THE HUMAN EMBRYO AS AN ORGANISM...AND ONE OF US.

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HISTORICAL INTRODUCTION

If a zygote obtained by in vitro fertilization is transferred at the pronuclear stage into the uterine mucosa, it is expected to develop into a baby - and not into any baby, but into one with definite family traits derived from the donors of the spem and ovum. The very existence of the IVF industry bears witness to the fact that the determination of the individuals of the human race is basically established just after the fusion of the gametes.

However the habit of handling embryos in manners entirely disregarding their human condition (freezing for example) has required for its justification the articulation of ideological discourses which would place the initiation of human life at diverse moments of embryonic development. These speculations tend to obscure the obvious implications of the discovery of the cellular phenomena of fertilization which for almost a century had been regarded as clear evidence that the life of the biological individual started with the fusion of the gametes.

In recent times however, repeated attempts have been made to establish some time point in the course of development before which adequate biological evidence could not be produced for the existence of a human individual. It is beyond the scope of this talk to make a review of the criteria that have been proposed. Two of them may however be recalled, namely the time of dissolution of the pronuclear envelopes when the chromosomes align for the fist cleavage division. (ca 24 hours) and the time of implantation in the uterine mucosa (ca 14 days). In the context of these proposals, old ideas of "delayed animation" have been once more put forward.

It seems therefore advisable to make a short historical introduction to remind how the early developments of the cell theory affected notions on the initiation of individual life.

The fact that fertilization involves the fusion of gametes was a discovery made within the framework of the early cell theory. The latter may not be said to have been properly stated before Remak and Virchow had established that cells take origin from other cells

(Virchow's dictum "omnis cellula a cellula", later "omnis cellula e cellula), and before Virchow himself had proposed that the functions of a living organism depend upon the activity of its anatomical units, the cells. (1852-1854). This is important in the context of the present talk because thirteen years before these advances, in 1839, Theodor Schwann who had first proposed the "Zellentheorie" (Cell theory) regarded the cells as small developmental units originated by some kind of crystallisation that took place within an amorphous unorganized living mass, the "cytoblastema". The cell was then not considered as the basic unit of the living organism, but as "Entwicklungsprinzip", (principle of development). The existence of an unorganized stage in the individual development was not alien to the biological thought of the time. In a much coarser and more primitive manner it had been held that the beginning of individual life was connected to the coagulation of some fluid (either menstrual or seminal). Within this perspective there was nothing unsound about the idea of a gradual establishment of individual life.

An end was put to this view by the maturation of the cell theory. It was shown by Hertwig that the start of individual development was marked by the fusion of two cells, and that it was therefore a very precise observational landmark. No evidence of an acellular or unorganized stage in individual development was ever found. In other words the idea that a developping organism went through a stage at which it was not really an organism, became untenable.

It is obvious that many philosophical or theological speculations on "delayed animation" which for centuries had been accepted because of grossly inadequate factual basis could not hold any longer. The ideas of delayed animation being voiced again may not claim support from old philosophical notions that were put forward in complete ignorance of the simplest facts of early development. As it is in the last twenty years the need to justify interventions against early human life has produced a rather confusing intermingling of scientific and philosophical discourse .

I think it is worthwhile to try and make a theoretical survey of the early stages of development in purely scientific terms, trying to remain separated from philosophical discourse as far as it may be possible to do so.

THE NOTION OF ORGANISM

My basic contention is that the human embryo is an organism of the human species, and that this condition is established at the moment of fertilization. I will start by a discussion of the notion of an organism, which shows how the embryo fits into this notion from the moment of the gamete fusion. The last minutes of the talk will be taken up by a brief overview of the stament that this organism is "one of us".

It is a matter of common knowledge **a)** that all stages of development take place within an enclosure i.e. within a space separated from the environment by a well defined physical boundary, and **b)** that every organism follows a "prescribed", robust, developmental pathway, i.e. that its state at any time point within its life course <u>can be predicted</u> with reasonable accuracy, if the species to which the organism belongs be known.

a) The requirement of the boundary is essential. It means that development takes place within an enclosure. The enclosure may be built up by a combination of biological structures. In extrauterine life one may think of the keratinized cells of the epidermis, the cell membrane of the digestive tube lining etc. The very early stages of development show boundaries built up by the cell membranes and the zona pellucida, etc. The functions of the boundary are related to mechanical integrity and to regulation of the internal milieu. The various means of ensuring the enclosure of the developping embryo start from the moment of incorporation of the sperm head. At this stage the zygote plasmalemma incorporates patches of the spermatozoon membrane, as can be demonstrated by suitable markers, while the zona pellucida becomes chemically modified by the cortical reaction, related to the blocking of polyspermy. The importance of the morphogenetic role of the zona pellucida may be inferred from the fact that spontaneous or induced alterations in its architecture may produce monozygotic twinning by interfering with the normal "hatching" process.

b) The boundary of the organism circumscribes the space where development takes place. Developmental pathways are the most important features of organic growth. The real difference between a zygote and any other cell is the fact that the structure and function of the zygote at any moment are points in a peculiar trajectory of development which includes partitioning of the space through cell multiplication, growth and differentiation into multiple varieties of cells. At any stage of development, the future state of a multicellular individual can be predicted with great accuracy from the experience obtained from other individuals of the species. These seemingly obvious considerations are disregarded when attention is given to the instantaneous features of a living form and its prospective features are left aside. The restrictive labelling of a zygote or an embryo as human "cell" or "tissues" is grosly misleading and does not fit the existing biological evidence.

The developmental pathway is made possible by the physicochemical conditions of the living matter. These should be given some attention at this moment.

From a thermodynamic point of view, organisms may be thought of as chemical reactors, which interact with the environment exchanging matter and energy whose constant influx maintains the chemical components at concentrations which are far removed from their points of equilibrium.

Two very distinctive processes in the life of organisms are body growth and the replacement of the component molecules by turn over. These are made possible by autocatalytic reactions, i.e. chemical reactions in which the reactants appear as products. A very simple instance could be written down as:

$A + B \quad 2A+C$

It has been shown that autocatalytic systems exhibit a spontaneous tendency to selforganization. In ordinary physicochemical systems however, these changes need not show the stability and self-regulation which are caracteristic of living systems.

Stability and self-regulation which are fundamental properties of living units, are especially evident in the course of developmental pathways.

This has been aptly formulated by P. T. Saunders (The organism as a dynamical system in "Thinking about biology", Stein and Varela editors, Addison Wesley 1993) referring to embryonic development: "One of the most characteristic properties of the developmental process is that it is stable... What is stable is not the state of the embryo at any one time but its pathway of development".

One necessary condition for the existence of stable developmental pathways (as well as of other regulated life phenomena) is that the thermodynamic living system be the seat of orderly dynamics.

With computational modelling it can be inferred that very complex systems formed by large number of units may exhibit orderly dynamics provided each of the units interacts only with a few others. If interactions are established bertween large numbers of units, the behaviour of the system becomes chaotic. (S. Kaufman)

Starting from the behaviour of these models, it is interesting to speculate upon the nature of the chemical species that would be likely to become involved in orderly dynamics. The main components of organisms are macromolecules, mainly nucleic acids, proteins and polysaccharides. These biopolymers are formed by linear arrays of monomeric units whose number and sequence determine the functional properties of the polymer. An enzyme for instance depends critically on the aminoacid sequence at the active site and other accessory emplacements, such as the allosteric site. If the polymers be taken to be a sequence of symbols, there is a definite number of yes/no decisions which are necessary to establish unambiguously a particular sequence. In this sense biological macromolecules have a high information content <u>with regard to the particular function they perform</u>. As a consequence they will be likely to be involved only in a limited number of reactions.

This goes to suggest that the presence of stable (homeorhetic) predictable trajectories in the course of the life of an organism may be regarded as one of the consequences of the fact that this system is a complex non-linear dynamical system made up mainly of molecules with high informational content. Therefore a stable pathway of development might be thought of as one direct consequence of the physicochemical features of the system. Conversely it may be said that its physicochemical nature requires that the diachronical dimension be considered essential for the characterization of an organism at any moment of its lifetime.

The evolution of complex systems is quite sensitive to initial conditions. On the other hand their behaviour is remarkably stable in face of many isolated changes even though some intercurrent alterations may become amplified in the course of its evolution.

PATHWAYS IN EARLY DEVELOPMENT

An overview of the course of some developmental phenomena will show, **i)** that early developmental processes involve dynamic interaction and cooperation of chemical constituents coming from several sources, i.e. both gametes and embryo, the whole of which become interwoven into one chain of events, so that well defined morphological or biochemical events in the course of development have no other meaning than that of discernible points within an ongoing process, no discontinuity being observable; and, **ii)** that the main features of the developmental process are intrinsically robust.

The continuous chain of the developmental processes and the relatively artificial nature of its segmentation into discrete events may be illustrated by several examples. I will choose the evolution of the pronucleus, the first segmentation division; compaction and initiation of protein synthesis; while **ii)** the robustness of developmental processes may be illustrated by chimaerism and genetic mosaics

i) Continuity in developmental pathways.

Evolution of the male pronucleus.

The expression "male pronucleus" is sometimes taken to mean that this structure is really a derivative of the spermatozoon head, and this misconception is in turn applied for placing the fertilization event at the moment of meeting of the chromosomal contents of both pronuclei. Actually the "male pronucleus" is a transient organelle of the fertilized egg. This is made clear from a consideration of its composition and origin.

As the sperm head enters the egg, its nuclear envelope becomes almost entirely disintegrated and lost into the cytoplasm which is thus brought into direct contact with the chromatin. Morphologically a decondensation of the chromatin is observed, while the biochemical counterpart is a reduction of disulphide bonds and a massive replacement of the sperm protamines by egg histones. The growth of the pronucleus seems to be associated with egg cytoplasmic factors and is followed by DNA synthesis and chromosome formation. The latter phenomena are known to require a protein complement, especially cyclins which are likely to be provided by the egg cytoplasm. Just before the formation of the pronucleus a recondensation of chromatin takes place.

The pronucleus is bounded by a structure which has all the morphological features of a regular nuclear envelope, including pores. This pronuclear envelope becomes formed mainly from endoplasmic reticulum membranes.

It is thus clear that the majority of the components of the pronucleus are formed or incorporated <u>after fertilization</u>. Only one half of the DNA could rightly be said to have been introduced with the sperm head.

The formation and evolution of the pronucleus is thus a complex morphogenetic process involving interaction between components of both gametes. These take part in a common morphogenetic pathway from the moment that it is a sequence of predictable steps, which may be thought of as the expression of an orderly dynamic system.

<u>First segmentation division.</u>

A well-defined morphogenetic pathway is seen at operation even before embryonic gene expression has reached any important degree.

Cytokinesis or actual cell division is observed ca 24 hours after penetration of the sperm, but events directly leading to it have been taking place for several hours before. Synthesis of DNA starts at the pronuclear stage , and is first observed in the "male pronucleus". The formation and orientation of the spindle involve the action of the sperm centrosome as microtubule organization center for egg tubulin as a result of which fact the mitotic potential of the human zygote is inherited from the male gamete.

<u>Compaction</u>

An equally clear instance of interaction between sperm and egg biomolecules is shown by the chain of events leading to compaction.

This striking event takes place at a time from the eight- to the sixteen- cell stage in the mouse. The blastomeres which until the four cell stage found themselves loosely bound within the space limited by the zona pellucida, come to form a compact mass of intimately adhering cells. Two types of junction make their appearance. Tight junctions are the first step toward the establishment of cell polarity and blastocoelic cavity. Gap junction formation mediates the onset of new intercellular connections, demonstrable through ionic coupling and transcellular passage of medium sized molecules.

This very noticeable point of development is only the culmination of a succession of events first detectable several cell cycles before. The work by Kidder and McLachlin shows that compaction is embryonically rather than maternally programmed, the necessary transcription being completed in the mouse by the four cell stage. The blocking of DNA replication by aphidicolin applied for ten hours at the two cell stage inhibits compaction two cycles later. Moreover as has been pointed out by Kidder, transcription of most genes is not temporally linked to compaction.

The main agent in cell adhesion is the protein E-cadherin, which is already present at the surface of the unfertilized egg. The earliest evidence of its synthesis de novo has been reported at the two cell stage. Activation of E-cadherin at the time of compaction is usually adscribed to post-translational changes .

Biosynthesis of gap junction components is similarly timed. mRNAs for Connexion 43, are fiirst expressed at the four cell stage .

Immunoblot analysis detects ZO1 a characteristic component of tight junctions at the late four cell stage.

These facts are in keeping with the statement by Kidder that the large majority of genes in the mouse whose mRNAs are present in the blastocyst are already being transcribed in the four cell stage.

The morphological event of compaction is thus seen as one step in a continuous chain of biochemical events.

Protein synthesis.

This is a gradually established function which at its earliest stages requires the cooperation of biomolecules of diverse origins.

As has been mentioned there are important events in the developmental pathway which make their appearance before any significant degree of activation of the embryonic genome can be demonstrated.

Kidder has remarked that most genes that are expressed during preimplantation are already being transcribed in the four cell stage, and some even earlier, at the two cell stage. This leads him to conclude that temporal regulation (expressed in cooperation to morphogenesis) is posttranscriptionally effected. Therefore transcripts and proteins formed at one stage become functional after several cleavage divisions .

The transition period between development dependent on maternally derived gene transcripts and the initiation of transcriptional activity by the embryonic genome occurs in the mouse during the two cell stage. But several studies demonstrate instances of protein synthesis even in the one cell stage at which moment the acquisition by the cytoplasm of a transcriptionally permissive state has been reported. Bouniol has shown that endogenous trascription in the mouse begins in the male pronucleus, and it has been demonstrated that the latter is capable of expressing firefly luciferase which has been incorporated into transgenic mice. On the other hand, zygote genome activation occurs in other species at various stages of cleavage, with four (pig), twelve (cow) and sixteen (sheep) cells .

Human embryos have been shown to synthesize new polypeptides at the four cell stage and sexual differentiation of growth rates has been detected at the the same period..

Taken together these points suggest that no single developmental event may be considered as separate from a pathway which precedes and follows its appearance. It is also apparent that as far as they can be identified, the various processes overlap in time, so that attempts to determine points of discontinuity in development do not seem to fit together with the biological evidence.

ii)<u>Robust pathways.</u>

Robustness is a characteristic of many types of orderly dynamics. Early embryonic development shows some striking examples of stability of evolution (homeorhesis in the expression coined by Waddington, see 40).

Genetic chimaeras are formed spontaneously and they can be manufactured by various experimental procedures. A full discussion of the subject would be far too lengthy, but I think that some of the results reported by Mintz are particularly illuminating in that they illustrate the robustness of embryonic developmental pathways.

The injection of a single genetically marked cancer cell into the blastocoelic cavity may result in a genetic mosaic in which the marker is distributed throughout the adult animal in tissues derived from all blastodermic sheets. Indeed a rough estimate has been made that about one third of the animal tissues would derive from this single injected cell. This finding is especially interesting in view of the fact that the inner cell mass (ICM) is made up of no more than fifteen cells. Markert has presented evidence from experimental chimerism obtained by fusion of early zona-free embryos that no more than three cells from the inner cell mass will eventually go into the formation of the embryo proper, while the rest is destined to originate extraembryonic tissues. It is therefore likely that one third of the embryo originating cells can be replaced by foreign tissue without substantially altering the continuous developmental pathway.

THE POINT OF DISCONTINUITY

There is but one point in the developmental pathway that is in a sense qualitatevely different from all the others, <u>and this is when the developmental pathway starts</u>, i.e. when through the fusion of gametes, a new evolving dynamic system becomes enclosed by a physical boundary. As has been stressed along this paper this moment is marked by the membrane fusion of the gametes and the cortical reaction of the oocyte. At earlier moments the developmental pathways of the gametes were essentially independent from one another. Immediately after fusion both cells integrate into a single trajectory where they interact in an orderly and predictable way which is essentially the same for all individuals of the species.

The idea is here presented that the developmental pathway which is one main distinctive feature of an organism in general, is prominent in very early stages of embryonic life. Order and robustness are characteristics both of developmental pathways and are the behaviour to be expected of certain dynamic systems. At this period of incipient differentiation it becomes easier to connect development to orderly dynamics resulting from the interaction of molecules of high informational content even though they are of diverse origins.

It is obvious that as development proceeds, the most important information carrying molecules in action will be those of the genome. Their functions of replication and transcription ensure that the proper information is passed for the continuing synthesis of biomolecules adequate for ongoing development and function. However the discussion about the moment in which the embryonic genome takes over, important as it certainly is, should not make us forget that coordinated, predictable and robust pathways of development leading eventually into the adult animal, are present much before zygote genome activation takes place.

Starting at the moment of fertilization, the human embryo shows one fundamental property of a living organism which is a predictable, stable, robust developmental pathway. "A living organism of the human species" is another way of saying "a living human body" with all the philosophical implications which this expression carries with itself.

ONE OF US

The developmental pathway in an organism leads always through states that are proper to all individuals of the species concerned. It may be true that various biological entities (for example a tumour) go through stages of development. What is proper to any developmental pathway in an organism however is precisely that it is inscribed in the constituent process of the organism itself. Higher brain functions for instance form a continuous developmental line beginning far back in the early genome expression which if interfered with will result in an impaired or destroyed function. This developmental pathway is intertwined into all the rest of pathways of the organism, just as was seen a moment ago for several embryonic development lines. It is not an arbitrary expectation to think that the proper choice of gametes to be fused will result in definite skin colour, height or body build, intelligence quotient etc. Just as one might rightly speak of a two cell embryo as belonging to the white race, one might speak of it as being rational, because there is a reasonable presumption that at a definite and predictible time it will be exerting higher brain functions as any member of the human race of his /her age.

This amounts to saying that there is an important difference between developmental pathways in an organism and the evolution of other systems biological or otherwise. The organism repeats the development of every individual of the species: the appearance of a higher function may not be regarded as one of the possible outcomes of an evolution. Indeed, if this appearance does not take place, one may speak of an abnormality of development. Once the zygote is established, a definite series of changes is expected to unfold.

It is considerations such as these that form the basis for a proper consideration of our relation to the embryo. An organism of the human species, even though it find itself at a different stage of development when compared to the observer, is really and fully one of us. There is no deformation of the facts in stating that the embryo is a rational individual embarked upon a unique course of life, following a unique destiny, and that this course of life and destiny are interwoven into those of all human creatures. In this sense the embryo provides a privileged place where to look for some fundamental human relations..

Even before any interpersonal contact is possible the presence of man introduces a new dimension into the world around. I remember the old tales about a solitary inhabitant of a remote oceanic island, who has been for years living in entire isolation and one morning discovers the traces of human steps on the sand in the beach. For years he has known pleasure and fear before his environment. It is not pleasure or fear which are the new feelings before these disembodied marks of human presence which profoundly alter his life and outlook. He is aware of an independent freedom. For better or for worse he knows now that there is around some unknown and that there is a mutual responsibility for each other, and that each has a right to be "received" by the other. Responsibility toward one and recognition of one are new ingredients in a world where joy and pain and fear had already their large places. The new situation is that The "Other" has made his appearance, and wakes up responsibility and demands an attitude oif hospitality.

The embryo is not a possible organism of the human species. He is an actual organism even thoughmany of its functions have not yet attained visible presence. In a sense it is evidence of the very certain presence of a human being. The appearance of the embryo reshapes the world around it. One is responsible for it and before it, and one is demandae to receive it, on exactly the same basis as one expects to be received and demands of the others to be responsible for one.

I prefer this way to look at things rather than the often employed term "respect", or "proper respect". These do not often convey the sense of an absolute command regarding the behaviour toward the other which is inherent to the consideration of the human person. One may respect the works of man: one is responsible only before man and God. One may enjoy the beauties of nature: hospitality is accorded only to the fellow man.