Magnetic field effects on selected biological tissues

by

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INTRODUCTION

Humans as well as all other living organisms are subjected to constant magnetic fields (CMFs) and alternating magnetic fields (AMFs) (Zharov, 1979; Ketchen et al., 1978; Marino and Becker, 1977; Chen and Rukspollmaung, 1977; Presman, 1970). The geomagnetic field (GMF) of 0.1 to 0.6 Gauss (G) and the magnetic fields (MFs) associated with electromagnetic radiation are the most common sources. The electromagnetic spectrum is shown in Figure 1. The frequencies of the visible region of the electromagnetic spectrum trigger the nerve responses involved in the process of vision, whereas waves with frequencies outside this range normally do not. In addition to the extensive studies of the effects of visible electromagnetic radiation on living organisms, the electromagnetic field (EMF) effects of other frequencies have been studied in the past century. Experiments have been conducted to see how living organisms respond to these fields, as well as to determine what intensities and/or frequencies are hazardous.

Previous studies of EMF effects can be divided into two categories: (1) macroscopic studies and (2) microscopic studies. Macroscopic studies deal with exposing the whole living organism to the EMF and then studying the behavioral and structural changes. An example is the pioneering work of D'Arsonval (1893) who reported that flashes of light may be seen when the head is placed in an AMF. This phenomenon is called a magnetic phosphene and is produced by a 10 to 100 Hz AMF with an intensity of 200 to 1000 G. Microscopic studies consider changes in the characteristic

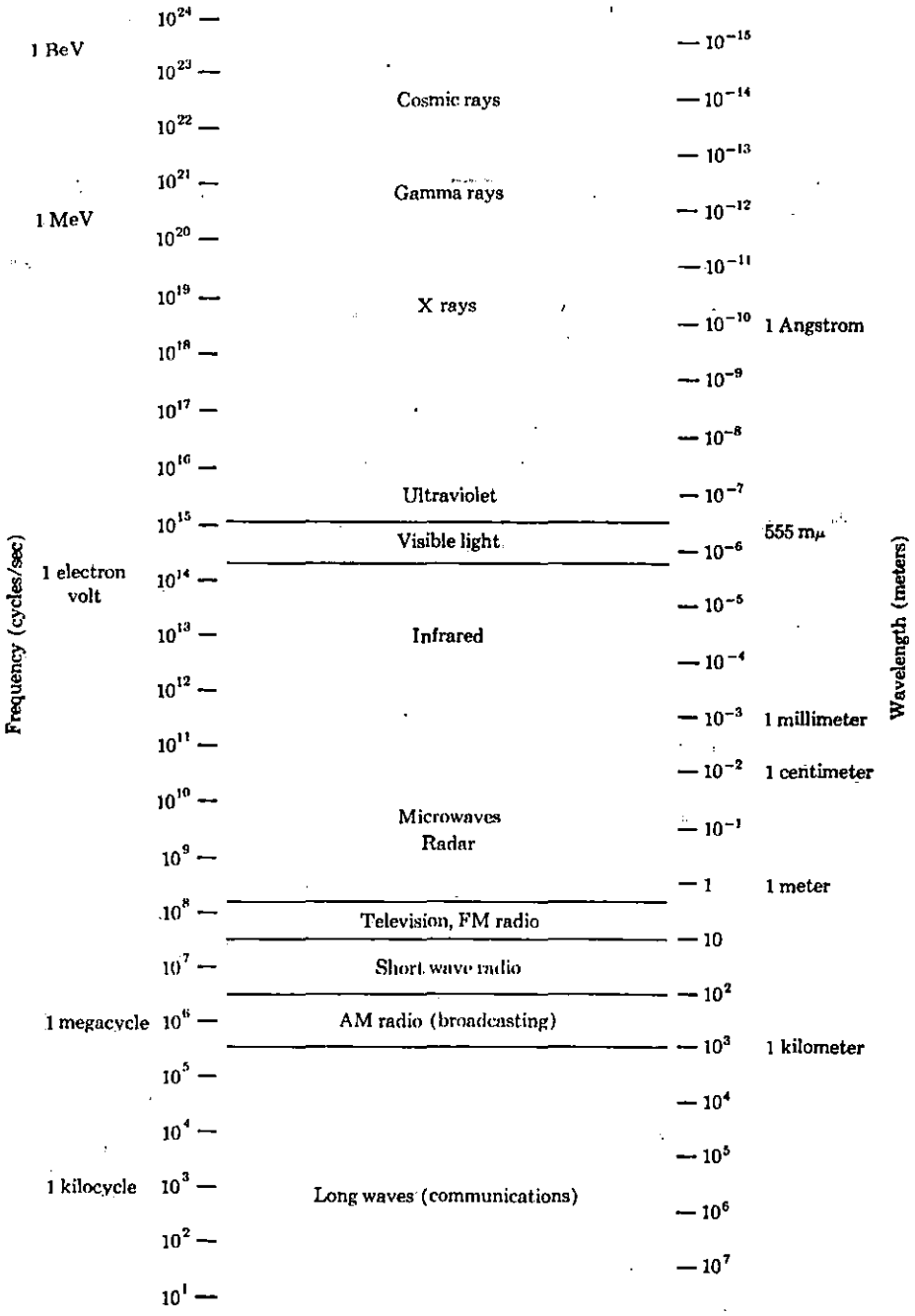

Figure 1. Electromagnetic spectrum.

movements of ions and consequently the susceptibility of excitable membranes to MFs.

Two major difficulties arise when attempting to interpret previous studies of EMF effects. First, different researchers have chosen very different intensities and frequencies of EMF and this makes it difficult to compare the reported changes in the living organisms. Second, most studies do not determine whether the magnetic component or the electrical component of EMF caused the observed changes.

In the research reported in this thesis, EMF effects on two biological systems were examined by the microscopic studies approach. Experimental work involved: (1) subjecting the giant axons of earthworms to an inhomogeneous AMF (60 Hz, 234-237 G r.m.s.) and (2) exposing pieces of frog skin to an inhomogeneous CMF (30-55 G). The well-known permeability properties of these tissues in the absence of an EMF were used to evaluate the changes that took place in the presence of the EMF.

LITERATURE REVIEW

Observations of the effects of an altered GMF on biological systems, the known interaction between magnetic and electrical forces and the importance of electrical parameters in biological systems, and a general interest in what types of stimuli can be transduced by biological systems have led to extensive experimentation aimed at determining how and to what extent magnetic fields affect biological systems. This literature review cites many of these experiments; unfortunately, great diversity in experimental methods and materials deters discussion of and generalization from these studies. Consequently, this section is divided into subsections in which different magnetic effects on similar biological systems are reported. A subsection on the Ussing method for studying isolated frog skin also is included. This method has been used in a few previous MF studies and is used extensively in the experiments reported in this thesis.

Altered GMF and Pathology

Soviet scientists have reported that an interplanetary MF exists and that a change in the intensity of the GMF occurs when the interplanetary MF changes sign (Zharov, 1979). Disruption of the GMF by cosmic factors has been correlated with pathological changes in humans (Petrova and Stefanov, 1977). Figure 2 shows a direct relationship between GMF variation and cardiac emergency cases (Malin and Srivastava, 1979).

Correlations between pathological situations and altered GMF activity due to solar changes, sunspots and magnetic storms were studied in Kiev in

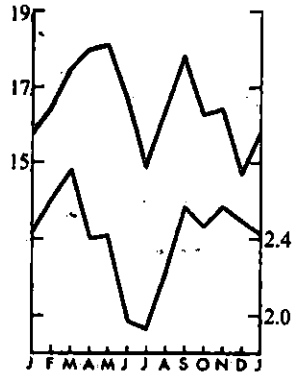

Figure 2. Monthly mean values of the daily sums of magnetic activity indices, Kp (upper graph) and the daily admissions of cardiac emergency cases (lower graph). The Kp index is a measure of the geomagnetic effect of solar particle flux

the Ukrainian Soviet Socialist Republic area of the Union of Soviet Socialist Republics. Data on the monthly distribution of 187,031 cases of cardiac emergencies between 1968-1973 and the daily distribution of 34,078 cases in 1973 indicated that the frequency of cardiac emergencies depended on changes in the earth's field intensity (Bardov et al., 1977). During seasonal changes in the GMF, cardiovascular diseases increased especially in the winter-spring period (Bardov et al., 1977; Gauquelin and Gauquelin, 1975). Bardov et al. (1977) noted a biotropic effect whenever the gradient of field intensity was 60% or more above normal, and the incidence of cardiovascular diseases increased in proportion to the increase in the gradient of GMF.

Kursh in the USSR has a magnetic abnormality which produces a GMF two to three times greater than normal. Neuropsychic diseases and hypertonia are 1.5 times more common in Kursh than in adjacent regions with lesser GMF strength (Fedorov and Nevstrueva, 1971).

Perception of MF by Living Organisms

Several reports exist which indicate humans and other animals are sensitive to and perceive MFs. Experiments by Harvalik (1978) were designed first, to confirm the phenomenon of magnetic sensitivity in the human body and second, to determine the anatomical position of the sensory receptor. He exposed 14 male skilled dowsers to a low power MF (1 Hz to 1 MHz) generator which produced MFs randomly. The presence or absence of a MF was signaled by the dowsers. Right answers were counted as positive responses and wrong answers were counted as negative responses. In 694 trials, 691 positive responses were recorded. By shielding different parts of the dowsers' bodies and then exposing them to the AMF he observed that when the head or kidney area was shielded correct responses failed to occur; therefore, he suggested the MF sensor was located in the renal vicinity as well as in the brain.

A variety of animals seem to be able to sense the earth's MF (Yorke, 1979) and to use it as at least a secondary source of orienting information in the absence of sunlight or visual cues (Emlen, 1975; Wallcott and Green, 1973; Emlen et al., 1976). Many homing pigeons are able to find their home direction after being released at unfamiliar sites. Research on both caged birds (Emlen, 1975; Emlen et al., 1976) and free flying birds (Emlen, 1975; Wallcott and Green, 1973) showed that they prefer directions determined by a CMF of the same order of magnitude (approximately 0.5 G) as that of the earth. This suggests that MF information is used for orientation. Similarly, when Wiltschko et al. (1971) tested the ability of European robins to orient using an artificial MF outdoors, they concluded

that the mean direction indicated that the birds' orientations were due to the MF. MF orientation behavior was more pronounced on dark and cloudy nights than on nights which were only partly dark and cloudy. Orientation behavior to certain directions of the GMF led Yorke (1979) to suggest that pigeons have a MF transducer to use as a compass. Wallcott et al. (1979), using a squid magnetometer, showed that in each pigeon tested there was permanent magnetic material located either in a small (1 by 2 mm) piece of tissue between the dura and the skull or too closely associated with the skull to be separated from it. They concluded that this material could be the magnetic transducer.

MF orientation also has been studied in honey bees. Gould et al. (1978) listed four conclusions from other authors suggesting that a MF can have an effect on the bees:

> (1) In the bee dance, they convert the angle flown to the food with respect to the sun into an angle danced with respect to gravity. In this conversion, bees make small regular errors which depend largely on the orientation of the dance with respect to the earth's field. Canceling the field causes the error to disappear. (2) When the comb is turned on its side so that the bees must dance on a horizontal surface, depriving them of their usual gravity cue, some bees stop dancing while others dance in a disoriented fashion. After several weeks, however, those dances that do occur become oriented to the four cardinal points of the magnetic compass. Canceling the earth's field eliminates this reorientation. (3) When a swarm of bees is placed into an empty cylindrical hive and otherwise deprived of orientation cues, they are reported to build their comb in the same magnetic direction as it was in the parent hive. (4) In the absence of all other cues, bees seem to set their circadian rhythms by the regular daily variations in the earth's MF. An abnormally strong field disrupts the rhythm.

Gould et al. (1978) searched for magnetic material in bees by inducing MFs on dried and freshly killed bees and measuring the remanence of MFs in the bee bodies. They found that almost all the magnetic material was located

in the front of the abdomen and suggested that this material served as a magnetic transducer.

Elasmobranch fish show a high sensitivity to electrical and MFs. The distribution of this sensitivity over the body surface corresponds to the morphological disposition of the receptor apparatus, the ampullae of Lorenzini. Transection of the nerves connected to the ampullae causes a sharp decrease in electrical as well as magnetic sensitivity (Broun et al., 1972). Brown et al. (1979) showed that geomagnetic variations created electrical currents which could be detected by the Lorenzini ampullae.

Cave salamanders are able to perceive the GMF and change their direction due to the field (Phillips, 1977).

Generally, three theories are suggested to explain the ability of animals to detect MFs: (1) Movement of the animal causes the MF to induce an electric current. An alternating MF also would induce an electric current. (2) The detector is a paramagnetic and/or diamagnetic material, and any change in the external MF produces a change in the MF of the magnetic material. This change can be detected by some other organ. (3) The detector is a magnetic material that can twist in an attempt to align with the MF, thereby producing a torque.

MF Effects on Growth

There have been several studies to determine the effect of MFs on the growth and reproduction of animals. In one study, guppies (Lebistes reticulatus) were continuously subjected to a 500 G homogeneous MF. The

experiment which continued through several generations had the following results: (1) in the first generation, the brood size was normal but the gestation period was reduced by 30%; (2) the second generation had an average reduction of spawn rate of 50% and a reduction of the gestation period of 30%, (3) in the third generation, reproduction was completely inhibited as long as the fish remained within the MF; and (4) there was a significant change in size between different generations, as shown in Figures 3 and 4. When the man-made MF was removed and the fish experienced only the GMF, after two generations their offspring were normal in size and like the controls (Brewer, 1979). Chabre (1978), using rabbits, also found that MF effects on size disappeared after a couple of generations in the absence of man-made MFs.

Davis and Rawls (1974) observed the different effects of south pole (SP) compared to north pole (NP) MFs on embryo growth, behavior and life span. Chicken eggs were incubated while located in the SP or NP of a 2500G MF. The temperature during incubation was 80°F. The incubation period was two or three days less than the normal time for the SP treated eggs, but the incubation period was one or two days longer than the normal time for the NP treated eggs. The chicks from the SP treated eggs grew faster and stronger than those from the NP treated eggs. Davis and Rawls (1974) also indicated that exposing mice and rats to a 2500G MF caused a shortening of the gestation period and the duration of parturition in the SP treated mice and rats, but a lengthening of the gestion period in the NP treated animals. The SP treated mice and rats were larger than the NP

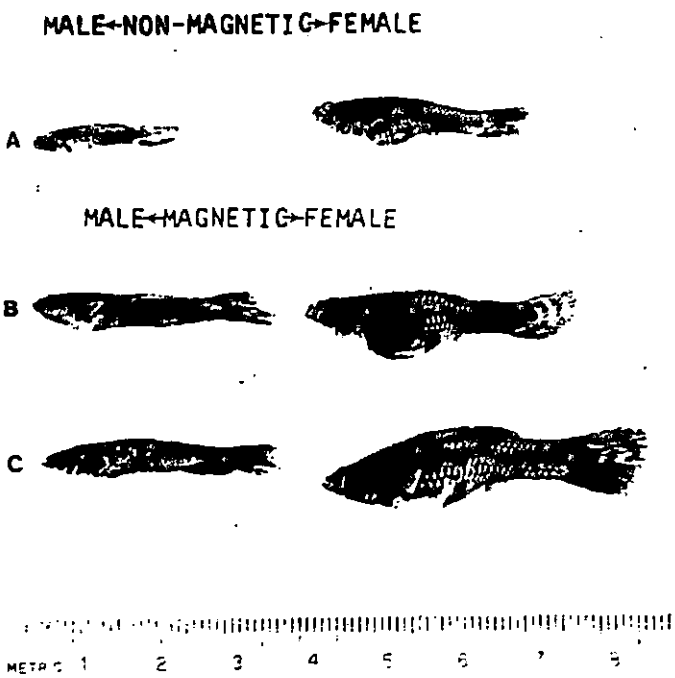

Figure 3. Lateral view of the Lebistes reticulatus after 180 d. (A) Male and female nonmagnetic environment control group. A, B, and C denote generations. (B) Male and female first generation magnetic field environmentally treated offspring. (C) Male and female second generation magnetic field environmentally treated offspring

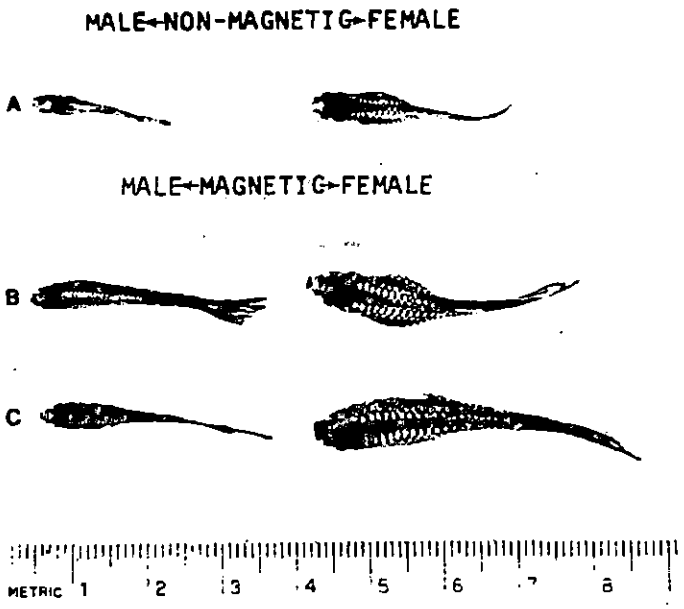

Figure 4. Dorsal view of the Lebistes reticulatus after 180 d. (A) Male and female nonmagnetic environment control group. (B) Male and female first generation magnetic field environmentally treated offspring. (C) Male and female second generation magnetic field environmentally treated offspring

treated mice and rats, and the SP mothers were stronger and parturition required less effort.

Mulay and Mulay (1964) showed that MF strength was very important in the production of deformities. When fruit flies (Drosophila) were exposed to 3000-4000 oersted (oe) for more than one generation, the frequency of deformities increased, but when a MF with 100-160 oe or even 1500 oe was applied for two or more generations the frequency of deformities did not change.

Kopanev et al. (1979) studied the influence of a hypogeomagnetic environment on the growth and development of warm blooded animals. Embryogenesis of rabbits and their development up to one month of age were

monitored in a condition of 600 times decreased GMF. This condition was created by shielding the chamber containing the animals. The data showed support for the influence of the GMF in normal development. Distrophic disorders in liver, in cardiac muscle and in the alimentary canal were found and attributed to the decreased GMF environment. It was observed that the motor activity of the test animals was markedly changed, that the neuromuscular apparatus was not normally developed, and that the mortality of the test animals was higher than that of the controls.

MF Effects on Behavior

Davis and Rawls (1974) described behavior differences between chickens incubated and hatched while exposed to each pole (NP or SP) of a CMF (2500 G). During hatching, eggs from each group were placed separately in cages containing a real horse shoe magnet and a false magnet, each with a gap distance of 2 1/2 inches. As soon as the SP treated chicks were half dry from leaving the eggs, they took turns, and one at a time moved between the poles of the real magnet. Each chick remained in between the real magnet poles for about two minutes, then left it and went as far as possible. Meanwhile, another chick entered and began the same process. The NP treated chicks followed the same process except they stayed in the field longer, up to three minutes. The control chicks which were not exposed during incubation waited until they were dry before moving between the magnet poles; they remained 2.5 to 3.5 minutes before leaving. In all of these experiments none of the chicks laid down between the poles of the false magnet. This indicates the chicks can perceive and are attracted to the MF.

The SP treated chickens ate more, even the flesh of the hens and other chicks and their intelligence was lower in all respects than that of the other two groups. They were strong and were found chasing dogs, cats or even attacking each other. In contrast, the NP treated chicks were light eaters and sensitive to all surrounding noises, heat, cold, sun and weather (Davis and Rawls, 1974).

Davis and Rawls (1974) also studied the behavior of mice and rats after exposing them to either the NP or SP of a CMF (2500 G). Mice and rats exposed to a NP field were very neat housekeepers and spent a lot of time washing and keeping themselves and their cages, nests and huts clean, but those exposed to a SP were always dirty, stained and careless. The NP treated mice and rats were highly sensitive while the SP treated mice and rats were bold, strong and nonfearful. The sex life of the NP treated animals was limited and less active than that of the controls, but they had a longer life span. In contrast, the SP animals showed a more active sex life but a shorter life span.

Cymborowski (1975) reported that the circadian rhythm of the locomotor activity in male crickets changed under AMF. Initially, inhibition of the level of locomotor activity is the most important effect. This lasts for several hours, but disturbance of locomotor activity lasts for 5 days after the application of an AMF.

Many behavior studies have dealt with the alteration of reaction time in humans, as well as other animals, exposed to AMFs. Friedman et al. (1963) reported a MF effect on the time taken by the subjects (seated in the MF created by two Helmholtz coils adjacent to their heads with 5-11 G

at 0.1-0.2 Hz) to release a telegraph key in response to a light flash. The results showed reaction time was about 30 msec longer at 0.2 Hz than at 0.1 Hz.

MF Effects on Biostructures

Hryhoryan et al. (1977) reported that a MF (from 2 K to 20 K oe) caused morphological and structural changes in the bacteriophage 62. The effect of a weak MF (26×10^{-5} G) was studied by Verkin et al. (1976) on several bacteria: Escherichia coli var; Communior, Bacteriam prodigiosum [Serratia marcescens]; Staphylococcus aureus 209 and anthracides. They observed that cultivation of the bacteria in the mentioned conditions for a long time resulted in changes of their pigment, morphological, cultural and biochemical properties.

Friedman and Carey (1972) observed no neuropathology in squirrel monkeys exposed to a homogeneous CMF (200 G) for 10 days with a 4 hr exposure each day. However, Kvakina et al. (1974) reported morphological changes in the hypothalamus of 32 white rats exposed to an AMF (300 oe, 1 Hz with a gradient in the hypothalamus region of 15-20 oe/mm). These changes were described as a dilation of the capillaries and activation of microglia and satellite glia. However, these effects characterize the response to moderate physiological excitation and a nonspecific reaction to stimulation of medium strength.

Aristarkhov et al. (1974) showed that exposure of mongrel white mice to a CMF (5000 oe) for 7 to 14 days caused development of tumors in the animals. The tumor development was accompanied by intense Fe absorption

by the tumor cells. However, it also has been reported that a MF can cause rejection of tumors. Barnothy (1964) experimented with the rejection of malignant tumor transplants in mice. In mice exposed to a 3000 G CMF, the tumors were rejected in 12 to 46 days, while the controls died from the effects of tumor growth in about 23 days.

MF Effects on Water

The influence of weak MFs on biological specimens may be through the action of the MFs on water, the principal component of living systems. According to Dudoladov and Trinccher (1971), water is a unique substance, present in a state of continuous phasic transition between crystal-liquid and liquid-crystal. The conversions between crystal-liquid and liquid-crystal are explained by the transfer of molecules with excess energy from the liquid micro-region to the crystalline. The crystalline lattice breaks down and the liquid microphase molecules rearrange under the influence of near action forces and are converted to the crystalline microphase. Intracellular water has special electrical forces and constitutes a kind of dynamic segnetoelectric.[1] The polarizing fields of protein molecules order the water,and the destructive action of thermal motion again takes it into the liquid phase. The crystallization waves spread along the system. Dudoladov and Trinccher (1971) used a theoretical approach to suggest that the transition of intracellular water to the quasicrystalline might be by the process of ordering of the dipolar

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[1]Segnetoelectric is a term used to denote substances which below a certain temperature (Curie point) are in the polarized state and have an excess dipole moment.

molecules of water by the polarizing fields of protein. The rotation of these dipolar molecules leads to the appearance of an intracellular water MF. Consequently, the structure of water in a cell may significantly change under the influence of an external MF.

Patrovsky (1976) discovered that water exposed to a CMF (3700 G) contained a small amount of hydrogen peroxide (H_2O_2). He claimed that magnetically treated water may be prepared in various ways, for example, by movement of a strong magnetic pole over the water surface or a better way, by rapidly passing the water through an inhomogeneous MF of strength at least 1600 G. Speed was not too critical, but some tests indicated that a higher speed increased the content of hydrogen peroxide.

Lielmezs et al. (1976) noted a slight increase in the viscosity of distilled, as well as tap, water when a MF (ranging from 0 KG to 10 KG) was applied at 25°C. The increase in viscosity was very smooth and small and did not exceed 0.25% at an applied MF strength of 10 KG. The theory which was presented to describe the viscosity change was an internal molecular energy (IME) change due to the applied external MF. They showed that the IME could be calculated from $\Delta E = -1/2X_m H^2$. For water with a susceptibility (X_m) equal to -13×10^{-6} e.m.u. (electromagnetic unit) and an applied MF strength (H) equal to 10 KG, the IME change (ΔE) had a value equal to 1.55×10^{-5} cal/mol or 1.079×10^{-21} ergs. This increase in energy causes angular and structural distortion, resulting in the 0.25% increase in viscosity.

Paulsson et al. (1977) showed that in solutions containing macromolecules much of the imposed microwave power was absorbed in the bound

water surrounding the molecules. This gave a heating effect, probably involving short thermal time constants, concentrated in the vicinity of the molecules.

MF Effects on Biochemical Reactions, Metabolism and Endocrine Function

MFs can affect the level of glucose-6-phosphate dehydrogenase (G6PD) in cells. In the case of experimental animals (young rabbits) shielded from the GMF, the observed decline of G6PD activity could indicate a decreased biosynthesis of enzymes of the pentose-phosphate route or a reduction in their activity (Shakula and Galeyev, 1979). At a MF intensity of 200 oe (50 Hz), Udintsev and Khlynin (1978) showed an incease in the G6PD one hour after exposure of rats to an AMF (200 oe and 50 Hz) for 24 hours, but the level decreased below control values one day after cessation of the MF exposure.

Hefco et al. (1969) noted that liver glycogen increased significantly, but muscle glycogen decreased significantly in guinea pigs after 10 days of treatment with a CMF (an average of 60 oe). However, Chernysheva and Kolodub (1975) observed a decrease in glycogen content in the liver and accumulation in the heart muscle of rats while the experimental animals were exposed to the chronic action of an AMF (7.5 KW/m^2).

An increase in lactic acid content and a slight decrease in ATP content in the cerebral hemispheres of rats exposed to 3000 oe for 3 hours has been reported (Nosova and Kurkina, 1979). This implies an increased metabolic activity in the brains of the experimental rats. A decrease of

lactic and pyruvic acids in both muscle and liver of guinea pigs exposed to a nonhomogeneous MF (average of 60 oe) was observed (Hefco et al., 1969).

Sakharova et al. (1976) described the levels of epinephrine (EPI) and norepinephrine (NEPI) of both the blood and the adrenal gland (AG) and the dopa content of the AG as significantly increased in rats immediately after they were exposed to a MF (200 oe and 50 MHz). The EPI and NEPI levels decreased during the first 12 hr after removal of the MF but remained higher than in the controls. The dopa level in the adrenal gland decreased sharply at one hour and then increased again by 12 hours. After 24 hours, the catecholamine (CCA) content of the blood was slightly decreased and the CCA content of the AG was lower than the control level, but the dopa content was approximately the same as the control level. After two days, the CCA content of both the blood and the AG were further decreased below the control levels. However, the CCA level was returning toward its normal level by approximately seven days and was completely normal by 14 days.

Sakharova et al. (1977) extended their previous study to an analysis of the mechanisms of the reaction of the sympathoadrenal mediation on the central (brain stem and hypothalamus) and peripheral (heart, liver and spleen) systems in rats when they were exposed to an AMF (200 oe and 50 Hz) for 24 hours. Phased changes were established in the catecholamine and dopa content in the central and peripheral mediators after a one time exposure. In the first phase immediately after the one day exposure, there was increased use of dopamine and EPI in the brain stem and NEPI in the hypothalamus. Increased EPI transfer from the blood to the heart and liver

and higher levels of NEPI in the liver and spleen were noted. Also, dopamine in the hypothalamus as well as dopa in the spleen were increased. In the second phase (one day after exposure), the EPI level remained low in the brain stem tissue and NEPI was high in the liver and spleen, while NEPI in the heart and hypothalamus was restored to normal levels. In the third phase (two days after exposure), CCA content decreased in all organs except the heart. Almost all of these indexes had returned to normal at 7-14 days after exposure.

Fedorov and Nevstrueva (1971) studied the effects of a CMF on the sympathico-adrenal system in rabbits. After a 24 hr exposure to a MF (1000 oe), the rabbits showed a reduction in the NEPI content in the hypothalamus and myocardium but no change in the EPI content in the adrenal medulla.

MF Effects on Orientation and Susceptibility of Biology Materials

Lorant (1977) suggested that the high sensitivity of biological structures to even small MFs, like the earth's MF (0.1 G to 0.6 G), must be due in some way to biological processes which involve both the solids and liquids which make up biological systems. Two hypotheses have been suggested to interpret the MF effects: (1) kinetic influence of the MF on biochemical reactions involving free radicals, and (2) diffusion-oriented influence of the MF on the association of diamagnetic-anisotropic molecules of biopolymers in solution (Aristarkhov, 1979).

Abashin and Yevtushenko (1975) stated that behavior in a MF can be used to divide all substances into three classes: a) ferromagnetic material in which there is marked increase in magnetization in an independently established MF; b) paramagnetic material whose relative permeability is slightly greater than unity such that when it is exposed to a MF the internal MF of that material will be more than the imposed MF; and c) diamagnetic material which has a relative permeability less than unity and a negative susceptibility, causing the internal MF of that material to be less than an imposed MF. Each of the categories is the result of the properties of a crystal of atoms. Ferromagnetic substances in biological systems are metals of the transitional group such as Co, Ni and Fe. Their concentrations are so low (less than 10 atoms per molecules or complex) that in biological systems, ferromagnetic substances appear to be absent. In biological paramagnetic materials such as potassium and oxygen (Hayt, 1974), the mechanical and magnetic moments of orbits are quenched by the intramolecular electrostatic field and therefore only the orientation of the spin magnetic moment can be considered. Consequently, Abashin and Yevtushenko (1975) concluded that biological effects will be observed only in very strong (of the order of 10^5 G and higher) MFs. Abashin and Yevtushenko (1975) described the interaction of the diamagnetic molecules, such as, hydrogen, sodium chloride and sulfur (Hayt, 1974), both in low molecular weight and macromolecules, with a MF and concluded that there was not any appreciable influence on the kinetics of biochemical reactions.

Orientation phenomena have been seen in different biological preparations. Chalazonitis et al. (1970) discovered that there is a threshold for orientation of outer segments. The percentage of material oriented and the angle of orientation are dependent on the strength of MF. These relations are shown in Figures 5 and 6. Hong et al. (1971) also observed orientation of retinal rods in a homogeneous MF (10 KG). The rods tended to orient themselves with their long axes parallel to the field. Time was required for the alignment of the single rods. Lecithin, which is an essential ingredient of biological membranes, has been observed to rotate and align under a homogeneous MF of 15 KG. The translational and rotational movements of lecithin in the MF are shown in Figure 7 (Becker et al., 1978; Boroske and Helfrich, 1978).

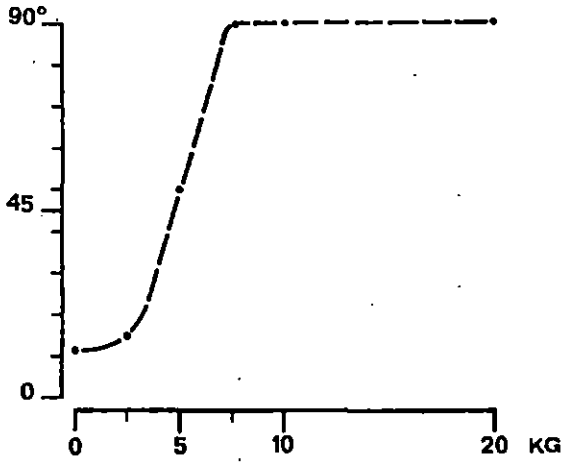

Figure 5. Angle of rotation of the segment as a function of the intensity of the magnetic field. The angle of rotation is measured in degrees beginning with perpendicular to the lines of the field and the initial axis of the segment. The measurements were made with different values of intensity of the field

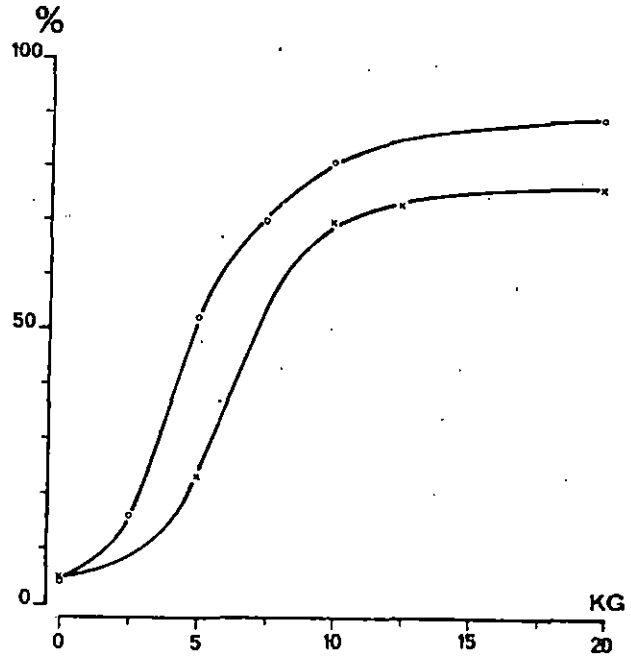

Figure 6. Segments, oriented parallel to the direction of the magnetic field as a function of its intensity, in percent of the total segments in the field. Upper curve: pure suspension of outer segments. Lower curve: melanine particles from the pigmented epithelium present among the segments

The orientation of small structures in a MF occurs because of the anisotropy of the material (Worcester, 1978) and the torque resulting from a MF acting on paticles or molecules the have anisotropic magnetic susceptibility requires several KG to produce a measurable rotation (Cope, 1973).

The existence of a threshold for orientation of biological preparations under the MF (Chalazonitis et al., 1970) suggests that as the magnetic forces become stronger than electrostatic forces, the molecule becomes able to orient in a different direction. When the orientation of biological structures is governed by the strength and direction of mag-

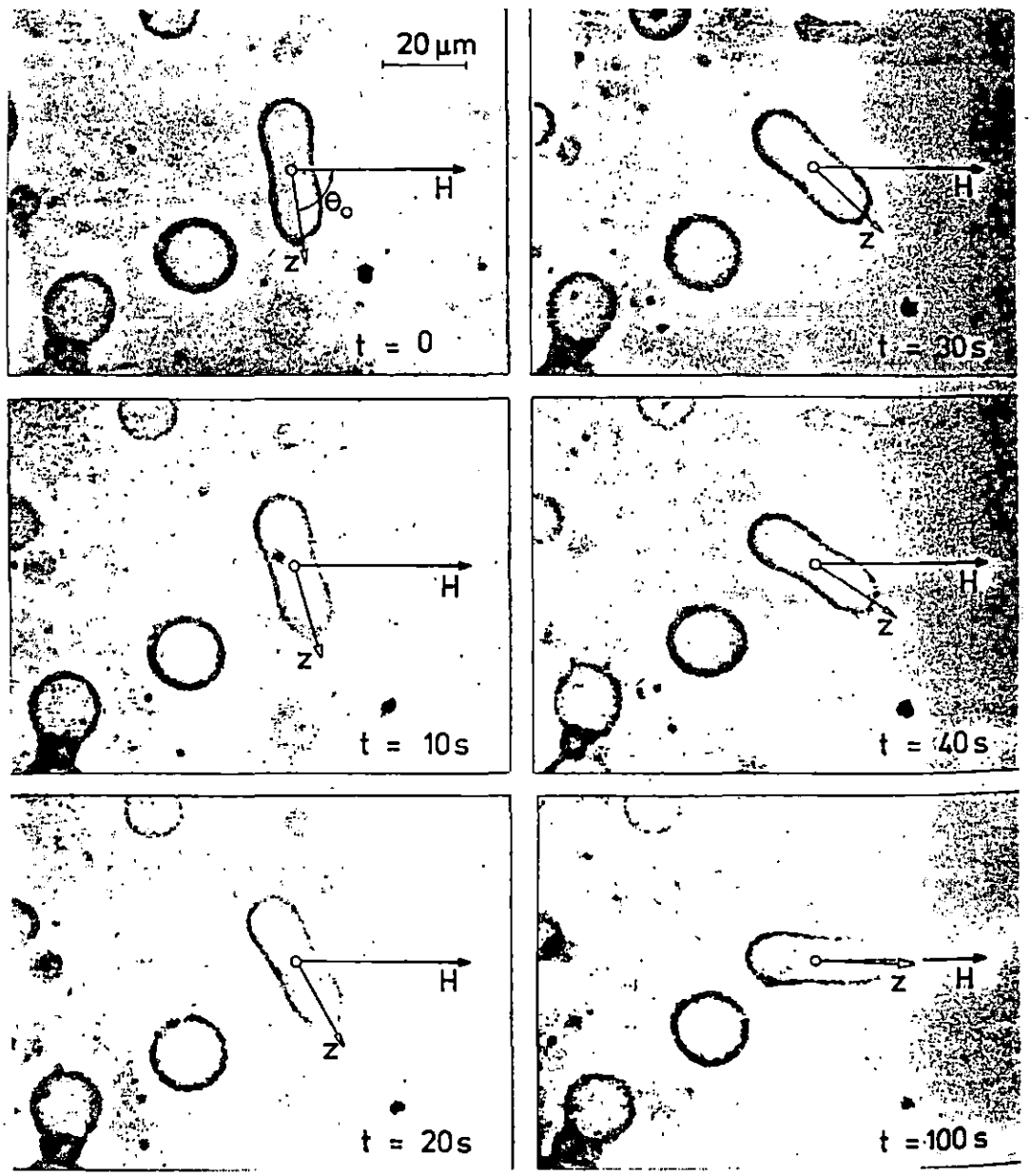

Figure 7. Time sequence of alignments of a cylindrical vesicle in a magnetic field, H

netic forces, the alignment will not be instantaneous.

Arnold et al. (1958) studied the magnetic asymmetry of muscle fibers as well as the magnetic material in the muscle. They based their experiments on the observation that an object with no permanent magnetic moment, when suspended in a horizontal MF, experiences four different torques around the vertical axis:

1) When the object is anisotropic, with K_1 as the volume susceptibility in one horizontal direction greater than K_2 in the other, there will be a torque tending to turn the object so as to make K_1 parallel to the field.
2) Unless the object is a sphere, it will have a different demagnetizing factors in each direction. This effect gives a torque tending to set the long axis of the sample parallel to the field. However, since this torque depends on the square of the susceptibility, it has never been detected for diamagnetic objects because of thier small susceptibilities.
3) The poles of the sample will form magnetic images in the iron of the pole pieces but because the torque depends upon the square of the susceptibility, it can be neglected except for ferromagnetic samples.
4) Unless the MF is homogeneous, the forces that tend to move a diamagnetic material from a region of strong field to a region of weak field can produce a torque on any nonspherical sample.

Using 3000 G homogeneous and inhomogeneous MFs, they showed that a fresh sample of rabbit psoas muscle had an asymmetrical diamagnetic material. A piece of muscle, several times longer than it was wide, was suspended in the inhomogeneous MF. It tended to set its long axis at right angles to the direction of the field. This is the behavior expected from a diamagnetic object. But a muscle fiber with its length one and one-half times its width was found to set its long axis parallel to the field. This showed the muscle fibers were anisotropic in magnetic properties (Arnold et al., 1958; reported from unpublished experiments of Szent-Gyorgyi, 1956). Such an orientation in a field is due to the difference in magnetic susceptibility Δx between the axial (x_a) and radial (x_r) principal volume susceptibility and the Δx may depend on differently oriented anisotropic susceptible molecules (Chagneux et al., 1977). Arnold et al. (1958) also observed that if the muscle sample was allowed to dry slowly, the diamagnetic behavior decreased during this time and some samples even became paramagnetic, but the magnetic anisotropy still remained. These experiments were repeated with both nerve and tendon taken from the rabbits; the asymmetrical magnetic properties were present in these tissues also.

Kolta (1973) studied the interaction of frog sciatic nerve with an inhomogeneous CMF (maximum intensity of 580 oe). This experiment was arranged as shown in Figure 8. A 35 mm long axon bundle was suspended from a hook; a thin silk cord was the control. He observed that the nerve was

attracted or repelled when the CMF direction changed. When the experiments were repeated with olfactory nerves from pike and peripheral nerves from rats, he observed the reactions shown in Figure 9. The magnetic properties of the nerves persisted after treatment in distilled water, 10% NaCl, 10% formaldehyde solution or after air drying, but the magnetic effect disappeared irreversibly when the nerves were soaked in a one-to-one mixture of chloroform and methyl alcohol for 2 minutes. This experi-

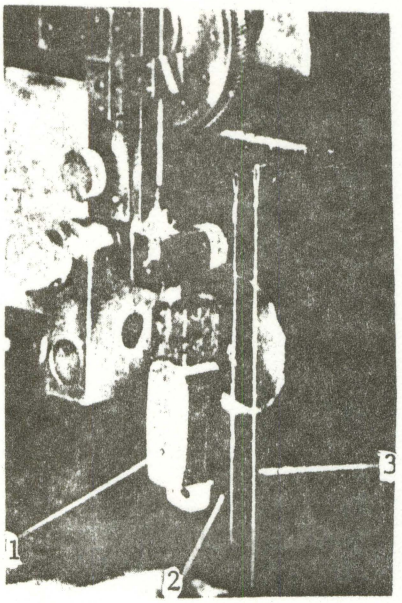

Figure 8. Experimental arrangement 1: permanent magnet; 2: sciatic nerve; 3: control cord

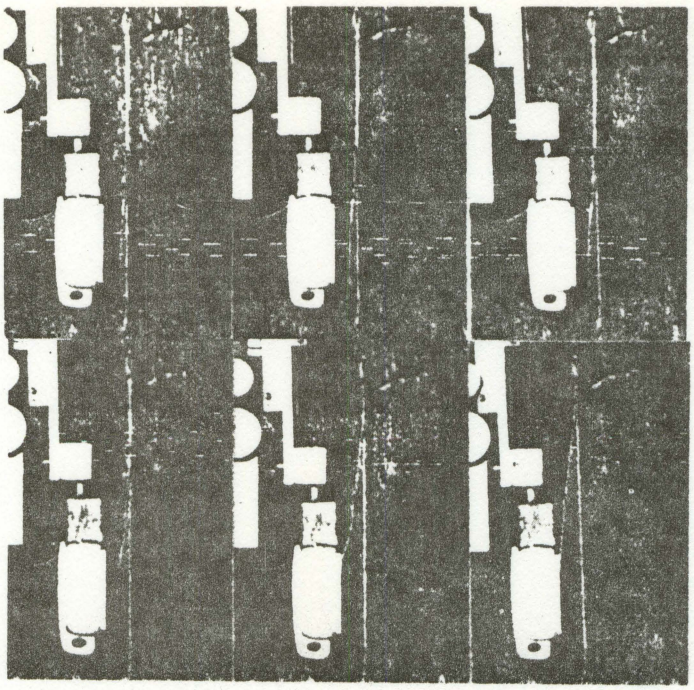

Figure 9. The magnetic interaction overcomes gravity. The permanent magnet pulls the axon-bundle to itself. The position of the control cord is unchanged

ment was repeated with other tissues: skeletal muscle, heart muscle, liver, intestine and bone, but they did not show similar magnetic characteristics.

MF Effects on Excitable Tissues and Active Transport Processes

Kolin et al. (1959), using a frog nerve-muscle preparation, recorded an intense tetanic contraction of the muscle whenever the magnet was on (60 Hz and 8740 G or 100 Hz and 2260 G) through the nerve muscle loop as shown in Figure 10. In another experiment they put the nerve-muscle preparation into a Petri dish filled with Ringer solution, with the AMF perpendicular to the Petri dish. They saw a contraction in the muscle and

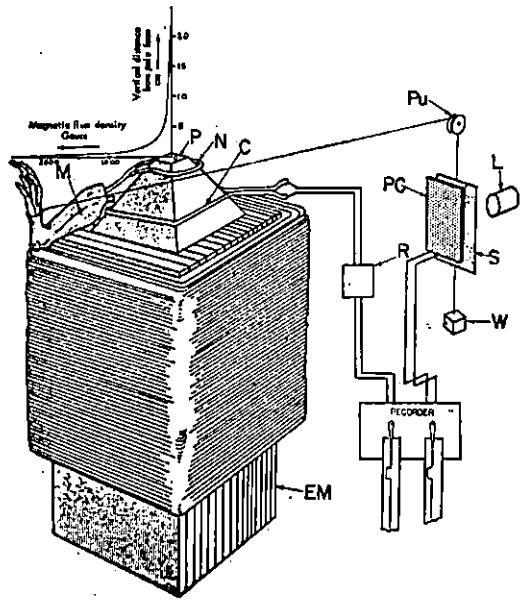

Figure 10. EM: laminated bar electromagnet generating a nonhomogeneous alternating magnetic field the axial component of which is represented in the diagram as a function of distance from the pole face P. N: nerve forming a closed loop in magnetic field. M: frog's gastrocnemius muscle. C: wire loop surrounding pole tip of the magnet. R: rectifier which rectifies the a.c. signal induced in loop C. The rectified signal is recorded by one of the 2 recorder channels. PC: photocell (International Rectifier Corp. type B-10) of the photoelectric device recording muscle contraction. L: light source. S: shutter which can be lifted by the string attached to frog leg. The shutter is actually flush with bottom of photocell PC. It is represented somewhat longer in the diagram to show the point of attachment of the string to which weight W is attached. When muscle contracts, shutter S and weight W are lifted thus exposing an increasing area of the photovoltaic cell PC to light from the source L. Voltage output of photocell is recorded by the second channel of the 2-channel recorder

showed that "Stimulation of the irritable tissues by the eddy currents induced in the contents of the Petri dish showed a dependence on their position in the dish as well as on their orientation. The maximum effect was observed when the muscle was placed near the rim of the Petri dish and no effect was seen when the muscle was at the center." Ueno et al. (1978) studied the relationship between the strength and duration of the voltage pulse creating a MF inside a core and the contraction in a frog nerve-muscle preparation. Their results are shown in Figure 11. Stimulation of the frog nerve-muscle preparation also has been reported using the arrangement shown in Figure 12 (Oberg, 1973; Ueno et al., 1978).

Schwartz (1978, 1979) studied lobster giant nerve fibers exposed to a strong CMF (1200 G). The results showed the CMF was not able to trigger action potentials in the nerves and had no effect on the conduction velocity, action potential amplitude and permeability of the nerves. Under voltage clamp conditions, the giant axons were not affected either by a parallel field or by a perpendicular field. The membrane potential and transmembrane current were fairly stable during the entirety of each experiment. In a different study (Liberman, 1959), it was shown that exposing a single myelinated nerve fiber of a frog to a CMF (1000 oe) did not change the action potential threshold.

Andrianov et al. (1974) showed that the electroreceptor in Black Sea skates (Trygon postinaca) responded to the induced electrical currents which were produced by an AMF. In their experiment, almost all observed neurons responded to the AMF, whereas a CMF failed to have any effect.

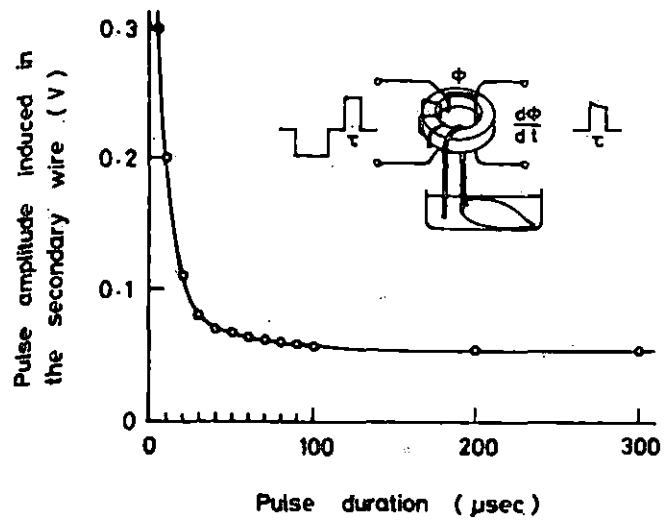

Figure 11. The strength-duration curve of the voltage pulse necessary to stimulate frog nerve muscle. τ is the pulse duration in primary and secondary wire

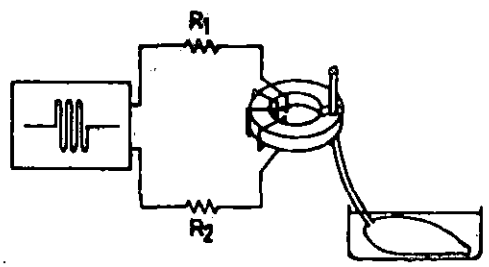

Figure 12. Experimental setup for nerve stimulation. A nerve bundle is set in an airgap of a ferrite core, and the core is driven by burst signals

Several experiments have been performed to determine the effect of an external field on active transport. Bresler et al. (1975) showed that MFs with strengths of 1000-28000 oe caused reversible changes in the structure of the membrane thereby preventing active transport of fluorescein in the proximal tubules of frog kidneys. However, a study of fluorescein transport in choroid plexuses isolated from rabbits (Bresler et al., 1978) showed that the active transport was increased 1.86-1.88-fold by exposing the choroid plexuses to a 22×10^3 oe CMF. Batkin et al. (1978) analyzed the sodium pump activity in kidney cortex and diaphragm of normal mice (A/J) after 11 days of exposure to an AMF (55-60 G and 60 Hz). The results indicated a significant reduction in the enzyme (Na^+-K^+) ATPase.

There has been a report of a Na^+ permeability change in the skin of frogs exposed to a MF. Muller and Jitariu (1969) exposed frogs to an AMF (250-300 G and 50 Hz) for three hours and then sacrificed them and measured the permeability of the abdominal skin using the Ussing method. The data showed that the permeability of the skin increased; there was a significant increase in short-circuit current. They suggested that the MF might have a local effect and directly change various processes in the skin cells, or that it might have an indirect effect via the nervous system and through hormones. Bianchi et al. (1963) attempted to test whether the sodium active transport across frog skin might be affected by a CMF. They used a 2 cm^2 cross-section core mounted on each side of the skin. The CMF across the skin was 650 G when the magnet poles were 1 mm apart and 250 G when they were separated by 22 mm. The results showed

that increasing the applied MF reduced both the influx and efflux of sodium by 10-30%. In the short circuit experiment, the current appeared to be altered within 1 sec of increasing the field.

Behavior of Isolated Frog Skin

The ability of many fresh water animals to take up ions, especially Cl^- and Na^+, from the surrounding medium was established by Krogh (1937). This uptake is through different organs in different animals; for example, the gills of fish, special organs in mosquito larvae and through the entire skin of frogs (Barker et al., 1946). Krogh (1937) found that frogs which had lost salt during several days stay in repeatedly renewed water took up NaCl when they were placed in a dilute NaCl solution. Frogs which had not lost salt did not show any net uptake of salt under similar conditions.

There is normally a potential difference between the exterior surface of the frog skin and the underlying connective tissue; the connective tissue is positive compared with the epithelial side (Ottoson et al., 1953; Ussing and Zerahn, 1951). Different values have been reported for this potential difference (PD) across the skin. For example, 100 mv was reported by Ottoson et al. (1953) and 150 mv by Guillermo (1964), but in most of these reports, the researchers did not mention at what time after preparing the frog skin the reported PD had been measured. This PD across frog skin has been studied by several researchers. Ussing (1949) reported that when the inside of an isolated frog skin was bathed with Ringer and the outside with a dilute NaCl solution, the inside was still positive

relative to the outside, and that the PD could still be detected in an isolated frog skin even when both sides were bathed in Ringer solution. Huf (1935) suggested this PD was closely related to the transport of salt from the outside to the inside. This connection was further indicated in another study on isolated frog skin in which both sides were bathed with the same solution. It was reported that sodium ions were passing in both directions through the skin, but that the influx of sodium ions was higher than the efflux. This experiment was performed using radioactive sodium 22 and sodium 24 (Levi and Ussing, 1949).

Francis (1933) reported that the mechanism in the cells which produced electrical current needed energy. Lund and Stapp (1947) suggested that this energy was produced as a result of an oxidative mechanism in the polar cells of the skin and that there was a continuous output of current throughout the life of the skin. They showed the requirement for oxygen by using two symmetrical skin halves, one in an oxygen free solution and the other in an oxygenated solution. The current produced by the skin in the oxygen free solution dropped much more rapidly and reached a lower value than that produced by the skin in the oxygenated solution. Ottoson et al. (1953) reported that the maintenance of the potential difference was closely related to oxidative metabolic processes in the epithelial cells. Ussing (1949) reported that the Na^+ influx went down and attained a minimum in the second one hour period after the isolated skin was poisoned by injecting a NaCN solution into the inside solution. Therefore, he showed the major part of the Na^+ influx was normally due to an active uptake which could be poisoned with cyanide. A hypothesis was

proposed based on these observations. This was that the PD was due mainly to an active and specific transport of Na^+ ions inward giving the inside solution a positive charge which in turn would attract Cl^- ions (and repel K^+ ions). The net results would be a transfer of NaCl from the outside to the inside and, to a lesser extent, an exchange of outside Na^+ for K^+ (Ussing and Zerahn, 1951).

Since the early studies on isolated frog skin, scientists have used a special chamber. This chamber has a pair of cups, with the frog skin in between, and two electrodes in each cup, one close to the skin (in the front of the cup) for measuring the voltage across the skin and the other one at the end of the cup for inducing current. Solutions in each cup are circulated by bubbling a gas into a reservoir. The chamber used by Ussing is shown in Figure 13. With the same Ringer solution on both sides of the skin, a potential difference was measured at P; however, when a counterpotential was supplied by D, both sides of the skin were at the same potential. Under this condition, no net passive transfer of ions could take place. On the other hand, the ions that were actively transported would continue to flow. The current running through the short circuit was equal to the resultant of all the net active transport processes.

In order to measure the skin current and voltage accurately, the electrodes had to meet certain special requirements. The induced current usually presented a much greater problem than the voltage measurement. Lund and Stapp (1947) suggested the following characteristics should be met: 1) the half cells should be isoelectric, 2) the electrodes should

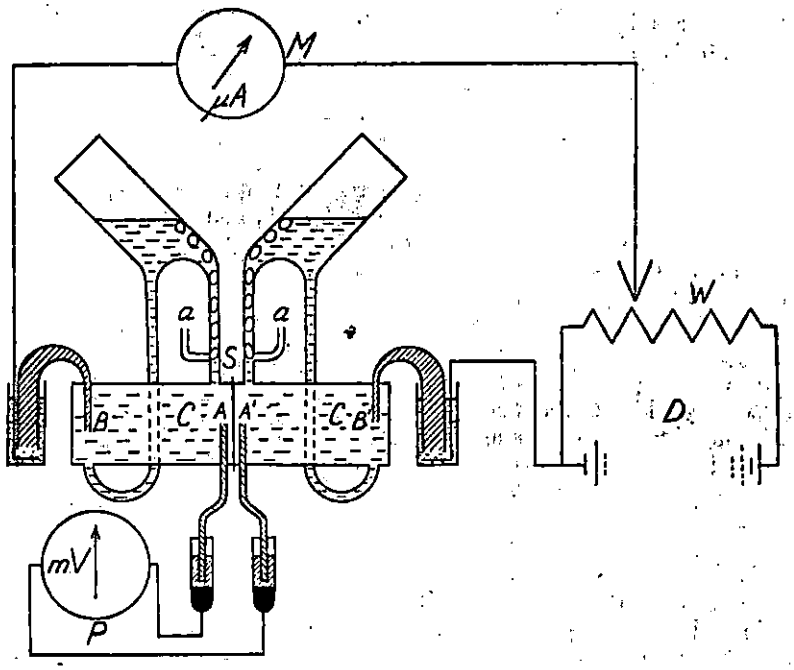

Figure 13. Diagram of apparatus used for determining Na-flux and short-circuit current

C: Celluloid chamber, containing, on each side of the skin, 40 ml Ringer
S: Skin
a: Inlets for air
A and A': Agar-Ringer bridges, connecting outside and inside solutions, respectively, with calomel electrodes
B and B': Agar-Ringer bridges used for applying outside E.M.F.
D: Battery
W: Potential divider
M: Microammeter
P: Tube potentiometer

not be polarized by the passage of direct currents of the magnitude produced by the tissue, 3) they should be of low resistance so the current produced in the external circuit can be maximum and they should have no appreciable capacity or inductance, 4) they should be nontoxic to the skin, and 5) they should not interfere with oxygen in the chamber. As a result calomel electrodes with agar bridges were used for measuring the voltage across the skin because they polarize less than silver-silver chloride (Ag/AgCl) electrodes, but silver-silver chloride electrodes with agar bridges were used for inducing current into the chamber because they have less resistance than calomel electrodes.

Lund and Stapp (1947) studied the effects of varying electrodes, Ringer solution flow and frog skins. First, the calomel or Ag/AgCl or both electrodes with agar bridges were tested for polarization by passing continuous currents (from 100 to 300 microamperes for 15-20 hours) through them and recording the voltage across them. The observed voltage was always very close to the voltage calculated from Ohm's law and it remained practically constant over the periods of observation. The results indicated that there was not any appreciable polarization in these electrodes. Second, the Ringer flow rate was studied. Since they observed that increasing the rate of flow in both sides in a similar manner did not have any significant or consistent effect either on the potentials across the skin or on the skin current, they considered that such variations in the flow rate as sometimes occurred under the experimental conditions did not influence the results obtained to a measurable extent. Third, they studied the effect of using frogs from different conditions. They

found that the skin from frogs which had recently been brought into the laboratory produced noticeably higher currents, both initially and throughout the experiment than those from frogs that had been in captivity for about a month or longer. Other experiments in their laboratory showed that the isolated skins from previously fed frogs generated a higher PD than the isolated skins of previously starved frogs. In general, they found that the skin current response as a function of time showed an initial sharp drop in an average of one hour and then the skin current curve tended to level off. However, the subsequent course of the curve was variable from individual to individual. The two symmetrical halves of the same frog skin were found to vary in the amount of current produced, but in general, the behavior by one-half closely paralleled that of the other half.

INSTRUMENTS AND EQUIPMENT

Temperature Measurement

It is necessary to record any temperature changes that occur in a biological preparation during exposure to a MF. For this purpose, three thermistors[1] were used in an instrument with three channels. The circuit diagram is shown in Figure 14. According to the thermistor catalog[2], the response of these thermistors to temperature is fast (between 1 and 9 seconds). This allows one channel to be used, while the other channels are not being used, thereby reducing the temperature error from electrical heat caused by the current passing through the thermistor's resistance. Operation of a channel is shown by a lighted lamp whenever the switch for that channel is closed. In each channel, a thermistor is used as a resistance in a bridge circuit. Any change in the thermistor resistance due to environmental temperature change creates an unbalanced voltage in the bridge circuit. This voltage, after passing through a voltage follower, is amplified by a differential amplifier. A selector switch connects a voltmeter to the output of the differential amplifier in each channel. The bridge circuit design was based on the thermistor manufacturer's[3] suggestions for more sensitivity in the temperature range likely to be encountered in subsequent experiments. Two variable resistors were used

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[1]Fenwal Electronics Co., Framingham, Massachusetts 01701.

[2]Thermistor manual. Copyright 1974 by Fenwal Electronics.

[3]Thermistor manual EMG6. Fenwal Electronics, Division of Walter Kidde and Co., Inc., 1974.

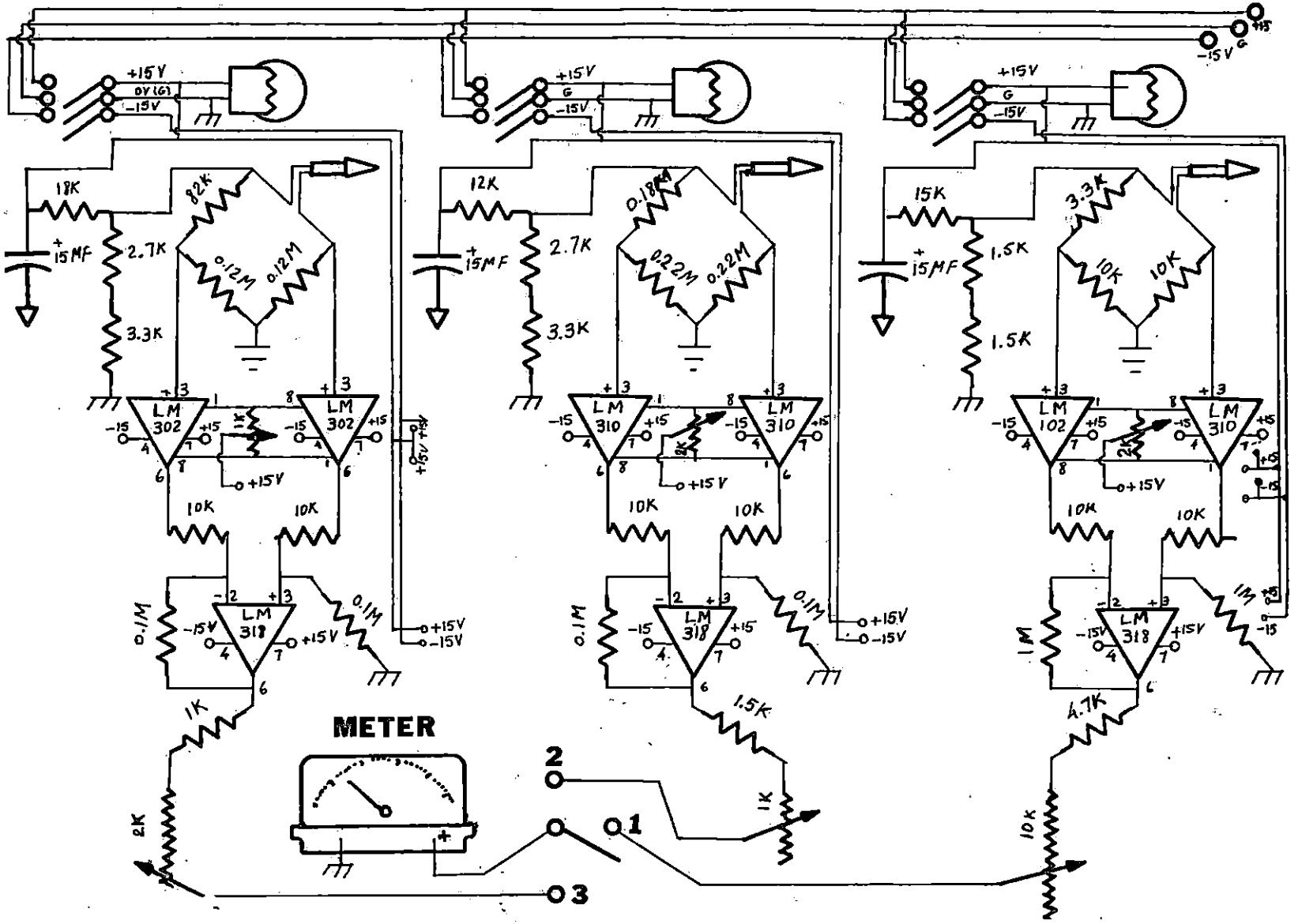
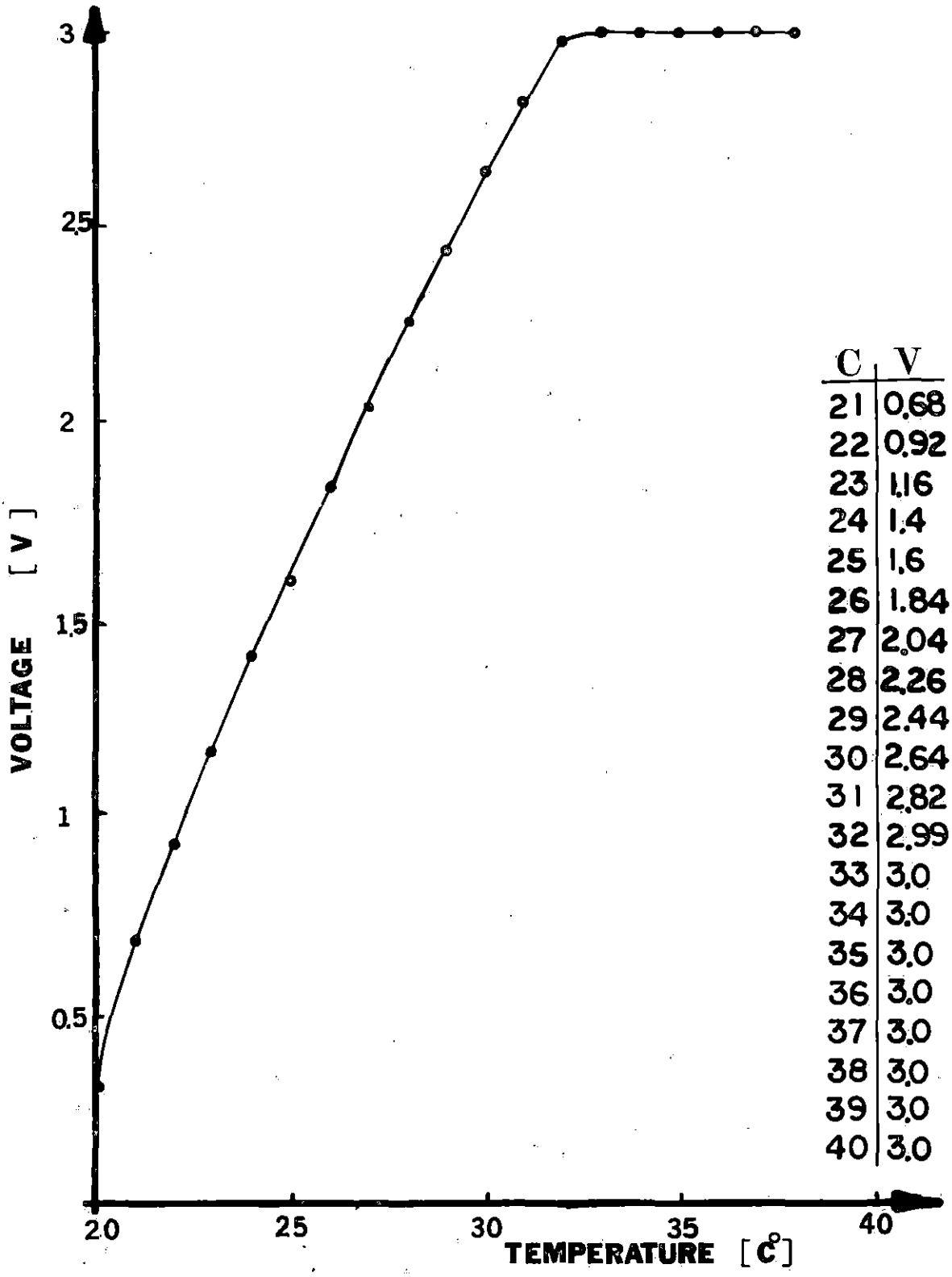


Figure 14. Circuit diagram of three channels for temperature monitoring

for calibration and zero adjustment of the voltmeter range. The three thermistors had nominal resistances at 25°C of 6500, 75000, 15000 ohms; these were in the first, second and third channels, respectively. Their performances as a function of temperature are shown in Figures 15, 16 and 17.

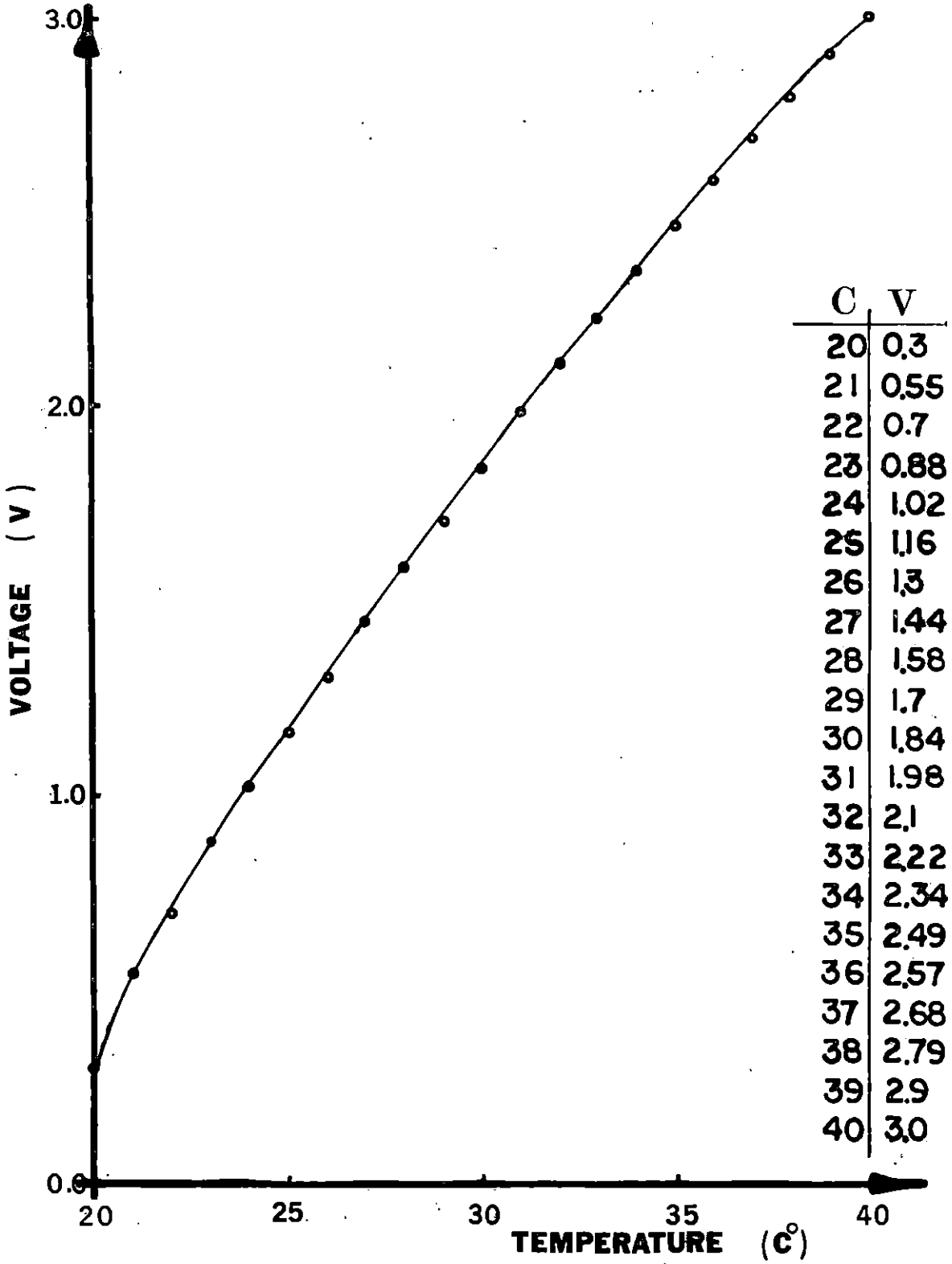
Chambers

Chambers were constructed for studying the effects of MFs on the permeability of frog skin. Three different designs were used to allow different MF directions. Drawings of these chambers are shown in Figure 18. The designs were based on the original chamber suggested by Ussing in 1949. The chambers were made of Plexiglas and a mirror image pair of chambers was held together by a special holder which also was made of Plexiglas. A rubber gasket was mounted between the two chambers of the pair to prevent saline leakage. Two ports were fabricated on each end of each chamber to allow access to the interior of the chambers. Saline was circulated between a rear inlet and a front outlet in each chamber. The other rear port in each chamber of the pair was used to induce current from one chamber to the other. Finally, the other front port of each chamber was used for measuring the voltage across any membrane that was mounted between the two chambers. Another front inlet (not shown in the picture) was used to insert a thermistor in one chamber of a pair. A slope was made inside each chamber to decrease the amount of turbulence which increases the fluid resistance while a solution is circulating inside. The difference in surface size at the rear and the front of the



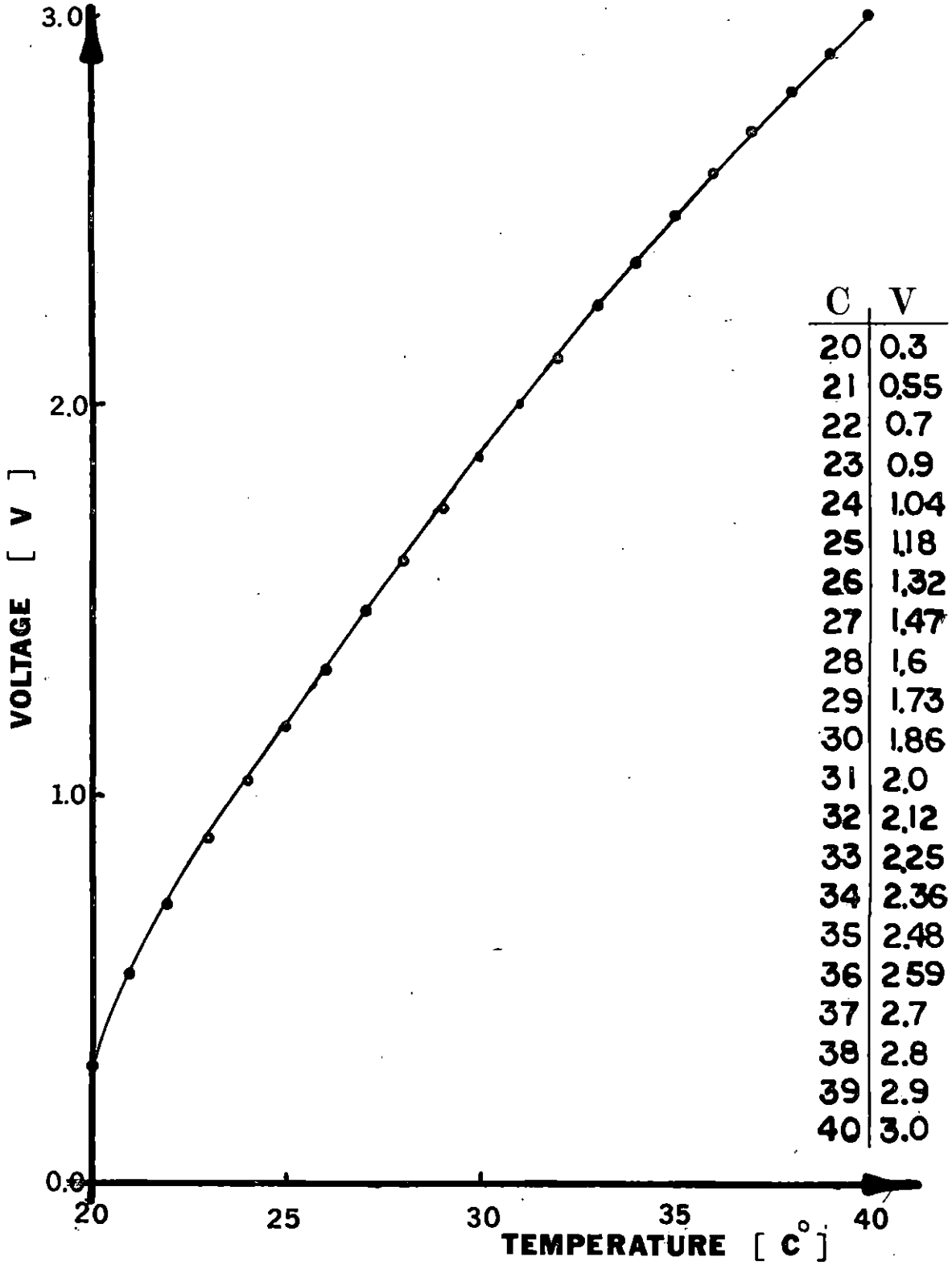
C	V
21	0.68
22	0.92
23	1.16
24	1.4
25	1.6
26	1.84
27	2.04
28	2.26
29	2.44
30	2.64
31	2.82
32	2.99
33	3.0
34	3.0
35	3.0
36	3.0
37	3.0
38	3.0
39	3.0
40	3.0

Figure 15. Calibration curve for the thermistor in the first channel



C	V
20	0.3
21	0.55
22	0.7
23	0.88
24	1.02
25	1.16
26	1.3
27	1.44
28	1.58
29	1.7
30	1.84
31	1.98
32	2.1
33	2.22
34	2.34
35	2.49
36	2.57
37	2.68
38	2.79
39	2.9
40	3.0

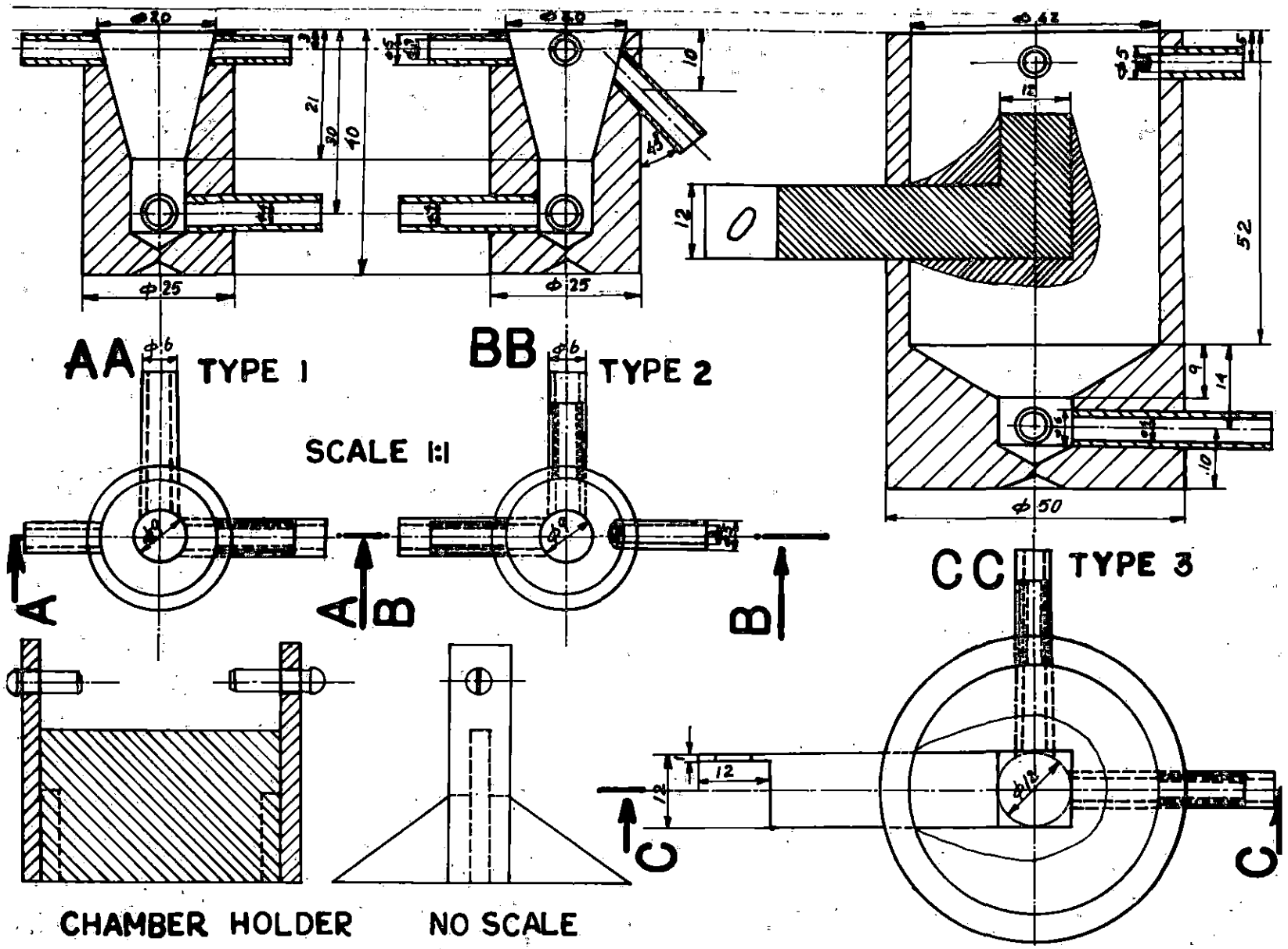
Figure 16. Calibration curve for the thermistor in the second channel



C	V
20	0.3
21	0.55
22	0.7
23	0.9
24	1.04
25	1.18
26	1.32
27	1.47
28	1.6
29	1.73
30	1.86
31	2.0
32	2.12
33	2.25
34	2.36
35	2.48
36	2.59
37	2.7
38	2.8
39	2.9
40	3.0

Figure 17. Calibration curve for the thermistor in the third channel

Figure 18. A schematic of the three chambers and a chamber holder. The scale is one-to-one except for the chamber holder which does not have a scale. All the dimensions are in millimeters


AA
TYPE 1
BB
TYPE 2
SCALE 1:1
CC
TYPE 3
CHAMBER HOLDER
NO SCALE

inside of each chamber allows the solutions to have a well mixed and uniform chemical concentration.

The type 1 chamber was used to study the effects of the parallel direction of the MF on the tissue. The type 2 chamber was used to study the effects of a closed magnetic flux on the tissue, a wire was inserted through the angled inlet-outlet and the tissue. In the type 3 chamber, the core of a magnet was arranged within the chamber. As shown in Figure 18, the inside part of the core was given an aerodynamic shape. The aerodynamic shape was fabricated with wax and the wax was painted to prevent it from dissolving in the saline. The type 3 chamber was used to study the effects of the perpendicular direction of the MF on the tissue.

Several items were necessary to induce current between the chambers and read voltage across the tissue between the chambers. An Eveready battery model NEDA200 was used as the source of the required current. The amount of current flowing from the 67.5 volts battery to the chambers was controlled and adjusted by two potentiometers used as the coarse and fine adjustments. The total current was read by measuring the voltage drop across a known resistance.

The current did not pass directly into the chamber; first, it went to a Ag/AgCl electrode which was mounted in a container of frog saline, then it passed to the chamber by an agar bridge. To make these bridges, a 3% agar (granular) Ringer solution was brought almost to the boiling point and then sucked into plastic capillary tubes. While they were cooling, the tips of the tubes were immersed in the agar solution to prevent drying of the agar inside the capillary. This concentration of agar was chosen

to minimize the resistance in the current pathway while maintaining a mechanically stable agar column. Since it was required that the agar tubes form a stable resistance in the current pathway, the position of the bridges in the chambers had to be stable during each experiment and for different experiments. Each capillary tube was passed into the chamber through a septum for water-tightness.

The voltage across the tissue was measured using a pair of capillary tubes located two or three millimeters from the tissue. These electrodes, which were located on the front side of the chambers, were similar to the current passing agar bridge electrodes located on the back side of the chambers. However, away from the chamber, they ended in calomel electrodes. The voltage was read on a potentiometer type K-3 universal potentiometer.[1]

Pure oxygen was bubbled in the saline to oxygenate the tissue and mix the saline for a uniform concentration of ions. This was done via the back inlet and the front outlet in each chamber.

The arrangement of this equipment is shown in Figures 19, 20 and 21 for the first, second and third chambers, respectively. Calibration of each chamber was needed to determine the offset potential between the two voltage measuring electrodes and the resistance in the current pathway. Calibration was done by applying a current and measuring the voltage between the two recording electrodes without any tissue between the chambers. These data are shown in Figures 22, 23 and 24. These curves show that the

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[1] Leads and Northrup Co., Philadelphia, PA.

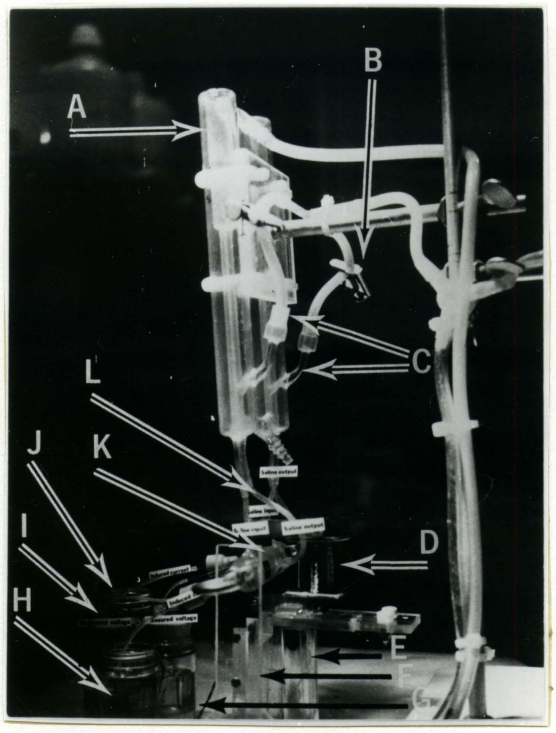


Figure 19. First chamber arrangement

A: Perfusion reservoir into which oxygen is bubbled
B: Oxygen flow control
C: Oxygen inlets
D: Type A magnet
E: Magnet core holder
F: Chamber holder
G: Right side of voltage measurement apparatus with agar bridge into calomel electrode. The capillary tube can be seen attached to the chamber via a septum.
H: Right side of connections for inducing current which reaches the chamber via a Ag/AgCl electrode and an agar bridge.
I: Left side of voltage measurement apparatus
J: Left side of connections for inducing current
K: Type 1 chamber
L: Saline outlet

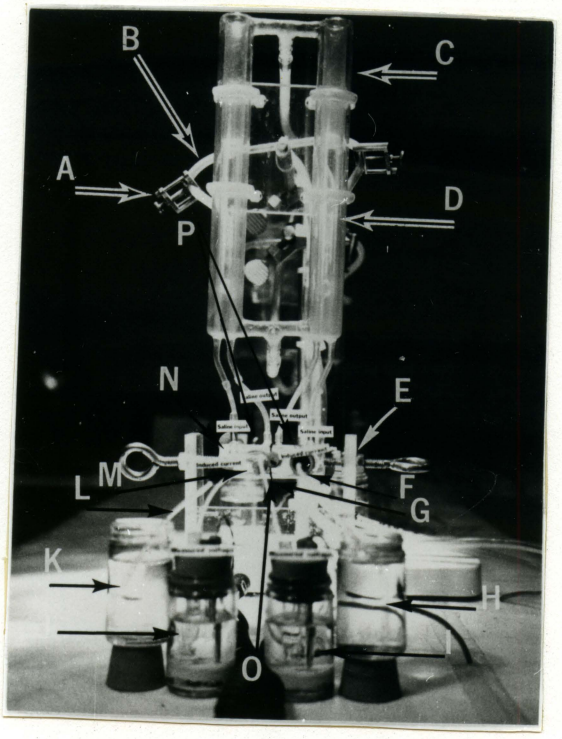


Figure 20. Second chamber arrangement

A: Oxygen flow control
B: Tube for oxygen flow
C: Perfusion reservoir
D: Saline inlet and outlet connected to perfusion reservoir
E: Chamber holder
F: Right side of induced current apparatus
G: Right side agar bridge to measure voltage
H: Right side saline pot for induced current
I: Right side calomel electrode
J: Left side calomel electrode
K: Left side saline pot for induced current
L: Left side agar bridge for induced current
M: Left side agar bridge to measure voltage
N: Type 2 chamber
O: Rubber gasket
P: The angled inlet and outlet for the magnet wire

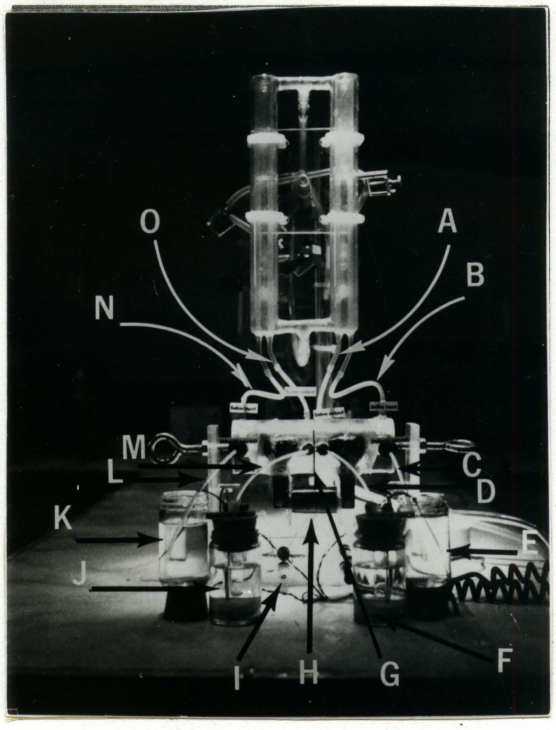
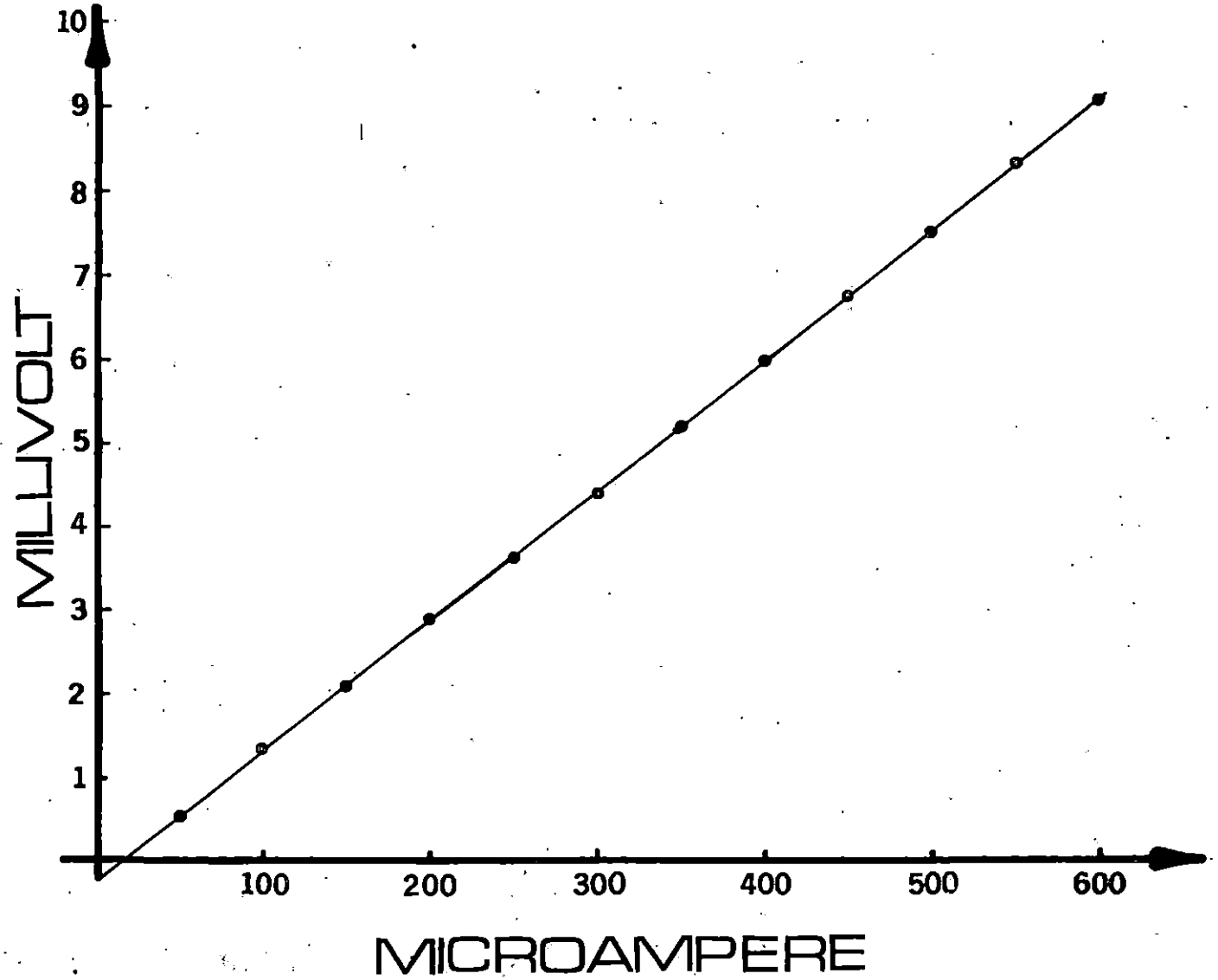


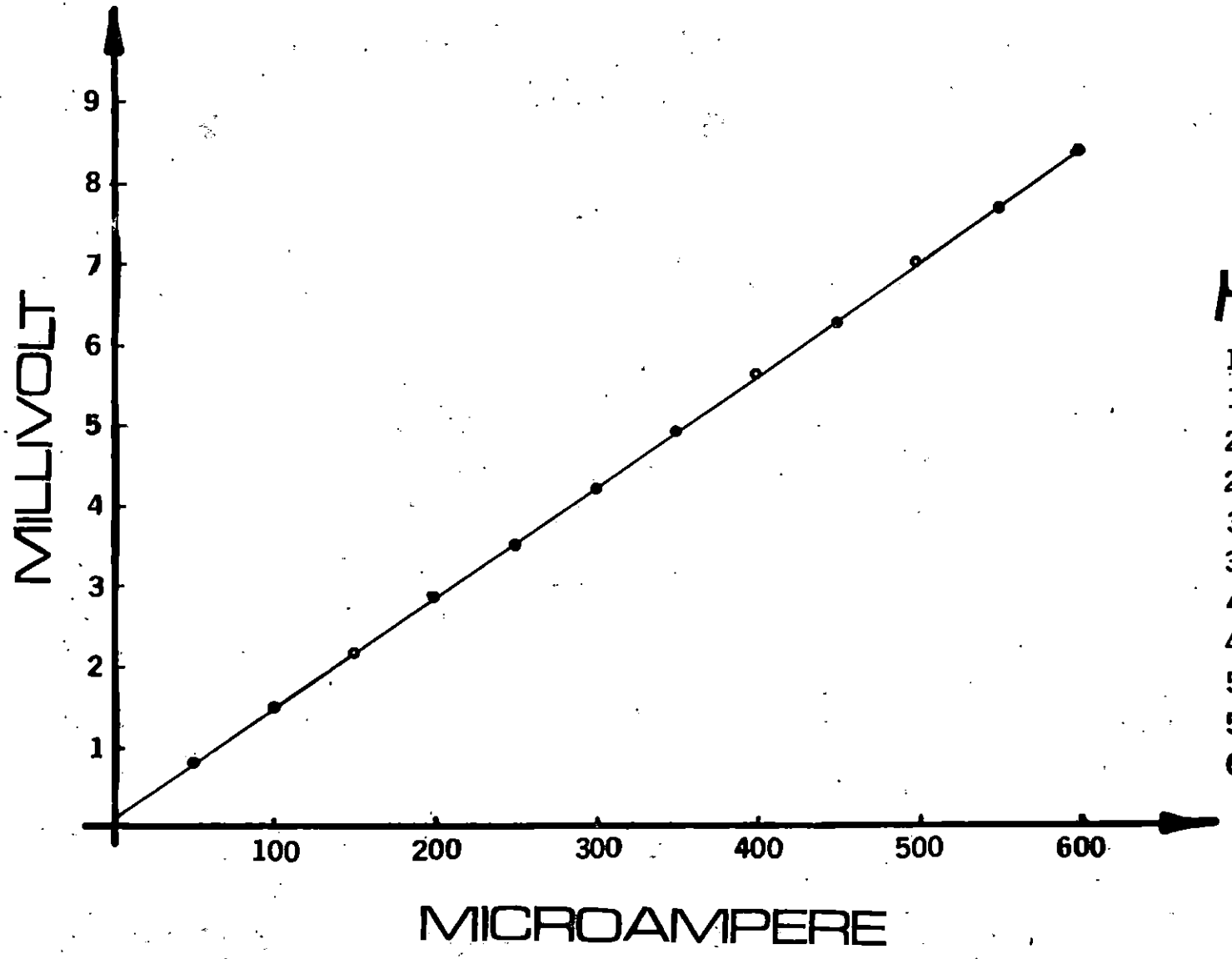
Figure 21. Third chamber arrangement

A: Right side outlet of circulated saline
B: Right side inlet of circulated saline
C: Right side agar bridge for induced current
D: Right side agar bridge to measure voltage
E: Right side saline pot
F: Right side calomel electrode
G: Rubber gasket
H: Type B magnet
I: Chamber holder
J: Left side calomel electrode
K: Left side saline pot
L: Left side agar bridge for induced current
M: Left side agar bridge to measure voltage
N: Left side inlet of circulated saline
O: Left side outlet of circulated saline



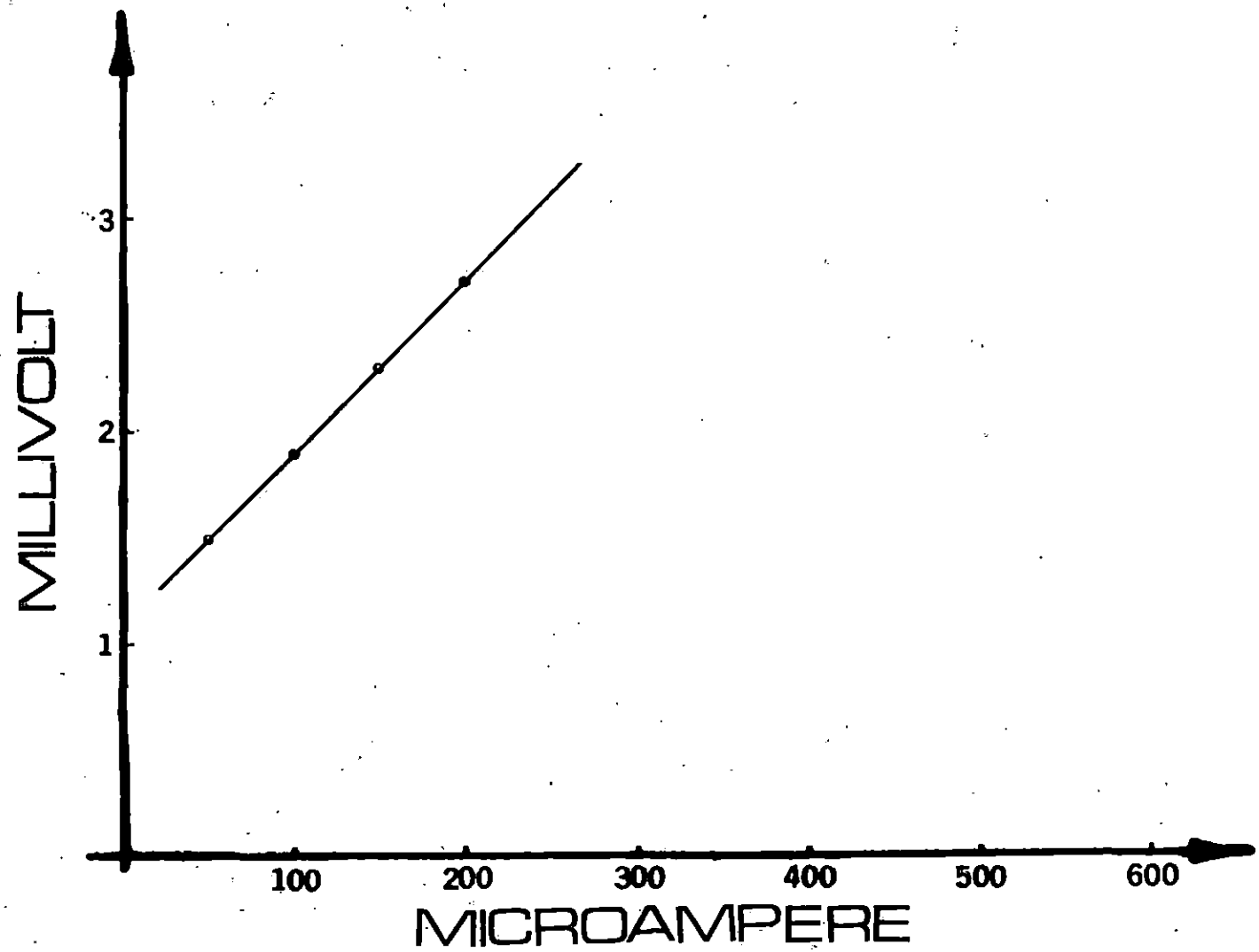
μA	mV
50	0.57
100	1.35
150	2.1
200	2.88
250	3.64
300	4.42
350	5.2
400	5.99
450	6.77
500	7.53
550	8.32
600	9.03

Figure 22. First chamber calibration curve



μA	mV
50	0.81
100	1.49
150	2.16
200	2.86
250	3.53
300	4.24
350	4.91
400	5.61
450	6.3
500	7.01
550	7.68
600	8.36

Figure 23. Second chamber calibration curve



μA	mV
50	1.5
100	1.9
150	2.3
200	2.7

Figure 24. Third chamber calibration curve

pathway resistance is fairly stable for each chamber. This resistance for the first chamber was 15.6 ohms, for the second one, 13.7 ohms and for the third, 8 ohms. The offset potential between the two recording electrodes was not the same for all chambers. For the first one, it was -0.2 millivolts, for the second one +0.1 millivolts, and for the third one +0.6 millivolts.

Nerve Chamber

The chamber used to study nerve behavior during exposure to the MF is shown in Figure 25. This nerve chamber was made with Plexiglas. The dimensions were chosen to allow the chamber to be located in the gap of a magnet core. The cross pieces which hold the nerve were made of string except for the ones used to stimulate and record and as the ground. String was used to minimize the metal contacting the nerve and decrease the possibility of creating eddy currents with the MF. A thermistor was placed in parallel with and at the same level as the nerve holders to monitor the temperature of the nerve.

Magnets

Three different magnets were made. The basic design used was wrapping magnet wire around a closed core with an air gap. Laminated iron cores from choke devices were used. The initial coil was unwrapped and rewrapped as necessary for each experiment.

The MF generated at the center of a coil theoretically is equal to

$$B = \mu NI/L$$

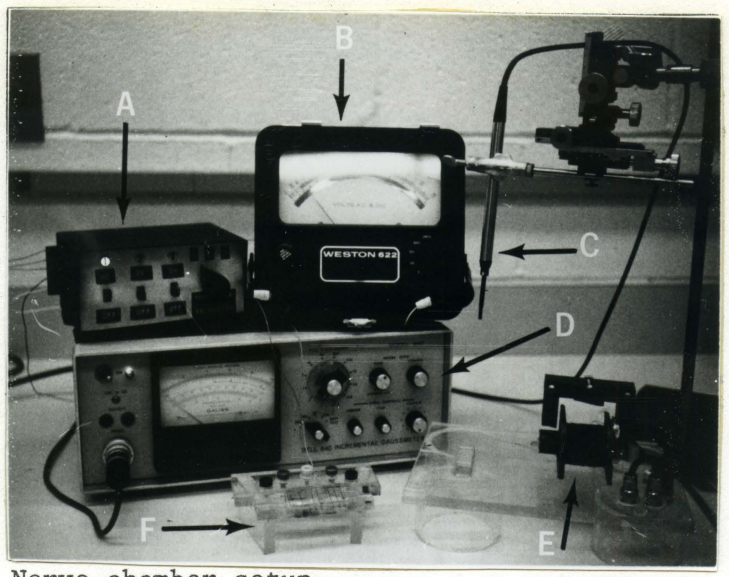


Figure 25. Nerve chamber setup

A: Temperature monitor with three channels
B: Voltmeter which indicates the temperature change
C: Gauss meter probe
D: Gauss meter recording instrument
E: Type C magnet
F: Nerve chamber; string supports can be seen as black lines

where B is normally called the magnetic induction; μ is the permeability constant ($4\pi x10^{-7}$ henry/meter, for vacuum); N is the number of turns of wire around the core; L is the length of the turns across the core; I is the electrical current.

Brancazio (1975) described the MKS unit of magnetic induction as the tesla, but in practice the CGS unit is more often used and is called the gauss (1 tesla = 10^4 gauss). Halliday and Resnick (1962) explained the MF that is independent of medium is called MF strength (H) and is equal to B/μ_0 for a vacuum. The MKS unit of MF-strength is ampere-turn per meter, but the CGS unit is called the oersted (1 ampere-turn per meter = $4\pi x10^{-3}$ oersted).

Fabricating a magnet of desired intensity by choosing theoretical parameters is essentially impossible; many problems appear during fabrication which are not considered in the theoretical formula. Welsby (1950) described different practical parameters that influence the results. With short coil length and a fat solenoidal coil (multilayer wrapping coil), there is no longer a uniform field inside the coil and inductance becomes a function of the ratio of length to radius of the wrapped coil. In addition, the eddy currents caused by an alternating current take power to dissipate so the field intensity drops. Since researchers have not determined the MF level necessary to modify biological behavior, no specific strength of MF was chosen before fabricating the magnets. Making magnets with strengths of several thousand gauss is not an easy task; therefore, magnets with lower field strengths were produced.

A gauss meter was used to measure the generated MF of each magnet and the GMF at the laboratory. A Bell 640 incremental gauss meter[1] using a transverse probe model T-6001 was used to record the MF. This has an accuracy of ±1.5% in the range of 30 KG.

The type A magnet, shown in Figure 19, had 9000 turns of 0.136 mm diameter wire wrapped around a laminated iron core which was 11x11 mm^2 in cross section with an average length of 36 mm and an average thickness of 7 mm. This solenoid coil had a DC resistance of 674 ohms and a 25 mm air gap. The responses of this coil were measured for 5, 10, 20 and 40 volts with and without saline present. Data are summarized in Tables 1 and 2 for one

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[1]Bell Inc., Columbus, Ohio.

Table 1. The MF generated by applying various voltages to the type A solenoid coil; no saline was present

Applied voltage	5	10	20	30	40
Magnetic induction (gauss)	14	28	55.5	81.5	102

Table 2. The MF generated by applying various voltages to the type A solenoid coil; saline was present

Applied voltage	5	10	20	30	40
Magnetic induction (gauss)	14	27	54	81	102

location of the gauss meter probe, because the chamber was too small to measure the MF at other locations. The response curve with saline present shown in Figure 26 is fairly linear.

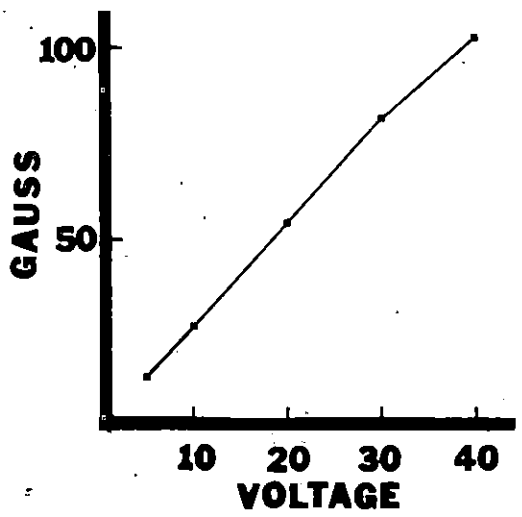

Figure 26. The magnetic induction as a function of voltage. Measurements were taken in the middle of the type A solenoid coil gap with saline present

The type B magnet was made by wrapping 8700 turns of a magnet wire (diameter 0.136 mm) around a laminated iron core with a cross section of 12x12 mm^2. The average length of the solenoid coil was 42 mm, the average thickness was 5 mm and the air gap was 34 mm. The total DC resistance of the wrapped magnet wire measured 653 ohms. The core was located inside a chamber as shown in Figures 18 and 21. The MF generated in the gap of the core of this coil was measured in different locations in the chamber. Measurements were taken in the middle, at the far right, at the far left and at the top. These measurements, taken using 5, 10, 20, 30 and 40 volts, are summarized in Tables 3 and 4. These tables show the MF created by the core alone; the GMF had been suppressed. The data in Table 3 were taken when no saline was in the chamber; those in Table 4 were taken when saline was present. A graph of the MF in the middle of the gap (saline present) as a function of voltage is shown in Figure 27. This graph shows the relationship is quite linear.

The type C coil, shown in Figure 25, was wrapped with 15000 turns of 0.195 mm diameter magnet wire. The solenoid coil had an average length of 38 mm and an average thickness of 10 mm. The coil had a DC resistance of 293 ohms and generated a MF in a laminated iron core with a cross section of 18x12 mm^2 and an air gap length of 11.5 mm. This magnet was used to generate a constant and an alternating MF in air. Data for different locations of the gauss meter probe (in or near the air gap) and different applied voltages are indicated in Table 5. A response curve relating the MF in the middle of the two poles to the voltage is shown in Figure 28; it indicates that the generated magnetic induction is not linear with voltage.

Table 3. The MF generated by applying various voltages to the type B solenoid coil; no saline was in the chamber

Voltage (v)	Magnetic induction (gauss)			
	Above	Middle	Right	Left
5	9	16.5	14.25	15
10	15.6	27.9	24.3	25.5
20	30	53	46	48
30	42	74.5	65	67.5
40	53	95	82.5	86

Table 4. The MF generated by applying various voltages to the type B solenoid coil; saline was in the chamber

Voltage (v)	Magnetic induction (gauss)			
	Above[a]	Middle	Right	Left
5	-	15.9	13.35	14.4
10	-	28.5	24.3	25.95
20	-	54	46	48.9
30	-	74	64.5	67
40	-	94.5	82	85.5

[a]Saline did not reach the level that was used for this measurement in Table 3.

An AMF also was generated in the type C solenoid coil using the 60 Hz wall outlet as the power supply. The magnetic inductions at various positions in the air gap were measured as shown in Table 6.

A plain magnet wire with a diameter of 0.54 mm also was used to generate a MF. This wire passed through an angled inlet-outlet in the type two chamber as shown in Figures 18 and 20. When connected to any

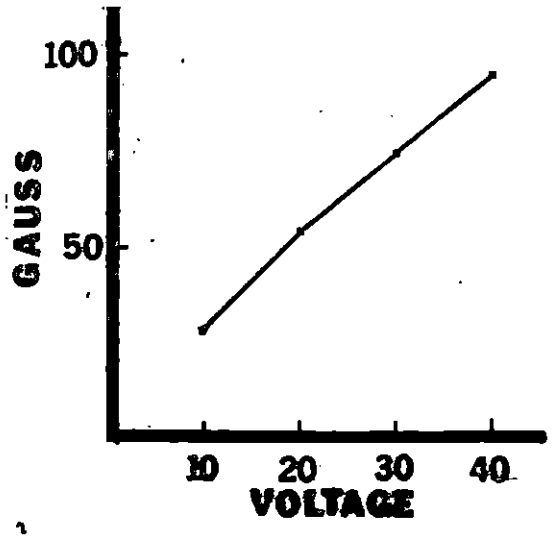

Figure 27. The magnetic induction as a function of voltage. Measurements were taken in the middle of the type B solenoid coil gap with saline present

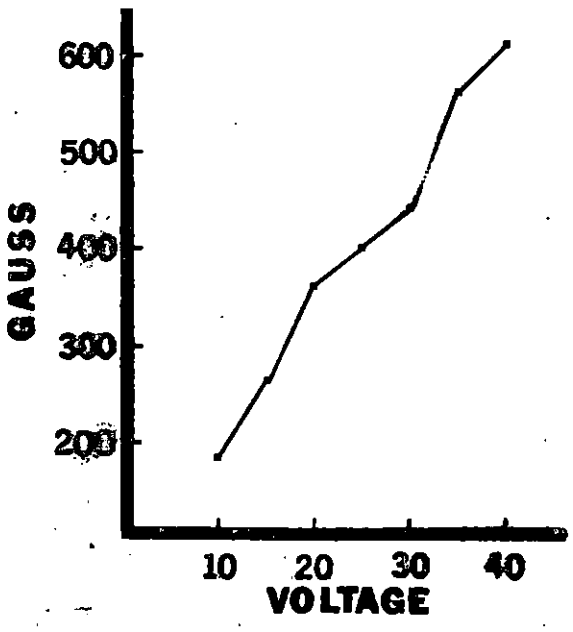

Figure 28. The magnetic induction as a function of voltage. Measurements were taken in the middle of the type C solenoid coil gap

Table 5. The MF generated by applying various voltages to the type C solenoid coil

Voltage	Magnetic induction (gauss)					
	Above	Below	Middle	Left	Right	Front
10	201	174	183	108	93	78
15	285	282	264	159	135	111
20	380	340	360	200	180	150
25	440	390	400	250	210	170
30	480	470	440	290	250	205
35	600	530	560	330	280	230
40	680	600	612	370	310	260

Table 6. The AMF generated by wall outlet power supplied to the type C solenoid coil

	Right	Right-middle	Middle	Middle-left	Left	Above	Below
Magnetic induction (G rms)	111	234	237	231	147	252	228

power supply it made a short circuit. The magnetic induction generated by this wire was 0.12 gauss (suppressed GMF) for all the voltage range.

Solutions

Frog saline with the following concentrations in one liter: NaCl 7.01 g; KCl 0.19 g, $CaCl_2$ 0.2 g, $NaH_2PO_4H_2O$ 0.09 g and Na_2HPO_4 0.16 g was used. The pH of the solutions was 7.2. A different saline solutions was required for the earthworm studies. Several saline solutions have been prepared to study the

giant nerve fibers of the earthworm (Rushton, 1945; Bullock, 1945). Drewes and Pax (1974) formulated a new earthworm saline solution which is much nearer to the ion composition of the body fluids than the earthworm salines used by previous investigators. The following concentrations are in one liter of distilled water: NaCl 1.461 g, KCl 0.298 g, $CaCl_2$ 0.666 g, $MgCl_2$ 0.203 g, Na_2SO_4 3.693 g and sucrose 18.826 g. Trizma acid (HCl) 0.25 g and Trizome base were used to adjust the pH to 7.5 at 25°C.

MF EFFECTS ON NERVE CORDS OF EARTHWORMS

Introduction

Every earthworm has a nerve cord located in the ventral part of its body. Three giant nerve fibers, one medial and two lateral, run the entire length of the dorsal side of the nerve cord. The fibers are not continuous throughout their length, but are divided into segments by membranes which apparently resemble the surface membrane of the fibers (Eccles et al., 1933). The two segment membranes are separated by an oblique septum with a gap of 5 nm (Coggeshall, 1965). The septum represents essentially a synapse with unusual properties (Bullock, 1945) such as conducting impulses in either direction (Eccles et al., 1933; Tashiro and Kuriyama, 1978) and offering little impedance to conduction in both directions (Tashiro and Kuriyama, 1978). The morphological and electrophysiological properties of these synapses indicate that the septa constitute electrical synapses; there is a specialized low resistance connection between the pre- and post-synaptic cells (Hama, 1961).

It has been shown that instead of the three action potentials (APs) which might be expected from these three fibers when an isolated earthworm nerve cord is stimulated, actually only two APs are recorded (Rushton, 1945). This is because the lateral fibers are connected by a conducting bridge in each segment (Bullock, 1945; Rushton, 1945). Rushton (1945) and Bullock (1945) also established that the AP in the medial giant fiber has a higher conduction velocity and a lower threshold than the AP in the lateral giant fibers.

At the head end of the nerve cord, the threshold stimulus for setting up the fast impulse is much lower than that for evoking the slow impulse, while at the tail end, the difference is always less. Also, the amplitude of the AP of the fast impulse at the head end is much greater than that of the slow impulse, but at the tail end the AP amplitude of the lateral nerve fibers may exceed that of the medial fiber (Eccles et al., 1933).

Method and Materials

Earthworms were collected locally and were kept in separate soil-filled containers in a refrigerator. In preparation for surgery, each earthworm was placed on a board and pinned through the lateral portion of its skin to stabilize it. With the aid of a dissecting microscope, fine scissors were used to make a dorsal longitudinal incision about the middle of the earthworm. After loosening the skin, the gut was removed and the nerve cord was visible. The nerve cord was separated carefully from its branches and approximately five cm of nerve cord were removed. The nerve cord was transferred to a Petri dish containing earthworm saline and allowed to stabilize for a few minutes. Then, it was placed in the nerve chamber (see nerve chamber section) and moistened with saline. The chamber was covered with a No. 1 1/2 Corning cover glass and sealed with stopcock grease.

The nerve chamber, containing the nerve cord, was placed under the type C magnet (see Tables 5 and 6) such that a pair of electrodes was located in the middle of the magnet poles. The experiments were performed in a Faraday cage which eliminated some undesirable MFs. The GMF meas-

ured in the absence of a MF generated by the type C magnet had a maximum value of 0.7 G which was quite small compared with the strength of the AMF (111-237 G rms) generated using the 110 V wall outlet power and the CMF (310-612 G) generated using 40 VDC. Most of the experiments were done with the AMF.

Experiments were divided into a series of 15 minute intervals. Each interval included exposing the nerve cord to the MF for approximately thirteen minutes, turning off the type C magnet to prevent the MF interfering with recording, then recording while stimulating the nerve with a S5 stimulator[1] adjusted to give a pulse with a duration of 0.07 msec and a frequency of 0.2 Hz. Biphasic recording utilized a type 502 oscilloscope with a differntial amplifier.[2] Usually, the nerve cord was stimulated at the site of exposure to the MF and recordings were taken 25 mm away. However, in some experiments, recordings were taken from the exposed region in response to stimulation 25 mm away. Some experiments were arranged as controls. The procedure in control experiment was the same except that the nerve chamber was not exposed to the type C magnet.

Results

In the absence of the MF, the depolarization characteristics of the medial and lateral nerve fibers remained constant for three hours as shown in Figures 29, 30 and 31. Also, in the control experiments, switching the stimulating and recording electrodes did not have any effect.

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[1] Grass Medical Instruments, Quincy, Mass.

[2] Tektronix Inc., Portland, Oregon.

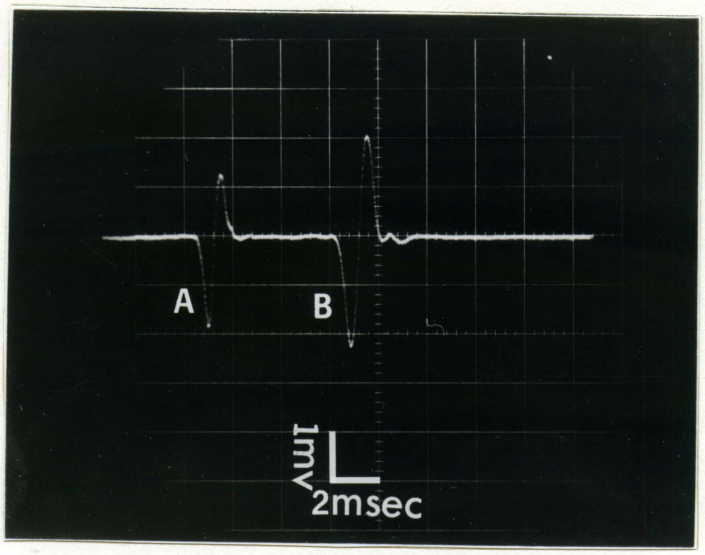


Figure 29. The APs in the nerve cord in the absence of a MF

A: AP in the medial nerve fiber
B: AP in the lateral nerve fibers

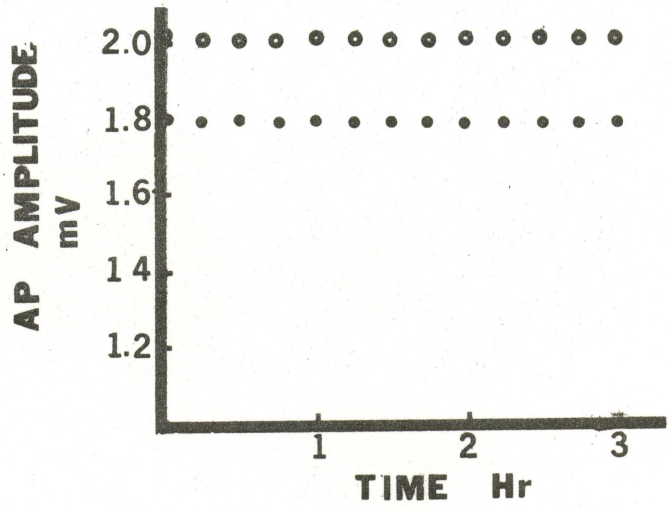


Figure 30. The time effect on the action potential amplitude of the earth worm nerve cord

○: Action potential amplitude in the lateral nerve fibers
●: Action potential amplitude in the medial nerve fiber

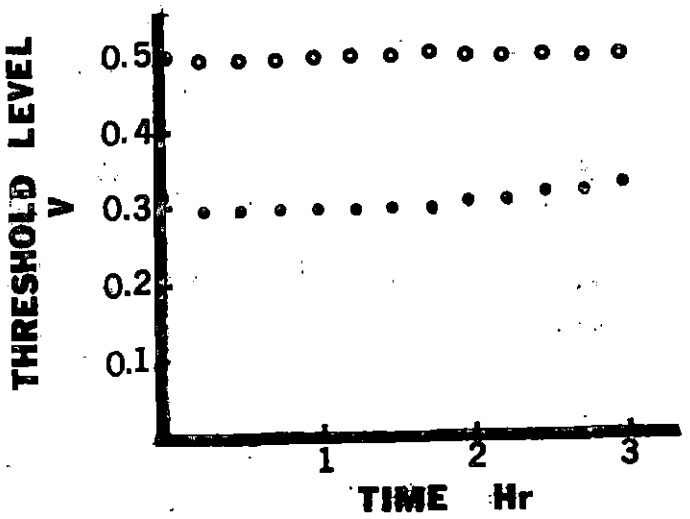


Figure 31. The time effect on the threshold level of the earthworm nerve cord

○: Threshold level in the lateral nerve fibers
●: Threshold level in the medial nerve fiber

After 15 minutes (in some experiments 30 min) of exposure to the MF (CMF or AMF), the nerve cord would not respond to stimulation no matter what stimulus strength was used. When inactivation of a nerve cord occurred, one of the following procedures was used: (1) removal of the nerve cord from the nerve chamber and placing it in the Petri dish with fresh saline for 15 minutes, or (2) leaving the nerve cord and the nerve chamber in position but with the MF turned off. It should be mentioned that although the MF was off, there was a small remanence MF in the type C magnet core. This was in the range of a few gauss. When a nerve cord which had been placed in the Petri dish was returned to the nerve chamber and stimulated, APs could be recorded (one observation with CMF and four observations with AMF). However, nerve cords which had remained in the nerve chamber were still inactive after 15 minutes. Even extending the time without the MF

to three or four hours, with testing every 15 minutes, failed to restore the excitability of the giant fibers (one observation with CMF and seven observations with AMF).

When a nerve cord was stimulated during the first 15 minutes of exposure to an AMF, the APs, as seen in Figure 32, were comparable to those recorded in the absence of a MF (Figure 29).

During exposure of the nerve cord to the CMF and AMF, it was noticed that some vapor accumulated under the Corning cover glass. The first channel of the temperature measurement device (see temperature measurement section) was used to determine whether this was due to a temperature rise. The thermistor was inserted very close to the electrodes under the MF and at the same level as these electrodes. The nerve chamber was prepared as before except that a nerve cord was not in it. The nerve chamber was placed in the gap of the magnet poles, in the absence of the MF, and allowed to reach its steady state temperature. After that, the MF was turned on. This experiment was repeated with CMFs generated by 20 VDC and 40 VDC, as well as a 60 Hz AMF generated by the wall outlet power. The results showed that the stronger the MF, the faster the temperature rise (Figure 33).

The temperature rise during MF exposure in a nerve chamber containing an earthworm nerve cord was not measured because a significant temperature rise was not expected and the temperature measurement device was not constructed until after the earthworm experiments. However, the tempera-

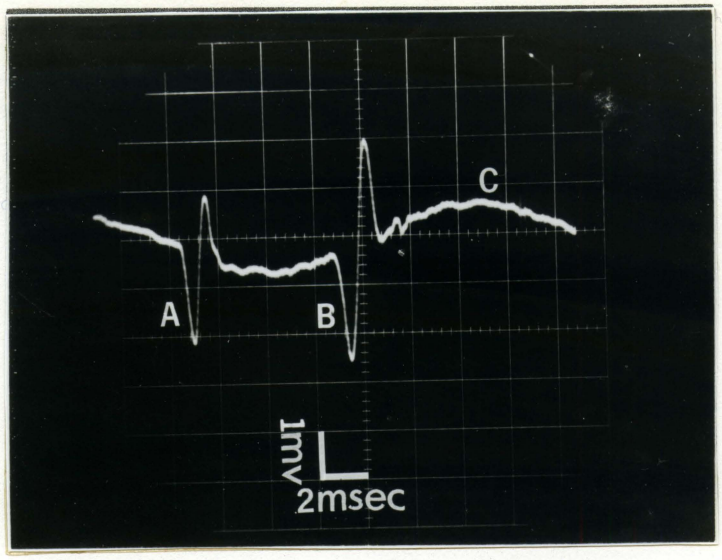


Figure 32. The APs in the nerve cord during exposure to the AMF

A: AP in the medial nerve fiber
B: AP in the lateral nerve fibers
C: The 60 Hz voltage induced in the recording electrodes

ture rise during MF exposure in a nerve chamber containing a dog sciatic nerve was monitored. The results were consistent with those obtained without a nerve cord.

To check whether this temperature rise was because of the Foucault currents or due to the MF power, air was blown with an air gun on different parts of the magnet core. No change in temperature rise occurred when air was blown on the magnet core or on the solenoid coil, but when air was blown directly on the nerve chamber, the temperature rise stopped.

Discussion

An important observation was the inactivation of the nerve cord during exposure to the CMF or AMF. Recovery of the excitability in the nerve cords which were treated with the first procedure suggested that the

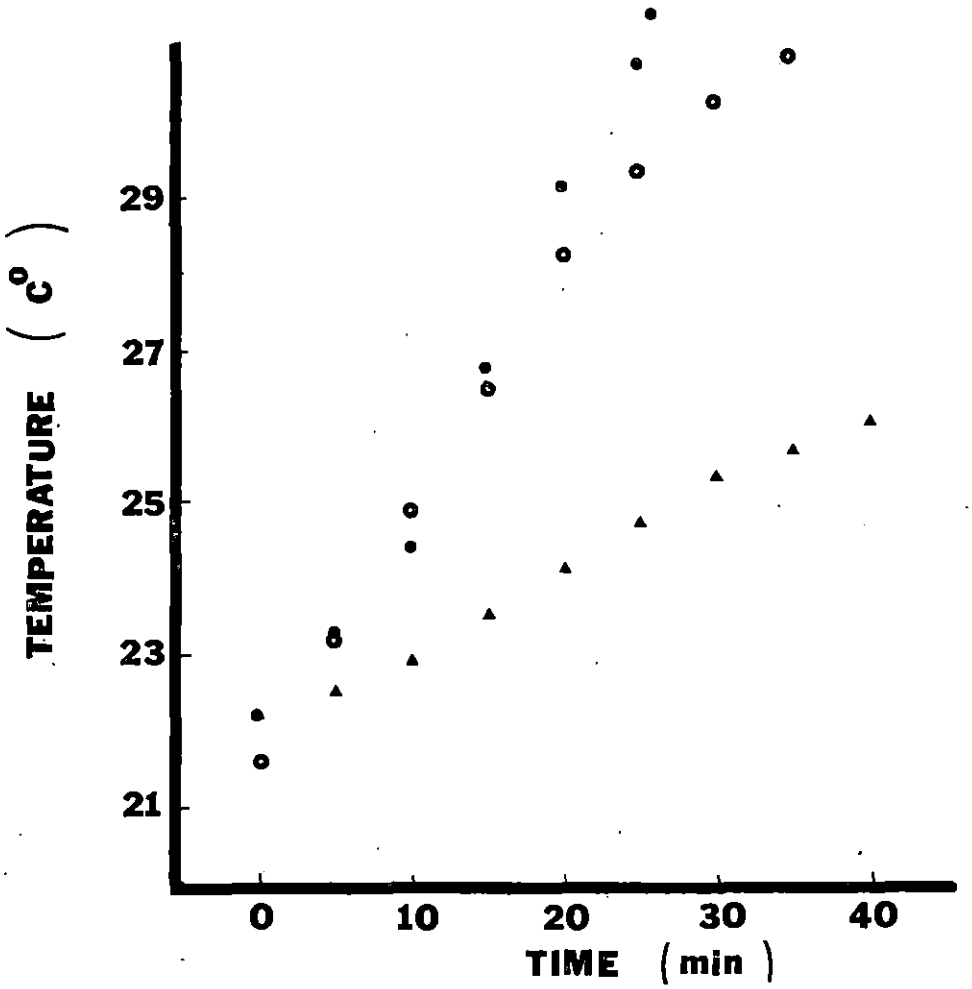


Figure 33. The temperature rise in the nerve chamber during exposure to the MF

▲: CMF generated by 20 VDC
●: CMF generated by 40 VDC
○: AMF generated by 60 Hz wall outlet power

inactivation did not result from orientation of any membrane molecules by the MF. While there are many parameters that may be involved in inactivation, one of the most effective might be the heating effect of the MF. Figure 33 shows that there is an increase in temperature from 21-22°C (room temperature) to 26-27°C during the first 15 minutes exposure time and up to 30°C for the second 15 minutes exposure time. It is possible that the proteins of the nerve cord had begun to denature at 30°C or even 27°C. However, recovery of the excitability of those nerve cords which had been transferred to the Petri dish and then retested needs to be explained. It is possible that the longer exposure to an elevated temperature completely inactivated the nerve cords which were left in place, while transfer to a cooler environment allowed the other nerve cords to recover.

Another possibility is that inactivation is due to changes in the constituents of the saline. Formation of hydrogen peroxide (Patrovsky, 1976) and increased water viscosity (Lielmezs et al., 1976) have been reported to result from exposure to MFs. These could cause inactivation because the former decreases the pH of the solution and the latter may decrease the mobility of active ions. Data to differentiate between these possibilities are not available; however, it seems unlikely that the magnitude and duration of MFs used here would produce sufficient changes in either the formation of hydrogen peroxide or solution viscosity to produce complete inactivation. Therefore, it is suggested that the generation of heat from the MFs caused the inactivation in the earthworm nerve cord.

A second important observation was that exposing the nerve cord to a MF did not trigger an AP in the nerve cord. This result agreed with the

findings of Schwartz (1978, 1979) on the giant nerve fiber of lobster. Using nerve-muscle preparations, Ueno et al. (1978), Kolin et al. (1959) and Oberg (1973) claimed that an AMF can trigger the nerve and cause contraction of the muscle. The MF had to be a time varying one for the muscle to contract. Although they recorded a muscle contraction, they did not mention measuring the nerve AP. An AMF produces a current in a circuit perpendicular to the magnetic flux and in the experiments of Ueno et al. (1978) and Kolin et al. (1959) (see Figures 10 and 11) it is possible that the induced currents reached the muscle and caused the contraction. In fact, Kolin et al. (1959) mentioned that the contraction was caused by eddy currents which were produced in the saline. Since current strength is directly related to the MF strength and its frequency, it is not surprising that the relatively small AMF used in the earthworm experiments did not produce sufficient current to trigger the earthworm nerve cord.

A third observation concerned stimulus threshold. In the range of the instrument accuracy, the MF had little, if any, effect on the threshold level. This is in accord with the results of Liberman (1959). Small threshold level changes could be due to variation in nerve cord position and electrode contact.

MF EFFECTS ON ISOLATED FROG SKIN

Introduction

Isolated frog skin (IFS) is able to transport salt from the outside to the inside even when both sides are bathed with Ringer, and this transport is closely related to the potential difference measured across the skin (Huf, 1935). This transport has been proven to be via a sodium pump (Ussing, 1949). Since the movement of ions across frog skin is easier to measure than the movement of ions across neuron membranes, MF effects on this system were studied.

Method and Materials

Three different types of chambers (see chambers section) were used, and before each experiment, the following checks were made in each chamber:

1. Saline circulation was checked by injecting food dye in the perfusion reservoir for each chamber. This allowed the circulating saline pathway to be traced easily.
2. The battery condition was checked by recording the maximum current that was induced in the chamber when the coarse and fine resistance controls were at their minimum positions.
3. The stability of the total resistance in the peripheral battery pathway was checked by calibrating each chamber.
4. The offset potential between the two voltage measuring electrodes was checked by recording the voltage difference across these two

electrodes when the induced current was zero.

5. All connections were checked to see if they were water-tight.
6. The lengths of the agar bridges were checked. The lengths were kept the same for all experiments to keep the total resistance of the pathway constant.
7. The positions of the capillary tubes and their lengths inside the chambers were made the same for all experiments. Any deviation from their original positions would change the resistance.
8. The voltage measuring capillary tubes were positioned as close as possible to the tissue by puncturing the septa at an angle and pulling the capillary tubes to the center of the chamber at a distance of 1 to 3 mm from the tissue.
9. The amount of oxygen bubbling in each side of the chamber was made approximately the same by adjusting the flow.
10. The chambers were checked for and freed of air bubbles after they were filled with saline.

Grass frogs were purchased and then maintained in an aquarium prior to use. To begin an experiment, a frog was pithed and its dorsal skin removed. The skin was placed in a saline filled Petri dish for washing. Then the frog skin, with a rubber gasket on each side of it, was placed between a pair of chambers. Sometimes stopcock grease was used between the rubber gasket and the skin to insure a water tight seal.

The saline inlets and outlets in a pair of chambers were connected to the perfusion reservoirs. The thermistor was fixed in the chamber to touch the skin and the agar bridges were inserted into the chambers. Then

the chambers were filled to the same level with saline. This insured equal pressure on the two sides of the skin. Pure oxygen was bubbled from the lower part of the glass tube which connected the saline outlet on the chamber to the perfusion reservoir (shown in Figure 19 under label C). These bubbles created a suction and caused the saline to circulate. Finally, the agar bridges were trimmed to the appropriate length; this also removed the dried part of the agar. Since the exterior side of the skin always faced the right chamber, the positive calomel electrode was connected to left chamber and negative calomel electrode was connected to the right chamber. In addition, the positive current was induced from the right chamber to the left chamber.

Data were taken first by measuring the voltage across the skin with a voltmeter. This reading included the voltage across the membrane plus the offset voltage and the voltage drop across the saline between the two electrodes. To measure the short circuit current (SCC), first, the voltmeter was adjusted to eliminate the offset voltage, then the induced current was increased until the voltmeter read zero. The amount of current recorded on the ammeter was then the same as the current produced by the electrogenic pump, because the potential produced by the induced current was equal and opposite to that produced by the membrane current.

The time that the frog was killed was designated zero and the time between killing the frog and beginning data taking was called idle time. Idle time included the time for surgery, washing the skin in saline and placing the skin in the chamber. An attempt was made to keep the idle time the same for all the experiments. The time interval for taking data

was always 10 minutes.

In the first experiments, the MF direction was parallel to the frog skin. The type 1 chamber and type A solenoid coil powered by 20 VDC were used. In these experiments, some of the skins were exposed to the MF for the entire time of the experiment but other skins were used as controls and never exposed to the MF. To produce a MF perpendicular to the frog skins, the type 3 chamber and type B solenoid coil powered by 20 VDC were used. In these experiments, each skin was exposed to the MF during parts of the experiment, but not during other parts of the experiment. In some skins, the pattern was two hours with exposure and two hours without exposure and then this sequence was repeated (as: MF - no MF - MF - no MF). For other skins, the order of exposure to the MF was changed: two hours without exposure followed by two hours with exposure and then the sequence repeated (as: no MF - MF - no MF - MF) for a total of 8 hours.

Since the magnet core was inside the type 3 chamber, it caused a nonuniform distribution of induced current inside the chamber. For example, the current density close to the chamber walls was much higher than at the middle of the chamber. Since this would directly affect the data, the opening of the type 3 chamber was reduced by using a rubber gasket with a small opening (1.6 cm inside diameter).

Attempts to use the type 2 chamber with a wire passing through the isolated frog skin were unsatisfactory. The skin did not seal around the wire, and thus a low resistance pathway was created through the skin.

The maximum GMF of the laboratory was measured as 0.5 G. This was judged to be insignificant compared to the experimental inhomogeneous

CMFs of the type A (maximum 54 G) and type B (maximum 54 G) solenoid coils.

Statistical Approach

The measured voltage across the skin and the short circuit current (SCC) obtained from each isolated frog skin (IFS) were plotted versus time. Two different statistical approaches were used to analyze the data from the IFSs exposed to different directions of the MF.

For IFSs exposed to the parallel MF direction, an optimum polynomial representing the IFS response as a function of time was fitted to each plot by the least squares method:

$$Y = a_0 + b_1 t + b_2 t^2 + b_3 t^3$$

where Y is the measured voltage or the SCC; t is the independent time variable; a_0 is the intercept; b_1, b_2 and b_3 are the coefficients of first, second and third degree.

A statistical method called Individual Curvature Analysis was used to determine the effect of the MF on the IFS. In this method, four major steps were followed:

1. Construct a table in which in each row the first four columns were the intercept and the coefficients of first, second and third degree of a fitted polynomial, the fifth column was the idle time and the last column was a vector called λ.[1]

---

[1] Where each component of λ is a fixed number called a comparison between those experiments exposed to the MF and those that were not exposed to the MF, λ has one value for all experiments which were done with a MF, and a different value for all experiments which were done without a MF and $\Sigma\lambda_i = 0$.

2. The last column was fitted to the first five columns to test the effect of the MF on the IFS. An analysis of variance table was prepared. However, the idle time effect had to be removed.
3. The last column was fitted to the fifth column and its analysis of variance prepared to eliminate the idle time effect.
4. Snedecor F test was calculated after removing the idle time effect as:

$$\text{Snedecor F test} = \frac{(S_1 - S_2)/(D_1 - D_2)}{M_1}$$

where S_1 is the sum of squares of models from the analysis of variance table introduced in the second step; S_2 is sum of squares of model from the analysis of variance table introduced in the third step; D_1 is degree of freedom of model from the analysis of variance table introduced in the second step; D_2 is degree of freedom of model from the analysis of variance table introduced in the third step; M_1 is mean square for error from the analysis of variance table presented in the second step.

The calculated F value was compared with tabulated F values presented by Snedecor and Cochran (1967) to determine whether the parallel MF had any effect.

For the IFSs exposed to a perpendicular MF, an optimum polynomial function was fitted to each two-hour time interval (MF on or off) of the plotted curves and the area under the curve for each interval was calculated. In each experiment, a contrast between the desired areas was calculated as $(A_2 + A_4)/2 - A_3$ where A_2 is the area under the curve during the second time interval; A_3 is the area under the curve during the third

time interval; and A_4 is the area under the curve during the fourth time interval.

A t-test was used to determine whether for each experimental condition $(A_2+A_4)/2$ was significantly different from A_3. The t value was defined as the mean of the contrasts $(A_2+A_4)/2-A_3$ divided by the square root of the variance of the sample mean. Four t values were obtained: one each for the area contrasts for the SCC and voltage curves from the experiments that started with MF exposure and one each for the area contrasts for the SCC and voltage curves from the experiments that started without MF exposure. These t values were compared with tabulated t values to determine the significance of the difference between $(A_2+A_4)/2$ and A_3. In addition, the contrasts from the SCC curves from experiments which started with MF exposure were compared to those from experiments that started without MF exposure, and the contrasts from the voltage curves from experiments which started with MF exposure were compared to those from experiments that started without MF exposure. Each these tests resulted in an analysis of variance table that contained an F value that could be compared with tabulated values to determine whether the contrasts for those experiments that started with MF exposure were significantly different from the contrasts for those experiments that started without MF exposure.

Results

The data for the parallel MF effects on the IFSs are shown in Figure 34 for the SCCs and in Figure 35 for the voltages across the membrane. Six

Figure 34. Responses of the short circuit current of isolated frog skin. Curves drawn with Δ and X represent the control experiments and the rest represent experiments with parallel CMF exposure

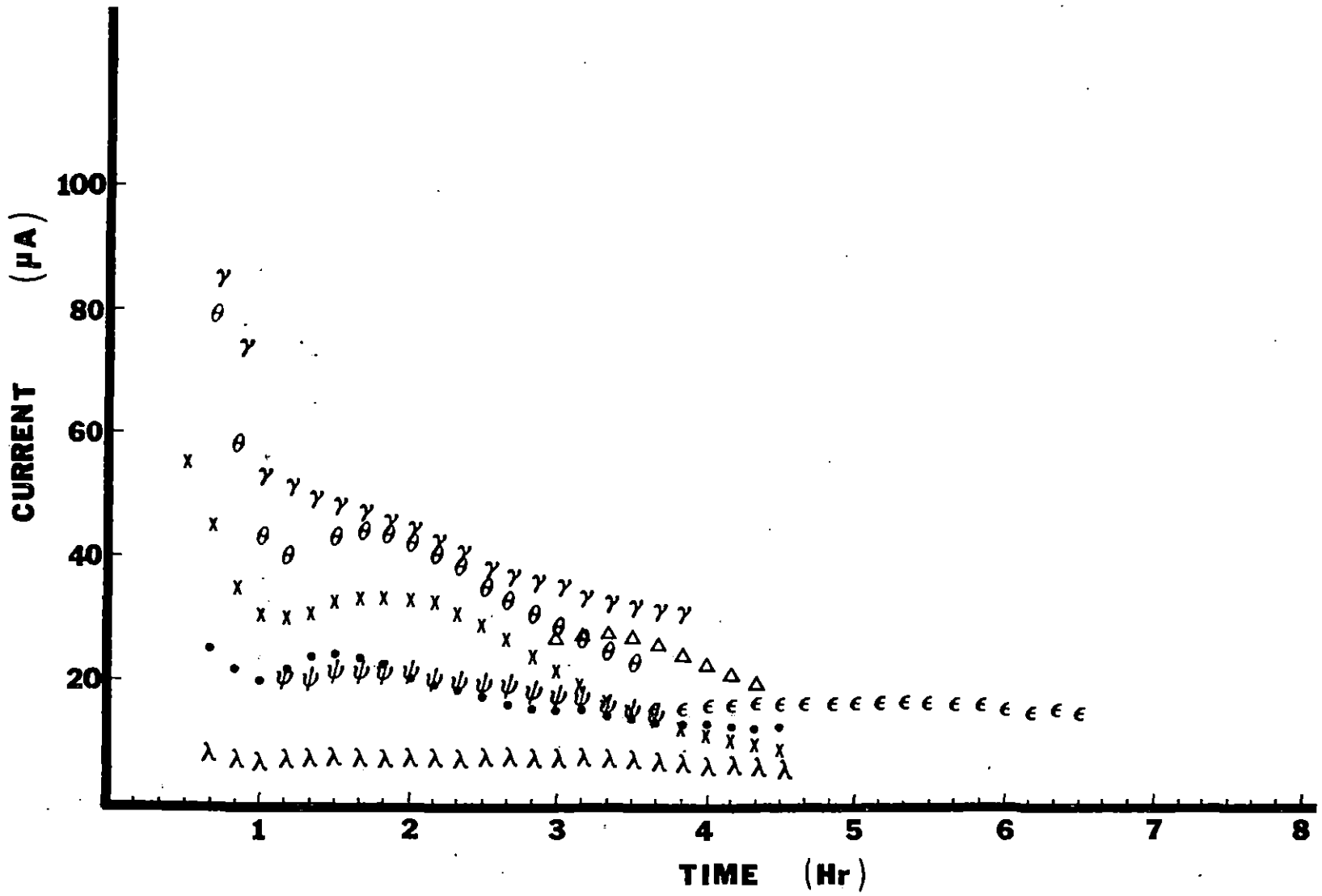
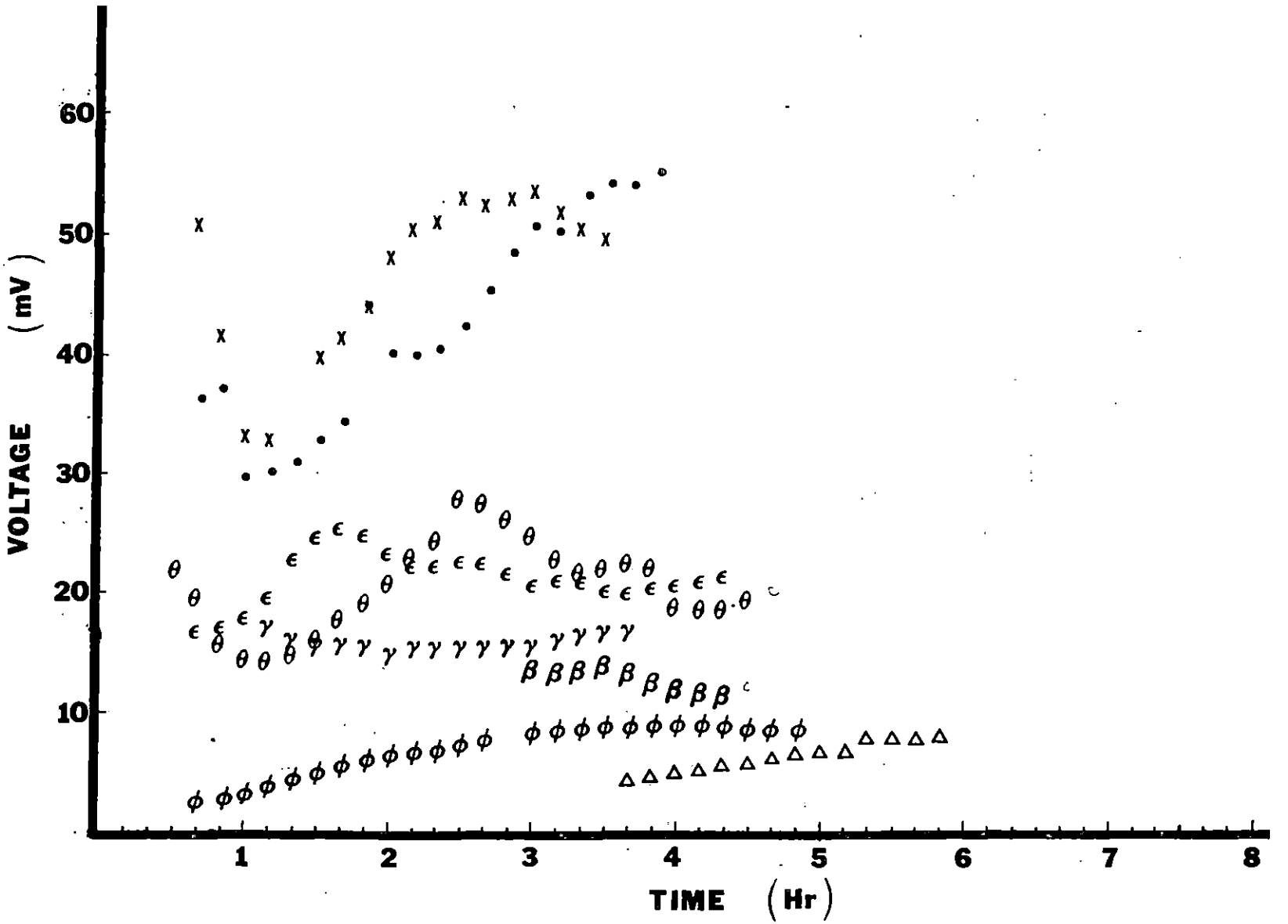

CURRENT (μA)
100
80
60
40
20
1
2
3
4
5
6
7
8
TIME (Hr)

Figure 35. Responses of the potential difference across isolated frog skins. Curves drawn with θ and β represent the control experiments and the rest represent experiments with parallel CMF exposure


VOLTAGE (mV)
60
50
40
30
20
10
1
2
3
4
5
6
7
8
TIME (Hr)

curves in each figure show the IFS behavior with the parallel MF present and two curves show the control experiments.

The temperature change was monitored in this experiment. The temperature in the type 1 chamber rose from 24-25°C (room temperature) to 27-28°C at the end of all the experiments.

The statistical analyses for the above curves resulted in two variance tables each for SCC and voltage across the membrane. These tables were calculated from their Individual Curvature Analyses and are shown in Tables 7 and 8 for the SCC and in Tables 9 and 10 for the voltage across the membrane. These tables come from steps 2 and 3 of the procedure outlined in the Statistical Approach.

The F value was calculated for each of these two different measured parameters as follows:

1. From the variance tables of SCC measured through the skin:

$$F = \frac{(73.54 - 4.4)/4}{11.23} = 1.54$$

2. From the variance tables of voltage measured across the skin:

$$F = \frac{(38.41 - 4.4)/4}{28.8} = 0.3$$

When these F values were compared with tabulated ones (19.25 for 95% significance), both results demonstrated that the parallel MF had no significant effect on the SCC or potential difference measured for the IFSs.

The effects of the perpendicular CMF on the SCCs and the voltages across the skins are shown in Figures 36 and 37 for the former and in Figures 38 and 39 for the latter. Figures 36 and 38 are from the experiments that

Table 7. The variance analysis of the effect of polynomial coefficients and idle time on the short circuit current for the exposed and control conditions

Dependent variable: Y			
Source	DF	Sum of squares	Mean square
Model	5	73.54299846	14.70859969
Error	2	22.45700154	11.22850077
Corrected total	7	96.00000000	

Table 8. The variance analysis of the effect of idle time on the short circuit current for the exposed and control conditions

Dependent variable: Y			
Source	DF	Sum of squares	Mean square
Model	1	4.40037418	4.40037418
Error	6	91.59962582	15.26660430
Corrected total	7	96.00000000	

Table 9. The variance analysis of the effect of polynomial coefficients and idle time on the voltage measured across the skin for the exposed and control conditions

Dependent variable: Y			
Source	DF	Sum of squares	Mean square
Model	5	38.40758675	7.68151735
Error	2	57.59341325	28.79620663
Corrected total	7	96.00000000	

Table 10. The variance analysis of the effect of idle time on the voltage measured across the skin for the exposed and control conditions

Dependent variable: Y			
Source	DF	Sum of squares	Mean square
Model	1	4.40037418	4.40037418
Error	6	91.59962582	15.26660430
Corrected total	7	96.00000000	

started with MF exposure and Figures 37 and 39 are from the experiments that started without MF exposure.

The t-test of the area contrasts between area intervals number 2, 3 and 4 was performed using each SCC and voltage curve. The results are shown in Table 11.

Table 11. t values of the area contrasts

	When the experiment started with the MF exposure	When the experiment started without the MF exposure
Voltage across the skin	8.12	0.58
Short circuit current	16.93	0.24

The tabulated t value for the first column is 2.776 (with 95% significance level and degree of freedom of 4) and for the second column is 2.6 (with 95% significance level and degree of freedom of 5).

Figure 36. Responses of the short circuit currents of isolated frog skins when the experiments started with perpendicular magnetic fields exposure. MF indicates the intervals when the magnetic field was on

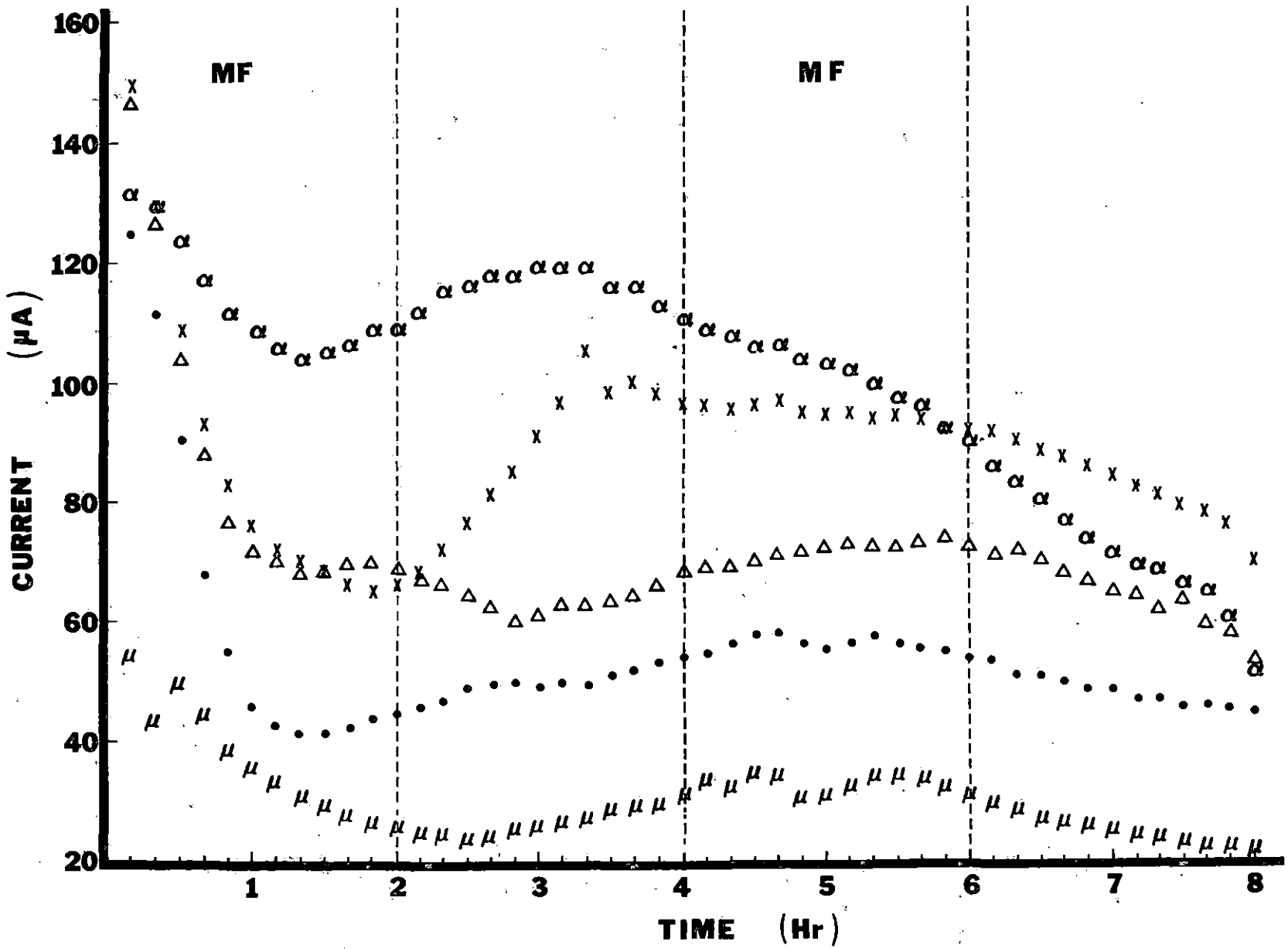

MF
MF
160
140
120
100
80
60
40
20
CURRENT (μA)
1
2
3
4
5
6
7
8
TIME (Hr)

Figure 37. Responses of the short circuit currents of isolated frog skins when the experiments started without magnetic field exposure. MF indicates the intervals when the magnetic field was on

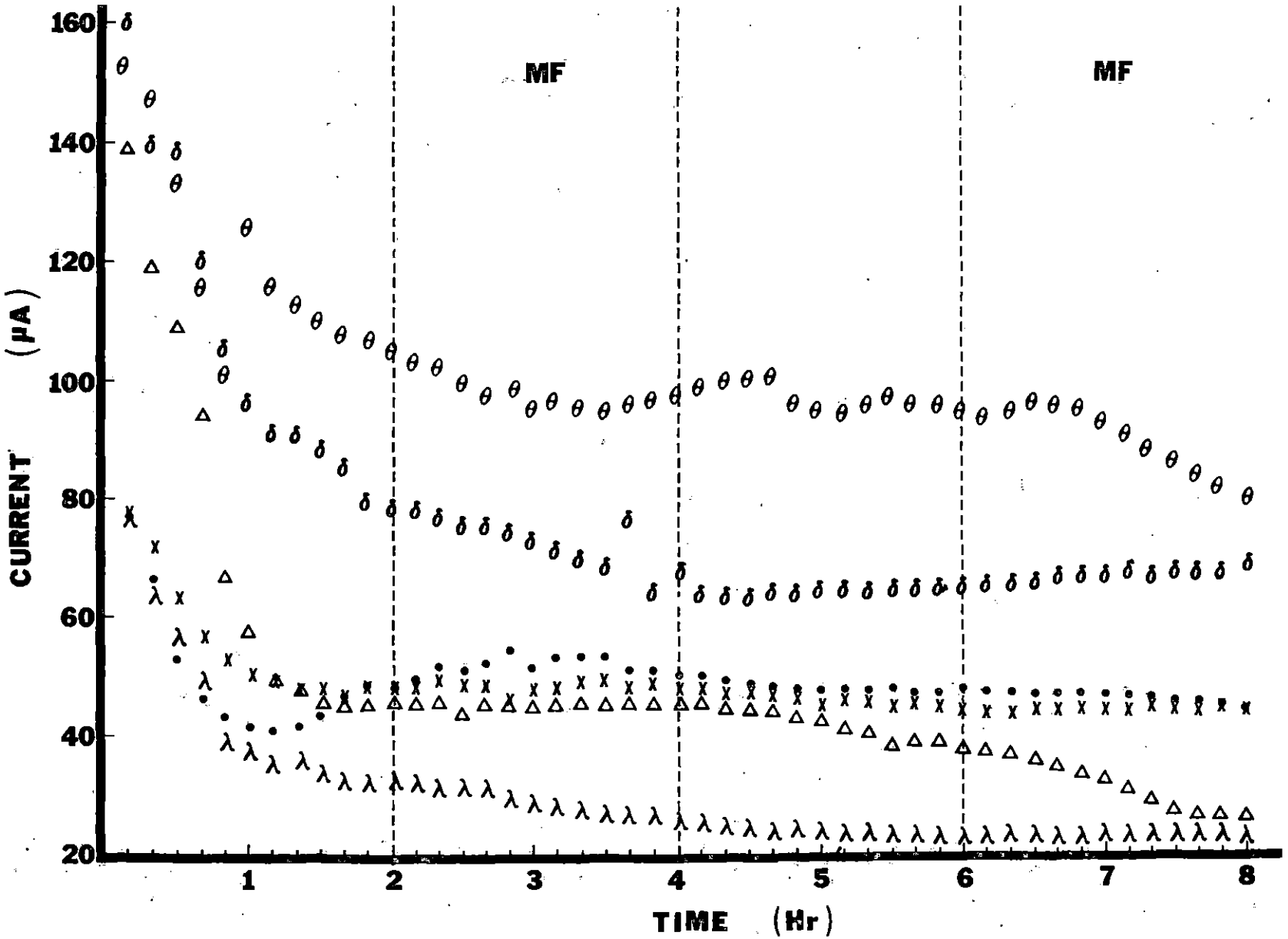

MF
MF
CURRENT (μA)
TIME (Hr)
160
140
120
100
80
60
40
20
1
2
3
4
5
6
7
8

Figure 38. Responses of the potential across the isolated frog skins, when the experiments started with magnetic field exposure. MF indicates the intervals when the magnetic field was on

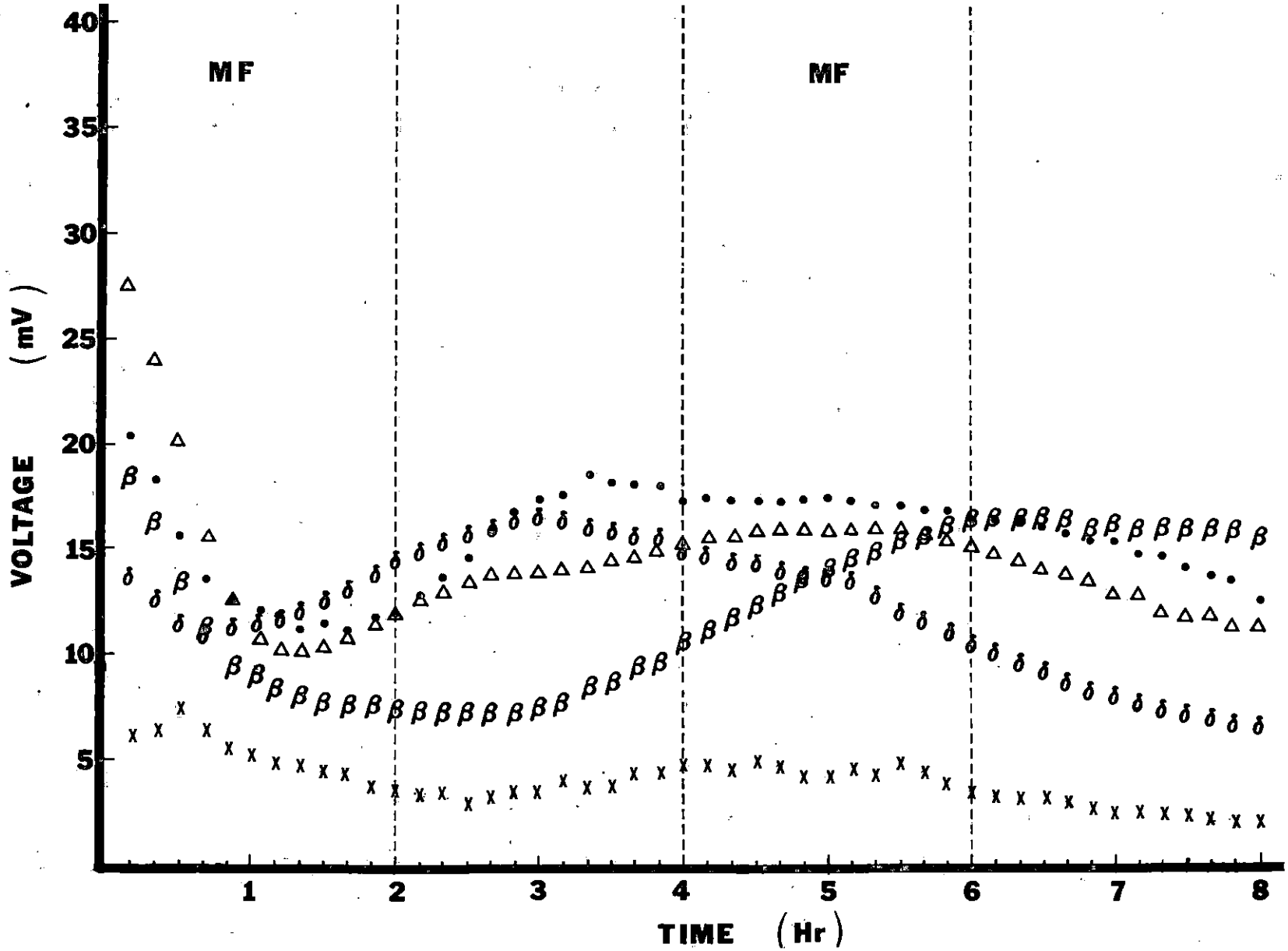
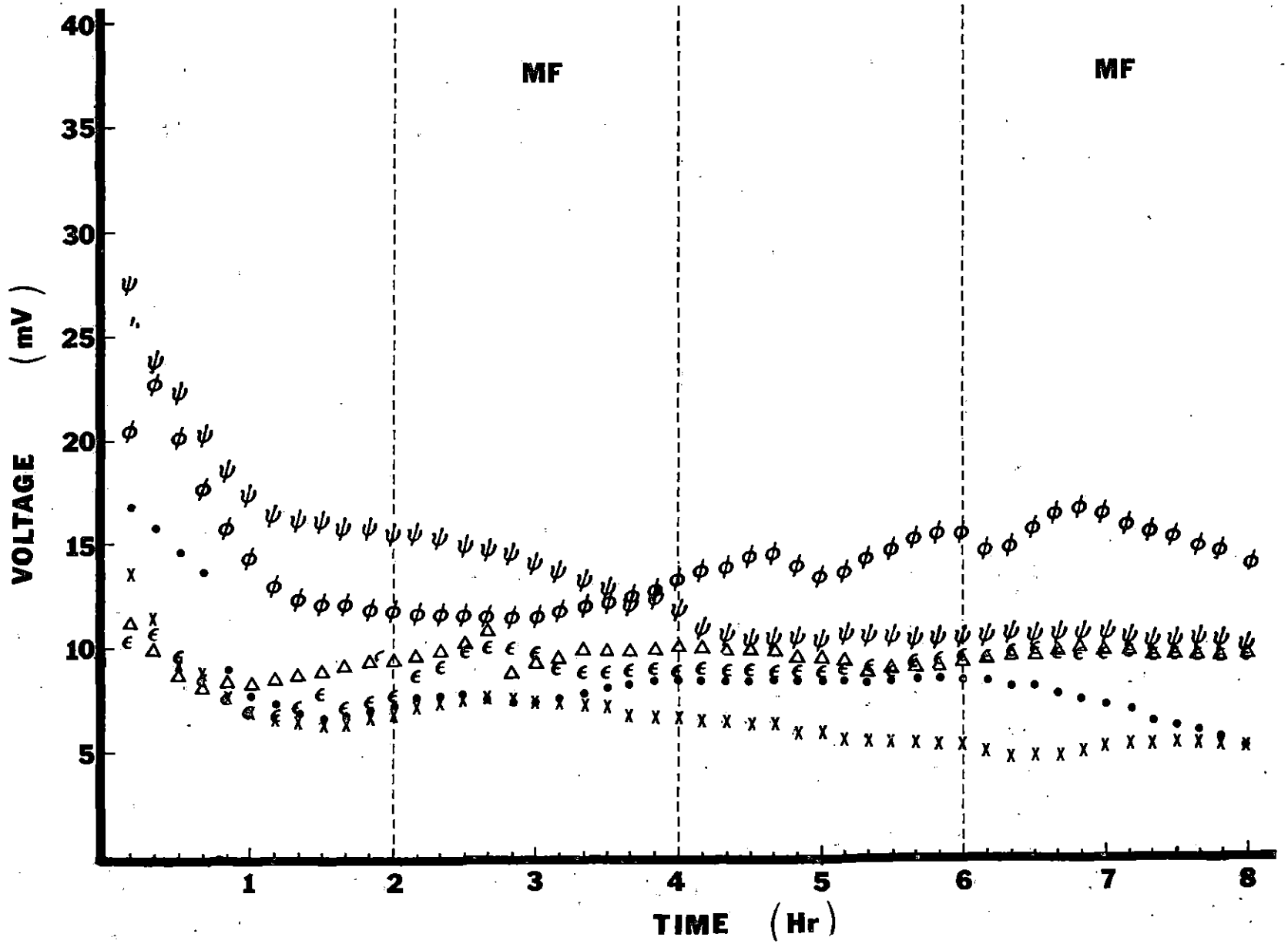

MF
MF
VOLTAGE (mV)
TIME (Hr)
40
35
30
25
20
15
10
5
1
2
3
4
5
6
7
8

Figure 39. Responses of the potential across the isolated frog skins when the experiments started without magnetic field exposure. MF indicates the intervals when the magnetic field was on


MF
MF
VOLTAGE (mV)
TIME (Hr)
40
35
30
25
20
15
10
5
1
2
3
4
5
6
7
8

When the calculated t-test values were compared with tabulated t values, the results showed that the area contrast between the area intervals number 2, 3 and 4 was different in experiments that started with the MF exposure. But the area relation was not different when the experiments started without a MF. These observations suggested the possibility that the perpendicular MF had an effect on the skin behavior. To check the significance of the difference between the two treatments, Individual Curvature Analyses tables were arranged for SCC and potential difference. F values were calculated from the variance analyses of these tables. This is shown in Table 12 (F=17.20) and 13 (F=24.6). comparing the calculated F values with tabulated F value (F=13.62) showed that the difference between the experiments that started with MF exposure and experiments that started without MF exposure was at 99.5% significance level.

Table 12. The variance analysis of the area contrast for short circuit current

Dependent variable: Y

Source	DF	Sum of squares	Mean square
Model	1	216.64134377	216.64134377
Error	9	113.35865623	12.59540625
Corrected total	10	330.00000000	

Table 13. The variance analysis of the area contrast for potential difference measured across the skin

Dependent variable: Y			
Source	DF	Sum of squares	Mean square
Model	1	241.65316135	241.65316135
Error	9	88.34683865	9.81631541
Corrected total	10	330.00000000	

Discussion

When an IFS was located inside the chamber with the same Ringer solution on both sides, there was a potential difference and a net current across the skin. These have been reported (Ussing, 1949; Ussing and Zerahn, 1951) to result from active transport. The measured voltage and short circuit current declined in time and this has been explained in terms of an efflux process resulting from concentration and voltage gradients, an influx of Cl^- ions, as well as a decrease in the energy available for active transport (Huf, 1935; Levi and Ussing, 1949; Ussing and Zerahn, 1951).

When IFSs were exposed to the perpendicular MF, the second, third and fourth intervals of the current and voltage curves showed greater fluctuation in the experiments that started with exposure to the MF than in experiments that started without exposure to the MF. This suggested that the effects of the MF needed time to be expressed. This idea was strengthened by the results that the second intervals for those experiments that started without MF exposure generally do not show an increase

in voltage and short circuit current, while the second intervals for those experiments which started with MF exposure do show an increase. It is possible that perpendicular MF caused reorientation of a small portion of molecules in the IFSs and this orientation allowed a change in permeability. Changes in permeability could alter ion concentrations and consequently the activity of the pump. It is possible that the pump activity was increased by: (1) a changed ion concentration on the inside side of the IFSs, or (2) a changed ion concentration inside the cells of the IFSs. For the first possibility, if Na^+ permeability increased, the efflux of ions would increase and the pump would be stimulated to maintain the $[Na^+]$ on the inside side of the skins at the previous level. For the second possibility, the intracellular $[Na^+]$ increased as a result of the increase in permeability and this stimulated the pump to maintain the previous internal ion concentration.

In the experiments that started with exposure of IFSs to the perpendicular MF, the permeability increased in the first interval; therefore, the sodium pump worked harder and consequently there was an increase in the voltage across the skin and in the SCC in the second interval. On the other hand, in the same experiments but in the third and fourth intervals, the MF caused an increased permeability but the sodium pump did not have the energy to respond; therefore, the voltage across the skin and the SCC dropped.

In the experiments that started without exposure to the perpendicular MF, when the MF was on during the second interval, the voltage or current did not increase significantly in the third interval but they were main-

tained. This again suggested that the MF required time to produce effects. The maintained level instead of an increase in the third interval could be explained by decreased energy for the pump.

In spite of a strong electrostatic bond (6000-10000 V/cm) between two molecules in a biological membrane (Abashin and Yevtushenko, 1978), Bresler (1978) reported that

> . . . up to 5 percent of organic substances have a temperature region of the liquid crystalline state. [For more information, see Appendix.] And what is most important, the lipids of which the biomembranes consist precisely belong to substances forming smectic liquid crystalline states at temperatures at which living organisms find themselves. . . . On the other hand, investigation of liquid crystals by irreproachable physical methods has shown that they, as diamagnetics, are oriented by the MFs of medium strength (of the order of several thousands of oe) and form ideally oriented layers. Since into the membranes are incorporated and orientated in space, thanks to their hydrophilic properties, many of the most important enzymes, for example, respiratory, ATPase and also protein carriers of ions and different water soluble metabolites, then it is quite understandable that a change in the orientation of the molecules of lipids will be significantly reflected in the orientation and activity of the enzymes and transport carriers.

It is possible that some of the phenomenon described by Bresler (1978) occurred in the IFSs under the perpendicular MF.

The results from the perpendicular MF experiments confirmed those of Muller and Jitariu (1969) who reported an increase in the pump activity of IFSs from frogs that had been exposed to an inhomogeneous AMF (50 Hz and 250 to 300 G). They concluded that the increase in the active transport might be because of the MF having a direct effect on the cells by changing various processes in the frog skin cells or that it might be because the MF brought about its effect through the nervous system or through hormones. Since the MF exposures reported in this thesis were done on the

isolated skin, effects on the nervous system or hormones can be ignored, leaving only the possibility of effects on processes in the skin cells.

In another report, Bianchi et al. (1963) claimed that active transport decreased when the perpendicular MF intensity increased. They measured the SCC in the presence of a permanent MF and reported that the SCC decreased when they switched from a 250 G MF with 22 mm separation between the two magnet poles to a 650 G MF with 1 mm separation between the two magnet poles. However, by using this procedure to increase the MF intensity, they automatically decreased the effective surface through which the induced current could pass. Consequently, a smaller amount of current was needed to shunt the voltage across the skin in the 650 G MF experiment than in the 250 G MF experiment. Therefore, the decreased SCC was not necessarily due to changed IFS behavior under the MF, but could have been due to a change in the experimental design.

The IFS results that were presented in this thesis may suggest that diamagnetic materials are located across the skin. When the IFSs were exposed to the inhomogeneous perpendicular MF, the diamagnetic materials tried to oriented themselves to receive the least effect of the MF (that is, perpendicular to the MF). The orientation of diamagnetic materials inside the skin would not be complete because of the influence of the other forces in the skin. When the IFSs were exposed to an inhomogeneous parallel MF, the diamagnetic materials did not show any displacement because they were already oriented perpendicular to the MF; therefore, the frog skins did not change behavior when exposed to the parallel MF.

SUMMARY

Important characteristics of a MF are its ability to impose a force on a moving charged particle or to produce a torque on a magnetic dipole[1] (Lee, 1970; Williams, 1931). Biological substances exposed to a MF need time to respond; the required time depends on the molecules involved and environmental conditions in the exposed biological substance. The observed effects might be different in different tissues or in the same tissues from different species. Also, a MF can create heat; the amount of heat produced is directly related to the MF strength. The reports reviewed in previous sections showed that a MF can affect biological systems, but its effects are dependent on the MF strength and direction as well as the structure of the exposed biological tissue. Specifically, it was found that: (1) an earthworm nerve cord exposed to an AMF (111-237 r.m.s G, 60 Hz) or a CMF (310-612 G) was inactivated; this inactivation was probably due to heat generated by the MF, (2) no action potentials were generated in earthworm nerve cords by the AMF or CMF, (3) no significant change in action potential threshold level was observed during MF exposure, (4) MFs (54 G) parallel to IFSs did not have a significant effect on permeability or active transport, (5) MFs (54 G) perpendicular to IFSs have a significant effect on IFS active transport, probably as a result of permeability changes.

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[1] Magnetic dipole refers to any MF which has two different poles. The poles in an atomic orbital result from a nonuniform movement of electrons.

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APPENDIX: LIQUID CRYSTALS

The term liquid crystals designates a state of matter that is intermediate between the solid crystalline and the liquid phases. Liquid crystals are true liquids which have surface tension and the ability to flow and adopt the shape of their containers, yet they have some of the optical properties of crystals and exhibit anisotropic properties as do solid crystals. Since the name liquid crystals is sometimes misleading because the substances are neither liquids nor crystals, they also are called mesophases or mesomorphic phases. Liquid crystals (mesophases) are of two major types: the thermotropic and the lyotropic.

The thermotropic mesophase state is dependent on the temperature (Priestley et al., 1975); they are formed when the temperature is varied (Meier et al., 1975). Materials showing thermotropic liquid crystal phases are usually organic substances with their molecular structures classified in three different categories: smectic, nematic and cholesteric. The smectic structure is stratified with the molecules arranged in layers with their long axes parallel to each other. The molecules can move in two directions in the plane and they can rotate about one axis. In the nematic structure, the molecules maintain a parallel or nearly parallel arrangement to each other. They are mobile in three directions and can rotate about one axis. The cholesteric liquid crystals are mostly derivatives of cholesterol and their molecules pack in layers about 2000 Å thick. Although most of the molecules in the cholesteric state are essentially flat, side chains project upward from the plane of each molecule with some hydrogen atoms extending below. Thus

the direction of the long axis of a molecule in a given layer is slightly displaced from the direction of the axis in adjacent layers and this produces a helical structure. Brown et al. (1971) illustrated schematically the different categories of thermotropic mesophases as is shown in Figure 40.

The term lyotropic liquid crystallinity is used to describe the formation of a thermally stable system by the penetration of a solvent between the molecules of a crystal lattice. Lyotropic liquid crystals are biologically important for they can contain two or more components (for example, lipid-water, lipid-water-protein systems) of which water is an integral part. Lyotropic liquid crystals are obtained by dispersing one compound with another compound and they are rod-like structures usually larger than typical thermotropic mesophases. A schematic presentation of a number of lyotropic liquid crystalline structures prepared with amphiphilic lipids and water is shown in Figure 41 (Brown and Wolken, 1979).

Mishra (1975) showed that many proteins, nucleic acids, lipids and polysaccharides in water exhibit liquid crystalline structures; these are shown in Table 14. Brown and Wolken (1979) reported that chitin, polynucleotides, polypeptides, proteins, DNA and transfer RNA exhibit cholesteric liquid crystalline structures.

Liquid crystals are mentioned here because of their great participation in biological structures, especially in membranes. They possess both mobility and structural order. Brown and Wolken (1979) and Brown et al. (1971) described the structural state of a liquid crystal as very sensi-

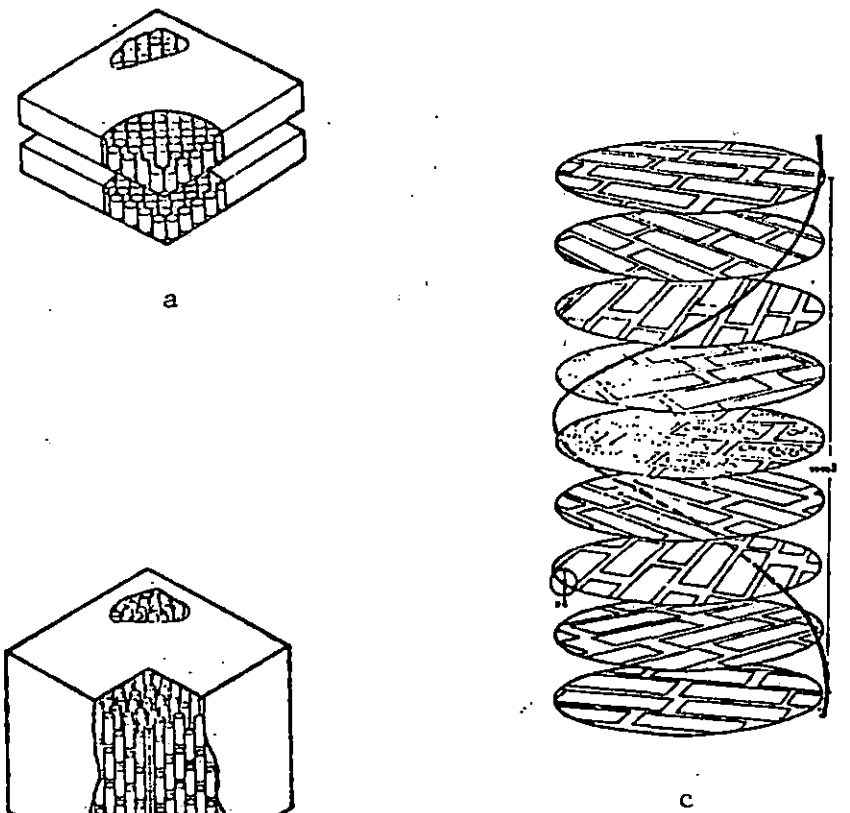

Figure 40. A schematic illustration of the three different categories of thermotropic mesophase molecular arrangements

a: Smetic structure
b: Nematic structure
c: Cholesteric structure

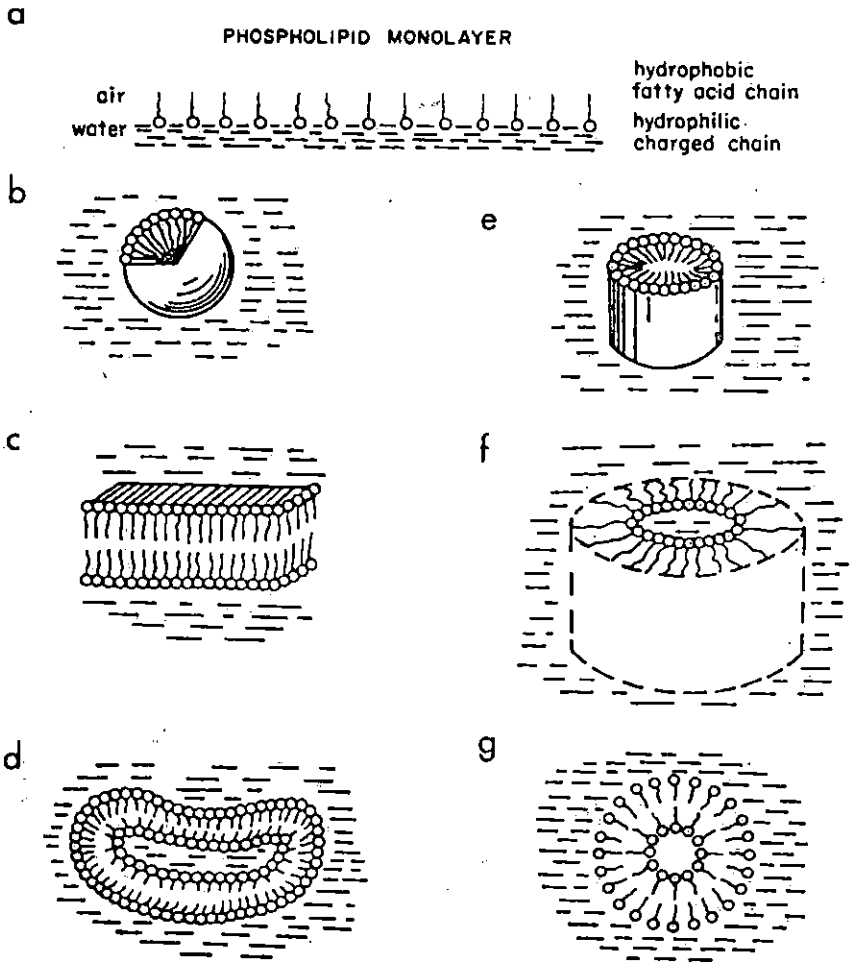

Figure 41. Schematic patterns representing lyotropic mesomorphism of amphiphilic lipids. (a) Monolayer at the air-water interface; (b) spherical particle; (c) lamellar phase in water; (d) lamellar phase: section through a sonicated liposome; (e) hexagonal phase 1 (oil in water); (f) hexagonal phase II (water in oil); (g) cross-section of cylinder with a bilayer packing. The circles represent the polar heads of the lipid molecules

tive to external forces as well as boundary conditions. Many external stimuli affect their physical and optical properties. These external stimuli are: light, sound, mechanical pressure, temperature, electric and magnetic fields, as well as changes in the chemical environment.

Table 14. Molecules in living structures claimed to exhibit liquid crystalline properties

Lipids
Lecithin
Sphingomyelin
Cephalin
Monoglycerides
Cholesterol esters
Various phospholipids
Proteins and polypeptides
Myosin
Hemoglobin
Trypsin
Poly-γ-benzyl-L-glutamate
Poly-γ-methyl-L-glutamate
Poly-γ-ethyl-D-glutamate
Poly-β-benzyl-L-aspartate
Poly-α-L-glutamic acid
Poly-α-L-glutamic acid
Poly-L-lysine hydrochloride
Nucleic acids
Deoxyribonucleic acid (DNA)
Ribonucleic acid (RNA)
Polysaccharides
Chitin