

A review
Historical evolution of preformistic versus neoformistic (epigenetic)
thinking in embryology

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To the memory of Professor Doctor Lucien Vakaet sr

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ABSTRACT. In the classical embryology there exist two main concepts to explain the rising of a living being. At one hand there exists the theory of preformation i.e. all the parts of the future embryo would already exist preformed during the preembryonic or early embryonic period. On the other hand there is the epigenetic view in which it is propounded that all parts arise by neoformation from interacting (induction) previously existing, apparently simpler structures. We found evidence that initially a kind of preformation (or pre-existence), disposed in concentric circular layers, exists in the full grown oocyte and early germ. After normal development these circular layers will settle successively, from the centrum to the periphery, into the central nervous system, the notochord, somites, lateral plates (coelom) definitive endoderm. Under influence of mechanical and gravitational forces an early epigenetic development starts by unequal oblique uptake (unidirectional chaos) and segregation of ooplasmic determinants in the germ. So an epigenetic cascade of early general body plan formation occurs in the vertebrate embryo. Recently, we demonstrated by hemi-sectioning of avian unincubated blastoderms that both a kind of preformational (mosaic) as epigenetic (regulation) behaviour depends on the spatial, ooplasmic distribution of Rauber's sickle material, homologous to the also sickle-shaped Wnt expressing gene region in ascidians. This clearly brings preformation and epigenesis much closer together since there is a common influencing ooplasmic factor. An ooplasmic continuity bridging the premature oocyte ooplasm to the embryonic primordial germ cells of the following generation is demonstrated. Both nuclear and ooplasmic continuity in the oocytes, present in the ovary of birds and mammals, is shown by radioactive premeiotic DNA labelling.

KEY WORDS : preformation, mosaicism, epigenesis, predispositions, ooplasmic determinants.

INTRODUCTION

Among all biological topics, embryology (developmental biology) presents most uniformity in the animal and even in the vegetal kingdom. So, not with standing the pronounced structural differences between species, it forms a common link among all living organisms. All embryological processes are based on cell biological, genetical and biochemical principles since every organism is formed by isolated or associated cells. In embryology one studies the very complex processes by which these cells differentiate and associate from one single initial more or less voluminous cell: the egg. The egg is the link between two generations and already by its volume it gives the impression to contain more than an ordinary cell. Our mind needs, already from its earliest manifestation, to know how a young animal, a child or a plant makes its appearance. It is still one of the first questions that children ask for when they begin to think and realize their own existence. Moreover their own origin, as is the case for most mammals is most difficult to observe since the mammalian embryo is completely hidden from direct observation. By contrast, avian embryos are more accessible to direct observation. For instance chickens lay eggs, incubate them by their own body heat and after approximately 20-21 days, as touched by a magician, hatching of a young bird takes place. It is easy to open the eggs at the successive stages of development, sometimes

even without killing the embryo. This has been and is still one of the bases of modern embryology.

EARLY HISTORY OF IDEAS IN RELATION WITH THE ORIGIN AND NATURE OF THE GERM

Ancient Arabs and old Germans thought that only mothers have the possibilities to give origin to the development of a child, whilst people in Greece considered that the father was only responsible (SHORT, 1977). Greek natural philosophers generally interpreted female seminal fluids as menstrual blood. The female usually was considered inferior to the male: due to a lack of internal heat, this menstrual blood did not form true seed. This did happen in males, leading to male seed or sperm, which also was thought to form out of blood (generally conceived as a life giving principle). Hippocrates (460-380 B.C.) "the father of medicine" was the first to control theories with real experiments. He made from embryology a separate science and made comparative embryological studies between the chicken and the human embryo. He accepted that both the female as the male contributed equally to the formation of the germ. ARISTOTELES in the 4th century before J.C., also called "father of the natural history", was the first to describe the "punctum saliens", the early beating heart of the avian embryo and followed its evolution, day by day during further incubation. While he observed

the evolution of the chicken embryo and its appearing organs, he concluded that the organs appear successively and not at the same moment. Therefore he proposed the so-called epigenetic development in contrast to the preformation hypothesis. Aristoteles did not consider uterine blood but the male seed as the essential factor for germ formation. Aristoteles saw it as an unmaterial principle of movement “triggering” the menstrual blood to further selforganize. The female “seed” is to be considered as the material cause of any embryological process. Ancient people must have observed the existence of egg sacs with large eggs in the ovaries from birds. The yellow aspect of the large follicles in the ovary and a similar aspect in the laid egg were the first indication that eggs are formed in the ovary. So the link between ovarian oocytes and the avian germ in the laid egg (oviparity) was established. But the principle of viviparity (development of an embryo in the female organism) could originally not be explained. ARISTOTELES in the 4th century before J.C. has made embryological observations on much different kinds of animals. He followed not only methodically the development of the chicken embryo but has also observed the embryos of dolphins and larval stages of insects (CAULLERY, 1957). The role of the male and female respectively in the generation process could be explained according to Aristoteles by the example of the chicken. Indeed, the latter taken apart from the rooster still continues to lay eggs in which however no embryos develop. Therefore he concluded that generally speaking the female furnished the constituting material of the embryo whilst this material was vitalized by the male. The place where this occurred in the chicken egg would be the cicatricular region where the germ disc, forming the base of the future embryo develops. Departing from Aristoteles’s natural philosophy, VAN SPEYBROECK et al. (2002) have historically shown that epigenesis gained alternating attention from the 17th century onwards. It was considered to be the opposite of the preformationist tradition. Where preformationism stated that the germ cells of each organism contain preformed miniature adults that unfold during development, epigenesis held that the embryo forms by successive gradual exchanges in an amorphous zygote. CLAUDIUS GALENUS (131-201) one of the founders of anatomy and physiology was born in Pergamon. He made dissections of animals, mainly apes, because it was not allowed to study human corpses. His influence has been very important until in the 16th century. Galenus described the female ovaries as testes and propounded that the male sperm was secreted by the testes. So the notion of gonads (genital glands) male or female was established. Galenus holds a very naturalistic view on the embryological processes in terms of growth, nourishment and genesis (change and shaping). After Galenus during 13 centuries, science and particularly embryology were completely influenced by religious ideas and no new discoveries were made.

PERIOD OF THE EARLY ANATOMISTS

Fabricius Ab Aquapendente (teacher of William Harvey) had a chair of anatomy at Padua and made an enormous contribution to embryology (ADELMANN, 1942). He



Fig. 1. – Preformation concept during the 17th century: drawings of the early developmental stages of the chicken embryo in which, according to Fabricius Ab Aquapendente, already the adult form could be seen.

was probably the first investigator to give an exact account of the role of the ovary in the formation of the hen’s egg, for he observed that besides that the yolk was formed in the ovary that the egg white, the shell membrane and the shell were all formed during the transit of the egg down the oviduct. This key observation was the first indication that the egg might be produced directly by the female rather than as the result of the union of male seed “and female soil” as supposed by Aristoteles. He also thinks that the avian egg is fecundated by the so-called “aura seminalis” (an emanation from the semen of the rooster). FABRICIUS was also a preformationist since he pretended that a “little birdie” was visible in the egg during the earliest developmental stage (1637) (Fig. 1). However the initial developmental stages of most of the embryos have a size which makes them invisible to the naked eye. The first microscopes appear in the 17th century, but only in the 19th century a higher degree of perfection permitted their real use for embryological observations. Embryos in advanced developmental stages are more or less visible to the naked eye and already have more or less the form and structure of the adult animal. This suggested the attractiveness of the theory of preformation i.e. that development was only the result of the progressive enlargement of the germ, having from the beginning its final constitution and complexity. The preformation theory seemed to be first propounded by the arab scientist, AVERROES (also called Ibn-Roschd) who was a teacher at Cordoba (1115-1198). Contrary to this theory, the theory of epigenesis was formulated: the complex structure of the definitive adult forms and even the embryo is only realized progressively by transformation of more simple structures. In the course of the history of embryology, as a research discipline, it appeared that a kind of progressively build up preformation (pre-existence) exists in the egg (already before fertilization), which is indispensable for early embryonic development. Initially, epigenesists defended the view that the embryo progressively forms out from a homogeneous matter.

Aristoteles, but also HARVEY (1653) claimed that it is most certain that in the egg there is no prepared material at all. It takes until the studies of Albrecht Von Haller (1708-1777) first adhering to epigenesis, later becoming a strong ovist-preformationist, to conclude that the egg does contain differences in viscosity.

EARLY EXPERIMENTATORS AND USE OF MICROSCOPES

WILLIAM HARVEY (1578-1657), medical doctor from the king (Charles I) of England, discovered the blood circulation (1628) and made dissections from female deer's after coitus and states that the young germs of the mammals are present in the form of eggs. In 1653 appears his book: "Exercitationes de generatione animalium".

He did point out a place where fecundation would occur, but at the same time, his research on deer did not allow him to conclude that the male semen made any material contact with the egg (or left any visible trace) on the female egg. According to him the little white cicatricula on the top of avian eggs is the place where fecundation occurs. After incubation this region extends and forms a semi-liquid mass (colliquamentum) in which soon the first traces of the embryo and particularly the heart appear. From him comes the aphorism "ex ovo omnia": every living being develops from an egg (1651). REGNIER DE GRAAF (1672) who was an excellent experimenter (LINDEBOOM, 1973) observed that after killing, female rabbits at different moments after coitus present a progressive evolution in the vesicles bulging at the surface of the ovary, followed by scars after ovulation. These "eggs" (sic) were found afterwards in the uterus. He thinks erroneously that the vesicles bulging at the surface of the ovary before the coitus were really the eggs themselves (SAWN, 1997). He observed that once they were found in the rabbit uterus they are first small but later larger, adhere to and become fixed into the wall of the uterus (implantation). Now we know that the ovarian vesicles described by Regnier De Graaf are not the eggs but are follicles surrounding the real, much smaller eggs. These follicles are now still called Graafian follicles in modern literature. At nearly the same moment, thanks to the use of the first microscopes, spermatozoa were seen in semen (perhaps by HAMM, or HARTSOEKER, 1694; fig. 2) but they were surely seen, drawn and described by the Dutch researcher ANTONIE VAN LEEUWENHOEK (1678; 1683). The main discovery of Van Leeuwenhoek was his finding in 1677 of "little animals" (animalcules) in semen (LA BERGE, 1999). He found these animalcules in semen from men but also from roosters and in the semen of different mammals. So Van Leeuwenhoek became an "animalculist" or "spermist" who thought that later the embryo was formed from one of these little animals (Fig. 2) or spermatozoa (Von Baer used this word). According to Van Leeuwenhoek, De Graaf's egg, if it did anything, provided no more than nourishment to the embryo. During the next century little further research was done on the topic, although there were much theoretical discussions

between the animalculists and the ovists (who considered the egg as the location in which the preformed embryo resides). De Graaf's ideas on the egg as being the precursor of the embryo fell in some disfavour because the evidence for the animalculists was easy to obtain by simple observation of semen under the microscope, which seemed to demonstrate the animalculist preformation theory (LINDEBOOM, 1973). The ovistic hypothesis of De Graaf was on the contrary more difficult to control. It was in fact only much later, 100 years after De Graaf's death, that CRUIKSHANK repeated and confirmed his studies on embryonic development in rabbits. Marcello Malpighi (1628-1694) described the capillary circulation in the lungs of frogs and also studied the development of the chicken embryo. His study "De formatione pulli in ovo" appeared in 1672. This biologist used systematically a microscope (fabricated by JANSSEN in 1590). He described the cicatricula as the most important part of the avian egg for the development of the future embryo after incubation. With Malpighi the microscope became an indispensable tool for biological research (LAMS, 1935; LÜTHY, 1996) and he adhered to ovism. In 1740, at the age of 20, CHARLES BONNET (Switzerland) describes for the first time the phenomena of natural parthenogenetic development (from a virgin egg) in a plant-louse. These observations of BONNET on virginal reproduction were confirmed by RÉAUMUR and BEGUELIN, TRIMBLEY and ALBERT VON HALLER. This discovery of parthenogenesis was strongly in favour of the ovist theory of preformation. However, the eventual "emboîtement" of the successive generations could hardly be explained. We must also mention the theory of organic molecules developed in 1745 by MAUPERTIUS (DOLLANDER & FENART, 1973). Seminal liquids both from the mother as from the father, coming from all parts of the body, should mix after mating in the uterus. So, Maupertius tries to explain that some characteristics of the father or mother are recognized in the children. By contrast both ovists and spermists (or animalculist) believed in the theory of preformation i.e. that the egg or the spermatozoon contains a miniature individual with all the parts of the adult. FABRICIUS AB AQUAPENDENTE (1637) and MALPIGHI (1669) were convinced preformists and according to them the avian germ disc contains a miniature organism already in its definitive form (Fig. 1) (ADELMANN, 1942; 1966). By growing, its different parts unfold progressively into an adult form. Albertus Von Haller (1753) had a more evolved idea (with epigenetic background) about preformation. He wrote: "nulla est epigenesis, nulla in corpore animalis pars ante aliam facta est et omnes simul creatae existunt". He found no recognizable organs in the chicken germ but considered that only their Anlagen were present and that these will progressively develop into their adult form. Von Haller was an authority in embryology and studied the chicken embryo at early stages after incubation. He wrote a book in French and described the development of the heart, the eye and respiratory phenomena (see the title page of his book: Fig. 3). He uses the chicken embryo to explain the formation of some anatomical structures in the human.

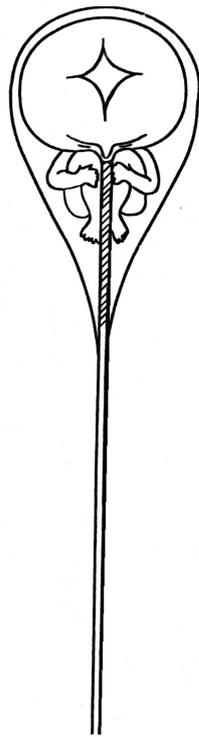


Fig. 2. – Drawing of the microscopic aspect of a human spermatozoon of animalculist Hartsoecker, in which he seems to see a miniature baby as was also accepted by Van Leeuwenhoek.

FORMATION

DU COEUR

DANS LE POULET;

SUR L'OEIL; SUR LA STRUCTURE
DU JAUNE &c.

PREMIER MEMOIRE.

EXPOSE' DES FAITS.

PAR

MR. DE HALLER,

*President de la Societé Royale des Sciences de
GOTTINGUE, Membre de l'Académie
des Sciences de PARIS, de celle de Chirurgie,
de la Societé Roy. de LONDRES, &c.*



A LAUSANNE,

Chez MARC-MICH. BOUSQUET
& Comp.

MDCCLVIII.



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Fig. 3. – Tittle page of the book of Von Haller (1758) in which he still accepts original preformation concepts.

EVIDENCE FOR EPIGENETIC DEVELOPMENT

The old preformistic view dominated in embryology up to the middle of the 18th century. During that period a new view on embryonic development was introduced by C. F. Wolff (1734-1794). He was born in Germany where he started his research in anatomy and embryology. This resulted in the publication of a dissertation entitled “Theoria Generationis”, published in Germany in 1759. In this study, C. F. Wolff developed and defended a so-called epigenetic view based on careful microscopical observations of early embryonic development. According to Wolff the chicken embryo is not formed by the accumulation of juxtaposed parts but the organs arise progressively the one after the other from more simple structures. Thus the gut is composed first from a flat membrane which later forms folds and finally has the general cylindrical aspect of a digestive tract. In the same way he describes the development of the neural groove followed by the medullary cord. So in the early germ nothing exists “in facto” but all “in potentio”. Thus from already pre-existing structures new and more complicated structures and organs were formed.

Wolff did not so much stress the existence of pre-existing structures. He did argue in favour of “an active nature”. This is however contrary to the preformistic concept of nature as a dead mass unto which blind mechanical forces work. It is in this regard that Wolff talks about “inorganic matter”, as the heterogeneous, but still unorganized, matter out of which an embryo self-organizes. At that time however this theory of epigenesis was not accepted in German scientific circles. So Wolff could not find a place in the German universities and immigrated to Russia. At the end of 1766, the St. Petersburg Academy of Sciences invited him to work as academician in the anatomical department. Finally he settled in St. Petersburg where he worked in the field of embryology, teratology and anatomy. The discovery of organic transformation and neoformation by C. F. Wolff was misunderstood by his contemporaries. According to Blumenbach’s unordered matter does not have the power to order itself, thus life cannot spring from non-live. The organization one sees in life, is due to a physiological impecunious principle of internal correspondence (Bildungstrieb), ungraspable to the human ratio. This principle is not equal to the mechanical formative power or Bildungskraft that inorganic matter also possesses. Blumenbach’s *nisus formativus* or “Bildungstrieb” is not entirely the same as Wolff’s *vis essentialis*. Whereas Wolff’s “wesentliche Kraft” is single in nature, producing but one effect, varying only through the influence of the surrounding context, Blumenbach’s *nisus formativus* was a multiple active force which could produce many different things by itself, making it by itself sufficient to generate a new organism. This makes Blumenbach much more into a vitalist than Wolff.

For Wolff the *vis essentialis* produces the different parts of the organic body no longer merely through itself and according to its nature, but rather with the help of countless other concurring causes, “and what it does through itself alone, becomes a completely simple effect, as attraction or repulsion, and is worlds apart from the

building of organic bodies” (WOLFF, 1789 in ROE, 1981: 117). This point is very important, since it was and still is often thought that Wolff deduced the total formation of matter from the *vis essentialis*. Even his opponent, Von Haller, did not grasp this point fully: “why does this *vis essentialis*, which is one only, forms always and in the same places the parts of an animal which are so different, and always upon the same model, if inorganic matter is susceptible of changes and is capable of taking all sorts of forms? Why should the material coming from a hen always give rise to a chicken and that from a peacock give rise to a peacock? To these questions no answer is given” (VON HALLER in NEEDHAM, 1959: 202). Wolff asserted several times that people paid too much attention to his *vis essentialis* and that his theory of attraction and solidification would have been the same without it. A follower of Wolff’s epigenetic theory was TREDERN DE LÉZÉREC, a usually forgotten pioneer of chick embryology. He submitted his thesis in Jena (1808). The importance of the male semen, next to the female egg for reproduction was shown during the same period by Lazzaro Spallanzani (1729-1799). Using amphibian eggs, SPALLANZANI applied the artificial insemination method used by JACOBI (published in the Hannover magazine in 1763) for fecundation of trouts or salmon. This method of Jacobi is still used at present to populate waters. Spallanzani did not believe in the “aura seminalis” theory of Harvey, as he was convinced of the necessity of material contact between egg and semen (more specifically spermal fluid). He demonstrated that the fecundation capacities of the semen of an amphibian (*Rana temporaria*) disappears after filtration or heating. That fertilization is the result of the effect of spermatozoa on an egg was concluded by PREVOST and DUMAS in 1824. They repeated the experiments of Spallanzani and demonstrated unequivocally that the spermatozoa are the real fecunding elements in the semen. The idea that the spermatozoa play a major role in fertilization by penetration, as was propounded by Prevost and Dumas, was not accepted by all biologists in that time. TH. W. BISCHOFF (1807-1882), in 1842 and J.P. MULLER (1801-1858) in 1844 doubted about this. Bischoff claims in “Entwicklung des Hunde-Eies (1845): “ich habe nie im Inneren eines Eies einen Spermatozoide auffinden können. Die Wirkung des Saamens auf das Ei halte ich dann zunächst für eine chemische”. By contrast, L’ALLEMAND believed strongly that fecundation consist of the union of two living parts: “un fluide ne peut évidemment transmettre la forme de la vie qu’il ne possède pas”. The fusion of spermatozoa with eggs has been really observed only for the first time clearly in Ficus by THURET (1854). The development of the embryology has also been influenced by evolutionistic concepts during the 19th century by LAMARCK (1802) and DARWIN (1859). In succession of F. MÜLLER (1844), HAECKEL formulated the so called biogenetic fundamental law, according to which ontogenesis summarizes phylogenesis. This means that the development of an individual being is a partial recapitulation of the evolution of his ancestors. The checking of this hypothesis has been at the origin of an important boom in descriptive embryology during the second period of the 19th century. Although Wolff’s epigenetic ideas in embryology were not accepted in Germany, his influence was great on the founders of the Russian embryological

school, C. H. Pander and K.E. Von Baer (SANDER, 1996; MIKHAILOV, 1997). Pander, a Russian zoologist born in Riga (12/7/1794) made a thorough description of the developing chick embryo with the three layers forming the body. His schemes of chick embryonic, development at different stages, surprisingly resemble modern classic descriptions. Pander demonstrates for the first time in history that a bird embryo develops from three germ layers (Fig. 4). PANDER (1817) distinguishes clearly in the cicatricula (germ disc region) of the unincubated avian egg the superficial “blastoderma” (now called blastoderm) and the central underlying “nucleus cicatriculae” (now called nucleus of Pander). Pander observed that the tinny membranes which will form the germ also contain a great generative power (*nisus formativus*) already described by BLUMENBACH (1789). Karl Ernst Von Baer (1792-1876) was born in Piep (Estonia) and, after graduating from the university, he started his career as zoologist and embryologist. He worked as professor of Anatomy at the University of Königsberg. His microscope was already much better then from his predecessors. Later he worked in the St. Petersburg academy of Sciences, where he worked for nearly 30 years and published more than 400 manuscripts. His scientific work was as he described himself influenced by two persons: “So dürfen die vorliegenden Untersuchungen sich rühmen, eine Folge jener für die Naturwissenschaft ewig denkwürdigen Verbindung zu sein, in welcher ein in physiologischen Forschungen ergrauter veteran (Döllinger), ein von Eifer für die Wissenschaft glühender Jüngling (Pander) sich verbanden um durch vereinte Kräfte eine feste Grundlage für die Entwicklungsgeschichte des thierischen Organismus zu gewinnen” (cited by VAKAET, 1965; page 137).

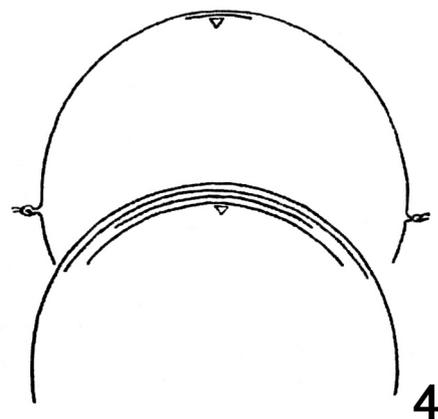


Fig. 4. – Early composition and disposition of the avian blastoderm and cicatricular region on the avian egg yolk ball, represented by PANDER in 1817: the so-called nucleus of Pander is seen as a triangular mass below the unincubated blastoderm (upper figure) and after incubation below the three germ layers (lower figure), clearly indicating epigenetic phenomena (neoformation).

In 1821, for the first time, VON BAER detected mature unfertilized eggs (oocytes) in the ovary of the female dog. In his paper “De ovi mammaliae et hominis genesis” (1827) he notes that they were identical to the ova found in the oviducts. Later, Von Baer started to analyze ovaries in rabbits, pigs and humans and described the human egg

and the structure of the Graafian follicle. Von Baer developed and enlarged Pander's ideas about germ layers and studied their fates during early embryonic development. He was able to demonstrate that the so-called "cutaneous" layer (external layer) transformed into superficial epidermis and central nervous system, that the so-called "muscular" layer (now merely somites) formed muscles, skeleton and connective tissues; that the internal "covering" layer of the digestive tract developed from the so-called "mucous" layer. From these three germ layers all body structures are formed according to an orderly process. By

the study of chick embryos he detected a new embryonic organ for the first time i.e. the backbone "cord" (chorda dorsalis or notocord). Based on detailed comparative studies of embryonic development in different vertebrate embryos he formulated some conclusions: during embryonic development more specified characteristics appear later than the more general features and the general features of a large family of animals appear earlier than the features of a species. Von Baer recognizes a certain similarity only between the early embryos of different animal groups.

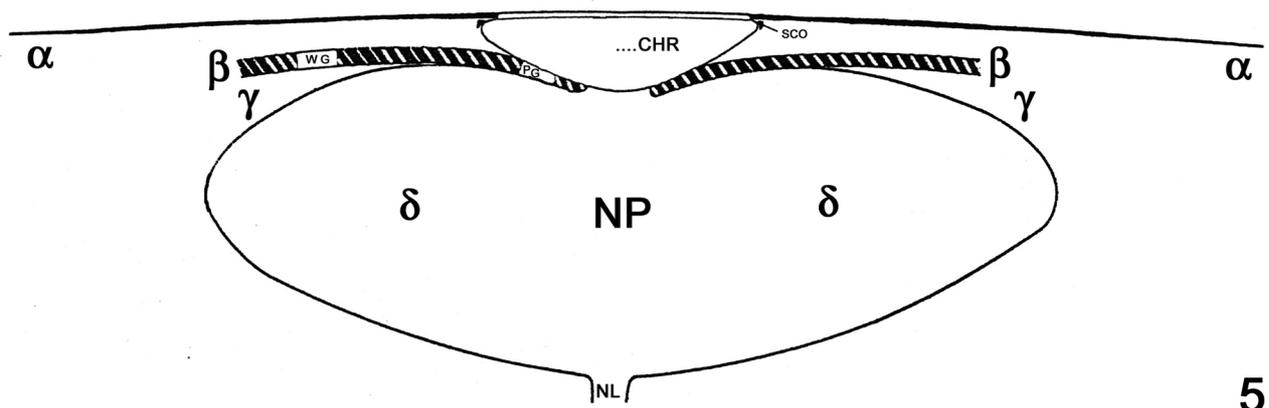


Fig. 5. – Schematic drawing representing the onion peel-like localization of the four ooplasmic (α , β , γ , δ) at the end of oogenesis (modified after CALLEBAUT, 1975): the ooplasmic will play a fundamental role, during the early development of the blastoderm; from peripherally and superficially to more centrally and deeply we see: the α ooplasm which after a centripetal movement functions during the cleavage stage (see Fig. 6); the β ooplasm is mainly incorporated in the primitive streak and in the embryo proper; a part of the γ ooplasm, surrounding the nucleus of Pander (NP), is spatially obliquely taken up caudolaterally in the blastoderm and forms Rauber's sickle (see also Fig. 8); part of the superficial part of the δ ooplasm (from the nucleus of Pander) is also taken up in a spatially oblique manner by the more central part of the blastoderm and forms the sickle shaped endophyll and primordial germ cells (see also Fig. 8); CHR: spherical postlampbrush chromosomes seen in the centre of the flattened germinal vesicle; SCO: perinuclear subcortical cytoplasmic organelles or ticos; WG: wedge granules layer; PG: polar granules; NL: latebra neck.

The germinal vesicle (the large nucleus of the oocyte) in the large ovarian oocytes of birds is transparent (Fig. 5) in contrast to the white opaque structure of the surrounding cicatricula. PURKINJE (1830) describes for the first time the avian germinal vesicle and gives it the name of "vesicula germinativa": Habet itaque cicatricula ovi ovarii partem specialem et sibi propriam, vesiculam sphaericam subcompressam, membranula tenerrima constantem, lympa propria, fors generatrice repletam (inde vesiculam germinativam appellaverim), in fossam cumulo albo mammaeformi e globules composito. (Eggs in the avian ovary present in their cicatricular region a special characteristic structure with a spherical, partly compressed aspect. It contains its own liquid which is always surrounded by a tinny membrane and it is localized in a mammiform mass of globules, it has the power to generate a future germ (sic), therefore I give it the name of germinal vesicle). Thus for Purkinje the germinal vesicle was the germ-generating-structure. By contrast for Von Baer (1828) it was not yet clear if the avian embryo was only formed from the disintegrated germinal vesicle together with the spermatozoa, or that also material from the surrounding cicatricula (ooplasm) plays a role in germ formation. He speaks from "Umbildung" i.e. a series of transformations from simple towards more complex. It was COSTE (1850) who described for the first time the cleavage furrows in the avian blastoderm, formed in the

cicatricula shortly after fertilization. So he demonstrated that the oocytal ooplasm of the cicatricula also gives rise to the avian germ. The germinal vesicle in birds, reptiles and cephalopods is according to COSTE (1850) always included in a fine granular layer, which forms the substrate for the later blastoderm from which the embryo will develop. SCHWANN (1839), who developed the general cell theory, was the first to understand that the oocyte (unfertilized egg) of a mammal must be considered as one cell. Owing to their enormous volume he still considered the large oocytes in the ovary of birds as multicellular. HOYER in 1858 clearly observed that the yolk of large avian oocytes contained no cells. Thus large intraovarian avian oocytes are single giant cells.

In 1865 JOHAN MENDEL discovered the fundamental laws of genetics. However his study has been negated and ignored for more than half a century (KRUMBIEGEL, 1957). The reason seems to be that in that period; Darwin described and propounded the variability of species whilst the work of Mendel just demonstrated a constant previsible evolution (DE VEER, 1969). Chronologically we can distinguish three steps in the pairing mechanisms of animals, resulting in the formation of a germ: pairing of the chromosomes during meiosis in the germ cells of the gonads of the parents, copulation of the parents, pairing of the gametes (unfertilized eggs with spermatozoa) at the moment of fertilization. By progression of the cytological

techniques, at the end of the 19th century, chromosomes in meicytes (during oogenesis or spermatogenesis) were seen as most prominent structures. So VAN BENEDEEN (1883) in the eggs of *Ascaris megalocephala* and VAN BAMBEKE (1885) found, that at the end of the meiotic division, only half of the number of chromosomes was found in the gametes (mature oocytes and spermatozoa). At the moment of pairing of the gametes (fertilization) the specific number of chromosomes is restored in the zygote. This demonstrated for the first time that both the father and the mother afford the same quantity of chromosomal material during the formation of the zygote, which gives rise to all the cells of the embryo. According to Van Beneden (University of Liège) and Van Bambeke (University of Ghent) this suggested that embryonic development is bound to chromosomal material charged with hereditary characteristics. However it is only in 1902 that SUTTON makes the link between the meiotic dissociation of chromosomes and the segregation of hereditary characteristics described by Mendel. The localization of genes on the chromosomes of *Drosophila* by MORGAN (1910) permitted further development of genetical knowledge. In recent decades the study of expression of genes during early developmental processes has become a powerful tool. Evolutionary and developmental biologists have joined forces to create a new field, unravelling the mysteries of evolution by studying the genes that control how an embryo develops (DEPEW & WEBER, 1995).

EXPERIMENTS ON EGGS

The theory of epigenesis, based on neoformation, as has been proposed by C.F. WOLFF since 1759, considers the egg not as a mosaic of territories with an achieved fate but as a progressive realization of stages which are conditioned by an earlier more simple stage (VAN SPEYBROECK et al; 2002). According to this epigenetic hypothesis the half of a germ can still give rise to a complete normal embryo of a smaller size. The egg is thus capable to regulate development of its parts. However in some species there is evidence for preformistic behaviour as the result of factors or territories present in the ooplasm. The link between ooplasmic structures (before fertilization) and structures in the germ (after fertilization) is not always obvious and differs from species to species. So the first experimental investigations in embryology by CHABRY (1887) and ROUX (1888) and CONKLIN (1905) suggested prelocalization – preformation mechanisms by mosaicism. Indeed in some species (ascidian or amphibian) an isolated hemisected egg (containing one of the two first blastomeres) will develop only in the corresponding half (left or right) of the embryo. The term mosaic development is used as originally defined by CONKLIN (1905) in ascidian species: each region of the whole fertilized egg would be able to form more or less independently on its own. The development of the entire embryo was regarded as being the sum of the development and interaction of the individual parts. Here the concept of “preformation” more particularly mosaicism no longer refers to the strong preformationistic theory described earlier in this review (pre-existence of parts), but as a soft preformation or pre-existence, inclining to a more sophisticated version of

preformation, due to ooplasmic determinants. OSCAR HERTWIG introduced in 1916 the concept of “preformed epigenesis”: the development of multicellular organisms from a fertilized egg is an epigenetical process whose species-specific course is firmly determined by the preformed hereditary substance which serves as its basis. In other species (echinoderms) an isolated half of an egg will produce a complete miniature embryo (DRIESCH, 1891) as the result of so-called regulation phenomena. This can however be explained by the all or not existence in the isolated halves or parts of all the different kinds of ooplasm in the blastomeres. Indeed during a study of the early embryonic avian development (CALLEBAUT, 1987), I found preformation evidence for the role of the four ooplasmic (α , β , γ , δ) which present an onion-peel distribution in the oocytal germ disc region (Fig. 5). I demonstrated that α ooplasm plays a fundamental role during the cleavage stage by penetrating along with the cleavage furrows into the underlying ooplasmic (Fig. 6). The β ooplasm originally mainly localized in the peripheral region of the area centralis becomes concentrated in the primitive streak by converging phenomena. The γ -ooplasm finally gives rise to Rauber’s sickle. So for instance in the large blastomeres of the early avian germ disc the four fundamental ooplasmic can still be observed as is the case in the whole younger germ (CALLEBAUT, 1987) (Figs 5; 6). That the caudocephalic axis of a vertebrate embryo is not preformed in the egg but can develop under influence of gravity was probably first observed by WINTREBERT (1922) in Selachians. Indeed, Wintreburt described the relationship between the spatial orientation of the early Selachian blastodisc on its egg yolk with respect to the vertical and the ensuing development of its caudo-cephalic axis. Also in the Selachian egg any part of the periphery of the blastodisc can give rise to the embryonic caudal edge: the only condition is that it should correspond temporally to its highest point. Still in the 20th century preformistic views concerning the craniocaudal axis of the future vertebrate embryo, persisted. So BARTELMEZ (1912; 1918) claimed that the craniocaudal axis of the future pigeon embryo was already established in the ovarian primordial follicle, visible as the long axis of the oocyte. According to VAKAET (1953; 1955) and FAUTREZ & VAKAET (1954) the plane of bilateral symmetry of the ovoviparous teleostean fish, *Lebistes reticulatus*, is already predetermined in the oocyte during previtellogenesis. This view, is contested, however by CLAVERT & FILOGAMO (1957 a; b). Indeed, after fertilization the *Lebistes reticulatus* egg becomes movable within its follicle and undergoes an orienting rotation by gravity whereby the future germinal disc is turned to the upper pole of the egg. By contrast to vertebrates the cranio-caudal axis of the *Drosophila* embryo seems to be preformed by the localisation of ooplasmic determinants within the oocyte (ST. JOHNSTON & NÜSSLEIN-VOLHARD, 1992). Bicoid mRNA localised in the cranial pole is translated after fertilisation to give rise to a morphogen gradient of Bicoid protein that patterns the head and thorax (BERLETH et al., 1988). Similarly, oskar mRNA is localised into the caudal pole of the oocyte where it directs the assembly of the pole plasm, which contains the caudal and germ line determinants (EPHRUSSI & LEHMANN, 1992). J. BRACHET (1933; 1941; 1961) and CASPERSON (1941) discovered

the role of RNAs in the synthesis of proteins. In 1953, Watson & Crick found the basis of our knowledge about the replication mechanism of DNA synthesis, giving rise to the development of molecular biology. Particularly in oocytes, intense synthesis of RNA's and proteins takes place (BRACHET, 1941). These molecules (messenger RNAs) will in part pass into the ooplasm of the zygote after fertilization and will play a fundamental role during early embryonic development. According to the hypothesis of DALCQ & PASTEELS (1937) the original localization of the ooplasmic constituents in the egg, even not genetically determined, can influence early stages of embryonic development. Thus a kind of molecular preformism, present in the ooplasm, must be accepted. The term epigenetics was introduced by C.H. WADDINGTON (1940; 1952) over 60 years ago as the study of those processes involved in the unfolding of development. The discovery of the role of DNA in inheritance has cast a shadow over this discipline for decades (CAVALI, 2006). It was found that molecular machines act on the chromatin to regulate gene expression. These epigenetic regulators play a crucial role in the global shaping and maintenance of developmental patterning. Histone modifications seem to play a major regulatory role. Epigenetics has then been redefined as the study of heritable traits that are not dependent on the primary sequence of DNA (CAVALI, 2006). Ooplasm remains indispensable for embryonic development, since until now in the absence of ooplasm no cloning of individuals can be obtained. The transplantation of somatic nuclei into maturing amphibian oocytes by GURDON (1968; 1969) demonstrated the fundamental effect exerted by the ooplasm and eventually the nucleoplasm of the germinal vesicle on an implanted nucleus for further embryonic development.

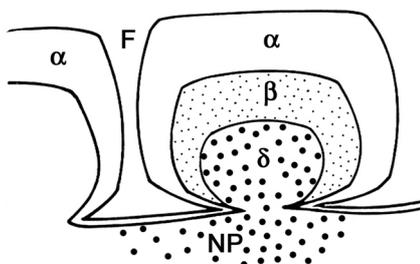


Fig. 6. – Schematic representation of the localization of the ooplasm in the first large “closing” blastomeres; the forming subgerminal space is lined by a narrow α layer; F: cleavage furrow; NP: nucleus of Pander containing δ ooplasm (after CALLEBAUT, 1987).

PERSISTENCE OF FEMALE GERM LINE CELLS FROM EMBRYO TO ADULT

Until the years '60 of the previous century numerous investigators claimed that the primitive sex cells in the ovary sooner or later all degenerate and completely disappear (FIRKET, 1914). Indeed a great part of the germ cells in the ovary degenerate and their investing follicular cells have a hormonal influence. Therefore it was thought that the definitive germ cells of the adult ovary develop secondarily by neoformation from somatic cells in the so-called germinal epithelium covering the ovary and

develop epigenetically through transformation of the common coelomic epithelial cells (BLOOM & FAWCETT, 1962). This seems not to be the case. Indeed in the case of birds, by radioactive labelling with ^3H -thymidine of the chromosomes of embryonic oocytes during the ultimate premeiotic DNA synthesis period (CALLEBAUT, 1967), I could demonstrate that the labelling persisted into the chromosomes of the adult oocytes. The adult oocytes are thus derived from the embryonic oocytes, present already long before hatching (CALLEBAUT, 1973). In mice similar results were obtained by PETERS et al. (1962), LIMA DE FARIA & BORUM (1962) and CRONE et al. (1965). This demonstrates that birds and mammals contain a final stock of oocytes, at the end of their embryonic development, which will be exhausted progressively during further life [part of the hypothesis of WALDEYER (1870)].

DISCOVERY OF EMBRYONIC INDUCTION

In 1924, SPEMANN & MANGOLD describe the induction phenomena i.e. an embryonic structure functions as an organizing inductor on another structure (reactor) in its neighbourhood and modifies this reactor into a new structure without itself affording a cellular contribution to this new structure. SPEMANN & MANGOLD (1924) discovered the so-called “Spemann organizer” in amphibian embryos. They observed that after transplantation of a piece of dorsal blastopore lip of the early gastrula to the ventral side of another embryo, a secondary embryonic axis developed on this side. So the existence of induction phenomena between different associated parts of embryonic tissues demonstrated epigenetic development. The Spemann’s organizer redeterminates the fate of a part of the host cells and induced them to form axial structures, more particularly a central nervous system. The formation of a neural plate which gives rise to the central nervous system was considered to be the result of an inductive influence from the underlying mesoderm with organizer property.

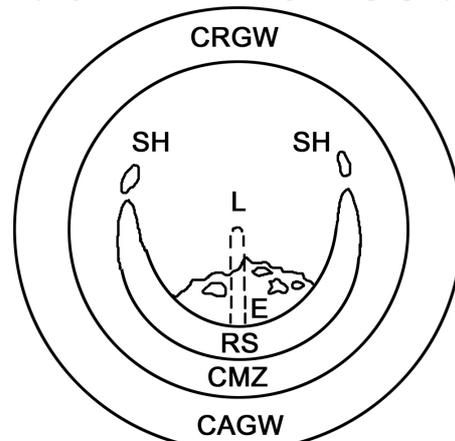


Fig. 7. – Schematic representation of the components of an unincubated avian blastoderm seen from its deep side after removal of the subgerminal ooplasm, ready for culture; CRGW: cranial germ wall; CAGW: caudal germ wall; CMZ: caudal marginal zone; E: incomplete endophyll sheet; L: lacune in the deep layer; RS: Rauber’s sickle with its fragmentary sickle (SH) enclosing the area centralis. The vertical interrupted double line represents the future localization of the PS after incubation.

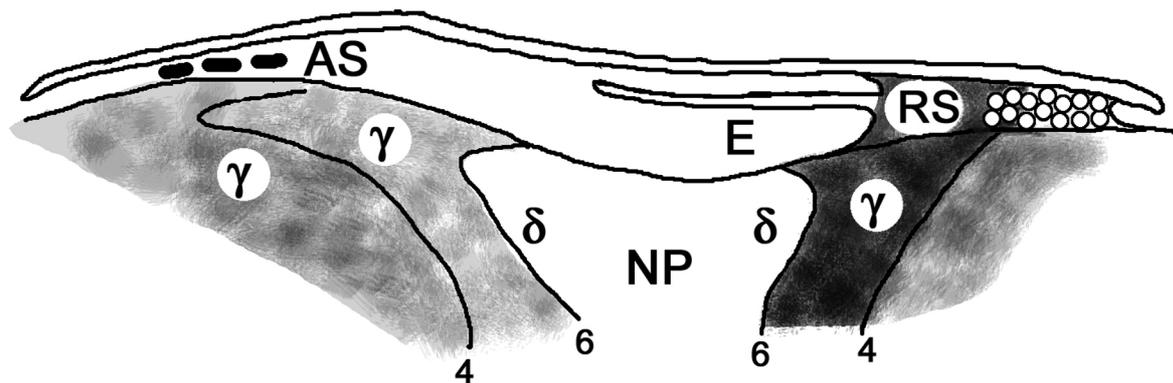


Fig. 8. – Schematic representation (simplified after CALLEBAUT, 1993b) of the localization of two intraoocytally radioactively labelled layers (respectively 4 or 6 days after a maternal radioactive injection) in the γ and δ ooplasm on a midsagittal section through an uninucleated avian blastoderm (presenting a sickle-shaped bilateral symmetrization). Note the permanent boot-shaped deformation of the ooplasmic layers around the nucleus of Pander (NP) composed of δ ooplasm (white) and in the surrounding γ ooplasm (grey). The toe-shaped part of both γ and δ layers are expanded and horizontally flattened. They remain in the underlying ooplasm below the anti-sickle region (AS) and they have lost contact with the blastoderm. Caudally, in the heel-shaped part, the γ ooplasm is more condensed and adheres vertically to the upper layer, forming Rauber's sickle (RS); the heel and middle part of the layers containing δ ooplasm of the nucleus of Pander (NP) are taken up in the caudal part of the area centralis of the blastoderm and later segregate progressively as endophyll (E) which also contains δ ooplasm (white) (CALLEBAUT, 1987; 1993a); the superficial upper layer is mainly composed of β ooplasm (after CALLEBAUT, 2005).

OOPASMIC DETERMINANTS IN SOMATIC TISSUES

During the last decades it became clear that induction phenomena not only occur as the result of interaction between embryonic structures (in the sense of SPEMANN & MANGOLD, 1924) but also extraembryonic ooplasmic structures, all or not nucleized, play a fundamental role in early embryonic induction phenomena. Indeed in 1969 NIEUWKOOP discovered that during early blastulation in amphibians, signals are required from a region which is localized vegetal to the prospective dorsal blastopore lip, to initiate development of the mesoderm (Spemann's organizer). This vegetal region is now designated as Nieuwkoop's centre. In amphibians, Wnts seem to be the primary axis (formation of head and trunk-tail regions) inducing substances. Indeed in *Xenopus*, microinjection of several Wnts into the ventral cells of the early embryos leads to complete duplication of the body axis (CADIGAN & NUSSE, 1997; DEARDORFF et al., 1998). This duplication is believed to arise from the formation of a second Nieuwkoop's centre (dorsal vegetal cells) of the early blastula which then induces overlying tissue in order to it might become the Spemann's organizer (homologous to the avian primitive streak and nodus, WADDINGTON, 1932). Indeed in birds and mammals the properties of the Spemann's organizer are performed by the node (Hensen's node in birds) which is the rostral end of the full grown primitive streak. We found that in birds, RAUBER's sickle (1876) (Figs 7; 8) has a homologous function as Nieuwkoop's centre in amphibia. It functions indeed as the primary major organizer, initiating gastrulation phenomena and primitive streak formation (CALLEBAUT & VAN NUETEN, 1994; CALLEBAUT et al., 2003a). The localization and function of Rauber's sickle present a strong similarity with the localization and function of the ascidian Wnt gene, Hr Wnt-5 from *Halocynthia roretzi* (SASAKURA et al., 1998). Indeed HrWnt-5mRNA is present in

the vegetal cortex of unfertilized eggs. After fertilization, HrWnt-5 moves to the equatorial region to form a sickle-shaped structure after which this mRNA is concentrated in the most caudal region of the embryo. That Vg1 (the axis inducer) present in the avian Rauber's sickle material (inclusive in the sickle horns) and not in the caudal marginal zone has recently been demonstrated (BERTOCCHINI & STERN, 2007). Hensen's node must be considered as a secondary major organizer linked to Rauber's sickle via a rostral outgrowth of the latter i.e. sickle endoblast. At the moment of the sickle-shaped bilateral symmetrization (characterized by the appearance of Rauber's sickle), there occurs a spatially oblique, sickle-shaped uptake of γ and δ ooplasm by oblique position in utero which become incorporated into the deeper part of the avian blastoderm (Fig. 8). This provokes an unidirectional chaos (radial symmetry breaking) in the ooplasm according to the principle of PRIGOGINE (PRIGOGINE & LEFEVER, 1968) which is indispensable for further development. These ooplasmic determinants (CALLEBAUT, 2005) which initiate (perhaps by Wnt signalling) either early gastrulation or neurulation phenomena by positional information (CALLEBAUT et al., 2003a). First we found (cytological) evidence for a radial predisposition (preformation) in the premature avian oocyte i.e. radially symmetric and concentric distribution of groups of mitochondria (CALLEBAUT, 1972) (Fig. 9). After the eccentric sickle shaped tilting of the yolk and ooplasmic layers in the fertilized egg by oblique positioning (CALLEBAUT et al., 2000), we observed the presence of predisposed sickle-shaped anlage fields in the upper layer (CALLEBAUT et al., 1996). There is a strong similarity with the gastrulation and neurulation phenomena described by VANDEBROEK (1969) in selachian germs (also developing on very large eggs). Indeed, in the latter vertebrate group there exist also analogous sickle-shaped anlage fields, localized in the upper layer in the same succession order as in birds. These fields are localized in the

concavity of Rauber's sickle in unincubated diblastic avian blastoderms which thus present a sickle-shaped bilateral symmetry (Fig. 10). Finally from this sickle-shaped bilateral symmetric disposition a primitive streak and neural plate will develop by convergent extension movements under influence of signalling molecules secreted by Rauber's sickle (CALLEBAUT et al., 2003a) (Fig. 11) so a triblastic one-axis-containing embryo is formed. The early neural plate inducing structure which forms a deep part of the blastoderm is the δ ooplasm-containing endophyll (primary hypoblast) (Fig. 8). Together with the primordial germ cells it is derived from the superficial centro-caudal part of the nucleus of Pander which also contains δ ooplasm. It is indeed known that the pre-laid nucleus of Pander (as is also the case for the endophyll) can induce the upper layer to form an early neural plate (CALLEBAUT et al., 2004b). The other structure (γ ooplasm) which is incorporated into the caudolateral deep part of the blastoderm forms Rauber's sickle (Figs. 7; 8). It induces first gastrulation (intramuros) and later blood island and coelom formation (extramuros). Rauber's sickle develops by ingrowth of blastodermal cells into the γ ooplasm (CALLEBAUT, 1994), which surrounds the nucleus of Pander (Fig. 8). The Rauber's sickle constitutes the primary major organizer of the avian blastoderm. Rauber's sickle generates only junctional and sickle endoblast and by positional information, organizes and dominates the whole blastoderm (first gastrulation, neurulation, and later coelom and cardiovascular system formation) (CALLEBAUT et al., 2003b). Fragments of the horns of Rauber's sickle extend far cranially into the lateral quadrants of the unincubated blastoderm, so that often Rauber's sickle material forms three quarters of a circle (Fig. 7). This explains the so called regulative capacities of isolated blastoderm parts with the exception of the anti-sickle region and/or the central blastoderm region, where no γ ooplasm and no Rauber's sickle material is present (which again demonstrates the influence of the γ ooplasm) (CALLEBAUT, 2005). Recently we demonstrated that in the one and the same species (*Gallus domesticus*) and the same unincubated blastoderm both mosaic development or regulation phenomena can be obtained (CALLEBAUT et al., 2007). Indeed after hemi-sectioning of unincubated chicken blastoderms and culturing both halves formatted *in vitro*, two kinds of development can be discerned: 1. When the unincubated blastoderms were hemi-sectioned according to the plane of bilateral symmetry, going through the middle region of Rauber's sickle (Fig. 12), we obtained two hemi-embryos (a left and a right one) containing each a half primitive streak (starting from the most median parts of Rauber's sickle) giving rise to a half mesoblast mantle and a half area vasculosa, which differentiate incompletely thus indicating mosaic development. This (mediosagittal) hemi-sectioning of the avian blastoderm is comparable with the unilateral destruction experiments of the first two ascidian blastomeres by CHABRY (1887) and CONKLIN (1905). Indeed, the ascidian two-cell embryo already presents a left-right asymmetry visualized by the natural mediasagittal cleavage plane through the caudal sickle-shaped Wnt gene expressing zone (SASAKURA et al., 1998), homologous with Rauber's sickle. 2. When the unincubated blastoderm is hemi-sectioned more obliquely going through a

more lateral part of Rauber's sickle (sickle horn), two complete bilaterally symmetrical miniature embryos will form indicating so-called regulation phenomena (Fig. 13). We demonstrated that those two types of development are in reality due to the different spreading and concentration of Rauber's sickle tissue around the area centralis (CALLEBAUT et al., 2007). Embryonic regulation must thus not be considered as a kind of totipotent regeneration capacity of isolated parts of the unincubated avian blastoderm but depends on the spatial distribution of a kind of extra-embryonic tissue (Rauber's sickle) build up by the late oblique uptake of γ ooplasm at the moment of bilateral symmetrization (CALLEBAUT, 1994; CALLEBAUT et al., 2000) forming an ooplasmic mosaic. Thus not only embryonic gene expression phenomena take place during early development but also uptake of extraembryonic preformed ooplasmic determinants play a fundamental role for the initiation of gastrulation, neurulation and cardiovascular development. So finally three ooplasmic determinants are respectively found in the three elementary tissues (not germ layers!) of the early, unincubated avian blastoderm (Figs 7; 8): the upper layer from which the embryo proper develops containing mainly β ooplasm (forming the embryonic stem cells), the Rauber's sickle containing γ ooplasm and the endophyll (primary hypoblast) containing δ ooplasm both forming the extraembryonic tissues. In mammals also the embryonic ectoderm (upper layer) constitute the real stem cells which under influence of the surrounding extraembryonic tissue transform in mesodermal and neural cell lines (HADJANTONAKIS & PAPAIOANNAOU, 2001). So the general body plan (bilaterally symmetric) is established in the diblastic germ. This confirms, in the case of birds, the existence of a similar master plan for the early development as was proposed for all chordates by EYAL-GILADI (1997). According to the hypothesis of Eyal-Giladi, the speed at which axialization of the embryo proper takes place, depends on the translocation speed of oocytal determinants from the vegetal pole towards the future dorsocaudal side of the embryo. After arrival at their destination, the activated determinants form in all chordates, an induction center homologous to the amphibian "Nieuwkoop center" and the ascidian Wnt expressing sickle shaped region, which later organize the formation of the intraembryonic "Spemann's organizer". Thus in birds by using radioactive or trypan-blue induced fluorescence oocyte labelling we could demonstrate that a kind of evolutive preformism exists which follows more or less the main evolution of the animal kingdom: from a radial symmetric disposition in the premature oocyte (as exists in coelenterates) via a sickle-shaped diblastic bilateral symmetric germ to a triblastic one-axis-containing embryo (gastrulation induced in aves by Rauber's sickle). Finally, we observed the formation of the coelomic cavity with associated cardiovascular system (typical for coelomates; DOLLANDER and FENART, 1973) also induced by Rauber's sickle material (CALLEBAUT et al., 2002; 2004a). An ooplasmic influence on spatial patterning of the mouse blastocyst has been demonstrated by GARDNER (1997), by using the localization of the polar body as a marker for the animal pole. Also PIOTROWSKA and ZERNICKA-GOETZ (2001) showed that the sperm entry position products the plane of initial cleavage of the mouse egg and can define embryonic and abembry-

onic halves of the future blastocyst. In addition, the cell inheriting the sperm entry position acquires a division advantage and tends to cleave ahead of its sister.

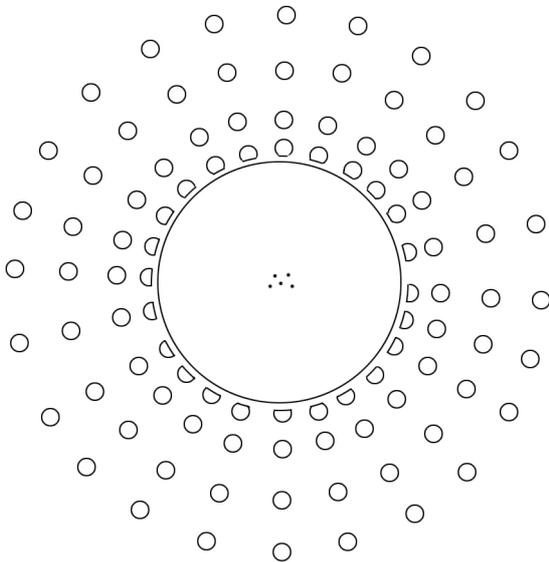


Fig. 9. – Schematic drawing of the ooplasmic radial symmetry in the premature quail (*Coturnix coturnix japonica*) oocyte, visible by the presence of RNA-rich subcortical cytoplasmic organelles (aggregates of mitochondria represented by small circles) (CALLEBAUT, 1972; D'HERDE et al., 1995).

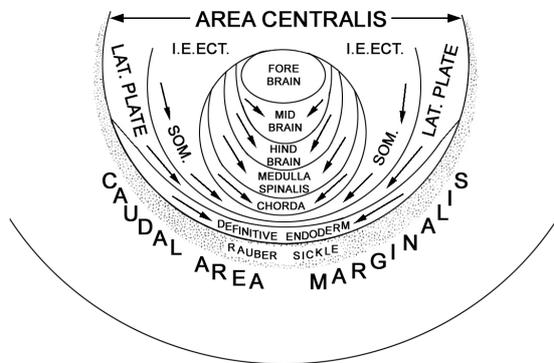


Fig. 10. – Schematic representation of the mean localization of the predisposed (not definitively committed) anlage fields (in good order but with possible partial overlapping of neighbouring parts) in the upper layer of the area centralis of a chicken (*Gallus domesticus*) unincubated blastoderm (slightly simplified after CALLEBAUT et al., 1996). Note the general eccentric sickle-shaped aspect of the anlage fields in the area centralis after the radially symmetry breaking eccentricity of the subgerminal ooplasm. There is an obvious parallelism between the sickle shape of the anlage fields in the UL and the ovoid central subgerminal ooplasmic layers (CALLEBAUT et al., 2000). The curved arrows on the anlage fields indicate the logically previsible converging movements of the upper layer during the ensuing gastrulation (WETZEL, 1929) and neurulation (BORTIER and VAKAET, 1992).

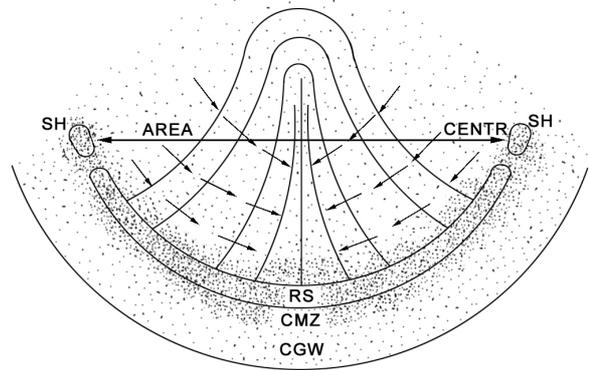


Fig. 11. – Combined schematic drawing representing: 1. the hypothetical diffusion of morphogens or signalling molecules (indicated by small dots) emanating from Rauber's sickle (RS) and its sickle horns (SH) into the neighboring tissues of the avian blastoderm, i.e., into the area centralis (AREA CENTR), into the caudal marginal zone (CMZ) and into the caudal germ wall (CGW), where they can influence (induce or inhibit) ectopically placed structures (endophyll, Rauber's sickle fragments, sickle or junctional endoblast) to form or not to form a second streak; 2. the broad movements (indicated by curved arrows) of cell groups in the upper layer of the area centralis, in the direction of the median primitive streak (partially after WETZEL, 1929). The curved legs of the U-shaped lines indicate moving fronts of cell groups (corresponding to local temporal primitive streak anlagen) that will ingress after fusion into the final median primitive streak.

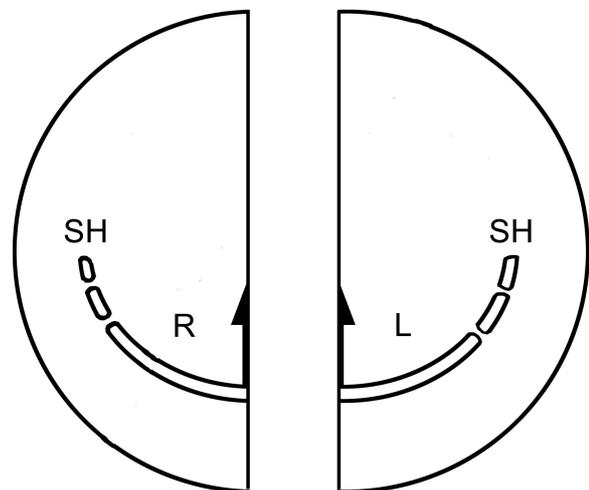


Fig. 12. – Schematic representation of the hemi-sectioning through the middle of the sickle of Rauber (double lined) of an unincubated blastoderm; both halves of the sectioned blastoderm are represented at some distance (ready for culture); SH: sickle horns of Rauber's sickle extending far cranially; R: right half blastoderm and L: left half blastoderm will each transform in a right and left half embryo, indicating mosaicism; the thick half arrows represent the formation of hemi-primitive streaks at the cut edge of the blastoderm after incubation.

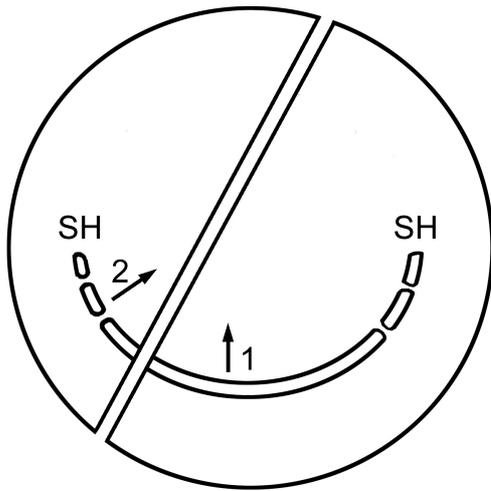


Fig. 13. – Schematic representation of the oblique hemi-sectioning through the lateral part of the Rauber's sickle of an unincubated avian blastoderm; the arrows 1 and 2 indicate the place where after culture a bilateral symmetric primitive streak will appear respectively under the influence of the middle part of the Rauber's sickle (double lined) or under influence of the remaining fragmentary sickle horn (SH), indicating regulation.

OOPASMIC DETERMINANTS IN PRIMORDIAL GERM CELLS

NUSSBAUM (1880; 1901) and WEISMANN (1892) were the first to propose the preformation thesis in birds i.e. that the primordial germ cells have inherited and retained for long periods the yolk from the precursor oocyte from which their egg yolk ball and blastoderm are derived. In birds DANTSCHAKOFF (1908) observed so called "endodermal Wander Zellen" in the space between the upper layer (epiblast) and deep layer. The primordial germ cells (PGC's) are indeed only first unequivocally distinguishable in the 1-8 somite chicken blastoderm between epiblast and endophyll of the germinal crescent (SWIFT, 1914). BOUNOURE (1939) describes a kind of "germ plasm" in the vegetal pole of Anura eggs. By irradiation this germ plasm could be inactivated, giving rise to sterile individuals. Only cells which inherited part of this germ plasm gave rise to PGC's. It was thus the kind of ooplasm which determined the fate of the involved cells. The germ plasm thus contains so-called "Keimbahn determinants". Because PGC's in birds are found close to the endophyll and seem to immerge from it, VAKAET (1962) supposed that they were derived from the endophyll. DUBOIS (1967; 1969) also concluded that the endophyll is at the origin of the formation of PGC's in birds. The precursors of the PGC's which are often initially morphologically indistinguishable from the surrounding somatic cells in earlier stages, are called presumptive primordial germ cells (pPGC's). These divide mitotically to produce one PGC containing "Keimbahn determinants" and one somatic cell. Thus in general it was accepted that chicken germ cells originate from the primitive deep layer. By contrast, EYAL-GILADI et al., (1981) concluded by using chick-quail chimeras, made before primitive streak formation (i.e. stage XIII: 10-12h incubation), that avian PGC's

were from epiblastic origin. Avian PGC's were then thought to arise through a gradual epigenetic process. However, in these older blastoderms the deep layer is no longer composed of endophyll but mainly formed by sickle endoblast, derived from Rauber's sickle (CALLEBAUT et al., 1997). Indeed the endophyll and associated PGC's are then already displaced cranially and adhere to the deep cranial part of the epiblast and to the there present hemicircular fibrous bands (ENGLAND, 1983). They will form part of the endophyllic crescent in older stages. The experiments of CUMINGE and DUBOIS (1992) seemed to confirm the thesis of Eyal-Giladi et al., but they also investigated similar old blastoderm stages which greatly differ from the unincubated blastoderm. By using trypan blue induced fluorescent labelling of the ooplasmic yolk layers of quail oocytes during their final post-lampbrush stage, I could demonstrate that primordial germ cells together with the endophyll contain yolk from the deep central region of the germ disc i.e. δ ooplasm from the superficial part of the nucleus of Pander (CALLEBAUT, 1984; 1987). So nearly 95% of the PGC's can be labeled 6-7 days after one single injection of trypan blue to the mother quail. Oocytal yolk labeling, 1 to 4 days after an injection gives no labeling of the primordial germ cell yolk, but gives labeling of more superficial somatic cells which contain more superficial ooplasm (β or γ). The observed trypan blue induced fluorescent yolk labelling in the caudally in the area centralis localized endophyll of the unincubated quail blastoderm (CALLEBAUT, 1987) is in agreement with the observed localization of the pPGC cells (also containing δ yolk) after transection experiments (FARGEIX, 1967; ROGULSKA, 1968; DUBOIS and CROISSILLE, 1970) i.e. mainly in the caudal region of the unincubated blastoderm. The original deep and central localization of pPGC material has recently been confirmed by the use of a chicken vasa homologue (TSUNEKAWA et al., 2000). Chicken vasa protein forms part of the mitochondrial cloud in younger chick oocytes and localizes to the central cleavage furrows (which extend into the δ ooplasm of the nucleus of Pander) until stage IV (EYAL-GILADI and KOCHAV, 1976). At that moment 6 to 8 cells of the approximately 300 blastomeres containing germ, present vasa protein and are probably pPGC's. (TSUNEKAWA et al., 2000). The data of CALLEBAUT (1984) and TSUNEKAWA et al. (2000) thus indicate that a kind of deep preformation may be the mechanism for germ cell specification in birds. As in *Xenopus*, in the quail there are two known populations of oocytal mitochondria which become finally localized in the early embryo: one population becomes localized in the vegetal pole where it forms a component of the germ plasm in *Xenopus* (MIGNOTTE et al., 1987; TOURTE et al., 1984) and a component of the nucleus of Pander (δ ooplasm) in the quail (CALLEBAUT, 1984; D'HERDE et al., 1995). The other population of mitochondria is localized much more superficially and forms the obvious radially and concentrically disposed group around the germinal vesicle both in *Xenopus* (MIGNOTTE et al., 1987) as in the quail (TICOS: CALLEBAUT, 1972; 1983; D'HERDE et al., 1995). These mitochondria will populate the somatic tissues of the offspring (in *Xenopus*: DAWID and BLACKLER, 1972) (in quail: WATANABE et al., 1985). Our conclusion is in agreement with WOLPERT (1998) and EXTAVOUR and AKAM

(2003) that epigenetic germ cell development (derived from somatic stem cells) is an exception and that most animals use localized ooplasmic determinants to specify the germ line.

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