Three Mice "Cloned" in Switzerland

Reports of the first successful nuclear transplants in mammals revive interest in the cloning of higher animals

The possibility of cloning always attracts attention. But the prospect of making multiple copies of higher organisms—like mice or men—arouses unusual interest.

A recent report in *The New York Times* headlining the "First cloning of mammals . . ." sparked a current flurry of interest. The experiment* in question was performed at the University of Geneva, Switzerland, by Karl Illmensee of that institution and Peter Hoppe of the Jackson Laboratory in Bar Harbor, Maine. It is the first time anyone has been able to produce mammals by transplanting embryonic nuclei into eggs, although several investigators have successfully produced amphibians, including frogs, in this manner.

Illmensee and Hoppe transplanted nuclei taken from embryonic mouse cells into recently fertilized mouse eggs whose own nuclei were removed. The donor embryos were from two strains of mice, one gray and the other agouti, and the recipient eggs were from black mice. Since the nuclei of the eggs were removed, only the genetic information in the transplanted nuclei would direct the development of the embryos, producing appropriately colored mice.

After growing the eggs bearing the nuclear transplants in culture, Illmensee and Hoppe implanted 16 of the resulting embryos into the uteri of white mice, together with 44 white mouse embryos that had not received nuclear transplants. (These embryos were included to bring the litter sizes up to normal.) All told, the foster mothers gave birth to 35 animals, 3 of which developed from eggs that had had transplants. These animals were readily distinguished on the basis of their gray or agouti coats. Their 32 littermates were all white.

Illmensee and Hoppe completed this experiment more than 18 months ago, and Illmensee described it at a symposium held during the summer of 1979 at the Jackson Laboratory to celebrate the 50th anniversary of the founding of that institution. A number of reports† of the work appeared then without exciting extraordinary interest.

Although the distinction is a rather fine one, the nuclear transplantations achieved by Illmensee and Hoppe did not quite produce clones. "Strictly speaking," says Bernard Talbot, who is a special assistant to the director of the National Institutes of Health, "they produced no clones since they produced no two identical animals." The three mice that developed from the eggs with the transplanted nuclei included a gray female, a gray male, and an agouti female. Talbot continues, however, "The method has the potential of producing clones."

It is analogous to the techniques used for cloning amphibians, a line of investigation extending back to 1952, when Robert Briggs of Indiana University and Thomas King, who is now director of the Kennedy Institute for the Study of Human Reproduction and Bioethics at Georgetown University, achieved the first nuclear transplants in frog eggs. Since then, they and other investigators, notably John Gurdon of Oxford University, have greatly extended the amphibian work and shown that it is possible to produce clones of adult animals by nuclear transplantation methods.

To obtain true clones, two or more nuclei from the same embryo must be transplanted into eggs, which then go on to develop into animals. This did not happen in the first phase of the transplant experiments done by Illmensee and Hoppe, although it might have. They performed 363 transplants of nuclei taken from the inner cell mass of mouse blastocysts, the early embryonic forms used in their experiments. (The inner cell mass is the part of the embryo that develops into the fetus; the remaining blastocyst cells form the membranes and other structures needed for maintenance and nourishment of the fetus.) Of these 363 recipients, only 48 formed blastocysts when cultured. The investigators chose 16 apparently normal embryos from among the 48 blastocysts to be implanted in foster mothers. No two of the three mice that subsequently developed originated from nuclei of the same embryo.

According to another *Times* report, however, Illmensee has said that in more recent work he and Hoppe produced mice that are true clones. Neither Hoppe, who has refused all interviews with the press, nor Illmensee, could be reached for comment on this report.

Whether or not mouse clones were produced in any experiment, many investigators consider the work to be a significant technical achievement. One prominent embryologist, who preferred not to be identified, dissented, saying,

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Three mice produced by nuclear transplantation

The three mice thus produced included two gray mice and one with the agouti color (right).

*The results are to be published in the January issue of *Cell*. †For example, see *Science News*, 28 July 1979, p. 68.

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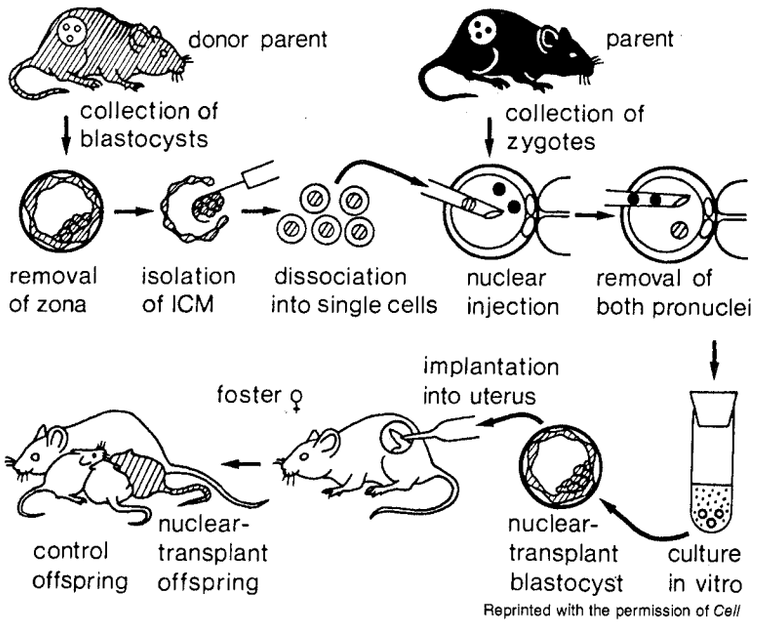

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Diagram of the nuclear transplantation experiment

"This is a media event, not a scientific event." Nevertheless, until the work of Illmensee and Hoppe there had been no reliable reports of successful nuclear transplants in mammalian eggs although there were a number of unreliable ones. Briggs and Fotis Kafatos of Harvard University both describe the current mouse work as a tour de force. And King concurs, saying that the "technical developments are considerable."

Despite the 28-year history of nuclear transplantation with amphibians, success with mammals has been slow in coming because of the many technical difficulties in working with mammalian eggs. They are produced in relatively few numbers and are much smaller and more fragile than frog eggs. Moreover, the latter are normally fertilized and develop outside the body, whereas manipulated mammalian eggs must be grown in culture until they reach the embryonic stage at which they will implant in the uterus. Then the embryos must be put into a foster mother to develop. The techniques for performing these steps have only become available within the past few years.

A prime motivation for developing nuclear transplantation methods in amphibians, and now mammals, is to study the relative contributions of nucleus and cytoplasm to the developmental patterns of the embryo. Of particular interest is the manner in which genes are turned on and off during the course of development as cells become committed to producing one kind of tissue, such as muscle, and not another.

The amphibian transplantation work has shown that the genetic material is altered, apparently irreversibly, during this commitment. Some of the genetic information is either lost or, more likely, switched off during development and cannot be turned on again, even when the nucleus is placed in an egg where the environment would otherwise favor expression of all the genes needed for development.

This effect can be seen quite early in amphibian development. Briggs points out that "75 percent of the nuclei taken from the frog blastula [equivalent to the mammalian blastocyst] give tadpoles or adult frogs. By the next embryonic stage this figure falls to 15 to 20 percent." In no case has anyone been able to grow an adult animal by transplanting a nucleus taken from adult cells, although Gurdon has shown that eggs bearing such transplants may develop through several embryonic stages.

A second possible application of nuclear transplantation is to produce multiple copies of an animal for use in biological research in general. The necessity of starting with embryonic cells means that the characteristics of the animals thus produced will be unpredictable, because the embryo's potential is an unknown, unless the nuclei are taken from an embryo of an already inbred strain. Members of inbred strains, although perhaps not as identical as members of a clone, already possess enough genetic uniformity to be satisfactory for most research applications, however.

The loss of genetic potential is currently an insurmountable obstacle to anyone who is enough of a megalomaniac to want to clone him- or herself. But even if the nuclear transplantation work in mice and amphibians were to show that it is possible to overcome this obstacle, the bioethical considerations would remain. King says, "I would find it extremely difficult to justify the nuclear transplantation experiment in humans."

One of the biggest problems, in his view, is the production of abnormal embryos. In the Illmensee-Hoppe experiment, only 48 of 363 transplants developed into blastocysts, and not all of these appeared normal. "What would you do with the human debris?" King asks. "Then you get into the debate on when life begins." This issue would be no less thorny in the context of nuclear transplantation than it already is in the continuing abortion controversy.

King also points out that having the same genetic composition would not guarantee that a clone would be just like the donor, because environmental factors will have an influence. "You cannot reproduce the environment of the donor. It is already past."

> "I would find it extremely difficult to justify the nuclear transplantation experiment in humans," says Thomas King.

One investigator, Kafatos, has already called for a ban on nuclear transplantation experiments with human and even with nonhuman primate materials. He does not think any information on mammalian embryogenesis could be obtained from these species that could not be obtained just as well from mice. "The mouse is a good experimental animal for the work," Kafatos maintains, "because of the degree with which its genetics and embryology is known." In any event, Kafatos reflects the view of many observers that the time to examine the issues raised by mammalian cloning is now, before the work is extended to humans.—JEAN L. MARX