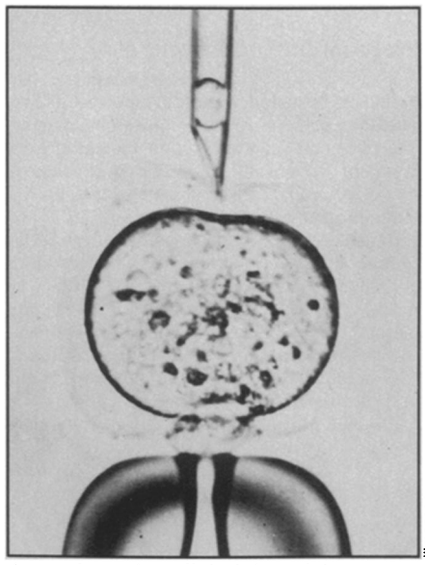
On the Way to a Clone

When writer David Rorvik claimed that a wealthy businessman had had himself cloned, biologists responded that the story was a hoax. Among their reasons was that no scientist had succeeded in cloning even a laboratory mammal (SN: 3/18/78, p. 164). Now Karl Illmensee of the University of Geneva reports a major advance in experimental embryology that makes cloning of mammals more feasible. He presented his results last week in Bar Harbor, Maine, at the Fiftieth Anniversary Symposium of the Jackson Laboratory.

Illmensee reported that he has transferred the nucleus of one mouse embryo cell into an egg cell of another mouse. He removed the egg's original genetic material and, with the aid of a mouse foster mother, obtained a normal adult mouse. This feat of micromanipulation is the first successful nucleus transplant in a mammal, Illmensee says. He has not yet done experiments to determine whether nuclei transplanted from adult mouse cells can also function in an egg to direct normal development. This would be cloning in the full sense of the word.

Biologists expect animal cloning experiments to answer questions about how gene expression is regulated during development. The findings will have implications for understanding the mechanisms of aging and cancer. In addition, cloning techniques are also expected to provide genetically identical animals for use in learning how the immune system develops its diverse responses. Illmensee cautions, however, that such experiments should be done in mice, not in humans.

In experiments with frogs and fruit flies, biologists earlier had succeeded in replacing egg genetic material with genetic material from another cell. But all attempts with laboratory mammals had failed. Illmensee attributes his recent success to several modifications in procedure.



Illmensee

An embryonic cell is injected into a recently fertilized mouse egg.

The donor nucleus for Illmensee's experiments comes from a mouse embryo at the blastocyst stage. Although of an early embryo, blastocyst cells are already differentiated into an outer layer and an inner cell mass. Each blastocyst cell is no longer able to develop by itself into a complete animal.

The recipient cell in the experiments is an egg so recently fertilized that the pronucleus containing genetic material from the female has not yet merged with the pronucleus from the male. After injecting a cell from a blastocyst inner cell mass, Illmensee uses the same fine pipette to remove the two pronuclei. Mild treatment with a chemical "relaxes" the egg's cytoplasmic structures to allow the manipulations. The donor blastocyst cell ruptures during the operations, leaving only the nucleus intact. Previous attempts at mammalian cloning had used unfertilized eggs or had removed the pronuclei before introducing the donor nucleus.

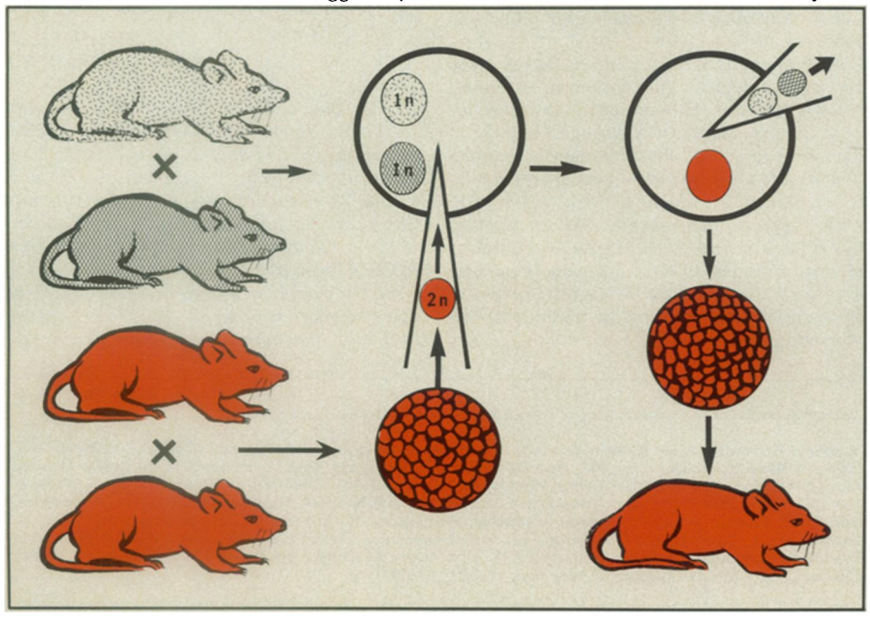
Illmensee watches the egg develop into an early embryo under laboratory conditions and tests single cells for an enzyme present in a characteristic form in cells developing from the transplanted nuclei. The embryos are then transferred to pseudo-pregnant mouse foster mothers to complete embryological development. So far, three mice born of this procedure produced only the donor form of the identifying enzyme. Thus these mice are genetically the offspring of an embryonic cell, rather than of an egg and a sperm.

In another approach to manipulating mammalian development, Illmensee and Peter Hoppe of the Jackson Laboratory removed but did not replace genetic material. Immediately after fertilization, they took one pronucleus out of a mouse egg and with a chemical treatment stimulated the remaining genetic material to duplicate. The result, after development in a foster mother, is a mouse with a pair of identical genes (homozygous) at each position on its chromosomes.

Because the biologists can distinguish in the fertilized egg the pronucleus contributed by each mouse parent, Illmensee and Hoppe can produce offspring derived entirely from either the sperm or the egg. Some scientists had speculated that completely homozygous mammals would not survive, because they would express harmful genes that would otherwise be paired with overriding normal genes. This problem may be reflected in the low success rate of the manipulations, Illmensee says, but several of the completely homozygous mice are now two years old and apparently healthy. Illmensee points out that if the manipulation is repeated on eggs of the homozygous mice, and if the paternal pronucleus is removed, the offspring of each mouse will be genetically identical animals, as in a clone.

James Crow, a geneticist from the University of Wisconsin, said in summarizing Illmensee's report, "Karl Illmensee has the embryological equivalent of a green thumb. He actually does the experiments everyone else talks about." □

Genetic material of a fertilized egg is replaced with a nucleus from another embryo.



John R. Ellis