in this area with human volunteers exposed for lengthy periods of time to a markedly reduced field at the center of three large mutually perpendicular coils at the Naval Ordnance Laboratory, White Oak, Maryland.⁽²²⁾ This apparatus was capable of maintaining a steady-state field of less than 50 gammas in the occupied area. However, the time constants of the automatic regulating systems were such that the micro-pulsations (range 0.1 to 10 cps) of the geomagnetic field were, at least in part, not negated. A gradual decrease in the subjects critical flicker fusion frequency was noted with continuing exposure to low-field conditions while a variety of psychological tests demonstrated no statistical differences between experimental and control subjects. At this time, no experiments have

been reported on human exposure to null fields produced by resistive shielding methods. The interest in this procedure is that all field components, steady-state and modulated, would be reduced to the same extent. This may be of considerable importance in the light of Friedman's report on the production of temporary reaction-time alterations only by low-strength fields modulated in the 0.1–0.2 cps range. The micropulsation activity of the geomagnetic field was present in Beischer's experiment and in those of Brown involving the natural field. The possibility exists that while the amplitude of the micropulsation activity is quite small, the frequency range of the major components is perhaps biologically significant (0.1 to 10 cps). Furthermore, alterations in the frequency and amplitude accompany magnetic storm activity⁽²³⁾ and there appears to be a diurnal rhythm in the micropulsation activity.⁽²⁴⁾ Therefore, the possibility that the biological effects of the geomagnetic field are in part associated with this modulation activity, rather than with its steady-state level, cannot be dismissed at this time.

While all these reported observations of the effects of magnetic fields on neural structures appear to be so diverse as to be unrelated, I believe a tenable simplification is that low-field strengths are productive of subtle behavioral alterations without demonstrable effects upon the measurable electrical activity, while high-strength fields are related to observable alterations in electrical activity. Since no attempts have been made to assess subtle functional changes with high-strength fields, these may be present and as yet undetected. In addition, there appears to be considerable evidence indicating a vectorial relationship between the field direction and the neuraxis and some evidence for the geomagnetic field exerting an effect on the function of higher neuronal centers.

For a number of reasons, it is important to determine the actual mode of action of the magnetic field on neural structures. Such knowledge could lead to testable hypotheses and possible therapeutic uses as well as increasing our knowledge of neural functioning itself. In considering this, I believe that we can discount any possibility of the effect being primarily upon the action potential *per se*. Liberman, for example, has reported no alterations in a variety of action-potential parameters with exposure to high-strength fields.⁽²⁵⁾ It is conceivable that effects at the molecular level (via dipole moments, *etc.*) could produce alterations of the membrane characteristics; if this is so, such effects appear to be nonproductive of major functional changes in the action potential at least of isolated nerve fibers. Nevertheless, the intact CNS with its complexities of anatomical arrangement and multiplicity of synaptic connections (whose sensitivity to magnetic effects has not

been determined *in vitro*) could theoretically be sensitive to this effect. Such consideration, *a priori*, would indicate that no specific vectorial relationship between the field and CNS would be necessary for a detectable effect with fields of sufficient strength. Some specific, as yet undescribed, effect at synaptic junctions would similarly be nonvectorial in nature. While some observers have reported alterations of electrical activity with high-strength random oriented fields, others have specified a definite vectorial relationship and most reports of low-field effects have made similar specifications. In addition, the well documented behavioral effects of low-strength fields, including the geomagnetic, are impossible to explain on such a generalized effect. I believe that the bulk of observations reported indicate an interaction between the applied magnetic field and some active functional property of the CNS that is both acutely sensitive to such a modality and associated with the overall functional-organizational pattern of the CNS.

There are certain aspects of the DC potentials of nerve tissue that indicate their possible role as the target mechanism. The DC or steady-state potentials display analog-type variations with certain basic stimuli and also are related in possibly a causal fashion to the efficiency of the action-potential system.⁽²⁶⁾ It would seem tenable to propose that they serve as a primitive data-transmitting and control system which regulates the ability of the CNS to process data via the more sophisticated action-potential system.⁽²⁷⁾ It is interesting that Von Neuman discussed the need for an analog-type of data system, additional to the action-potential system, on a cybernetic basis some time ago.⁽²⁸⁾ Representatives of all animal phyla possessing even a rudimentary CNS have been found to have evidences for a DC system, in each case displaying a field pattern expressing the overall anatomical arrangements of the CNS itself.⁽²⁹⁾ Certain evidences have been obtained in our laboratory indicating that this DC system is based upon some solid-state, possibly semiconduction, property of the tissue organization generating and transmitting the steady-state potentials.⁽³⁰⁻³²⁾ From a theoretical point of view, the existence of standing potentials in a conducting network implies a current flow sufficient to maintain the potential. If such current flow is semiconducting in nature, the interaction between the charge carriers and an applied magnetic field, the Hall effect, would be many orders of magnitude greater in this case than in the case of such interaction between similar magnetic field and similar current values in a metallic conductor with an even greater difference over an ionic conduction system of the same current value.⁽³³⁾ Thus, if our thesis that the DC-field system is basically semiconducting is correct, then we have a system exerting some regulatory effect upon the overall functioning of the CNS that is at the same

time acutely sensitive to applied magnetic fields. Some evidence has been acquired for the existence of the postulated interaction with a vectorial relationship between the applied field and the neuraxis.⁽³⁴⁾ In addition, observations in our laboratory of frequency modulation of the cerebral DC potentials during changes in the state of consciousness served to indicate the appropriate frequency range for Friedman's experiments.⁽²⁰⁾ In our experience, the DC systems of the lower phyla appeared to play a larger role in CNS functioning and to be more acutely sensitive to external fields. Thus, the observations of Brown on the variations in the biocyclic activity of planarians, etc. produced by extremely low-strength fields are possibly explainable.

While the foregoing is proposed primarily as a working hypothesis, it seems to lend itself well to explaining the majority of the reported phenomena. In addition, it provides a testable hypothesis subject to experimental verification. Provided exposures to magnetic fields are proven to be without the production of pathological lesions, the hypothesis leads one to some conclusions of therapeutic interest such as the induction of sleep or anesthetic states by properly applied and modulated magnetic fields, and provides us with another means of exploring the role played by the DC potentials in integrated neural functioning.

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INVESTIGATIONS OF THE REACTIONS OF MAMMALIAN BRAIN TO STATIC MAGNETIC FIELDS

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The central nervous system plays a significant role in the reactions of organisms to static magnetic fields (SMF). [See Kholodov (1964, 1966), Becker (1963), Vjalov (1967), among others.] Our present aim was to investigate the initial reactions of the mammalian brain to a SMF by conditioned reflex, electrophysiological, and histological methods.

Conditioned reflexes to an SMF in fish were investigated by Lissman (1958) and Kholodov (1958). The measurement of the conditioned reflexes to SMF in pigeons [Orgel and Smith (1956); Kholodov (1964)] and in rabbits [Kholodov (1966)] is rather difficult. We think that the use of SMF as a conditioned stimulus in rabbits was unsuccessful because (a) the SMF was not strong enough; (b) the number of trials was not large enough; or (c) the latent period of the reaction was very long.

To examine these assumptions we observed in four rabbits the electrode-defensive conditioned reflex to a 1000-Oe SMF. The delay of the reinforcement (above threshold electrical stimulus on the left hind leg) was 20 sec. The animal's legs were fixed during the experiment. The head of the rabbit underwent a 22-sec exposure to this horizontal SMF generated by an iron-core electromagnet. We recorded on the electroencephalograph the leg's movement and EEG of the optic, acoustic, and sensomotoric regions of the cerebral cortex. We gave the electrical stimulus (2 sec) only when the conditioned movement was absent. The magnetic and electrical stimuli were terminated simultaneously.

Three rabbits as controls received an acoustic stimulus. We made about

20 trials in an experiment with intervals of about 1–6 min. We gave magnetic and acoustic stimuli at random but in equal numbers.

It was found that the conditioned reflex to an SMF can be produced in each rabbit. The magnetic conditioned reflex occurred on the average after 40.0 ± 2.3 trials; it was established (the criterion being five successive times) after 248 ± 5 trials and it had a latent period of 8.50 ± 0.1 sec. In the same rabbits the corresponding numbers of the acoustic conditioned reflex were: 26.0 ± 3.4 trials, 137.0 ± 2.8 trials, and 4.90 ± 0.09 sec.

The formation of the magnetic and acoustic conditioned reflexes in rabbit No. 1 is shown in Fig. 1. One can see that the acoustic conditioned reflex occurred earlier and was more stable than the magnetic conditioned reflex.

These results confirm our previous conclusion that an SMF is a weaker stimulus than the optical or acoustic stimuli. The considerable delay of the unconditioned stimulus causes some difficulty in the formation of the acoustic conditioned reflex. An additional series of experiments (two rabbits) showed that the shorter delay (6 sec) accelerates the formation of both the acoustic and magnetic conditioned reflexes. Hence it follows that our previous failure to observe the magnetic conditioned reflex to an SMF in rabbits [Kholodov (1966)] may be explained by too few trials (70) and too weak SMF (300 Oe).

Thus, a 1000-Oe-strong SMF can produce a positive conditioned stimulus in rabbits, though this reflex acts slowly. All properties of the magnetic conditioned reflex are weaker than the same properties of the acoustic conditioned reflex. It is possible that these peculiarities of the magnetic

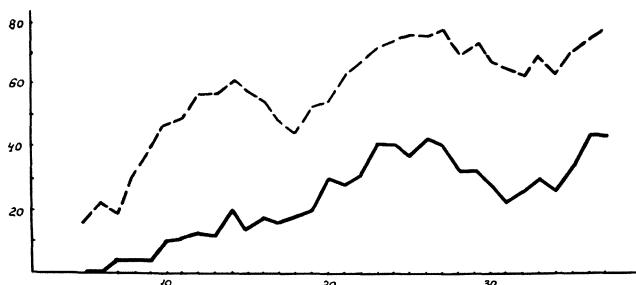


Fig. 1. Formation of the electrodefensive conditioned reflex in rabbit No. 1 to the static magnetic field (full line) and to sound (dashed line). The ordinate gives the number (in %) of the movemental reactions to the conditioned stimulus during one experiment; the abscissa gives the numbers of the experiments. Data obtained by moving average method.

conditioned reflex combine with the production of an inhibitory effect in the central nervous system during the exposure to an SMF.

This assumption supports the observations of numerous authors about a reduced sensitivity of organisms to various stimuli in an SMF, and also our electrophysiological investigations of the patterns of the reaction of the mammalian brain to the SMF.

We observed on the EEG of rabbits (150 animals) which underwent about 1- to 3-min exposure to a horizontal SMF (200–1000 Oe) an increase in the number of spindles and of slow waves. These EEG patterns are indicative in appearance of a certain phase of sleep in the mammalian brain.

The example of the EEG reaction of the sensomotor region of the cerebral cortex of the rabbit is shown in Fig. 2. One can see that the EEG reaction consists of an increase in the number of spindles. This reaction continues for a short time after the electromagnet has been switched off. We have also observed a similar EEG reaction in cats.

The automatic frequency analysis of this EEG reaction confirmed the data of visual analysis and showed that in the SMF the EEG of the rabbit increases the proportion of slow frequencies and the frequencies which form spindles. Moreover, we noted in the EEG an increase in the proportion of the rapid frequencies (27–30 impulses/sec) which we did not observe in visual analysis.

We investigated the effect of the SMF (600 Oe) on the EEG of rabbits

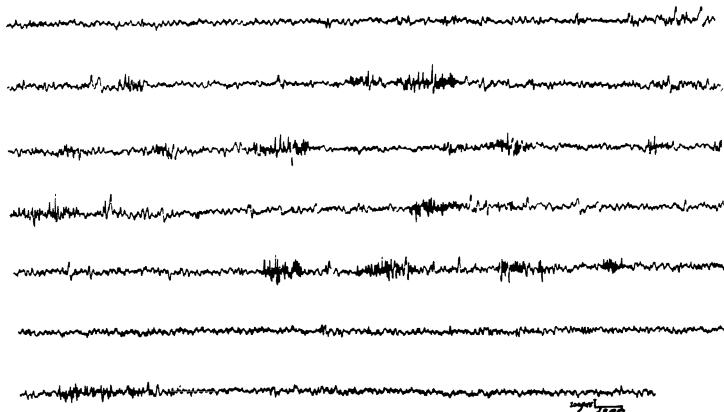


Fig. 2. Changes in the rabbit's EEG during exposure of the animal's head to a static magnetic field of 1000 Oe. Continuous (3-min) recording of the EEG of the sensomotor area of the cerebral cortex of the left hemisphere. The arrows show the moments of switching the electromagnet on and off.

using a digital computer for automatically switching the electromagnet on and off, and for automatic analyzing of the amplitude of the EEG. It was shown that the SMF increases the magnitude of the amplitude of the EEG ($p < 0.001$) during 1-min exposures to the SMF and for 1 min after the electromagnet has been switched off.

Thus the various methods of analyzing our data confirm that the SMF increases the amplitude and often decreases the frequency of the biopotentials of the mammalian brain.

Similar changes of the EEG in an SMF have been noted in reptiles [Becker (1963)], in pigeons [Gualtierotti (1963)], in rabbits [Kholodov (1964)]; Ivanov-Muromsky *et al.* (1967)], in monkeys [Knepton and Beischer (1966)], and in man [Vjalov (1967) and others]. Therefore the EEG reactions to an SMF are a reliable measure of the effect of an SMF on the brain of various vertebrates.

It should be noted that the character of the EEG reaction to an SMF depends on the initial functional state of the central nervous system and on the individual peculiarities of an animal. The EEG reaction to an SMF increases when caffeine or adrenaline are administered and decreases for nembutal or aminasine. We did not observe the adaptation or the summation when the exposure to the SMF was 1–3 min and the intervals between the exposures were 5–20 min, but we observed the summation (the prolonged increase of the amplitude in the EEG) when a 3-hr exposure to the SMF was repeated every day.

Stimulation by the SMF of a synchronous reaction in the EEG is similar to the reaction of electrical low-frequency stimulations of the thalamical region of the mammalian brain. It was supposed that the structures of non-specific thalamus responses to the SMF predetermined the character of the EEG reaction. But according to results of the experiments with lesions, as mentioned above, and with the isolation of the cerebral cortex, we assume that the exposure of an animal to the SMF may cause a response of every part of the brain, and that a reaction of the whole brain to the SMF is determined by the combined interaction of local responses from various regions of the brain. Therefore we investigated further the local response of the cerebral cortex to the SMF.

We fastened small permanent magnets (1–3 g) using stiracryl on the skull's bones above the sensomotor area of the cerebral cortex for 2–40 days; the strength of the SMF was about 200 Oe at the surface. Dummy magnets of the same weight were attached to control animals. It was found that the EEG of the cortex area under the magnet differed from the EEG of other cortex areas by a predominance of the slow high-amplitude bio-

potentials. Thus, local and continuous exposure of the SMF induces a local reaction in mammalian brain [Kholodov (1968)].

We investigated the response of individual neurons by the microelectrode method during exposure of the rabbit's head to a 1000-Oe SMF. From an extracellular recording of the electrical activity of the neurons we found that the SMF decreases the discharge frequency of neurons in 36% of the cases; increases it in 24% of the cases, and no changes occurred in 40% of the cases.

It should be noted that in most cases the neurons increase the discharge frequency during exposure to adequate stimuli (sound, light). A predominantly inhibitory effect of the SMF on the spontaneous electrical activity of neurons has been discovered for the mammalian brain on an isolated ventral nerve chain of the crayfish [Kholodov *et al.* (1966)] and on a subesophageal ganglion from the American cockroach [Sittler (1966)].

We noted more often inhibitory effects of the SMF on the electrical activity of neurons when we investigated the activity of neurons stimulated by light or sound. We recorded the response of 67 neurons in the optical area of the cerebral cortex of rabbits to a 1000-Oe SMF for a flash of light of 1-msec duration, for sounds, and for various combinations of these stimuli.

The effect of the SMF on the electrical neuronal activity stimulated by light was shown in 53% of all cases, and in 30% of all cases this effect was inhibitory, that is, there was an increase in the latent period of the neuronal

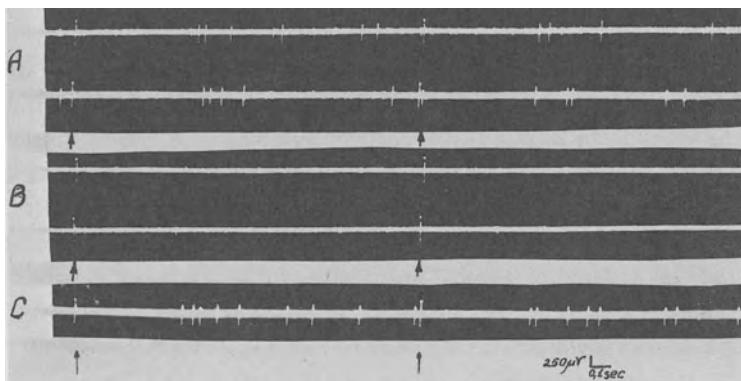


Fig. 3. Inhibition of neuronal reaction to light in the visual area of a rabbit's cerebral cortex during exposure of the animal's head to a static magnetic field of 1000 Oe. Extracellular recording of the electrical activity of the neuron before (A), during (B), and after (C) exposure to the static magnetic field. The arrows indicate the flash of light.

reaction to the flash, a decrease in numbers of spikes in the response to the flash, and sometimes an absence of the neuronal response to the flash (Fig. 3). The responses reappeared after the electromagnet was switched off.

The effect of the SMF on neuronal activity stimulated by sound was often similar (53% of all cases) but the strength of the inhibition was larger. During exposure to the SMF the neuronal reactions to the complex stimulus sound plus light had also been inhibited. It is interesting that the ratio of the exciting neurons to the inhibiting neurons on exposure to light, sound, or sound plus light in the SMF was equal to about $\frac{2}{3}$. These data show that the effect of an SMF is nonspecific in character and that this effect is independent of the nature of the testing stimulus [Lukjanova (1968)].

Thus, the SMF inhibits predominantly the spontaneous and stimulated neuronal activity of the whole rabbit brain. However, this conclusion applies also to experiments with the neuronally isolated cerebral cortex of a rabbit brain. It was shown that during exposure of the head of a rabbit to a 1000-Oe SMF the neuronal activity was changed in 72% of the cases (in the intact brain in 58% of the cases) and was inhibited in 50% of the cases (in the intact brain in 33% of the cases). Therefore, the isolation of the cerebral cortex increases both the number of neurons responding to the SMF and the number of inhibiting neurons.

From all the facts it was inferred that the SMF acts simultaneously on the exciting and on the inhibiting structures of the brain, and, moreover, that the inhibitory effect occurs more often. This fact plays an important role in the process of the formation of the magnetic conditioned reflex.

The analysis of the EEG reaction of the rabbit to the SMF during the formation of the magnetic conditioned reflex confirmed our assumption. During the establishment of the magnetic conditioned response, the spindles in the EEG were absent in most cases. Only in 15 (2.6%) cases out of 573 were the spindles in the EEG preceded by the magnetic conditioned reflex. But in these cases the conditioned movement had the largest latent period and the least strength and usually was accompanied by reinforcement. Consequently, the appearance of the spikes in the EEG of the rabbit prevents the conditioned movement and makes it difficult to form conditioned magnetic reflexes.

The main peculiarity of the reaction of the mammalian brain to an SMF is the predominance of the inhibitory process, and the second is its latent period. These play a part both in the formation of the magnetic conditioned reflex in the rabbit and in the recording of slow neuronal electrical activity in the mammalian brain. When the latent period of the reaction to an adequate stimulus was 10 msec, the latent period of the reaction of

the mammalian brain to an SMF was a few seconds, or sometimes a few tens of seconds. Since the latent period of the brain's reaction to an SMF was large, we assumed that electrically activated elements can be involved in the reaction as well as the electrically inactive elements (glia or vessels).

To verify the above assumption experimentally we investigated the reaction of the brain to an SMF by a histological method. The rabbits were sacrificed by air embolism immediately after the exposure to an SMF of 200–300 Oe. The control animals were sacrificed by a similar method. The reactions of the glia and neurons were investigated by morphological methods: astrocytes were stained according to Cajal, oligodendrocytes and microglia according to Alexandrovskaya, and nerve cells according to Nissl. After staining the sensomotor cortical area we counted the number of glial cells in the field of a microscope ($\times 200$ magnification). The astrocytes were investigated in more detail.

It was found that after 3 min of exposure of the rabbit's head to the SMF the number of staining astrocytes was increased from 9.0 ± 0.5 (control) to 16.7 ± 0.4 . The numbers of cells of oligodendroglia and microglia also increased. The neurons remained intact, with a well defined nucleus and nucleolus and a clear tigroid [Alexandrovskaya and Kholodov (1968)].

This histological reaction is simultaneous with the EEG reaction to the SMF. We believe that the EEG reaction forms by means of the glia.

After 1-hr exposure to the SMF the number of staining astrocytes in the cerebral cortex increased further to 23.0 ± 0.5 . Cells with hyperplasia and hypertrophy of the cell walls were noted. The increased number of staining astrocytes after the exposure of the rabbit's head to an SMF is shown in Fig. 4. The numbers of the staining cells of other kind of glia also increased. Neurons remained intact. It was seen that the histological reaction was increased with an increase in the exposure time. The number of the staining astrocytes after 1 hr of exposure was greater ($p < 0.01$) than after 3 min of exposure.

By increasing the time of exposure to the SMF we observed the productive-dystrophic lesions of the neuroglia and neurons. The picture of hypoxic encephalopathy was established morphologically. This conclusion leads to the assumption that on the biochemical level the SMF changes the oxidative processes in the brain.

It is known that the staining of nerve tissue by silver depends on the level of oxidative-reduction potentials. We assumed that the increase in the number of staining astrocytes did not reflect a true variation in the number of astrocytes, but this process was probably due to changes in

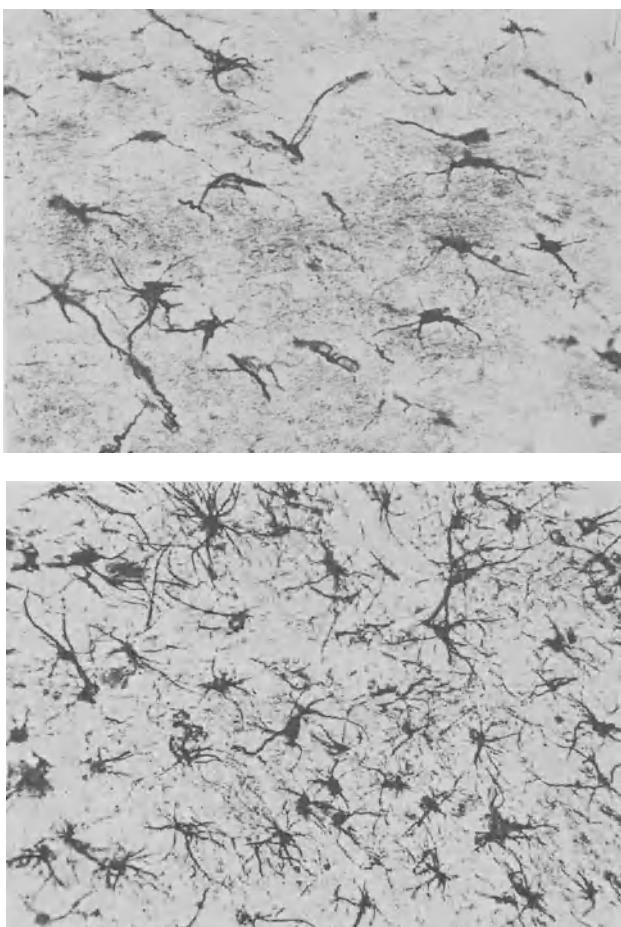


Fig. 4. Increase in the numbers of astrocytes in the sensomotor area of the cerebral cortex of the rabbit after 1-hr exposure to a static magnetic field of 300 Oe. Stain according to Cajal. Magnification $\times 220$. (A) Number of astrocytes in controls; (B) in rabbits exposed to SMF.

the metabolism of the glial cells which increased the number of astrocytes being stained by silver.

It may be noted that numerous investigators have observed changes in the processes of biological oxidation in an SMF [Shyshlo and Shimkevich (1966); Tarakanova (1966); Pereira *et al.* (1967) and others].

Taking into consideration that oxygen starvation affects the brain

TABLE I

	Control group		Magnet groups		
Number of mice	30	30	30	30	12
Time of magnetic treatment, prior to oxygen starvation experiment, min	0	0	15	60	120
Average survival time of mice in the sealed container	24.5 ± 0.8	22.6 ± 0.8	19.3 ± 0.9	19.3 ± 1.1	20.0 ± 0.3
Probability of difference	$p > 0.1$	$p < 0.001$	$p < 0.005$	$p < 0.001$	

[Petrov (1967)], we investigated the effects of an SMF on the survival time of 192 adult white mice (weight 23–25 g) during oxygen starvation. We placed the animals in a sealed container and determined their time of death in the SMF and without field in controls (no measures were taken to absorb the developing CO_2).

The results obtained with exposures of different lengths to a 400-Oe SMF prior to the oxygen starvation experiment are summarized in Table I.

In a 2000-Oe SMF the sealed container used had about half as great a volume, and the duration of the magnetic treatment prior to the oxygen starvation experiment was 15 min. The average survival time of the control group (30 mice) was 13.0 ± 0.4 min; that of the magnet group (30 mice) was 11.0 ± 0.4 min. The difference 2.0 ± 0.56 has a probability level $p < 0.001$.

It is interesting that whereas Pereira *et al.* (1967) noted a constant value of the effect on oxidative processes by increasing the strength of the SMF, we noted a constant value of the effect by increasing the length of the exposure. It must be supposed that exposure to an SMF of a certain strength and for a certain time changes the oxidative processes, and a further increase in the strength and/or duration of the exposure does not affect this reaction.

It is possible that the SMF caused a hypoxia in the tissue of the mammalian brain and in this way decreased the survival time of the mice. This conclusion can be confirmed from indirect data on the changes in the electrical activity of the intact brain and in the neuronally isolated cortex of a rabbit during experimental hypoxia [Malkin *et al.* (1966)]. These changes were similar to reactions of the same region of the brain to an SMF (Kholodov, 1966).

The threshold of the strength of the SMF in experiments with cellular respiration [Pereira *et al.* (1967)] was about 80 Oe, and in our experiments with visual and automatic analysis of the recording of the electrical activity of rabbit's brain it was about 100 Oe. The nearly equal values of these thresholds lend additional support for an interaction between the EEG and the oxidative processes in the brain in an SMF.

The third important peculiarity of the reaction of the brain to the SMF is a long-term effect which we noted. During the investigation of tracing reactions of various sorts of glia in the sensomotor area of the cerebral cortex of the cat, after 6 hr of exposure to a 200- to 300-Oe SMF it was found that the increasing number of astrocytes had a fluctuating character, and we noted a considerable increase 14 days after a 4-hr exposure. The increase of numbers of other sorts of glia also had a fluctuating character but with different parameters of time [Alexandrovskaja (1966)].

It may be noted that the above-mentioned reaction of the glia to the SMF is accompanied by the presence of intact neurons. Probably the neuroglia play the principal role in the tracing reaction of the brain after an exposure to weak injuring agents. We believe that these tracing reactions of the brain after exposure to the SMF combine with restoring processes which cause stress reactions in the organism.

The presence of the histological reaction of neuroglia in the cat's brain 20 days after a 6-hr exposure was confirmed by other investigators. The morphologists [Toropzev (1968)] discovered a continuous reaction of the brain and other organs in the guinea pig after 6 hr of exposure to a 200-Oe SMF. This SMF can cause continued breaks in the regulating processes of the organism.

It may be inferred that these various methods of investigation confirmed our previous data on the high sensitivity of the central nervous system to the SMF. The peculiarities of the reactions of the mammalian brain to an SMF are predominantly inhibitory effects of prolonged latent periods and lead to long-term after-effects. Further investigations, particularly on the neurochemical level, will define more precisely the primary mechanism of the reaction of the brain to an SMF and peculiarities of the nervous activity during the action of the fields.

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EFFECT OF A CONSTANT MAGNETIC FIELD ON INVERTEBRATE NEURONS*

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INTRODUCTION

Investigations of possible effects of magnetic fields on neurons have been carried out with varied degrees of systematic experimentation and success. McKendrick⁽¹⁾ in studying the effects of a magnetic field on the sciatic nerve of a frog indicated that the magnetic field reduced the irritability of the nerve-muscle preparation. The results of his experiments were not conclusive. Erdman⁽²⁾ conducted experiments which indicated that magnetic fields (greater than 1000 Oe) initially decreased, and then increased, the chronaxie of the frog sciatic nerve-muscle preparation. Liberman *et al.*,⁽³⁾ in an attempt to verify Erdman's results, concluded that a 10,000-Oe magnetic field did not affect single fibers of the sciatic nerve. Young and Gofman⁽⁴⁾ observed that the isolated frog vagal heart preparation exhibited a decrease in the duration of vagal inhibition, work per cycle, contractility, and a high frequency of irregularity after cessation of magnetic fields of 4,000–15,000 Oe.

Reno and Beischer⁽⁵⁾ in studying turtle hearts exposed to magnetic fields (3,400–15,000 Oe) observed a decrease in the amplitude of contractions followed by incomplete relaxation during diastole. Alteration of the excitability of neurons in rabbits, birds, and fish has been reported by Kholodov.⁽⁶⁾

In a clinical report by Hansen⁽⁷⁾ it was indicated that a constant magnetic field reduced the sensation of pain and the histamine flush reaction (reflex action in the autonomic nervous system) in humans. It has also been reported that under certain conditions a flash of light may be seen when

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man places his head in an undulating magnetic field. This phenomenon, called a magnetic phosphene, has been documented by the works of Dunlap,⁽⁸⁾ Barlow *et al.*,⁽⁹⁾ and others. It appears that this well-documented effect can be explained in terms of electrical currents induced in the photo-sensitive cells of the retina by the varying magnetic field.

The primary objective of this investigation was to systematically determine whether or not a magnetic field would affect the spontaneous activity of neurons located in the subesophageal ganglion from an invertebrate. The secondary objective was to determine the type and magnitude of the effect.

MATERIALS AND METHODS

The nervous tissue used was the subesophageal ganglion from the male American cockroach, *Periplaneta americana*, (L). The invertebrate nervous system is constantly in a state of spontaneous nervous activity; therefore, no stimulating apparatus was needed for the experiments in this study. The insects were grown in the laboratory and maintained on identical diets of dogmeal and water. The cockroaches were anesthetized with carbon dioxide and decapitated. The ganglion was dissected from the insect's head and mounted onto an Ag-AgCl wire-glass electrode system which was connected to a Grass P-5 preamplifier. The electrode system was placed in the glass

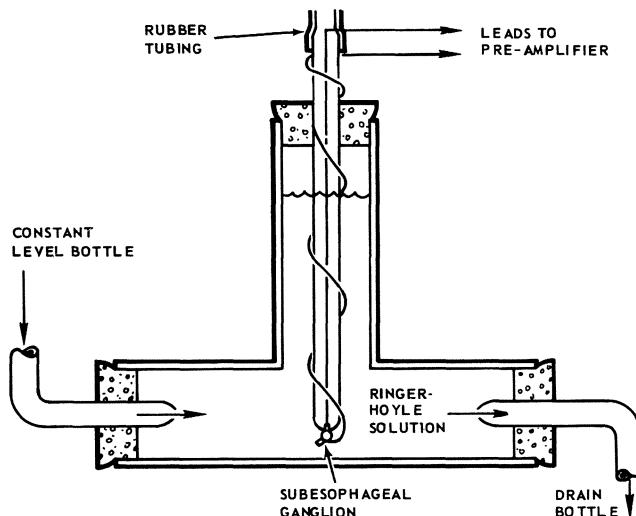


Fig. 1. Schematic of T tube.

T as shown in the schematic in Fig. 1. Ringer-Hoyle solution was passed through the T-tube to maintain a constant environment. The temperature of the solution was maintained at $21 \pm 1^\circ\text{C}$ and the flow rate at 5.0 ± 0.5 ml/min. A schematic of the apparatus used to maintain the constant flow rate is illustrated in Fig. 2.

A magnetic field of 6600 Oe was obtained by using a permanent magnet whose tapered poles had both an air-gap and pole-face diameter of 10 mm. The magnetic field around the preparation was varied by moving the T-tube along a 1.3-m aluminum track which was oriented perpendicular to the magnetic lines of flux.

The preparation was placed alternately in the field and out of the field for 5-min intervals as long as the preparation remained active (1–2 hr), and the spontaneous neural activity was amplified and recorded on magnetic tape. Analysis of the bioelectrical data was carried out as follows: The recorded pulses were played back, amplified, and converted into appropriate

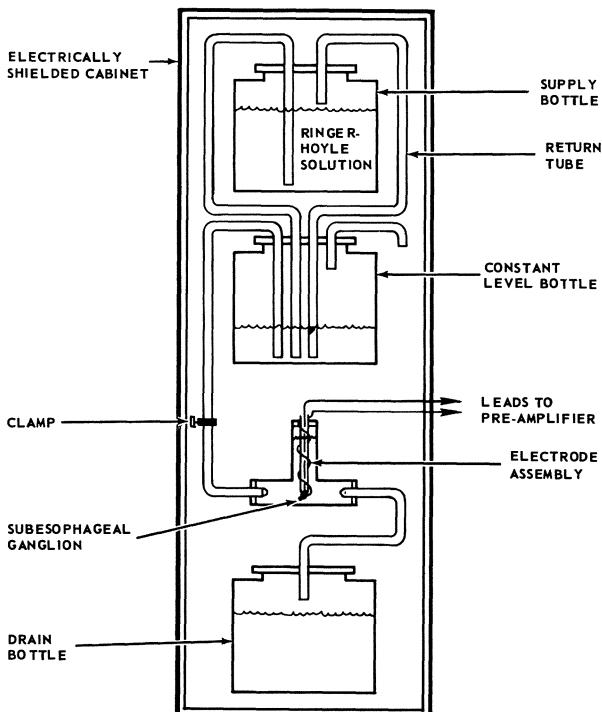


Fig. 2. Schematic of apparatus used to maintain constant flow rate.

input signals for a Nuclear Data, Model 130, 512-channel pulse-height analyzer. The gain was adjusted so that the recorded signals corresponding to the $10\text{-}\mu\text{V}$ noise level would not be detected by the analyzer. Recorded impulses were counted during the second and third minutes of each 5-min recording interval, after which the total number of impulses were summed and the sum printed out. The number of impulses per 2 min during each exposure to the magnetic field was compared with the number of impulses per 2 min during the field-free intervals just preceding and just following the exposure. The ratio of pulse rate out of the field to pulse rate in the field was calculated. These ratios are denoted activity ratios. A ratio equal to one indicates no change in neural activity, whereas a ratio less than one indicates a stimulating effect of the magnetic field on the ganglion, and a ratio greater than one indicates an inhibition of neural activity.

Any ratio of two numbers that is less than one is between 0 and 1, whereas any ratio that is greater than one is between one and infinity. This would tend to make the mean value for ratios of pairs of numbers taken from a set of random numbers greater than one. However, if the logarithms of the ratios were used, then the mean value of these logarithms of ratios of pairs of numbers from a set of random numbers would be zero since $\ln(0) = -\infty$; $\ln(+1) = 0$; and $\ln(+\infty) = +\infty$. For this reason, the natural logarithms of the activity ratios were calculated and used to evaluate the average effect of the magnetic field on spontaneous firing rates.

RESULTS

Nerve impulses from ten subesophageal ganglia were analyzed and are presented in Table I. The impulses were recorded on ten magnetic tapes which were numbered consecutively to correspond with the number of the experiment. The number of activity ratios for each tape is listed in the second column of Table I. The average values of the logarithms of the activity ratios for each tape are listed in column three. The fourth column shows the root-mean-square deviation (standard deviation) of the set of logarithms of activity ratios from the mean value for each of the ten tapes. The fifth column gives the deviation of the value of M for each tape listed in the third column from the average value \bar{M} for all ten tapes, which was calculated to be 0.24. The average value \bar{M} for all ten tapes with the standard error for the data was calculated to be 0.24 ± 0.06 . Applying Student's t test to 0.24 ± 0.06 places a 0.3% level of significance on this value, and establishes that the 6600-Oe magnetic field caused a 17 to 27% inhibition in spontaneous firing rates.

TABLE I
Summary of Activity Ratio Data from Ten Ganglia

Tape No.	No. of activity ratios	$\bar{\ln N} = M$	RMS Dev.	$D = \bar{M} - M$
1	15	0.023	1.253	-0.212
2	15	0.035	0.138	-0.200
3	13	0.195	0.623	-0.040
4	13	0.474	1.889	0.239
5	13	0.498	0.841	0.263
6	15	0.293	0.565	0.058
7	17	0.308	0.710	0.073
8	15	0.375	0.673	0.140
9	15	0.099	0.541	-0.136
10	15	0.052	0.219	-0.183

Activity curves plotted for each experiment were similar in shape to the one illustrated in Fig. 3, where total number of counts per 2 min is plotted against time.

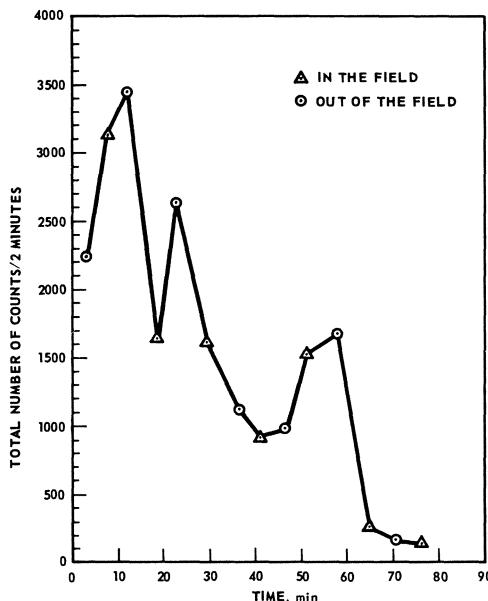


Fig. 3. Activity curve.

DISCUSSION

There are several mechanisms which might be contributing to the inhibitory effect exhibited in these experiments. If the quantity of transmitter substance available to interact with receptor sites were reduced, either by a decrease in rate of production or by decreased permeability of the presynaptic membrane for this substance, an inhibitory effect would result. Another possible explanation for the inhibitory effect is that the magnetic field might cause an increased production, or activity, of an enzyme responsible for inactivating the transmitter substance. Another consideration would involve the effect the magnetic field could have on an interaction between the transmitter substances and the receptor sites. A constant magnetic field might cause a change in the configuration of the receptor sites, thereby making a poor fit between the transmitter substance and the receptor sites. The smaller the number of receptor sites interacting with transmitter substance would tend to decrease the probability of the post-synaptic membrane's being depolarized.

An extension of this work is necessary to determine if any of the above-mentioned mechanisms are acting in the observed inhibitory effects on the spontaneous activity of invertebrate neurons.

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PREFERENCE OF MICE TO CONSUME FOOD AND WATER IN AN ENVIRONMENT OF HIGH MAGNETIC FIELD*

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INTRODUCTION

Information on the biological capacity of mammals and other biosystems under anomalous conditions is necessary in assessing the biological cost of a task or occupation. The fact that man has resided in magnetic fields without experiencing obvious harmful effects should not preclude a detailed, systematic assessment of magnetic environments and their possible bioeffects. Interest in magnetic shielding of astronauts against cosmic radiation raises the question of bioeffects of strong magnetic fields. High-intensity magnetic fields can be expected in connection with ion and other advanced propulsion systems. Even with proper shielding and arrangement of such fields, the possibility of fringe and/or accidental exposure requires experience with bioeffects of high-intensity magnetic fields.⁽¹⁾

Barnothy⁽⁴⁾ has performed experiments which indicated biomagnetic effects on activity and food consumption in mice. In one experiment he subjected ten, 70-day-old female C₃H-strain virgin mice for 4 weeks to a homogeneous field of 4200 Oe. Two-hundred twenty days after removal of the exposed mice from the field and the 30 controls from the dummy magnets, the activity of the mice was continuously monitored from the age of 320 days to 509 days. The mean activity of the magnetically treated mice was (36.3 ± 4.5)% higher than that of the untreated mice. Barnothy also monitored the weekly food consumption and noted that for the magnetically treated mice the food consumption was (26 ± 1)% lower than for the controls.

* This work was supported by the General Dynamics Corp. IRAD program.

Activity data from Jennings and Ratner⁽⁵⁾ revealed no significant difference in activity of mice during exposure to a 200- to 3500-Oe magnetic field and the controls.

The purpose of this study was to detect possible biomagnetic effects on activity and food-water consumption in small animals.

MATERIALS AND METHODS

The T-shaped Lucite cage (see Fig. 1) was designed and constructed to facilitate the investigation on possible effects of a permanent magnetic field of 1100 Oe on activity and food-water consumption by white mice. *M* denotes the permanent magnet and *D* the plaster dummy made to simulate the permanent magnet. Both *M* and *D* were wrapped in electrical tape and sprayed with black enamel paint to prevent the animal from being repelled or attracted to either *M* or *D* because of a difference in color or odor. The entire apparatus was covered with an opaque light-tight black box, not shown in the figure, during an experimental run to minimize extraneous distractions.

Temperature and humidity at the *M* and *D* ends were continuously recorded with two Belfort Hygro-Thermographs. The temperature was measured within $\pm 0.25^{\circ}\text{F}$ and the percent relative humidity between $\pm 0.25\%$.

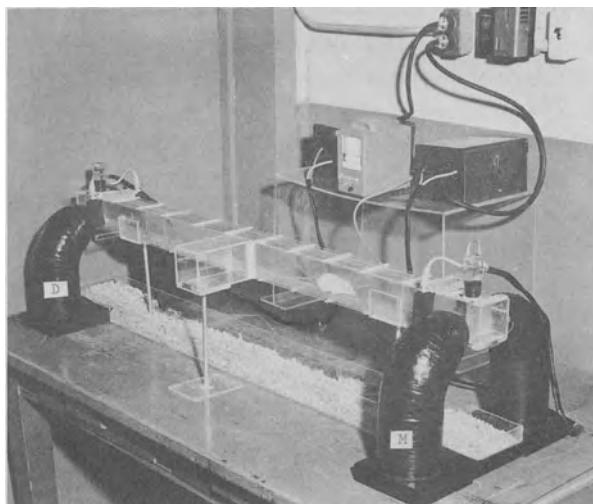


Fig. 1. Lucite cage with photocell system, permanent *M* and dummy *D* magnets.

The range of temperatures was 72–75°F in the boxes and the relative humidity 30–32%. Whenever a change in temperature and/or humidity occurred, it occurred at the same time at both *M* and *D*. Measurements showed that there was no temperature or humidity gradient between one end of the box and the other. Identical removable food and water containers were located between the pole faces of *M* and *D*.

A photocell system placed adjacent to the pole faces of both *M* and *D* was connected to a strip chart recorder mounted on the shelf behind the activity cage and outside of the opaque black box.

The test animals, white albino Swiss female mice approximately 12 months old, were placed individually into the T-cage through the door which is located equidistant from *M* and *D*. Each of the 11 test animals was subjected to four different configurations of the apparatus as presented in Fig. 2. In each configuration the entry door or *D* and *M* are reversed. Each of the test animals was monitored in each of the four configurations for 22 hr. The mice were not deprived of either food or water prior to being placed into the experimental set up. A mouse was placed in the T-cage, for example, at 11 a.m., Monday, and removed at 9 a.m., Tuesday. Thereafter the dummy magnet and magnet were exchanged and the same mouse placed back in the T-cage at 11 a.m., Tuesday, until 9 a.m., Wednesday. Then, the cage was turned 180° for the Wednesday-to-Thursday run and finally dummy magnet and magnet were exchanged for the Thursday-to-Friday run. At the beginning and end of each run the food was weighed and the volume of water was measured to determine consumption rates. No record was made on animal weights for this study.

Activity was recorded on four of the animals during the last 16 runs. Activity was detected by the photocell system shown in Fig. 1. Activity is expressed as the total number of trips each animal made to *M* or *D* and also as the length of time, in minutes, the animal remained at either location.

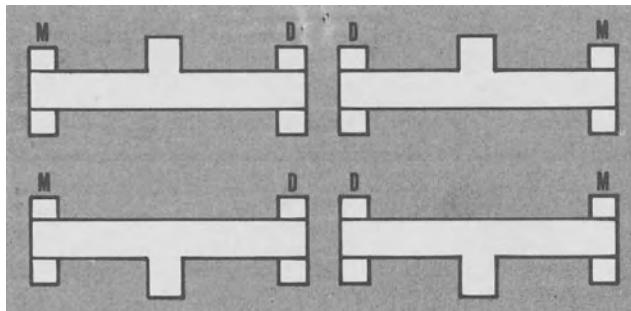


Fig. 2. Schematic representation of the four T configurations.

TABLE I
Food and Water Consumption by Mice in a High Magnetic Field Environment M Versus a Low Magnetic Field Environment D

Animal number	T configuration	M_f , g	D_f , g	$F = M_f - D_f$	M_w , ml	D_w , ml	$W = M_w - D_w$	Totals
1	$D \perp M$	3.869	0.723	3.146	1.86	8.86	($M_f = 12.627$)	
	$M \perp D$	2.141	0.408	1.733	2.85	2.298	$D_f = 2.298$	
	$D \top M$	2.428	0.670	1.758	3.91	1.19	$M_w = 16.31$	
	$M \top D$	4.189	0.497	3.692	5.71	1.55	$D_w = 7.45$	
2	$D \perp M$	1.104	1.122	0.853	0.79	2.04	1.58	$M_f = 4.688$
	$M \perp D$	1.050	0.696	0.354	2.20	0.56	$D_f = 3.835$	
	$D \top M$	1.643	0.925	0.718	2.29	1.26	$M_w = 6.69$	
	$M \top D$	0.891	1.092	-0.201	1.41	1.25	$D_w = 5.11$	
3	$D \perp M$	1.198	0.914	0.343	2.90	2.03	2.23	$M_f = 3.977$
	$M \perp D$	0.838	0.964	-0.126	2.41	3.00	$D_f = 3.634$	
	$D \top M$	1.239	0.518	0.721	5.25	1.40	$M_w = 13.38$	
	$M \top D$	0.702	1.238	-0.536	2.82	4.72	$D_w = 11.15$	
4	$D \perp M$	2.546	0.705	1.841	3.079	2.56	3.27	$M_f = 6.115$
	$M \perp D$	0.801	0.863	-0.062	2.85	0.98	$D_f = 3.036$	
	$D \top M$	1.462	1.033	0.429	1.79	2.90	$M_w = 9.55$	
	$M \top D$	1.306	0.435	0.871	2.35	2.01	$D_w = 9.16$	
5	$D \perp M$	1.475	1.026	0.387	8.95	2.68	8.16	$M_f = 4.518$
	$M \perp D$	1.605	1.612	-0.007	4.22	3.69	$D_f = 4.131$	
	$D \top M$	0.499	0.194	0.305	1.29	0.46	$M_w = 19.02$	
	$M \top D$	0.939	1.299	-0.360	4.56	4.03	$D_w = 10.86$	
6	$D \perp M$	1.028	1.817	0.261	1.66	2.84	2.95	$M_f = 4.279$
	$M \perp D$	1.035	1.212	-0.177	2.02	1.70	$D_f = 4.018$	
	$D \top M$	0.982	0.143	0.839	2.66	0.68	$M_w = 9.45$	
	$M \top D$	1.234	0.846	0.388	3.11	1.28	$D_w = 6.50$	

TABLE I (continued)

Animal number	T configuration	M_f , g	D_f , g	$F = M_f - D_f$	M_w , ml	D_w , ml	$W = M_w - D_w$	Totals
7	$D \perp M$	1.570	1.420	1.143	4.05	5.18	4.05	$M_f = 4.813$
	$M \perp D$	0.560	0.416	1.29	0.56	2.21	$D_f = 3.670$	$D_f = 3.670$
	$D \top M$	1.540	0.928	3.80	4.84	1.98	$M_w = 13.98$	$M_w = 13.98$
	$M \top D$	1.143	0.906	1.227	3.10	2.07	$D_w = 9.93$	$D_w = 9.93$
8	$D \perp M$	2.058	0.649	1.421	2.13	1.43	$M_f = 4.299$	$M_f = 4.299$
	$M \perp D$	1.238	0.483	0.755	1.73	1.46	$D_f = 3.072$	$D_f = 3.072$
	$D \top M$	0.424	0.932	-0.508	2.42	0.94	$M_w = 9.38$	$M_w = 9.38$
	$M \top D$	0.579	1.008	-0.429	2.43	3.42	$D_w = 5.90$	$D_w = 5.90$
9	$D \perp M$	0.788	0.824	-0.036	2.14	2.15	$M_f = 7.120$	$M_f = 7.120$
	$M \perp D$	3.549	0.950	2.599	6.63	2.86	$D_f = 4.657$	$D_f = 4.657$
	$D \top M$	0.658	1.198	-0.540	1.56	5.35	$M_w = 16.22$	$M_w = 16.22$
	$M \top D$	2.125	1.685	0.440	5.89	2.44	$D_w = 12.80$	$D_w = 12.80$
10	$D \perp M$	1.022	0.861	0.161	0.558	2.36	$M_f = 2.680$	$M_f = 2.680$
	$M \perp D$	0.553	0.330	0.223	1.94	1.06	$D_f = 2.122$	$D_f = 2.122$
	$D \top M$	0.537	0.552	-0.015	1.90	2.03	$M_w = 7.92$	$M_w = 7.92$
	$M \top D$	0.568	0.379	0.189	1.72	1.19	$D_w = 6.07$	$D_w = 6.07$
11	$D \perp M$	0.878	0.851	0.083	2.94	0.81	$M_f = 2.856$	$M_f = 2.856$
	$M \perp D$	0.498	1.179	-0.681	1.87	0.93	$D_f = 2.773$	$D_f = 2.773$
	$D \top M$	0.972	0.563	0.409	1.99	2.42	$M_w = 8.85$	$M_w = 8.85$
	$M \top D$	0.508	0.180	0.328	2.05	2.34	$D_w = 6.50$	$D_w = 6.50$
				$\bar{F} = \frac{\Sigma F}{10} = 1.040$	$\bar{W} = \frac{\Sigma W}{11} = 3.57$			
				$\bar{F} + S.E.* = 1.04 \pm 0.29$	$\bar{W} + S.E.* = 3.57 \pm 0.76$			
				Probability level	1 : 170			
						1 : 150		

* S.E., standard error.

RESULTS

Table I contains data on food (g) and water (ml) consumption where M_f is the food consumed in the magnetic environment; M_w is the water consumed in the magnetic environment; D_f is the food consumed in the nonmagnetic environment; and D_w is the water consumed in the nonmagnetic environment.

The total food and water intake given in column six, Table I, indicates that each of the 11 test animals consumed more food and water in the magnetic field environment than out of the field. However, in some cases the difference was small.

Table II shows the activity data for mice numbered 8, 9, 10, and 11. The data on total time at M or D show that the animals spent more time at M during half of the runs and at D during the other runs. A total of 2130.8 min were spent at D as compared to 1839.5 min at M .

TABLE II
Activity of Mice in a High Magnetic Field Environment M Versus a Low Magnetic Field Environment D

Animal number	T configuration	Total number of trips		Total time in minutes	
		Magnet M	Dummy D	Magnet M	Dummy D
8	$D \perp M$	1790	1123	58.6	391.1
	$M \perp D$	1694	924	92.3	43.1
	$D \top M$	267	522	17.5	12.0
	$M \top D$	1686	889	712.2	46.6
9	$D \perp M$	1343	1048	63.5	33.7
	$M \perp D$	1425	1107	82.1	256.0
	$D \top M$	1917	1346	79.9	40.3
	$M \top D$	1801	1739	111.9	365.4
10	$D \perp M$	1256	1163	48.7	161.9
	$M \perp D$	526	1685	79.3	428.5
	$D \top M$	2410	628	203.8	20.5
	$M \top D$	1292	2130	120.2	178.5
11	$D \perp M$	1025	552	28.3	18.4
	$M \perp D$	638	919	26.5	92.3
	$D \top M$	557	626	17.0	17.4
	$M \top D$	614	643	97.7	25.1
Totals		20,241	17,044	1839.5	2130.8

The animals showed more activity (total trips) to the high magnetic environment *M* than to the low magnetic environment *D* on 10 of the 16 runs. The total number of trips for all four animals showed that 20,241 trips were made to *M*, compared to 17,044 trips made to *D*. The average time per trip at *M* was 5.5 sec as compared to 7.5 sec at *D*.

CONCLUSIONS

An apparatus was designed and constructed to study the activity and food-water intake as influenced by a permanent magnetic field of 1100 Oe as compared to a low magnetic field environment.

The results showed that all eleven animals used in this study had increased food and water intakes in presence of the high magnetic field environment as compared to the low-field environment. The animals spent more time (2130.8 min) in the low magnetic field environment as compared to 1839.5 min in the high magnetic field environment. As a measure of animal activity, it was found that the animals made 20,241 trips to the high magnetic field environment compared to 17,044 trips to the low-field environment.

The results of this study indicate that the animals showed a preference toward increased activity and increased food-water intake in the high magnetic field environment. Additional numbers of animals and experimental runs on animals confronted with the same environments are needed to further verify this preference. It is also recommended that similar experiments should be conducted on higher mammals.

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VECTORCARDIOGRAM AND AORTIC BLOOD FLOW OF SQUIRREL MONKEYS (*Saimiri sciureus*) IN A STRONG SUPERCONDUCTIVE ELECTROMAGNET*

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INTRODUCTION

In a previous study by Beischer and Knepton,⁽¹⁾ wherein squirrel monkeys were exposed to magnetic fields up to 70,000 Oe, a striking increase in the *T*-wave amplitude of the electrocardiogram (ECG) was observed and found to be proportional to the field strength, with a value of 0.05 mV per 10,000 Oe. An influence of the magnetic field on the repolarization of the heart was advanced as a tentative interpretation of this observation. Recently, Togawa *et al.*⁽²⁾ showed, in experiments with rabbits in a field of 10,000 Oe, that the electromotive force (emf) of blood flow was superimposed on the ECG. The emf of flow was small, but at least two peaks were observed between the *S* wave and the end of the *T* wave of the ECG. The connection of this potential with blood flow was demonstrated by reversal of the sign of the flow emf at inversion of the polarity of the magnetic field. In the present study with squirrel monkeys, the use of a superconductive magnet with high field strength and application of vectorcardiographic leads furnished much stronger and clearer signals than those of the previous studies.^(1,2) The observed increase of the *T* wave has thus been confirmed to be a superimposition on the ECG of the emf generated by blood flow, as first suggested

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The animals used in this study were handled in accordance with the "Principles of Laboratory Animal Care" established by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences, National Research Council.

by Togawa. The magnetic method provides, in a single record, information on the electrical and mechanical activity of the heart and represents a new, noninvasive method of studying cardiac performance.

APPARATUS AND PROCEDURE

A superconductive magnet built by Radio Corporation of America was made available by the Magnetics and Cryophysics Branch, National Aeronautics and Space Administration, Lewis Research Center, Cleveland, Ohio,⁽⁸⁾ and was operated by personnel of that center. The large snow-covered cylindrical container, the nitrogen Dewar, enclosed the helium Dewar with the magnetic coil (Fig. 1). The magnet itself was about 13.5-in. high, with an outside diameter of about 20 in. and an inside bore of 6 in. It consisted of 22 modules, or subcoils, and energy sinks, and allowed

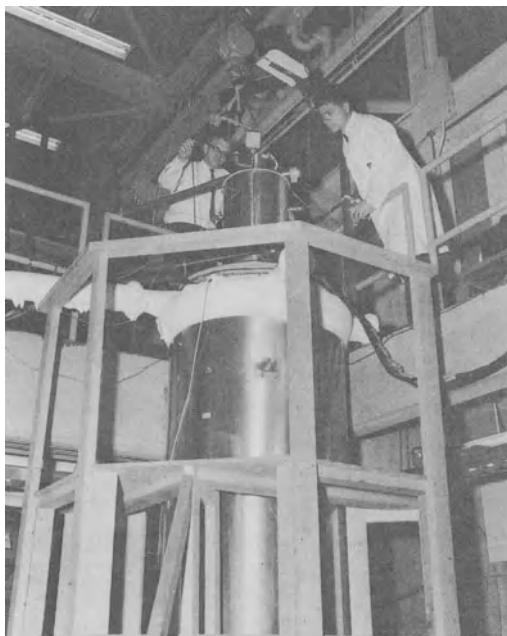


Fig. 1. RCA superconductive electromagnet at Lewis Research Center, Cleveland, Ohio. Outside Dewar is snow-covered on top. Operators look at opening of inside Dewar.

generation of a maximum field strength of 150,000 Oe in the center of the coil; up to 100,000-Oe fields only were used in the present experiment. To generate a field of this dimension and strength in a water-cooled magnet requires several megawatts of power and extensive cooling facilities. The field-distribution curve along the axis of the superconductive magnet had a bell-shape form, with the field strength constant to less than 1% in a space of 1 in.³, cocentered with the center of the magnetic field. A finger Dewar of 4.5-in. inner core diameter provided access at room temperature to the magnetic field. The maximum of the field was located 90.5 in. below the top of the finger Dewar. An automatically regulated heating coil in the lower part of the finger Dewar held the temperature in the experimental space constant at 25°C.

Two healthy, adult squirrel monkeys, gothic arch race *Saimiri* from Leticia, Brazil, held at the Institute colony for more than a year prior to the experiments, were used as test animals. Their body weights at the time of the tests were 600 g (#72) and 708 g (MJ), respectively. Electrode placement followed the system described by Frank⁽⁴⁾ for human clinical vectorcardiography (Fig. 2). The electrodes were small silver cups of 6 mm diameter

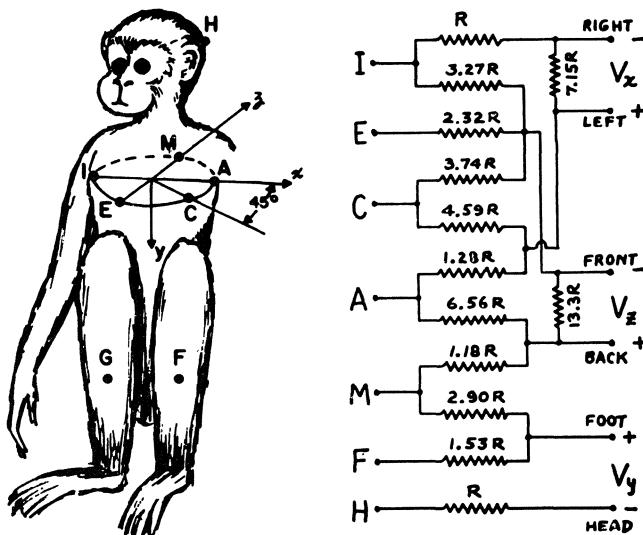


Fig. 2. Frank lead system of vectorcardiography as applied to squirrel monkey. The same resistor proportions with reference to $R = 100,000 \Omega$ as proposed by Frank for man were used for the monkey. Letters *A* through *M* designate Frank electrodes, and their placement is indicated on the depicted monkey. (Figure adapted from Frank⁽⁴⁾).

(Grass EEG electrodes). A drop of solder was applied to the concave side and shaped to form a knob which fitted in the holes of a rubber band. A small plastic sponge, freshly soaked with Sanborn Redux electrode paste, was placed in the cup of the electrode. The animals were prepared by shearing a band of pelt around the chest, the lower extremities between knee and ankle, and the dorsal part of the head. The five electrodes with Frank designations *I*, *E*, *C*, *A*, and *M* were placed in proper position by insertion of their knobs into the holes of the rubber band fastened around the thorax of the animal below the nipples. The remaining three electrodes were placed on the left leg *F* (between knee and ankle), right leg (ground), and on the head *H*, and fixed by adhesive tape. Great care was taken in placement of the electrodes since, due to the small size of the animal, small variations might have introduced errors in the measurements. During placement of the electrodes the nonanesthetized animal was held by an assistant, then placed into a contour-fitted Fiberglas container 11 in. long and $3\frac{7}{8}$ in. in diameter, and secured by Velcro bands. The container with the animal was attached to a long wooden rod which was used to insert the animal into the magnet and as a solid support for the leads. The leads were shielded by grounded nonferromagnetic metal shields extending nearly all the way from the electrodes to the lead selector, leaving unshielded only 4-in. lead sections close to the electrodes. To reduce artifacts the leads were tightly fastened to the container and to the insertion rod. An effort was made to avoid loops in the leads in order to reduce artifacts due to movement of the leads in the strong magnetic field.

The leads were connected to a Frank Lead Selector (Model 461-162B Sanborn, Waltham, Mass.). A Sanborn High Gain Amplifier (350-2700) and a Visicorder Oscillograph (Model 1108 Honeywell, Denver, Colorado) were used to amplify and record the biosignal. After calibration of the three channels with a 1-mV test signal, a control vectorcardiogram was recorded with the animal outside the magnetic field. The monkey was then inserted into the field by lowering it at a velocity of about 1 in./sec into the magnet operated at the desired field strength. The heart of the monkey was located in the 1-in.³ space of homogeneous field strength. After the vectorcardiogram was recorded the animal was retracted from the field and another measurement made. Changes of the field strength of the superconductive magnet could be made only slowly, and measurements at the three field strengths used in the experiment (40, 65, and 100 kOe) were performed on three successive days, using the two squirrel monkeys as experimental subjects each day in at least five insertions each. Samples of tracings recorded with this procedure are shown in Fig. 3A (control) and

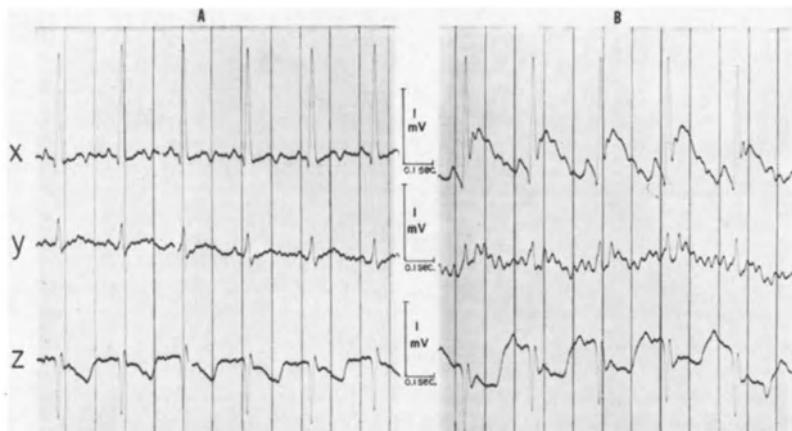


Fig. 3. Segments of vectorcardiogram (Frank lead system) of squirrel monkey (#72). (A) Control outside the magnetic field. (B) Animal in magnetic field of 100,000 Oe.

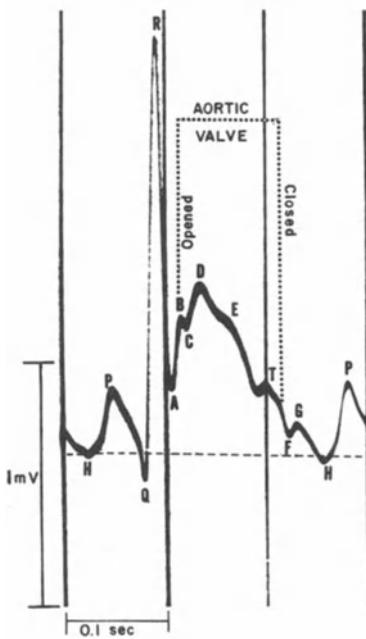


Fig. 4. Single complex of X tracing of frontal plane electrocardiogram (single points P, Q, R, S, and T) with superimposed flowgram. (See text for explanation of points A through H).

3B (magnetic field of 100,000 Oe). Details of the third complex of the *X* tracing in Fig. 3B are seen in Fig. 4.

The heart rate was determined from the ECG by counting the number of *QRS* complexes falling within 3 sec, and respiratory rate was derived from the frequency of shift of the base line. Figure 3A and B show about one respiratory cycle each.

From the three values of the *X*, *Y*, and *Z* traces of the vectorcardiograms (Fig. 3A and B) triaxial displays of ECGs were constructed and used to determine peak magnitudes and direction of the *T* wave as well as the flow vector.

The dimensions and the anatomic position of the aorta were determined by two different procedures. Open-chest caliper measurements of the aorta were made in the deeply anesthetized animal. These measurements were supplemented by aortograms photographed after injection into the heart of 0.1 Hypaque-M 75% Brand (Winthrop Laboratories, New York, New York). An aortogram of a squirrel monkey (nonexposed subject weighing 710 g) is presented in Fig. 5. The caliper measurements were performed in two and the aortograms in four nonexposed monkeys. These six monkeys were comparable in weight and size with the animals used in the magnetic exposure experiments.

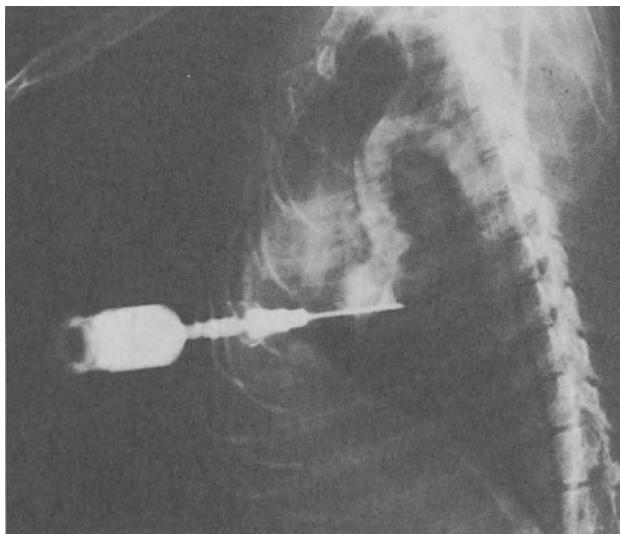


Fig. 5. Normal aortogram (sagittal plane) of a squirrel monkey (prepared by H. H. Khalil). Distance from root of aorta to peak of aortic arch was 2 cm.

RESULTS

Even a casual glance at the tracings presented in Fig. 3 revealed major effects of the strong magnetic field on the ECG, visible clearly in the complex following the *QRS* wave. The *T* wave was completely overshadowed by the strong signal generated by the aortic blood flow in the magnetic field. Subtraction of the control values from the field values, using a single tracing like the *X* tracing in Fig. 3 allowed characterization of the flow potential in great detail.

The characteristics of the flow potential were especially well-manifested in the *X* tracing in Fig. 3B, as compared to the control tracing (Fig. 3A) where the complex following *QRS* deviated little from the baseline. The flow signal had a fast ascending and a gradually descending branch, characteristic of graphs for the aortic blood velocity as measured by a flowmeter on the aorta. The vector of the flow potential during its total duration was roughly pointing in the direction of the *+X* axis with a slight anterior inclination. This direction was predominant in both exposed animals and was observed in all experiments at the different field strengths.

The maximum scalar value of the flow potential was linearly related to the strength of the magnetic field. Maxima of flow potentials measured in fields of 40,000, 65,000, and 100,000 Oe are plotted in Fig. 6. These potentials were considerably influenced by the respiratory cycle; the values plotted in Fig. 6 represent the mean of the highest value measured at each field strength in 25 respiratory cycles during inspiration and the mean of the lowest values in the same number of cycle measured during expiration. The respiratory periods are well defined by the baseline shifts as seen best in the *Z* trace of Fig. 3B. A respiratory rate of about 50 to 60 cycles was observed in the control measurements; this rate remained the same during exposure of the animals to the different magnetic field strengths.

Superimposed on the ascending and descending branch of the flow potential, some interesting details were noticed. At certain time intervals the *X*, *Y*, and *Z* tracings showed well-defined peaks and notches (Fig. 3B) which carry letters from *A* through *H* in the single complex seen in Fig. 4. Their recurrence at regular time intervals in cycle after cycle was so typical that these singular points of the potential curve must represent characteristic events in the course of the blood flow. The notches in the flow curve were seen in the records of both monkeys at all field strengths studied. Table I presents a characteristic example of the time intervals between singular points of Fig. 4.

These time intervals were determined with an accuracy of about 1 msec.

TABLE I
Time Intervals Between Lettered Events in Fig. 4

Events	Interval, sec
<i>A</i> to <i>B</i>	0.010
<i>B</i> to <i>C</i>	0.005
<i>C</i> to <i>D</i>	0.015
<i>D</i> to <i>E</i>	0.030
<i>B</i> to <i>F</i>	0.110
<i>F</i> to <i>G</i>	0.007
<i>Q</i> to <i>Q</i>	0.240 (heart rate 250)
<i>Q</i> to (<i>S</i>)	0.028

The accuracy of measurement was especially high at points where the curve changed abruptly (points *A*, *B*, and *C*) and was limited by the width of the recording trace.

The configuration of the electrocardiograms of squirrel monkeys in strong magnetic fields was so much distorted by the flow potential that values for some complexes and intervals were difficult to determine. The following results will provide material for discussing the question of whether the flow potential is the only factor modifying the ECG tracings, or if a direct influence of the strong field on the electrical processes in the heart can be demonstrated.

In the present study the exposure period to the high magnetic field was so short, less than 15 min, that the slowly developing decrease in the heart rate and increase in the degree of sinus arrhythmia which were previously described⁽¹⁾ were not noticed. Heart rates of the two squirrel monkeys ranged between 240 and 280. Because the rate was not always the same in the control and experimental measurements, the rate should be considered in comparison of complexes and intervals of the ECGs.

The *P* wave in the control record (Fig. 3A) had a small amplitude of about 0.1 mV in the *X* trace and was scarcely noticeable in the other two traces. Thus, the *P* vector extended mainly in the + *X* axis, with a slight inclination towards the + *Y* axis. The duration of the normal *P* wave in the squirrel monkey was about 0.02 sec, and the *P-R* interval in the control measurement was about 0.045 sec.

The *P* wave of the monkeys in the strong magnetic field (Fig. 3B) appeared to be influenced by the field, as the amplitude and duration showed variations with respiration. In expiration the amplitude of *P* was similar

to that of control conditions (0.1 mV) and increased during inspiration to about 0.3 mV in the *X* tracing. The direction of the vector of the *P* wave was not changed from that of control conditions. The duration of the wave varied between 0.02 and 0.05 sec, and the *P-R* interval between 0.045 sec in expiration and 0.06 sec in inspiration. The *P* wave in the magnetic field was superimposed on a stronger flow signal that started to increase earlier than the control *P* wave and lasted longer. This flow signal was most likely connected with venous return and the filling of the atrium. It was directed in the + *Z* axis of the animal (posteriorly).

The *QRS* complex in the control tracing (Fig. 3A) had an average duration of 0.035 sec. The electric axis of the *QRS* complex was directed to the left (+ 30°), anteriorly, indicating a vertical heart position. The strongest signal was observed in the *X* tracing (+ 1.6 mV); next in intensity was the signal in the *Z* tracing with - 0.8 mV, and then the signal in the *Y* tracing (+ 0.35 mV). In the strongest field (100,000 Oe) (Fig. 3B) neither duration, axis, nor amplitude of the *QRS* complex was changed. This observation holds true for both animals at all field strengths tested and is in agreement with earlier results.⁽¹⁾

The axis of the *T* wave in the control measurement (Fig. 3A) was directed anteriorly (+ 90°) and slightly inferiorly (10°). Amplitude of the *T* in the *Z* tracing of Fig. 3A was 0.3 mV. The *S-T* segment was about 0.03 sec, and the *Q-T* interval was 0.15 sec.

The *T* wave in the strongest magnetic field (Fig. 3B) is seen as a peak superimposed on the flow potential curve and is marked in Fig. 4 as such. The three traces in Fig. 3B demonstrate that the *T* wave was directed anteriorly as in the control tracing and had probably the same amplitude, but accurate measurements were not possible due to the predominant flow potential. The *S-T* segment could not be determined, but the time interval from *Q* to *T* (peak) could be measured with good accuracy and was the same as in the control record.

Notch *F* and peak *G* in Fig. 4 were most probably connected with the closing of the aortic valve. The *Q-F* interval was about 0.16 sec (average of 10 measurements), and the time interval from point *B* to point *F* (points representing opening and closing of the aortic valve, respectively), 0.11 sec.

Aortogram measurements in three monkeys showed a mean diameter of the ascending branch of the aorta close to the root of 5 mm. The mean length of this branch from the root to the culmination of the aortic arch in the same animals was 20 mm. These measurements hold true for the squirrel monkey with a body weight of about 700 g.

DISCUSSION

Superconductive Magnet in Electrophysiology

A superconductive magnet was used for the first time in VCG studies of primates in very high magnetic fields. Squirrel monkeys had previously been exposed to very high magnetic fields in modified Bitter magnets,^(1,5) but experimental difficulties were experienced in ECG and EEG measurements. Ripple current from the generator and mechanical vibration were recognized as artifact producing factors. A certain amount of ripple current could not be avoided in a magnet of low inductance supplied by a mechanical generator and it interfered with electrophysiological measurements. The strong stream of cooling water in the Bitter magnet caused vibrations which moved the magnetic field relative to a stationary subject and probably exposed it to an alternating magnetic field. The enormous power consumption in the megawatt range characteristic of the Bitter magnet limited the exposure period to economically feasible time periods and prohibited long-term physiological observations. These adverse factors were absent in experiments with the superconductive magnet. Such magnets can be operated free of ripple current and vibration and need only comparatively small power generators. During operation in the persistent mode the superconductive magnet does not need an outside power supply, although the cost of liquid helium which can be reduced by a regeneration facility may be a limiting factor for extended operation. All things considered, the use of superconductive magnets in generation of high magnetic fields for electrophysiological experimentation is highly recommended.

Difficulties which are inherent to electrophysiological measurements in strong magnetic fields remain to be solved. The electrical leads in connection with the animal body formed a conductor loop, and a change of the magnetic flux density in this loop induced an electromotive force in the conductor. This force is proportional to the flux and to the mode of change of the loop area; in a field of 100,000 Oe an area change of 1 cm² will induce about 1 mV in the conductor loop. It was expected that respiratory, cardiac, and other movements would open and close the conductor loop to a certain extent. To counteract the effect of such movements the lead wires were placed as parallel as possible to the magnetic flux and solidly attached to the animal container and to the insertion rod. An estimate of the electromotive force induced by change of the chest diameter during respiration could be made by comparing the baseline amplitudes in the field with the corresponding control data. The maximum amplitude in the

field was less than 0.1 mV larger than the maximum control amplitude. Cardiac action through chest-diameter change was expected to induce a still smaller voltage in the lead loop. Thus, under the given experimental conditions the electromotive force produced by motion of the lead conductor in the magnetic field may be disregarded at least as far as the ECG is concerned.

Frank Lead System for Small Primates

Application of the Frank⁽⁴⁾ lead system for spatial vectorcardiography on squirrel monkeys needs some comment. This lead system with orthogonal image vectors projected onto anatomical body axes was developed specifically for the torso shape of man and has found extensive clinical use. Scaling this system down to a small primate like the squirrel monkey would require careful analysis, and the experiments of Frank should have been repeated with an accurate three-dimensional homogeneous torso model of the squirrel monkey. However, such an extensive investigation was not feasible within the limits of the present study; therefore, it was decided to apply the Frank lead system in its present form to the squirrel monkey and judge its merits from the results. The record sample in Fig. 3A of a normal squirrel monkey indicated that the *QRS* complex as well as the configuration of the *P* and *T* wave was similar to records received from normal man in a sitting position, and no gross distortions of amplitude or vector direction were noted.

Theoretically, no flow potential should have been recorded in the *Y* tracing since the body axis was parallel to the direction of the magnetic field. However, the recording of the *Y* trace (Fig. 3B) shows a trace which at first glance appears to contain electronic interference but which actually registers events also seen in the other two traces. This is a clear indication that the resistor proportions selected by Frank for man should have been corrected for use in the squirrel monkey. Later discussion will show that the vector of the flow potential derived from the *X* and *Z* tracings had, in general, the expected spatial orientation in relation to the known orientation of the aorta, which justifies the application of the Frank lead system in monkeys in first approximation. Incidentally, the use of a strong magnetic field would also allow a sensitive test of the validity of the selection of resistors in the Frank lead system in its use for measurement of the vectorcardiogram in man. If the resistors have been selected correctly no flow signals should be observed in a tracing with its axis parallel to the field direction.

Interpretation of Flow Potential

General Principles

The electromotive force registered immediately following the *QRS* complex was generated by blood flowing in the presence of a magnetic field. The vector of this electric field is directed perpendicularly to the direction of the magnetic field as well as the direction of the movement of the blood. The polar direction of the emf is found by applying the right-hand rule. In the present study the magnetic field was applied parallel to the long axis of the animal; thus, all blood flow in the direction of the animal long axis did not generate an emf, and only the component of blood flow directed perpendicularly to the magnetic field contributed to the emf. In the case of the aorta, an emf was generated by blood flowing through the curving aorta to the degree to which the ascending branch deviated from the direction of the body axis. The maximum was reached when the blood passed the peak of the arch where the flow is perpendicular to the magnetic field. As the descending branch approached a direction parallel to the field and the body axis, the electrical signal diminished and reached zero when complete parallelism was achieved.

In a discussion of the flow signal it should be kept in mind that the technique of using external electrodes rendered at any given time the vector sum of the flow potentials generated throughout the body. Under certain conditions branching flow, as in the pulmonary arteries, will generate in the branches electromotive forces which are equal in intensity but opposite in sign, and the two signals will cancel at some distance from the sources. No signal from such branching flow was expected to reach the external electrodes. This distinguished the present experimental method from the magnetic flowmeter in which a cuff is applied with electrodes positioned locally on the vessel wall and in which the flow in this specific vessel is determined by measurement of the emf generated in a magnetic field.

The form of the external flowgram (Figs. 3B and 4) resembled the well-known left ventricle ejection curve with a steeply ascending and gradually descending branch. Not much can be learned from the rough outlines of this curve. However, the peaks and notches which appear in great regularity in the *X* and *Z* tracings (Fig. 3B) offer most interesting information and a challenging new approach to a discussion of the electrical and mechanical events in cardiac activity.

Cardiac Blood Flow During Isometric Contraction

The *QRS* complex was not quite completed at point *A* (Fig. 4) when the flow potential started to rise. For all practical purposes, point *A* cor-

responded on the time-scale to point *S* of the ECG, as an extension of line *R-A* to the baseline demonstrates. The potential rose steeply in 10 msec until point *B* was reached. It is postulated that this part of the curve (*A-B*) indicates movement of the blood in the heart during the isometric cardiac contraction and that point *B* indicates the opening of the aortic valve.

The period *A-B* (10 msec) represents 4% of the total time of one cardiac cycle of the squirrel monkey (240 msec). In man the aortic valve opens about 30 msec after point *S* of the ECG, which is 3.75% of the total cardiac period (800 msec). Thus, it appears justified to associate point *B* of the flowgram with the opening of the aortic valve.

Other explanations of the discontinuity of the flowcurve at point *B* were considered and discarded. Branching of the blood stream at the root of the truncus brachiocephaliens, which is parallel to the magnetic field, would reduce the flow signal at point *B*. However, the branching point is anatomically so closely approximate to the peak of the aorta that the time period of 20 msec appears too long for movement of the pressure wave between these two landmarks (*B* and *D*). Furthermore, extension of the line from *B* to beyond *A* to the isoelectric line would place the starting point of the flow emf and thus the opening of the aortic valve well into the *QRS* complex. Opening of the aortic valve in the squirrel monkey at such an early time in the cardiac cycle appears most unlikely.

The electromotive force vector during the period of isometric heart contraction (*A-B* in Fig. 4) was directed anteriorly and to the left of the animal, indicating that the blood in the heart during this period had a component of movement directed to the left and posteriorly. This is about the same direction as that found for the blood flow in the aorta. Under the assumption that a general cardiac contraction was in the direction apex to base during the isometric phase, most of the blood flow would be directed in the body axis and would not be registered in the magnetic field. A lateral component would have been expected to register to the right and not to the left as was actually seen.

The concept of an absolutely isometric contraction of the ventricle during the first part of ventricular systole was clarified by Rushmer's experimental work on systolic deflections of cardiac walls. Rushmer⁽⁶⁾ found that "a considerable quantity of blood must be displaced within the enclosed ventricular cavity to produce these large early systolic deflections." It is this displacement of blood which is most probably recorded as a sharp upstroke of the *A-B* segment in Fig. 4. While cardiac pressure and displacement measurements have been made numerous times during the period of "asynchronous contraction,"⁽⁶⁻⁸⁾ actual flow of blood in the

heart during this period could be demonstrated by the present method for the first time.

It should be noted in Fig. 4 that the flow signal did not drop to zero before the aortic valve opened at point B; thus, the blood flow in the heart during asynchronous contraction continued into the aorta. This observation opposes the presently accepted theory of left ventricular ejection into the aorta which states that the blood starts its movement at the aortic valve from zero velocity and is accelerated rapidly by the pressure differential between ventricle and aorta. The flowgraphs of the present study suggest that a pulse wave was initiated in the ventricle during the isometric phase of contraction, reached the aortic valve, opened the valve, and passed into the aorta. Rushmer depicted ventricular ejection as a piston striking a mallet. According to that picture the piston is first struck, not at the opening of the aortic valve, but earlier in the left ventricle. Numerous flowgrams of other authors,^(7,8) which show aortic flowgraphs starting from zero, are not in contradiction with the present view since the aortic flowmeters register the passage of the pulse wave at the site of the meter. Information on the origin of the pulse wave cannot be obtained with the conventional flowmeter method. By the magnetic method using external electrodes flow of blood in the heart as well as in the large vessels is registered, and a continuous record which could be easily timed by the superimposed ECG is provided.

Arterial and Venous Blood Flow

The slight decrease of the emf in section B-C was probably caused by the entry of the blood into the ascending branch of the aorta, which was practically parallel to the magnetic field and thus would not contribute to signal generation. Narrowing of the lumen of the blood column from the diameter of the heart (about 1.0 cm) to the diameter of the aorta (0.5 cm) would also reduce the flow signal strength at point B.

The emf increased again as the blood reached the arch of the aorta and passed through a maximum at point D while the blood flowed through the highest point of this vessel. At point D the blood had a direction perpendicular to the electrical flow signal. The flow points posteriorly with a small deviation to the left, as expected from the direction of the aortic arch.

From point D (Fig. 4) the signal decreased as more and more blood passed through the descending branch. The singularity in this section at point E could not be explained. The next peak on the downward slope of the flow curve was identified as the T wave superimposed on the flowgram. The aortic valve closed, probably shortly before point F, and the steep

descent to *F* may be connected with the backflow of blood after closure of the valve. The peak of flow potential at point *G* is probably connected with blood flow in the inferior vena cava where a maximum of flow occurs in early diastole.

The flow signal superimposed on the *P* wave was most probably generated by venous return and filling of the left ventricle starting at point *H* (Fig. 4) with opening of the mitral valve and initiation of the rapid filling phase. The strong dependence of this flow signal on the respiratory cycle was probably connected to the small pressure differential between left atrium and left ventricle characteristic for this phase. An increase of pressure in the chest cavity during expiration would be expected to decrease the velocity of filling of the left ventricle.

Thus the method gives information on arterial as well as venous blood flow in addition to the ECG. The following tongue twister designation for the records may be appropriate: Electro-magneto-cardio-arterio-venogram (EMCAVG).

New Quantitative Method to Determine the Velocity of Aortic Blood Flow

Under the assumption that point *B* in Fig. 4 indicates the opening of the aortic valve, an interesting direct method of determination of the flow velocity in the ascending branch of the aorta is suggested: According to the data in Table I the pulse wave travels from point *B* to *D* in 0.02 sec and advances during this time from the aortic valve to the peak of the aortic arch. This distance was measured in aortograms of several monkeys and found to be 2 cm. Thus, the pulse wave travels 2 cm in 0.02 sec, which corresponds to a mean pulse wave velocity of about 100 cm/sec in the ascending branch of the aorta of the squirrel monkey. The results of this method were based exclusively on a time and a distance measurement, the basic components of a velocity determination. The literature offered no values of the blood velocity in the aorta of small animals of less than 1 kg weight. Spencer and Greiss⁽⁷⁾ found a range of maximum velocities from 52 to 150 cm/sec in dogs of a mean weight of 17 kg. Hence, the velocity of 100 cm/sec found in the squirrel monkey by the magnetic method fell within a reasonable range.

Flow Dipole Used as Electrical Reference Source

An equation given by Kolin,⁽⁹⁾ that is,

$$V = H \cdot d \cdot \nu \cdot 10^{-8}$$

can be used to calculate the electrical potential generated on the walls of the aorta of a squirrel monkey. With a diameter d of the aorta of 0.5 cm, a magnetic field H of 100,000 Oe and a blood velocity v of 100 cm/sec, the potential in volts was calculated as follows:

$$V = 10^5(5 \times 10^{-1}) \cdot 10^{-2} \cdot 10^{-8} = 0.05$$

With external electrodes a maximum flow potential of 0.75 mV was found experimentally (Fig. 6). Thus, the externally measured flow potential is considerably lower than the emf of 50 mV calculated by the formula of Kolin for the wall of the aorta (factor 1 to 70).

With the site of the aorta and the site of the heart about equidistant from the surface of the body, the factor 1 to 70 found in the flow measurements was used to calculate the emf at the site of the heart from the amplitude of the R wave in the frontal plane ECG (1.6 mV). A value of about 110 mV was found which is of the order of the action potential of the ventricular membranes. The principle of this approach may be of more general interest. The magnetic field in connection with pulsating blood flow allows generation of an artificial pulsating electrical dipole of known direction and strength inside the chest cavity, and other electrical activity in this cavity can be studied in reference to the flow dipole.

A comparison of the action potential and the flow potential at the site of generation, along with the corresponding values measured externally,

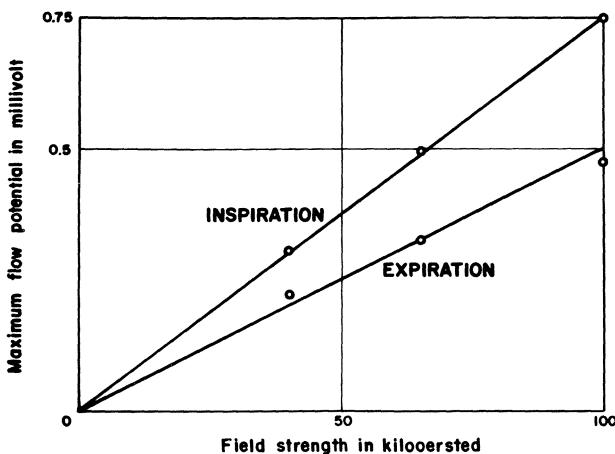


Fig. 6. Maximum flow potentials generated by the different field strengths (40, 65, 100 kOe) in the part of the aorta during inspiration and expiration.

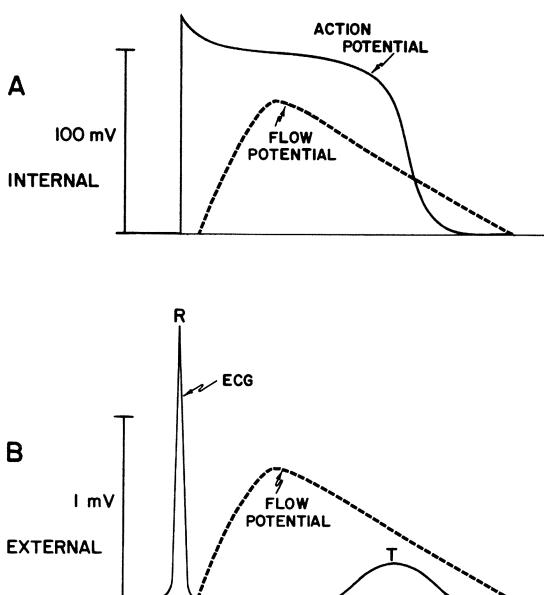


Fig. 7. Relationship between internal (A) and surface values (B) of the cardiac potential (—) and the flow potential (---).

is given in Fig. 7. It is readily seen that the emf of the flow potential has a constant relationship to the externally measured signal. The flow potential is a current-supporting source during the total period of action. The action potential is current supporting only during its periods of main change which are measured externally as the *R* and *T* wave. This schematic comparison presents nothing principally new, but it may help to understand and illustrate the relationship of the action potential to the externally measured ECG.

Possible Cardiac Stimulation by the Flow Potential

The flow potential which reached about 50 mV at its source in the heart and the large vessels, and is a current-supporting source of sufficient duration, should be considered a potential cardiac stimulus. However, no sinus arrhythmia or extrasystoles were observed in short-duration experiments with monkeys. The fact that the main part of the flow potential fell into the refractory period of the heart obviously prevented stimulation. The process of the repolarization of the monkey heart, as indicated by the *T*

wave, also was not noticeably influenced by the flow potential. However, the shape of the *T* wave could not be determined with sufficient accuracy to exclude completely a possible effect of the flow potential on repolarization.

In a previous study⁽¹⁾ where the squirrel monkeys were exposed to magnetic fields up to 70,000 Oe for a period of about 1 hr, a decrease of heart rate was observed. It appears possible that, during longer exposure to the magnetic field, the flow potential stimulated the aortic and the carotid sinus pressoreceptors which, via vagal branches and the parasympathetic system, eventually decreased the heart rate. The emf of blood flow was generated so closely to the pressoreceptors that an influence of this pulsating stimulus on the regulation of the heart rate seems possible. In future experiments an attempt will be made to study this relationship more extensively.

The experiments with squirrel monkeys were performed in preparation for a systematic study of the possible effects of strong magnetic fields on man. Even though man probably will not be exposed in the laboratory or by industrial magnets to the extreme fields used in the present study, the results obtained from studying lower primates in very strong magnetic fields should help in the evaluation of possible hazards of strong magnetic fields to humans. With regard to future human exposure it is reassuring to know that the flow potential generated in the magnetic field by cardiac and large vessel blood flow did not immediately affect the cardiac activity of the experimental animals. However, in short- and long-term human exposure to strong magnetic fields heart rate and blood pressure should be observed carefully.

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MAGNETIC SUSCEPTIBILITY OF BIOLOGICAL MATERIALS

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INTRODUCTION

Although the field of biomagnetism is a relatively young branch of science, an extensive bibliography on various aspects of the subject⁽¹⁾ has developed within recent years. If one examines this bibliography, it is evident that a rather small part of this work has been expended in obtaining fundamental quantitative magnetic data on biological materials, such as magnetic susceptibilities of tissue, cell fluids, etc. In part, this is due to the nature of the material. Biological specimens are usually very complex and variable, and hence it is difficult to attach a meaningful quantitative value to such specimens. Moreover, biological specimens generally contain considerable water and other fluids which make significant measurements more difficult to obtain than on solid samples. Nevertheless, if the science of biomagnetism is to progress, one must interpret and explain the ever-increasing number of biological effects produced by magnetic fields. Ultimately one must examine these results in the light of the magnetic properties of the constituents of tissue, cells, blood, etc., and the biochemicals which constitute them.

It is the purpose of this chapter to review the measurements of static magnetic properties of biological materials that have been made up to 1968. For the most part, these results have given us a deeper insight into the structure of the biochemical compound in question, but, as yet, the

TABLE I
Relation of the Spin-Only Moment μ , the Number of Unpaired Electrons, and the Molar Susceptibility χ_M

Number of unpaired electrons, N	S (1 electron, $S = \frac{1}{2}$)	χ_M^* (emu $\times 10^6$)	$\mu_{\text{eff}}^{\dagger}$ (theor.), B. M.	$\mu_{\text{eff}}^{\dagger}$ (obs.), B. M.	Representative metal ions
0	0	0	0	~0	Cu(I), Zn(II), Low-spin Fe(II), and Co(III)
1	$\frac{1}{2}$	1,250	1.73	1.73–2.5	Cu(II), Low-spin Fe(III), Co(II), and Mn(IV)
2	1	3,340	2.83	2.75–3.5	Octahedral Ni(II)
3	$\frac{3}{2}$	6,260	3.87	3.8–5.2	Fe(V)
4	2	10,000	4.91	4.7–5.0	High-spin Fe(II) and Mn(III), Fe(IV)
5	$\frac{5}{2}$	14,600	5.92	5.7–6.0	High-spin Fe(III) and Mn(II)

* At room temperature, $\sim 298^\circ\text{K}$.

† $\mu_{\text{eff}} = \sqrt{N}(N + 2)$.

data are far too scanty to aid in the explanation of some of the biomagnetic phenomena described elsewhere.⁽¹⁾

Most biological materials are diamagnetic, some are paramagnetic, and a few occurrences of apparent ferromagnetism and antiferromagnetism have been reported. Compounds having no unpaired electrons are diamagnetic with negative susceptibilities which are field and temperature independent. Free radicals and certain coordination compounds containing transition metal ions with unpaired electrons are paramagnetic with positive temperature-dependent, field-independent susceptibilities. Some metals and inorganic compounds have ferromagnetic and antiferromagnetic properties which exist below certain temperatures (Curie and Neel points), and often exhibit complex field and temperature variations. The comparative magnitudes of typical magnetic moments and susceptibilities are shown in Table I. Several good summary articles⁽²⁻⁵⁾ dealing with the general theory of magnetism are available. There are also a number of excellent texts on the subject,⁽⁶⁻¹⁰⁾ which describe methods of measurement.

TYPE OF INFORMATION OBTAINED BY MAGNETIC STUDIES

To ultimately understand biomagnetic phenomena, magnetic studies on biological specimens and also on purified biochemicals will have to be made. Depending on the specific problem, both kinds of information are a necessary adjunct to a fuller understanding of the science of biomagnetism. In reviewing the past work, we will make what seems to be the logical division between biological and biochemical data. It is difficult to perform valid experiments on biological specimens such as tissue or individual cells which will yield useful information. Consequently only a few experiments of this nature have been carried out. A number of studies, however, of the magnetic properties of purified biochemicals have been made. By far the greatest effort has been made on the biochemical derivatives of hemoglobin. Considerable work has also been carried out on the nucleic acids, vitamins, enzymes, etc.

Simple room-temperature magnetic susceptibility measurements of biological specimens are generally not too instructive. One can, for instance, tell if there is a paramagnetic constituent, in a tissue, say, by making susceptibility measurements before and after lyophilization. Fresh biological specimens are usually diamagnetic. If the specimen becomes less diamagnetic or paramagnetic upon removal of water, there is a good probability that a paramagnetic constituent is present. Susceptibility measurements

made on a specimen throughout a series of temperatures would be much more useful. It is difficult, however, to measure biological specimens separated from an organism throughout a series of temperatures without incurring unwanted changes in the specimen. To some extent this problem can be circumvented by fast freezing or freeze-drying a specimen prior to the magnetic measurement. True diamagnetism is temperature independent, whereas paramagnetism is temperature-dependent, and hence a paramagnetic component is more easily detected. Paramagnetic biological materials are generally considered to follow the Curie law, i.e., the paramagnetism is inversely proportional to the absolute temperature. Abnormal deviations from these relationships can often be interpreted in terms of the history of the specimen, e.g., oxygen adsorption, denaturation, etc.

Magnetic-susceptibility measurements of purified biochemicals have been shown to be much more fruitful. For instance, the chemical structure can often be ascertained through such measurements. The magnetism of diamagnetic biochemicals can generally be calculated using the methods devised by Pascal.⁽¹¹⁾ Comparison of the experimental susceptibilities with those calculated from several assumed structures can be used to infer true structure. With regard to paramagnetic biochemicals, much emphasis has been directed to the study of those compounds containing one of the transition elements, such as iron, copper, or molybdenum. There are a large number of biochemical compounds of this type whose structure comprises one or more groups having a central transition metal atom. The paramagnetism of the group will depend greatly on the position and type of bonding of this atom to the diamagnetic moiety of the molecule. For example, one of the most studied coordination complexes of this type are those compounds containing a heme group. By changing the ligands attached to the heme, such as by oxygenation or changing pH, the bonding and hence the susceptibility will usually be altered.

Finally, from a knowledge of the ion's energy levels, or in some cases where this is not experimentally known, the temperature dependence of the magnetic moment can be calculated. Very accurate, wide temperature range susceptibility data, especially on single crystals are capable of furnishing molecular symmetry and parameters, such as the zero field splitting parameter D .

It is appropriate to mention how the effective magnetic moment of a typical transition metal ion in a molecule is calculated. The observed susceptibility per gram of paramagnetic ion χ_g , corrected for the diamagnetic moiety of the molecule, is converted to the molar susceptibility χ_M by multiplying by the atomic weight of the paramagnetic ion. The effective

magnetic moment μ_{eff} , in Bohr magnetons, can then be calculated from the equation

$$\mu_{\text{eff}} = 2.84 \sqrt{\chi_M T}$$

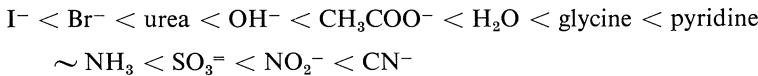
where T is the absolute temperature. This equation assumes that the Curie law holds at temperature T . For most biological specimens this is probably true at room temperature, but in certain cases the form

$$\mu_{\text{eff}} = 2.84 \sqrt{\chi_M (T - \theta)}$$

where θ is the Weiss constant is a better representation of the data.

If the orbital angular momentum is quenched, the number of unpaired electrons N is related to μ_{eff} by the relation $\mu_{\text{eff}} = \sqrt{N}(N + 2)$. The quantity χ_g should be measured at constant temperature as a function of field strength to note the presence or absence of trace ferromagnetic impurities which can lead to erroneous moments.

Many earlier workers have interpreted their results in terms of Pauling's magnetic criterion of bond type.⁽¹²⁾ Thus, the observation of five unpaired electrons in an iron compound indicated Fe^{3+} ionically bonded to its ligands, whereas one unpaired electron denoted covalent Fe(III). However, recent experimental and theoretical work⁽¹³⁾ shows that there is no definite relationship between the number of unpaired electrons found by bulk susceptibility measurements and the ionic-covalent character of the metal-ligand bond. We will adopt the usual convention that a species with the maximum number of unpaired electrons be called a high-spin (or low-field) complex, whereas observed electron pairing is termed low-spin (or high-field) compounds. This will avoid any implications of bond type which are implicit in the earlier work. The properties of metal ions and ligand atoms that give rise to various types of magnetic behavior are fairly well-known and have been extensively reviewed.⁽¹³⁻¹⁵⁾ Of particular biochemical importance is the spectrochemical series, which orders ligands in their ability to produce spin-paired or spin-free complexes. For octahedral coordination, the tendency toward spin-paired complexes generally falls in the order⁽¹⁵⁾



Following the convention of Pauling and Coryell,⁽¹⁶⁾ the molar magnetic susceptibilities and moments of iron in hemoglobin derivatives are calculated

per heme, rather than for the entire molecule. The susceptibilities are given in electromagnetic units (cgs units), the moments in Bohr magnetons, and temperatures in °K.

STUDIES OF BIOLOGICAL SPECIMENS (TISSUE, CELLS, ETC.)

Tumor and Normal Tissue

In 1960, Barnothy⁽¹⁷⁾ pointed out some of the remarkable, but inexplicable, results obtained by exposing tumor-containing animals to magnetic fields. Under certain conditions, mitosis is hindered when an animal is exposed to even moderate fields. The growth of some types of cancer cells is likewise inhibited by use of magnetic fields.⁽¹⁸⁾ To obtain some quantitative data, Senftle and Thorpe⁽¹⁹⁾ have made a comparative study of the magnetic susceptibility of transplantable hepatoma and normal liver tissue in rats (see Table II). They found that the normal tissue was less diamagnetic than either the tumor tissue or the liver tissue from the tumorous host and that this increase probably reflects the increase in water content ($\chi_{H_2O} = -0.72 \times 10^{-6}$ emu/g). Measurements on the same specimens were also made down to liquid-nitrogen temperatures, and, as shown⁽²²⁾ in Fig. 1, distinct differences were found between the normal and tumor tissue. Their initial measurements of the magnetic susceptibility of pure ice throughout the same temperature ranges strongly resembled those

TABLE II
Magnetic Susceptibility of Some Specimens of Tumor and Normal Tissue

Specimen	Number of specimens	Magnetic susceptibility (emu $\times 10^6$ /g)	Temperature °K
Normal human larynx	3	-0.57 ± 0.007	302
Tumorous human larynx	3	-0.61 ± 0.008	302
Transplanted hepatome (Morris No. 3683)	5	-0.688 ± 0.0046	263
Liver tissue from tumor-bearing rat	3	-0.670 ± 0.0012	263
Liver from normal control animals	3	-0.637 ± 0.0059	263

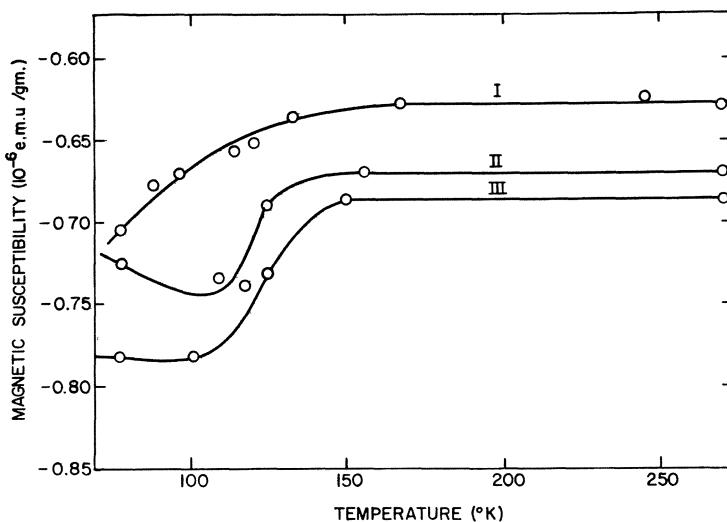


Fig. 1. Magnetic susceptibility as a function of temperature of normal liver tissue in control animal (I) and liver (II) and transplanted hepatoma Morris No. 3683 (III) from tumor-bearing rats (Ref. 22).

obtained from the tumor tissue, i.e., an increase in diamagnetism below 150°K. This led them to believe that the characteristic change in susceptibility of the tumor cells observed between 77° and 150°K was due primarily to free water in the tumor cells. The water in the normal cells, they hypothesized, was more strongly bound and hence did not freeze in the same manner as free water. Normal diamagnetism is not temperature-dependent, however, and further measurements were made of the magnetic susceptibility of ice at low temperatures in an attempt to determine some extraneous cause of the increased diamagnetism. Subsequent experiments^(21,22) showed that the increase in diamagnetism below 150°K was due to the chemisorption by the ice of oxygen, an impurity in the helium surrounding the specimen. Of course, the question now arises, if ice adsorbs oxygen below 150°K, why did the tissue-fluid ice adsorb more oxygen in the tumor cells than in the normal cells?

The answer to this question will in the final analysis require further experimental work. However, some speculation may be worthwhile. When water is frozen at a moderately fast rate, the ice crystals do not get a chance to grow, and hence only small crystals are formed. By extremely fast freezing of very thin films of water, crystallization can be reduced to a point where only amorphous or glassy ice can form.⁽²³⁾ However, such rapid

freezing cannot be accomplished readily in bulk specimens. On the other hand, it is known⁽²⁴⁻²⁶⁾ that when certain organic substances are dissolved in water, the rate of crystallization is reduced, and that under conditions of only moderately fast freezing, crystallization can also be impeded to the extent of forming noncrystalline or vitreous ice in bulk specimens. Thorpe and Senftle⁽²⁷⁾ have made additional experiments on the freezing of bulk specimens of concentrated egg albumen and alcohol-water solutions of certain concentrations and show that vitreous ice is formed on freezing moderately fast, whereas only hexagonal ice is formed when the same solutions are slowly frozen. Their magnetic-susceptibility data on concentrated egg albumen suggested that oxygen adsorbs more readily on hexagonal ice than on vitreous ice. Although oxygen is ordinarily paramagnetic, in the process of chemisorption it will be held by ionic or covalent bonds, and there will be no unpaired electrons.⁽²⁸⁾ Oxygen under these circumstances is diamagnetic, and consequently the diamagnetism of ice increases with adsorbed oxygen. They propose the hypothesis that the cell fluids in the normal cells contain some organic substance in sufficient concentration to prevent the formation of normal hexagonal ice under the conditions of freezing. On the other hand, if the concentration of this substance in the cell fluid of a tumor cell is too low, owing to dilution, hexagonal ice will be formed and adsorption will take place. When both types of cells are exposed to oxygen at low temperatures ($< 150^{\circ}\text{K}$) the cells with the hexagonal ice will adsorb oxygen and will thus increase in diamagnetism. Apparently the tumor-cell fluid contains too low a concentration of some organic constituent to prevent formation of the hexagonal form of ice in contrast to the amorphous form obtained in normal cells.

Blood and Blood Serum

Although blood should be more properly discussed as a biological specimen, so much work has been done on the biochemical components of blood and their derivatives that it is more convenient to discuss it in the section below.

Single Biological Cells

Very little work has been carried out on single biological cells, as most methods used for magnetic-susceptibility measurements require at least milligram quantities of sample. Gill *et al.*⁽²⁹⁾ have developed a unique method of measuring magnetic susceptibility based on the change in the average velocity of a particle in a magnetic field. Their technique is capable of

measuring the susceptibilities of particles from 1 to 100 μ in diameter. Cultures of yeast (*Saccharomyces cerevisiae*) were grown and measured over a period of 90-odd hr.⁽³⁰⁾ The gram susceptibility for 13 individual yeast cells 6 to 10 μ in diameter varied nearly 15% about an average value of -0.830×10^{-6} emu/g. Using the Gouy technique, Sugiura and Koga⁽³¹⁾ studied the susceptibility of resting, metabolizing, and heat-treated cells of the above yeast. They observed that the diamagnetic susceptibility increased slightly upon the death of the cells, in contrast with the results of Bauer and Raskin.⁽³²⁾ The latter noted a large (4%) increase in diamagnetism upon the death of the bacterial cells *B. coli* and *B. proteus*, and also yeasts. They hypothesized that some protoplasmic molecule was in an excited (paramagnetic) state during life and reverted to a diamagnetic ground state upon death. Measurements have also been made on red blood cells.⁽³⁰⁾ In a 1% saline solution, blood cells have a biconcave disc form. The average susceptibility of this form was -0.741×10^{-6} emu/g for individual cells with a 9% variation over 15 cells. When the disc form was converted to a spherical form by adding a small amount of lecithin to the solution, the same average susceptibility was found. However, in the later case a variation of 38% was noted in the susceptibility over 16 individual cells. The authors suggest the interesting possibility that the hemoglobin molecules within the disc form have a definite orientation, whereas those in the spherical form are not so well-organized.

Eggs and Sperm of the Sea Urchin

Perakis⁽³³⁾ in his studies on cell division in a magnetic field has made some interesting observations on the magnetic properties of eggs and sperm of the sea urchin. The nonfertilized eggs and the fresh sperm which were thoroughly washed free of NaCl were found to have susceptibilities of about -0.70×10^{-6} emu/g. When the eggs were further washed in alcohol and dried in ether, the diamagnetism decreased to -0.41×10^{-6} emu/g at room temperature. At lower temperatures this susceptibility increased, indicating a paramagnetic component. After calcination the susceptibility of the ashes went up to $+ 4.3 \times 10^{-6}$ emu/g and traces of iron were found. Eggs which had been fertilized for several hours showed no change in iron content.

In Vivo Measurements

Bauman and Harris⁽³⁴⁾ have explored the possibility of using magnetic susceptibility to estimate *in vivo* hepatic storage iron. *In vitro* studies were made on the livers of depleted, normal and iron-loaded rabbits. The induc-

tion technique used did not distinguish between depleted and normal livers, whereas an increase in susceptibility with above-normal tissue iron content was noted. *In vivo* measurements on iron-loaded rats showed increases in diamagnetism in the rats' abdomen which roughly paralleled the added iron concentration. They suggest the possibilities of this method to detect hepatic iron overload in humans.

STUDIES OF BIOCHEMICAL COMPOUNDS

Blood and Related Compounds

As early as 1845, Faraday reported⁽²⁰⁾ on the diamagnetic properties of whole blood. Shortly afterward Plücker⁽³⁷⁾ showed that the blood corpuscles were more strongly diamagnetic than the fluid plasma. More than 50 years passed before further work was reported by Gamgee⁽³⁸⁾ who investigated the magnetic properties of hemoglobin and its derivatives. He found that the oxyhemoglobin and other blood-coloring derivatives of hemoglobin were diamagnetic, whereas the hematin and heme were positively magnetic. Gamgee was the first to recognize a profound transformation that occurred in the magnetic susceptibility of hemoglobin when it was decomposed in the presence of oxygen. In spite of this discovery, however, he did not recognize the fact that the magnetic susceptibility of arterial and venous blood should be substantially different, a fact pointed out by Pauling and Coryell⁽¹⁶⁾ at a much later date. No further studies of the magnetic properties of blood seem to have been undertaken until the mid-1930's when several investigations⁽³⁹⁻⁴⁴⁾ were reported. The work of Pauling, Coryell, and their co-workers^(16,45-52) which followed shortly afterward, initiated a series of papers on the magnetic properties of blood components which have contributed substantially to our knowledge of blood chemistry.

Owing to some confusion in the past concerning the nomenclature used in blood chemistry, it is appropriate to discuss briefly the names and chemical structures of the principal components of blood. We will follow the names suggested by Pauling and Coryell.⁽¹⁶⁾ The hemoglobin in blood is made up of a protein called globin, and four iron-bearing group called heme. Globin is diamagnetic, and hence the chief magnetic properties of hemoglobin will be centered about the heme. Heme is an iron-protoporphyrin complex. As the porphyrin nucleus is diamagnetic [$(-0.46$ to $-0.54) \times 10^{-6}$ emu/g],⁽⁵⁶⁾ the paramagnetic properties of hemoglobin and its derivatives will essentially depend on the chemical state of the iron. Depending on whether the central ion is Fe(II) or Fe(III), the iron-por-

phyrin complex is called ferroheme or ferriheme. If the iron is Fe(III) and is attached to a hydroxyl ion, the compound is called ferriheme hydroxide, formerly known as hematin; if compounded with chloride it is called ferriheme chloride or hemin. When four hemes, either ferroheme or ferriheme, are combined with native globin, ferrohemoglobin (reduced hemoglobin), or ferrihemoglobin (methemoglobin), respectively, are formed. Both types of hemoglobin can be reacted with other anions or molecules to form a large number of hemoglobin derivatives. Myoglobin contains one heme per molecule and the nomenclature is similar. The work on the magnetic properties of the hemoprotein derivatives up to 1945 has been reviewed by Hartree.⁽⁵³⁾ Later data, which to some extent will influence the older interpretations, have been added in the following summary.

The Porphyrin Complexes

A porphyrin is a cyclic conjugated tetrapyrrole pigment. When a metal ion, generally one of the transition elements, is bonded to the four nitrogen atoms of the pyrrole groups, a metalloporphyrin is formed. The porphyrin structure is fundamental to a great many biochemical substances such as hemoglobin and its derivatives, myoglobin, in certain oxidase enzymes, and chlorophyll. Several monographs are available on porphyrin chemistry.^(35,36)

Iron Porphyrins

Hemin

It has been known for many years that if blood is treated with acetic acid and NaCl, crystalline ferriheme chloride or hemin can be isolated. X-ray diffraction studies on various porphyrins, metalloporphyrins, and hemins show that the porphyrin nucleus is not always planar, nor does the metal ion invariably lie in the plane of the four pyrrole nitrogen atoms.⁽⁵⁴⁾ Many workers have studied the susceptibility of hemin (Table III). The results of single-temperature measurements depend on an accurate knowledge of the diamagnetism of the porphyrin and the validity of the Curie Law at the particular temperature. Havemann *et al.*⁽⁵⁵⁾ have tabulated the single-temperature data on hemin, and note that a variety of diamagnetic corrections have been applied by different workers to the same porphyrin, leading to different effective moments. Havemann *et al.*⁽⁵⁶⁾ have made extensive measurements on the diamagnetism of porphyrins. Several groups⁽⁵⁷⁻⁵⁹⁾ have shown that hemin follows the Curie Law from liquid-nitrogen to room temperature. They report moments of 5.93 and 5.79 B.M., showing that hemin is a high-spin Fe(III) complex. However, hemin deviates

TABLE III
Representative Magnetic Moments of Iron Porphyrins

Form	μ , B. M.	Temperature (°K)	Reference
Hemin			
Solid	5.97	80-300	58
	5.93	80-300	57
	5.81	84, 192, 294	55
	5.94	293	59
Hematin			
Hemin in 0.2N NaOH	3.6	292	65
Hemin in 0.01N NaOH	5.7	294	66
Hemin in sucrose-0.2N NaOH	5.56	291	45
Solid	5.63	80-300	58
Hemichromogen			
Hemin in 20% pyridine-0.2N NaOH	1.97	278	65
Hemin in 50% pyridine-0.05M NaOH	2.3	278	66
Solid diimidazole tetraphenylporphine ferric chloride	2.36	4.2-50	67
Ferroheme			
Hemin in sucrose-0.2N NaOH reduced with dithionite	4.83, 5.02	291	45
Hemochromogen*			
Dipyridine hemochrome	dia	291	45
Dicyanide hemochrome	dia	291	45
Globin hemochrome	dia	291	45
CO-pyridine hemochrome	dia	291	69

* In solutions.

markedly from the Curie law⁽⁵⁸⁾ from 300° to 600°K. From electron spin resonance (ESR) data⁽⁶⁰⁾ it was suggested that hemin is ferromagnetic at 4.2°K, whereas Moessbauer experiments⁽⁶¹⁾ give no indication of this effect. Helium temperature susceptibility measurements can resolve this question. The analogous phthalocyanines have quite different magnetic properties.⁽⁶²⁾

Magnetic-susceptibility results^(55,63) of similar hemin-like complexes in which the porphyrin or coordinated anion are varied show moments that are close to the spin-only value for high spin Fe(III). Harris⁽⁶⁴⁾ has theoretically calculated the effective moments of ten hemin-like complexes at

298°, 77°, and 4°K. In agreement with experiments, the moments are fairly temperature independent above 77°K, and further studies are needed to substantiate the predicted variations at lower temperatures.

Hematin

When hemin is dissolved in NaOH solutions, ferriheme hydroxide or hematin is formed. Rawlinson⁽⁶⁵⁾ obtained a moment of about 3.6 B.M. in 0.1N NaOH, which was shown to increase⁽⁴⁵⁾ to 5.6 B.M. upon the addition of sucrose. Blauer and Ehrenberg⁽⁶⁶⁾ found that very dilute solutions of hemin from pH 10–12 gave moments indicating that the presumably dimerized hematin was spin-free Fe(III). Iron porphyrins tend to aggregate at high concentration and pH, and the sucrose possibly acted as a deaggregating agent. At constant pH, the addition of high concentrations of electrolytes as NaCl or NaClO₄ decreased the observed moments from 5.8 to around 3 B.M. Since the anions do not produce a sufficient field to spin-pair Fe(III), the low moments were interpreted as resulting from the interaction of molecules of ferriprotoporphyrin within aggregates or micelles produced by the salts. No work on the susceptibility of hematin compounds in the presence of detergent solutions, which presumably⁽³⁶⁾ solubilize and monomerize porphyrins has been reported. Schoffa and Scheler⁽⁵⁸⁾ report a moment of 5.63 B.M. for solid hematin from 98°–300°K, with marked deviations from Curie behavior above 300°K. The room-temperature moments of solid hematins prepared from nonaqueous solutions approximate high-spin Fe(III) values, whereas the hematins precipitated from 5–10M aqueous KOH solutions vary from 2.4 to 5.70 B.M. The species giving these moments are not well-understood, and could be oxy-bridged dimers.

Hemichromogen

The susceptibility of hemin in pyridine solutions varies with the solvent composition and concentration (Table III). Blauer and Ehrenberg,⁽⁶⁶⁾ Rawlingson,⁽⁶⁵⁾ and Havemann *et al.*⁽⁵⁶⁾ all observe moments in the range of 1.97 to 2.74 B.M. in pyridine–NaOH solutions. The species present is presumably a hemichromogen (parahematin), in which two pyridines occupy the iron porphyrins axial positions. The observed moments are in the usual range found for spin-paired d^5 Fe(III) systems. The crystalline diimidazole complex of tetraphenylporphyrin ferric chloride had an effective moment⁽⁶⁷⁾ from 4.2–50°K of 2.36 B.M. with a Weiss constant of -1.6°K . Hemin with poly- α,L -lysine in NaOH was shown to be a low-spin Fe(III) complex.⁽⁶⁸⁾

Related although somewhat different is verdohemochromogen, a green

pigment formed by oxidation of ferriheme chloride. Craig and Mellor⁽⁹³⁾ studied the magnetic properties of three rather impure specimens of this compound. They obtained a magnetic moment of 2.16 B.M. on the purest sample and concluded that the central iron in verdohemochromogen was probably a low-spin ferric ion.

Ferroheme and Hemochromogen

Upon reduction of hematin in basic solution with dithionite, Pauling and Coryell⁽⁴⁵⁾ found moments of 4.83 to 5.02 B.M., roughly corresponding to the spin-only moment of 4.89 B.M. for spin-free Fe(II). The addition of strongly coordinating ligands such as CO,⁽⁶⁹⁾ pyridine and cyanide⁽⁴⁵⁾ to high-spin ferrohemes form hemochromogens. The two added ligands have fields sufficiently strong to produce diamagnetic spin-paired Fe(II).

Other Metalloporphyrins

Loach and Calvin⁽⁷⁰⁾ have studied the susceptibilities of manganese(II), (III), and (IV) porphyrins as possible models for the participation of manganese in the oxygen evolution phase of photosynthesis. Blumberg and Peisach⁽⁷¹⁾ have shown by ESR techniques that copper uroporphyrin from the feathers of the African touraco bird contained d^9 Cu(II). Theoretical treatments^(64,72) of the magnetic properties of metalloporphyrins have been made, and susceptibilities of many derivatives⁽⁵⁷⁾ have been reported from liquid-nitrogen to room temperature.

Hemoglobin and Myoglobin

Ferrohemoglobin (deoxygenated), formed by the reduction of hemoglobin using sodium dithionite, was found by Pauling and Coryell⁽¹⁶⁾ to behave magnetically as a spin-free $3d^6$ Fe(II) compound. They determined a magnetic moment of 5.46 B.M. per heme, which is greater than $\mu = 4.90$ required for four unpaired electrons per heme. Somewhat later, Taylor and Coryell⁽⁵²⁾ confirmed their magnetic-susceptibility data using a superior technique and obtained a magnetic moment of 5.43 B.M. at 297°K (see Table IV). This later work was done both on blood and hemoglobin solutions with the same results, and showed that hemolysis of the blood corpuscles had no effect on the magnetic susceptibility of the ferrohemoglobin.

The theoretical effective magnetic moment was calculated by assuming that the Curie law holds and that the iron in each of the four hemes orients itself in the applied field independently of the iron in the other hemes.

TABLE IV
Effective Magnetic Moment of Iron in Hemoglobin and Myoglobin at Approximately 297°K

Form	μ_{eff} , B. M.	Reference
Ferrohemoglobin (cow)	5.46, 5.435	16, 52
Ferrohemoglobin (cow)	4.9*	74
Ferrohemoglobin (horse)	5.43	52
Ferrohemoglobin (sheep)	5.46	52
Ferrohemoglobin (human)	5.35	52
Ferromyoglobin	5.46	73
Ferrihemoglobin (cow)	5.80, 5.83	47, 48, 99
Ferrimyoglobin	5.85	73
Ferrihemoglobin (Form I)	5.46	48, 50
Ferrihemoglobin (Forms II, III)	5.77	48, 50
Hemoglobin in 0.5N NaOH	0.56†	84
Ferrimyoglobin fluoride, I	5.80	84
Ferrimyoglobin fluoride, II	5.80	84
Ferrimyoglobin fluoride, III	5.91	84
Ferrimyoglobin peroxide, III	2.88	94
Ferrimyoglobin methyl peroxide, III	2.66	90

* A more accurate value for ferrohemoglobin in view of the recent diamagnetic correction for globin (see reference 74).

† Volume susceptibility, emu/cc (see reference 42).

This leads to a predicted theoretical moment of 4.90 B.M., a somewhat lower value than that determined experimentally. Pauling and Coryell⁽¹⁶⁾ reasoned that, because of the strong quenching effect of the adjacent nitrogen atoms in the porphyrin complex, the difference between the experimental and theoretical values was not due to an orbital contribution as is found in some other ferrous compounds, but to an interaction between adjacent hemes which tends to stabilize the states with parallel heme moments as compared to those with opposed heme moments. The oxygen-equilibrium value of the interaction energy of adjacent hemes is of the right order of magnitude to justify such an interpretation. This theory, however, was subsequently found to be in error by Coryell *et al.*⁽⁴⁶⁾ who by a magnetic titration technique showed that the proposed interaction between hemes had but a very small effect.

Taylor⁽⁷³⁾ first reported the magnetic susceptibility of ferromyoglobin which is shown in Table IV. The susceptibilities are, within experimental error, the same as those obtained for ferrohemoglobin. This result is quite

important for the following reason. In myoglobin, there is only one heme per molecule and, as the hemes will be well separated in dilute solution, no interaction between hemes is expected. Yet the results are almost the same as found in hemoglobin. Thus the observed magnetic moment of iron in myoglobin showed that the heme-heme interaction hypothesis was untenable. The problem of the high magnetic moment of ferrohemoglobin has been solved by the work of Havemann *et al.*,⁽⁷⁴⁾ who redetermined the diamagnetic contribution of globin and found a value of -0.54×10^{-8} emu/g compared with the value of -0.71×10^{-6} emu/g inherent in the calculations of Pauling and Coryell.⁽¹⁶⁾ As a result they obtain a magnetic moment of 4.9 B.M. which corresponds with the theoretical value and again makes the hypothesis of a heme-heme interaction unnecessary. For ferrihemoglobin (methemoglobin), Coryell *et al.*⁽⁴⁷⁾ found a moment of 5.80 B.M. at 298°, slightly below the spin-only moment for an $S = \frac{5}{2}$ state. The authors attributed the low moment to a heme-heme interaction, which in this case tended to stabilize those configurations in which the heme moments are opposed. However Taylor's⁽⁷³⁾ value of 5.85 B.M. for metmyoglobin and the more recent work of Havemann *et al.*⁽⁷⁴⁾ imply the absence of an interaction. Attention should be called to an explanation by Griffith⁽⁷⁵⁾ to explain the low moments of certain cobalt compounds. The paramagnetism of hemoproteins are generally corrected for basic diamagnetism by using an analogous diamagnetic compound on the assumption that the paired *d* electrons do not contribute to the paramagnetism. Griffith has theorized that the magnetic field polarizes the electrons in the diamagnetic comparison compound resulting in a small induced paramagnetism, i.e., an induced orbital, not spin, contribution. The diamagnetism is thus less than it should be, yielding a correction which is too small, and hence a moment smaller than the true moment. His estimates of this effect, however, while in the right direction are too small to explain the low moment observed for ferrihemoglobin.

In their studies of ferrohemoglobin from blood of cows, horses, and sheep, Taylor and Coryell⁽⁵²⁾ were able to show that the magnetic susceptibility of human ferrohemoglobin was slightly low in one isolated measurement, but this was not considered significant. From their measurements they were also able to show the presence of ferric compounds in ferrohemoglobin and oxyhemoglobin. Hence, they proved that the capacity of the blood for oxygen absorption was not a reliable measure of either the hemoglobin or iron content. In their bovine blood specimens, an average of 5.7% of the total iron did not combine with oxygen, a value in close agreement, with the results of Klumpp.⁽⁷⁶⁾ The form of this ferric iron is interesting

and one wonders if it may be attached to the protein in much the same way as the ferric iron found in DNA or ferritin which are discussed more fully below.

As noted earlier, wide-temperature-range susceptibilities on frozen solutions, powders, and especially single crystals are capable of giving atomic parameters. The work of Kotani,⁽⁷⁷⁾ Schoffa,⁽⁷⁸⁾ Griffith,⁽⁷⁹⁾ and Weissbluth⁽⁸⁰⁾ and co-workers are examples of this approach applied to biological systems. Weissbluth's elegant monograph on the physics of hemoglobin reviews the work in this area.⁽⁸⁰⁾ Kotani⁽⁸¹⁾ and Schoffa *et al.*⁽⁸²⁾ have measured the susceptibility of various heme proteins down to low temperatures, and they note that important information comes from the deviations from the Curie law below 20°K. Kotani⁽⁸³⁾ has recently measured the anisotropy in susceptibility of ferrimyoglobin.

Electrode potential and magnetic susceptibility studies by Coryell and Stitt⁽⁴⁸⁾ and Coryell and Pauling⁽⁵⁰⁾ established the existence of three different types of acid groups interacting with the heme in ferrihemoglobin depending on the pH of the solution. Three forms of ferrihemoglobin were found that correspond to pK values of 5.3, 6.65, and 8.10, respectively. The magnetic properties of each form, uncorrected for the 4 to 8% ferric iron impurities which do not react with oxygen, are shown in Table IV. Coryell and Pauling⁽⁵⁰⁾ postulated that the heme-linked acid group, Form I, is a histidine imidazolium ion in poor position for electrostatic coordination with the basic form of the iron atom. The group in Form II is the imino group of a histidine residue whose number three nitrogen atom is strongly coordinated with the iron atom. Form III is due to the iron atom itself which, acting as an acid group, may add a hydroxide ion, or a water molecule coordinated to the iron atom.

Theorell and Ehrenberg⁽⁸⁴⁾ have studied the spectrophotometric and titrimetric properties of myoglobin along with the magnetic properties. Coryell and Pauling⁽⁵⁰⁾ found a change in the magnetic susceptibility of iron in ferrihemoglobin of from $12,430 \times 10^{-6}$ to 8250×10^{-6} emu/mole in going from an acid to basic solution. Theorell and Ehrenberg, however, found that the susceptibility of iron in ferrimyoglobin varied little between a pH of 5 to 7.5, but changed from $13,690 \times 10^{-6}$ to $11,040 \times 10^{-6}$ emu/mole in going from a neutral to alkaline solution. Three forms of ferrimyoglobin exist that are similar to ferrihemoglobin. Form I is established for both compounds at pK = 5.3. Forms II and III of ferrimyoglobin have somewhat higher pK values than ferrihemoglobin. In ferrimyoglobin fluoride, Theorell and Ehrenberg have established magnetic transitions in the region of pH 6, 8, and 10, respectively (see Table IV), corresponding to the

three forms. As a result of their investigations, they conclude that the same chemical configuration is present in the oxygen-attached side of the heme plane in myoglobin as in hemoglobin.

Derivatives of Hemoglobin and Myoglobin

If the R group on one side of the porphyrin-globin complex is replaced by different ions or molecules, the magnetic and spectral properties change considerably. These changes have been studied in some detail and have been very useful in unraveling the chemistry of hemoglobin derivatives.

Coryell *et al.*⁽⁴⁷⁾ have shown that the magnetic susceptibility of ferrihemoglobin undergoes a pronounced change when the pH of the solution changes. By using a novel magnetic titration method when preparing hemoglobin derivatives, they showed that the intermediate compounds formed were linearly related to the number of hemes which had undergone reaction. Further, they were able to confirm that the conversion from ferrihemoglobin (acid methemoglobin) to ferrihemoglobin hydroxide (alkaline methemoglobin) was first-order in hydroxyl ion, a fact which was earlier pointed out by Austin and Drabkin⁽⁸⁵⁾ from spectrophotometric studies.

The data of Austin and Drabkin on ferrihemoglobin hydroxide showed that the substances at intermediate pH values approximately correspond to the following equilibrium*



An alternative explanation which has some merit from the magnetic standpoint has been suggested by Coryell *et al.*⁽⁴⁷⁾ If iron in ferrihemoglobin is octahedrally coordinated, as is apparently the case in ferrihemoglobin hydroxide, then they suggest the equilibrium reaction



At higher pH values, the transition to ferrihemoglobin hydroxide would then be accomplished by a bond change and the loss of a proton rather than the addition of a hydroxyl ion. The pK values for a number of heme-proteins have been tabulated.⁽⁸⁶⁾

The magnetic measurements made on ferrihemoglobin hydroxide do not indicate the nature of the oxidation state of the central iron atom with as much certainty as in some of the previously mentioned compounds. As

* Following Coryell, Stitt and Pauling, the symbols Hb^+ , HbOH , etc. represent that amount of ferrihemoglobin or its derivative containing one heme.

TABLE V

Effective Magnetic Moment of Iron per Heme of Some Hemoglobin Derivatives at Approximately 297°K

Form	μ , B. M.	Reference
Ferrihemoglobin hydroxide	4.47	47
Ferrimyoglobin hydroxide	5.11	84
Ferrihemoglobin hydroxide	4.45, 4.48	48, 99
Ferrihemoglobin fluoride	5.92	47
Ferrihemoglobin cyanide	1.50, 2.50	47, 99
Ferrimyoglobin cyanide	2.35	84
Ferrohemoglobin cyanide	0	51
Ferrihemoglobin hydrosulfide	2.26	47
Carbonmonoxyhemoglobin	0	16, 74
Carbon monoxymyoglobin	0	84
Oxyhemoglobin	0	16, 74
Ethanol ferrihemoglobin	5.89	48
Ferrihemoglobin azide	2.84	48
Ammonia: ferrihemoglobin hydroxide	2.98	48
Ethanol: ferrihemoglobin hydroxide	5.39	48
Imidazole ferrihemoglobin	2.2 (av.)	49
Nitric oxide ferrihemoglobin	1.75	46
Ethylisocyanide ferrohemoglobin	0	49
Globin hemochromogen	0	45
Pyridine hemochromogen	0	45
Nicotine hemochromogen	0	45
Dicyanide hemochromogen	0	45

shown in Table V, the magnetic moment is approximately equivalent to about three unpaired electrons ($\mu = 3.88$), if the orbital contribution is taken as approximately 0.4 B. M.

In high symmetry octahedral or tetrahedral mononuclear Fe(III) complexes, two types of magnetic behavior are usually observed. The complexes are either low-spin with one unpaired electron [i.e., $\text{Fe}(\text{CN}_6)^{4-}$] or high-spin with five unpaired electrons [i.e., $(\text{NH}_4)_3\text{FeF}_6$], and have characteristic temperature-susceptibility variations. In these environments $S = \frac{3}{2}$ ground states are theoretically forbidden.⁽⁸⁷⁾ There are, however, examples of complexes of lower symmetry having $S = \frac{3}{2}$ ground states. Several complexes of higher symmetry⁽⁸⁸⁾ have low moments that have been traced to a thermal equilibrium between high- and low-spin states. Here a sensitive balance between the energy of the two states is required. George *et al.*⁽⁸⁶⁾

TABLE VI

Magnetic Moment in Bohr Magnetons of Iron in Ferrihemoglobin and Ferrimyoglobin Derivatives at 293°K[†]

Ligand	Ferrihemoglobin, μ	Ferrimyoglobin, μ
F ⁻	5.76	5.77
F ⁻	5.8*	—
H ₂ O	5.65	5.73
H·COO ⁻	5.44	5.69
CH ₃ ·COO ⁻	5.44	5.70
OCN ⁻	5.40	4.41
OCN ⁻	5.5*	—
SCN ⁻	5.06	5.47
SCN ⁻	5.1*	—
OH ⁻	4.66	5.04
NO ₂ ⁻	4.13	5.12
NO ₂ ⁻	4.2*	—
SeCN ⁻	3.88	4.89
Imidazole	2.87	2.44
CN ⁻	2.50	1.96
CN ⁻	2.3*	—
N ₃ ⁻	2.35	3.30
N ₃ ⁻	2.9*	—
NH ₂ ·CH ₃	2.3	—

[†] Data taken from references 97 and 98, except where marked with *; the latter data are from reference 94.

argue that such is the case in ferrihemoprotein hydroxides. By assigning the magnetic moments of $\mu_{hs} = 5.92$ and $\mu_{ls} = 2.24$ to pure high- and low-spin complexes, they were able to calculate the fraction of high-spin ($1 - \alpha$) and low-spin (α) forms present from a knowledge of the observed moment μ_0 , using the relation $\mu_0^2 = \mu_{ls}^2\alpha + \mu_{hs}^2(1 - \alpha)$. The results are shown in Table VII. To prove that a spin-state equilibrium did exist, it was shown that over a small temperature range the high- and low-spin forms of unmixed hemoproteins approximately followed the Curie law whereas the mixed-spin-state hemeprotein hydroxides did not. They derived an equilibrium constant for the process, in which the fraction of high-spin form increased with temperature. These susceptibility measurements also parallel the absorption spectra changes.

This mixture of spin states explains the intermediate moments previously attributed to three unpaired electrons with an orbital contribution. Although

TABLE VII
Spin-State Equilibria in Some Ferrihemoproteins at 293°K*

Form	Percent Low Spin ($S = \frac{1}{2}$)
Ferrimyoglobin-X	
OH ₂	8
OH	31
N ₃ ⁻	78.5
Imidazole	90.5
Ferrihemoglobin-X	
SCN ⁻	40.5
OH ⁻	64
NO ₂ ⁻	71.5
N ₃ ⁻	95.5

* Data from ref. 86; also see refs. 94 and 96.

many workers had suggested the notion of a thermal equilibrium,⁽⁸⁶⁾ the first definitive evidence among biochemical derivatives was that found with ferrihemeprotein hydroxides. This concept is extremely helpful in explaining the magnetic properties of ferrihemochromes where the hydroxide is replaced by other ligands.

Some of the extensive data on the susceptibilities of ferrihemeproteins having various ligands in the 6th position are listed in Table V and VI.* They can formally be placed in three classes. Compounds with moments in the range 5.6–5.9 B.M. are essentially high-spin Fe(III) derivatives, as noted in the hemoglobin F⁻ and OH₂ derivatives. Those with values between 2.0–2.8 B.M. are low-spin Fe(III) types, as the CN⁻ and imidazole complexes. If the susceptibilities are between 2.8–5.6 B.M., mixed spin states ($S = \frac{1}{2}$ and $S = \frac{5}{2}$) are indicated. However, as George *et al.*⁽⁸⁶⁾ point out, the temperature variation of the moment must be used to give evidence for a thermal mixture of spin states. Such equilibria have definitely been established for the complexes in Table VII. It is noted that the same added ligand produces different thermal distributions depending on the particular ferrihemeprotein. The ligand's tendency to spin-pair shows obvious correlations with its position in the spectrochemical series. Havemann *et al.*⁽⁵⁵⁾

* The redetermined diamagnetic contribution of globin⁽⁴⁾ should be taken into account in these tables.

have discussed the fact that the magnetic properties of hemin type complexes are fairly insensitive to both the nature of the porphyrin and added weak field ligand.

Coryell and Stitt⁽⁴⁸⁾ have used an interesting magnetic titration technique to establish the presence of certain complexes. The susceptibility of alkaline ferrihemoglobin solutions increased upon the addition of fluoride, typical of the formation of a higher-susceptibility species, given by the asymptotic value. Methanol was found to have slight effects on the magnetic properties whereas *n*-propanol led to the denaturation of the species.

The most comprehensive study of ferrihemoglobin and ferrimyoglobin derivatives has been carried out by Scheler *et al.*^(97,98) They have compared the light absorption and paramagnetic susceptibility of a great many derivatives as shown in Table VI. In general, the pattern is about the same as that determined earlier and shown in Table IV. They found the magnetic susceptibility to be strongly correlated with the Soret (violet) bands, but weakly correlated with the red and green bands. The red bands, however, were more intense for those ferrihemoglobin complexes with the higher susceptibilities. The absorption bands in those complexes with low susceptibility were shifted toward the longer wavelengths. Havemann and Haberditzl⁽⁹⁴⁾ have also made an extensive study of hemoglobin derivatives and have obtained about the same results except for the azide derivative. Brill and Williams⁽⁹⁵⁾ have attempted to systematize the relationships between absorption spectra and magnetic moments, and were able to set up some diagnostic rules which permit the recognition of the nature of unknown ligands from the properties of the iron-porphyrin complex.

Up to this point we have been concerned with those hemoglobin derivatives which have paramagnetic susceptibilities. We will now discuss those which, although they contain iron, have been observed to be diamagnetic. Ferrohemoglobin cyanide and ethylisocyanide ferrohemoglobin⁽⁴⁹⁾ have been found to be completely diamagnetic, indicating a low-spin $3d^6$ configuration. The more important diamagnetic derivatives, however, are oxyhemoglobin and carboxyhemoglobin. These are similar to ferrohemoglobin except that the added group attached to the iron is substituted for by O_2 and CO. Their structure is particularly interesting from the magnetochemical point of view. It will be recalled that iron in ferrohemoglobin has four unpaired electrons while the O_2 molecule in its normal state has two unpaired electrons. Pauling and Coryell⁽¹⁶⁾ have shown that oxyhemoglobin is diamagnetic, indicating a profound change in the electronic structures of both molecules upon reaction, while the diamagnetic CO simply spin-pairs the spin-free Fe(II). Most important is the fact that the hemoglobin- O_2

reaction is reversible, the Fe(II) is spin-paired and not oxidized to Fe(III), as happens with Fe(II) porphyrins. The structure and nature of the iron-oxygen bond has been widely discussed. Reviews on hemoglobin⁽⁸⁸⁾ and other synthetic oxygen carriers⁽⁸⁹⁾ should be consulted.

Soybean leghemoglobin has the highest oxygen affinity of any heme-protein and combines with one mole of N₂ per mole of protein. Ehrenberg and Ellfolk⁽¹⁶⁸⁾ showed that the CN⁻ derivative was pure high-spin Fe(III), and the F⁻ and acetate forms were pure low-spin Fe(III). The acidic form was 60% high-spin at 300°K and 35–50% high-spin at 77°K. The alkaline form was 25% high-spin at room temperature. This behavior was similar to the hydroxide form of myoglobin but the opposite to that found in the RHP proteins.

The peroxides of myoglobin have also been studied by Theorell and Ehrenberg.⁽⁹⁰⁾ Magnetic-susceptibility measurements of the peroxide and methyl peroxide derivatives of myoglobin indicate that both compounds contain two unpaired electrons. The conversion reduction in magnetic moment as the peroxide is formed has been observed by electron spin resonance.⁽⁹¹⁾ Gibson *et al.*^(91,92) have reported the formation of a free radical associated with the peroxide during the action of H₂O₂ on myoglobin, which theoretically can exist up to 9% of the peroxide formed. Under reducing conditions the amount of free radical diminishes.

Gersonde *et al.*⁽¹⁰⁰⁾ have shown that metmyoglobin and methemoglobin add 25 and 11 moles per chain of lauryl pyridinium chloride, respectively. The methemoglobin compound was red with a moment of 3.34 B.M., indicating a spin-state mixture with 25% high spin-Fe(III). The methemoglobin complex, having a moment of 2.16 B.M. was a spin-paired Fe(III). The red complex after adding more ligand molecules turned green; however, the magnetic properties were not affected.

Dehydrated Hemoglobin and Myoglobin

Havemann and Haberditzl⁽¹⁰¹⁾ have studied the susceptibility of ferrihemoglobin as it was dehydrated. The anhydrous form was diamagnetic like that of oxyhemoglobin or carboxyhemoglobin. Its paramagnetic characteristics were again observed on hydration of the dried form. In contrast^(97,102,103) reconstituted metmyoglobin ($S = \frac{5}{2}$) changes to a low-spin $S = \frac{1}{2}$ state upon dehydration (anhydromyoglobin).

Dehydrated deoxyhemoglobin was studied by Moessbauer and magnetic techniques.⁽¹⁰³⁾ The susceptibility from 4°–60°K gave an effective moment of 3.5 B.M., and was interpreted as a mixture of 50% high-spin Fe(II)

($S = 2$) and 50% low-spin Fe(II) ($S = 0$). The two quadrupole-split doublets were in agreement with this interpretation.

In a similar vein, the spectrum and susceptibility of methemoglobin and metmyoglobin change in a fashion similar to a dehydration process in strong solutions of electrolytes.⁽¹⁰⁴⁾ The spectrum resembles parahematin and the moment decreased from 5.3 to 3.8 B.M. upon the addition of $MgCl_2$ to metmyoglobin. The latter revealed similarities to the free porphyrin system mentioned earlier.⁽⁶⁶⁾

Cytochromes

The cytochromes are heme type compounds combined with a protein, and are involved in electron transfer. By analogy with the ferrous hemochromogens, Theorell⁽¹⁰⁶⁾ felt it was very probable that ferrous and ferric cytochrome c would also be diamagnetic. Experimental verification, however, of the five spectral types of ferricytochrome, ferricytochrome fluoride, ferricytochrome cyanide, and ferrocyanochrome carbon monoxide exhibited a wide range of magnetic susceptibilities. As expected, the ferrocyanochrome carbon monoxide was diamagnetic, but the ferricytochrome compounds were all paramagnetic. Moreover, their magnetic susceptibilities bore a strong resemblance to the hemoglobin compounds reported by Coryell, Pauling, and their colleagues (compare Tables IV, V, and VIII). Boeri *et al.*⁽¹⁰⁶⁾ later reexamined the magnetic properties of ferricytochrome c at various chloride ion concentrations in the neutral to acid range. Their new preparations were of higher purity, and hence the new susceptibility values tended to be somewhat lower. Upon acidification of the neutral cytochrome, they were able to show an uptake of two protons resulting in a compound ferricytochrome c- $2H^+$. By addition of chloride ions to a solution of this new compound they found another compound ferricytochrome c- $2H^+ - 2Cl^-$ showing an uptake of two chloride ions. The susceptibility measurements indicated that iron in the Cyt- $2H^+$ was Fe(III) high-spin, whereas in Cyt- $2H^+ - 2Cl^-$ three unpaired electrons were indicated. In the latter compound the chloride ions take up positions close to the iron forming a dipole chain. Ferricytochrome c, Type II, shown in Table VIII presumably was a combination of these two new compounds. As the enzymic activity is lost at high acidity, the acid form, Type I, may include a significant amount of a denatured product.

As pointed out by Lumry *et al.*⁽¹⁰⁷⁾ the studies of Theorell and his co-workers, similar to previous work, showed how the susceptibility due to the central iron atom in the heme can change by altering the ligands in the fifth and sixth positions. To obtain new information, Lumry and his co-

TABLE VIII
Effective Magnetic Moment of Iron in Some Cytochromes at Approximately 295°K

Form	μ , B. M.	Reference
Ferricytochrome fluoride	5.91	105
Ferricytochrome cyanide	2.34	105
Ferrocytochrome carbon monoxide	dia	105
Ferricytochrome c, Type I (pH = 0.7)	5.92	105
Ferricytochrome c, Type II (pH = 1.4)	5.18	105
Ferricytochrome c, Types III and IV (neutral)	2.80	105
Ferricytochrome c, Type V (pH = 13.5)	2.13	105
Ferricytochrome c (neutral)	2.24	106
Ferricytochrome c (-2H^+)	6.17	106
Ferricytochrome c ($-2\text{H}^+ - 2\text{Cl}^-$)	3.81	106
Ferricytochrome c, dehydrated	3.55	106
Ferricytochrome c, lyophilized	4.11	106
Ferrocytochrome c, dehydrated	2.03	106
Ferrocytochrome c, lyophilized	2.70	106
Ferriporphyrin c-peptide (pH = 1.5)	5.20	108
Ferriporphyrin c-peptide (pH \approx 3.8)	4.56	108
Ferriporphyrin c-peptide (pH > 10.5)	2.20	108
R. rubrum RHP (oxidized)	5.15	109
pH = 12.1	2.06	109
(reduced)	5.12	109
Chromatium RHP (oxidized)	5.30	109
pH = 12.0	2.96	109
(reduced)	5.50	109
Rps. palustris RHP (oxidized)	5.10, 5.17	109
(reduced)	4.91	109
Chromatium cytochrome c (oxidized)	2.71	109
(reduced)	dia	109

workers have restudied the magnetic susceptibility of ferrous and ferric cytochrome c without changing the ligands (two imidazole nitrogen atoms of the globin complex). They have accomplished this by varying the amount of bound water held by the protein on the assumption that this will cause significant modification in the protein conformation, and hence the electronic and magnetic properties of the central iron atom, while at the same time not removing the ligand imidazole groups in the protein. As a control they measured the change in the magnetic susceptibility of trypsinogen as a function of the amount of bound water, and found that the susceptibility of protein-bound water is identical to that of free water. Based on this fact

both the oxidized and reduced form of cytochrome c were found to be more paramagnetic in the very dry (lyophilized) state than in the simply dehydrated state. Although the exact reason for this difference is not known, Lumry and his co-workers suggest that the observed differences may be due to the altering or displacement of one or more imidazole groups by water resulting in the pairing of electrons. When the water is removed by the lyophilization process, one to three electrons become unpaired in ferricytochrome and from zero to two electrons become unpaired in the ferrocyanochrome. This change would lead to the observed increase in susceptibility shown in Table VIII.

Paleus *et al.*⁽¹⁰⁸⁾ have converted cytochrome c to ferriporphyrin c-peptide by peptic digestion. They studied the magnetic susceptibility of this degradation product at different acidities, and compared the data with that of the parent compound. In HCl solution ($\text{pH} = 1.5$) the effective magnetic moments of both the parent and the peptic compounds, they suggested, are compatible with the presence of five unpaired electrons. However, their data in Table VIII show that the magnetic moment corresponds better with the presence of four odd electrons with an orbital contribution of about 0.4 B.M. They explain that the low moment of the peptide may be due to heme-bonded groups of a different nature. In alkaline solution they found both the ferrichrome c and ferriporphyrin c-peptide to have about the same susceptibility, and conclude that both are octahedrally coordinated low-spin complexes. The close analogy which appears to hold for the acid and alkaline forms of ferricytochrome c and ferriporphyrin c-peptide does not seem to exist for the intermediate compound.

Ehrenberg and Kamen⁽¹⁰⁹⁾ have measured the susceptibilities of *chromatium* cytochrome c and several RHP (modified type c cytochrome) heme proteins shown in Table VIII. The ferro RHP proteins had four unpaired electrons while the oxidized forms were mixtures of spin states, as shown by optical data. In contrast to the hydroxide forms of myoglobin, leghemoglobin, and acid hemoglobin, but similar to acid myoglobin, the proportion of high-spin forms of RHP increased as the temperature decreased. Upon addition of base, the spectra of *R. rubrum* and *chromatium* RHP changed to a hemichrome type with a parallel susceptibility transition to low-spin states.

Cytochrome oxidase contains cytochrome a, a₃, and copper. Ehrenberg and Yonetani⁽¹¹⁰⁾ deduced that cytochrome a is similar to cytochrome c, in that both the oxidized and reduced forms are low-spin. Cytochrome a₃ is a high-spin Fe(II) derivative when reduced, and the susceptibility of the oxidized form (7900×10^{-6} cgs emu) indicates a thermal equilibrium mix-

ture containing more than 50% low-spin character. The ESR spectrum indicated the majority of copper was Cu(II).

Catalase and Peroxidase

Catalase and peroxidase are two enzymes of the iron-porphyrin-protein type. The former catalyzes the decomposition of hydrogen peroxide into water and molecular oxygen, while the latter catalyzes the oxidation of various biochemical compounds in the presence of hydrogen peroxide. The magnetochemistry of these two enzymes has been studied to some extent.

Michaelis and Granick⁽¹¹⁸⁾ found a moment of 4.6 B.M. at 300°K for beef liver catalase, which corresponds to three unpaired electrons; a conclusion which Michaelis himself apparently held reservations about. Theorell and Agner⁽¹¹⁹⁾ determined the susceptibility of horse liver catalase and a number of its derivatives. Owing to the small amount of iron in catalase, the diamagnetic correction which they used was relatively large (30%). The correction was later shown to be too large⁽¹¹⁹⁾ and hence their data tends to be high. Nearly a decade later Deutsch and Ehrenberg⁽¹²⁰⁾ repeated the measurements which are essentially in agreement with those of Theorell and Agner after the proper corrections are applied. The results are shown in Table IX.

TABLE IX
Effective Magnetic Moment of Iron in Catalase, Peroxidase, and Their Derivatives
at Approximately 293°K

Form	μ , B. M.	Reference
Catalase (pH 4.8-10.4)	5.66	122
Fluoride catalase	5.60	120
Azide catalase	5.36	120
Cyanide catalase	2.29	120
Hydrogen-sulfide catalase	2.51	120
Catalase azide peroxide in CO	0	90
Catalase azide peroxide in N ₂	0	90
Ferriperoxidase (neutral)	5.47	90
Ferriperoxidase (alkaline)	2.67	121
Ferroperoxidase	5.19	121
Peroxidase fluoride	5.92	121
Peroxidase cyanide	2.24	121
Peroxidase hydrogen sulfide	2.41	121
Carbomonoxy-ferroperoxidase	dia	121
Horse Radish peroxidase	5.27	122

TABLE X
Effective Magnetic Moments of Some Hemeproteins and Their Derivatives*

Form	μ , B. M.
Catalase	5.66
Catalase I MeOOH	3.90
Catalase II MeOOH	2.93
Horse radish peroxidase	5.27
Horse radish peroxidase I MeOOH	3.99
Horse radish peroxidase II MeOOH	3.53
Horse radish peroxidase IV MeOOH	5.80
Myoglobin	5.73
Myoglobin III MeOOH	2.85
Myoglobin III HOOH	2.97
Cytochrome c peroxidase	5.50
Cytochrome c peroxidase II ETOH	4.1 (3.7) [†]

* Data from ref. 122 and 123.

[†] Corrected for free radicals.

Unlike horse or beef catalase, horse erythrocyte catalase contains only heme iron, and, as there is no biliverdin iron,* the interpretation of the magnetic data is much simpler. The data are similar to methemoglobin derivatives having states $S = \frac{5}{2}$, $S = \frac{1}{2}$, and mixtures. Neither catalase nor its fluoride showed any change in susceptibility with change in pH of 4.8 to 10.4. Denaturation occurs in more alkaline solutions and is accompanied by a sudden drop in susceptibility. In contrast with ferrimyoglobin fluoride, no dissociation of the heme-linked groups was observed at specific pK values. Other derivatives of horse erythrocyte catalase have been examined by Theorell and Ehrenberg⁽⁹⁰⁾ as shown in Table IX.

Theorell⁽¹²¹⁾ was first to measure the magnetic properties of peroxidase and some of its derivatives. He also measured several complexes of horse-radish peroxidase and hydrogen peroxide, but his results were not corrected for dissolved oxygen and hence are a little low. Measurements of these complexes were later repeated^(90,119) and the new data are shown in Table IX.

Ehrenberg and co-workers^(122,123) have measured the susceptibility of a number of peroxy derivatives of the hemoproteins shown in Table X. The compounds are distinguished by the visible absorption spectra type and named accordingly (I, II, . . .). Ehrenberg has given an excellent discussion

* Biliverdin iron in liver catalase is essentially high-spin iron⁽¹¹⁹⁾.

of the magnetic properties of these compounds in relation to their reactivity.⁽¹²²⁾ He considers catalase as a thermal mixture ($S = \frac{5}{2}, \frac{1}{2}$), Cat I as a formal Fe(V) ($S = \frac{5}{2}$) high-spin form, and Cat II as a formal Fe(IV) low-spin ($S = 1$) complex. The Cat I complex, he argues, can be formed and dissociated without a large contribution to the activation energy due to electron redistribution, and so should react faster than those of Cat II, which must undergo a low-spin \rightarrow high-spin transition. Similarly, myoglobin and myoglobin III have a spin mismatch leading to sluggish reactions. This analysis parallels that given for V(II)-V(III) and Cr(II)-Cr(III) electron exchange rates. Magnetic changes have been used to follow several peroxy reactions.⁽¹²²⁾ It is also noted that the total measured susceptibility does not take into account the sometimes significant amount of free radicals present in the system.

Copper Proteins

Very few magnetic studies have been made on the vast number of copper proteins,^(111,112) presumably because of their complexity, and greater tractability by ESR methods. Hemocyanine, a copper-containing protein important in the respiration of some mollusks and arthropods, combines with oxygen in the ratio $2\text{Cu}/\text{O}_2$. While it has been stated that the deoxygenated hemocyanine⁽⁸⁸⁾ contains diamagnetic Cu(I), there is no general agreement on the spin states of the blue oxygenated species.

Nakamura⁽¹¹³⁾ has made some initial susceptibility measurements on apo, oxidized, and reduced laccase, extracted from crude latex. By using differential calculations to correct for diamagnetism, he found that $\mu_{\text{ox}} - \mu_{\text{apo}} = 1.9$ B.M./copper while $\mu_{\text{ox}} - \mu_{\text{red}} = 2.2$ B.M. This indicated that the oxidized form was $d^9\text{Cu}(\text{II})$ while the reduced form was diamagnetic Cu(I). Reoxidation restored the original susceptibility indicating a reversible reaction involving one unpaired electron per atom of copper. Since laccase contains four copper atoms per molecule, these measurements indicated confirmation of Nakamura's previous chemical data⁽¹¹⁴⁾ which showed that the four cuprous atoms could be oxidized by one molecule of oxygen.

However, later ESR studies on laccase⁽¹¹⁵⁾ indicated that only 43–48% of the total copper in the resting protein was Cu(II). A subsequent magnetic study by Ehrenberg *et al.*⁽¹¹⁶⁾ showed that the ratio of the observed susceptibility ($550\text{--}570 \times 10^{-6}$ emu) to the calculated susceptibility based on total copper as Cu(II) ($14,000 \times 10^{-6}$ emu) was 40%, in agreement with the ESR experiments.

Ceruloplasmin is a copper serum protein, containing diamagnetic Cu(I)

in the reduced state. Ehrenberg *et al.*⁽¹¹⁶⁾ showed that, as in laccase, oxidized ceruloplasmin contained 40% of the total copper as Cu(II). Aisen *et al.*⁽¹¹⁷⁾ noted that in Ehrenberg's single temperature study, the paramagnetism of Cu(II) contributed only 0.5% to the total room-temperature susceptibility. More information on copper interactions and a temperature independent paramagnetism (TIP) correction could be obtained by low-temperature data. They determined the susceptibility of ceruloplasmin from 2° to 4.2°K and at 77°K. The protein followed the Curie law above 2°K, no TIP term was found, and no Cu-Cu interactions were indicated. They showed that 44% of the total copper was Cu(II), which favored a formulation of seven copper atoms/mole of ceruloplasmin, three being Cu(II).

Noheme Iron Proteins

Ferredoxin

C. pasteurianum ferredoxin contains seven iron atoms and is presumed to be important in electron transfer processes. The susceptibility of this protein was measured in solution⁽¹²⁴⁾ by nuclear magnetic resonance techniques. The shift of the proton resonance line of an inert reference substance (tetramethyl ammonium chloride) due to a paramagnetic species is given by the expression $\Delta H/H = (2\pi/3) \Delta K$ where ΔK is the change in volume susceptibility. The average of five susceptibility determinations in solution gave a moment of 2.0 ± 0.2 B.M./iron. Solid-state susceptibilities by the Faraday method showed that when the sample was compacted, the erratic results indicated that the iron coordination and/or oxidation state had changed. A moment of 2.3 B.M./iron was found for an unpressed sample. There are four combinations of spin states of seven low-spin iron atoms that could produce the observed moment: 6Fe(III) + 1Fe(II); 5Fe(III) + 2Fe(II); 4Fe(III) + 3Fe(II); and 7Fe(III). Another possibility was 5Fe(II) low-spin + 2Fe(III) (high-spin). The Moessbauer spectra were interpreted as two overlapping doublets, indicating at least two nonequivalent iron environments. Combined with chemical evidence, this ferredoxin was postulated to exist as a linear chain of seven low-spin Fe(III) atoms, the outer two and inner five being equivalent. However, preliminary X-ray data indicated that there is no linear chain.⁽¹²⁶⁾

Ehrenberg and Kamen⁽¹⁰⁹⁾ found that the *chromatium* high-potential iron protein was diamagnetic Fe(II) in the reduced state, and had the strange value of 1.46 B.M./iron in its oxidized form. The reason that the moment was lower than that of one unpaired electron was not known. In agreement with the magnetic data, a Moessbauer study⁽¹²⁴⁾ showed the reduced form

to be low-spin Fe(II). It was indicated that the four iron atoms in the oxidized state were identical, with a possibility of two or three electrons delocalized over the four iron atoms.

Spinach ferredoxin contains two iron atoms that the Moessbauer data⁽¹²⁴⁾ indicate to be in nearly equivalent environments in the oxidized ferric form. Upon reduction with sodium dithionite, and in agreement with chemical evidence, the spectra indicated that only one of the iron atoms was reduced to a high-spin Fe(II) state. The susceptibility of the oxidized form⁽¹²⁶⁾ was 1015×10^{-6} esu/iron atom and in the reduced state was 2285×10^{-6} esu/iron atom possibly indicating some spin unpairing upon reduction. These susceptibilities were stated to be inconsistent with most current models of spinach ferredoxin, which predicted pure $S = 0$, $S = \frac{1}{2}$ behavior.

Hemerythrin is an iron-containing respiratory pigment. Kubo⁽¹²⁷⁾ presented preliminary susceptibility evidence showing that the magnetic moment of the species decreased upon oxygenation; and Manwell⁽⁸⁸⁾ has postulated four resonance forms to account for this observation. Kubo interpreted his data as indicating the deoxygenated species was high-spin Fe(II) changing to diamagnetic Fe(II) upon oxygenation. Bayer and Schremann⁽⁸⁹⁾ have also commented on this substance.

Xanthine Oxidase

Xanthine oxidases are capable of oxidizing a variety of nitrogen-containing heterocyclic compounds. Milk xanthine oxidase is a complicated system, one form of which contains 8Fe : 2Flavin : 2Mo, all of which are capable of being reduced.⁽¹²⁸⁾ Although initially thought to be totally diamagnetic,⁽¹²⁹⁾ later work⁽¹²⁸⁾ showed the diamagnetism to be half that obtained on authentic diamagnetic metalloproteins. The resting enzyme gave no ESR signals from iron, molybdenum, or flavin, and the initial susceptibility was attributed to two spin-paired Fe(III) atoms with the other six iron atoms being in diamagnetic $S = 0$ Fe(II) states.⁽¹²⁸⁾ The low-spin Fe(III) signal was assumed too broad for detection. A marked susceptibility increase upon reduction was attributed to the transformation of low-spin Fe(III) (one unpaired electron) to high-spin Fe(II) (four unpaired electrons). The reduced enzyme has a temperature dependent signal at $g = 1.9$, and Ehrenberg and Bray⁽¹³⁰⁾ showed a correlation coefficient of 0.98 between the intensity of the signal and the increase in susceptibility upon reduction. This showed that the signal was due to iron, as the coefficients of 0.68 and 0.52 were found for the Mo and flavin signals, respectively. They point out that the susceptibility increase could be due to a reduction

in another part of the molecule (not iron) producing conformational changes which decrease Fe-Fe interaction, producing higher susceptibilities, as noted in iron fructose.⁽¹³¹⁾ However, Beinert⁽¹³²⁾ argues that the complexity of the system does not rule out an eight-atom diamagnetic Fe(II) initial state being reduced to a formally low-spin Fe(I) (one-electron) final configuration. He suggest more studies on simpler protein systems that do not have the added complexity of the reducible Mo and flavin components.

Ferrochrome and Ferroverdin

Weisel and Allen⁽¹³³⁾ made the interesting observation some years ago that when the rust fungus *Ustilago sphaerogena* was grown in a medium containing yeast extract, the cells were pink and displayed the spectrum of cytochrome c. Neilands⁽¹³⁴⁾ showed that the cytochrome-c production was accompanied by the formation of a nonprotein crystalline organoiron pigment which he called ferrochrome. He later isolated a second iron-bearing pigment, ferrochrome A, from the same fungus. Both ferrochromes have but one iron atom per molecule which is trivalent in the oxidized forms. Ehrenberg,⁽¹³⁵⁾ in order to elucidate the structure of these complexes, has measured the magnetic susceptibility of both the oxidized and reduced forms. His data shown in Table XI correspond to the five unpaired electrons of Fe(III) for the oxidized forms, and to the four unpaired electrons of Fe(II) for the reduced forms. Ferrichrome A was later shown to be a cyclic hexapeptide by chemical and X-ray analysis.⁽¹³⁷⁾ The compound obeys the

TABLE XI
Magnetic Moment of Iron in Some Complexes at Room Temperature

Compound	μ_{eff} , B. M.	Reference
Ferrichrome	5.68	135
Ferrochrome	4.93	135
Ferrichrome A	5.73	135
Ferrochrome A	4.95	135
Dihydroxo-dileucino-ferric chloride	4.08	139
Dihydroxo-diisoleucino-ferric chloride	4.14	139
Dihydroxo-divalino-ferric chloride	4.14	139
Dihydroxo-dimethionino-ferric chloride	4.20	139
Dihydroxo-diphenylalamino-ferric chloride	5.18	139
Dihydroxo-dilysino-ferric chloride · 7H ₂ O · 2HCl	5.87	139

Curie law from 1°–4.2°K, and its ESR and Moessbauer spectra have been analyzed.⁽¹³⁶⁾

Ehrenberg⁽¹³⁵⁾ also studied the magnetic properties of ferroverdin, an organoiron pigment isolated from a type of streptomyces. He found this pigment slightly paramagnetic in methanol and glycol solutions, and, after making corrections for basic diamagnetism, he concluded that the iron in ferroverdin was spin-paired Fe(II).

Ferritin

Michaelis *et al.*^(137,138) have studied an interesting iron-storage protein called ferritin, which can be extracted from horse spleen. In spite of the very high iron content of this protein (~ 23%), they were not able to detect any ferromagnetism. Their measurements showed the iron to be in the ferric state with a magnetic moment of 3.78 B.M. The iron in ferritin is easily extracted with NaOH and results in a ferric hydroxide precipitate. The effective magnetic moment of this precipitate was shown to be 3.77, the same value as the iron in the ferritin itself. After detailed studies of the susceptibility of various preparations of ferric hydroxide these authors concluded that the crystal lattice of ferritin was made up of the iron-free protein molecules (apoferitin) with micelles of ferric hydroxide interspersed in the intermolecular spaces. The form of the iron in this protein is interesting, especially when contrasted to the form of iron normally present in blood. Rawlinson and Scutt⁽⁶³⁾ have made a study of the bonding of iron in various ferriheme hydroxide derivatives compared to simple iron hydroxides and ferritin. Their results furnished evidence for low-spin iron in alkali solutions although specific bonding of the iron could not be established. Ribonucleic acids are also thought to be closely associated with colloidal iron hydroxide. Perhaps the incorporation of an inorganic iron compound by a larger protein molecule or micelle is a more common occurrence in biological materials than at first thought.

Bayer and Hauser⁽¹³⁹⁾ have studied the magnetic susceptibility of ferritin as a function of temperature down to liquid-nitrogen temperatures. Unlike many other biochemicals, the susceptibility of this substance does not follow a straight line relationship with temperature and hence is not governed by either the Curie or the Curie–Weiss law. At 22°K they obtained a susceptibility of 6200×10^{-6} emu/mole of iron in ferritin, which corresponds to an effective moment of 3.84 B.M., agreeing with the earlier measurements.

Schoffa⁽¹⁴⁰⁾ has measured the susceptibility of ferritin from 4.2°–300°K and finds antiferromagnetic behavior with a Neel temperature of $20^\circ \pm 3^\circ\text{K}$. The results were interpreted in terms of antiferromagnetic exchange be-

tween isolated clusters of two iron atoms in the micelles, and an iron-oxide-iron superexchange mechanism. Low-temperature Moessbauer studies⁽¹⁴¹⁾ have distinguished between the γ -FeOOH and FePO₄ forms of iron in ferritin. Magnetic ordering was observed at helium temperatures.

In the range 279°–316°K, Ehrenberg⁽¹²²⁾ finds ferritin to have a Weiss constant of $-93^\circ \pm 17^\circ\text{K}$ and an effective moment of 2.9 B.M. The θ value agrees with that found by Bayer and Hauser.⁽¹³⁹⁾ He also comments on the consistently low susceptibilities found in frozen solutions of heme proteins.

Conalbumen

Conalbumen is an egg white protein of molecular weight 76,600 which binds two ferric ions per molecule.⁽¹⁴²⁾ It has been suggested that the three tyrosine phenolic groups are coordinated to each iron, but this has been questioned.⁽¹¹¹⁾ Wishnia⁽⁹⁹⁾ has studied the proton relaxation time and magnetic susceptibility of iron conalbumen. The single-temperature-corrected susceptibility of $14,600 \times 10^{-6}$ cgs units corresponds to a moment of 5.92 B.M., indicating an $S = \frac{5}{2}$ Fe(III) system.

Transferrin

Transferrin is an iron transporting serum protein of molecular weight around 90,000 that can combine with two iron atoms. Ehrenberg and Laurell⁽¹⁴³⁾ found a positive susceptibility of $15,700 (\pm 500) \times 10^{-6}$ cgs units at 293°K corresponding to an effective moment of 6.08 ± 0.1 B.M. The iron was interpreted as being in a high-spin Fe(III) form with possible weak interaction between the two atoms in the molecule. A discussion of other magnetic possibilities arising from two iron atoms per molecule is given. When iron transferrin was reduced with dithionite, the decolorized solution had a susceptibility of $11,000 \times 10^{-6}$ cgs units (5.1 B.M.). This is close to the spin-only moment for high-spin Fe(II).

Iron-Bearing Amino Acids

Some of the iron-bearing amino acids are structurally similar to the iron in heme. Bayer and Hauser⁽¹³⁹⁾ have studied the magnetic properties of several of these complexes having the general structure



Their results on compounds in which various substitutions were made for R are shown in Table XI. When only the R's are substituted, the complex

has a susceptibility corresponding to about 3 B.M. Presumably it has the square-planar arrangement consisting of four ligands and partial low-spin character. When, however, both the R's are substituted and at least one HCl molecule is added per iron atom, then the structure tends toward spin-free systems, *i.e.*, complexes are formed with different coordination numbers. Bayer and Hauser further showed that for the high-spin iron, the Curie law was obeyed, whereas for the partially low-spin compounds a Curie-Weiss law was obeyed with a θ -value of -40.

Amino acids which do not have a central paramagnetic atom are diamagnetic. Havemann *et al.*⁽⁷⁴⁾ have measured the magnetic susceptibility of a series of the more common amino acids, and their results compare favorably with the susceptibilities calculated by Pascal's method.

Adsorbed Chlorophyll

The susceptibility of the diamagnetic magnesium complex chlorophyll⁽¹⁴⁴⁾ was found to change in a complex manner to large paramagnetic values upon adsorption onto alumina and silica gel from alcohol solutions. Positive susceptibility values were found regardless of the degree of surface filling, as measured by adsorption isotherms.

Vitamins

Woernley⁽¹⁴⁵⁾ has made a fairly comprehensive study of the magnetic susceptibility of many of the vitamins. With the exception of vitamin B₁₂, the vitamins do not contain any transition elements, and they are all diamagnetic. Woernley, using Pascal's systematics⁽¹¹⁾ has compared the calculated susceptibilities with the experimental values (Table XII). For the most part the comparison is very good. From these results, it appears that his approach of comparing the experimental data with values calculated on the basis of several possible structures is a powerful tool for determining the correct formula. For instance, he was able to confirm the currently accepted structure of ascorbic acid (III) by comparing the experimental value with the calculated susceptibilities based on the following three possible structures:

- I. COOHCOCH₂COCHOHCH₂OH
- II. COOHCOH:CHCOCHOHCH₂OH
- III. COCOH:COHCHCHOHCH₂OH
 └──O──┘

TABLE XII
Molar Magnetic Susceptibilities χ_M of Some Vitamins at Room Temperature

Material	χ_M , emu $\times 10^6/\text{mol}$	Reference
Abscorbic acid	- 93.2	145
Biotin	-140.5	145
Calcium pantothenate	-262.1	145
Folic acid	-220.7	145
2-Methylnaphthoquinone (menadione)	- 81.3	145
Nicotinic acid	- 63.8	145
α -Tocopherol	-332.95	145
Riboflavin	-188	145
Carotene	-379*	145
Vitamin A acetate	-221*	145
Vitamin B ₁₂	-854 [†]	148
Vitamin B ₁₂	-750	147
Vitamin B _{12a}	-147*	149
Vitamin B _{12b}	-800 [†]	148

* Calculated.

† Sample impure, approximate value.

The calculated susceptibilities of the forms I, II, and III ($\times 10^5$) are $(-0.415, -0.473, \text{ and } -0.533) \times 10^{-6}$ emu/g, respectively. The experimental value for ascorbic acid is -0.53×10^{-6} emu/g, thus confirming form III to be correct.

Vitamin B₁₂ is unique among the vitamins in that it contains the transition element cobalt, i.e., it is a cobalt complex. The magnetic properties of vitamin B₁₂ were first investigated by Diehl *et al.*⁽¹⁴⁶⁾ and Grün and Menasse.⁽¹⁴⁷⁾ Although their specimens were not very pure, they did show that it was diamagnetic. Wallmann *et al.*⁽¹⁴⁸⁾ also studied six preparations of vitamin B₁₂. They too found impurities in their samples, but three specimens were apparently pure enough to be clearly diamagnetic and possibly low-spin d⁶ Co(III).

These authors also measured several specimens of vitamin B_{12b}, but again due to impurities, their samples varied from diamagnetic to slightly paramagnetic. Diehl *et al.*⁽¹⁴⁹⁾ have made more recent measurements on a purified preparation of vitamin B_{12a}. They consider both vitamin B_{12a} and B_{12b} to be the hydroxyl form of cobalt-organic complex, based on other data,^(150,151) but their susceptibility value for vitamin B_{12a} (-0.114×10^{-6}

emu/g) is substantially different from that obtained by earlier authors on vitamin B_{12b}.

Compounds Related to Cancer Studies

Under this classification we shall include not only those chemicals which are known to produce cancer, but also the nucleic acids which are important in providing the information about the constitution of normal and tumor type cells. Magnetic-susceptibility measurements have been made on many of these substances and have served as a useful tool in analyzing the data.

About a quarter of a century ago, Schmidt⁽¹⁵²⁾ suggested that there might be a relationship between the electronic configuration and carcinogenic activity of polycyclic hydrocarbons. This theory was later extended by other investigators⁽¹⁵³⁻¹⁵⁵⁾ and reviewed by Badger⁽¹⁵⁶⁾ and by Coulson⁽¹⁵⁷⁾ on the basis that carcinogenic hydrocarbons contain a localized charged region called the K region, whereas those hydrocarbons displaying little carcinogenic activity have a normal electron distribution. When the benzene rings of the hydrocarbon are connected side-to-side, as in anthracene, the electron distribution is considered normal, whereas if the rings are staggered as in phenanthrene, that part of ring which is offset has an abnormal electron distribution which is known as the K region. The diamagnetism of an organic molecule depends on the electronic structure, and hence magnetic studies have been very helpful in delineating those compounds having active K regions. If the experimental diamagnetism coincides reasonably well with that calculated on the assumption of normal electron distribution, the compound should not have a highly charged K region and hence should not be carcinogenic. On the other hand, if there is a difference of several percent between the calculated and experimental values, a charged K region is present, and according to the theory the compound should display carcinogenic activity. As a result of this theory, the magnetic susceptibilities of many carcinogens have been measured^(158,159) as shown in Table III. With a few exceptions the comparison of the calculated and experimental values is very good. On the basis of X-ray, magnetic, and biological data the correlation between the K region and carcinogenic activity is not as strong as originally thought. Mayr and Rabotti⁽¹⁶⁰⁾ have also studied the magnetic susceptibility of certain chemotherapeutic agents known to exhibit antineoplastic action. As shown in the table these substances have experimental susceptibilities which are higher than the calculated values. It is too early to say whether or not these differences are significant. Although a

TABLE XIII
Calculated and Experimental Gram Magnetic Susceptibilities for Carcinogenic and Chemotherapeutic Compounds at Room Temperature

Compound	Magnetic susceptibility		
	Calculated emu × 10 ⁶	Experimental emu × 10 ⁶	Reference
Carcinogenic			
3-Methylcholanthrene	-0.724	-0.707	159
	-0.759	-0.68	158
Anthracene	-0.715	-0.729	159
Cholesterol	-0.744	-0.741	159
Ergosterol	-0.700	-0.705	159
Calciferol	-0.694	-0.689	159
Dihydroisoandrosterone	-0.642	-0.644	159
Estradiol	-0.676	-0.685	159
Disoxycholic acid	-0.681	-0.693	159
Cholic acid	-0.674	-0.691	159
Colchicine	-0.539	-0.534	159
3,4-Benzyrene	-0.736	-0.59	159
Acenaphthanthrocene	-0.766	-0.73	159
Acetylaminofluorene	-0.8	-0.63	159
Perylene	-0.73	-0.69	159
1,2,5,6-Dibenzofluorene	-0.04	-0.69	159
3,4,5,6-Dibenzphenanthrene	-0.72	-0.73	159
O-Aminoazotoluene	-0.6	-0.61	159
Chemotherapeutic			
Di-(2-chloroethyl)-methalamine hydrochloride	-0.68	-0.81	160, 165
Di-(2-chloroethyl)-β-naphthylamine	-0.68	-0.74	160, 165
2,4,6-Triethyleneimino-1,3,5-triazine	-0.61	-0.74	160, 165
Ethyleneimine picrate	-0.40	-0.58	160, 165
N,N'-dicycloethylene-carbanyl-hexamethylene-diamine	-0.61	-0.82	160, 165
Mercaptopurine	-0.46	-0.64	160, 165
Butyl-1,4-di(methylsulfanate)	-0.55	-0.69	68, 165

TABLE XIV
Calculated and Experimental Gram Magnetic Susceptibilities of Nucleic Acid Constituents and Related Compounds*

Compound	Form	Magnetic susceptibility	
		Calculated, emu × 10	Experimental, emu × 10
Cytosine	Enol	−0.484	−0.503
	Keto	−0.427	
Uracil	Enol	−0.465	
	Keto	−0.352	
	Keto-enol	−0.409	−0.416
Thymine	Enol	−0.507	
	Keto	−0.407	
	Keto-enol	−0.457	−0.453
Adenine		−0.461	−0.447
Guanine	Enol	−0.451	−0.450
	Keto	−0.409	
Uric acid	Enol	−0.431	
	Keto	−0.318	
	Keto-enol	−0.393	−0.394
	Keto-enol	−0.356	
<i>D</i> -Ribose	Enol, ring	−0.548	−0.564
	Keto	−0.506	(30 g/100 g H ₂ O)
<i>D</i> -Glucose	Enol, ring	−0.548	−0.567
	Keto	−0.513	
Sucrose	Enol, ring	−0.546	−0.553
			−0.558
			(40 g/100 g H ₂ O)
Cytidine	Enol, ring	−0.516	−0.509
	Keto	−0.490	
Uridine	Enol, ring	−0.508	
	Keto	−0.456	
	Keto-enol	−0.482	−0.479
Adenosine		−0.502	−0.515
Guanosine	Enol, ring	−0.494	−0.527
	Keto	−0.472	
Phosphoric acid	Liquid (86.3%)		−0.447

* After Woernley (159).

strong correlation with carcinogenicity or antineoplastic action has not been found, the magnetic studies of these compounds have established a valuable technique which can be useful in the examination of the electronic configuration of molecules.

Woernley⁽¹⁵⁹⁾ has calculated the susceptibilities of a number of nucleic acid constituents and related compounds using Pascal's systematics,⁽¹¹⁾ and a comparison of the calculated values with his experimental values is shown in Table XIV. He based his calculations on the various tautomeric forms of the compound and, as shown in the table, it is clear which form he measured. This magnetic technique is very useful in settling questions of tautomeric form. Veillard *et al.*⁽¹⁶¹⁾ have also made similar calculations on some of the same compounds using a somewhat different method. These results, however, are considerably different from those calculated by Woernley and do not compare well with his experimental data.

Woernley⁽¹⁶⁹⁾ also attempted to study the magnetic properties of ribonucleic and deoxyribonucleic acid, but found that all preparations of these substances contained an appreciable and variable amount of iron (> 0.05%). Owing to the high iron content he was unable to measure the susceptibility, but on the basis of the known formula of ribonucleic acid he calculated a value of -0.48×10^{-6} , assuming no iron present. The presence of iron in nucleic acids has been confirmed by electron spin resonance.⁽¹⁶⁶⁾ The fact that denaturation or charring does not destroy the electron spin resonance signal indicates the existence of an inorganic form of iron.⁽¹⁶⁸⁾ Walsh *et al.*⁽¹⁶⁴⁾ have shown by electron microscopy that small platelets ($\sim 1000 \text{ \AA}$) of an inorganic iron compound are imbedded in the nucleic acid and hence cannot be removed by ordinary methods. Although they were not able to positively identify the compound, it has properties which closely parallel those of colloidal iron oxide-hydroxide. Walsh *et al.*⁽¹⁶⁴⁾ conclude from their experiments that the iron in nucleoproteins is present in living cells in highly disseminated form and that in the process of extraction or storage there is a nucleation of the iron into a ferromagnetic compound.

Perakis and Kern⁽¹⁶⁶⁾ studied the susceptibility of two preparations of DNA from calf thymus from 80–300°K at several field strengths. The relation $(\chi - X_0)T = a(1 - 5X/3)$ was obtained, where X_0 and a are constants, $X = 1$ for the high-polymer DNA sample, and $X = 0$ for the low molecular weight form.

The problem of iron in nucleic acids and the apparent ferromagnetism of these compounds has recently been discussed by Blumenfeld.⁽¹⁶⁷⁾

CONCLUSIONS

As is evident from the above review of the magnetic-susceptibility measurements of biological materials, probably the most important application of these data is the determination of chemical structure and the solution of certain specific problems. From the point of view of elucidating the biological effects of magnetic fields, the results are not immediately rewarding. If one examines the data as a whole, however, some useful information is evident.

In general the following observations appear to be true. It appears that in biological systems the range of paramagnetism is about as large as the range of diamagnetism, i.e., there do not seem to be any notable extremes of paramagnetism or diamagnetism in biochemical compounds. In a magnetic field, then, there will be, on the average, about the same magnitude of force exerted on both diamagnetic and paramagnetic components. Although these forces will be small, a significant separation might conceivably be effected and could upset some enzyme reaction. From the experiments that have been performed, it is clear that the changes in magnetic properties, and hence in the structure, are highly sensitive to the mobility of electric charge. Also, both the observed paramagnetism and diamagnetism are large enough in some cases to cause significant orientation and distortion of the biological components when placed in a magnetic field. The combination of these electron distribution effects may well bring about or be the initial cause of many of the biological effects which have been observed in magnetic fields. It appears, therefore, that the immediate effects of the magnetic field on the electron distribution in a particular biochemical will ultimately be shown to be more important in explaining biomagnetic effects than the differences in the magnetic susceptibilities themselves.

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