Cell Fusion And the New Genetics

By Joshua Lederberg

The union of cells from dissimilar organs or animal species may help to explain their great diversity

A reliable technique for fusing cells from man and other mammals has ignited a worldwide explosion in genetic research. Scientists have been combining specialized cells such as those of the liver with those of the blood. Even species lines are no barrier—hybrids of human and mouse cells were among the first produced. Human cells have also been fused with those from hamsters, rats, chickens, and other animals.

Classical genetic experiments, the kind that most people learned about in school, have revealed virtually all that we know of genetics. But, these experiments focus only on the offspring that develop from the fusion of a male and a female sex cell, such as the egg and sperm of higher organisms. Scientists discovered long ago, however, that all cells in the body contain the basic units that determine an organism's characteristics. These units, called genes,

The contents of one cell explode into another in an early stage of induced cell fusion.

are biochemical chains so small that they cannot be seen even through the most powerful microscopes. In most organisms, the genes are found on structures called chromosomes. The chromosomes, which are large enough to be seen through a simple light microscope, are located in the nucleus of the cell.

Classical experiments are useless for any direct study of genetic changes in the new organism's body cells after the egg is fertilized. Yet, the body cells comprise nearly all of the living creature, and they are the center of the most important and scientifically challenging processes of life. One such process is differentiation, through which embryonic cells develop into specialized cells such as those of the brain, skin, and blood. By fusing differentiated cells and examining the activities of the hybrid cells, scientists are beginning to understand better why the parent cells differ from one another. Ultimately, this research should also reveal much about how the body's organs perform their vital processes. Learning, which is possible only because of brain differentiation, and immunity, an extension of lymphoid tissue differentiation, are only two examples. Furthermore, some diseases, such as cancer, are examples of differentiation gone awry, and can be studied through cell-fusion experiments.

Cell-fusion has also proved to be a powerful tool in one place where classical genetics has uncovered information very slowly. Elaborate experiments with many generations of test animals have been used for gene mapping—determining the chromosomes on which specific genes are located. Scientists only rarely found human beings with family histories that could reveal what experiments reveal in animals. And controlled crossbreeding of people was, of course, unthinkable. But through cell fusion, a scientist can crossbreed human cells, and follow a single chromosome and its genes through the generations of cell division that follow.

The most important factor in fusing cells is an inactivated virus called inactivated Sendai virus (ISV). The ISV causes fusion, ordinarily a rare and random event, to become common and predictable. Pathologist Henry Harris of the University of Oxford in England developed the technique of adding ISV to mixtures of cells. He had read of research that seemed to indicate that cells fused in tissues that were infected with various viruses. Harris selected the Sendai strain after testing many viruses and grading their ability to promote fusion. He also discovered that ultraviolet light inactivates the virus, destroying its ability to infect but not its ability to induce cell fusion. Thus, experimenters have been able to use the virus without worrying about possible confusing side effects from virus infection of the cells.

In most experiments, the cells to be fused are from tissue cultures in which all cells are genetically identical and have been reproducing for many generations. Cells from such strains are the easiest to work with because they have adapted to growth under controlled conditions, yet they still maintain many of the characteristics of the tissue from which

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they came. However, cells taken directly from the organs of laboratory animals or human beings can also be fused.

Fusion begins when each inactivated virus attaches itself to the surface of two cells. Anywhere from two to dozens of cells may be clumped together. Then, the viruses open the walls of adjacent cells, forming channels between them. Next, the channels widen and the cells fuse, forming one large hybrid cell. At this point, the hybrid cell is multinucleate—that is, it has one nucleus from each of the cells that formed it.

After the fused cells incubate for a few days, some of them begin reproducing through mitosis, a process in which a cell divides and forms two new cells. Each of these daughter hybrid cells has a single nucleus containing chromosomes from the several nuclei in their parent cell. Some of the new, single-nucleated hybrid cells may then reproduce. After several generations, some of the daughter cells form strains of identical cells that can be kept alive and reproducing in cultures.

The simplest and most useful hybrid strains are those produced after only two cells are fused. But the nature of even these hybrids is often not as predictable as one might expect. For example, the cells of a strain originating from the fusion of a mouse cell and a human cell would be expected to have 86 chromosomes, because each mouse cell has 40 and each human cell has 46. However, stable strains of mouse-human hybrid cells rarely have 86. This is because the original hybrid and its offspring tend to lose chromosomes in the mitotic divisions that precede the emergence of a stable strain. In mouse-human hybrid cells, more human than mouse chromosomes are lost. This may be because the human chromosomes are geared to a slower mitotic rate than are those of the mouse.

Pathologists were the first scientists to suspect that it would be possible to fuse cells. They had seen and reported multinucleated cells, particularly in diseased tissue, as early as 1875. However, genetics was not then a full-fledged science, and, in the years that followed, pathologists and geneticists exchanged little information. As a result, cell fusion was virtually unknown to geneticists, the scientists who could best use it.

It was not until the 1950s that geneticists took a real interest in cell fusion. At that time, genetics was in the midst of a revolution triggered by the recruitment of bacteria and viruses as experimental organisms.

Investigators believed that only very closely related body cells, such as those from the same organ of two individuals of the same species, might fuse. But, because such cells are so much alike, it would be hard to tell the fused from the unfused cells. Researchers needed strains of cells that were closely related but that had distinguishable features that might show up in a fused cell. Such features are known as genetic markers because they are usually caused by a mutated gene. Scientists could use such cells to prove fusion by locating a single cell with the effects of the mutant genes from more than one cell strain. However,

almost no genetic markers were then known for closely related cells. Nevertheless, in 1956 and 1957 some scientists reported experiments in which they used markers to detect genetic exchange between cells. These markers, however, were technically difficult to detect with the tools then on hand. Although the researchers were on the right track, their results were inconclusive.

Then, in 1960, a group of French researchers approached the problem from another angle. Certain strains of mouse cells grown in culture for many years had developed many visible changes in the size and shape of some of their chromosomes. The French researchers reported that when cells from the strains were mixed and cultured together, single cells appeared that had mixtures of the unusual chromosomes from the different strains. This showed that cells from different cell strains had fused. To many scientists, this seemed an all too easy solution to such a long-standing problem—to prove that cell fusion could occur. But several other investigators soon corroborated the findings.

The studies created optimism and renewed interest in cell fusion. Some of the researchers affected were pathologists who focused their attention on the influence of certain viruses. Following this line of investigation, Harris and a co-worker J. F. Watkins fused human and mouse cells with the aid of ISV in 1965. It was this that opened the floodgates of cell-fusion research.

Fusing cells from species so widely separated by evolution completely eliminated the problem of detecting fused cells. The chromosomes are perhaps the best markers in mouse-human cells, because mouse chromosomes are shaped like Vs and human

Two cells, top background, are connected by a channel that was induced by one of the small sphere-shaped viruses. The hybrid cell produced, left center, has two nuclei, each with chromosomes forming in preparation for reproduction through mitosis. When mitosis begins, bottom left, the chromosomes align on mitotic spindles. (Mouse chromosomes look like Vs; human chromosomes like Xs.) Spindle fibers pull chromosome halves to opposite ends of the cell, bottom center. But some human chromosomes do not split, causing a deficiency of human chromosomes in the upper of the two daughter cells about to form, right foreground.

chromosomes are shaped like Xs. Genes that can act as markers are also abundant in such a fusion.

In 1970, biophysicists Theodore T. Puck and Fa-Ten Kao of the University of Colorado reported a study in which they fused human cells with Chinese hamster cells. Chinese hamster cells contain fewer and much larger chromosomes than do mouse cells. The large chromosomes are particularly easy to distinguish from human chromosomes, which are about the same size as those of a mouse.

One of the most exciting areas in cell-fusion research is fusing cells from two different, fully differentiated strains. One of the first such experiments was performed by geneticist Boris Ephrussi and coworkers at Case Western Reserve University in Cleveland. The scientists fused deeply pigmented hamster cells with unpigmented mouse cells. The mouse cells were known to have the genes for pigmentation, because some of the mouse's skin cells were pigmented. The cell strains produced by cell fusion would answer some questions about how the pigmentation genes are regulated in differentiated cells. For example, would the pigmented cell turn on the genes for pigmentation in the unpigmented cell?

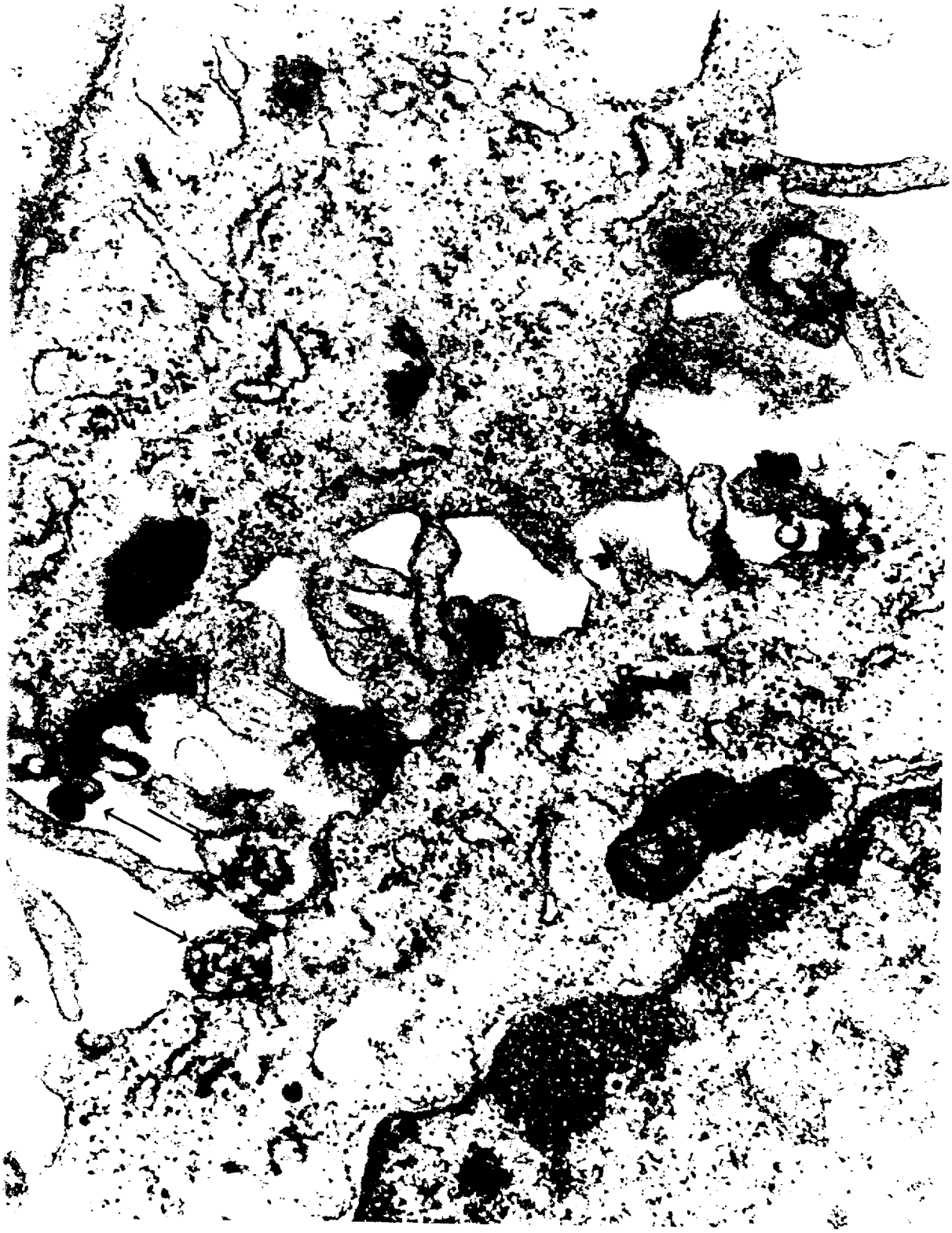
The hybrid cells formed no new pigment and soon were totally unpigmented. However, they produced many other hamster gene products. This indicated that although the genes of the unpigmented mouse cells had inhibited the pigmentation genes of the hamster cells, the mouse genes did not inhibit all the hamster genes.

This experiment raises many questions. The more obvious ones include: Are the hamster pigment-forming genes irreversibly repressed? Will these pigmentation genes reassert themselves if one particular mouse chromosome is removed? That is, can researchers identify a particular mouse chromosome as source of the pigmentation-gene inhibitor and, therefore, the gene that produces it? Is the absence of pigment caused by a specific gene or by a general imbalance of the whole hybrid chromosome set?

In another experiment, Harris used cell fusion to study differentiation. In birds, red blood cells retain a nucleus throughout their lifetime in the circulation. However, this nucleus is apparently genetically dormant—that is, its genes are not operating. Harris set out to learn if the nucleus keeps the full repertory of normal genetic information. He chose the bird red blood cell because its nucleus can be separated from its cytoplasm and, with the aid of ISV, fused with a cell from any of a number of species. This eliminates any factors in the blood cell's cytoplasm that might influence the dormant genes.

Harris fused the nuclei of chicken red blood cells with mouse cells. The first thing he saw indicating change in the dormant nucleus was

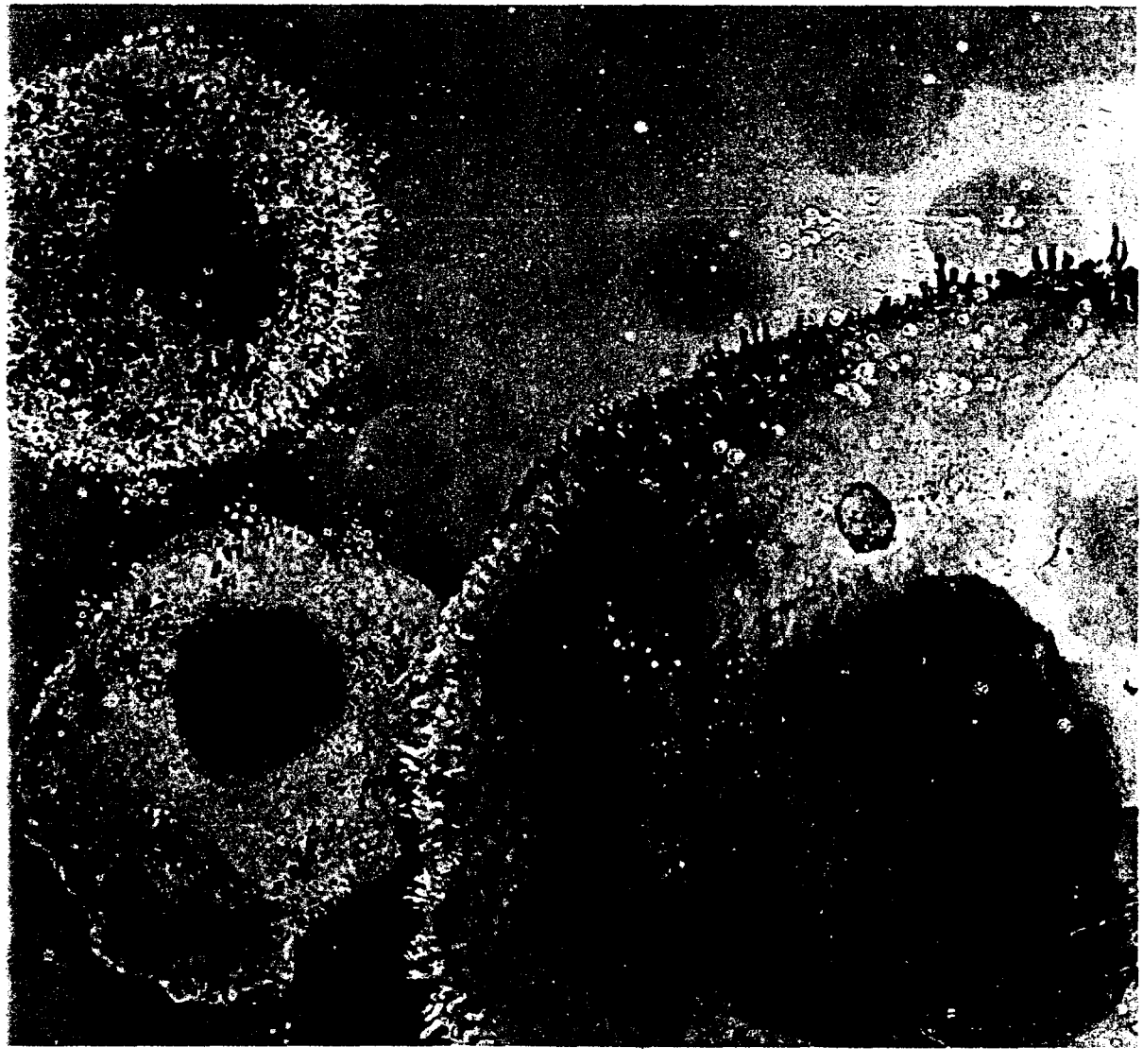
A photomicrograph of the edges of two cells about to fuse shows inactivated viruses (arrows), each of which can start a fusion channel.



In differentiation study, two cells, extreme left, contain pigmentation genes, but lower one is unpigmented. Cells fuse, center, forming binuclear hybrid cell, far right. The pigment begins to deteriorate, and no new pigment is produced. The original unpigmented cell probably contained a gene that inhibits pigment-producing genes.

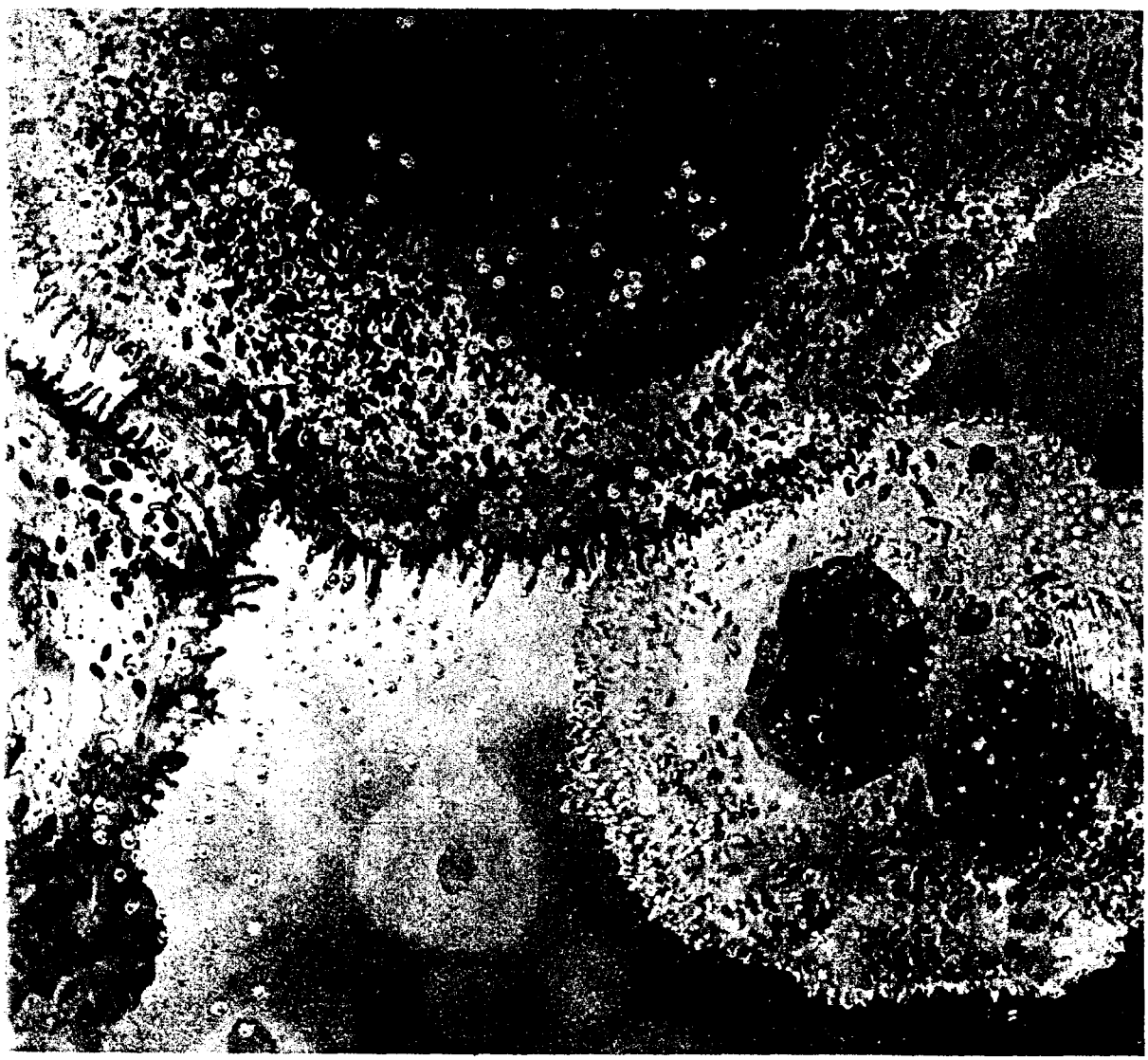
a 30- to 40-fold increase in its size. He then found bird gene products, including certain antigens and enzymes, in the hybrid cells. This proved that the dormant nucleus was activated by the nucleus and cytoplasm of the cell with which it was fused. It is particularly interesting that the genes in the dormant cell nucleus of a bird can be activated by the cells of such a biologically distant organism as a mouse.

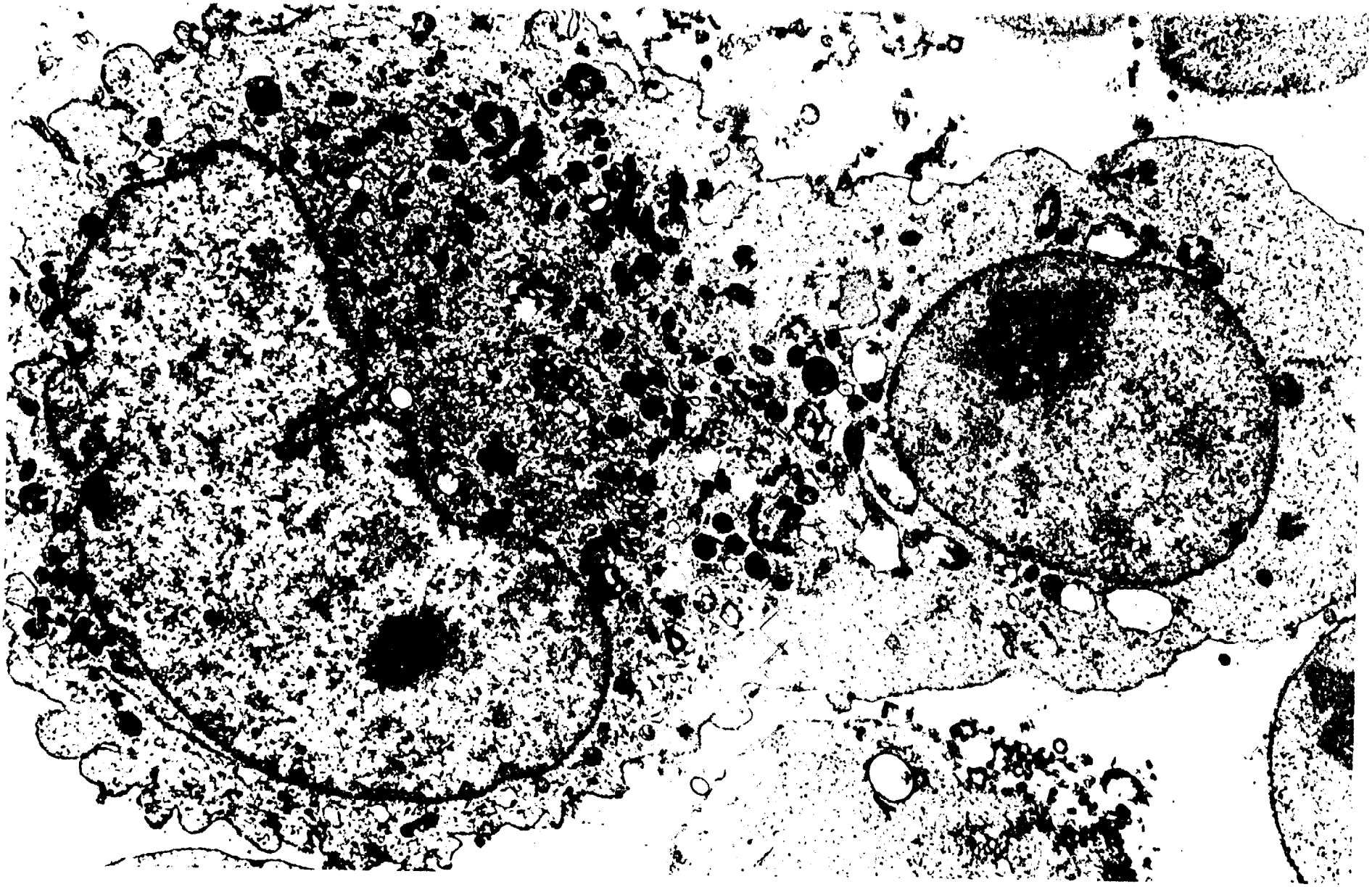
To study cancer, researchers, primarily in England, France, Israel, Sweden, and the United States, have fused cancer cells and normal cells. One aim is to determine if malignancy is dominant or recessive. In classical genetics, these terms are used to indicate which of two different genes for the same characteristic will be expressed when they are present in a single organism. For example, a child who inherits a



gene for blue eyes from one parent and a gene for brown eyes from the other, will have brown eyes. The gene for brown eyes is dominant; the one for blue eyes, recessive.

By mid-1971, the results of the cancer-normal hybrid cell experiments appeared inconsistent. In some of the studies, the hybrids have been normal—that is, they have not caused cancer when injected into test animals. This seems to indicate that genes on some of the chromosomes from the normal cells were dominant to those that caused cancer. Further support for this view arose from experiments in which some of the cancer-normal hybrid cells produced cancerous daughter cells after losing some of the chromosomes that came from the normal cell. In addition, the nonmalignant, cancer-normal hybrid cells proved





to be a vaccine against cells from the original cancer. Evidently, the nonmalignant hybrid cells retained the cancer-cell antigens, causing the animals injected with them to manufacture cancer antibodies.

In 1969 and 1970, however, Harris and co-workers reported studies that yielded hybrids of some kinds of normal and malignant cells that acted quite differently when injected into test animals. Even without the loss of chromosomes, these cells had some tendency to multiply and invade normal tissue, as do malignant cells.

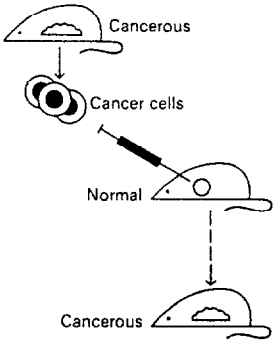
Thus, although the cancer studies provide much to think about, they have not answered the original question–is malignancy dominant or recessive? But the fault is with the question. Genes are properly classified as dominant or recessive only when they are in pairs on two chromosomes, as they are in normal cells. Because the genetic arrangements in hybrid cells are much more complex, this terminology is inapplicable. The data from the fusion work, however, should prove useful to those who will formulate more meaningful questions.

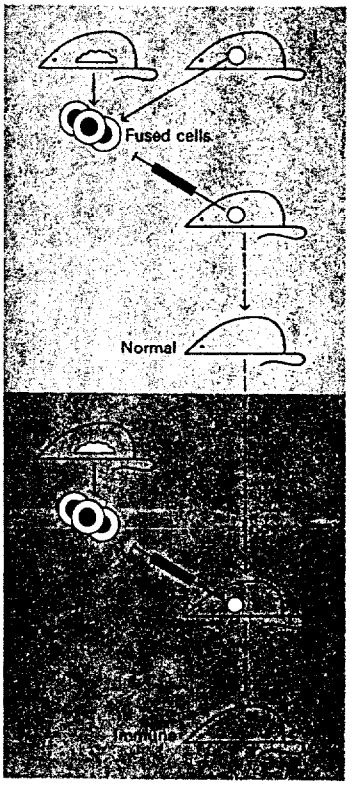
Several other groups of scientists have used cell fusion to study another aspect of cancer. The virus called SV40 is one of many that can transform normal cells into cancer cells by injecting its genes into them. Human cells made cancerous by SV40 were used to start cultures of human cancer cells. Such cultures continue to produce cancer cells generation after generation without further use of the SV40 virus. Presumably this is because SV40 genes attach to human chromosomes and are reproduced along with them. When the scientists fused the human cancer cells with normal mouse cells, the initial hybrids were malignant. However, those hybrids that had lost all but three or fewer human chromosomes after several mitotic divisions were not malignant. It did not seem to matter which of the human chromosomes were lost. This indicates that the cancer-causing viral genes were incorporated into not just one, but many of the chromosomes of the originally infected cells. This also supports other work indicating that the transformed cells carried many copies of the viral genes.

Further study showed that some hybrids of normal cells and human cancer cells from SV40-infected strains suddenly liberate complete SV40 viruses fully capable of infecting new cells. As far as researchers could determine by examining the human cancer-cell cultures, the original viruses that made the parent cells malignant had disappeared many generations before. Their reappearance indicated that copies of the entire genetic content of SV40 were incorporated into the chromosomes of the original transformed cells, and were reproduced and passed on from generation to generation. Other experiments, however, showed that not all of the SV40 viral genes need be incorporated in the chromosomes to make some cells malignant. In these cancer cells,

Fused Cells Stop a Cancer

The malignant cells of a mouse with abdominal cancer caused cancer in a normal mouse. However, malignant cells fused with normal cells did not cause cancer in a normal mouse, and made it immune to cancer cells.

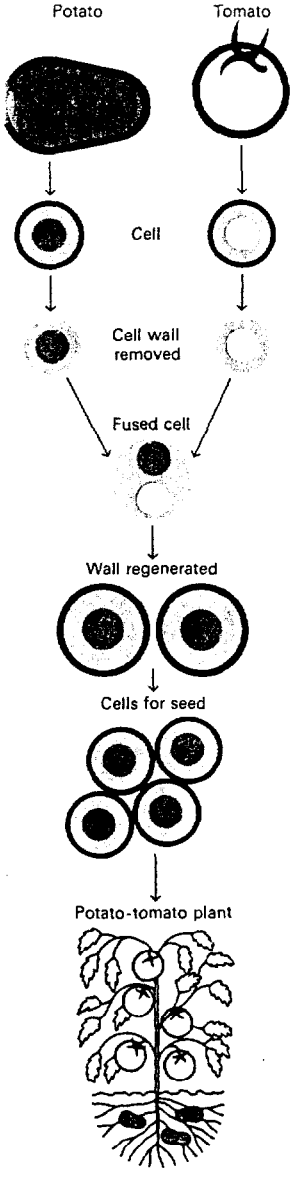




A photomicrograph, *opposite page,* shows the final stages of fusion of a chicken embryo's cell, top, with the cell of an adult mouse.

Creating Seeds For a New Plant

Because plants can be grown from single cells, and plant cells can be fused, some scientists envision unique new crop plants grown from the fused cells of two existing species.



though, complete SV40 viruses can be liberated after massive infection with other kinds of viruses that probably provide substitute genes for those that are missing.

The implications of all this go far beyond what we may learn about cancer. These experiments prove that a virus can add some or all of its genes to a human or animal chromosome. They also indicate that a bottomless reservoir of new viruses may exist in the genes in cells of men and animals–viruses that we will not know about until either cell fusion or some other stimuli activate them. Such dormant viruses known to exist in some bacteria can be awakened by radiation and many components of air and water pollution.

The special value of cell fusion for gene mapping is based upon the chromosome loss that occurs as the hybrid cells reproduce. As early as 1967, researchers used this technique to find out which human chromosome contains the gene that produces the enzyme thymidine kinase. They fused human cells with mouse cells of a strain that cannot produce the enzyme. Then, they put the hybrid cell in a specially prepared nutrient solution that would kill cells unable to produce thymidine kinase–those that during mitosis had lost the human chromosome containing the gene for the enzyme. The scientists then found surviving cells that contained only one human chromosome. This chromosome had to be the one that carries the thymidine kinase gene.

A more generalized gene-mapping method similar to this technique would be to use cells from several hybrid strains that have only a few human chromosomes. A microscopic examination of cells from each strain would reveal which human chromosomes they contain. Scientists then could merely examine cells in each line through a microscope and analyze them for various chemicals. It is then relatively simple to correlate the retention or loss of a trait or chemical from the human cell with the retention or loss of a specific human chromosome.

In 1970, two teams of scientists used a related technique to prove that the genes for two specific enzymes are linked–that is, on the same chromosome. One team was led by geneticist Walter F. Bodmer, then at Stanford University, the other by F. H. Ruddle of Yale University. The scientists fused human and mouse cells and analyzed the hybrid cells from a series of resulting strains. Each team looked for about 15 different human enzymes. The scientists reasoned that enzymes produced by linked genes would always be present in those cells that retained the genes' common chromosome and absent in those cells that had lost the chromosome. The enzymes peptidase B and the B subunit of lactate dehydrogenase, which were among the enzymes that both teams were checking, were consistently absent or present together. This indicated that the genes for their production are linked.

Ultimately, the knowledge derived from cell fusion promises to be of incalculable use to man. For example, much of what scientists foresee for genetic engineering, the purposeful manipulation of chromosomes and genes, will require knowing at least on what chromo-

some a gene is located. The proof that viral genes can be incorporated into a human chromosome strengthens the hope of scientists that, ultimately, they may use harmless viruses to introduce useful new genes into human cells. The first genes selected for such treatment will probably be those that will repair single-gene defects like phenylketonuria, which causes brain damage, and hemophilia, in which the blood does not clot normally.

Also, many products of differentiated cells, such as specific enzymes and antibodies, could become important in medicine if we could produce them in larger, predictable quantities. Cell fusion should enable scientists to learn to increase the rate at which such substances are produced by cells in culture. This has already been achieved with bacterial cells. Researchers have developed strains of bacteria that commonly produce up to 100 times their normal amount of a specific biochemical. For example, several such strains were used to produce the enzymes that were added to detergents.

The manipulative possibilities of cell fusion may see their first dramatic use in the most ancient arena of genetic engineering–agriculture. A single plant cell can grow into an entire plant that is genetically identical to the plant from which the cell was taken. Perhaps the cells of two desirable species can be fused to produce a form that cannot be produced by cross-pollination, the customary method of hybridization in plant breeding.

The cellulose wall in which most plant cells are generally encased are barriers to fusion. These must be removed without injuring the cells. In some plants, this can be easily achieved with an enzyme that destroys the cell wall. In 1970, botanist Edward C. Cocking of the University of Nottingham in England reported that he had fused plant cells for the first time. Cocking used the enzyme to remove the walls of corn and wheat cells, and suspended the cells in solution. He triggered the fusion by adding sodium nitrate to the solution.

It is not far-fetched to predict that similar fusions will produce some extraordinary results, perhaps sooner than a responsible scientist dare guess. Cocking already envisions a new plant that would grow tomatoes above ground and potatoes below. If this seems frivolous, consider other crops that cell fusion might produce. For example, rice is both high-yielding and a very popular food in many overpopulated countries. Unfortunately, however, its nutritive value is low. Through cell fusion, we might someday produce a ricelike plant that has as much nutritional value as, say, soybeans. This would clearly establish the practical value of the new genetics, for cell fusion would then have a tremendous impact on our crowded and poorly fed world.

For further reading:

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