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SUMMARY PAGE

THE PROBLEM

Becker reported a reduced growth rate of Staphylococcus aureus in a magnetic field environment lower than the geomagnetic field. A confirmation of this observation is of fundamental importance with respect to the propagation of life outside the magnetosphere of the Earth.

FINDINGS

The observation could not be confirmed. No significant differences were noted between the growth of S. aureus in the geomagnetic field and in a field reduced by a factor of 1000. Pigmentation, mannitol fermentation, gelatinase activity, coagulase production, and catalase activity were also not influenced by the low magnetic field.

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INTRODUCTION

During the last decade the singularity of the magnetosphere of the Earth has been established by numerous space probes. The geomagnetic field dwindles from a value of about 50,000 gamma (0.5 gauss) on the Earth's surface to less than 10 gamma at a distance from the surface of the Earth of about 10 Earth radii. Moon and Mars have been found to be free of an intrinsic magnetic field, and recently during the Apollo 12 Moon landing a field of less than 40 gamma was measured on the surface of the Moon. The question arises as to whether life which has developed on Earth in the nearly continuous presence of a magnetic field can be exposed without adverse effects for prolonged periods of time to the practically zero magnetic fields outside the magnetosphere of the Earth. Experimental work on the effects of near-zero magnetic field strength upon biological systems has recently been reviewed by Conley (4) who cited about a dozen original studies ranging from algae to man. In that review positive effects of the null magnetic field on biological material were quoted repeatedly, but none of the experiments had been duplicated by independent investigators nor were the results verified.

It is of special interest that positive effects of the low-intensity magnetic field environment have been observed at the cellular level of organization. Halpern (7) observed accelerated reproduction of Euglena and Chlorella in very low magnetic fields, and Becker (1) described a reduction in size and number of colonies of Staphylococcus aureus grown in a field reduced by shielding to 1/10 of the field strength of the geomagnetic field. Since such a decrease of growth caused by a small reduction of the geomagnetic field is striking, it appeared of interest to perform growth experiments with S. aureus in a field of about 1/1000 of the geomagnetic field, simulating conditions outside the magnetosphere of the Earth. In these experiments no significant differences were observed between the growth of S. aureus in the geomagnetic field and in a field below 50 gamma field strength.

PROCEDURE

EQUIPMENT

Room-sized Magnetic Shield Facility

The low field strength magnetic environment was provided in a shielded room originally erected for the exposure of human subjects to a null field (2). The facility resembled a shielded room (5) erected at the Jet Propulsion Laboratory, Pasadena, California, according to a principle originally developed by Patton and Fitch (9). The magnetic shield resembled a large box with a wall thickness of 1 foot and continuous Moly-permalloy cladding of the outside and inside as well as the doors of the lock used for entrance. The inside dimensions of the shielded room were 8 x 8 x 8 feet, and ample space was available for experimentation. All gear and personal belongings of the experimenter were checked to make sure that no ferromagnetic materials entered the room.

The field strength inside the shield was measured by a Hewlett-Packard probe (3529A) calibrated in a standard coil where each milliampere of energizing current corresponded to 1-gamma field strength in the center of the coil. The field strength of the room depended on the shielding factor of the facility and on induced remanence of the inner shield ("perming of inner shield"). This perming can be changed by moving an electromagnet judiciously over the inner wall. In the present experiments the field strength at the location of the experiment was 47 gamma with a gradient of 2.5 gamma/foot. The polarity of the field inside the room was NNE with 30-degree inclination.

Provisions for Constant Temperature

Two special nonferromagnetic incubators were constructed, one for housing of the experiment inside the shielded room and one for the control in the geomagnetic field. The incubators were double-walled copper cylinders (radius 6 inches, height 11 inches) with double-walled covers placed within wooden containers (17" x 17" x 14"), insulated with glass wool. A Lauda Constant-Temperature Circulator, Model WB-20/R, was used to circulate water through both incubators, including the covers. The same length of hose connected the circulator to each incubator. The temperature inside the incubators was held at 37° C ± 0.1° C and was measured by a Yellow Springs Instrument Company Thermistor Probe No. 402. Sanborn Model 760-53 Calibrated Temperature Bridges, Sanborn Carrier Amplifiers Model 350-1100B, Esterline-Angus Graphic Ammeters Model AW, and Esterline-Angus Recorders were used in recording of the measurements. The temperature held constant for 96 hours and no significant variation between the two incubators was observed. The temperature dropped only briefly by about 1° C during opening of the incubators for sampling.

MATERIALS

The organism was Staphylococcus aureus DA No. 2208-8 which was gelatinase+, coagulase+, pigmented, fermented glucose and mannitol, and phage Type 80 (courtesy B. Pittman, National Communicable Disease Center, Atlanta, Ga.).

The media and solutions were made up of the following: Difco Brain Heart Infusion (BHI) broth twice filtered through Gelman Metricel GA-6 filters, pore size 0.45μ before sterilization. Difco BHI agar. Staphylococcus 110 agar (Difco). Sodium chloride 0.85% (filtered as above). Bromcresol purple (Fisher) indicator. Saturated ammonium sulfate solution. Difco Bacto-Coagulase Plasma. Phosphate buffered formaldehyde (18.5%). H_2O_2 3%.

METHOD

A slant of BHI agar was streaked from a stock culture of S. aureus 2208-8 and incubated overnight. A small amount of this growth was emulsified in sterile 0.85% NaCl solution and used to inoculate 50 ml of sterile BHI broth in a 250-ml flask. This suspension was in turn incubated for 16 hours. Five-ml portions of the resulting cell

suspension were used to inoculate each of four flasks containing 400 ml of BHI broth. The inoculum was thoroughly dispersed in the BHI broth by swirling of the flasks. Two 10-ml samples were taken from each of the four flasks and used as the zero-hour samples. Two of the flasks were placed in the control incubator and the other two in the incubator within the shielded room. Two additional 10-ml samples were taken from each of the flasks at 2, 4, 6, 8, 10, 12, 14, 24, 48, 76, and 96 hours after initiation of the cultures. The flasks were swirled each time prior to sampling to distribute the cells evenly. The samples were transferred to sterile, stoppered spectrophotometer tubes. One ml was removed from each tube immediately, diluted and plated. Two-tenths ml of buffered formaldehyde were added to the remaining suspension to stop the growth. These solutions were stored in a refrigerator for subsequent optical density and Coulter Counter analysis.

Measurement of Growth

Three methods of measuring the bacterial growth were used: 1) Light transmission measurements were performed at 660 mμ using Coleman 19 x 150 mm tubes with the Coleman Junior Spectrophotometer. 2) Bacteria in suspension were counted using a Coulter Counter Model F with a 30μ aperture and 50μ manometer, sensitivity settings of 0.500 and 8.0, and thresholds of 5 or 7; counts were made with appropriate dilutions of the samples. 3) The numbers of colony forming units (CFU) were determined by the pour plate method; the colonies appearing on BHI agar after 24 to 48 hours at 37° C incubation were counted.

Growth Characteristics on Staphylococcus 110 Agar

In addition to these counting procedures other growth characteristics of Staphylococcus aureus were investigated. An overnight culture of S. aureus 2208-8 was diluted and 1-ml amounts of the resulting suspension spread uniformly over the surface of Staphylococcus 110 agar plates. After incubation for 48 hours under control and experimental conditions the resulting isolated colonies were examined for size, pigmentation, mannitol fermentation, and gelatinase activity. A drop of bromcresol purple indicator was added to the area from which a colony was removed to detect the fermentation of mannitol, indicated by a change in the color of the indicator. Gelatinase activity was detected by flooding the plates with 5 ml of a saturated solution of ammonium sulphate. The activity was estimated by the cleared zones around the colonies after standing 10 minutes at 37° C.

Coagulase production was tested by adding 0.1 ml of a 48-hour BHI broth culture to 0.5 ml of Difco Bacto-Coagulase Plasma and incubating at 37° C. The tubes were read at 30 minutes, 1 and 2 hours. Catalase activity was determined by the method of Janzen and Cook (8). One ml of a 48-hour BHI cell suspension and 0.5 ml of 3% H_2O_2 were incubated in a syringe assembly at 37° C. The volume of oxygen released was determined after 20 minutes' incubation. A mixture of 1-ml buffer and 0.5 ml 3% H_2O_2 served as control.

Since the growth experiments of Becker (1) were performed at room temperature with no provisions for temperature control, the following experiments were also conducted at room temperature (about 26° C). An overnight culture of S. aureus 2208-8 was diluted and used for making pour and streak plates on BHI agar and on Staphylococcus 110 agar medium, each time in duplicate. The plates were divided into two groups and placed inside cardboard boxes to exclude light; one box was placed inside the shielded room and the other one on a table outside the room. Using Becker's procedure, we examined the plates for numbers and size of colonies after 72 hours.

RESULTS

In three different experiments separated by several months the growth curves of samples in and outside the shielded room were found not to differ significantly. This was true for the results of all three methods of measurement. Figure 1 presents characteristic growth curves obtained from one of these experiments when growth was measured by determining the numbers of colony forming units and per cent transmittance.

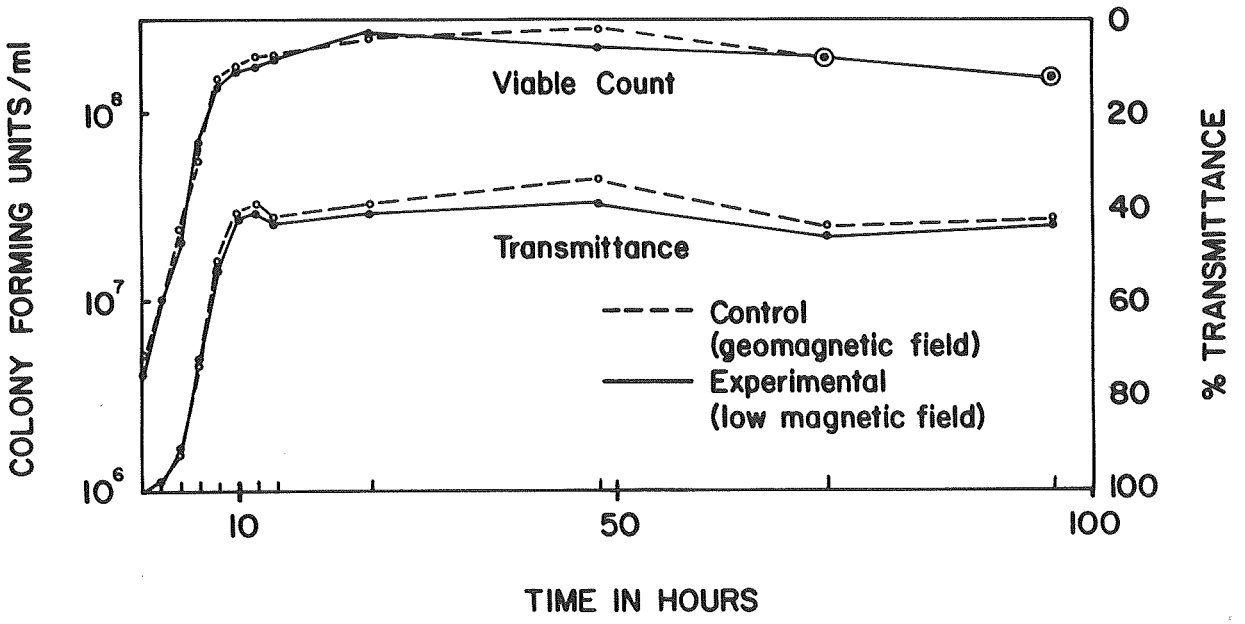

Figure 1

Growth Curves Comparing Numbers of Colony Forming Units and Per Cent Transmittance of Cultures Grown in Low Magnetic Field and in Geomagnetic Field

S. aureus 2208-8 colonies grown on Staphylococcus 110 medium for 48 hours in the shielded room were similar to their counterparts grown in the geomagnetic field: well-pigmented, fermented mannitol with a positive gelatinase reaction. There was no noticeable difference in appearance or size of the colonies or change of other characteristics between control and experimental colonies.

No differences between the control and experimental cultures was noticed in coagulase and catalase activities.

Pour and streak cultures on BHI and on Staphylococcus 110 agar media exposed for 72 hours to the low field environment without temperature control did not differ significantly from control cultures in number or size of the colonies.

DISCUSSION

The negative findings of this report contrast with Becker's preliminary findings (1) of a fifteen-fold reduction in number of colonies as well as some reduction in size of colonies of S. aureus in a magnetic field reduced in field strength by a factor of 10 compared with the geomagnetic field. Becker's findings have been cited repeatedly in review articles (3, 4) without mention of their preliminary nature, which may leave the impression of well-documented observations indicating positive bioeffects of low magnetic fields. At present, no satisfactory explanation for the considerable variance in results of seemingly similar experiments can be given, but the following differences in experimental procedures may be pointed out.

Different strains of Staphylococcus aureus were used in the two experiments. While the strain of the present study, S. aureus DA No. 2208-8 (NCDC, Atlanta, Ga.), is well characterized, the strain of Becker's original experiments could not be identified beyond the fact that it had a moderate growth rate at room temperature (1). It appears unlikely that a difference in strain could cause the marked difference in growth at low magnetic fields.

The magnetic field strength in the two approaches was not the same. Becker (1) reported both average field strength and magnitude of fluctuation to be reduced by a factor of 10. However, in personal communication he has stated that the resultant field diminution was not measured. The field applied in the experiments of the present study was well characterized in field strength, gradient, and vector (47 gamma, 2.5 gamma/foot gradient, and pointed NNE with 30-degree inclination) and is probably lower than the field used by Becker by a factor of 10 to 100. If the absence of a magnetic field is the determining factor for biological changes, greater effects should have been observed in the present study with its lower fields. It appears unlikely that a "window" of effective field strength exists at 1/10 geomagnetic field. The negative results speak strongly for complete absence of a "null" magnetic field effect on the growth of S. aureus.

Becker's experiments were apparently not performed at regulated temperature. The "room temperature" may have differed somewhat at the location of the shielded and the control samples. A special attempt was made in the present study to keep the temperature constant within 0.1° C during the total course of the experiment and to keep control and experimental samples at the same temperature (37° C). A slight temperature differential between control and experimental samples in Becker's experiments might explain a difference in colony size, but would not adequately explain the marked reduction of colony number observed. The temperature may have played a role in still another respect. While all of Becker's experiments were performed at "room temperature," the results of all experiments in the present study with one exception were recorded at higher temperature (37° C), the optimum growth temperature for S. aureus. The rate of growth and therefore the duration of the period of exponential growth of a bacterial culture is proportional to temperature between the minimum and optimum growth temperatures. The exponential phase of growth, the period most likely to be sensitive to the magnetic field, would be longer at lower temperatures, and the extended period of sensitivity might result in greater effects. Since no effects at all were found at 37° C, one growth experiment was performed at "room temperature." No effects were observed at this lower temperature either.

A last possible explanation for the difference in observations may result from errors in procedure. The plating procedure used by Becker has a large (about 10%) margin of error, but even such an error could not explain his results.

No other bacteriological systems have been studied in low magnetic fields; however, some lower plant and animal systems have been observed in very low magnetic fields. Halpern (7) found the reproduction of Euglena and Chlorella accelerated in fields below 100 gamma. The growth of Paramecium was accelerated under similar conditions. Gibson, Isquith, and Goodman (6) observed a growth enhancement in the protozoan Blepharisma intermedium in a magnetic field 1/500th of the geomagnetic field. These two studies indicate that there may be a biological effect of null magnetic fields on primitive animals and plant-like organisms. A repetition of these experiments would, however, be well advised before final conclusions are drawn.

It seems reasonable to conclude that effects of low magnetic fields on lower forms of life cannot be ruled out completely, but such effects cannot be demonstrated easily.

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No significant differences were observed between the growth of S. aureus in the geomagnetic field and in a magnetic field with a field strength reduced by a factor of 1000. Pigmentation, mannitol fermentation, gelatinase activity, coagulase production, and catalase activity were also not influenced by the low magnetic field. The results are discussed in their relation to previous findings of R. O. Becker who observed a reduction of growth rate in fields of lower field strength than the geomagnetic field.

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