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Nature and status of the human embryo and its sanctity of life: biomedical points of view

First of all let me thank you for your kind invitation to participate in this symposium which is indeed of great interest to me. Concerning the title of my talk I regret the printing error that occurred in the announcement: I will indeed not be able to address anything else than some biomedical points of view, and I feel perfectly unable to cover the philosophical-ethical points of view since I am a simple physician and biologist. I will be very happy, on the other hand, if I can stimulate the experts in the fields of philosophy and ethics assembled here to think about some of the problems that I wish to raise.

What I would like to do in the limited time available is to concentrate on the early human embryo, not the fetus. Why bringing up the issue of the nature and status of the early human embryo with respect to sanctity of life now after so many discussions have already been held on this in recent years, stimulated by the problems of prenatal diagnosis, abortion laws, and in Germany in particular in connection with the German Embryo Protection Law?

It is because I feel new technical developments are forcing us to take a new look at this complex problem, namely the recently reported development of pluripotent or even totipotent stem cell lines in primates, cell lines that can be propagated apparently unlimited in vitro, and that have a Janus-faced character:

- bearing great promise as a perhaps ideal source of material for transplantation biology and medicine;

- and on the other hand showing the ability to differentiate in vitro to embryo-like structures and perhaps even to complete embryos as it appears, which, I feel, may possibly prohibit their use.

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I feel that these cell lines present features that are so remarkable that they should deserve much more attention than many of the news that have flooded the press recently like cloning experiments (sheep Dolly: Wilmut et al. 1997).

What are embryonic stem (ES) cell lines like? Before addressing these cells directly let me first go back briefly in history and describe features of a related cell type, the so-called embryonal carcinoma or EC-cells and I will come back to the ES cells later on. These EC cells are derived from tumors as occur spontaneously in the human and in animals but can also be produced experimentally in animals. In the latter case they are typically produced from early embryos and from germ line cells in the mouse. In human pathology a very strange type of tumor, not a malignant but a benign one, can be found in the ovary that has always astonished the observers: the so-called dermoid cysts. The tumorous mass that these cysts contain is typically composed of differentiated cell types representing various tissues in the body, may contain skin with hair, bones, cartilage, nervous tissue and so on. Sometimes the shape of a grossly malformed fetus can be reached as in the case illustrated here. What is typical, however, is that the regular organization and order of a well-formed harmonious fetus is not attained, and the various types of tissues are intermingled in an unpredictable way as histology reveals. This is an example from a so-called teratoma, a related type of tumor. The histologist notes a chaotic arrangement of well-differentiated tissues. Much excitement came up in the biomedical research society when it was reported years ago that such tumors can be produced easily experimentally by simple manipulation in certain strains of mice. As it turned out, in such mouse strains eggs in the ovary initiate embryonic development parthenogenetically and the tumors are derived from these atypically activated eggs. From these cells, cell lines can be produced in vitro and can be propagated. When transplanted to host animals they show very high growth rates and malignant characteristics, produce massive tumors without structural order, and kill the host animal within short time. In the light of this, experiments became famous in which such malignant embryonic carcinoma cells were transplanted into normal early embryos of the mouse like in this example where a group of such cells is being transplanted into the cavity of a blastocyst. A chimera is produced in this way. As also known from experiments with normal early embryonic cells, the transplanted cells can then participate in the formation of various organs of the chimera thus produced, and it is unpredictable to what extent they will contribute to the various organs. In this example

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where black coat color is used as a genetic marker it is shown that they can contribute to various degrees to the skin and hair of the animal but of course also to other organs. They can even enter the germ line. In the next slide a mouse is shown that was produced this way from a normal embryo plus EC-cells, and that this particular mouse is made up predominantly of transplanted, tumor-derived cells while the normal host cells remain in the background. In summary, an EC cell can, according to the experimental conditions, either just multiply and remain in a rather undifferentiated state, or develop into a teratoma, a tumor composed of differentiated cell types in addition to undifferentiated stem cells, but without any structural order (you remember the tumors I described in the beginning), or cells can be forced to behave in a rather normal and benign way by being introduced into a normal host embryo. This had raised hopes in certain circles of researchers in the seventies that these EC-cells could possibly be used at least as a model in transplantation biology and cloning experiments. It turned out, however (and this was not really a surprise), that EC-cells maintained the tendency to produce tumors in high incidence in vivo, meaning that their inherent malignant character cannot be eradicated totally in spite of their temporarily normalized behaviour, by transplantation into a normal embryo.

While this naturally cooled down the excitement, a new model of pluripotent cell types was developed in more recent years, the modern counterpart, so to say: ES-cells. They are benign , not malignant cells and are derived from normal early embryos in vitro, and if certain precautions are taken they can continue dividing without differentiating in vitro and can maintain at least pluripotency for a long time or perhaps forever. Mouse ES-cells are being produced in many laboratories routinely these days, and laboratory handbooks are available that describe the technique in detail. These cells are predominantly used as a means to introduce genes into the germ line of the mouse in order to study gene function. Mouse ES-cells can differentiate into any type of cells, individual ES-cell lines showing different of such traits. With a special experimental design it has been possible to demonstrate that ES cells can, on principle, even form a complete mouse, not only contribute to parts of it. In this case, ES cells were transplanted to host embryos much in the same way as described above for EC cells, but it was made sure (by a genetic trick) that the host embryo cells would be unable to survive any longer than the very early embryonic stages but would degenerate therafter, so that only the transplanted ES cells would survive and form alone all parts/organs of the developing mouse (Nagy et al., 1993). This experiment proves that mouse ES cells can show

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totipotency in the sense that they can form all derivatives of the embryoblast, and even a harmonious complete embryo proper (if helped at least temporarily by a host embryo). On the other hand, what has not been described in mouse ES-cell lines is the additional ability to form also the extra-embryonic cell types normally derived from an early embryo, including for example trophoblast in addition to embryoblast derivatives. Also spontaneous development of high structural order in vitro has not been described in the mouse, nor has been the autonomous formation of a complete embryo.

Attempts at producing ES-cell lines in farm animals are being made since years, here predominantly for cloning purposes. Success is still limited. Last year, however, a publication appeared on the production of such an ES-cell line in a primate, the marmoset monkey (Thomson et al., 1996). I like to draw your attention to this publication because the features reported on this cell line are most remarkable. When propagated on a feeder cell layer as usual for ES-cell culture, the cells can be maintained in an undifferentiated state. Remarkably, however, when these cells are allowed to differentiate by simply letting them grow at high density they develop embryo-like structures in vitro. The authors have published a picture of such a spontaneously developed early embryo-like structure that is nearly identical with a normal embryo as found in vivo. What can be seen here is the embryonic shield, the amnion and the amnionic cavity, the yolk sac. An early human embryo would appear nearly the same, and here is a schematic drawing of such an early human embryo showing the primitive ectoderm or epiblast, the embryonic cavity and the amnion, the yolk sac and the surrounding trophoblast. Evidence for the differentiation of trophoblast, the cell type that normally forms the fetal part of the placenta, was also found in the monkey ES-cell line so that apparently every cell type is produced here in vitro. Even more importantly, a primitive streak is also formed in vitro. The primitive streak is the structural equivalent of the formation of mesoderm and definitive endoderm and of the body axes. That a normal-looking primitive streak is formed in these embryo-like structures in vitro shows on one hand that they have probably to be addressed as real embryos. The primitive streak has been of utmost importance in all discussions about cloning, abortion and experimentation with human embryos. Formation of the primitive streak is thought to mark the onset of individuality, since monozygotic twinning is not possible after the primitive streak stage has been reached. Development of the embryo-like structures (or embryos) in the monkey cell line has not been followed further in vitro, as far as was reported in literature, but from the published data can be no doubt that development

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could have proceeded to even more advanced stages and there seems to be a high probability that well-formed embryos rather than teratomas can grow in this case in vitro, and, of course, these could be in large numbers of identital , cloned embryos.

ES-cells could be an ideal source of material for transplantation purposes in the human. We will of course never have enough donated human cells and organs to meet all transplantation needs, in spite of all efforts that are being made. Xeno-transplantation poses immunologic and animal protection problems. ES-cells are attractive because they can be influenced by various cytokines to differentiate into certain directions, e. g. into the direction of haematopoietic stem cells (bone marrow cells) that are of utmost interest in transplantation medicine (for animal experiments see e.g. Forrester et al., 1991; Palacios et al., 1995). Thus there can be no doubt that people will be much interested in producing human ES-cell lines.

Traditionally mammalian and human embryos are of course always thought to be necessarily derived from eggs. All discussions of the legal status of the embryo and of moral implications of embryo-related technology have this type of embryo in mind. Eggs are a rare type of cells, since only few egg cells are produced during reproductive years of a woman. Even when follicle growth and egg cell production in the ovary are stimulated hormonally remains the number of produced eggs relatively limited. [In parenthesis it may be added that our attitude seems to be different with respect to gametes that are produced in abundant quantity (namely sperm)]. What appears also relevant to our traditional thinking and arguing is the limited capacity of the female genital tract which can permit growth and give birth to only one or only a few embryos at a time. I feel that the public attention that has been payed and is being payed for example to experiments on cloning farm animals like the sheep Dolly (see above) using technologies based on egg-derived embryos may not be justified, at least not in comparison with the potential that lies in ES-cells, since all these egg - based procedures still meet considerable technical problems, need a lot of time and effort and can therefore be done only at a very limited scale due to the limited availability of only a few eggs. The situation would obviously be different if the same type of thing would be done with embryonic stem cells that can be produced in unlimited quantity. Similarly, attempts to use fetal cells and tissues for transplantation purposes necessarily meet restrictions as long as these materials have to be obtained from traditional, egg-derived embryos or fetuses, and even if one approves of such

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procedures for human use it is clear that the number of embryos or fetuses for example from abortions will still always be limited. I do not want to overburden this talk, and since also time is short I will not discuss the legal and ethical problems generally connected with fetal transplants.

Let me briefly discuss the properties and the status of ES cells as compared to eggs from the biological point of view and point out the following:

Transplantation experiments as desribed above for EC cells had revealed a major role of structural order. When EC cells were transplanted to ectopic sites, teratomas (lacking structural order although exhibiting all types of differentiated cells) developed. However we have seen that the same type of cells can contribute to the formation of perfectly normal organs if they are put into the fields of factors of a normal embryo.

This suggests that a major organizing element in an egg or embryo may be positional information, structuring cues, asymmetric distribution of molecules, and these direct the formation of axes (dorsovertral, anterior-posterior, proximo-distal)**.** That this can theoretically be achieved by very simple physicochemical mechanisms has been shown since many years in computer models as described by Meinhardt (for recent reviews, see Meinhardt, 1989, 1996). Very related are the modern concepts about axis formation in the early insect embryo (St. Johnston and Nüsslein-Volhard, 1992). Genes and gene products are being discovered that appear to be the main players in this game. There is evidence that these genes seem to be basically similar in vertebrates including mammals and man.

With respect to a comparison of ES cells and eggs one would expect, on this background, that basic molecular events and developmental mechanisms are similar in both systems. But the specific feature of the egg seems to be that it provides a very simple pre-pattern and by this way secures a degree of structural order for the orthotopic formation of body axes and so on so that also the subsequent patterning of all tissues in the emerging embryo will lead to the formation of a harmonious, functional whole. It is a peculiar feature of eggs, on the other hand, that they can regulate defects and restitute such a harmonious whole, and it is probably due to these mechanisms that , as we have seen in the example of ES cells in culture, order can also develop spontaneously in a cell monolayer, not dependent on an egg-derived pre-

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pattern. In this case probably stochastic molecular events (of the type that can perhaps be described best by chaos theory) generate a first simple asymmetry. Eggs, early embryonic cells (blastomeres) and ES cells have the ability to initiate a wave of hierarchically dependent (and vectorial) processes of differentiation, cell movements and cell-to-cell interactions that is probably identical in all these cases and leads to the development of more complex order.

An old aphorism, based on one of the founders of experimental embryology, Carl Ernst von Baer, states: „Entwicklung ist Umbildung von einer Gestalt zur anderen“ (as formulated by Seidel, 1960). It may have to be re-formulated or re-interpreted in the light of the remarkable pattern-forming abilities of the ES cells.

The German Embryo Protection Law has ruled:

> „Als Embryo im Sinne dieses Gesetzes gilt bereits die befruchtete entwicklungsfähige menschliche Einzelle vom Zeitpunkt der Kernverschmelzung an. Ferner jede einem Embryo entnommene totipotente Zelle, die sich bei Vorliegen der dafür erforderlichen weiteren Voraussetzungen zu teilen und zu einem Individuum zu entwickeln vermag.“
> *(Gesetz zum Schutz von Embryonen, ESchG, von 1990, § 8)*

Obviously this definition includes, without saying, human ES cells. Let me , at the end, simply state that I am personally supportive of this law.

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