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The embryo as an organism.

In the years that followed the introduction of artificial fertilization techniques, a number of attempts were made to redefine the moment of initiation of "individual human life". This was felt to be required by legal and ethical reasons related to the admissibility of manipulations such as cryopreservation or microsurgery.

The idea was 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 article to make a review of the criteria that have been proposed. Two of them may however be recalled, namely the time of implantation in the uterine mucosa (ca 14 days), and the time of dissolution of the pronuclear envelopes when the chromosomes align for the fist clevage division. (ca 24 hours). These choices seemed more closely related to the uses that were envisaged for "embryonic material" than to unequivocal biological evidence.

It appears that a more accurate characterization of the embryo as a biological object might be useful toward answering the question of the time of beginning of its life course. I attempt here to sketch some lines for this characterization , purposely avoiding any arguments that might seem alien to normal scientific discussion. It is expected that this approach should prove useful in providing a ground of debate that is common to developmental biologists, to members of the medical profession and to bioethicists.

The following discussion is restricted to the development of mammals because of its direct relevance to human embryonic life. It will however be apparent that it could be partly extended to other living forms.

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I propose here to explore the meaning and consequences of the statement that the human embryo is an organism belonging to the human species. With this purpose it will first be necessary to describe some general features of mammalian organisms relating them to the basic organization of living matter. Once a general picture is established it will be possible to determine how the embryo fits into it.

It is a matter of common knowledge **a)** that every organism follows a "prescribed" robust(16), developmental pathway, i.e. that its state at any time point within its life course can be predicted with reasonable accuracy; and **b)** that the states along this pathway take place within a discrete unit of living matter, i.e. within a space separated from the environment by a well defined physical boundary.

A.- The "pathway" requirement has some interesting implications.

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 2A+C

It has been shown that autocatalytic systems exhibit a spontaneous tendency to bifurcation in the phase space and to symmetry breaking, in other words to selforganization (36,37). In ordinary physicochemical systems however, these changes show none of the stability and self-regulation which are prominent in living systems.

The latter characteristics have been aptly summarized by Saunders (40) 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."

It seems reasonable to think that a stable trajectory of development corresponds to underlying orderly dynamics. It might therefore be asked under what conditions might a chemical reactor exhibit such dynamics.

An interesting insight into this question is given by the analogy with Boolean dynamical networks (24,25,45,46).

A very simple yet instructive instance is shown in Figures 1, 2 and 3. In Fig. 1 a "string" of "cells" is portrayed each of which may find itself either in an "on" or an "off" state. The state of each cell at t=m depends exclusively on the state of its two neighbors at t=m-1. In the case under consideration the system follows a very simple "exclusive or" rule. This means that C_i at t=m will be "on" only in the case that either C_{i-1} or C_{i+1} was "on" at t=m-1. If on the contrary C_{i-1} and C_{i+1} were <u>both</u> "on" or <u>both</u> "off" at t=m-1 then C_i will be "off" at t=m.

The analogy is obvious with the model of a genome where gene products would act exclusively on the neighboring genes according to an "exclusive or" rule. (24)

However at this moment I want to stress a different view of the model. Figure 2A shows the result of iteration of the simple rule explained. It can be seen that a very definite pattern develops, which if followed long enough will repeat itself which may be interpreteted by saying that the trajectory of the system entered the basin of a cyclic attractor. This behaviour exhibits a remarkable stability. Figure 2B shows the case of a more complex set of rules with the same initial

conditions. (For a detailed discussion of the behaviour of this kind of systems, see the above mentioned references).

A close consideration of this model throws some light on the significance of orderly dynamics in organic development. Even the apparent simplicity of the "exclusive or" rule is quite deceptive. The very fact that any "cell" can be influenced only by two others suggests a high selectivity of the interaction rules. If the "cells" are thought of as reacting chemical species, this means that there is a high specificity of reactions , each of the chemical species present being able to react only with another two. Such a behaviour might be expected for instance of enzymes whose chemical configuration allows them to take part in a very limited number of different reactions. As mentioned below this may be expressed by saying that each of the "cells" forming the string represents a molecule with high informational content .

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

A counterexample will render the meaning clearer. If instead of having only two "cells" determine the state of each member of the string, one may define a different "wiring" whereby a large number (in the extreme, the totality) of the "cells" have influence on the behaviour of each one. In this limit all "cells" are equivalent to one another i.e. they have a low informational content. In this case (K=N in the symbolism employed by Kauffman) and instead of orderly trajectories, the system will exhibit chaotic behaviour.(24,25)

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> (see 10). 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 expression 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 a 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.

This approach is quite generic. It applies equally to a single cell and to a multicellular organism.

B.- The "discrete unit" condition means that an organism is separated from the environment by a well defined physical boundary. This is obvious for a fully developped organism. In the case of the very early embryo, the boundary is built up both by the the plasma mebrane and the zona pellucida until "hatching" time, under conditions which are briefly discussed below. (6, 9 17)

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The basic requirements discussed above seem to be met by the zygote from the very moment of membrane fusion between sperm and egg.

A.-Selforganization through interaction between components of the former gametes begins almost immediately and proceeds in a remarkably orderly fashion. Conclusive evidence for the early start and the robustness of the developmental pathway is of course afforded by the success attained in producing live fetuses and new born individuals after embryo transfer carried out as early as the pronucleus stage.

Furthermore, it is clear that the main traits of the early steps of development are relatively independent of the presence of the normal environment. This is shown by the work of Hsu who cultivated embryos "in vitro" until the stage of ten somites with beginning organ differentiation. (20,21).

B.- As for the requirement of the boundary, it is clearly fulfilled by various means from the moment of incorporation of the sperm head onwards. The early steps of development proceed within physical barriers established by the plasma membrane and the zona pellucida . Both of these structures become modified during the earliest phases of development. The zygote plasmalemma has incorporated patches of the spermatozoon membrane, as can be demonstrated by suitable markers (47), while the zona pellucida has been chemically modified by the cortical reaction, related to the blocking of polyspermy. (7)

The importance of the morphogenetic role of the zona pellucida may be inferred from the fact that spontaneous (2) or induced (17,22) alterations in its architecture may produce monozygotic twinning by interfering with the normal "hatching" process.

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An overview of the course of some developmental phenomena will show, **i)** 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; **ii)** 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, and, **iii)** that the main features of the developmental process are intrinsically robust.

These statements may be illustrated by **i)** the first segmentation division; **ii)** compaction, **iii)** protein synthesis and **iv)** chimaerism and genetic mosaics.

A.- First segmentation division.

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

Cytokinesis is observed ca 24 hours after penetration of the sperm. (35) Events directly leading to it have been taking place for several hours before. They involve not only biomolecules but also structures directly provided by the gametes, which through their interaction initiate a common developmental pathway.

These aspects are clearly brought forward by the beginning of the S phase of the first cleavage division and by the formation and orientation of the spindle.

Synthesis of DNA starts at the pronuclear stage ( ), and is first observed in the male pronucleus. In this latter case it will necessarily involve an interaction of the male chromatin with egg enzymes. Prior to this moment, the formation of the male pronucleus has been preceded by decondensation of the sperm chromatin (14), and its subsequent recondensation (1). These processes involve a replacement of sperm protamines by egg histones.

An equally clear instance of interaction between sperm and egg structures is shown by the action of the sperm centrosome as microtubule organization center for egg tubulin (41), as a result of which fact fact the mitotic potential of the human zygote is inherited from the male gamete (Palermo, 38)

B.- Compaction.

This striking event takes place at a time from the eight- to the sixteen- cell stage in the mouse.(8) The blastomeres which were loosely bound together at the four cell stage, 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 (18). Gap junction formation mediates the onset of new intercellular connections, demonstrable through ionic coupling (12) and transcellular passage of medium sized molecules (3,12).

Biomolecular studies show that morphogenesis of these structures is inserted in a gradual process.

The work by Kidder and McLachlin (28,32,43) shows that compaction is embryonically rather than maternally programmed, the necessary transcription being completed in the mouse by the four cell stage. However as has been pointed out by Kidder (27), transcription of most genes is not temporally linked to compaction. Furthermore the blocking of DNA replication by aphidicolin applied for ten hours at the two cell stage inhibits compaction two cycles later. (27).

The main agent in cell adhesion is the protein E-cadherin (12,13,18,19,26,29), which is already present at the surface of the egg. The earliest evidence of its synthesis de novo has been reported at the two cell stage (12). Activation of E-cadherin at the time of compaction is usually adscribed to post-translational changes (18,19)

Biosynthesis of gap junction components is similarly timed. mRNAs for Connexin 43 are fiirst expressed at the four cell stage (3,27).

Immunoblot analysis detects ZO1 a characteristic component of tight junctions at the late four cell stage (13).

These facts are in keeping with the statement by Kidder (27) that the large majority of genes in the mouse whose mRNAs are present in the blastocys are already being transcribed in the four cell stage.

The morphological event of compaction is thus seen as one step in a continuous developmental pathway.

C.- Protein synthesis.

This is a gradually established function which at its earliest stages certainly 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 (27) 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 (27,41). But several studies demonstrate instances of protein synthesis even in the one cell stage (30) at which moment the acquisition by the cytoplasm of a transcriptionally

permissive state has been reported (31). Bouniol (4), has shown that endogenous trascription in the mouse begins in the male pronucleus, and it has been demonstrated (34) 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 (42).

Human embryos have been shown to synthesize new polypeptides at the four cell stage (5) and sexual differentiation of growth rates has been detected at the the same period. (39)..

Taken together the three points brought forward under A.-, B.- and C, 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.

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D.- Robust pathways.

Robustness is a characteristic of many types of orderly dynamics. Early embryonic development shows some striking examples of stability of evolution (16) (homeorhesis in the expression coined by Waddington, see 40).

Besides the already mentioned development of cultured embryos (20,21), I would like to bring forward the case of chimaeras and genetic mosaics.

Genetic chimaeras are formed spontaneously (44) and they can be manufactured by various experimental procedures. (11,15,23,33). 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. (23). 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 (33) has presented evidence from experimental chiamerism 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.

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The point of discontinuity.

There is but one point in the developmental pathway that is in a sense qualitatevely different from all the others, and this is when the developmental pathway starts, 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.

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A postscript.

The idea is here presented that the developmental pathway which is the foremost 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 of 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 action ensures 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 some essential features of living organisms are present in embryos even before that event.

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.