The Emergence of Life and Form!

Living in the 21st century as we gingerly grind towards a future of genetically engineered humans, it is perhaps a good time to mull over the journey of our quest to understand the beginning of new life. From ancient Arabs and old Germans postulating that mothers alone were responsible for the origin of newborns to ancient Greeks placing the burden of origin on fathers[1], indeed, humanity's interest in early embryonic development is of immense antiquity. Hippocrates (460-380 BC), the ancient physician, imagined that a mother's breath had the power to endow form[3,4]! In the early years of Scientific Revolution, the smartest men and women of the day grappled with the big questions of the origin of new life. In the absence of tools for a systematic enquiry, early philosophical debates seeking to explain the emergence of organic forms gave rise to two intensely contended ideas - Preformationism and Epigenesis, that applied to all organisms - plants and animals.

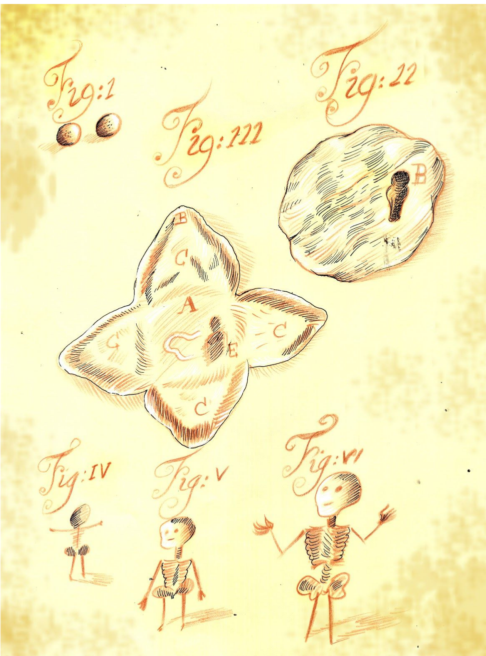

Figure 1: Preformationism- this image is called 'origin of man' and has been adapted from the original woodcut by the Dutch anatomist Thomas Kerckring. It was first published in 'A ground-plan of the origin of man' (Anthropogeniae ichnographia) in 1671. Within the figure itself Fig I shows 'two humane eggs of different bigness', fig II 'an Embryo of three, or at the most four days after Conception', and fig III 'the Hepar uterynum [placenta] with the Veins and Arteries ... dispersed through the substance of it'. Fig IV 'represents to the eye a gristly Skeleton of an embryo of three weeks', fig V 'an Embryo of one month', and fig VI, to the modern eye the diminutive skeleton of a child, 'an Embryo of six weeks'.

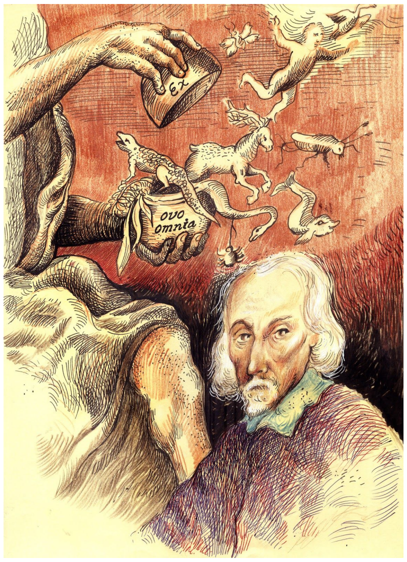

Figure 2: William Harvey and preformationism. Image at the back is adapted from the original etching by Richard Gaywood, titled Ex ovo omnia, and is inspired by William Harvey's preformationism. The frontal figure in this image is the portrait of William Harvey.

'Preformationism' ('Emboitement' - as called in the 18th century) argued that every organism pre-existed as a fully formed miniature individual ('homunculus' for humans, 'animalcule' for other animals) as either the egg (the 'ovist' view) or the sperm (the 'spermist' view) [5]. From the Greek philosopher Pythagoras (570 BC to 495 BC) to the evolutionary biologist Ernst Haeckel (1834-1919), many naturalists were familiar with the concept of young animals hatching from eggs. It implied that other animals that gave birth to their young might also have an egg stage during early development.

Figure 3: Dutch Biologist Jan Swammerdam and his study on the development of a butterfly. Image in the thinking bubble is adapted from the original drawing by Swammerdam

In his seminal work of 1651, 'On the Generation of Animals' ('*DeGeneratione Animalium*'), prominent witchcraft-sceptic and '*Physician Extraordinary*' to King James-I, William Harvey, denounced spontaneous generation and advanced the doctrine of '*ex ovo omnia*' by proposing that all life originated from eggs ('*Omne vivum ex vivo*'). Harvey, one of the most prominent 'Ovists' of his time used chicken eggs as a model system that King Charles-I had graciously allowed him to kill in the royal forests! [7]. His avowed critic, Dutch biologist Jan Swammerdam (1637-1680), author of '*Histoire des insects',* however, studied frogs, butterflies and moths and concluded that each developmental stage (egg, larva, pupa, adult), even if it underwent "astonishing transformations" during development, was already 'nested' within the previous one and was therefore pre-formed[8,9]. Their contemporary and eponymous discoverer of the graafian follicle and corpus luteum in rabbits, Regnier de Graaf (1641-1673), established the egg as the undisputed source of life, when he published '*De mulierurn organis generationi inservientibus*' ("The Generative Organs of Women"). Even if he was not a preformationist and also performed exciting experiments on male reproductive organs, he did believe that the egg contained the germ of the future organism [10], inadvertently giving 'Ovism' a shot in the arm.

Figure 4: Regnier de Graff's experiment with the ovary. Drawing of the ovary on the board has been adapted from the original drawing done by de Graff which was drawn while experimenting with a cow. De Graff used cows, rabbits and dogs for anatomical dissection studies.

Eighteenth century naturalist Charles Bonnet (1720-1793) was a frail man who at the tender age of 20, while still a student of law started growing plant louse (aphids) in seclusion. The blind naturalist chanced upon the strange phenomenon of 'parthenogenesis', thus pushing 'virgin birth' into the realm of science [6,7].This was a definitive triumph for 'ovism' that made Bonnet one of its brightest stars! Around the same time, Lazzaro Spallanzani '*Magnifico*' (1729-1799), was on one hand weakening the defense of Needham's spontaneous generation and on the other, he was chopping up salamander's tails only to put them back together. Elegant and technically difficult experiments were beginning to reveal the beautiful regenerative capabilities of animals and scientists were hard pressed to explain them. The 18th century was perhaps the golden age of regeneration research and strangely, prominent scientists seemed to conflate the two ideas of reproduction and regeneration. Bonnet even had a 'germ' theory to explain both phenomena - 'germs' in the body mediated reproduction and regeneration! Spallanzani, a preformationist, was finding it difficult for 'germ' theory to explain some results of regeneration. However, he was a persistent skeptic and even published results that went against his understanding of preformation. His letters to Bonnet, a stalwart at the time, are invaluable for embryologists! [28]

Figure 5 (left): Antony Leeuwenhoek and his experiment with sperm. The drawing of the sperm in the background has been adapted from the original drawing by Leeuwenhoek. His drawing on sperm was published in The Philosophical Transaction (journal of the Royal Society) in 1678, which is widely regarded as the beginning of systematic study of sperm biology via publication in a peer reviewed journal.

Figure 6 (right): Nicolaas Hartsoeker and his sperm drawing. This human figure in the head of a spermatozoon has been adapted from the original drawing, published by Nicolas Hartsoeker. The man in the image is a portrait of Hartsoekar! The drawing of the sperm was first published in Essay de dioptrique in 1694.

The Ovist view was challenged in the late 17th century after the Dutch microscopist AV Leeuwenhoek, a committed preformationist, discovered spermatozoa. In his words,"*all manner of great and small vessels, so various and so numerous that I do not doubt that they be nerves, arteries and veins…*". He argued that humans originated from animalcules present in semen![12]. Inspired by this discovery, Nicolas Van Hartsoeker (1656-1725), a mathematician and inventor of the screw-barrel simple microscope, wrote '*Essai de Dioptrique*' in 1694, where he proposed and sketched a tiny human curled up inside the sperm - thus providing us with the iconic illustration of preformationism! [13] This hypothesis relegated the egg to merely a receptacle that was waiting for the mighty sperm to kickstart embryo development! While probably resonating well with patriarchal notions of the time and receiving considerable support, it is sexist by today's standards in addition to being incorrect. However, for many who engaged in deep philosophical debates on religion and God, the idea that they were previously encased in strange looking motile 'worms' coupled with the implied wastefulness where millions of lives had to be lost for one conception, was blasphemous.

An unintended effect of 'Preformation' was that this view of embryogenesis suited the ruling class well. By putting lineages inside each other like Russian Matryoshka dolls, Preformation implicitly legitimized the anti-democratic dynastic system of the day [11]. Even if preprogrammed encasement of successive generations was the central tenet of preformation, the organization and amount of encasement underwent several revisions through the years. So much so that in the course of a century (mid-1600s to mid-17OOs), preformationist hypotheses evolved from completely pre-formed individuals to pre-existing fundamental parts. Interestingly, in its final form, it came tantalizingly close to our present models in developmental biology. But that leap, when put in the context of available technology at the time, was impossible to make!

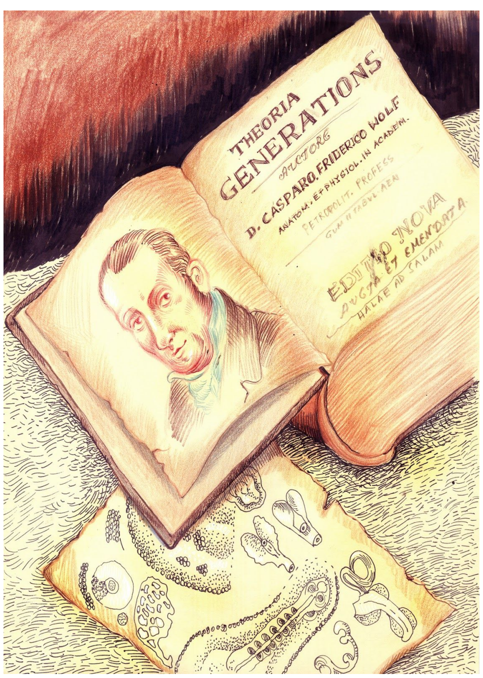

Figure 7: Casper Friedrich Wolf and his drawings of Wolffian bodies.

Discovery of cells by Robert Hooke (1635-1703) and emergence of 'cell theory' in 1839 from the work of Theodor Schwann, Matthias Schleiden and Rudolf Virchow challenged the Preformation hypothesis. In 1759, a young student Caspar Friedrich Wolff reported primitive kidneys (or "Wolffian bodies") in his dissertation '*Theoria Generationis*' and described embryonic development as a process involving 'layers of cells' [14]. It was a provocative idea and generated so much controversy at the time that it turned out to be a pivotal moment in the re-emergence of epigenesis as a conceptual framework of embryogenesis.

Figure 8: Aristotelian epigenesis- the man in the image is a representation of Aristotle working on chick embryo. The series of images depicting stages of egg development has been adapted from the original woodcuts published in Rueff's textbook illustrating the gradual coagulation of male and female seeds into a child. The egg-shaped mass covered with three membranes gradually develops blood vessels and organs such as the liver and heart that resembles the human form and finally turns into a child (right side of the figure).

'Epigenesis' (or 'Neoformism') was the antithesis of preformation. Greek stalwarts Aristotle and Heraclitus argued that form gradually emerged out of developmental free will, in steps - relying on environmental cues. From his observation of developing chick embryos at different stages, Aristotle (384BCE-322BCE), teacher to Alexander - the conqueror, concluded in Book-2 of the 5-book '*De Generatione Animalium*' that form and organs were acquired gradually to ultimately form an organism [15]. Unfortunately in the West, for the next several centuries after Aristotle, science and especially embryology were influenced by religious doctrines and no major advancements were made. On the other hand, ancient Indian texts like '*Carakasamhitā*' (composed between 400BCE to 200CE), Jain texts like the '*Tandulaveyāliya*' and Buddhist texts '*Garbhāvākrāntisūtra*' (composed within the first few centuries of the Common Era) contain medical or pseudo-medical details of step-wise fetal development during human gestation[16,17]. Sanskrit texts like '*Agnipurāna*', composed between the 7th-11th century and one of the 108 Sanskrit upanishadic texts – '*Garbhopanishad*', contain almost month-to-month details of a growing fetus in its uterine environment [16]. These medico-religious texts did not deliberate on modes or mechanisms of embryogenesis, however, some among them, especially, '*Carakasamhitā*' and '*Garbhāvākrāntisūtra*' emphasized that a growing embryo gradually developed over time and could therefore be considered 'epigenetic' in their outlook.

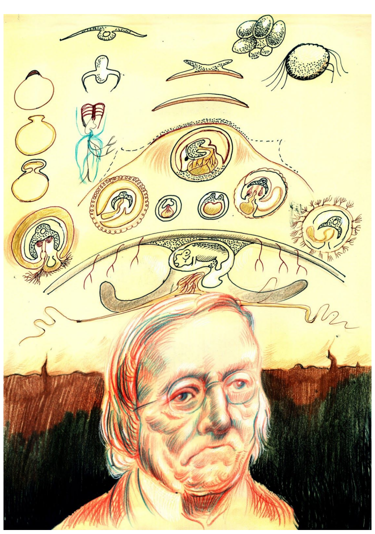

Figure 9: Ernst Von Baer and the stages of mammalian oocyte development. The background image depicts oocyte development and vertebrate body plan as illustrated by Von Baer. The original image was published in "On the History of animal development", by K E Baer, 1953.

During his stay at Konicsberg, an old Prussian city, Karl Ernst Von Baer (1792-1876) observed mammalian oocytes for the first time in the ovaries of his collaborator's dog. However, before him, Swammerdam, Van Horne and De Graaf had worked with reproductive organs and

Swammerdam had injected wax into soft tissues to preserve and study their structure and had claimed to have identified ovaries – so we know that the concept of female reproductive organs was known even during the 16th century [8]. Baerian ideas on comparative embryology gave rise to "Von Baer's Laws" that emphasized sequential movement of organismal development from homogeneous to heterogeneous, thus making it 'epigenetic' [8,18].

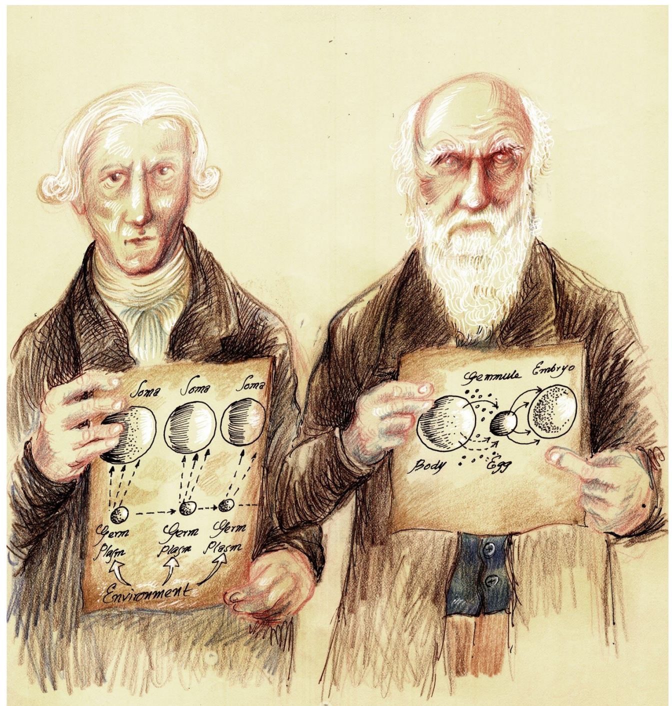

Figure 10: Lamarck (left), explaining the role of environment on embryo development and Darwin (right), explaining his theory of Pangenesis.

Prominent naturalists and evolutionary biologists of the 18th-19th century, Erasmus Darwin (1731-1802) and Jean B Lamarck (1744-1829), strongly believed in the overarching role of the environment in patterning of the early embryo. An influential critic of Lamarckian inheritance, German evolutionary theorist August Weismann (1834-1914), proposed the 'germplasm theory' that sought to establish a stark distinction between functions of somatic- and germ-cells. He envisioned germ cells to be impervious to environmental and somatic influences[20]. However, in support of Lamarckian ideas, Erasmus Darwin's famous grandson Charles Darwin (1809-1882), postulated the idea of "pangenesis". In his book 'Variation in Plants and Animals under Domestication', he argued for the ability of environmental cues to induce somatic cells to shed "gemmules" or "pangenes" that accumulated in germ cells and transferred information to the next generation [19]. It is indeed remarkable how Lamarck's ideas on the 'theory of inheritance of acquired traits' was initially disfavored and then regained legitimacy as the cornerstone of transgenerational epigenetics!

Figure 11: August Weismann and his germplasm theory.

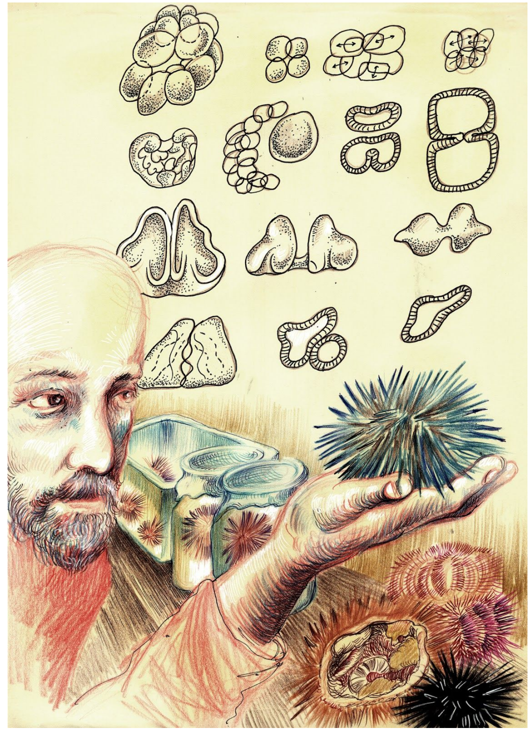

Figure 12: Hans Driesch and his experiment with Sea-urchin embryos. The background images of this figure have been adapted from an original drawing by Hans Driesch, published in 1902.

However, in general, the later part of the 19th- and turn of the 20th-century saw extreme positions giving way to interactionist models of development. Excitement was brewing as chromosomes were being described for the first time in the German laboratories of Theodor Boveri (1862-1915), Oscar Hertwig (1849-1922) and others. Haeckel's student, the German embryologist Hans Driesch (1867-1941), made epochal observations with sea urchin embryos and generated initial ideas on cellular potency that provided important refutation of Preformationism [21-23]. His conclusions, however, were in contrast with another of Haeckel's proteges, Wilhelm Roux (1850-1924), whose experiments with frog embryos led him to conclude that embryonic development was entirely a consequence of factors internal to the embryo[21-23]!

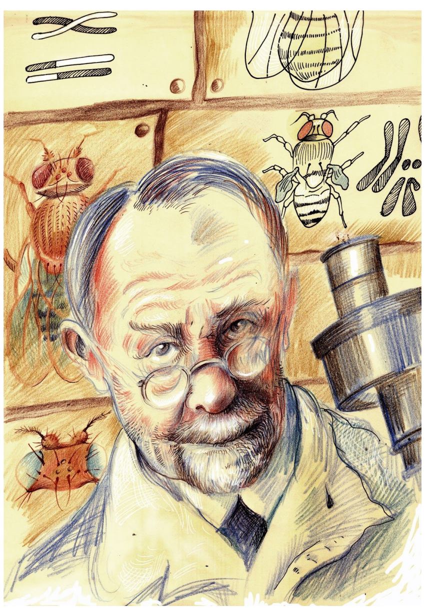

Figure 13: TH Morgan and his drosophila drawings.

Driesch's colleague, TH Morgan, the embryologist who later spearheaded 20th century genetics using fruit-flies, did not initially see a role for chromosomes in explaining acquisition of form [24]. In 1907, Morgan went so far as to remind readers of *Science* how the idea of chromosomes being passed down as heritable particles closely resonated with the old problem of Preformation! However, considering groundbreaking evidence from his own lab, he published the conceptual work, '*Mechanisms of Mendelian Heredity*', in 1915 - which explained his shift in allegiance and laid the foundation of what came to be known as 'new-preformationism'. However, Morgan had insisted that "we have two factors determining characters: heredity and modification during development" [21-25]. Interestingly, the cloning of 'Dolly' in 1997 by Ian Wilmut's team [26] and development of human ES-cell lines by Thomson and Gearhart [27] emphasized the flexibility and plasticity of early development and laid the foundation of 'new epigenesis'.

Epigenesis could explain variations and direct observations of organogenesis better but preformation excelled at addressing the phenomena of continuity across generations. It is also remarkable how experimental model systems influenced attitudes towards understanding organismal development. Even if technological advances of the last few decades made stunning and dramatic insights possible in developmental biology, it is indeed quite extraordinary that the profound debates through centuries provided theoretical foundation for it. Given the rich traditions of imagination, discussions and discourse over millennia, perhaps it was just a matter of time before cloned animals, iPS-cells and CRISPR-babies became a reality.

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