ml) were detected in approximately 0.75 ml of medium removed from around the trophoblastic outgrowths of embryo 1 between 216 and 288 hours after insemination. If we assume that there were 500 trophoblastic cells in this outgrowth, then each cell produced 4 mIU of β-hCG per 24 hours during this period; in vivo, each human trophoblastic cell produces 14 mIU on day 10 and 0.7 mIU on day 16 (*7, 8*).

If plasma volume in an average female is 1600 ml, then the concentration of plasma β-hCG from the outgrowths of embryo 1 in vitro at 288 hours would have been 3.8 mIU/ml, assuming no loss in vivo. This concentration is much lower than expected; levels between 10 and 30 mIU/ml have been detected in plasma 8 days after ovulation (*9*). Growth obviously was arrested during development in vitro, affecting metabolism and secretion. The amount of hCG detected in the culture media may be less than the amounts secreted. Proteinases are produced by mammalian blastocysts (*10, 11*), and proteinaceous substances secreted by mouse blastocysts can be destroyed in the presence of embryos (*12*). More frequent sampling of the culture medium and the use of radiolabeled precursors to study the turnover of secreted products are necessary to accurately determine the amount of hCG synthesized by human embryos. At present, we do not know whether hCG is secreted by cytotrophoblasts or syncytioblasts in vitro.

The secretion of hCG by implanting embryos can be delayed during pregnancy. It was not detected in the urine of some patients until 16 or 17 days after ovulation (*13*)—much later than in most pregnancies. This delay could arise through an arrest at implantation or a delay in the synthesis of hCG. Several of these patients have already delivered healthy babies.

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References and Notes

1. R. B. Heap, A. P. Flint, J. E. Gadsby, *Br. Med. Bull.* **35**, 129 (1979).
2. R. G. Edwards, *Conception in the Human Female* (Academic Press, London, 1980).
3. S. B. Fishel, in *Human in Vitro Fertilization and Early Embryonic Development* (Bari Congress, SIS, Rome, in press).
4. E. T. Ross, *Ciba Found. Symp. No. 64* (1979), pp. 191–201.
5. R. G. Edwards and J. M. Purdy, *Human Conception in Vitro* (Academic Press, London, 1982).
6. R. G. Edwards, in *The Use of Non-Human Primates in Research on Human Reproduction*, E. Diczfalusy and C. C. Standley, Eds. (Karolinska Institutet, Stockholm, 1972), pp. 131–134.
7. G. D. Braunstein, J. Rasor, D. Adler, H. Danzer, M. E. Wade, *Am. J. Obstet. Gynecol.* **126**, 678 (1976).
8. K. J. Catt, M. L. Dufau, J. L. Vaitukaitis, *J. Clin. Endocrinol. Metab.* **40**, 537 (1975).
9. A. G. Ahmed and A. Klopper, *Br. J. Obstet. Gynaecol.* **90**, 604 (1983).
10. D. Dabich and T. J. Andary, *Fertil. Steril.* **25**, 954 (1974).
11. H.-W. Denker and U. Petzoldt, *Cytobiologie Z. Exp. Zellforsch.* **15**, 363 (1977).
12. S. B. Fishel, unpublished manuscript.
13. ———, R. G. Edwards, J. M. Purdy, in *Fertilization of the Human Egg in Vitro: Biological Basis and Clinical Application*, H. M. Beier and H. R. Lindner, Eds. (Springer-Verlag, Berlin, 1983), pp. 251–269.
14. We thank P. C. Steptoe and J. Webster for their help during this study.

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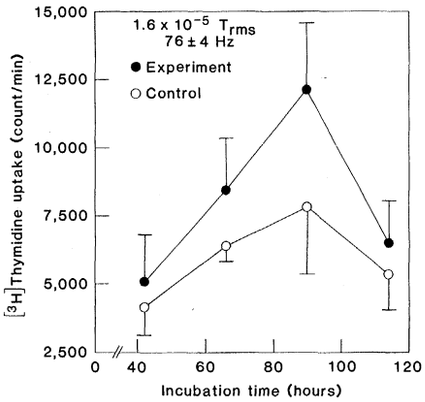
Time-Varying Magnetic Fields: Effect on DNA Synthesis

Abstract. *Human fibroblasts have exhibited enhanced DNA synthesis when exposed to sinusoidally varying magnetic fields for a wide range of frequencies (15 hertz to 4 kilohertz) and amplitudes (2.3×10^{-6} to 5.6×10^{-4} tesla). This effect, which is at maximum during the middle of the S phase of the cell cycle, appears to be independent of the time derivative of the magnetic field, suggesting an underlying mechanism other than Faraday's law. The threshold is estimated to be between 0.5×10^{-5} and 2.5×10^{-5} tesla per second. These results bring into question the allegedly specific magnetic wave shapes now used in therapeutic devices for bone nonunion. The range of magnetic field amplitudes tested encompass the geomagnetic field, suggesting the possibility of mutagenic interactions directly arising from short-term changes in the earth's field.*

Most studies of the effect of static magnetic fields on living systems have yielded negative or inconsistent results (*1*); the exception is studies of species that incorporate ferromagnetic materials as geomagnetic sensory elements (*2*). Experiments on time-varying magnetic fields have been fewer and more difficult to interpret (*3*). A therapeutic technique for accelerating repair in bone nonunions that subjects the site to a time-varying magnetic field (*4*) has achieved a measure of acceptance in the clinical community (*5*). However, the mechanism underlying this procudure is still largely unexplained, in part because the narrow and repetitive pulse shape of the wave form, allegedly specific to the therapy, makes it difficult to perform and interpret in vitro cellular experiments (*6*).

We sought to determine the effect of sinusoidally varying magnetic fields on DNA synthesis in cell culture, particularly the frequency and intensity response, if any. A pair of matched incubators were fitted with modified Helmholtz coils (0.5 m, inside diameter; 0.25 m long), providing a horizontal magnetic field with an active field uniformity no less than 3 parts out of 17. Either could be an experimental or control (no field) unit. Periodically the roles were switched and, at other times, both were used simultaneously as controls. An au-



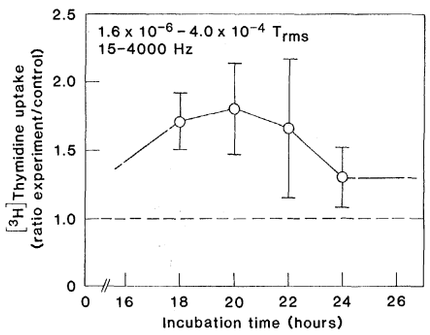


Fig. 1 (left). Tritiated thymidine uptake (mean counts per minute ± standard error) in human foreskin fibroblastic cells exposed to a magnetic field of amplitude 2.3×10^{-5} T oscillating at 76 ± 4 Hz in the horizontal plane (experiment) and uptake in unexposed cells (control). Mean levels differ at $P = 0.0001$, as determined from the pooled variances. The ambient 60-Hz magnetic field in the area of the incubators was $\lesssim 1 \times 10^{-7}$ T_{rms}. Note that the approximate intensity of the geomagnetic field is 5×10^{-5} T. Fig. 2 (right). Mean ratios and standard errors of [^{3}H]thymidine uptake in exposed cells to that in control cells for ten different combinations of frequencies and fields. The dotted line indicates the expected response if there is no effect due to a magnetic field. The peak at 20 hours corresponds to the middle of the S phase of the cell cycle. The dashed lines below 18 and above 24 hours represent the expected, but inexact trends, in these data.

dio amplifier fed by a function generator supplied 0 to 1.0 root-mean-square ampere to either coil, enabling us to generate magnetic fields up to 1.0×10^{-3} root-mean-square tesla (T_{rms}) at frequencies ranging from 15 Hz to 20 kHz.

Two independent series of experiments were completed, both with human embryonic foreskin fibroblasts (*7*) as the test cell line.

In the first series, one frequency (76 ± 4 Hz) and one intensity (1.6×10^{-5} T_{rms}) were used for all runs. Cells were seeded into eight 96-well culture plates (*8*) at densities of approximately 1.0×10^6 cells per milliliter, and the plates equally, but randomly, distributed between the control and experimental incubators. Following exposures of 24, 48, 72, and 96 hours, [^{3}H]thymidine (*9*) was added to each well and the plates returned to their original incubators for 18 hours, after which all cells were washed twice in isotonic saline and collected on a filter strip by an automated sample harvester (*10*). The strips were dried, and the individual filter disks corresponding to each well were counted in a liquid scintillation counter. In 45 out of 48 paired plates, the amount of incorporated thymidine was higher for cells exposed to the magnetic field (Fig. 1).

In the second set of experiments, the cell cultures in the experimental incubator were subjected to various frequencies between 15 Hz and 4 kHz and to intensities between 1.6×10^{-6} and 4×10^{-4} T_{rms}. Tritiated thymidine was added to the cell medium at the time of seeding. The initial inoculation density was limited to 5×10^4 cells per milliliter. Paired (24-well) plates (*11*) were removed from both incubators at various times during a 24-hour period. Cell exposure was limited to 24 hours following inoculation to enhance cell synchrony. Selection of plates was not random as it was in the first experiment; seeding and harvesting of these cells were arranged beforehand. Corresponding wells in both plates were sequentially seeded, collected, and compared. In this manner, a fundamental data unit for a given frequency, intensity, and exposure time consisted of the ratio of thymidine uptake in an experimental well to that for a matched control. Averages of these units for all such pairs provided uptake ratios. Preliminary trials indicated that a peak in this ratio occurred after about 20 hours of exposure. Subsequent data collection was then restricted to four exposure times: 18, 20, 22, and 24 hours (Fig. 2).

In separate experiments to determine cell growth as a function of time, the peak in uptake was found to correspond to the middle of the S phase of the cell cycle. Although the enhancement of thymidine uptake was, on average, no greater than 80 percent (Fig. 2), ratios two and three times this occasionally resulted in individual experiments. Figure 3 shows, for two separate exposure times, the relative uptake as a function of the product of frequency ν and root-mean-square field strength B (B_{rms}); this product is directly proportional to the time derivative of the magnetic field. As such, the slopes of the lines plotted will test whether Faraday's law is implicated in the results.

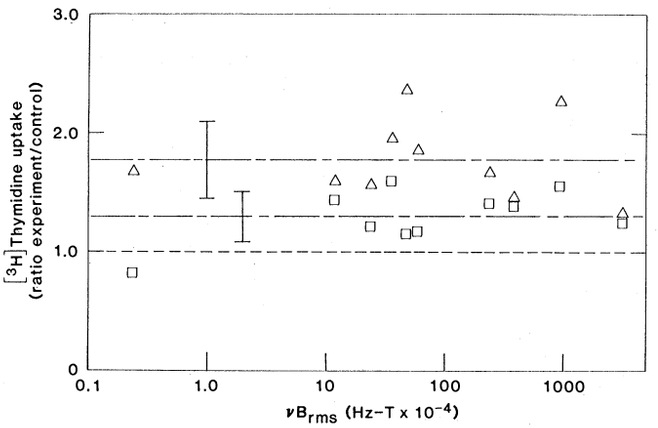


Fig. 3. The mean ratios (from Fig. 2) as a function of the product of frequency and magnetic field intensity. Exposure times of 20 hours (△) and 24 hours (□) are shown. Note the apparent lack of variation in response for four orders of magnitude, indicating that this interaction is independent of the time derivative of magnetic field, $\partial B/\partial t$. The two upper lines represent the mean levels for the 20- and 24-hour exposures, respectively.

These experiments show that there is an interaction, albeit a subtle one (the results from Fig. 2 represent approximately 100 trials), between time-varying magnetic fields and DNA synthesis. Inasmuch as our fields varied sinusoidally, some measure of doubt is introduced as to whether the particular wave shape now used to accelerate bone fusion is indeed as necessary as has been claimed. An alternative, though not a likely one, is that two separate cellular interactions occur at low-frequency magnetic fields.

Although it is reasonable to suggest that Faraday's law is involved in inducing extracellular and intracellular currents according to $\nabla \times \mathbf{E} = -\partial \mathbf{B}/\partial t$ (where $\mathbf{E}$ is the induced electric field and t the time), it should then follow that the observed response will scale as $|\partial \mathbf{B}/\partial t| = 2\pi\nu B$. The lack of such a response (Fig. 3) may mean that induced eddy currents do not play a role in this phenomenon, or it may indicate that the effect is a saturable phenomenon—for example, either a configurational effect or a self-limiting shift in the onset of the S phase.

Our most interesting result relates to the magnitude of the magnetic intensities that were studied. The lowest level of field strength that we used is at or below the intensity of the geomagnetic field (approximately 5×10^{-5} T). In surveying the experimental area, it was found (*12*) that the 60-Hz ambient field is only two orders of magnitude lower. We estimate the threshold for this effect to be $\nu B_{rms} \simeq 5$ to 25 μT/sec, close to the value reported (~ 10 μT/sec) (*13*) as interfering with normal chick embryo development. Therefore, care should be taken when studying cell culture that there are no perturbations from local 60-Hz magnetic fields, such as those resulting from fans, motors, and fluorescent lights in close proximity to the culture.

Finally, we can also remark on the potentially mutagenic response that may accompany this phenomenon, if indeed chromosome replication is affected. This would constitute a new type of mutational force and perhaps could be used to explain, in a rather direct fashion, the interrupted speciation accompanying geomagnetic reversals (*14*).

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References and Notes

1. M. F. Barnothy, Ed., *Biological Effects of Magnetic Fields* (Plenum, New York, 1964 and 1969); vols. 1 and 2; I. L. Silver and C. A. Tobias, *Space Radiation Biology and Related Topics*, C. A. Tobias and P. Todd, Eds. (Academic Press, New York, 1974); G. I. Malinin, W. D. Gregory, L. Morelli, V. K. Sharma, J. C. Houck, *Science* **194**, 844 (1976); R. J. Newman, P. D. Wilkinson, G. D. W. Curtis, J. Clair, M. J. O. Francis, *IRCS Med. Sci. Biochem.* **10**, 606 (1982).
2. R. B. Frankel, R. P. Blakemore, R. S. Wolfe, *Science* **203**, 1355 (1979); C. Walcott, J. L. Gould, J. L. Kirschvink, *ibid.* **205**, 1027 (1979); J. L. Gould, J. L. Kirschvink, K. S. Deffeyes, *ibid.* **201**, 1026 (1978).
3. T. Huzella, *Arch. Exp. Zellforsch. (Jena)* **15**, 250 (1934); M. T. Marron, E. M. Goodman, B. Greenebaum, *Nature (London)* **254**, 66 (1975).
4. C. A. L. Bassett, R. J. Pawluk, A. A. Pilla, *Ann. N.Y. Acad. Sci.* **238**, 242 (1974); *Clin. Orthop.* **124**, 128 (1977); J. Watson, *IEEE Proc.* **128**, 329 (1981), part A.

5. C. A. L. Bassett, S. N. Mitchell, S. R. Gaston, *J. Am. Med. Assoc.* **247**, 623 (1982); editorial comment in *ibid.*, p. 669; J. S. Kort, *et al.*, *Clin. Orthop. Relat. Res.* **165**, 124 (1982); M. L. Sutcliffe and A. A. J. Goldberg, *ibid.* **166**, 45 (1982).
6. G. A. Rodan, L. A. Bourret, L. A. Norton, *Science* **199**, 690 (1978); L. A. Norton, *Clin. Orthop. Relat. Res.* **167**, 280 (1982); R. A. Luben, C. D. Cain, M. C. Chen, D. M. Rosen, W. R. Adey, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4180 (1982); R. Goodman, C. A. L. Bassett, A. S. Hendersen, *Science* **220**, 1283 (1983).
7. Cell line HFS-15, HEM Research, Inc., Rockville, Md. (catalog number 4-404).
8. Costar, Cambridge, Mass.; seeding in 0.02 ml of Delbecco's minimum essential medium (DMEM) plus 10 percent fetal calf serum (FCS) (Gibco, Grand Island, N.Y.) per well; incubation at 36.5° ± 0.4°C, 5.0 ± 0.1 percent CO_2, and 95 percent air.
9. New England Nuclear, Boston, Mass. (1 μCi per well).
10. R. J. Hartzman, M. L. Bach, F. H. Bach, G. B. Thurman, K. W. Sell, *Cell. Immunol.* **4**, 182 (1972).
11. Costar, Cambridge, Mass. Seeding in 1.0 ml DMEM plus 10 percent FCS per well; incubation at 36.5° ± 0.4°C, 5.0 ± 0.1 percent CO_2, 95 percent air.
12. These measurements were made by J. Gauger (personal communication).
13. J. M. R. Delgado, J. Leal, J. L. Monteagudo, M. G. Gracia, *J. Anat.* **134**, 533 (1982).
14. J. D. Hays, *Geol. Soc. Am. Bull.* **82**, 2433 (1971).
15. We acknowledge the technical assistance of C. Wintling and A. Alicea, as well as advice from L. Homer. This research was supported by the Naval Medical Research and Development Command Research Work Unit 3M161102BS10.BA.436 and MR0410100-5.0004.
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Cardiac Atria of BIO 14.6 Hamsters Are Deficient in Natriuretic Factor

Abstract. *The hearts of 220-day-old hamsters of the BIO 14.6 strain are deficient in atrial natriuretic factor; saline extracts of atria produce one-third the natriuretic and diuretic effects of extracts of atria from age-matched normal hamsters. BIO 14.6 hamsters are known to develop congestive heart failure with edema when they are about 200 days old, and the venous congestion and edema are preventable by parabiosis with normal hamsters. The humoral mediator, the deficiency of which causes venous congestion and edema in BIO 14.6 hamsters, may be atrial natriuretic factor.*

The BIO 14.6 strain of Syrian hamsters is subject to hereditary cardiomyopathy (*1–3*). The cardiac degeneration results in decreased mechanical myocardial performance at all ages (*2*). BIO 14.6 hamsters develop congestive heart failure with cardiac dilation and edema by about 200 days of age (*1–3*). Parabiosis of BIO 14.6 hamsters with normal hamsters prevents the development of venous congestion, heart failure, and edema but does not prevent the myocardial degeneration (*1*). The life-span of the parabiotic BIO 14.6 hamsters is nearly tripled (*1*). These observations imply that BIO 14.6 hamsters are deficient in a humoral mediator, which is present in normal hamsters and transmissible to BIO 14.6 hamsters. They also imply that the humoral mediator increases the ability of the BIO 14.6 animals to excrete salt and water; that is, the congestive heart failure and edema are not the result of cardiomyopathy per se, but, rather, the result of a deficient ability to excrete salt and water.

Exogenous aldosterone causes increased renal sodium reabsorption in normal human subjects (*4*). The kidneys escape from the influence of aldosterone within a few days, however, and sodium excretion returns to normal (*4*). Aldosterone also causes increased sodium reabsorption from the tubular fluid in sweat glands, but sweat glands do not escape (*4, 5*). Thus renal escape is not part of a general tachyphylaxis to aldosterone; rather, renal escape seems to represent activation of another mechanism that overrides the renal effect of aldosterone. The escape phenomenon has caused an intense search for a salt-losing hormone (*6*). But renal escape from the influence of aldosterone does not always occur, particularly not in human congestive heart failure (*7*). Human patients with congestive heart failure have elevated aldosterone concentrations and retain sodium. It is possible that the edema and extracellular fluid volume expansion of congestive heart failure reflect a deficiency of the normal natriuretic-diuretic system, which accounts for the failure of the kidneys to escape from the influence of aldosterone.

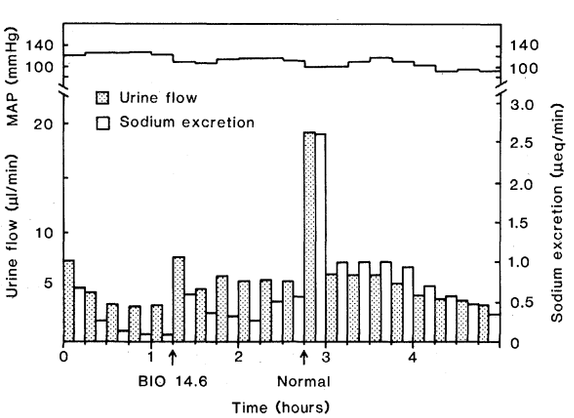

Fig. 1. Urine flow, sodium excretion, and mean arterial pressure in one assay rat during administration of BIO 14.6 and normal atrial extracts from pair 3 shown in Table 1. Each column represents the mean value for a 15-minute collection period. All collection periods are shown through the termination of the assay. Abbreviation: *MAP*, mean arterial pressure. The extracts (0.2 ml) were injected intravenously at times indicated by the arrows.

Since 1961 there has been a quest for a natriuretic hormone that could increase the renal excretion of salt and water (*6*). Natriuretic material (NM) has been extracted from the blood and urine of a variety of species under a variety of circumstances (*6, 8*). Typically the concentration of NM is increased when the volume of blood or extracellular fluid is expanded experimentally (*6, 9*). However, no known tissue origin or physiological role has been found for this natriuretic substance in blood and urine (*8*).

In 1956 Kisch (*10*) and in 1964 Jamieson and Palade (*11*) described specific (protein) secretory granules in mammalian cardiac myocytes in the atria, but not the ventricles. Twenty-five years passed before the observation was made that saline extracts of mammalian atria, but not ventricles, produce potent natriuretic and diuretic effects when injected intravenously (*12, 13*). This material—atrial natriuretic factor (ANF) (*14, 15*)—is not identical to NM extracted from blood and urine: (i) ANF has a molecular weight of about 5000 (*16*), and NM may be less than 1000 (*8*); (ii) ANF is sensitive to protease (*15, 17, 18*), whereas NM may be relatively insensitive (*8*); and (iii) ANF does not inhibit Na^+- and K^+-dependent adenosine triphosphatase (*18*), but NM does (*8*). Still, they may be part of the same system, much as renin, angiotensin II, and aldosterone are parts of a system for salt and water conservation (*19*).

We sought to determine whether BIO 14.6 hamsters are deficient in ANF. We speculated that failure to produce sufficient ANF by the degenerating hearts of BIO 14.6 hamsters is the cause of inadequate renal sodium and water excretion and of the syndrome of congestive heart failure.

Fourteen BIO 14.6 hamsters 220 days old were sorted into seven pairs. Each pair was then paired with two age-