tively, a large pool of proto-*onc* genes may exist but are not readily available for transduction. Readily transducible proto-*onc* genes might be those that are active in many cells at many stages and thus available for retroviral integration or recombination as, for example, genes required for mitosis or basic cellular functions. Other proto-*onc* genes may rarely be activated and thus not readily transduced by retroviruses.

The close relation between sequences of viral *onc* genes and cellular proto-*onc* genes does not imply that cellular proto-*onc* genes are cancer genes. It is emphasized that the *mht-raf*, the *fps-fes*, the *fgr-yes*, and probably the *sis*, *abl*, and *ras* homologies described above encompass only a fraction of the respective viral *onc* genes and proto-*onc* genes (*1*). It is likely that the sequences shared by related viral *onc* genes and the respective proto-*onc* genes encode a shared functional domain. The virus-specific sequences of viral *onc* genes may provide functions required for transformation, whereas the cell-specific sequences of proto-*onc* genes may provide functions essential for normal cells. Furthermore, the virus-specific *onc* gene elements of viruses carrying related *onc* genes appear to affect the oncogenic potential of the viruses. Examples are the different transformation capabilities of MH2 and MSV 3611 and the different transformation capabilities of Abelson murine leukemia virus (which primarily causes acute leukemias) and HZ2-FeSV (which is only known to cause sarcomas) (*11*, *23*).

We conclude that (i) the number of cellular proto-*onc* genes is limited because MH2 and MSV 3611, as well as several other viruses of different taxonomic groups, have transduced the same *onc* gene sequences from different cell species, and (ii) specific deletions and linkage of the same proto-*onc* sequences to different retroviral vector elements affect the oncogenic potential of the resulting viruses.

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24. Supported by NIH grant CA11426 (to P.H.D.). Complete sequence analysis of MSV 3611 was completed by G. Mark and U. Rapp. After submission of this report we received a preprint of a paper by M. W. Jansen, R. Lurz, K. Bister, T. Bonner, G. E. Mark, and U. Rapp in which homology between MSV 3611 and MH2 is shown by molecular hybridization.
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Human Chorionic Gonadotropin Secreted by Preimplantation Embryos Cultured in vitro

Abstract. *Human oocytes were collected by laparoscopy and fertilized and cultured in vitro. Human chorionic gonadotropin was detected in the medium surrounding two embryos cultured for more than 7 days after fertilization.*

Human chorionic gonadotropin (hCG) is a widely studied embryonic secretion, but its specific function and the stage of embryonic development when its secretion begins are unknown (*1–3*). It has been found in plasma and urine as early as 6 to 9 days after conception (*2–4*), that is, very soon after attachment of the blastocyst to the endometrium (implantation). We now report the detection of β-hCG in culture fluids surrounding preimplantation human embryos after fertilization and culture in vitro.

Oocytes were obtained from two patients undergoing in vitro fertilization for the treatment of infertility. The embryos were "surplus" to the number replaced in the mother. The ethical procedures for such embryos recommended by the Bourn Hall Ethical Committee are similar to those that were issued by the Royal College of Obstetricians and Gynaecologists in 1982. In the current procedure a maximum of three embryos are replaced in the mother, and the others are observed for their growth in vitro. In our study the embryos were periodically transferred to fresh medium and the spent culture medium was used for analysis.

The first patient was 32 years old and had undergone bilateral salpingectomy. She was treated with 50 mg of clomiphene citrate on days 2 to 6 after her last menstrual period and with 5000 IU of hCG (Pregnyl, Organon) on day 11 to induce follicular maturation. The second patient was 34 years old and had an occluded left fallopian tube; her right fallopian tube was absent. She was treated with 100 mg of clomiphene citrate on days 2 to 6 after her last menstrual period and with two ampoules of human menopausal gonadotropin (Pergonal, Serono) daily from days 2 to 9 of her cycle. Each ampoule is reported to contain 75 IU of follicle-stimulating hormone and 75 IU of luteinizing hormone. She was then given 5000 IU of hCG on day 10 of the cycle. Laparoscopy was performed 34 hours later and an oocyte was collected from each of the six follicles observed.

Recovery of oocytes by laparoscopy, fertilization in vitro, and culture of cleaving embryos have been described in detail by Edwards and Purdy (*5*). The oocytes were aspirated from their follicles 33 and 34 hours after the injection of hCG into patients 1 and 2, respectively, and were fertilized in ~ 0.1-ml droplets of culture medium held beneath liquid paraffin (mineral oil) (British Drug

Houses). The culture medium was Earle's culture medium modified by the addition of sodium pyruvate, penicillin, and 8 percent (by volume) heat-inactivated maternal serum. The gas phase was 5 percent CO_2, 5 percent O_2, and 90 percent N_2. Oocytes from patients 1 and 2 were exposed 37 and 36 hours after laparoscopy to 1.6×10^5 and 1.4×10^5 spermatozoa per milliliter of medium, respectively. Surrounding cumulus cells were removed from the oocytes on the morning after they were exposed to spermatozoa and the fertilized eggs were transferred to droplets of medium identical to that described above but containing 15 percent maternal serum. This medium was used for the remaining period in culture.

Many embryos growing in prolonged culture fail to "hatch" from the zona pellucida. To avert this problem the zona pellucida was removed at the blastocyst stage by incubating the embryo from patient 1 in acid Tyrode's solution (*p*H 2.0) for 25 seconds and the embryo from patient 2 in acid Earle's solution (*p*H 2.15) for 1 minute 47 seconds. Both embryos were immediately washed extensively in culture medium containing 15 percent maternal serum. The embryo from patient 1 was then transferred in a droplet containing modified Earle's solution with 15 percent maternal serum and the embryo from patient 2 was treated in the same manner, except the concentration of serum was 25 percent. The transfer procedure was repeated several times over succeeding days, with fluid from the "spent" droplet being carefully collected. These fluids were stored at −20°C until they were assayed for β-hCG (Amersham Amerlex β-hCG radioimmunoassay kit). The medium used for control assays was Earle's medium containing 15 or 25 percent maternal serum and was incubated for the same period of time in the same petri dish as the medium containing the embryos. The standards for the radioimmunoassay kit were calibrated against the second international standard for hCG (61/6). The coefficient of variation ranged from 4.0 to 7.2 percent in the assays and from 4.6 to 8.8 percent between assays.

A single embryo from each patient was cultured in vitro for several days. The development of each embryo is shown in Table 1. After removal of the zona pellucida both embryos expanded, with embryo 1 attaching to the surface of the petri dish between 189 and 216 hours post-fertilization. Its organization was lost as outgrowths, mostly trophoblastic, formed. Culture was continued for a further 100 hours before the outgrowths were fixed for histological analysis. When embryo 2 was at the eight-cell stage it was used to test the method of replacing embryos into their mothers, as practiced at the Bourn Hall Clinic. It was transferred into Earle's medium containing 75 percent maternal serum and loaded into a replacement catheter for 14 minutes before being replaced in Earle's medium containing 15 percent serum for further culture. These manipulations did not interfere with the continued growth of the embryo; it formed a blastocyst and expanded, but became degenerate by 197 hours after insemination.

We did not detect β-hCG in the culture medium or in fluid removed from around each embryo 110 to 120 hours after insemination (Table 2) (*6*). Small amounts were found in fluid removed at 170 and 197 hours from around embryos 1 and 2, respectively. Large amounts (6033 mIU/

Table 1. Development of embryos 1 and 2.

Time (hours)	Stage of development	Comment
	Embryo 1	
0	Insemination	
50	Eight-cell	
54	Eight-cell	
76	Sixteen-cell	
100	Early compaction	
110	Compacted	
126*	Early blastocyst	
147	Blastocyst	Fully expanded
170	Blastocyst	Fully expanded with large secretory trophoblast cells
189	Blastocyst	Dense inner cell mass, elliptical, endoderm
216	Attached to petri dish	Large inner cell mass, reduced blastocoelic cavity
246	Trophoblast outgrowth	Fibroblastic cells migrating from inner cell mass
266	Trophoblast outgrowth	Outgrowth more extensive
288	Trophoblast outgrowth	Extensive trophoblast outgrowth
317	Trophoblast outgrowth	Difficult to judge development; tissue collapsed, fixed for analysis
	Embryo 2	
0	Insemination	
39	Four-cell	
61	Eight-cell	
68	Eight-cell	Transferred to 75 percent serum and replacement catheter
84	Twelve-cell	
94	More than 16 cells	
110	Compacted	
120	Early blastocyst	Small vesicles and blastocyst cavity forming
148	Blastocyst	Fully expanded
155*	Blastocyst	Fully expanded, very thin zona pellucida; transferred to 25 percent serum
170	Blastocyst	Distinct inner cell mass, possibly endoderm
197	Collapsed	Degenerate tissue

*Zona pellucida removed.

Table 2. Detection of β-hCG in the medium surrounding human embryos cultured in vitro. N.D., not detected.

Sample	Stage of development	Number of hours after insemination	hCG (mIU/ml)
Control 1			N.D.
Medium surrounding embryo 1	Compacted	110	N.D.
Medium surrounding embryo 1	Blastocyst	170	25
Medium surrounding embryo 1	Blastocyst	216	43
Medium surrounding embryo 1	Trophoblast outgrowth	288	6033
Control 2*			N.D.
Control 3†			N.D.
Medium surrounding embryo 2	Early blastocyst	120	N.D.
Medium surrounding embryo 2	Collapsed blastocyst	197	50

*Earle's medium containing 15 percent maternal serum. †Earle's medium containing 25 percent patient 2 serum.

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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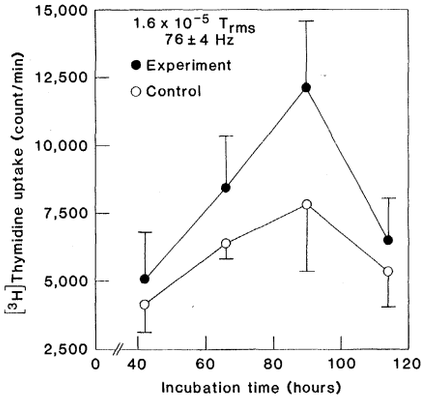
Time-Varying Magnetic Fields: Effect on DNA Synthesis

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-



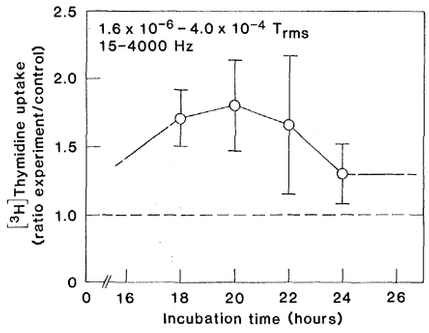


Fig. 1 (left). Tritiated thymidine uptake (mean counts per minute ± standard error) in human foreskin fibroblastic cells exposed to a magnetic field of amplitude 2.3×10^{-5} T oscillating at 76 ± 4 Hz in the horizontal plane (experiment) and uptake in unexposed cells (control). Mean levels differ at $P = 0.0001$, 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.