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A REVIEW OF THE BIOLOGICAL EFFECTS OF VERY LOW MAGNETIC FIELDS

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SUMMARY

The first studies of the effects upon living organisms of exposure to magnetic fields lower than that of the Earth were made within the present decade. In this review, ten of these are tabulated according to the taxonomic classification of the organism studied. Also included in the tabulation are eleven studies of the effects of fields in the geomagnetic range. Reports of studies of very low magnetic field effects were not found for fungi, echinoderms, amphibians or subhuman primates; specific studies of musculo-skeletal functions have not been reported, nor do ultrastructural techniques appear to have been applied to the study of very low magnetic field effects. A useful body of information should include such studies.

Nonetheless, some well controlled experiments reviewed herein appear to have established that certain lower invertebrates, protozoans, and plants are indeed sensitive to the vector of the ambient magnetic field in the geomagnetic range, and that in nearly null magnetic fields, the growth, reproductive, aging, behavioral and phagocytic functions of some species are affected.

Two studies of very low magnetic field effects done in the author's laboratory are described in detail; these show a reduced cyto-enzyme synthesis rate *in vivo* in mice, but no reaction rate change *in vitro*.

INTRODUCTION

Man's exploration of space is resulting in his prolonged separation from the terrestrial magnetic field. In figure 1, a typical distribution of the geomagnetic field at the Earth's surface is shown on a Mercator projection. Spacecraft, at the altitude and latitudes of the usual near Earth orbits, will be exposed for the most part to magnetic fields no lower than those around Rio de Janeiro at sea level. But in space flights carrying him more than 10 Earth radii (about 1/6th 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 (refs. 2 and 3) have revealed extremely low magnetic fields, that is, in the range of a few gamma (10^{-5} Oersted) of intensity, and planetary probes (refs. 4-6) show fields of considerably less

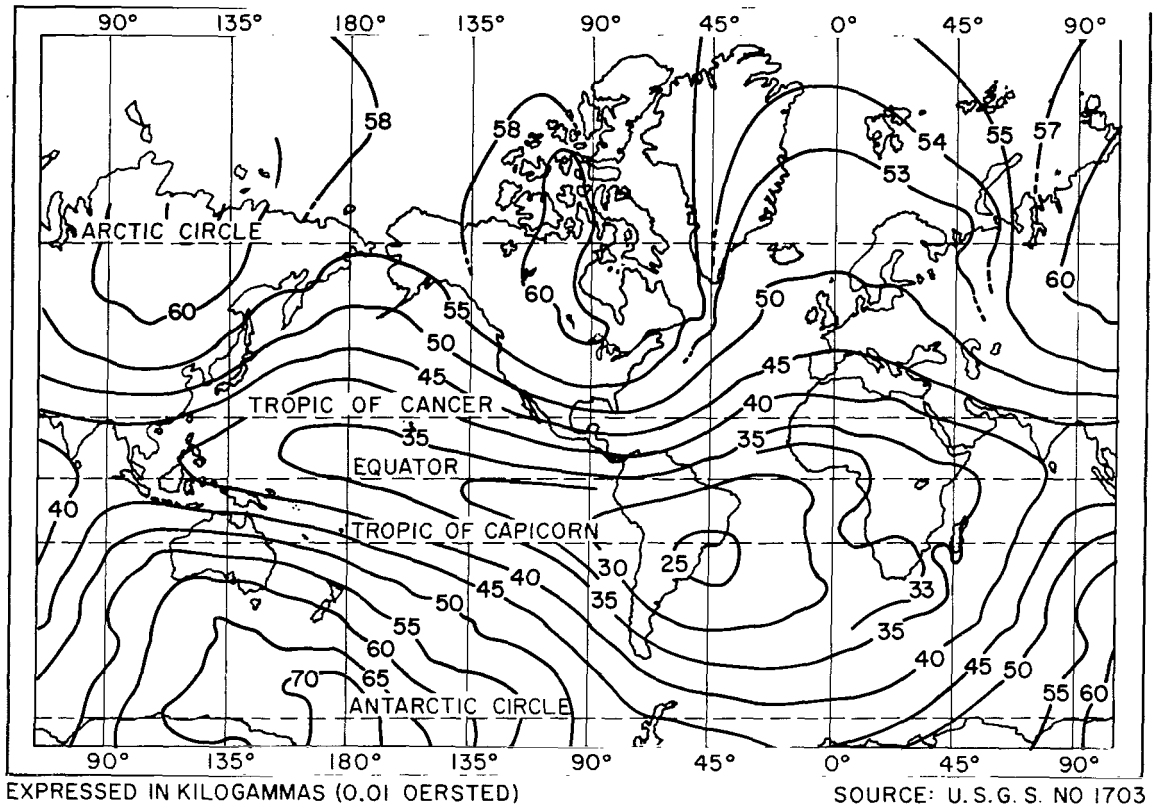


Figure 1.- The total intensity of the Earth's magnetic field. Expressed in kilogammas (0.01 Oersted). Source: U. S. Geological Survey no. 1703.

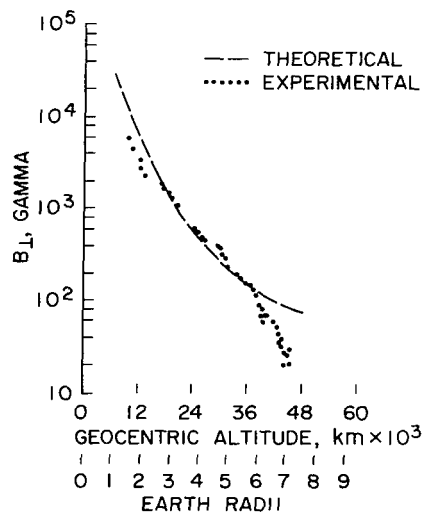


Figure 2.- Field magnitude measurements during a magnetic storm (Explorer VI) (ref. 1).

than 100 gamma near Mars and Venus, for example (table 1). 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.

TABLE 1.- SOME REPRESENTATIVE MAGNETIC FIELD INTENSITIES

[Adapted from ref. 7]

Alnico magnet surface	~1000 Oersted	~10 ⁸ Gamma
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

Beischer (ref. 8) 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 penalty." In this respect, the findings of Harrison and Funnel (ref. 9), recently confirmed by Watkins and Goodell (ref. 10), 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.

It is the purpose of this paper to review contributions to the understanding of the biological significance of magnetic fields of the intensities found in nature, especially in the extraterrestrial environment, and to present some original data.

PREVIOUS REVIEWS OF THE SUBJECT

It is to be remembered that in the temperate zones, the terrestrial magnetic field is of the order of 0.5 Oersted (i.e., 5×10⁴ gamma, see fig. 1). In discussing the research work cited here, the term *geomagnetic* intensity shall refer to fields of this order, or at the highest, fields of a few Oersted. These latter fields, sometimes referred to as *low* fields, have generally been applied by means of a small bar magnet in experiments concerned with the effects of the direction of the geomagnetic vector. In such studies the technique has thus been to superimpose a field just slightly stronger than the horizontal component of the Earth's field.

We are primarily concerned, however, with subgeomagnetic field intensities, since that is the condition in outer space. When the terms *very low*

near-zero, or *null* magnetic field intensity are used, they shall refer to fields of the order of a few gamma (i.e., about 10^{-4} Oersted). In the bibliographic table (table 3), wherever possible, the field intensities are given in milliOersted (mOe) to facilitate comparisons.

In one of the most thorough recent reviews, Busby (ref. 11) has organized the reports of high magnetic field effects on biological systems in tabular form. Of a total 148 references cited, 4 are to works concerned with low field effects, and 15 are to reports of very low field studies; no tabulation of these latter groups is presented. To Busby's low and very low field citations, only 13 can be added to date, indicating the small amount of published work of this type. But it should be remembered that until quite recently (ref. 7) there were no reported biological studies of magnetic fields below the intensity of geomagnetism.

An organized, analytical approach to the literature of biomagnetic effects appears in reference 12. Here, again, the paucity of very low field experiments is evident; there were no references to such studies out of a total of over 200 reports cited. However, Gross did cite 62 publications of studies in the geomagnetic range; of these 23 referred to bird navigation.

In a brief review related to space travel considerations, Beischer and Miller's (ref. 13) previously unpublished very low field experimental findings were cited as the only reference to subgeomagnetic work out of 29 citations.

Becker (ref. 14) published a well organized review with 44 citations, and although he himself is one of the few experimenters to have studied subgeomagnetic field effects (ref. 15), he did not cite this particular work, or any other such very low field studies, in his survey.

A thoroughly comprehensive review was published by Davis and co-workers (ref. 16) under the supervision of A. J. Jacobius for the Federation of American Societies for Experimental Biology. It is carefully annotated and has become a standard source. But, although it contains nearly 400 references, none are to work in subgeomagnetic fields. These reviewers have defined "low intensity" fields as less than 100 gauss, for their purposes. They do supply 80 references to studies of geomagnetic field effects; half of these concern bird navigational experiments and observations.

The review by Alexander (ref. 17) lists 40 references, but, again, none are to subgeomagnetic work. Dr. Alexander and his company are to be thanked for arranging the 1961 translation of the monumental "Course in Magnetobiology" given by Valentinuzzi at the School of Medicine of Montevideo (ref. 18). In this work there are 113 references, but, as could be expected, none to subgeomagnetic experiments.

In Leikind and Wiener's annotated bibliography prepared for the Office of Naval Research (ref. 19), there are 82 references, 22 are to bird navigational studies and 4 are to geomagnetic effects in other animals.

TECHNIQUES FOR PRODUCING LOW AND VERY LOW MAGNETIC FIELDS

Precise and reliable measurements of the fluctuations in the natural geomagnetic field are possible with modern equipment and can provide important data for correlations in observational studies of biological systems. There is a real need for expansion of this kind of work; it is exemplified by the studies of Alvarez (ref. 20), Düll and Düll (ref. 21), and R. O. Becker and co-workers (ref. 22) which show correlations between human factors, such as mental illness, and variations in the geomagnetic field. Even though such work is difficult to accomplish and interpret, modern computation equipment should facilitate it, and modern science demands it.

But we are primarily concerned here with the experimental alteration or reduction of the ambient magnetic field and shall expand on J. M. Barnothy's list (ref. 23) of 4 major categories of techniques for accomplishing this.

1. *Superimposition* of fields, a few Oersted 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 and co-workers (ref. 24).

2. *Astatisation* 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 gamma 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 the University of Illinois in Chicago, and was described recently in a paper presented by Cohen (ref. 25).

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 gamma/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 (ref. 26).

Such an arrangement of coils with a compensating electronic servo system has been constructed at Ames Research Center to provide a usable volume of some 3 cubic feet within which the magnetic field intensity can be kept in

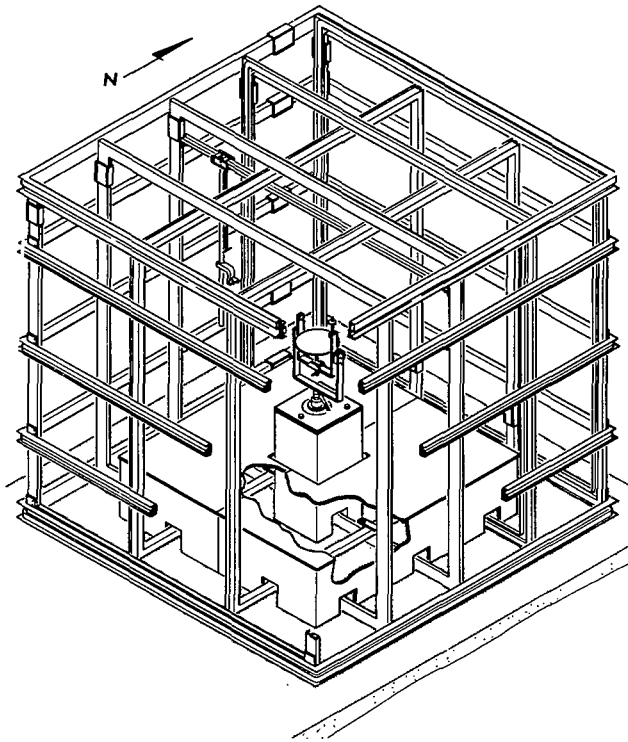


Figure 3.- Hypercubic coil assembly of space magnetic environment simulation laboratory at the NASA Ames Research Center (ref. 27).

the range of a few gamma or less, comparable to interplanetary levels. This is illustrated in figure 3 and is described in detail in reference 27. 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 such large facilities, including those mentioned in reference 23, 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.

the facility at the Institute of Geophysics and Planetary Physics of the University of California at Los Angeles (ref. 28). 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.

5. *Combinations* of Mu-Metal shielding and active electrical compensation are in use, for example,

As indicated in references 25 and 27, 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 (cf. ref. 29). It may well be that real appreciation of the magnetic facts of life has awaited the development of facilities capable of eliminating the overshadowing effect of Earth's natural field, however "weak" it is considered to be. Thus, while Baule (ref. 30) has indeed measured the magnetocardiogram of humans using only the simple precautions of an outdoor location and a wooden bed, his readings are of the order of a fraction of a gamma of magnetic field produced, and one suspects that the precision of his records could have been even greater in a more completely shielded environment.

Similarly, the measurements by Gengerelli and co-workers (ref. 31) of the magnetic fields associated with the propagation of nerve action potentials

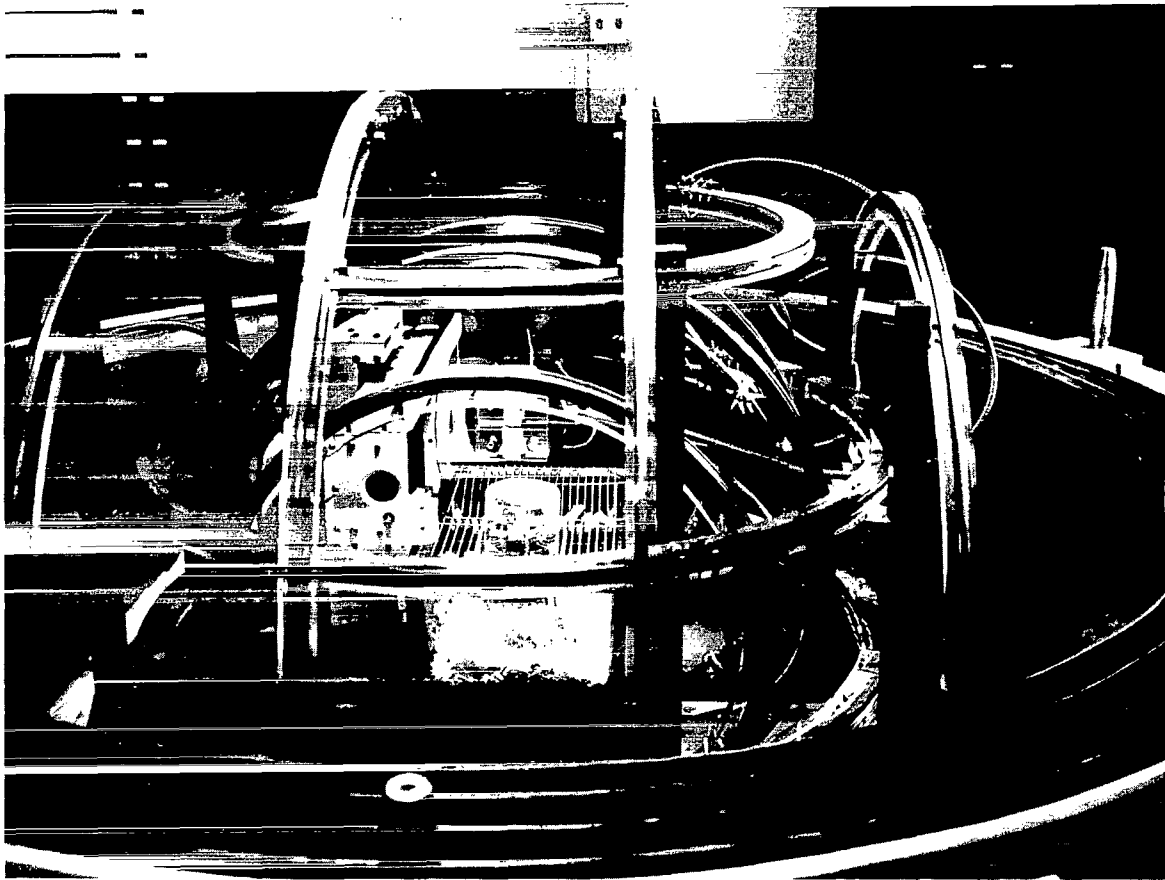


Figure 4.- Magnetic shielding tank composed of concentric cylinders of Mu-metal, opened to show the three-axis compensating coil assembly surrounding an experimental subject.

are calculated to be in the 100 gamma range, the range of very low fields. Such measurements would undoubtedly be more precise if taken in a reduced field environment.

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 comparable fidelity in analogous, ground-based simulation of the near-zero *gravitational* field of outer space.

THEORETICAL CONSIDERATIONS

There is a question as to the basis for organizing a review of reports of the interaction between biological systems and external environmental magnetic fields. Even a review intended to cover only the effects of low or

very low fields (those near or below geomagnetic intensity) meets with tremendous qualitative variation among all the experimental approaches and results. And, in the past, some physical scientists have registered dismay at reports of biomagnetic findings, protesting that there was no ready theoretical basis for their interpretation.

Fortunately, however, patterns are beginning to emerge among studies of biological responses to magnetic field changes. High magnetic field exposure has been shown to provide protection against biological damage due to subsequent, or even prior, exposure to ionizing radiation in a variety of species (e.g., refs. 32 and 33).

Possibly related are the *in vitro* findings of several workers that the exposure of certain enzymes to high magnetic fields prior to the introduction of the substrate enhances their reactivity (refs. 34-36).

In addition, several species of living organisms have by now been clearly shown to be sensitive to the vector of the ambient or applied magnetic field, especially at intensities near the natural, geomagnetic level (refs. 24 and 37-40).

It has thus become possible to hypothesize biomagnetic mechanisms, and although these theories are many and varied, they appear to center on two interrelated, major physical principles (cf. ref. 41).

The first is that paramagnetic and diamagnetic biological molecules are susceptible to applied magnetic force fields. Thus, the molecules are conceived of as being displaced (ref. 42) or, as in the case of the biologically ubiquitous liquid crystals, the molecules may actually become aligned (refs. 43-45).

The second principle forming a basis for many biomagnetic theories is Faraday's law of electromotive force, a special case of which is the Hall effect. Thus, the movement of ions along charged membranes in the presence of a magnetic field is invoked to explain a variety of biological events which follow alterations in the magnetic environment (refs. 46-48). The Hall effect is thought also to apply in the case of long organic polymer molecules which theoretically (ref. 49) may be capable of acting as superconductors even at body temperatures. It is possible that such molecules can be influenced by relatively modest magnetic fields, since the electrons they conduct are traveling at very high speeds.

But since most current biomagnetic theorizing is based on high field studies (cf. ref. 50) and, as such is not strictly within the scope of this paper, the reader is referred to the thoughtful correlation of modern hypotheses presented in reference 11.

REVIEW OF LOW AND NEAR-ZERO MAGNETIC FIELD STUDIES

The reports of low and very low magnetic field experiments are organized on a biological basis. In addition to near-zero field effects, the studies tabulated include work in magnetic fields of a few Oersted intensity.

Bird navigational studies, however, are a special case. Since they are of a limited scope biologically, and are so numerous, the citations included here are restricted to non-navigational bird studies. For navigational studies, the reader is referred to the scholarly review by Griffin (ref. 51). It is important to note, however, that Griffin's doubtful attitude concerning a magnetic basis for bird migration has periodically come under challenge as newer experimental methods have been applied. One example is the recent report of observations of homing pigeons by helicopter which clearly implicate cuing by the Earth's magnetic field (ref. 52).

In the organization of the review presented here, the taxonomic classification of the experimental subject (table 2(a)) forms the first category; the biological subject studied is placed under one of 14 headings which are essentially in order of increasing complexity (ref. 53).

The biological level of organization of the experimental subject (table 2(b)) is a category modified from reference 16, and has 6 terms.

The third category in the tabulation is by function and is shown in table 2(c).

From the tables of the three biological categories with their enumeration of pertinent low or very low magnetic field studies, it becomes obvious where work needs to be done in answering the question of whether or not such magnetic fields have a biological significance. Of the four taxonomic groups not represented by work reviewed herein, three (the fungi, echinoderms, and amphibians) lend themselves readily to prolonged exposure within the type of equipment required to produce low fields. The functional aspects of all four have been well studied in laboratories over the years, for example, the genetic function of the fungus *Neurospora*, and the embryonic development and cell replication phenomena of the sea urchin and of the frog. Small primates have made good subjects for high field studies (ref. 46), and some species of this order, besides, man, should make suitable subjects for low field work.

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 nor 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.

Table 3 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

TABLE 2.- BIOLOGICAL CATEGORIES OF EXPERIMENTAL SUBJECTS STUDIED
IN LOW OR NEAR-ZERO MAGNETIC FIELDS

(a) Taxonomic classification	Studies reviewed
Simple algae such as euglenophytes and chlorophytes	2
Schizomycophytes (bacteria)	1
Eumycophytes (true fungi)	0
Angiosperms (cotyledonous plants) and other embryophytes	2
Protozoans	2
Flatworms	2
Arthropods	2
Mollusks	2
Echinoderms	0
Amphibians and other lower chordates	0
Birds	3
Rodents and other lower mammals	3
Subhuman primates	0
Man	7
(b) Level of organization	
Subcellular level, anatomical and chemical	1
Cellular level	5
Tissue level, <i>in vitro</i>	4
Tissue level, <i>in vivo</i>	1
Organ level	0
Whole organism	14
(c) Biological function observed	
Life span - death	1
Aging	1
Growth, including body composition	10
Reproduction	3
Behavior, including locomotion	13
Nervous system functions, including special senses	3
Muscular system functions	0
Skeletal functions	0
Integumentary functions	1
Cardiovascular functions	2
Respiratory functions	1
Alimentary, including enzymic functions	2
Endocrine functions	1
Genito-urinary functions	0
Cytologic and reticulo-endothelial system functions	2

TABLE 3.- BIOLOGICAL STUDIES OF LOW OR VERY LOW MAGNETIC FIELDS

Biological classification	Specific common name	Level of organization	Function observed	Specific parameter measured	Magnetic field intensity ^a	Exposure duration	Effects observed	Author and year
Simple algae	<i>Euglena, Chlorella</i>	Cellular	Reproduction	Reproduction rate	< 1 mOe to 10 ⁶ mOe	1-3 weeks	Reproduction accelerated in very low fields, inhibited in high fields.	Halpern, 1966 (ref. 54)
	<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, 1963 (ref. 38)
Schizomycophytes	Bacterium <i>Staph. aureus</i>	Cellular	Reproduction	Size and number of colonies	51 mOe	72 hr	Reduction in size and number of colonies: 15-fold	Becker, 1963 (ref. 15)
Angiosperms	White clover (seeds)	Whole organism	Growth	Germination success	< 0.7 mOe	48 hr	10-20 percent increase in germination after 31 hr, -1000 seeds/batch	Halpern, van Dyke, 1966 (ref. 55)
	Winter wheat (seedlings)	Whole organism	Growth	Root orientation	Horizontal geo.-vector, equivalent (~200 mOe)	Growth period	Orientation parallel to geo., or applied field, all cases	Pitman, 1964 (ref. 39)
Protozoans	Ciliate (<i>Paramecium</i>)	Cellular (=whole organism)	Growth (reproduction)	Rate of reproduction	< 1 mOe to 10 ³ Oe	3 weeks	Growth accelerated under very low fields, inhibited under high fields	Halpern, 1966 (ref. 54)
	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 < 0.001)	Brown, 1962 (ref. 37)
Flatworms	Planarian (<i>Dugesia</i>)	Whole organism	Locomotion	Direction of migration and klinokinesis	170 mOe (geo-) to 10 ⁴ mOe	Approx. 16 sec	Orientation modified ca. 10° from mean path by altered geo.-field (p < 0.05 to < 0.005)	Brown, 1962 (ref. 37)
Arthropods	Fly (<i>Drosophila</i>)	Whole organism	Locomotion	Orientation in phototaxis and activity	2000 mOe	Approx 1 min	Applied field influenced degree of turning. (p < 0.001; all field configurations)	Picton, 1966 (ref. 40)
	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 < 0.001)	Schneider, 1963 (ref. 56)
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 < 0.005)	Brown and co-workers, 1960 (ref. 24)
	Snail (<i>Nassarius</i>)	Whole organism	Locomotion	Orientation during phototaxis	40 mOe to 10 ⁴ mOe	Approx. 1 min	Approx. 2° shift of mean path, max. at superimposed fields nearest geo.-levels (p to < 0.001)	Brown and co-workers, 1964 (ref. 57)

TABLE 3.- Concluded

Biological classification	Specific common name	Level of organization	Function observed	Specific parameter measured	Magnetic field intensity ^a	Exposure duration	Effects observed	Author and year
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 percent reduced diameter and 33 percent increase in N-S orientation in higher fields; appearance of anomalies, decreased Fe	Veneziano, 1965 (ref. 58)
	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 (ref. 59)
	Sparrow	Whole organism	Behavior	Amplitude and character of motor activity	Approx. 600 to 1700 mOe	2-9 hr	Increase and change in motor activity, 2-4 fold in 87 percent of cases	El'darov and Kholodov, 1964 (ref. 60)
Rodents	Mouse	Cellular, tissue <i>in vivo</i>	Cytologic (lysosomal enzyme activity)	Acid phosphatase content of peritoneal macrophages	< 0.8 mOe	18 hr	31 percent reduction in enzyme activity from very low field animals (p < 0.001)	Conley and co-workers, 1966 (ref. 61)
	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 (ref. 59)
	Mouse	Whole organism	Aging, growth, reproduction and behavior	Life span, litter size, activity, positioning and pathology	1.0 ± 0.5 mOe	1 year	Shortened life span (6 mo.), diffuse tissue hyperphasia, infertility (F ₄), cannibalism and supine positioning	van Dyke and Halpern, 1965 (ref. 62)
Primates	Man	Subcellular (chemical)	Human enzyme reactivity	Quantity of specific substrate converted	< 0.5 mOe to 5 kilo-Oe	Several minutes	No significant alteration of enzyme reactivity in high or very low fields	Conley and co-workers, 1967 (ref. 63)
	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 (ref. 59)
	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 (ref. 21)
	Man	Whole organism	Cardiovascular, cytologic	Blood pressure, leukocyte count	Geo.- (fluctuations)	1 year	Time correlation between biol. parameters in 43 subjects and geomag. fluctuations	Alvarez, 1935 (ref. 20)
	Man	Whole organism	Behavior	Rate of psychiatric hospital admissions	Geo.- (fluctuations)	1 mo	Positive correlation between geomag. intensity and admission rate (p < 0.001)	Becker and co-workers, 1961 (ref. 22)
	Man	Whole organism	Behavior, nervous, cardio., resp., alimentary, endocrine, cytol.	Psych. tests, C.F.F., E.E.G., E.K.G., B.P., resp. temp., weight, P.B.I., leukocytes	Approx. 0.5 mOe	10 days	Depression of C.F.F. (flicker fusion) threshold, otherwise no significant changes	Beischer and Miller, 1964 (ref. 13)
	Man	Whole organism	Behavior, nervous function, other functions	Psych. tests, C.F.F., motor tests	< 0.5 mOe	10 days	Decreased C.F.F. threshold (p < 0.001)	Beischer and co-workers, 1967 (ref. 8)

^a"Geo.-" or "Geomag." refers to intensities ≈ 500 mOe.

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 on 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.

ORIGINAL STUDIES (REF. 63)

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

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 in ref. 27) was located at the center of a 12-foot cube surrounded by open rectangular magnetic compensating coils, working in a manner analogous to the Helmholtz principle and balanced to maintain a total field of less than 80 gamma (0.8 mOe) during the incubation period. The coil system occupied one-half of an isolated building of 15 × 30 foot plan dimensions, 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 (fig. 5). Temperatures were recorded by calibrated hygrothermographs at each location.

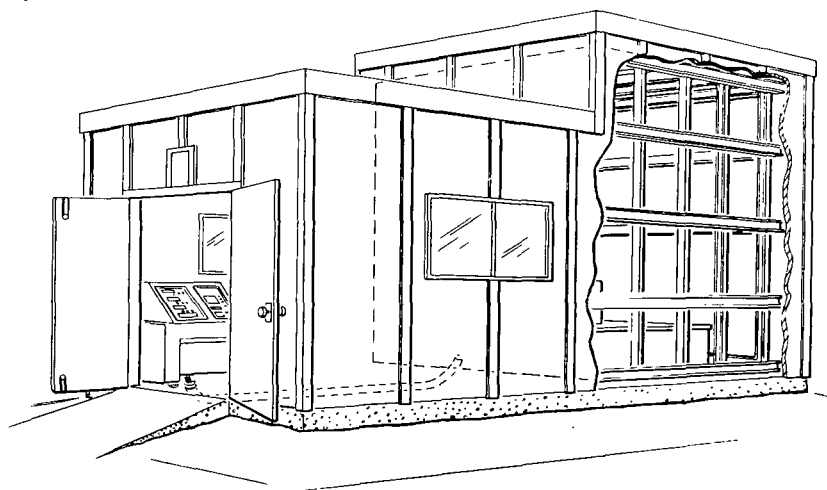


Figure 5.- Perspective of space magnetic environment simulation laboratory building showing field control console and three-axis cubic coil assembly.

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 hours starting immediately after injection.

Since the acid phosphatase activity of serosal macrophages is correlated with their phagocytic activity, this measurement was used 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 figure 6. Later, using the lipopolysaccharied of *Escherichia coli*, we found that among selected strains, the C₃H mouse exhibited the most distinctive quantitative peak in this reaction, and that it occurred after approximately 18 hours of incubation (fig. 7). Peritoneal macrophages were

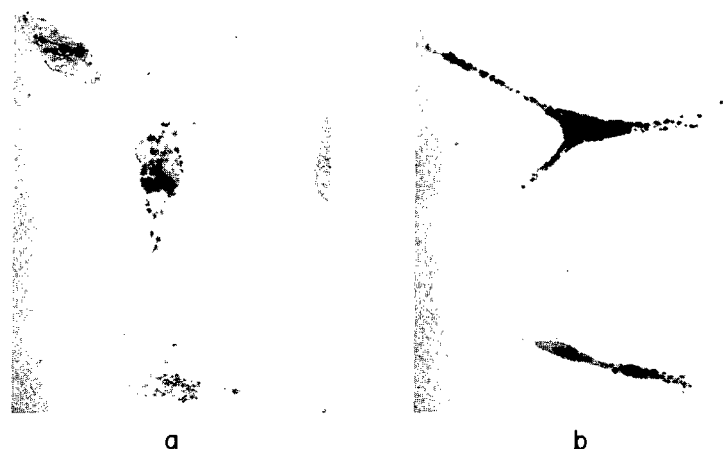


Figure 6.- Photomicrographs of serosal macrophages stained for acid phosphatase after harvesting by peritoneal lavage from (a) untreated control mouse, and (b) mouse injected 24 hours previously with a foreign protein, showing increased enzyme in cytoplasm. Gomori acid phosphatase stain, counterstained with nuclear red, $\times 1000$.

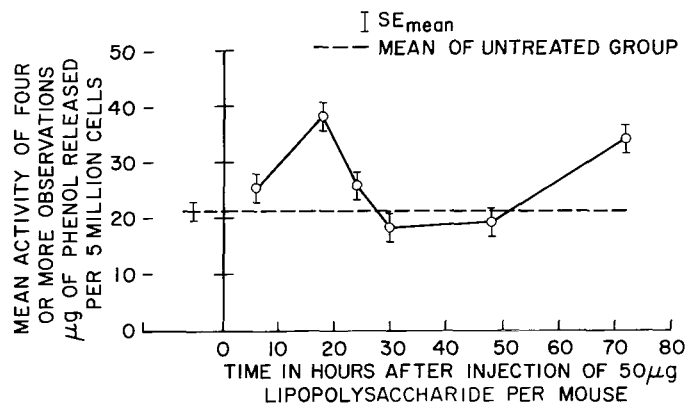


Figure 7.- Acid phosphatase activity in C₃H mouse macrophage suspensions following injection of foreign material; note peak activity after 18 hours of incubation.

obtained by saline lavage from all mice within 30 minutes after removal from the test chamber; a blind selection technique was used. Individual cell suspensions were assayed for total acid phosphatase activity by a modified Lowry spectrophotometric method (ref. 64). The activity was calculated in relation to the number 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.- Consistently lower acid phosphatase activity occurred in cell suspensions from animals 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 4) 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 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-percent 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 concomitant variable for the

TABLE 4.- ENZYME ACTIVITY *IN VIVO*: EFFECT OF EXPOSURE TO NULLED MAGNETIC FIELD

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 ^a
Control (geomagnetic field: ~ 500 mOe)	21.05	25.58	25.58	18.15	10.75	15.12	19.32 ^a

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

(Acid phosphatase activity in mouse peritoneal macrophage suspensions, expressed as micrograms of phenol released per five million cells)

particular day of experiment. The ambient temperature was found to be nonsignificant as a factor in response to treatment (fig. 8).

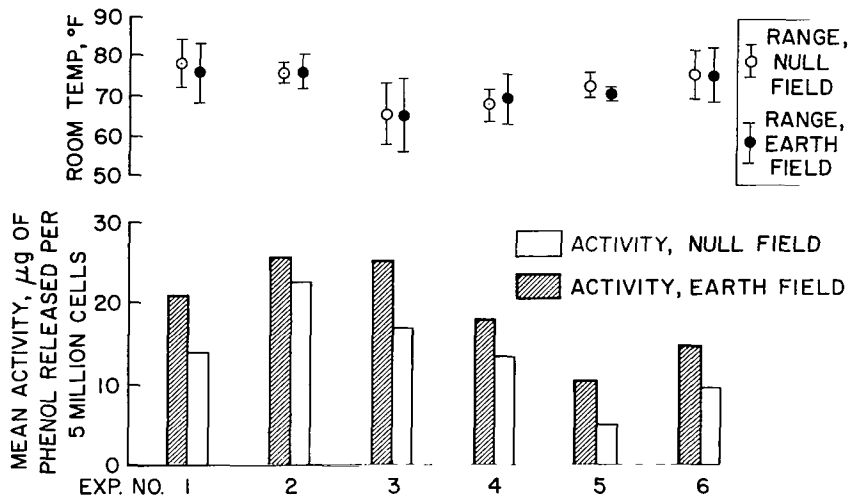


Figure 8.- Effect of a null magnetic field and the lack of an effect of room temperature upon the acid phosphatase activity of stimulated mouse peritoneal macrophages.

In addition, continuous records of the local geomagnetic field 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, a macrophage "priming" effect was achieved by the intraperitoneal injection of sterile, complete Freund's adjuvant a few weeks prior to the injection of the lipopolysaccharide. This preparation 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. 9, table 5).

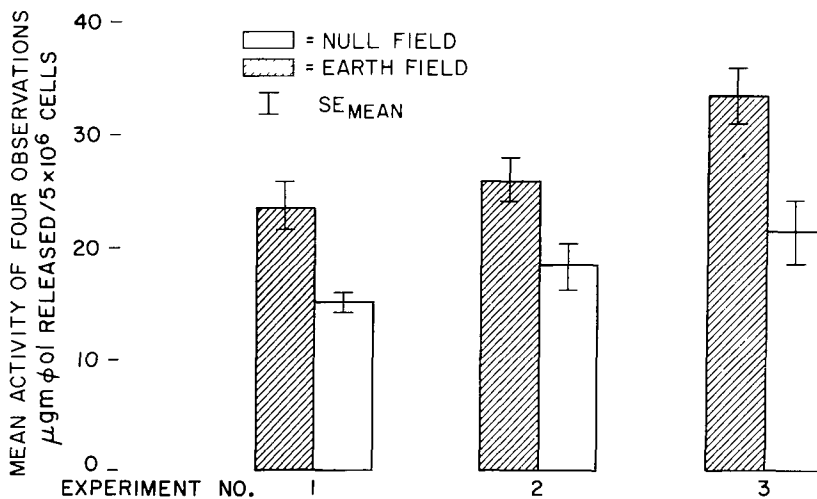


Figure 9.- Macrophage acid phosphatase activity: Effect of null magnetic fields after preparation of mice with Freund's adjuvant.

TABLE 5.- COMPARISON OF FREUND'S ADJUVANT PREPARED GROUPS
ANALYSIS OF VARIANCE TABLE

[Single classification]

Source of variation	Freedom degrees	Mean square	F-ratio	Significance
Treatment	1	642.37	12.74	p < 0.002
Within groups	21	50.39		N.S.

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.

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 changes nor with 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.

In Vitro Studies

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

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 (ref. 34), Akoyunoglou (ref. 35) and Wiley and co-workers (ref. 36) have shown enhancement of enzyme activity following exposure to high magnetic fields. But Maling and co-workers (ref. 65) found no effect when the enzyme-substrate systems they used were allowed to react in a 100 kilo-Oersted field. Since none of these studies involved acid phosphatase or the nulled field environment, we observed both nulled and high (kilo-Oersted) 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 with a Varian Model V-4007 6-inch water cooled electromagnet having V-4037 tapered pole caps with 3-inch faces, providing a uniform field of 5.7 kilo-Oersted with 95-percent homogeneity over a 2-1/2-inch diameter.

A closed plastic water bath held the experimental test tubes in groups of three within the uniform field area. The water bath 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, assayed by the Reitman-Frankel assay method. Thirty and 60 minute incubation times were used, respectively (cf. refs. 64 and 66).

Results.- The results in table 6 show the lack of any significant effect of either high or low field upon the *in vitro* activities of the three enzymes. These results are consonant with those of Maling and co-workers (ref. 65). But neither Maling's work nor ours (cf. ref. 63) should be construed to contradict the findings of the three other groups (refs. 34, 35, and 36), since

TABLE 6.- ENZYME ACTIVITY *IN VITRO*: LACK OF A SIGNIFICANT EFFECT OF MAGNETIC FIELD VARIATION

[Activity Level^a]

Enzyme Experiment number	Grouped by magnetic field exposure			Significance ^d t-values (df ≤ 18)
	Null: (<0.5 mOe)	Geomagnetic: (control: 0.5 Oe)	High: (5×10 ³ Oe)	
1 Acid phosphatase ^b	1.21 (±0.01)	1.22 (±0.02)		0.500
2 Acid phosphatase ^b		1.34 (±0.03)	1.36 (±0.04)	0.444
3 Alkaline phosphatase ^b	6.02 (±0.18)	5.83 (±0.27)		0.189
4 Alkaline phosphatase ^b		5.33 (±0.41)	4.90 (±0.18)	0.962
5 Transaminase ^c (glutamic-oxalacetic)	92.3 (±1.19)	93.2 (±0.99)		0.600
6 Transaminase ^c (glutamic-oxalacetic)		93.5 (±0.74)	94.6 (±0.88)	0.960

^aMeans (±S.E.)

^bLowry-Brock units

^cReitman-Frankel units

^dt-values < 1.0 with df = 18 indicate a lack of significance at the 70-percent level (p > 0.30)

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.

Conclusions.- In our *in vivo* experiments (ref. 61) 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 our results. 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 enzyme does not completely exclude the possibility that malformation or denaturation was an operative mechanism in the *in vivo* experiments. But the results do seem to favor 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 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 (ref. 67), 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 exerted by magnetic fields 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 (ref. 68) 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 biomagnetic 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 in 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.

Most importantly now are needed *in vitro* studies of any implicated biological systems, and experiments on *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 (the acquisition of more data) with great profit in many instances.

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National Aeronautics and Space Administration
Moffett Field, Calif., 94035, Sept. 26, 1969

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