in the 0- to 307-μsec time regime after photolysis (Fig. 2a) has a ≈ 3-eV shift to lower energies relative to that for MbCO; this result indicates that at this time a significant amount of the photolyzed CO has not yet recombined with the Mb. As can be seen from the magnitude of the edge shift in Fig. 2b, a sizable fraction of the CO has still not recombined with the Mb. The first indication of near complete recombination is seen in Fig. 2c (the 563- to 1203-μsec time interval). The data collected during the fourth and fifth time intervals are identical (Fig. 2d), demonstrating complete recombination.

Attempts have been made to obtain time-resolved x-ray absorption spectra in the temporal regime that we have considered. These experiments (*5*) were not successful, and it was suggested that there may be accelerator instabilities that precluded such time-resolved experiments with storage rings. Our data on the flash photolysis and recombination of MbCO demonstrate the feasibility of such time-resolved techniques. We believe that these data represent the first x-ray absorption spectroscopic observation made with the use of synchrotron radiation of dynamic charge and structural redistribution on a time scale of much less than a millisecond. The ability to perform such experiments on this time scale, which is consistent with many biological and chemical processes, bodes well for the general application of such atom-selective time-resolved techniques for the determination of oxidation state and structural alterations in the active sites of proteins and other chemical systems. We see no inherent reason why this scale cannot be decreased by several orders of magnitude.

The Mb absorption edge results we have obtained so far are consistent with data obtained by other techniques (*2*) at room temperature. Thus, in terms of the edge shift, we observed a deoxy conformation immediately (0 to 307 μsec) after the laser flash. Our initial data also suggest that the pre-edge bound state transition (1*s*-3*d*) may be changing its position by ≈ 5 eV on a time scale shorter than the MbCO recombination time inferred from the shift in the edge position. Future experiments should be aimed at accurately determining the energy position of the near-edge peak in order to allow detailed comparisons with calculations. We also plan to extend these investigations to lower temperatures and higher solvent viscosities. This work not only will be considerably less demanding experimentally because of the slower time scales involved but also should provide further information on the intermediate states in MbCO recombination. Additional extensions of the technique to the region beyond the edge, in which fine structure is observed on the x-ray absorption spectrum, will allow transient structural studies of the active site to be made by extended x-ray absorption fine-structure analysis. These experiments should eventually yield important time-resolved information on the chemistry and interactions of the active site atoms and also dynamic structural information with a resolution of better than 0.05 Å.

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References and Notes

1. H. Winick and S. Doniach, Eds., *Synchrotron Radiation Research* (Plenum, New York, 1980); C. Kunz, Ed., *Topics in Current Physics—Synchrotron Radiation, Techniques and Applications* (Springer, New York, 1979).
2. R. H. Austin, K. W. Beeson, L. Eisenstein, H. Frauenfelder, J. C. Gunsalus, *Biochemistry* **14**, 5355 (1975); H. Frauenfelder, G. Petsko, D. Tsernoglou, *Nature (London)* **280**, 558 (1979); D. Beece *et al.*, *Biochemistry* **19**, 5147 (1980); J. C. Hanson and B. P. Schoenborn, *J. Mol. Biol.* **153**, 117 (1981); J. Friedman, D. Rousseau, M. R. Ondrias, *Annu. Rev. Phys. Chem.* **33**, 471 (1982); E. R. Henry, J. H. Sommer, J. Hofrichter, W. A. Eaton, *J. Mol. Biol.* **166**, 443 (1983).
3. D. M. Mills, V. Pollock, A. Lewis, A. Harootunian, J. Huang, in *Proceedings of the Third National Conference on Synchrotron Radiation Instrumentation* (North-Holland, Amsterdam, in press).
4. S. I. Salem, C. N. Chang, P. L. Lee, V. Severson, *J. Phys. C* **11**, 4085 (1978).
5. D. R. Sandstrom, S. C. Pyke, F. W. Lytle, *Stanford Synchrotron Radiat. Lab. Activities Rep. April 1980 to March 1981* (1981); *Stanford Synchrotron Radiat. Lab. Rep. 80/01* (1980).
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A Common *onc* Gene Sequence Transduced by Avian Carcinoma Virus MH2 and by Murine Sarcoma Virus 3611

Abstract. *A common cellular sequence was independently transduced by avian carcinoma virus MH2 (v-*mht*) and murine sarcoma virus (MSV) 3611 (v-*raf*). Comparison of the nucleotide sequences of v-*mht *and v-*raf *revealed a region of homology that extends over 969 nucleotides. The homology between the corresponding amino acids was about 95 percent with only 19 of 323 amino acids being different. With this example, 5 of the 19 known different viral* onc *genes have been observed in viruses of different taxonomic groups. These data indicate that (i) the number of cellular proto-*onc *genes is limited because, like other viruses of different taxonomic groups, MH2 and MSV 3611 have transduced the same* onc *gene–specific sequences from different cell species and (ii) that specific deletion and linkage of the same proto-*onc *sequences to different viral vector elements affect the oncogenic potential of the resulting viruses. The difference in transformation capabilities of MH2 and MSV 3611 serves as an example.*

The transforming *onc* genes of retroviruses consist either of sequences derived entirely from cellular genes, termed proto-*onc* genes, or more often from sequences derived from cellular and essential retroviral genes (*1*). Thus, proto-*onc* genes have obvious oncogenic potential as progenitors of viral *onc* genes. However, despite the presence of proto-*onc* genes in all vertebrate cells and despite the presence of retroviruses in many animal species, transduction of proto-*onc* sequences by retroviruses is a rare event. The probable reason is that, since sequence relationships are often lacking between essential retroviral and proto-*onc* sequences, nonhomologous recombination is necessary to transduce proto-*onc* sequences. It is now believed that proto-*onc* genes also have oncogenic potential on their own as a result of mutations, rearrangements, or enhanced expression (*1–4*). The risk of transformation for a given cell by a proto-*onc* gene would, in each of these cases, depend on the number of its proto-*onc* genes.

It is difficult to estimate the number of proto-*onc* genes, because retroviruses with *onc* genes are rarely isolated from tumors. Nevertheless, the following arguments suggest that the number of proto-*onc* genes is limited. (i) Transduction of proto-*onc* sequences by retroviruses appears to depend on nonhomologous recombination, and therefore any cellular sequence can be a candidate for transduction. Since only about 19 different *onc* genes have been found in retroviruses to date, the number of proto-*onc* genes appears to be small. (ii) Sequences of the same or very closely related proto-*onc* genes have been found in indepen-

dently isolated oncogenic retroviruses of the same taxonomic group (*3, 5*). The taxonomic group of retroviruses is structurally defined by retroviral *gag* genes (encoding structural core proteins) and other essential genetic elements of the transducing nondefective virus and functionally by the viral host range. Examples of related *onc* genes are the *myc* sequence shared by avian MC29, MH2, CMII, and OK10 viruses (*5*), the *amv* (or *myb*) sequence shared by avian myeloblastosis and E26 viruses (*6*), the *fps* sequence shared by avian Fujinami, PRCII and UR1 sarcoma viruses (*5*), the *fes* sequence shared by Snyder-Theilen (ST) and Gardner-Arnstein (GA) feline sarcoma viruses (FeSV) (*7*), and the *ras* sequence shared by Harvey (Ha), Kirsten (Ki), rat, and BALB sarcoma viruses (*8*). Since each of these viruses sharing a related *onc* gene has a different genetic structure, it is likely that these viruses were generated by independent transductional events. However, it may be argued that viruses of the same taxonomic group with related *onc* genes arose from a common precursor by deletions and rearrangements. Alternatively, it may be that a large pool of proto-*onc* genes exists but selective activation favors a few proto-*onc* genes for transduction. This activation may involve spe-

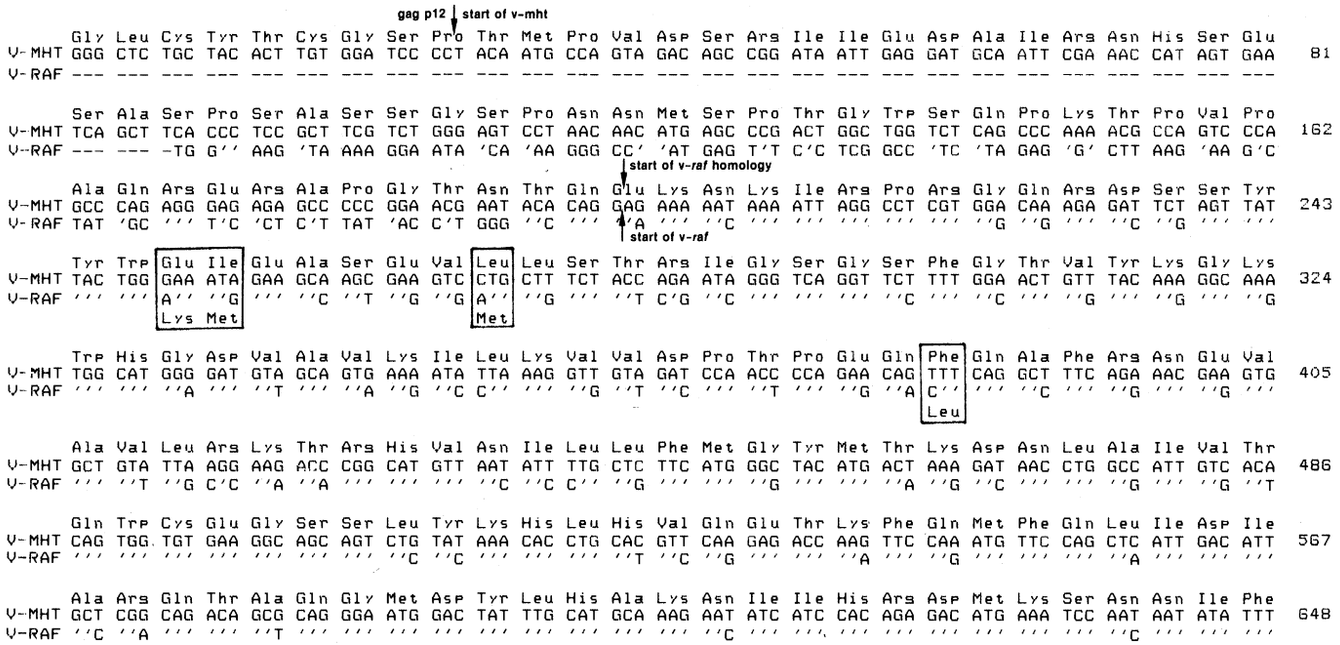

Fig. 1. Sequence homology between v-*mht* and v-*raf* genes. Dashes represent undetermined nucleotides, and, at positions 1150 to 1152, represent deletions; apostrophes represent identical nucleotides; and stars represent translation termination codons. The top line lists the amino acids deduced from the nucleotide sequence of v-*mht* gene. When different amino acids are encoded by v-*mht* and v-*raf* genes, the amino acid for v-*raf* is listed on the bottom line and the changes are shown in boxes.

cies-specific gene rearrangements or translocations (*4*). There are several examples that an *onc* sequence but none of the retroviral vector sequences is shared by viruses from different taxonomic groups. In these cases, independent transduction of the related *onc* sequence is almost certain because the RNA's of these retroviruses are not only very distantly related but they also have different host ranges (*3*). One example is the 3-kilobase (kb) *fps* sequence of avian Fujinami sarcoma virus of which about 1 kb is shared with the 1.5- and 1.2-kb *fes* sequences of ST- and GA-FeSV. The homology between *fps* and *fes* in this region has been estimated at 75 percent by sequence analysis (*7, 9*). In other cases, molecular hybridization has revealed that a part of the *abl* sequence of Abelson murine leukemia virus is shared with that of Hardy-Zuckerman 2 (HZ2) FeSV and that the *sis* sequence of simian sarcoma virus is shared with that of Parodi-Irgens (PI) FeSV (*10, 11*). The rat sarcoma virus (RaSV), which shares its retroviral vector elements with a rat leukemia virus and the *ras* sequence with Ha-, Ki-, and BALB-MSV's, also appears to be an example, because the vector elements of Ha-, Ki-, and BALB-MSV's are all derived from mouse leukemia viruses (*8, 12*). Nevertheless, preliminary sequence analyses indicate that the rat leukemia virus which probably transduced the *ras* sequence to generate RaSV is partially related to mouse leukemia viruses, in particular in the *gag* gene (*13*). Recently, 80 percent homology was reported between the specific sequence of *tgr* of Gardner-Rasheed feline sarcoma virus and the *yes* sequence of avian sarcoma virus Y73 (*13a*).

We report the sequence analysis of two newly discovered *onc* sequences that are shared by two viruses of different taxonomic groups—the avian carcinoma virus MH2 and the murine sarcoma virus MSV 3611. Although the two *onc* genes appear to be transduced from very different animal species (chicken and mouse) into very different vectors (avian and murine retroviral vectors), the homology between the two *onc* sequences is about 95 percent.

MH2 causes carcinomas, sarcomas, and acute leukemias in birds and transforms avian fibroblasts and hemopoietic cells in culture (*14, 15*). We have recently shown that MH2 contains two potential transforming genes or oncogenes. One is a 3.3-kb hybrid gene consisting of a partial retroviral *gag* gene and an MH2-specific sequence termed *mht* (*16, 17*). The other gene mostly consists of a 1.3-kb *myc* sequence that is shared with

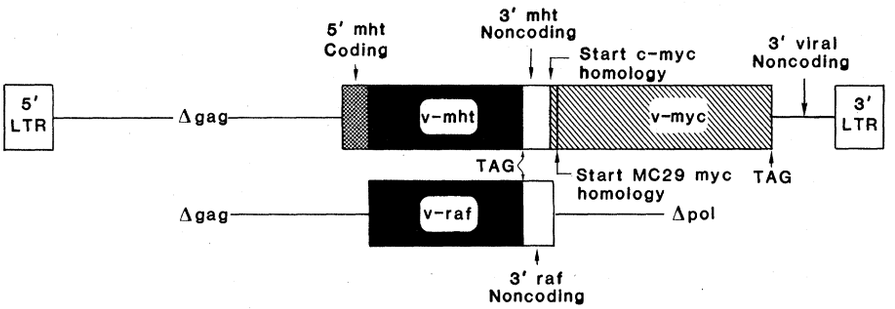

Fig. 2. Comparison of the genetic maps of v-*mht* and v-*raf* genes. The MH2 proviral genome is shown above the v-*raf* gene of MSV 3611. Boxes at both ends of MH2 represent long terminal repeats (LTR); boxes between the LTR's represent cell-derived sequences; and lines represent viral sequences. Solid areas indicate the highly homologous regions of v-*mht* and v-*raf* genes.

three other oncogenic avian retroviruses MC29, CMII, and OK10 (*5*). The two genes are linked on the 5.2-kb genomic MH2 RNA in the order 5′ Δ*gag-mht-myc* 3′ (*16*). The Δ*gag-mht* gene encodes a nonstructural *gag*-related protein of 100,000 daltons (p100) (*18*), probably via genomic MH2 RNA as messenger RNA (mRNA), and the *myc*-related gene encodes a 57,000-dalton protein (p57) via a subgenomic 2.4-kb mRNA (*19*). It is unclear whether these two MH2 genes cooperate in oncogenic transformation or whether each gene by itself may have oncogenic function.

MSV 3611 causes fibrosarcomas in mice and transforms murine fibroblasts and epithelial cells in culture (*20*). The virus belongs to the same taxonomic group as Moloney murine leukemia virus. MSV 3611 is thought to have a single Δ*gag-raf* hybrid *onc* gene that encodes two overlapping *gag*-related transforming proteins of 75,000 and 90,000 daltons (*20*). Thus MH2 and MSV 3611 differ in their genetic structures, oncogenic potential, and host ranges.

We have compared the newly discovered *mht* sequence of MH2 (*21*) with the sequences of other known *onc* genes, including the *raf* sequence of MSV 3611 (*22*). The *mht* sequence proved to be closely related to the *raf* sequence of MSV 3611 but not to other *onc* gene sequences. Comparison of the sequences of *mht* and *raf* (Fig. 1) provides the following information. (i) The region of homology between the two sequences includes 969 nucleotides. At the 5′ end, the homology starts 174 nucleotides downstream from the *gag-mht* junction of MH2 and at the *gag-raf* junction of MSV 3611. At the 3′ end, the homology ends with a common translation termination signal, TAG (T, thymine; A, adenine; G, guanine), at positions 1165 to 1167 in MH2 (Fig. 1), beyond which the *mht* and *raf* sequences diverge. (ii) Within the 969-nucleotide stretch of sequence homology, *mht* and *raf* differ in 190 nucleotides. Most of these changes are in the third base of a codon and result in the same amino acids; of these, only 19 alter the amino acids encoded by their respective triplets. The homology between amino acids is therefore about 95 percent. With the exception of one amino acid deletion the number of amino acids remains the same in both *mht* and *raf*. (iii) Beyond the 5′ border of overlap the two viral *onc* genes are different. The 2-kb Δ*gag* element of avian retrovirus MH2 is unrelated to the 1.5-kb Δ*gag* element of murine retrovirus MSV 3611. Moreover, the 5′ boundary of the portion of the *mht* that overlaps *raf* is preceded by an MH2-specific sequence of *mht* of 174 nucleotides (Fig. 2).

The observation that the *mht* sequence of MH2, a virus that contains two genes with oncogenic potential, is closely related to the *raf* sequence of MSV 3611, a virus that contains only one *onc* gene, supports the view that the Δ*gag-mht* hybrid gene of MH2 has oncogenic function. Pachl *et al.* (*19*) favored the view that the *myc* gene of MH2 rather than a *gag*-related hybrid gene carries the primary oncogenic function of MH2. Genetic analyses involving deletions of *mht* and *myc* sequences in MH2 may provide an answer to this question.

The high degree of amino acid homology (95 percent) between *mht* and *raf* is the closest yet observed among the *onc* sequences shared by different taxonomic groups of retroviruses. Since the essential viral genes of nondefective retroviruses that must transduce the *onc* sequences from cellular proto-*onc* genes are unrelated and have different host ranges (*3*), the *onc*-specific sequences of these viruses must have been independently transduced from the respective host cell species. Because closely related *onc* gene sequences occur in different taxonomic groups of retroviruses, we conclude that the number of proto-*onc* genes in vertebrate cells is limited to a small group of cellular genes. Alterna-

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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References and Notes

1. P. H. Duesberg, *Nature (London)* **304**, 219 (1983).
2. J. M. Bishop, *Cell* **23**, 5 (1981).
3. R. A. Weiss, N. M. Teich, H. Varmus, J. M. Coffin, Eds. *The Molecular Biology of Tumor Viruses* (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1982).
4. G. Klein, *Cell* **32**, 311 (1983).
5. K. Bister and P. H. Duesberg, in *Advances in Viral Oncology*, G. Klein, Ed. (Raven, New York, 1982), pp. 3–42; P. H. Duesberg, W. F. Phares, W.-H. Lee, *Virology*, in press.
6. M. F. Nunn, P. H. Seeburg, C. Moscovici, P. H. Duesberg, *Nature (London)* **306**, 391 (1983).
7. A. Hampe, I. Laprevotte, F. Galibert, L. A. Fedele, C. J. Sherr, *Cell* **30**, 775 (1982).
8. R. W. Ellis, D. R. Long, E. M. Scolnick, in *Advances in Viral Oncology*, G. Klein, Ed. (Raven, New York, 1982), vol. 1, pp. 107–126; P. R. Andersen, *et al.*, *Cell* **26**, 129 (1981).
9. M. Shibuya and H. Hanafusa, *Cell* **30**, 787 (1982).
10. P. Besmer, W. D. Hardy, Jr., E. E. Zuckerman, P. Bergold, L. Lederman, H. W. Snyder, Jr., *Nature (London)* **303**, 825 (1983).
11. P. Besmer, H. W. Snyder, Jr., J. E. Murphy, W. D. Hardy, Jr., A. Parodi *J. Virol.* **46**, 606 (1983).
12. S. Rasheed, M. B. Gardner, R. J. Huebner, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 2972 (1978).
13. S. Rasheed, personal communication.
13a. G. Naharro, K. C. Robbins, E. P. Reddy, *Science* **223**, 63 (1984).
14. R. W. Alexander, C. Moscovici, P. K. Vogt, *J. Natl. Cancer Inst.* **62**, 359 (1978).
15. C. Moscovici and L. Gazzolo, in *Advances in Viral Oncology*, G. Klein, Ed. (Raven, New York, 1982), vol. 1, pp. 83–106.
16. N. C. Kan, C. S. Flordellis, G. F. Garon, P. H. Duesberg, T. S. Papas, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 6566 (1983).
17. N. C. Kan, C. S. Flordellis, G. E. Mark, P. H. Duesberg, T. S. Papas, *ibid.*, in press.
18. S. S. F. Hu, C. Moscovici, P. K. Vogt, *Virology* **89**, 162 (1978).
19. C. Pachl, B. Biegalke, M. Linial, *J. Virol.* **45**, 133 (1983).
20. U. R. Rapp *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 4218 (1983); U. R. Rapp, F. H. Reynolds, Jr., J. R. Stephenson, *J. Virol.* **45**, 914 (1983).
21. T. S. Papas *et al.*, in *Cancer Cell: Cold Spring Harbor Conferences on Cell Proliferation and Cancer*, J. D. Watson, G. F. Vande Woude, A. Levine, W. Topp, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., in press).
22. G. E. Mark, and U. Rapp, in preparation.
23. S. P. Goff and D. Baltimore, in *Advances in Viral Oncology*, G. Klein, Ed. (Raven, New York, 1982), pp. 127–139.
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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