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Contribution of the Belgian school of embryology to the concept of neural induction by the Organizer

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When Spemann and Mangold's historic paper "Über Induktion von Embryonanlagen durch Implantation artfremder Organisatoren" was published in 1924, Belgian embryology was clearly dominated by the personality of Albert Brachet (Fig. 1), founder of the famous Brussels school of embryology (Mulnard, 1992). Having obtained his degree in medicine from the *Université de Liège* where he worked as an assistant, he accepted in 1904 the Chair of Anatomy of the *Université Libre de Bruxelles*. There he conducted very active and fruitful research concerning the descriptive embryology of vertebrates, and also experimental embryology, a discipline he had learned from G. Born during a stay in Breslau in 1895. He was to make this discipline, which he called causal embryology, a major one within the life sciences.

Having demonstrated the role played by the spermatozoon in symmetrisation of the anuran egg and in the distribution of materials within it (Brachet, 1904), he became interested in the role of this distribution, and particularly in the role of the grey crescent, which he suspected might be the site of formation of all the dorsal organs. He was convinced that localised destruction of parts of the grey crescent, one at a time, would be the best way to study its properties. Unlike other authors, he chose to work on advanced blastulae, i.e., ones at a stage immediately preceding the appearance of the dorsal groove of the blastopore. He conducted delicate experiments in which he selectively destroyed zones of the grey crescent with a heated needle. The results are described and discussed at length in a long article published in "Archives de Biologie", a journal that enjoyed, at the time, an international reputation. One of the significant figures of this paper is reproduced here (Fig. 2) (Brachet, 1923).

Some particularly pertinent conclusions are worth mentioning. Brachet observed that the median region of the grey crescent is an area of spontaneous differentiation. This was in agreement with previous work by Spemann on the newt egg, except that Brachet attributed to a specific point on the grey crescent of the blastula a morphogenetic power that Spemann attributed strictly to the dorsal lip of the blastopore (Spemann, 1918). Brachet also observed that this "primary self-differentiation centre" determines, upward from it, the differentiation of the material forming the anterior head and, downward from it, that of the material forming the posterior head, trunk, and tail. Lastly, he showed that this centre acts on neighbouring regions having virtual potentials, causing them to enter specific activity via production of a factor or "excitant" about whose nature the question arose: "harmozone" or "ferment"? This indirect evidence of the organisation centre of the axial organs in anurans received, as everybody knows, masterful confirmation and a final demonstration in Spemann and Mangold's transplantation experiments in the newt. A. Brachet mentioned this parallel in 1927 in an article devoted to a comparative study of germinal localisations in the eggs of anuran and urodele amphibians (Brachet, 1927). In it he also presented the encouraging results of his own attempts at

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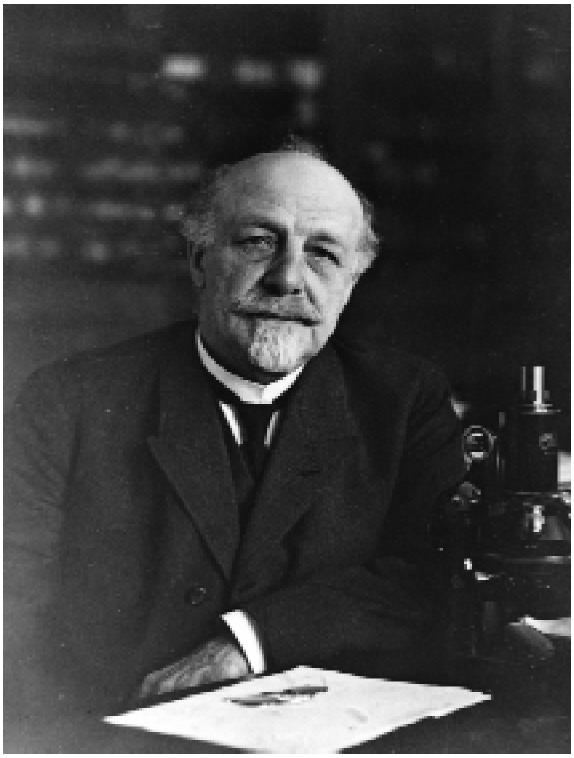

Fig. 1. Albert Brachet (1869-1930). *(Archives de l'Université libre de Bruxelles).*

transplantation in *Rana fusca*, such experiments being particularly difficult in this species.

It is thus fair to say that A. Brachet contributed significantly to the emergence of the organiser concept, as attested also by references to his work made by Spemann himself in an overview published in 1936 (Spemann, 1936). In addition to demonstrating the existence of an organisation centre in anurans, A. Brachet also demonstrated its early determination, since the chordamesodermal territory corresponding to the median zone of the grey crescent acquires organising activity well before the lip of the blastopore is formed. Yet the justified excitement surrounding Spemann et Mangold's work was such that all references to that of A. Brachet have totally disappeared from the scientific literature, even though citations of the German authors have increased exponentially without interruption since 1970 (Elison and Holowacz, 1995).

Albert Dalcq, successor and faithful disciple of A. Brachet (who died young in 1930), continued the work of the «untiring apostle» of causal embryology (Dalcq, 1931) by trying to solve the problem of the organiser's determination. He notably showed, in *Discoglossus*, that a double embryo forms as a result of a "latitudinal translocation" occurring when the animal hemisphere of the blastula, including the upper half of the chordamesodermal territory, rotates 180° (Fig. 3) (Dalcq, 1933). Yet what gave him for over a decade a preponderant place in the debate on the determination of early embryonic induction was a theoretical reflection, elaborated with his closest collaborator and future successor Jean Pasteels.

Their view was inspired by that of Child, who had worked on integrating the organiser into the theory of physiological gradients. Child postulated that at gastrulation, a new physiological gradient starting at the organiser replaces a primary gradient decreasing from the animal to the vegetal pole (Child, 1928; 1929). Dalcq and Pasteels, however, favoured the notion of a gradient of distributed constituents, making the animal-vegetal yolk gradient one of the factors responsible for morphogenesis. They also proposed the existence of a field with a dorsally situated focus, imposing differences according to dorso-ventral polarity. This hypothesis could explain certain experimental observations such as the indispensable character of yolk accumulated at the vegetative pole (Vintemberger, 1938a,b) or the effect produced when the yolk is displaced by maintaining the egg in an inverted position, compressed between two microscope slides (Penners et Schleip, 1928; Pasteels, 1938).

The model proposed by Dalcq and Pasteels can be summarised as follows: morphogenesis results from the interaction of the «dorsal field» with the yolk gradient, or rather from a chemical combination of a cortical constituent C of the field and a vegetal substance V. The concentration of product CV is what determines the fate of the different territories, a region's "morphogenetic potential" increasing with the CV concentration. Because the CV concentration shows a regular decrease dorso-ventrally in the chordamesoblastic layer, the highest morphogenetic potential is in the notochord, followed successively by the somites, the intermediary mesoderm, and the lateral plates. Segregation of the anlagen arises from a series of CV-concentration thresholds. Thus, for these authors, Spemann's organisation centre is formed by the combination of two factors, a yolk factor and a cortical factor linked to the grey crescent (Dalcq and Pasteels, 1937). First imagined for amphibians, this 'field-gradient-threshold' concept was extended to other vertebrates, the ascidians, and the sea urchin (Dalcq, 1938).

Pasteels later contributed to producing experimental evidence in favour of the existence of the hypothetical inducing substance CV, by centrifuging fertilised eggs and studying the influence of this treatment on their development. The major nervous-system deficiencies that he observed are easily explainable if the inducing substance, less dense than the yolk, moves towards the centripetal pole (Fig. 4) (Pasteels, 1940).

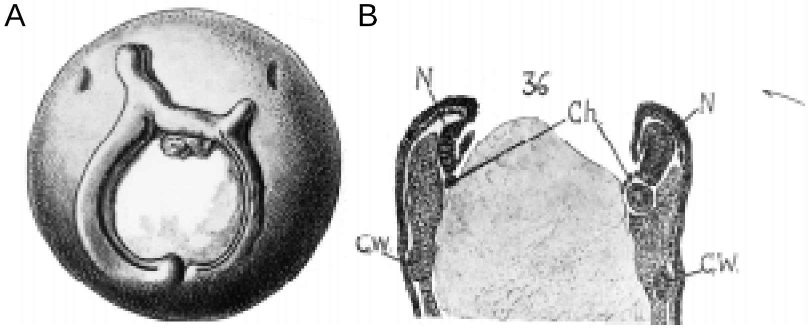

Fig. 2. Detail of a plate from the article by A. Brachet dealing with the effect of pricking specific regions of the grey crescent of the *Rana fusca* embryo with a heated needle. *Embryo fixed 48 hours after the intervention.* **(A)** *General view: the locations of the two notochords are represented by two black lines.* **(B)** *Cross-section: Ch, rudimentary chords; N, neuroderm; CW, Wolffian ducts (from Brachet, 1923).*

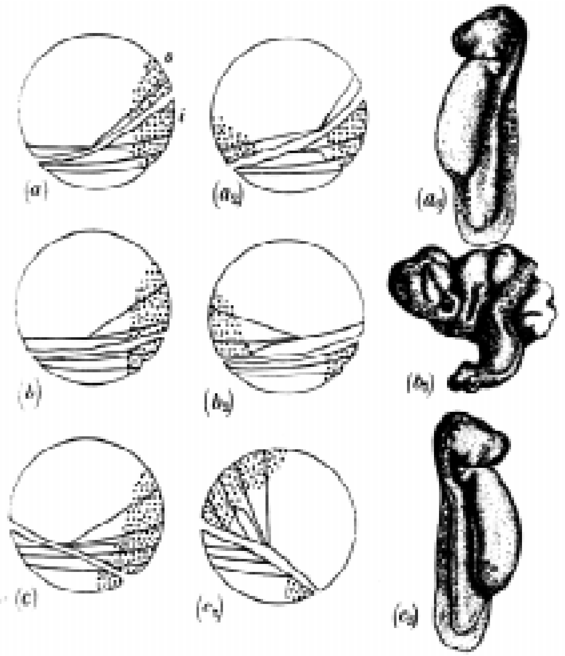

Fig. 3. 180° rotation of the animal hemisphere of a *Discoglossus* blastula. (a), (b), (c) *left side view of the egg with the general map of anlagen and the three types of sections, intersecting only the presumptive neuroblast and epiblast* **(a)**, *dividing the chordo-mesoderm into two parts* **(b)** *or attaining the level of the blastoporal groove* **(c)**. *A large Nile-blue mark was placed on the dorso-marginal territory (dots).* (a_2), (b_2), (c_2) *the same germ after the 180° rotation of the animal part.* (a_3), (b_3), (c_3) *the embryos obtained (from Dalcq, 1938).*

By the early nineteen-fifties, morphogenetic gradients no longer interested most embryologists, essentially for lack of clear qualitative arguments. Embryology by then had become resolutely chemical and was about to become molecular. Yet gradients were brought back to the forefront, if not rediscovered, in the eighties, when we became aware of their material existence in the form of messenger RNA molecules unequally distributed in the ovocytes of ascidians, *Drosophila*, echinoderms, and *Xenopus* (Bashirullah *et al*., 1998) or in the form of diffusible peptide growth factors establishing gradients of positional information throughout the pre-gastrulation embryo (Gurdon *et al*., 1998). The notions of gradients and thresholds proposed as the explicative basis of morphogenesis by Dalcq and Pasteels sixty years ago thus count among the topics that excite developmental biologists today.

As mentioned above, causal embryology became chemical under the determining influence of J. Needham, rightly considered the father of this new discipline (Gurdon and Rodbard, 2000). The aim was then to explain morphogenesis by biochemistry and cell physiology. Jean Brachet, younger son of Albert Brachet, was convinced of this. At the age of 20, in the second year of his medical studies, Jean Brachet became fascinated with ontogenesis which, as he was to write in 1944 in the preface to his first book «Embryologie Chimique», «enables one to witness the gradual passage from simple to complex, and to study how structures incessantly perfect themselves and how the most delicate organs build up and begin to function». His first works, carried out under A. Dalcq, were devoted to the behaviour and synthesis of thymonucleic acid during ovogenesis in various animal species, and during the development of the sea urchin egg (Brachet, 1929, 1931). He then wrote a series of articles on the metabolism of the frog egg, one of these dealing specifically with Spemann's organisation centre (Brachet, 1934). On the reprint he gave to his mother, and which I keep with respect, appears the following dedication: «To my dear Mother. In memory of him whom I do not forget and who taught me what the organiser is». This was his homage to his father Albert Brachet, who was convinced of the metabolic theory of the organiser in which he saw «a kind of catalyser, site of an intense metabolism whose energy radiates and is transmitted to the surrounding parts» (Brachet, 1931).

Jean Brachet actually undertook to verify this interpretation at a time when Bautzmann *et al.* (1932) and Needham *et al.* (1934) had not yet made their decisive contribution to the problem of induction, which they attributed to an inducing substance. Although he did not doubt that the organiser acts via a chemical substance, it seemed to him that comparative measurements of metabolism in different territories of the young gastrula could shed light on the reasons for the localisation of the inducing substance. This work, based on various techniques (colorimetry, titrimetry, manometry) led him to conclude that the blastopore region has higher metabolic activity than the other regions of the embryo (Brachet, 1934; Brachet & Shapiro, 1937). Today, however, the value of this work is purely historic. J. Brachet himself recognised, as only great scientists can, that the techniques he had used at the time were totally lacking in rigour and that his experiments had merely confirmed the increasing energy requirement of the differentiating embryo (Brachet, 1983). To get a clear picture it was necessary to await the work of Boell and Needham, with whom was associated the Belgian physiologist Henri Koch, professor at the *Université Catholique de Louvain*. These authors developed a delicate method for measuring the respiratory quotient (R.Q.) in fragments of Axolotl gastrulae. Using a Cartesian diver apparatus requiring exceptional manual dexterity, they showed that what characterises the dorsal lip of the blastopore is essentially an intense glucide catabolism and not a high oxygen consumption (Boell *et al*., 1939). Child's metabolic theory was thus permanently abandoned in favour of the inducer substance theory.

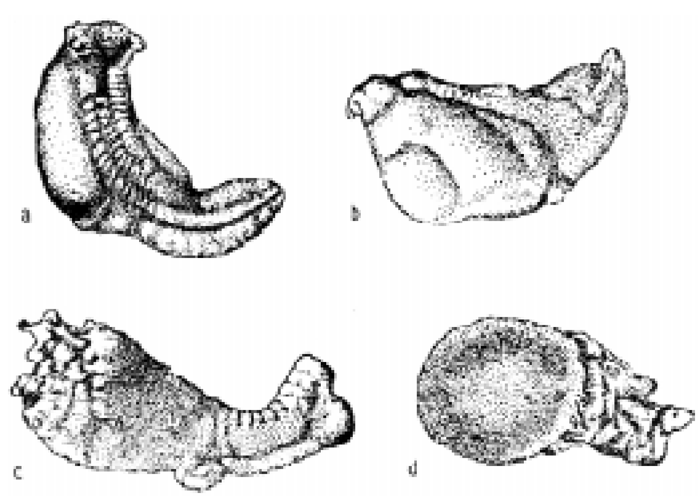

Fig. 4. Increased reduction of head development, from (a) to (d), after centrifugation of fertilised eggs *(from Pasteels, 1940).*

The idea that the organiser owes its inducing power to a chemical substance was readily accepted as soon as it was shown that the lip of the blastopore remains active when it is killed by heating, desiccation, freezing, or alcohol (Bautzmann *et al.*, 1932). J. Needham, the uncontested master of chemical embryology in the thirties, perceived the paramount importance of the discovery of the organiser and was thus naturally one of the first to orient his research towards identifying the inducing substance. He used his mastery of delicate amphibian-egg microdissection techniques, acquired in the laboratory of Albert Brachet during a stay in Brussels in 1930. Jean Brachet, who already appeared as one of the most eminent embryologists of his time (Gurdon and Rodbard, 2000), was one of J. Needham's young foreign collaborators, co-signing four articles with him. The first three dealt respectively with the metabolism of the *Rana fusca* egg, the activity of arginase, and the origin of urea in the chick embryo (Brachet and Needham, 1935a, 1935b; Needham *et al.*, 1935). The fourth dealt with a line of research that J. Needham and also C. Waddington, F.G. Fisher, L.G. Barth, and a few others were developing. It was based on the observation that an increasing number of organs of many different kinds, their extracts, and even diverse purified substances (sterols, glycogen, nucleotides, fatty acids…) can induce neurulation of the ectoblast, but without forming a typical organ. J. Needham, C. Waddington, and D. Needham (1934) thus suggested that one should speak of evocation rather than induction, the heterogeneous inducers being called *evocators* rather than *organisers*.

It must be hard for today's embryologist to perceive the importance of such studies and hypotheses, and to understand the enthusiasm they roused. Textbooks have totally forgotten them and all authors of this fascinating period of life science history are now deceased. Only those who founded schools were able to leave testimony to their passion, successes, and failures. This was the case for Jean Brachet, significantly associated with the attempt to clarify the problem of evocation. At the time there was intense controversy as to the nature of the inducer or "evocator". English authors thought it was a sterol; the German school defended the

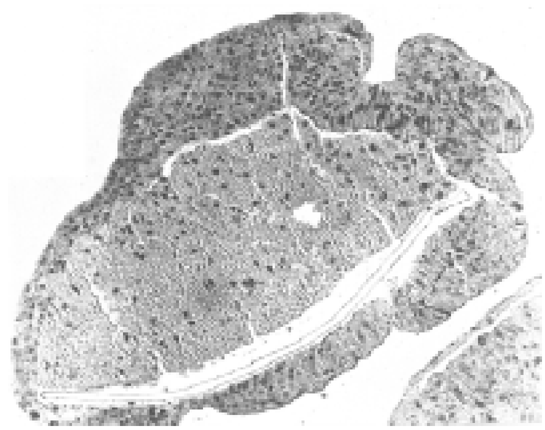

Fig. 6. Complete inhibition of induction by insertion of a cellophane membrane between the organiser and the ectoblast *(from Brachet, 1950).*

idea that the reaction was fairly unspecific and triggered by various acids (Fisher *et al.*, 1935) In collaboration with C. Waddington and J. Needham, J. Brachet showed for the first time that a molecule of non-biological origin, methylene blue, could induce neurulation of the ventral ectoderm (Waddington *et al.*, 1935). At first glance, this only added a name to the list, already long, of exogenous inducers, but the originality of this contribution was in the authors' theoretical interpretation. They proposed that methylene blue acts by unmasking locally the natural evocator, the latter being expressed uniformly in the embryo and inactivated by association with proteins and glycogen in a ternary complex. This totally original view was abundantly commented by several authors including S.C. Shen (1939), who produced a diagram representing this interpretation. J. Brachet used this diagram (Fig. 5) in the first edition of his book "Embryologie chimique" (Brachet, 1944a, Fig. 117, p 408).

At a time marked by the success of the heretical neural default model, which views neurulation as the consequence of inhibition by the organiser of ectodermal differentiation factors (Weinstein and Hemmati-Brivanlou, 1999), the similarly heretical hypothesis of Waddington, Needham, and Brachet cannot fail to strike us. This hypothesis already postulated the existence of an indirect induction mechanism, with the organiser activating neurodermal differentiation by releasing the evocator. The comparison must stop there, however, as the nature of the evocator was totally unknown and was to remain so for many years. The sterol theory was gradually abandoned in favour of other, equally unverifiable theories, to the extent that J. Brachet predicted that «the nature of the active substance within the living organiser will be very hard to determine» («Embryologie chimique», 1944, p. 427). History was to prove him right, since it took more than 40 years and the generalisation of *Xenopus* rearing in laboratories for the natural inducers to be identified at last (Gilbert and Saxen, 1993).

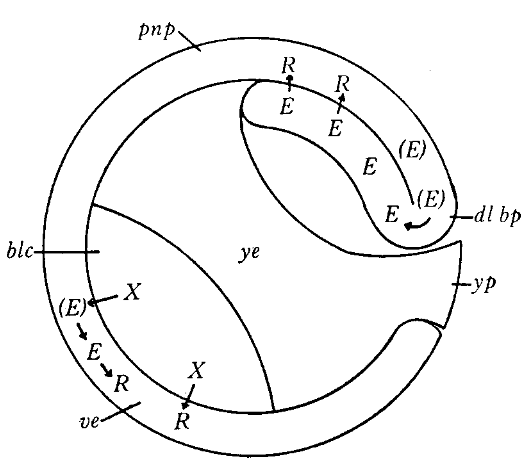

Fig. 5. Diagram to illustrate the induction hypothesis of Waddington, Needham and Brachet. *X, chemical substance; (E), masked organiser substance; E, free, active organiser substance; R, neuralisation response; blc, blastocoel; dl bp, dorsal lip of the blastopore; pnp, presumptive neural plate; ve, ventral ectoderm; ye, yolk endoderm; yp, yolk plug (from Shen, 1939).*

The name of Jean Brachet now appears in most books on the history of biology in the 20th century, but much more often in chapters dealing with protein synthesis than in ones devoted to molecular embryology. This is true even though he was one of the founders of the latter discipline (Alexandre, 1992). With Torbjörn Caspersson, he was the first to suggest the role of RNA in protein synthesis (Brachet, 1941). He is thus rightly considered one of the founding fathers of molecular biology (see H.F. Judson: The Eighth Day of Creation. The Makers of the Revolution in Biology. Jonathan Cape, London, 1979). Quite naturally, therefore, the embryologist Jean Brachet attempted in the early forties to show that RNA, or the ribonucleoprotein particles that had just been discovered, had inducing power. A first harvest of data having evidenced the inducing power of ribonucleoproteins of various origins, Brachet was led to imagine that RNA could be the active substance in induction (Brachet, 1942, 1944a,b; Brachet and Shaver, 1949). This interpretation was wrong, of course. He himself expressed doubt as to the real importance of RNA in the evoking power of ribonucleoprotein implants after observing, in contradiction to his previous experiments (Brachet, 1944a), that purified ribonuclease did not inhibit induction (Brachet *et al*., 1952). He then understood that the inhibitions observed were due to proteolytic impurities in his early preparations and not to the action of ribonuclease on RNA. He thus became convinced that the inducer must have a proteic nature. Two years earlier, he had made a significant contribution to the problem, in the form of a short communication published in *Experientia* (Brachet, 1950). In that paper he showed that neural induction is totally suppressed when a cellophane membrane is inserted between organiser and ectoblast explants (Fig. 6). He concluded that either direct contact or the passage of macromolecules ensures the success of induction (Brachet, 1950; 1960).

Later he gradually abandoned the study of the organiser, introduced to him for the first time in the twenties by his father, in favour of the study of biochemical interactions between the nucleus and the cytoplasm, the biological role of RNA in protein synthesis, and the role of macromolecules in differentiation.

His interest in RNA, amply justified, had thus led him to formulate a hypothesis concerning induction that turned out not to be true. It did, however, have the merit of stimulating research that was to make chemical embryology molecular.

Summary

Albert BRACHET, founder of the Brussels School of embryology, conducted delicate experiments in which he selectively destroyed zones of the grey crescent with heated needles. This allowed him to observe, in 1923, that the median region of the grey crescent of the blastula is a area of spontaneous differentiation and that this «primary self-differentiation centre» organizes the axial organs in anurans. It is thus fair to say that A. BRACHET contributed significantly to the emergence of the organizer concept. Albert DALCQ and Jean PASTEELS, successors of A. BRACHET, trying to solve the problem of the organizer's determination, proposed their famous quantitative theory of embryonic development resulting in the concept of *morphogenetic potential,* which increases with the CV concentration, a combination of a cortical constituent C and a vegetal substance V. Jean BRACHET, the younger son of A. BRACHET and one of the founding father of molecular biology and embryology, was soon convinced that the organizer owes its inducing power to a chemical substance. Being the first to suggest the role of RNA in protein synthesis, he first imagined that RNA could be the active substance in induction but became convinced afterwards that the inducer must have a proteic nature. His interest in the molecular aspects of induction stimulated research that was to make chemical embryology molecular.

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