# SOMATIC CELL HYBRIDS BETWEEN MOUSE PERITONEAL MACROPHAGES AND SV40-TRANSFORMED HUMAN CELLS

## I. Positive Control of the Transformed Phenotype by the Human Chromosome 7 Carrying the SV40 Genome*

By CARLO M. CROCE AND HILARY KOPROWSKI

*(From the Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania 19104)*

Using somatic cell hybridization techniques, we have recently assigned the genes for SV40 T antigen (1, 2) and SV40 tumor-specific transplantation antigen[1] and the SV40 genome (1, 3) to human chromosome 7 in SV40-transformed human cells. Since we observed a preferential retention of the human chromosome 7, carrying the SV40 genome, in more than 80% of hybrid cell clones obtained by fusing mouse L cells with SV40-transformed human cells, we hypothesized that the preferential retention of the human chromosome that contains the integrated SV40 genome was due to the fact that the retention of this chromosome in the hybrids determined a growth advantage (1, 4). In addition, we have recently shown that the fusion of SV40-transformed human cells with normal diploid human fibroblasts results in the formation of somatic cell hybrids that behave as transformed cells and that can be propagated indefinitely in tissue culture (5, 6). This fact indicated the dominance of the transformed phenotype in this type of hybrid (5), and suggested that gene products of the human chromosome 7 in which the SV40 genome is integrated, are responsible for the expression of the transformed phenotype and for the unlimited growth of the hybrid cells in cultures.

To demonstrate that the human chromosome 7, carrying the SV40 genome, codes for "transforming factors" responsible for the expression of the transformed phenotype, we decided to hybridize mouse peritoneal macrophages, which in our culture conditions are nondividing cells (7), with SV40-transformed human cells, deficient in hypoxanthine guanine phosphoribosyltransferase (HGPRT).[2] If the human chromosome 7 with the integrated SV40 genome is responsible for the unlimited growth of the human transformed cells and for the transformed phenotype, we should obtain hybrids between mouse macrophage and

* This work was supported in part by U. S. Public Health Service Research Grant CA 10815, RR 05540 from the Division of Research Resources, and the Commonwealth of Pennsylvania.

[1] Girardi, A. J., and C. M. Croce. Manuscript in preparation.

[2] Abbreviations used in this paper: HAT, hypoxanthine-aminopterin-thymidine; HGPRT, hypoxanthine guanine phosphoribosyltransferase; LN, human skin fibroblasts.

SV40-transformed human cells that grow indefinitely in culture and that are transformed when the human chromosome 7 carrying the SV40 genome is present.

## Materials and Methods

*Cells.* Peritoneal macrophage cultures derived from C57BL mice were kindly supplied by Dr. Gianni Garotta of the Wistar Institute, Philadelphia, Pa., and were prepared according to the method of Cohn and Benson (8) with some modifications. Mouse C57BL embryo fibroblasts were used as controls in some experiments. Human skin fibroblasts (LN) were derived from a patient with the Lesch-Nyhan syndrome (9), and were obtained from the Institute for Medical Research, Camden, N. J. LN-SV cells are SV40-transformed LN fibroblasts, which are deficient in HGPRT (1, 2).

*Cell Hybridization.* Mouse peritoneal macrophages were fused with either LN or LN-SV cells in the presence of β-propiolactone-inactivated Sendai virus at pH 8.0 according to the method described by Croce et al. (10). The fused cultures were selected in hypoxanthine-aminopterin-thymidine (HAT) medium (11). Hybrid cell colonies, growing in HAT selective medium were picked, propagated in HAT medium, and cloned. Each clone studied was derived from a different hybrid colony. Mass cultures derived from flasks containing more than 10 different colonies per flask were included in this study. At the time of this study, the hybrid clones derived from the fusion of mouse macrophages with LN-SV cells were subcultured for more than 30 passages.

In four experiments performed at different times, no continuously growing hybrid cells were obtained by fusing mouse peritoneal macrophages with LN fibroblasts. The details of the procedures used for cell culture, and cell cloning and subcloning are described elsewhere (1–3).

*Expression of the SV40 T Antigen.* Hybrid and parental cells were stained for SV40 T antigen by indirect immunofluorescence by described methods (12). The percentage of SV40 T-antigen-positive cells was determined by examining 2,500 cells in two different coverslips per each clone.

*Karyological Analysis.* Giemsa (G-) banding staining of the metaphase chromosomes of parental and hybrid cells was performed by a modification of the method described by Seabright (13, 14). The chromosome preparations were treated with a solution of trypsin (0.05%) and EDTA (0.02%) (Grand Island Biological Co., Grand Island, N. Y.) for 5 min, rinsed with medium containing 5% fetal calf serum, and then rinsed with Hanks' solution. The slides were then stained in 2% Giemsa (pH 6.8) for 5 min, rinsed with pH 6.8 buffer (made by dissolving one tablet of G. T. Gurr pH 6.8 buffer in 100 ml of water), and dried in humidified hot air. A minimum of 20 metaphases of each hybrid clone and of 40 metaphases of each mass culture of hybrid cells were photographed and analyzed.

*Cloning of Parental and Hybrid Cells in Soft Agar.* $10^4$, $10^3$, and $10^2$ of either parental or hybrid cells were resuspended in 3 ml of 0.3% noble agar in Eagle's MEM, and plated over an underlay of 5 ml of 0.5% noble agar in MEM in 60-mm plastic petri dishes. This method is a modification of that described by MacPherson (15). Colonies (larger than 0.1 mm) of cells growing in soft agar were scored 3 wk after seeding. 20 of such colonies (11 obtained from clone 1 and 9 obtained from clone 13) were picked from the petri dishes with fine Pasteur pipettes and plated in 25-$cm^2$ plastic flasks.

## Results

The fusion of mouse peritoneal macrophages with LN fibroblasts did not result in the formation of hybrid cell colonies in HAT selective medium (it is possible, however, that formation of hybrid cells between the two cell types occurred, but that these hybrid cells had a limited life span). The fusion of mouse peritoneal macrophages, which under our culture conditions do not synthesize DNA, with SV40-transformed Lesch-Nyhan fibroblasts (LN-SV), however, did result in the formation of hybrid cell colonies in HAT medium. The cells from each colony could be picked, grown, and, subsequently, cloned.

*Expression of the SV40 T Antigen in Mouse Macrophage-SV40-Transformed*

*Human Cell Hybrids.* As shown in Table I, all the hybrid cell clones obtained were SV40 T-antigen positive. In addition, three mass cultures of hybrids derived from three different original flasks (see Materials and Methods) were also T-antigen positive. 100% of the cells of each clone and of each mass culture were SV40 T-antigen positive. No segregation into SV40 T-antigen-negative hybrid

TABLE I

*Expression of the Transformed Phenotype in Hybrids between Mouse Peritoneal Macrophages and SV40-Transformed Human Cells*

| Cells | SV40 T antigen* | Contact inhibition | Human chromosomes present in the hybrid clones‡ |
|---|---|---|---|
| Mouse peritoneal macrophages | – | ND§ | |
| LN-SV | + | – | |
| Hybrids: | | | |
| cl 1 | + | – | 4,6,7,11,12,14,17,19,20,X |
| cl 2 | + | – | 4,6,7,11,12,17 |
| cl 6 | + | – | 6,7,17,20 |
| cl 7 | + | – | 4,5,7,11,12,17,18,20 |
| cl 11 | + | – | 4,6,7,11,12,17 |
| cl 12 | + | – | 4,7,11 |
| cl 13 | + | – | 7 |
| cl 17 | + | – | 6,7,11,12,14,17 |
| cl 19 | + | – | 1,4,6,7,11,12,17,19,20,21 |
| cl 23 | + | – | 7,12 |
| cl 28 | + | – | 4,5,7,11,12 |
| cl 35 | + | – | 2,4,7,11,12 |
| cl 41 | + | – | 7 |
| cl 46 | + | – | 4,7,11,12,20,21 |
| cl 47 | + | – | 4,6,7,11,12,14,19,20,X |
| cl 61 | + | – | 4,5,7,11,12 |
| cl 62 | + | – | 7 |
| cl 63 | + | – | 4,6,7,10,11,12,14,17,20,22,X |
| cl 75 | + | – | 4,7,11,12,17,19 |
| cl 81 | + | – | 7 |
| cl 82 | + | – | 7,11,12,17 |
| cl 87 | + | – | 7,12 |

* 100% of the cells of each hybrid clone were T-antigen positive. A minimum of 2,500 cells per each independent clone was analyzed for the presence of SV40 T antigen.

‡ A minimum of 20 metaphases per each clone was photographed and karyotyped. All the metaphases contained the human chromosome 7. The other human chromosomes were present in at least 5% of the metaphases analyzed per each clone.

§ Peritoneal macrophages are nondividing cells in these cultural conditions.

clones was observed. In addition, 100% of the cells of 45 subclones of clones 1 and 13 were SV40 T-antigen positive.

*Expression of the Transformed Phenotype in Somatic Cell Hybrids Between Mouse Peritoneal Macrophages and SV40-Transformed Human Cells.* As shown in Table I, all the hybrid clones did not display the density dependent

inhibition of cell division (contact inhibition) characteristic of normal cells and all piled up in culture. The saturation density of the hybrid clones was approximately five times that of human LN fibroblasts and about four times that of mouse diploid fibroblasts (Table II). When the parental and hybrid cells were resuspended at various densities in soft agar, only the SV40-transformed human cells and the hybrid clones gave origin to colonies of growing cells (Table III). 100% of the cells derived from each of the 20 colonies growing in agar were SV40

TABLE II

*Saturation Density of Parental and Hybrid Cells**

| Cells | Saturation density‡ |
|---|---|
| Lesch-Nyhan diploid fibroblasts | $0.4 \times 10^5$ cells/cm$^2$ |
| LN-SV | $2.0 \times 10^5$ cells/cm$^2$ |
| Mouse diploid fibroblasts | $0.6 \times 10^5$ cells/cm$^2$ |
| Hybrid clone 1 | $2.0 \times 10^5$ cells/cm$^2$ |
| Hybrid clone 2 | $2.1 \times 10^5$ cells/cm$^2$ |
| Hybrid clone 6 | $2.3 \times 10^5$ cells/cm$^2$ |
| Hybrid clone 17 | $1.9 \times 10^5$ cells/cm$^2$ |

* Parental diploid human and mouse fibroblasts and hybrid cells were counted 10 days after seeding the same number ($2 \times 10^6$) of cells in 75-cm$^2$ plastic Falcon flasks.
‡ The saturation density of mouse peritoneal macrophages was not determined since the macrophages are nondividing cells.

TABLE III

*Transformed Phenotype in Hybrids between Mouse Peritoneal Macrophages and SV40-Transformed Human Cells*

| Cells | Colony formation in soft agar* |
|---|---|
| Lesch-Nyhan diploid fibroblasts | – |
| LN-SV | + |
| Mouse peritoneal macrophages | – |
| Mouse diploid fibroblasts | – |
| Hybrid clone 1 | + |
| Hybrid clone 2 | + |
| Hybrid clone 6 | + |
| Hybrid clone 13 | + |

* The efficiency of colony formation in soft agar was between 1 and 5 colonies per 100 LN-SV or hybrid cells seeded. No colonies developed by plating either LN fibroblasts or mouse macrophages or mouse fibroblasts.

T-antigen positive. The growth of cells in agar has been considered a characteristic of transformed cells.

*Karyological Analysis.* As shown in Table I, the hybrid clones contained from 1 to 11 human chromosomes, which could be identified after Giemsa banding staining (Figs. 1 and 2). The presence of the human chromosome 7 was the common denominator of all the selected hybrid clones. 100% of the metaphases of each hybrid clone contained at least one human chromosome 7. Fig. 3

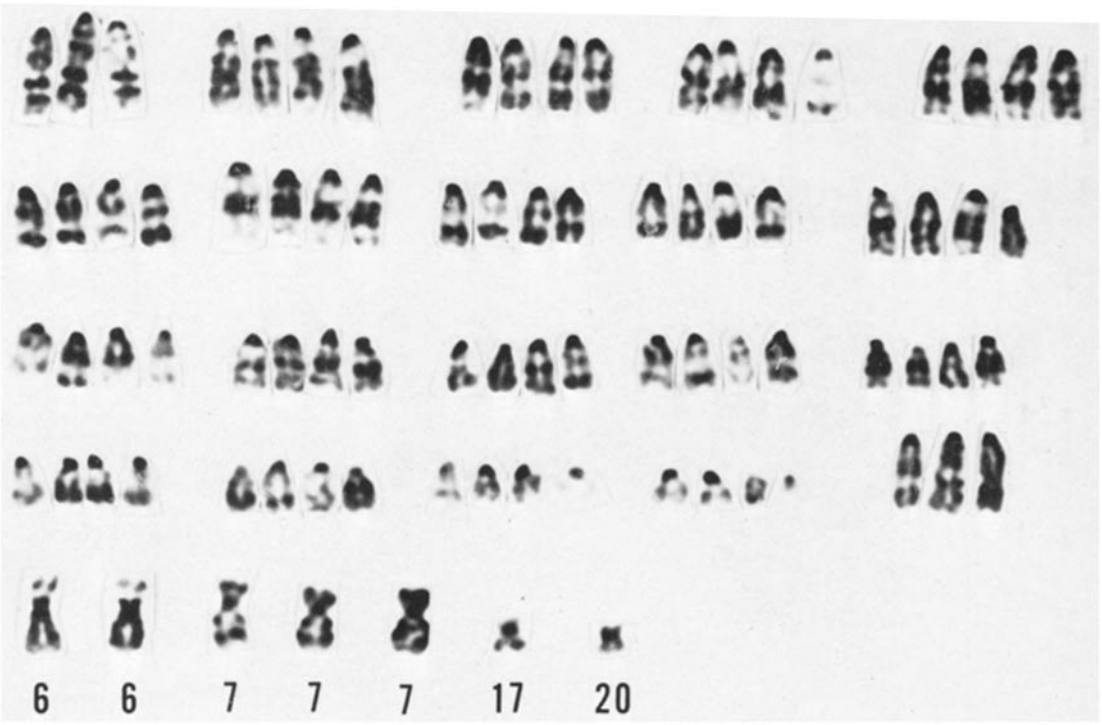


FIG. 1. Karyotype of the hybrid clone 6. The human chromosomes 6, 7, 17, and 20 are present in this clone, while all the remaining chromosomes are of mouse origin. The number of mouse chromosomes present in this clone is quasi-tetraploid.

summarizes the data presented in Table I and describes the positive correlation between the expression of the SV40 T antigen and of the transformed phenotype and the presence of the human chromosome 7.

Karyological analysis of the three different hybrid mass cultures also indicated that the human chromosome 7 was retained by all the hybrid cells. The number of mouse chromosomes present in the hybrid cell clones varied from clone to clone, but more than 75% of the hybrid clones contained a quasi-tetraploid number of mouse chromosome (Figs. 1 and 2). The hybrid clones which contained a near tetraploid or hexaploid number of mouse chromosomes were those which contained the greatest number of human chromosomes.

## Discussion

The results described in this paper indicate that it is possible to produce somatic cell hybrids between SV40-transformed human fibroblasts and primary mouse macrophages and that these hybrid cells behave as transformed cells in vitro. All hybrid clones and all the cells of each hybrid clone were SV40 T-antigen positive and contained the human chromosome 7 in which the SV40 genome is integrated. The possibility that cells other than macrophages obtained from the peritoneal

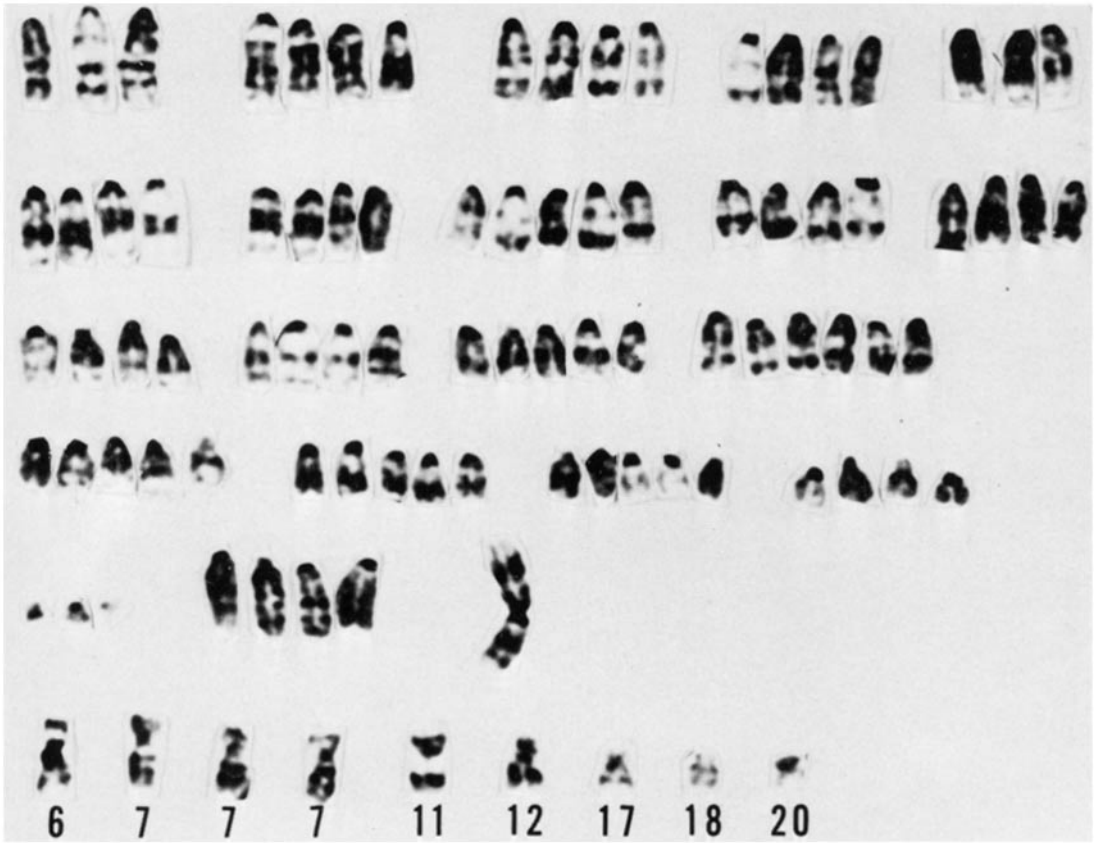


FIG. 2. Karyotype of a hybrid cell of a hybrid mass culture. The human chromosomes 6, 7, 11, 12, 17, 18, and 20 are present in this hybrid. All the other chromosomes are of mouse origin. The biarmed chromosome present in the fourth row is originated by the centric fusion of two mouse chromosomes.

cavity of the mice were spontaneously transformed in culture and hybridized with human cells can be excluded because: (*a*) no transformed mouse cells were found in the cultures, which contained only hybrids with 1 of 11 human chromosomes, and (*b*) no segregation into SV40 T-antigen-negative hybrid clones was observed. We have recently shown that hybrids between transformed mouse cells and SV40-transformed human cells segregate into SV40 T-antigen-positive and -negative clones (1, 2), but no segregation into SV40 T-antigen-negative clones has been observed with the mouse macrophage-SV40-transformed human cell hybrids, this fact indicating that SV40 T-antigen-negative cells can not be propagated in culture. It can be thus inferred that the human chromosome 7, which carries the SV40 genome, is essential for the indefinite growth of the hybrid cells in culture. The fact that the common denominator in all the hybrid clones was the presence of the human chromosome 7 which carries the SV40 genome, indicates that the human chromosome 7 with the SV40 genome contains a gene(s) responsible for the expression of the transformed phenotype. It can be

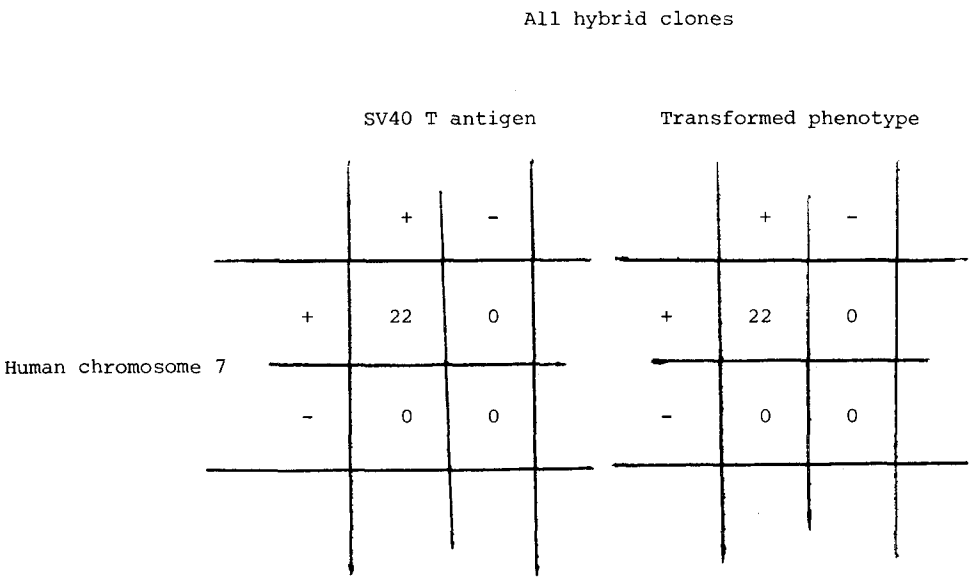


FIG. 3. Positive correlation between the expression of the SV40 T antigen, the expression of the transformed phenotype, and human chromosome 7 in a series of somatic cell hybrids between mouse peritoneal macrophages and SV40-transformed human cells. 100% of the cells of each clone were SV40 T-antigen positive.

postulated that if loss of the chromosome 7 from the hybrid cell occurs, the hybrid cells become incapable of cell division.

We have recently shown that somatic cell hybrids between normal and SV40-transformed human cells are transformed (5). The present results are in contrast with the hypothesis formulated by Harris, Klein, and colleagues (16–19), who stated that, as a rule, somatic cell hybrids between transformed cells and normal cells behave as normal cells, if the chromosomes derived from the normal parents are retained by the hybrids. Somatic cell hybrids between mouse normal cells and SV40-transformed human cells retain all the chromosomes of the mouse normal parental cells (the majority of the clones contain even a tetraploid number of mouse chromosomes), they behave as transformed cells in vitro, and they can be subcultured indefinitely in culture. The fact that the human chromosome 7 carrying the SV40 genome is essential for the growth of the hybrid cells and it is retained by the totality of the hybrids, suggests the hybrids between nondividing mouse cells and human transformed cells derived from cancer patients might retain the "oncogenic" chromosome in order to divide and to express the transformed phenotype. We are now investigating this possibility by using human cancer cells deficient in HGPRT.

## Summary

Fusion of mouse peritoneal macrophages with SV40-transformed human cells, deficient in hypoxanthine guanine phosphoribosyltransferase, resulted in the

formation of transformed somatic cell hybrids which contained, without exception, the human chromosome 7 carrying the SV40 genome. It is postulated that the hybridization of mouse nondividing cells with human cancer cells could permit the identification of the human "oncogenic" chromosome(s) present in human cancer cells, since such chromosome(s) should be retained by the totality of the mouse-human hybrid cells.

*Received for publication 4 June 1974.*

## Bibliography

1. Croce, C. M., A. J. Girardi, and H. Koprowski. 1973. Assignment of the T antigen gene of Simian virus 40 to human chromosome C-7. *Proc. Natl. Acad. Sci. U. S. A.* **70:**3617.
2. Croce, C. M., and H. Koprowski. 1974. Concordant segregation of the expression of SV40 T antigen and human chromosome 7 in mouse-human hybrid subclones. *J. Exp. Med.* **139:**1350.
3. Croce, C. M., K. Huebner, A. J. Girardi, and H. Koprowski. 1974. Rescue of defective SV40 from mouse-human hybrids containing human chromosome 7. *Virology.* **60:**276.
4. Croce, C. M., B. B. Knowles, and H. Koprowski. 1973. Preferential retention of the human chromosome C-7 in human-(thymidine kinase deficient) mouse hybrid cells. *Exp. Cell Res.* **82:**457.
5. Croce, C. M., and H. Koprowski. 1974. Positive control of the transformed phenotype in hybrids between normal and SV40-transformed human cells. *Science (Wash. D. C.).* **184:**1288.
6. Croce, C. M., and B. Bakay. 1974. Presence of two active X chromosomes in hybrids between normal diploid human fibroblasts and SV40-transformed fibroblasts derived from patients with the Lesch-Nyhan syndrome. *Exp. Cell Res.* In press.
7. Epifanova, O. I., and V. V. Terskikh. 1969. On the resting periods in the cell life cycle. *Cell. Tissue Kinet.* **2:**75.
8. Cohn, Z. A., and B. Benson. 1965. The differentiation of mononuclear phagocytes, morphology, cytochemistry, and biochemistry. *J. Exp. Med.* **121:**153.
9. Nyhan, W. L., B. Bakay, J. D. Connor, J. F. Marks, and D. K. Kelle. 1970. Hemizygous expression of glucose-6-phosphate dehydrogenase in erythrocytes of heterozygotes for the Lesch-Nyhan syndrome. *Proc. Natl. Acad. Sci. U. S. A.* **65:**214.
10. Croce, C. M., H. Koprowski, and H. Eagle. 1972. Effect of the environmental pH on the efficiency of cell hybridization. *Proc. Natl. Acad. Sci. U. S. A.* **69:**1953.
11. Littlefield, J. W. 1964. Selection of hybrids from matings of fibroblasts in vitro and their presumed recombinants. *Science (Wash. D. C.).* **145:**709.
12. Koprowski, H., F. Jensen, and Z. Steplewski. 1967. Activation of production of infectious tumor virus SV40 in heterokaryon cultures. *Proc. Natl. Acad. Sci. U. S. A.* **58:**127.
13. Seabright, M. 1971. A rapid banding technique for human chromosomes. *Lancet.* **2:**971.
14. Croce, C. M., G. Litwack, and H. Koprowski. 1973. Human regulatory gene for inducible tyrosine aminotransferase in rat-human hybrids. *Proc. Natl. Acad. Sci. U. S. A.* **70:**1268.
15. MacPherson, I. 1969. Agar suspension culture for quantitation of transformed cells. *In* Fundamental Techniques in Virology. K. Habel and N. Salzman, editors. Academic Press, Inc., New York. 214.

16. Harris, H., O. J. Miller, G. Klein, P. Worst, and T. Tachibana. 1969. Suppression of malignancy by cell fusion. *Nature* (*Lond.*). **223:**363.
17. Harris, H., and G. Klein. 1969. Malignancy of somatic cell hybrids. *Nature* (*Lond.*). **224:**1315.
18. Klein, G., U. Bregula, F. Wiener, and H. Harris. 1971. The analysis of malignancy by cell fusion. *J. Cell Sci.* **8:**659.
19. Wiener, F., E. M. Fenyo, G. Klein, and H. Harris. 1972. Fusion of tumor cells with host cells. *Nat. New Biol.* **238:**155.