Embryos, cells, genes – and Society

If one believes, as I do, that the task of science is not merely to understand the world, but to change it for the better, it is fortunate that determinism plays little part in biology, that the environment exerts such a profound influence on development, at all levels – the external environment, the maternal environment, the tissue environment, the intracellular environment. My own research has always been on mice, as the most appropriate animal model for our own species; it has never been directly applied, but there has always been some human relevance in mind. And I have always felt it important to tell people in general what I am doing and why, what other scientists are doing, and what are the implications (both good and bad) of scientific advances.

For twenty-two years I worked for the British Agricultural Research Council. First in London, Dr. Donald Michie and I kept mice at hot, cold and intermediate temperatures, and found that the temperature of rearing influenced not only external features (size, tail length, ear size) but also their variability. Then we used the previously pioneered technique of embryo transfer to analyse a maternal effect on number of lumbar vertebrae in crosses between two strains of mice. Was it the origin of the egg that was important, or the uterus in which the embryos developed? It turned out to be the uterus, and this remains one of the few good examples of the uterine environment influencing an anatomical character. Frustratingly it was impossible at that time to pursue the finding further: it would be thirty years before techniques for examining the molecular basis for gene expression during development came on line. With Dr. John Biggers, I used the same embryo transfer method to show that early embryos removed from the female and cultured for 24 hours in the laboratory would develop into normal fertile mice. That result was to have important consequences, which perhaps explains in part why I am here today.

Moving to Professor Waddington's Institute in Edinburgh, I worked for some years on implantation, seen at that time as a promising target for contraception. Although I made certain advances, I was again frustrated by the elusive nature of the crucial local signal for implantation that the embryo gives to the uterus, a signal that has still not been identified. At about the same time, Tarkowski invented his embryo-aggregation technique for making chimeras, and I realised that chimeras provided the ideal situation for examining the effect of a tissue environment of one genetic type on a cell of a different genetic type. With this in mind, I looked at various aspects of development, in particular hair colour and sexual differentiation. Today, of course, studies of cell-cell signalling can be conducted at the molecular level.

After Edinburgh, I worked for 18 years for the Medical Research Council, as Director of the newly constituted Mammalian Development Unit. Up to that time, the developmental biology of mammals had been a somewhat neglected and under-funded topic in Britain, at least from the medical point of view. Studies on sex determination with a number of wonderful colleagues led me to a consideration of the factors influencing the sex of germ cells, the all-important cells that eventually give rise to sperm and eggs. Throughout their lengthy and fascinating developmental history, germ cells have turned out to be closely dependent on their tissue environment. The unfertilised egg, the final product of female germ cell development, itself provides a unique cytoplasmic

environment, which can reprogramme a specialized cell nucleus to support development of a new cloned embryo.

If germ cells are removed from their normal environment and cultured in the presence of certain chemical factors in the laboratory, they change into stem cells, which will survive and proliferate indefinitely. These stem cells are pluripotent, meaning that they can give rise to any of the cell types in the adult body. We and others are concerned to find out how pluripotent stem cells can be induced to form pure populations of specialized cells, which in the human could be used to repair damaged or diseased tissues – for example, nerve cells for Parkinson's disease, heart muscle cells for heart disease, insulin-producing pancreatic cells for diabetes. With germ-cell-derived stem cells, it will be important to ensure that the marks imposed on the DNA of certain so-called imprinted genes during germ cell development do not cause any abnormalities in the specialized tissues to which the stem cells give rise. During the next ten to twenty years, stem cells derived from adults, from foetuses, or from early embryos, may well revolutionize the treatment of degenerative diseases. The ethical and social implications of this field are under active discussion in many countries. Japanese centres are in the forefront of the scientific advances and are also involved in this ethical debate.

Flexibility and patterning in early mammalian development: a dilemma for embryologists.

Mammals reproduce only sexually by fertilization of an egg-cell by a spermatozoon. Although both germ cells contribute equally to the genome of the resulting offspring, the egg is by far the more important partner: being much larger than the spermatozoon it contributes practically all the cytoplasm with all organelles (except a sperm-introduced centriole) and the cell membrane. The egg is a polarized cell, which means that its structural and molecular constituents are spatially arranged along the so called animal-vegetal polar axis in a unique way that insures development of an embryo, and finally of an individual, according to the stable and repeatable – from generation to generation – scenario. Another superiority of the egg-cell over the spermatozoon is that it is able to initiate development by itself (either spontaneously or as result of experimental intervention) and in some non-mammalian animals completes it effectively, i.e. gives rise to parthenogenetic progeny. The fate of unsuccessful spermatozoa is as a rule miserable – they pass away unnoticed.

The above arguments make it obvious why the egg is the most precious cell for the species in question and the most intriguing cell for embryologists. I have spent my scientific life on looking with the never-ending admiration on mouse eggs, mouse embryos and mice that developed from embryos that I had earlier subjected to various experimental treatments. My research has been always 'curiosity driven': at first I did not think of and probably even did not expect any practical applications of my embryological adventures. But with the passing time when more and more scientists became interested in mammalian gametes and embryos it became evident for me that experimental embryology can produce results that may turn out to be useful in animal breeding and human biotherapy.

Trying to recall the most important event that influenced and directed my research in experimental embryology, I came to the conclusion that it was a fortuitous observation of a two-cell mouse embryo in which one cell was accidentally destroyed. Immediately, I asked myself a question: would the remaining cell form a normal, though half-sized embryo, or would it form a handicapped and non-viable embryo? I transplanted the damaged embryo to the oviduct of a foster mother and in few days I knew the answer: at least up to the stage when embryos embed in the uterus a damaged embryo can develop normally. After many months of experimentation I produced a number of normal, adult fertile mice which developed from embryos in which I had intentionally destroyed one of the first two cells. When I learned that a part of the embryo can develop into a complete mouse, the next question I asked was whether two early embryos aggregated together would develop into one normal mouse or into a monstrous foetus (individual?)? The first option turned out to be true and we called thus produced animals – chimaeras. These two experiments – the first carried out in Poland and the second in Great Britain – proved that the early development of a mammalian embryo is extremely flexible, or – as the embryologists say – the embryos have great regulative capabilities. Further studies carried out upon my return to Poland have shown that up to the stage when the embryo is built of several cells, the fate of cells is not yet determined. At this stage cells have two developmental pathways to choose: either to become predecessors of the foetus and later the animal, or to contribute to the foetal membranes, the auxiliary structures that are discarded at birth. It was suggested that the decision as to the choice of one of these two ways depends on the position occupied by cells in the aggregation:

these situated inside will form the foetus, those located outside will built the auxiliary membranes. This idea is known as the 'outside-inside' hypothesis. During the next years many experimental embryologists have provided dozens of examples of great developmental flexibility of early embryos in several mammalian species. It has been shown that also single cells of the 4- and 8-cell embryos can develop into adult animals, and twins, triplets and quadruplets have been produced. Thousands of chimaeric animals have been created using different developmental stages and different techniques.

In recent years it has been pointed out that this great developmental flexibility of early embryonic cells is manifested only under experimental conditions *in vitro* and that when embryos are left undisturbed they develop according to a predetermined pattern which stem from the organization of the egg. There are thus two sources of information which apparently are in conflict, and have to be somehow reconciled. My proposition is that the mammalian egg and the early embryo have indeed a system of spatial organization and signalling that guarantees a repeatable course of developmental events. Lack of a morphogenetic pattern would result in the developmental chaos. However, each developmental system characterizes itself also by some (greater or smaller) regulative capabilities. In mammals these capabilities are very large but perhaps *in vivo*, i.e. in the maternal womb, embryos rarely make use of them for developmental repairs. But due to these abilities identical twins can spontaneously develop, and in this way Nature confirms the view of many experimental embryologists, including myself, that developmental patterning in early mammalian development is very labile. This, in turn, permits us to manipulate the embryonic development, hopefully to the benefit of biomedical sciences.