

Molecularizing Embryology: Alberto Monroy and the origins of Developmental Biology in Italy

BERNARDINO FANTINI

*Louis-Jeantet Institute for the History of Medicine.
Geneva Medical School, Geneva, Switzerland*

Introduction

In a paper written in 1977 for the Italian Encyclopaedia, the Italian embryologist Alberto Monroy distinguished two main periods in the history of embryology of the 20th Century. The first he defined as 'classical embryology' and the second 'molecular embryology', the result, in the early 1960s, of the synthesis between embryology, biochemistry and genetics (Monroy, 1977). A closer analysis shows other relevant scientific transitions during the same period, such as the passage from morphological experimental embryology in the first two decades of the century to chemical embryology in the late 1930s and later to biochemical cytology in the late 1940s and early 1950s, or the shift from molecular embryology of the late 1950s and 1960s to the developmental biology of the 1970s.

During the first half of this century, developing embryos were observed, manipulated, and conceptualized by scholars of very different backgrounds, including, naturally, embryologists, but also physiologists, morphologists, geneticists, physicians, followed by biochemists and soon molecular biologists. The different perspectives, methods and experimental apparatus lead to interesting alliances, tensions and controversies, reflected in a variety of disciplines of undefined boundaries: descriptive or experimental embryology, developmental mechanics, "embryologie causale" (Brachet, 1931), chemical embryology or developmental physiology.

However, the distinction introduced by Monroy corresponds to a deeper change, in the sense that the origins of molecular embryology do not coincide only with the introduction of new techniques or new problems, another shifting phase in a constant tradition, but represent the "introduction of a new way to consider the problems; the difference is in other terms conceptual" (Monroy, 1977, p. 444).

Historians have already devoted a lot of effort to the reconstruction of the molecular revolution and its impact on biology and medicine and in recent years an effort has been made to provide a comparative analysis of this revolution in different countries and cultural contexts.¹ In Italy, as well as in Belgium,² embryology played a major role in the emergence of molecular biology, as this discipline, in terms of institutions and scientists, inherited the scientific traditions of cellular physiology and above all chemical and cellular embryology. This was in large part due to the relevance and the international visibility of the embryological tradition in Italy, itself the result of a very particular historical situation. Because of

¹ Cfr. *Molecular Biology in Postwar Europe*, edited by Bruno Strasser and Soraya de Chadarevian, in press, Proceedings of a workshop held in Annecy - Center des Pensières, 29 June - 1 July 2000.

² On the history of molecular embryology in Belgium see the special issue of *History and Philosophy of the Life Sciences*, 19(1), 1997, 5-142, edited by R. Burian and D. Thieffry.

*Address correspondence to: Prof. Bernardino Fantini. Louis-Jeantet Institute for the History of Medicine. Geneva Medical School, CMU, Case Postale, 1211 Geneva 4, Switzerland. TEL: 41 22 702 5790. FAX: 41 22 702 5792. e-mail: Bernardino.Fantini@medecine.unige.ch

the presence of the Stazione Zoologica of Naples, a truly international research center, Italian zoology and in particular the universities in the southern part of the country, had always, in fact, been in contact with the leading biologists and with the newest techniques and experimental procedures. The presence in Naples, over many decades, of outstanding scientists, in particular embryologists, created the best conditions for the integration of several Italian embryologists into the international context, putting them in contact with the most advanced biological research. At the same time, the Italian zoological tradition also had itself a solid reputation, thanks to the schools created mainly in Rome, Naples and Palermo by zoologists such as Battista Grassi, who, in 1896, received the prestigious Darwin Medal from the Royal Society of London for his work on the biology of termites and on the reproduction and metamorphosis of the common eel, in addition to Federico Raffaele, Carlo Emery, Umberto Pierantoni and Andrea Giardina. This particular situation produced, in Italy, in the field of embryology at least, fully international scientific research within a strong national, cultural and institutional tradition.

The transition from 'classical embryology' to molecular embryology and developmental biology can be reconstructed from an analysis of Alberto Monroy's scientific career and achievements. His career began in the late 1930s during a period of critical transition, especially in embryology, and fully expanded in the 1950s and 1960s, when the molecular revolution changed the scientific style of biology. Furthermore, Monroy took a decisive part, as a leading scientist and science manager, in the 'molecular revolution', introducing the new methods and ideas to Italian research centers and participating very actively in the construction of national and European institutions in the field. Last, but not least, Alberto Monroy had solid roots in his country and its culture but was nevertheless cosmopolitan, and fully integrated in the international network of scientific institutions that played a fundamental role in the creation of developmental biology in Europe, America and Japan. He was, in other words, 'a member of the clan', of the small group of biologists that, in the 1950s and 1960s, built the scientific and institutional basis for molecular biology. Reconstructing Monroy's remarkable scientific odyssey is therefore a good way to understand the nature of the scientific transition produced by the molecular revolution and its institutional and personal implications.

An anatomist turns to embryology in the 1930s

Alberto Monroy was born July 26, 1913 in Palermo, Italy into a noble Sicilian family of Spanish origin, which descended from Hernán Cortés, the conqueror of Mexico. However, very few outside his close familial circle knew his social position, as he was totally unconcerned with rank and traditions. On the contrary, he was engaged, in a very liberal way, with the pursuit of excellence in science and culture, without regard for class or political boundaries. For his entire life he would remain personally engaged in many battles for social justice and the improvement of living conditions in his country, especially his beloved southern Italy. In the late 1970s he would also serve as a city councillor in the town of Naples, as an independent in a left-oriented government.

Monroy's interest was in biological research and for this reason he entered the medical school, at that time the only faculty that allowed a research practice in the field. His interest in embryology started in 1936 when he went to work for four months with Otto Mangold at Erlangen University, thanks to a fellowship from the

Italian Academy. His research interests became the experimental modification of development and his very first publications were quoted with very positive comments by Umberto D'Ancona (D'Ancona, 1937). In 1937 he studied cristallin regeneration in *Triton* (Monroy, 1937) in relation to morphogenesis, in particular the correlations in the development and growth of the eye by means of heteroplastic transplantation and the effect of different factors upon the rate of regeneration. With the support of O. Mangold, Monroy published his results in the eminent journal *Wilhelm Roux' Archiv für Entwicklungsmechanik*.

In July 1937 Monroy received an M.D. degree from the University of Palermo with honors and immediately after, joined the University of Palermo as an assistant professor of anatomy, with a salary covered by the Italian Consiglio Nazionale delle Ricerche (National Research Council). He was entering into an institution that already had a solid reputation in the field of comparative anatomy and embryology. The Palermo Institute of Comparative Anatomy bears the name of Andrea Giardina, a pupil of Federico Raffaele, who, in the early years of the 20th century, published a series of studies on the morphology and experimental embryology of insects and the diving water beetle *Dysticus*. He demonstrated that cystoblasts go through four rounds of synchronous mitosis to form an oocyte cyst of 16 cells, one of which differentiates into an oocyte while the others become nurse cells (Giardina, 1901). The oocyte was distinguished from its siblings as early as the 2 cell-stage because it contained a particular morphological ring in its interior. Later it was shown that Giardina's body is made of highly amplified rDNA and in 1998, M. de Cuevas and A.C. Spradling demonstrated that the same mechanism holds for *Drosophila*.³ By reinterpreting drawings in Giardina's 1901 paper, these authors attributed to him the discovery that the fusome and the Giardina's body are inherited by the same cellules and this determines which cell will become an oocyte.

In another well known paper, Giardina suggested a model experiment in order to attempt to penetrate the mechanisms by which the centrosome organizes the mitotic spindle (Giardina, 1903). At the beginning of the 20th century, biologists' curiosities were piqued by the 'mystery of the centrosome', considered to be absent in the sea urchin egg and present in the spermatozoon. Giardina devised an experiment to simulate the mechanisms of the movement of the sperm nucleus and its fusion with the egg nucleus in the fertilized sea urchin egg. He floated a small boat-shaped wax plate on water which simulated the sperm head. He placed a droplet of alcohol in a recess on the rear of the plate and diffusion of the alcohol caused the wax plate to advance rapidly. From this experiment he suggested that the centrosome was the site of diffusion of tensioactive substances that caused the movement of the sperm nucleus. Upon observing the events of the fusion of the two pronuclei, Giardina also noticed that the egg nucleus started moving towards the sperm nucleus upon being reached by the radiations of the sperm aster, acquiring an amoeboid shape. This he attributed to substances that diffused along the radiations of the sperm aster, lowering the surface tension of the nuclear envelope. These observations, however naïve, were considered by Monroy as the first attempt to offer a physico-chemical interpretation of the mechanics of the encounter and fusion of the two pronuclei in fertilization and provided a model for further investigations (Monroy, 1986).

When Monroy began his research career in the late 1930s, embryology occupied a central position in the sciences. Since the end of the 19th century and the origins of experimental embryology,

the fertilized egg had been regarded, not only as a model for the understanding of developmental processes in biology—particularly differentiation and regeneration, but it was also considered as an ideal experimental tool for the study of inheritance and cellular physiology, and as a consequence of the fundamental properties of life (reproduction, nutrition, respiration). In dealing with these aspects, biologists used two alternative models of scientific explanation. On the one hand, a strong tendency was in favor of a purely physical and chemical explanation of development and differentiation. On the other hand, another theoretical attitude insisted on the role of the complex organization of the cell and on the role of specific explanatory principles, not deductible from chemistry and physics. In the laboratory, the former looked for physical and chemical forces that could modify the standard process of embryonic growth and differentiation (changes in gravity, saline concentration, pressure, chemical or temperature gradients etc.); the latter used morphological observations and tissue transplantation as an experimental tool, following cell lines and the possible role played by morphogenetic forces or vital forces (such as the *entelechia* proposed by the German embryologist and philosopher Hans Driesch).

The situation of embryology in Italy in the 1930s and early 1940s

Among Italian biologists there was a generational change in the late 1930s and many of the young researchers gathered around the problems that were at the center of embryological research, that is to say, the search for the primary organizer and its chemical nature (Spemann, 1938), attempting to try to fill the gap between biochemistry and morphology.⁴ The attention given to new developments in genetics and experimental embryology was also great. For example, T.H. Morgan himself was asked to write the entry 'Genetics' in the new *Encyclopaedia Italiana*, the most important cultural enterprise in the early 1930s. Morgan's book *Embryology and genetics* (Morgan, 1934) was translated into Italian by O.M. Olivo already in 1938 (2nd edition in 1950). Thanks to the presence of the Stazione Zoologica, the new generation of embryologists were immediately in contact with the international leaders in the field.

All the leading embryologists and geneticists that would energize Italian biology in the 1950s were already producing their first results. The geneticist Adriano Buzzati-Traverso published relevant papers on the concept of the gene (Buzzati-Traverso, 1937), on radiogenetics (Buzzati-Traverso, 1939), on population genetics, in collaboration with C. Jucci and N.W. Timofeef-Ressovsky (Buzzati-Traverso, 1938) and later with Luigi Luca Cavalli Sforza (Buzzati-Traverso, 1945; Baldi, 1945).

The embryologist Giuseppe Reverberi began his career in Rome in the late 1920s, before moving first to the Naples Zoological Station and then to Palermo after WWII. He studied the development of Ascidian eggs, using, as well, experimental interspecific crosses, and the causal morphological in the chicken embryo. Silvio Ranzi working in Milan and Naples, also began his research in the 1920s, studying mainly the physiological basis of determination of the embryo in Echinoderms, in particular the physical-chemical changes that accompany histological differentiation (Ranzi, 1933; Ranzi, 1937). In the 1930s both Reverberi and Ranzi produced a cytochemical analysis of development and differentiation, showing, at the same time as Ries *et al.* (1937), a

difference between regulative and mosaic eggs, that was the localization of fermentative properties or affinity for some substances (Reverberi, 1940), and Ranzi demonstrated that induced changes in embryonic determination were related to changes in these properties (Ranzi, 1939).

Giuseppe Montalenti in Naples oscillated between embryology and genetics. He studied the embryological and genetic aspects of hybrids in amphibians (*Bufo vulgaris* x *B. viridis*) (Montalenti, 1932; Montalenti, 1933; Montalenti, 1938), the influences of hormones on bird plumage colour, the parthogenetical activation of Lampreda eggs, and the determination of sex (Montalenti, 1939). In 1939 he published the first coherent textbook on genetics (Montalenti, 1939), the result of three years of teaching at Bologna University, in the institute directed by A. Chigi, and in 1945, he published a textbook of embryology (Montalenti, 1945).

The post-war crisis in embryology. New research programs and hopes

After WWII, science and in particular the biological and medical sciences, evolved at an ever-increasing pace and on an ever-expanding institutional and economic scale. The number of scientists began to rise exponentially, and the costs of apparatus and materials increased rapidly. The era of romantic science was gone. The ideals of science changed from an individual enterprise linked to individual dreams and projects to a full-scale collective effort.

A new model of scientific activity imposed itself in post-war Europe, modelled on the successes of American science, and centered on three paradigmatic disciplines: chromosomal genetics, microbiology, biochemistry and structural chemistry, with hybrid corn, antibiotics and DDT as symbols of the practical successes of the new science. In this context, the general shift from German to English as the standard language of publication is only one aspect of a more general change in scientific references.

After the war, the three leading research programs that had dominated in the 1920s and 1930s were more or less in a period of crisis. Embryology had outlived the drama of the end of the search for the organizer; biochemistry could not contribute as it had hoped to the solution of the fundamental problems of biology, in particular evolution and development, and finally genetics, notwithstanding its great successes, was unable to find answers to the most fundamental question: what is a gene and how does it function?

The attitude towards this situation and the search for new insights depended on different personalities and also on differences between generations. At the individual level, both J. Runnström and Hörstadius, together with their pupils, T. Caspersson in Sweden, and Silvio Ranzi with Giuseppe Reverberi in Italy, continued to pursue their previous research, simply adapting to the new tools, in particular biochemical and biophysical methods. The

³ I owe thanks to Giovanni Giudice for signalling to me this publication, and for a very careful, critical reading of this paper.

⁴ This research program is marked by three books, the monumental treatise by Joseph Needham, *Chemical Embryology* (1930), the first synthesis *Biochemistry and Morphogenesis*, published by Joseph Needham in 1942 (Needham, 1942) and the first book by Jean Brachet, *Embryologie chimique*, published in 1944 (Brachet, 1944), and translated into English in 1950. These books are centered on the hope of finding unity between biochemistry and morphogenesis through a chemical explanation of morphological discontinuities during development and differentiation.

younger generation, on the contrary, looked elsewhere, in particular in the direction of genetics. Giuseppe Montalenti completed his own 'Morgan's deviation' and became the first full professor of genetics in Italy, at the University of Naples, creating a very active school whose first result would be the elucidation of the heredity of thalassamia, in collaboration with Marcello Siniscalco and the Roman pathologists Ezio Silvestroni and Ida Bianco (Silvestroni, 1954). Others, in the first place Jean Brachet in Belgium and Alberto Monroy in Italy, decided to give new impetus to embryological research by developing molecular embryology after the origin of molecular biology.

The search for unifying principles: the plasmagene theory and the primacy of the cytoplasm

In the late 1940s a new general biological theory emerged, which aimed at a general explanation of life, heredity, growth and disease, the 'plasmagenes theory'. According to this theory, which arose from many unconnected fields, non-nuclear self-reproducing units, normal constituents of cells, have, in common with genes, the properties of mutation and recombination. These structures were considered to be dependent on a specific gene constitution of the 'host' cell in order to be able to reproduce or mutate. The plasmagenes are produced by the nucleus, but then they migrate in the cytoplasm where they reproduce at the expense of the cytoplasm constituents, creating an equilibrium between different forms and determining specific protein synthesis and, as a consequence, different metabolic pathways and cellular function. This equilibrium is the result of a competition between different chemical entities in the cytoplasm, and can be interpreted in Darwinian terms. This general model can explain phenomena as different as the physiological action of the gene (Wright, 1945; Spiegelman, 1946), enzymatic induction (Spiegelman, 1945; Monod, 1949), cellular differentiation and viral infection. Leading scientists such as Sewal Wright, Sonneborn, Spiegelman, Delbrück, Monod, Cavalli-Sforza, Buzzati-Traverso took part in the construction of such a general theory (AA.VV., 1949).

The chemical heterogeneity of the cytoplasm, in particular its proteic composition and quantitative gradients, was explained as a result of variable synthesis of proteins along the gradients. Between 1945 and 1953 the theory of plasmagenes or 'self-reproducing units' (genes, virus, cytoplasmic microstructures) seemed to have provided a unified explanation of the self-replicating phenomena in microbiology, genetics, biochemistry and medicine, thanks to a generalized replicative property attributed to different chemical and morphological entities, composed mainly of proteins and nucleic acids. This was particularly true for embryologists in search of a causative explanation of nucleocytoplasmic relationships during differentiation (Brachet, Montalenti). The most accepted view considered nucleic acids as a necessary part of self-replicating structures (ribonucleoproteic granules), 'organized cytoplasmic particles,' analogues to the genes, plasmagenes and viruses, and responsible for differentiation and cell metabolism (Brachet, 1950). These self-reproducing particles were supposed to emanate from the nucleus but retain their replicative autonomy in the cytoplasm. Particular nucleoproteic granules, produced by the nucleus and then distributed along the morphogenetic gradient and able to self-reproduce, would be the seat of protein synthesis and hence differentiation. Notwithstand-

ing the hypothetical nature of such 'plasmagenes,' the idea of a 'chemical migration' of particular molecular species from the nucleus to the cytoplasm remains a guide for research, linking together genetics, cytology and embryology. Embryologists interpreted their experimental observations according to the new theory. Discussing a paper by Adriano Buzzati-Traverso on 'Elementary units, selection, and differentiation', John Runnström noted that:

The literature on plasma genes has been very stimulating for embryologists. According to my hypothesis, the animal and vegetal poles represent centers of activity, and I could very well imagine that these centers have their material basis in plasma genes and that plasma genes are formed within the nucleus during the long-lasting phase of preparation for the meiotic divisions, and become released only when the germinal vesicle breaks down. These plasma genes, however, become only active in a certain environment, in our case the most animal and the most vegetal part of the egg. Here they promote the formation of certain enzymes. Under the action of these animal or vegetal part diffusible substances are produced which interact and form the basis of the segregation of the pattern along the egg axis (Runnström, 1949).

The plasmagene theory was based on the idea of a 'material continuity' as the basis for heredity and development, within the classical tradition established by August Weismann. In fact, as Buzzati-Traverso writes in 1949 (Buzzati-Traverso, 1949, p. 205),

If we replace the hypothetical terms used by Weismann with the terms we use today for indicating experimentally determined entities, and we say 'elementary unit' instead of 'biophor', gene instead of 'ide', plasmagene instead of 'determinant', and if we include in this schema natural selection, "silently and insensibly working, whenever and wherever opportunity offers" (Darwin), we must remain deeply admiring of the prophetic spirit of these great men.

Monroy's professional choices in the 1940s

During the war Alberto Monroy remained on a double track. On one side he kept publishing on human anatomy (Monroy, 1944; Monroy, 1940; Monroy, 1944) but a large part of his research was devoted to embryology. In collaboration with his wife, Anna Monroy Oddo, he studied the classical problem of regeneration in amphibians and he began to work on the subject that would remain his lifelong interest, fertilization, observing the submicroscopical structure of the spermatozoon of the echinoderms (Monroy, 1944; Monroy, 1944).

Soon after the end of WWII in the south of Italy, Monroy went to Naples to head up the physiological laboratory of the Stazione Zoologica, giving up the secure institutional basis of a brilliant career at the University of Palermo for a more precarious job at the Zoological Station, which at the time was going through a very severe financial and institutional crisis. What attracted him was the international nature of this prestigious institution, the possibility to interact with the most important biologists, especially embryologists, of the time, and the possibility of working on his own research subjects without any constraints, in particular the physiological and biochemical study of fertilization.

At the Stazione, Monroy entered into contact with members of the Swedish School of Embryology (Runnström, Hörstadius) who used to spend long periods of research in Naples and he became one of the few to use biochemical approaches in the study of eggs and embryos. The sea urchin was naturally the model animal for study. He began an intense period of collaboration with John Runnström who seemed to share his positions and his own professional choices: "Almost from the beginning of my studies, my interest was directed towards problems of fertilization and early embryonic development. I was brought up in an institute devoted almost exclusively to comparative anatomy and found there rather little nourishment" (Runnström, 1959).

Monroy, during this experience, took on an even stronger conviction that the solution to most problems in developmental biology had to come from biochemistry. He became a pioneer in the field of 'chemical embryology', and a friend of the Belgian embryologist Jean Brachet, one of the leaders in the discipline, who spent long periods of research in Naples, first at the Stazione Zoologica and then, in the 1960s, in Monroy's laboratory at Arco Felice, before becoming the director of the Division of Molecular Embryology of the LIGB in 1964.

In 1948-49 Monroy spent a research period at the Wenner-Gren Institute in Stockholm and at the Swedish Zoological Station at Kristineberg with a fellowship from the Rockefeller Foundation. In 1949 he visited the United States for the first time, as a fellow of the Rockefeller Foundation at the Rockefeller University, collaborating with A. Mirsky and meeting many leaders in the fields of embryology and cytology, including Paul Weiss and Daniel Mazia. On this occasion, he also visited the Marine Biological Laboratory, Woods Hole, for the first time, where he would return almost every Summer for the next 30 years, eventually becoming a member of the corporation in 1955, then the first non-American member of the MBL Board of Trustees, and finally an Emeritus.

Monroy's research on fertilization at the Zoological Station (1944-1952)

The study of fertilization is the most important research program developed by Alberto Monroy in the period after WW2. For a number of years fertilization was almost considered to be a sterile field, at least in terms of the interaction between specific substances, but in the late 1940s, after a long period of silence, the problem of fertilization, thanks to the research of J. Runnström, M. Hartmann and A. Tyler, began to attract renewed interest.

In 1902 Boveri had published an important essay, which remained virtually unknown for a long time. In this essay Boveri indicated the three main conditions which must be fulfilled in order to ensure the success of fertilization:

1. The gametes must be in a 'repressed' condition, that is "they should not be able to develop by themselves, and in fact they should be blocked in such a way that inhibition is released by the other cell".

2. "the two cells must be able to find each other".
3. "the equivalence of the germ cell... in the same way we say that the spermatozoon fertilizes the egg, so we may say that the egg fertilises the spermatozoon". This means that in fertilization neither gamete behaves passively in the sense that one is the activator and the other the activated one; for successful fertilization the gametes must activate each other (Boveri, 1902a).

The study of the early stages of development promised to provide information regarding the organization of the egg and its physiological properties, which must be the determining elements of development. As C.O. Whitman put it: "The egg is the architect of its own destiny... every ontogenic form presupposes a preliminary arrangement" (Whitman, 1878; Whitman, 1887), in other words, 'everything must be in the fertilized egg', in particular what would later be called the 'program of development'.

According to Jacques Loeb in 1913, the process triggering the activation of the egg is a *transient surface cytolysis* brought about by a *lysin* borne by the spermatozoon. It was Loeb's discovery that prompted Warburg to use the sea urchin egg for the study of biochemical processes controlling cell division. Warburg was interested in the problem of how neoplastic cells escaped growth control and he used the developing egg as a model of normally controlled growth (Warburg, 1908; Warburg, 1910; Warburg, 1925).

A few years after Loeb's publication, Lillie formulated a theory of fertilization based on the interaction of specific chemical substances (fertilizin and antifertilizin) secreted by the gametes. The actual activation of the egg would result from the chemical encounter of gametes (Lillie, 1919), (Monroy, 1965b). Lillie drew attention to the analogy between gamete interaction and antigen-antibody



From the left: Anna Oddo Monroy, Alberto Monroy, Mrs. Bacci and Guido Bacci in the main room of the Villa Aquarium, the annex of the Stazione Zoologica on the island of Ischia. 1951.

reactions, and Lillie's model of fertilizin and antifertilizin was directly inspired by the Ehrlich model for the antigen-antibody reaction (Ehrlich, 1900) and to the 'lock and key' chemical model suggested by Emil Fischer (Fischer, 1894). This model implied the role of an agglutinating substance present in the jelly coat which surrounds the sea urchin egg. Lillie's ideas were taken up and further developed by A. Tyler and his students (Tyler, 1948a; Metz and Monroy, 1967) in the late 1940s and gave new support for a chemical interpretation of the processes involved in fertilization, that is: a) the role in fertilization of substances produced by the gametes, b) species-specificity in fertilization and c) the mechanism preventing polyspermy.

In the late 1940s and early 1950s these problems were studied within two different theoretical contexts and with two different sets of experimental tools. Alberto Monroy and his co-workers played a relevant role in both.

In the period 1943-1947 the question of the cortical changes occurring at activation is taken up again independently by Runnström and his co-workers in Sweden and by Monroy and Montalenti in Naples, mainly using examination with polarized light (Monroy and Montalenti, 1947). Furthermore, in 1945 Ruffo and Monroy show a relevant decrease of viscosity of egg extracts after fertilization (Ruffo and Monroy, 1945). In 1928 Runnström had shown that sea urchin eggs undergo some typical cyclical changes in sensitivity towards some cytolysing and plasmolysing agents in the lapse of time between fertilization and the first cleavage, with an alternance of 'smooth' and 'angular' plasmolysis (Runnström, 1928). In 1936, in *Lampreda* eggs, Montalenti had shown that fertilization produced in the egg a 'contraction wave', moving from the animal pole, the point of entrance of the spermatozoon, toward the vegetal pole, producing the detachment of the vitellin coat (Montalenti, 1936). Monroy and Montalenti demonstrated that this morphological behavior parallels the changes in birefringence (Monroy and Montalenti, 1946). The positive cortical double refraction of the unfertilized sea urchin egg disappears upon fertilization and with the beginning of the anaphase. This means that the cortical layer undergoes rhythmic variations of its submicroscopic structure and in 1947 Monroy suggested that fertilization produces a change in the molecular constitution of the cortical layer of unfertilized eggs and "in the cytoplasmic colloids" (Monroy, 1945; Monroy and Montalenti, 1946) (Monroy and Montalenti, 1947; Monroy, 1947).

In 1946 Anna Monroy Oddo studied variations in Ca and Mg in *Arbacia* eggs as a result of fertilization "in view of the great importance of mineral ion equilibrium in life processes in general and in the 'Entwicklungserregung' in particular". This observation suggested that an immediate effect of fertilization was the increase of the distances between the molecules of the cortical layer. As the results of Runnström showed that the cortical layer contains proteins and lipids, Monroy suggested that "the proteins are arranged in sheets parallel to the egg surface; the lipids are radially arranged" (Monroy, 1947, p. 108). The 'spermalysin' discovered by Runnström, S. Lindvall, S. and A. Tiselius in sperm extracts was regarded as the splitting agent.

Montalenti and Monroy (Monroy and Montalenti, 1947) regarded the whole process as follows: "the first step of the cortical reaction should consist in a broadening of the intermicellar spaces of the cortical layer.... The smooth plasmolysis of the egg just after fertilization shows that the cortical layer has become 'soft' compared with the condition of the unfertilized egg".

In 1948 Monroy delivered an important lecture on 'Cortical responses to activation in sea urchin eggs' at the special symposium of the IUBS on the occasion of the first international congress of pathophysiology of animal reproduction and artificial fertilization (Monroy, 1948). In this paper, Monroy discussed the changes which the cortical layer of sea urchin oocytes undergoes during maturation, considering them as "the reverse of those observed at fertilization". The submicroscopic structure of the cortical layer has "an important functional character, ... which seems to be strictly connected with the ripeness of the egg, i.e. with the responsiveness to activation. As soon as the cortical layer has reacted to a spermatozoon, the birefringence disappears, i.e. its submicroscopic structure is disarranged" (Monroy, 1948, p. 6). In order to clarify the proposed chemical mechanism, Monroy established an analogy with the thromboplastic protein which has a definite ultrastructure, consisting of leaflets of proteins with lipids arranged in between, a pattern which seems to be very important for the physiological activity of the protein itself, so that if the complex is broken down, the thromboplastic activity is lost.

At the end of 1947 Monroy began a direct collaboration with Runnström in Naples,⁵ that would continue during a visit by Monroy to the Zoological Station of the Swedish Academy of Science, Kristineberg, to conduct a common study. The two scientists performed certain experiments pertaining to the chemical changes occurring at the formation of the fertilization membrane of sea urchin eggs (Monroy and Runnström, 1948). They showed that the fertilization membranes of *Psammechinus microtuberculatus* and *Paracentrotus lividus* treated with thyoglycolic acid became softer and easily attacked by trypsin, whereas this enzyme was without any effect on normal membranes. Monroy inferred that disulfide linkages may be responsible for the mechanical properties of the membrane (Monroy and Runnström, 1948). This result suggested a keratin-like character of the membrane and the formation of 'clotting' with the formation of disulfide cross-links, a process which presents a general resemblance to the clotting system of blood (Monroy and Runnström, 1948).

An enzymatic mechanism had been suggested in order to explain how sperm were able to go through the jelly-coat in fertilization (Monroy and Ruffo, 1947; Vasseur, 1951) but Monroy and Luisa Tosi⁶ (Monroy and Tosi, 1952) showed the contrary and discussed the possibility of quite a different mechanism, that is a chemical reaction between sperm and jelly-coat substances, during which "molecules" of the latter adhere to the sperm surface. This same mechanism had been suggested by Tyler in 1948, using an immunological model. Following the multivalence theory of antigens and antibody of Heidelberger, Tyler suggested an interaction between 'fertilizin molecules' (i.e. jelly-coat molecules) and spermatozoa (Tyler, 1948a; Tyler, 1948b). This chemical reaction produces structural rearrangement of the protein molecules, a reorganization of the egg proteins which was confirmed using isotopes (Monroy, 1953a) and serological studies (Perlmann, 1953). Fertilization produces a 'complicated intramolecular reorganization combined with the formation of new links between submicroscopic structural units' (Monroy and Runnström, 1948, p. 5).

Using the protein models suggested by Mirsky and Lindstrom-Lang (Lindstrom-Lang, 1952), this structural rearrangement can be explained in three ways:



Alberto Monroy (left) and Giuseppe Montalenti (right) at a meeting on Electronic Microscopy organised by the Stazione Zoologica and held at Paestum in May 1951.

issue of *Experimental Cell Research* (Monroy, 1950):

The large number of biochemical studies on the activation of the sea urchin egg have mainly been concerned with metabolic aspects of the problem. Little attention has been centered on changes occurring at the protein level, although profound rearrangements of the protein pattern are suggested by observations on the viscosity, permeability, cortical changes etc. occurring in the egg on activation.

In order to isolate differences in protein structure, Monroy studied, in A.E. Mirsky's laboratory at Rockefeller University, the electrophoretic mobility of the water extracts of unfertilized and fertilized eggs, the same method used by Linus Pauling in 1948 for the discovery of the chemical difference of normal and sickle-cell haemoglobin (Pauling *et al.*, 1949). Certain components of the diagrams changed after fertilization, but the results were inconclusive, as there were too many variations. The combination of electrophoresis with ultracentrifugation was equally unsatisfactory but it clearly showed the use of the latest experimental technologies and apparatus for embryology (Monroy, 1950).

- a) the change in the configuration of the protein molecule (folding or unfolding);
- b) the aggregation of simpler units into more complex ones; or,
- c) the breaking-down of large complexes.

In 1952, a paper in collaboration with Peter Dohrn (Dohrn and Monroy, 1952) showed some evidence of the formation of complexes between jelly-coat substances and cytoplasmic nucleoproteins. The authors suggested that a similar complex may well be present in the cortical layer of the unfertilized sea urchin egg, the fertilizing spermatozoon being instrumental in breaking it down and thereby liberating the nucleoprotein.

The idea of a structural arrangement of the protoplasm was very popular in Italy in the 1940s. Based on Szent-Györgyi's concept of structural proteins, a network of fibrous proteins, as the myosin of the muscle, the idea that fibrillar or fibrillar folded proteins are involved in embryonic determination processes is often advanced, suggested by the behavior of cells in polarized light and with physico-chemical methods (changes in viscosity and permeability).⁷ Frey-Wyssling's book (Frey-Wyssling, 1938) was translated into English in 1948 and Italian in 1951, and represents the cell as a network of fibrous proteins, a network that in some cases is quite evident with the electron microscope. The determination processes are considered to be the transformations of this network. The cell in general is considered to be a network of fibrillar proteins (Ranzi, 1947; Cigada *et al.*, 1949) and fibrillar folded proteins, that can be easily extracted from the embryo, which are considered to be the fundamental cytoplasmic substance, involved in embryonic determination (Ranzi, 1952a; Ranzi, 1952b).

The two opposing theories concerning the chemistry of fertilization underlines the different paradigms at work. In the biochemical model, the changes in metabolism are responsible for morphogenesis. In the structural model, on the contrary, the changes in the molecular architecture of the egg at fertilization are responsible for the metabolic changes. This theoretical alternative is clearly expressed by Monroy in a paper published in 1950 in the very first

Protein synthesis and nucleic acids

In the following decade there were many attempts to explain the observed role of nucleic acids in protein synthesis. The nucleic acids were in turn considered to be a source of energy, a structural component of a respiratory subcellular structure or a catalytic effector. However, the most important working hypothesis in the early 1950s was a link between chemical processes and morphological self-reproducing unities (the plasmagene theory). In 1943 Albert Claude isolated, by progressive centrifugation of extracts of crushed cells, various fractions consisting of nuclei, mitochondria and other small granules (microsomes) (Claude, 1943).

The role of the small granules remained mysterious for a long time. As they contained a large proportion of ribonucleic acid (RNA) and as this substance seemed to play a part in protein synthesis (Brachet, 1941b; Caspersson, 1941), Brachet suggested in 1944 that microsomes were important agents in protein synthesis (Brachet and Jeener, 1944; Brachet, 1944). Such a suggestion was reinforced in the early 1950s by the results of experiments on the incorporation of labelled amino-acids into proteins by various cell fractions (Hultin, 1950a; Hultin, 1950b; Stern and Mirsky, 1952; Siekevitz, 1952; Gale and Folkes, 1953). These experiments clearly demonstrated that microsomes were

⁵ "Runnström suggested to the writer to investigate the chemical nature of the active substance and its eventual changes upon fertilization" (Monroy, 1950).

⁶ Luisa Tosi was at that time, at the University of Milan.

⁷ On the idea of the importance of cytoskeleton and ultramicroscopic particles in the cell physiology cfr. (Haraway, 1976; Olby, 1986).

more active in protein synthesis than other cell fragments, and that the integrity of RNA is essential for this process, as digestion of RNA by the enzyme ribonuclease leads to a strongly decreased incorporation of the labelled amino-acids into the proteins of the homogenates (Brachet, 1950). Even if “much more work will be necessary before the exact status of these particles ... can be evaluated” (Brachet, 1950, p. 867-868), the main idea being that if they have many characteristics in common with viruses and plasmagones, and that as a consequence morphogenesis can be the result of the action of this self-reproducing entity of macromolecular nature (granules).

In this context, reproduction of macromolecular entities seemed to be a general property of life. In Italy, G. Reverberi, in an analysis of ‘molecular reproduction’ (Reverberi, 1946), put together genes, viruses, plasmagones and ribonucleoproteic granules, stating that “molecules are able to reproduce themselves, either genes, viruses or nucleic acids. Reproduction is a property even of inanimate molecules”.

The Palermo years (1952-1969)

The reconstruction of embryological research in the late 1940s and early 1950s, delineated in the previous paragraphs, is essential for understanding the professional trajectory of Alberto Monroy and the revolutionary changes produced by the impact of molecular biology.

In 1952 Monroy returned to Palermo as full professor and head of the Institute of Comparative Anatomy. His laboratory at the Institute of Comparative Anatomy ‘Andrea Giardina’, became the seat of an intense scientific life, the first Italian ‘laboratory of molecular embryology’ and a center of excellence in the field.

In modest but efficient quarters on the historic site of the university on the narrow Via Archirafi, Monroy’s institute acquired a solid international reputation and attracted many students, young researchers and a stream of visiting scientists from around the world. Italian co-workers of this period included Luisa Tosi, G. Giardina, R. Maggio, F. Ajello, F.M. Vittorelli, N. Bosco, A.M. Rinaldi, and especially noteworthy, Giovanni Giudice, who later, after Monroy’s departure for Naples, would take on the responsibility of directing the laboratory of molecular biology in Palermo.

The foreign visitors included, notably, A. Tyler and E. Nakano. Monroy had already established many contacts with American scientists, and, from the 1950s, he established a solid and fruitful collaboration with Japanese scientists, contributing to the growth of the Italian-Japanese Biological Association. When Eizo Nakano, as the first visiting researcher from Japan after the war, visited Italy he worked at the Stazione Zoologica on the development of the sea urchin egg. Here he met Alberto Monroy, who invited him to Palermo to work in collaboration with him. At the same time Nakano received an invitation from John Runnström to go to Stockholm after his stay in Naples. At this time, Sweden was the center for research on sea urchin development, but Nakano was “extremely impressed by the personality of Alberto Monroy” and decided finally to go to Palermo instead of Stockholm (Nakano, 1987).

Collaboration with Tyler and Nakano played an important role in shaping the research programs in Palermo. The major discussion was on how to further the research on egg maturation, fertilization and early stages of embryonic development. In Palermo everyone seems to have shared the same attitude towards

modern biology (Nakano, 1987): to apply the new techniques that were spreading rapidly after the war in the various fields of biology, especially the use of isotopes.

Continuing with fertilization

The problem of fertilization remained at the core of Monroy’s research project, but with an enlarged scope including the problem of ‘activation’ of the egg by the spermatozoon which could be understood only in relation to the previous events, oogenesis, and the events that follow it, that is segmentation and the first stages of development (Monroy, 1974). The term ‘activation’ was used because fertilization encourages the egg to abandon a state of lethargy and become “apt to accomplish the formidable task of building up a new organism” (Monroy, 1954). The interaction between egg and spermatozoon was seen as the starting point of a complex series of biochemical events, in particular the regulated synthesis of new macromolecules, proteins and nucleic acids (Monroy, 1954). The aim was to understand the “biochemical and structural changes at fertilization”, bringing together morphology and biochemistry (Monroy, 1953b), as in the classic chemical embryological style.

The fact that a jelly-coat solution causes sperm agglutination suggested either a binding of the jelly-coat molecules to the surface of the sperm or at least a modification of the sperm surface induced by the jelly-coat (Monroy *et al.*, 1954). The observations confirmed that the organic material of a purified solution of sea urchin jelly-coat can be practically completely absorbed by sperm, as suggested already by Tyler in 1948 (Tyler, 1948b). In the early 1950s Monroy and A. Tyler attacked the problem of fertilization using immunological techniques and radioisotopic tracers. They demonstrated that the change in K⁺ conductance is one of the first events in sea urchin development after fertilization (Tyler and Monroy, 1956; Monroy and Tyler, 1958). The interaction between ions and high molecular compounds could explain the modification of the macromolecular pattern of the egg cytoplasm, as those produced by thiocyanate and lithium ions that alter normal development, a phenomenon already observed by Herbst at the end of the 19th century (Herbst, 1893) and widely studied in the early 1950s by the Swedish School of embryology and by Ranzi in Italy (Ranzi, 1957; Runnström, 1959).

The most important results concerning the metabolic changes at fertilization were obtained by Monroy in collaboration with Nakano. Three months after the Japanese scientist arrived in Palermo, to study the incorporation of ³⁵S-methionine in the cell fractions of sea urchin eggs and embryos, Monroy and Nakano produced the relevant finding that protein synthesis is highly activated at fertilization in the sea urchin egg (Nakano and Monroy, 1957; Nakano and Monroy, 1958). In collaboration with Giudice, they also showed that this phenomenon was equally present in eggs activated artificially (Nakano, Giudice and Monroy, 1958). These results were rapidly repeated by many researchers in various laboratories and became the starting point of a new research program, namely the study of the processes underlying the metabolic activation of the egg at fertilization, the causes of the inability of the unfertilized egg to carry out protein synthesis and the mechanism of the release of this inhibition at fertilization.

The origin of molecular embryology: was ‘Monsieur Jourdain’ a molecular embryologist?

The late 1950s and early 1960s saw the fusion of chemical embryology and molecular biology to form the new discipline of

'molecular embryology'. This process was considered by embryologists as a natural evolution of their previous research. Jean Brachet, T. Caspersson and Alberto Monroy⁸ suggested a substantial continuity during this period. As Jean Brachet put it (Brachet, 1986, p. 247): "In 1930, nobody spoke of Molecular Biology which was still at an early embryonic stage. Of course, people working at that time on nucleic acids already were molecular biologists; but they did not know it and were like Molière's M. Jourdain who discovers that he has been talking in prose for forty years without being aware of the fact!"⁹

In fact, a number of embryologists felt perfectly 'at home' in the new disciplinary context. They had been working on nucleic acids and proteins, in particular protein synthesis, for at least a decade and in the 1940s and early 1950s they had learned to use the same experimental apparatus that were at the heart of molecular biology, for embryological and cytological research: cytochemistry, UV absorption, isotopes, radioactive isotopes, chromatography, electron microscopy, ultracentrifuge, and a bit later differential centrifugation. Furthermore, the new vocabulary of molecular biology, based on information flow, control and regulation, was fairly familiar to embryologists; at least since the time of Boveri they had talked and thought about 'nuclear control', nucleocytoplasmic interactions and cytoplasmic regulations.

Molecular biology uses biochemical and biophysical tools, but it tries to solve traditional biological problems. Instead of being a 'chemical explanation of biology', it is in fact a 'biology applied at the molecular level'. Many traditional biological concepts, such as form, structure/function relationships, selection, specificity, induction, regulation and control, and especially reproduction, became essential parts of the explanatory structure of the new discipline.

Embryologists such as A. Tyler, T. Hultin, J. Brachet, E. Nakano and A. Monroy contributed relevant concepts and experimental information to the new field of molecular biology. Their studies on protein synthesis in unicellular organisms and egg fragments and on the role of proteins in cellular morphology and embryonic development participated in the 'molecular synthesis' that, in the early 1960s, defined the theoretical and social status of the new discipline and its ambitions. Embryology could show better than any other discipline that biochemists and geneticists had been working for many decades, without knowing it, on two different aspects of the same problem: the control of the synthesis of a specific protein (Brachet, 1960).

Embryology 'at the molecular level'

After the origin of molecular biology, and without a conscious cross-fertilization, embryology, biochemistry and molecular genetics began to fuse together. Embryology contributed to this fusion a large amount of experimental knowledge on classic problems: regeneration, determination, gradients, morphogenetic fields, induction, polyspermic eggs and embryonic hybridation; biochemistry brought metabolic studies, fractionation of chemical components of the cell, determination of enzyme activity, oxygen consumption and histochemistry; genetics, after the solution of the 'problem of the gene' by molecular biology, could bring the formidable theoretical framework of gene function and its role in the control of cellular activities.

Papers on gene expression and cellular regulation began to discuss cytoplasmic 'gradients' and embryologists started to use the new information vocabulary, speaking of 'gene regulation' and 'information flow'. This is realized by a sort of theoretical bricolage,



Alberto Monroy in the seventies.

using ideas and explanatory tools picked up from different disciplines and experimental contexts.

This process was not a 'natural evolution', but the result of theoretical and institutional choices. Many embryologists and embryological institutions remained linked to the traditional problems, and only a few institutes switched rapidly to the new scientific and institutional landscape.¹⁰ In Italy, the Institute of Comparative Anatomy of Palermo University was first, followed by the CNR Center in Arco Felice, Brachet's molecular laboratory at the International Laboratory of Genetics and Biophysics, also in Naples, and Monroy's laboratory at the Zoological Station which all become the protagonists of the molecular revolution. Probably because of the extraordinary scientific backgrounds, the main events in this field seem to take place in southern Italy, between Naples and Palermo.

In Palermo, Monroy along with his co-workers, continued the analysis of the process of activation of protein synthesis upon fertilization in eggs in different conditions of molecular activation. He showed that active synthesis of RNA takes place in the course of oogenesis but is halted at the onset of maturation (Monroy, 1965a). Autoradiographic studies also showed that the growing

⁸ Interviews and personal communications (1978-1980).

⁹ The same concept was told to the author in an interview in 1980: "J'ai fait de l'embryologie moléculaire comme Mr. Jordan faisait de la prose sans le savoir". Brachet added on that occasion that the change of the name from chemical to molecular embryology had been of simple opportunity: "In the late 1950s nobody would have given a grant to a 'chemical embryologist', as molecular biology was much more fashionable".

¹⁰ The public image of science changes equally, in the title of congresses, conferences journals, and books. For example, in the same year 1973 two leading embryologists published an important book, the first by S. Hörstadius bears the title *Experimental Embryology of Echinoderms* (Hörstadius, 1973), the second by G. Giudice is titled *Developmental Biology of the Sea Urchin Embryo* (Giudice, 1973).

Whenever I think of Alberto,
 he comes to me as a wooden figure.
 Not exactly the same,
 but somewhat like the one shown below.
 Even when he was actually speaking to me,
 or when he was on the other side of the earth,
 He comes to me as a wooden figure,
 somewhat like the one shown below.
 Is it because of his hair,
 his face or his figure?
 No, it's his atmosphere, I think,
 that makes me imagine him in this way.
 His warm-hearted person
 and his humorous temperament.
 He was a creative and productive biologist,
 so people would say, I'm sure.
 But to me, he was always
 a kind friend, a generous father
 Who overlaps with my dear memory
 of Napoli and the Stazione Zoologica.



"My image of Alberto Monroy". Poem
 and sketch by Marina Dan-Sohkawa.
 From *Bolletino Associazione Biologica
 Italo-giapponese*, X, 1987: 24-25.

oocytes are very active in incorporating amino acids into its proteins: but this incorporation becomes negligible in the mature egg (Monroy and Maggio, 1964). Both the ribosomal and the messenger RNAs used by the embryo during the first part of its development are those synthesized during oogenesis. Up until the time of gastrulation, no appreciable synthesis of ribosomal RNA occurs and the whole work of protein synthesis is carried out by the

ribosomes present in the egg at the time of fertilization, i.e. the ribosomes synthesized during oogenesis.

The problem that arises at this time is the following: if the egg ready to be fertilized is equipped with the necessary machinery to carry out protein synthesis, why is it unable to do so?

This inability begins to manifest itself at the onset of maturation and Monroy's experiments showed that both the activation of amino acids and the formation of the amino-acyl-sRNA could be carried out by the unfertilized egg (Monroy, Maggio and Rinaldi, 1965). However, the stimulation of the ribosomes of the unfertilized egg can be obtained by poly-U. (Wilt and Hultin, 1962). This seemed to suggest that the missing link in the protein synthesizing machinery of the unfertilized egg might be the messenger RNA. In 1963, in collaboration with A. Tyler, Monroy demonstrated that the activation of protein synthesis was due to the formation after fertilization of ribosome complexes (polysomes) (Monroy and Tyler, 1963), that are absent in the unfertilized eggs. This result suggested two alternative interpretations: (a) the mRNA synthesized during oogenesis is destroyed, leaving the unfertilized egg devoid of it and hence the formation of the particles active in protein synthesis, the polysomes, must await the synthesis of new messenger; b) the messenger is formed during oogenesis, is conserved and hence is present in the unfertilized egg, but for some reason the interaction with ribosomes is prevented.

A series of experiments supported the following alternatives: (a) development until blastula stage is normal even when RNA synthesis is blocked; b) non-nucleated fragments of sea urchin eggs, activated parthenogenetically, are able to incorporate amino acids into their proteins (Brachet, Ficq and Tencer, 1963); c) RNA extracted from unfertilized eggs is able to stimulate *in vitro* ribosomes of rat liver or sea urchin embryos to carry out incorporation of amino acids into proteins (Maggio *et al.*, 1964).

Fertilization was considered to be the "process which activates the use of information stored in the oocytes during oogenesis. Upon fertilization the egg recovers the ability to synthesize DNA, lost by the oocyte" (Monroy, 1977, p. 450).

The results obtained by Monroy and his co-workers on nucleate and anucleate fragments of sea urchin eggs¹¹ complemented perfectly the results obtained by Jean Brachet on *Acetabularia mediterranea*; the stimulation of protein synthesis at fertilization resulted from the translation of previously 'masked' mRNAs stored in the egg cytoplasm (Brachet *et al.*, 1963). The later part of oogenesis is mainly characterized by synthesis and accumulation of ribosomal and informational RNA. This synthesis is stopped when the oocytes reaches physiological ripeness, i.e. ability to be fertilized, in coincidence with a general depression of metabolism.

For molecular embryologists these results were the equivalent of what the natural philosophers called preformation in the 18th century; the egg cytoplasm contains the 'preformed' information necessary for the first stages of development; this information is stored during oogenesis under the control of the maternal nucleus; the synthesis of new mRNA species under genetic control after fertilization brings to the egg the fresh information required for epigenesis (Brachet, 1986).

Some comparative studies on the activation of protein synthesis in eggs carried out in Palermo (Monroy and Tolis, 1964) suggested that this mechanism was universal. The actual block of the protein synthesizing machinery was at the last step, the one that involved the activity of ribosomes. Monroy suggested the hypothesis that the ribosomes of the unfertilized egg were non-functional, that they

¹¹ This techniques had been introduced by E. B. Harvey in 1936 (Harvey, 1936).

had an 'anomalous conformation' produced by some repressor or chemical effector. In fact, preparations of ribosomes from unfertilized eggs even when combined with the supernatant fraction of embryos or of rat liver are unable to incorporate amino acids into proteins (Maggio *et al.*, 1968). In 1965 the presence in the unfertilized egg of ribosomes carrying an attached mRNA chain was demonstrated, but this complex was rendered inactive by a protein coat (Monroy *et al.*, 1965).

The presence of a stock of messenger RNA in the unfertilized egg to be used during early development poses the question of how the sequential and timely translation of the transcribed messages is operated and controlled and when new stocks of mRNA are synthesized during development. In the context of the new theoretical framework produced by molecular embryology, this means the control of gene expression during development. This was the research program developed in Palermo by G. Giudice, a pupil of Alberto Monroy, from the late 1960s onwards (Giudice, Mutolo and Donatuti, 1968).

Experiments involving x-ray irradiation showed that the period between fertilization and the blastula stage is independent of immediate nuclear control. On the other hand, nuclear activity during cleavage seemed to control gastrulation (Neifakh, 1960).

Electrophoretic analysis of the radioactive labelled proteins demonstrated that the overall protein synthesis remained unchanged during the period between fertilization and blastula. It appeared likely that the mRNA itself was conserved during the early period of development, stored for later translation. Most of the messages present in the unfertilized egg continue to be translated throughout this period of development. Very little or no synthesis of mRNA was needed until the blastula stage.

A tool for investigating gene activity in the course of embryonic development was provided by inhibiting RNA synthesis with actinomycin D (Gross and Cousineau, 1964). Fertilized eggs are able to develop normally up to the blastula stage in the presence of actinomycin to dose which almost completely suppresses RNA synthesis. The blastula also exhibits a normal respiratory activity (determined by the conventional method of Warburg).

In a series of elegant experiments Giudice and his co-workers treated developing sea urchin embryos with actinomycin for various lengths of time between fertilization and the mesenchyme blastula. Then the embryo were washed free of actinomycin and returned to normal seawater. This "pulse" treatment allowed for a comparison between the abnormal result in the various phases of development with the normal course. This method could provide information on three main events of development: 1. the ability of the sea urchin egg to develop to a blastula in the absence of RNA synthesis; 2. the control of gastrulation, and 3. the control of the differentiation of the skeleton.

The results demonstrated that it was during the second part of the considered period that most of the RNA needed for gastrulation was synthesized. The mRNA needed for gastrulation seemed to be made between the early and the late blastula stage, in agreement with the observation of Barros and Monroy (Barros, Hand and Monroy, 1966) in starfish. On the contrary, the mRNA necessary for the differentiation of the skeleton must be continuously produced during the period following gastrulation. The synthesis of mRNA necessary for the formation of the spicules seemed to take place continuously from some time before the early gastrula until completion of the growth of the skeleton.

New alliances and strategies

Alberto Monroy had a superb talent for defining the essence of a problem, for translating ideas and hypotheses into experimental tests and laboratory procedures, and at the same time he was very keen to transform an apparently modest observation into a general principle (Giudice, 1987). The search for 'general views' was the essence of his work, and from this point of view he was in tune with one of the main characteristics of molecular biology, a discipline that from the very beginning looked for the 'secrets of life' (Judson, 1979). Monroy was therefore in an ideal position to understand the relevance of molecular biology and very quickly began to apply the new theoretical tools to traditional embryological problems, first of all fertilization and early development. By combining the new theoretical framework with the embryological tradition he became one of the founders and leaders of developmental biology.

From the early 1960s Monroy's national and international activity was at the focus of the new 'molecular embryology'. He became 'a member of the club', looking for new alliances to assure the still fragile identity of the new discipline, selecting and discarding problems, establishing fruitful collaborations with scientists and institutions.

In the Summers from 1962 to 1968 he went to the Marine Biology Laboratory, Woods Hole, as a staff member of the NIH-supported training program on "Fertilization and Gamete Physiology". His book *Chemistry and Physiology of Fertilization*, published in 1965 at the apogee of the molecular revolution, rapidly became a cornerstone in the growing discipline of developmental biology and a catalyst for the new generation of biologists. Two more books on fertilization, written in collaboration with C.B. Metz, would be published in 1967 and 1985 (Metz and Monroy, 1967; Metz and Monroy, 1985) and would reinforce his leadership in the field.

The first volume of *Current Topics in Development Biology* was published in the Summer of 1966. The editors, Monroy and A. Moscona, decide to instigate this reference series in order to establish a review platform for the then fledging field of developmental molecular biology. Some twenty volumes would be published over about twenty years. The last volume published under Monroy's responsibility was number 23, devoted to *Advances in Mammalian Development* (McLaren and Siracusa, 1987), a result of his ever-present interest in fertilization and early development, in connection with the new developments in cell lineages (especially the destiny of fetal germ cells) and homeobox genes.

Monroy also spent a lot of energy strengthening European scientific cooperation. He was a member of the Euratom scientific board from 1961 to 1968 (president in 1963) and in the 1960s he was one of the founders of the European Molecular Biology Organization (EMBO) and the first Italian member of the council of EMBO (1970-1975), later becoming a member of that organization's Scientific Advisory Board (1978-1986).

He was a founder of laboratories, head of professional societies, convener of international congresses and symposia, editor of books and book series. He was also very active in several scientific societies, such as the International Cell Research Organization and the International Society of Developmental Biologists, becoming its president in 1969-1974, and played a primary role in bringing into the society many molecular biologists. As a visiting professor at prestigious universities around the world he was able to participate in the teaching and research of the new field: the University of Chicago (1972, 1975), the Rockefeller University (1949-1950,

1964), the University of Puerto Rico (1970), the California Institute of Technology (1956, 1960), the University of Nagoya (1961), and University College London (1974). He was also member of the editorial boards of many scientific journals and Editor-in-Chief of *Cell Differentiation* until 1981. His wide cultural interests pushed him to organize symposia on the history of biology, such as a symposium organized in October 1984 on the relationships between the Zoological Station and the MBL, and to begin new editorial activity at the Stazione, such as the journal *History and Philosophy of the Life Sciences* (1979). As stated by Moscona: "Monroy was a statesman of science, an ambassador of developmental biology, dedicated to serving the scientific community worldwide".

In November 1969 Monroy took on another challenge and accepted the proposal of the Italian National Research Council to move back to Naples in order to establish and direct a new Laboratory of Molecular Embryology at Arco Felice. It was the starting point of another scientific and institutional adventure that brought to the new discipline many young scholars and colleagues, including E. Parisi, S. Filosa, B. De Petrocellis, G. Augusti-Tocco, B. Baccetti, S. Denis-Donini, F. Rosati, R. De Santis. This passage coincided with another disciplinary transition that transformed molecular embryology into developmental biology.

The shift to developmental biology in the late 1960s and 1970s

The phrase 'developmental biology' became popular in the late 1950s when molecular biology was in a period of transition, characterized by a revival of interest in the problems of differentiation and embryonic development, which were again considered to be at the frontiers of biology. The first issue of the new journal *Developmental Biology* was published in 1959. The introduction of the new term was not only a new 'slogan' to recall or compete with 'cell biology' or 'molecular biology', but reflected also the broadening of interests and the integration of different biological disciplines, in particular genetics, biochemistry, classical experimental embryology and molecular biology.

The enthusiasm generated by the discoveries in the field of molecular biology, especially after the proposal by F. Jacob and J. Monod in 1958-1961 of the 'operon model' as a molecular explanation of the control of genetic expression, (Jacob and Monod, 1961; Jacob and Monod, 1961; Monod and Jacob, 1961) produced high hopes of finding the solution to the problems of differentiation thanks to extensive knowledge available on genetic regulation in microorganisms, with the same tools and the same theoretical strength used to solve the problem of the nature and function of the gene.

In the 1960s, several molecular biologists turned to the problem of cell differentiation and pattern formation in higher organisms, on the lookout for ways to extend their powerful conceptual apparatus and experimental techniques to embryonic development. Molecular biologists became aware that "in a funny way, what we have done so far is to work out Morgan's Deviation" (Brenner in Judson, 1979, p. 205), that is to concentrate on the problem of transmission of genetic information, leaving aside the problem of its expression and regulation.

In fact, at this moment, molecular biology is confronted with the problem of differentiation, in exactly the same terms as expressed by Morgan in his 1933 Nobel lecture:

If as is generally implied in genetic work (although not often explicitly stated), all of the genes are active all the time, and if the characters of the individual are determined by the genes, then why are not all the cells of the body exactly alike?... At every division of the egg, the chromosomes split lengthwise into exactly equivalent halves. Every cell comes to contain the same kind of genes. Why then is it that some cells become muscle cells, some nerve cells, and others remain reproductive cells? (Morgan, 1935).

The functional and structural complexity of the eukaryotic cell and the importance of cell-cell interaction during development posed new problems and developmental biologists began to realize that, as put brilliantly by Monroy, 'the egg is not a glorified *Escherichia coli*' (Monroy, 1970). According to Monroy, the enthusiasm generated by molecular biology does not justify "the belief that when we have sufficiently extensive knowledge of microorganisms, we will also find a solution to the problem of differentiation", as the complexities of the eukaryotic cells pose problems that are not encountered with microorganisms. Therefore, "the impact of molecular biology on the study of development [had] been essentially methodological and conceptual", suggesting the method of "asking clear questions and experimenting to find their solutions". In particular the "concrete definition of the term 'gene expression' marked the turning point in the study of both cellular and developmental processes".

Within the new paradigm, research concentrated on "gene controlled regulatory processes in the development of organisms" (Britten and Davidson, 1969) (Monroy and Augusti-Tocco, 1975) and in the 1970s development was reinterpreted in terms of gene-dependent pattern formation, based on clonal restriction and homeotic mutants in insects, coupled with a new revival in the already old thinking on gene regulation during ontogenesis established in the research on sea urchin morphogenesis (Davidson, Hough-Evans and Britten, 1982).

Monroy's research group, inheriting the now classical interpretation of fertilization as the result of the most exquisite process of cell recognition and interaction, centered on cell surface interactions in morphogenesis, on the exchange of effectors *via* cytoplasmic bridges, specialized junctions or other mechanisms, particular cell microstructures (microtubules), and on signals generated at the membrane, transmitted *via* specific receptor sites and intracellular mediators. Their work suggested that the binding of the spermatozoon to the inner glycoprotein coat (the vitelline coat), thanks to a highly specific molecular matching between receptors at the gamete surface, is the key event in the preliminary steps of development. Recognition between the egg and the sperm is a species-specific event and depends on the presence of specific "receptors"¹² at the surface of both gametes, because, as Boveri wrote in 1902 in a paper considered by Monroy almost as prophetic, the gametes "must be able to find each other" (Boveri, 1902a; Monroy *et al.*, 1986).

Using concanavalin A as an experimental tool, Monroy demonstrated, in 1973, that the characteristics of the cellular membrane can change completing after fertilization. The same tool was applied to the study of tissutal affinities in developing embryo. This research program had been initiated by J. Holtfreter, who had shown that different parts of the embryos differed in their tissutal affinities, showing either positive or negative affinities (Holtfreter,

1939). He demonstrated also that the different tissues, if dissociated, can reaggregate selectively (Holtfreter, 1943). Along the same line, Giovanni Giudice demonstrated in Palermo in 1969 that a mixture of cells from different developmental stages of sea urchin embryos could reaggregate selectively, forming normal plutei (Giudice, 1965). On the contrary, a mixture of cells from embryos of different species (i.e. *Paracentrotus* and *Arbacia*) segregate, forming two plutei, typical of the two species (Giudice *et al.*, 1969). This recognition process is species-specific and typical exclusively of embryonal and tumoral cells. The molecular organization of the membrane of the blastomeres is probably responsible for the control of the segregation process.

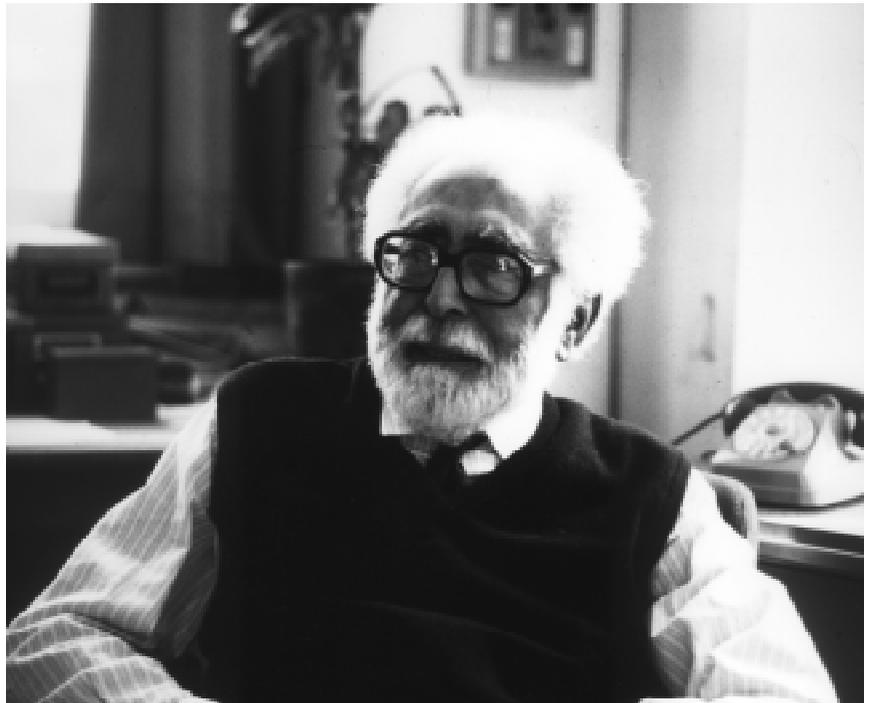
The new theoretical ideas and experimental tools allowed for a reinterpretation of the classic embryological problems, such as the formation of gradients, the establishment of polarity in the fertilized egg and the segregation of cell lines during segmentation.

The problem of polarity, the earliest and the most fundamental property to appear in a developing organism is the result of the acquisition of a differential organization of the egg components which results in the expression of different properties at the two opposite ends of the polarity axis. The animal-vegetal polar axis is acquired first, the dorsoventral axis appears later in the course of development (Monroy and Moscona, 1979). The question which arises is: what is the molecular basis of polarity? Does it depend on a molecular lattice of the egg cytoplasm which arises during oogenesis or after fertilization; or on the organization of the egg surface, or on a combination of both?

In the egg of *Xenopus laevis*, Monroy showed that the overall organization of the egg surface, as revealed by scanning microscopy, differs at the animal pole from that at the vegetal pole (Monroy and Baccetti, 1975). In this context, Monroy goes back to Giardina's ideas and suggests that the relationship of the oocyte to the ovarian wall causes a specific alteration of the oocyte surface.

In all multicellular organisms, the cleavage of the egg gives rise to cells which differ from each another and which, through successive cell divisions, will eventually give rise to homogeneous cell populations (cell lines), each endowed with its own specific developmental program. This implies: a) sorting out of molecules into various blastomeres and b) cell recognition and coordination at the level of rate of cleavage and metabolic activities. In 1979 Monroy and F. Rosati suggested that the process of segregation of cell lines consists of two main processes: 1. the progressive differential restriction of the developmental program in each line and 2) the differential expression of genes in the different lines. Both processes are expressed in a specific molecular organization of the surface of the cells belonging to a cell line and of their derivatives (Monroy and Rosati, 1979, p. 57).

As demonstrated by the classical experiments of Hörstadius, one of the interesting features of the development of the sea urchin embryo is the segregation at the fourth cleavage of a group of small blastomeres, called the *micromeres*, deriving from the unequal division of the four vegetal blastomeres (Hörstadius,



Alberto Monroy in 1986.

1949; Hörstadius, 1953). The segregation of the micromeres also inaugurates a vegetal-animal gradient of cell divisions. In a classic experiment by Reverberi and Minganti in 1946, at the Institute of Zoology of Palermo University, it was shown that in the ascidian embryo, substitution of the 4 b.1 blastomeres (presumptive ectoderm of the tail) for the 4 a.1 (presumptive cephalic ectoderm and brain and sense organs) results in an embryo lacking the nervous system (Reverberi and Minganti, 1947). Already at the 8-cell stage, the two animal anterior and the two animal posterior blastomeres appear to be irreversibly committed to differentiation along a well-defined pathway. These results were re-interpreted and defined with the new vocabulary of Developmental Biology: they imply "an irreversible restriction of the developmental program of each blastomere, and hence the irreversible silencing of certain genes" (Monroy and Rosati, 1979). Gene silencing is considered the most important event underlying the segregation of cell lines during cleavage in all the embryos, with a turning point at the blastula stage, confirming the experimental results obtained by F. Baltzer already in 1910 (Baltzer, 1910) in the study of nonviable hybrids.

Back to the Stazione Zoologica

In March 1976 Alberto Monroy went back to the Stazione Zoologica to become its director, in a difficult period for the 'old lady', devoting himself once again to the Renaissance of modern biology in Italy and the rise of developmental biology.

As director Alberto Monroy was first able to achieve fundamental results, avoiding the risk of transforming the Stazione into an ecological monitoring or administrative center, and pushing even

¹² The receptor is defined as "a very large molecular entity".

more the need to develop basic science. The scientific life of the Stazione became increasingly lively, in a context of permanent scientific collaboration. In his own research field, embryology, he started a new research project, directly linked to new trends in the discipline: the study of the molecular biochemical mechanisms of fertilization and development, in collaboration with his previous laboratory of molecular embryology in Arco Felice, and in particular with Jean Brachet.

Alberto Monroy was also conscious of another great tradition of the Stazione, its cultural atmosphere. He therefore placed new emphasis on the organization of public lectures, symposia and Summer courses devoted to the history and philosophy of the life sciences.

However, Alberto Monroy remained fundamentally a 'laboratory man', a creative scientist not always conscious of administrative problems. It was difficult to change the objectives and the functioning of the entire institute and at the same time bring in new blood for the different research projects. The growing financial and institutional constraints rapidly became a major difficulty for the scientific life of the Stazione. A new institutional and financial leap was needed. In 1980 Monroy resigned from the post and became head of the Stazione's Laboratory of Cellular and Developmental Biology. His laboratory as usual was full of brilliant co-workers and foreign guests. In collaboration mainly with F. Rosati, R. De Santis, M.R. Pinto, G. D'Alessio and E. Parisi, Monroy continued to produce relevant papers on sperm-egg interaction, in particular the sperm receptor and sperm-activating activities of the vitelline coat, on the segregation of the germ and somatic cell lines in the embryo.

Monroy's death at the MBL

Alberto Monroy died the night of 23rd of August 1986, in Woods Hole, an evening during a week-end filled as usual with research, reading at his table in the library, and meetings with colleagues and friends. He had been severely ill during the previous months, but did not want to miss his rendezvous, completing a cycle and bringing together once again the two countries that had played such a major role in his scientific life. His wife Anna and his colleagues in Palermo, in particular Giovanni Giudice, have recognized his wish and established a foundation that bears his name and favors the scientific exchanges between Italy and the MBL, in the field of molecular embryology and developmental biology.

With a very finely attuned historical sensibility, in the previous year, 1985, he had devoted the introductory lecture delivered at the MBL on 25 August 1985 on the occasion of the Symposium on Sea Urchin Development to Theodor Boveri, who had provided the ideas and suggested the problems for developmental biology to solve, and to his lifelong laboratory friend, the sea urchin egg. This paper, Monroy's very last, was accepted for publication by the *Biol. Bull.* on 21 August 1986, shortly before his death (Monroy, 1986). It bears the title "A centennial debt of developmental biology to the sea urchin" and stands as the concrete symbol of the debt of developmental biology to Alberto Monroy, his science, his culture, and his personality.

Acknowledgments

Special thanks are due to Christiane Groeben, archivist of the Naples Zoological Station, for the photos included in this paper.

References

- AA VV. (1949). *Unités biologiques douées de continuité génétique*. CNRS, Paris.
- BALDI, E., BUZZATI-TRAVERSO, A., CAVALLI, L.L. and PIROCCHI, L. (1945). Frammentamento di una popolazione specifica (*Mixodiptomus laciniatus* Lill.) in un grande lago in sottopopolazioni geneticamente differenziate. *Mem. Ist. Ital. Idrobiol.* 2: 171-216.
- BALTZER, F. (1910). Über die Beziehung zwischen dem Chromatin und der Entwicklung und Verebnungsrichtung bei Echinodermenbastarden. *Arch. Zellforsch.* 5: 497-621.
- BARROS, C., HAND, G.S.J. AND MONROY, A. (1966). Control of gastrulation in the starfish, *Asterias forbesii*. *Exp. Cell Res.* 43: 167-183.
- BOVERI, T. (1902a). *Das Problem der Befruchtung*. G. Fischer, Jena.
- BOVERI, T. (1902b). Über mehrpolige Mitosen als Mittel zur Analyse des Zellkerns. *Verh. der Phys. Med. Ges Würzburg* 35: 67-90.
- BRACHET, A. (1931). *L'oeuf et les facteurs de l'ontogénèse*. Gaston Doin, Paris.
- BRACHET, J. (1939). Etude du métabolisme de l'oeuf de granouille (*Rana fusca*) au cours du développement. V. Le métabolisme protéique et hydrocarboné de l'oeuf en relation avec le problème de l'organisateur. *Archives de Biologie* 50: 233-267.
- BRACHET, J. (1941a). La détection histochimique et le microdosage des acides pentosenucléiques. *Enzymologia* 10: 87-96.
- BRACHET, J. (1941b). La localisation des acides pentosenucléiques dans les tissus animaux et les oeufs d'Amphibiens en voie de développement. *Arch. Biol.* 53: 207-257.
- BRACHET, J. (1944). *Embryologie chimique*, II éd. 1945. Masson, Desoer, Paris, Liège.
- BRACHET, J. (1950). The localization and the role of ribonucleic acid in the cell. *Ann. N. Y. Acad. Sci.* 50: 861-869.
- BRACHET, J. (1954). Nuclear control of enzymatic activities. *Colston Papers* 7: 91-102.
- BRACHET, J. (1957). *Biochemical Cytology*. Academic Press, New York.
- BRACHET, J. (1960). Le contrôle de la synthèse des protéines. *Scientia* 54: 1-6.
- BRACHET, J. (1960). Ribonucleic acids and the synthesis of cellular proteins. *Nature* 186: 194-199.
- BRACHET, J. (1986). Early interactions between embryology and biochemistry. In *A History of Embryology (Symposium of the Bri. Soc. Dev. Biol.)* (ed. Horder, T.J., J.A. Witkowsky and C.C. Wylie), pp. 245-259. Cambridge University Press, Cambridge.
- BRACHET, J. and JEENER, R. (1944). Recherches sur des particules cytoplasmiques de dimensions macromoléculaires riches en acide pentosenucléique. I. Propriétés générales, relations avec les hydrolases, les hormones, les protéines de structure. *Enzymologia* 11: 196-212.
- BRACHET, J., FICQ, A. and TENCER, R. (1963). Amino acid incorporation into proteins of nucleate and anucleate fragments of sea urchin eggs: effect of parthenogenetic activation. *Exp. Cell Res.* 32: 168-170.
- BRITTEN, R.J. and DAVIDSON, E.H. (1969). Gene regulation for higher cells: a theory. *Science* 165: 349-357.
- BUZZATI-TRAVERSO, A.A. (1937). Il gene modello. *Boll. Soc. Ital. Biol. Sper.* 12: 585-587.
- BUZZATI-TRAVERSO, A.A. (1939). I nuovi orizzonti della radiogenetica. In *Atti della III riunione della Società Italiana di Genetica ed Eugenia (Bologna, 5-7 Settembre 1938-XVI)*, Roma.
- BUZZATI-TRAVERSO, A.A. (1949). Unità biologiche elementari, selezione e differenziazione. *Pubbl. Staz. Zool. Napoli* 21 Suppl.: 191-210.
- BUZZATI-TRAVERSO, A.A. and CAVALLI, L.L. (1945). Genetica di popolazioni in *Drosophila*. IV. Fenotipi e costituzione genetica in una popolazione di *Drosophila melanogaster*. *Mem. Ist. Ital. Idrobiol.* 2: 217-251.
- BUZZATI-TRAVERSO, A.A., JUCCI, C. and TIMOFEEFF-RESSOVSKY, N.W. (1938). Genetica di popolazioni. *Ric. Scient.* 9, n. 11-12: 30.
- CASPERSSON, T. (1941). Studien über den Eiweiszumsatz der Zelle. *Naturw.* 29: 33-43.
- CHANTRENNE, H. (1944). *Enzymologia* 13: 213.
- CHANTRENNE, H. (1947). Hétérogénéité des granules cytoplasmiques de foie de souris. *Biochim. Biophys. Acta* 1: 437-448.
- CHILD, C.M. (1928). The physiological gradients (Sammelreferat). *Protoplasma* 5: 447-476.

- CIGADA, M., CITTERIO, P., ORLANDI, A., RANZI, S. and TOSI, L. (1949). Ricerche sulle proteine tubulari. *Rend. Ist. Lomb. Sc. e Lett.* 82: 351-358.
- CLAUDE, A. (1943). Distribution of nucleic acids in the cell and the morphological constitution of cytoplasm. *Biol. Symp.* 10: 111-129.
- CONKLIN, E.G. (1929). Problems of development. *American Zoologist* 63: 31.
- DALCQ, A. (1951). Form and modern embryology. In *Aspect of Form* (ed. Whyte, L.L.). Humpries, London.
- DALCQ, A.M. (1941). *L'oeuf et son dynamisme organisateur*. Albin Michel, Paris.
- D'ANCONA, U. (1937). Studi e ricerche compiuti in Italia, durante l'anno XIV E.F. nel campo della Zoologia. *Atti della Società Italiana per il Progresso delle Scienze* 3: 293.
- DAVIDSON, E.H. (1968). *Gene Activity in Early Development*. Academic Press, New York.
- DAVIDSON, E.H., HOUGH-EVANS, B.R. and BRITTEN, R.J. (1982). Molecular biology of the sea urchin embryo. *Science* 217: 17-26.
- DOHRN, P. and MONROY, A. (1952). Analysis of the *in vitro* reaction between jelly-coat and a cytoplasmic component (antifertilizin) of sea urchin eggs. *Experientia* 8: 189-192.
- EHRlich, P. (1900). On Immunity, with special reference to cell life. *Proc. Roy. Soc. London B* 66: 424.
- FISCHER, E. (1894). Einfluss der Konfiguration auf die Wirkung der Enzyme. *Ber. Chem. Ges.* 27: 2985-2993.
- FREY-WYSSLING, A. (1938). *Submikroskopische Morphologie des Protoplasmas und seiner Derivate*, Engl. transl. New York, Elsevier, 1948.
- GALE, E.F. and FOLKES, J.P. (1953). Amino-acid incorporation by fragmented staphylococcal cells. *Biochem. J.* 55: xi.
- GIARDINA, A. (1901). Origine dell'oozite e delle cellule nutritive nel *Dysticus*. *Int. Mschr. Anat. Physiol.* 18: 417-484.
- GIARDINA, A. (1903). Note sul meccanismo della fecondazione e della divisione cellulare, studiato principalmente in uova di echini. 2. Sulla fecondazione. *Anat. Anz.* 22: 40-58.
- GIUDICE, G. (1965). The mechanism of aggregation of embryonic sea urchin cells; a biochemical approach. *Dev. Biol.* 12: 233-247.
- GIUDICE, G. (1973). *Developmental Biology of the Sea Urchin Embryo*. Academic Press, New York.
- GIUDICE, G. (1987). Alberto Monroy 23 August 1986. *Differentiation* 35: 163-164.
- GIUDICE, G., MUTOLO, V. and DONATUTI, G. (1968). Gene expression in sea urchin development. *Arch. f. Entw. mech. d. Organismen* 161: 118-128.
- GIUDICE, G., MUTOLO, V., DONATUTI, G. and BOSCO, M. (1969). Reaggregation of mixtures of cells from different developmental stages of sea urchin embryos. *Exp. Cell Res.* 54: 279-281.
- GROSS, P.R. and COUSINEAU, G.H. (1964). Macromolecular synthesis and the influence of actinomycin on early development. *Exp. Cell Res.* 33: 368-395.
- HARAWAY, D.J. (1976). *Crystals, Fabrics, and Fields: Metaphors of Organicism in 20th Century Developmental Biology*. Yale University Press, New Haven.
- HARRISON, R.G. (1937). Embryology and its relations. *Science* 85: 369-374.
- HARVEY, E.B. (1936). Parthenogenic merogony or cleavage without nuclei in *Arbacia punctulata*. *Biol. Bull.* 71: 101-102.
- HERBST, C. (1893). Experimentelle Untersuchungen über der Einfluss der veränderten chemischen Zusammensetzung des ungenbenden Mediums auf die Entwicklung der Thiere. II - Weiters über die morphologischen Wirkung der Lithiumsalze und ihre theoretischen Bedeutung. *Mitt. Zool. Stat. Neaples* 11: 136-220.
- HOLTFRETER, J. (1933). Eigenschaften und Verbreitung induzierender Stoffe. *Naturwissenschaften* 21: 766-770.
- HOLTFRETER, J. (1939). Gewebefähigkeit, ein Mittel der embryonalen Formbildung. *Arch. Exp. Zellforsch.* 23: 169-209.
- HOLTFRETER, J. (1943). *J. Exp. Zool.* 93: 251-323.
- HOLTFRETER, J. (1948). Concepts on the mechanism of embryonic induction and its relation to parthenogenesis and malignancy. *Symp. Soc. Exp. Biol.* 2: 17-49.
- HÖRSTADIUS, S. (1949). Experimental researches on the developmental physiology of the sea urchin. *Pubblicazioni della Stazione Zoologica di Napoli* 21, suppl.: 131-172.
- HÖRSTADIUS, S. (1953). Influence of implanted micromeres on reduction gradients and mitochondrial distribution in developing sea urchin eggs. *J. Embryol. and Exp. Morph.* 1: 257-259.
- HÖRSTADIUS, S. (1973). *Experimental embryology of echinoderms*. Clarendon Press, Oxford.
- HULTIN, T. (1950a). Incorporation *in vivo* of 15 N-labelled glycine into liver fractions of newly hatched chicks. *Exp. Cell Res.* 1: 376-381.
- HULTIN, T. (1950b). The protein metabolism of sea urchin eggs during early development studied by means of 15 N-labelled ammonia. *Exp. Cell Res.* 1: 599-602.
- HUXLEY, J.S. and De BEER, G.R. (1934). *Elements of Experimental Embryology*. Cambridge University Press, Cambridge.
- JACOB, F. and MONOD, J. (1961). Genetic Regulatory Mechanisms in the Synthesis of Proteins. *J. Mol. Biol.* 3: 318-356.
- JACOB, F. and MONOD, J. (1961). On the regulation of gene activity. *Cold Spring Harbor Symp. Quant. Biol.* 26: 193-212.
- JUDSON, H.F. (1979). *The Eight Day of Creation. Makers of the Revolution in Biology*, London.
- KAY, L.E. (1993). *The Molecular Vision of Life*. Oxford University Press, Oxford.
- LILLIE, F.R. (1919). *Problems of Fertilization*, 2nd ed. 1923, Reprint 1988, New York: Garland Publishing. The University of Chicago Press, Chicago.
- LILLIE, F.R. (1927). The gene and the ontogenetic process. *Science* 66: 361-368.
- LINDESTROM-LANG, K. (1952). *Proteins and Enzymes*. Stanford University Press, Stanford.
- LOEB, J. (1913). *Artificial Parthenogenesis and Fertilization*. The University of Chicago Press, Chicago.
- LOEB, J. (1919). The physiological basis of polarity in regeneration. *J. Gen. Physiol.* 1: 337-362.
- MAGGIO, R., VITTORELLI, M.L., CAFFARELLI-MORMINO, I. and MONROY, A. (1968). Dissociation of ribosomes of unfertilized eggs and embryos of sea urchin. *J. Molec. Biol.* 31: 621-626.
- MAGGIO, R., VITTORELLI, M.L., RINALDI, A.M. and MONROY, A. (1964). *In vitro* incorporation of amino acids into proteins stimulated by RNA from unfertilized sea urchin eggs. *Biochem. Biophys. Res. Commun.* 15: 436-441.
- McLAREN, A. and SIRACUSA, G. (Eds.) (1987). *Recent advances in mammalian development. Dedicated to the memory of Alberto Monroy*. In *Current Topics In Developmental Biology*. Vol. 23, a series edited by A. Monroy and A. Moscona..
- METZ, C.B. and MONROY, A. (Eds.) (1967). *Fertilization*, New York: Academic Press.
- METZ, C.B. and MONROY, A. (Eds.) (1985). *Fertilization*, New York: Academic Press.
- MONOD, J. (1949). Facteurs génétiques et facteurs chimiques spécifiques dans la synthèse des enzymes bactériens. In *Unités biologiques douées de continuité génétique*. C.N.R.S., Paris.
- MONOD, J. and JACOB, F. (1961). General Conclusions: Teleonomic mechanisms in cellular metabolism, growth and differentiation. *Cold Spring Harbor Symp. Quant. Biol.* 26: 389-401.
- MONROY, A. (1937). Über die Linsenregeneration bei Urodelen verschieden Alters. Unter besondere Berücksichtigung der Metamorphose. *Archiv für Entwicklungsmechanik der Organismen* 137: 25-33.
- MONROY, A. (1940). Ricerche sulla fine vascolarizzazione del timo (normale ed in involuzione) dell'uomo e di alcuni mammiferi. *Arch. Ital. Anat. Embr.* 45: 45 pp.
- MONROY, A. (1944a). Osservazioni sulla architettura funzionale delle valvole atrio-ventricolari e semilunari cardiache. *Atti R. Accad. Sc. Med. Palermo*: 18 pp.
- MONROY, A. (1944b). Prime osservazioni sull'architettura funzionale delle valvole semilunari aortiche e polmonari dell'uomo. *Monit. Zool. Ital.* 54: 81-90.
- MONROY, A. (1945). Di alcuni fenomeni che accompagnano la fecondazione e le prime divisioni dell'uovo di riccio di mare. *Experientia* 1: 1-3.
- MONROY, A. (1947). Further observations on the fine structure cortical layer of unfertilized and fertilized sea urchin eggs. *J. Cell. Comp. Physiol.* 30: 105-109.
- MONROY, A. (1948). Cortical responses to activation in sea urchin eggs. In *Sostanze interattive tra uova e spermatozoi e partenogenesi*. Simposio speciale dell'IUBS 21-22 giugno 1948 organizzato in occasione del primo Congresso Internazionale di Fisiologia-Patologia della Riproduzione Animale e di Fecondazione Artificiale, pp. 1-9.
- MONROY, A. (1950). A preliminary electrophoretic analysis of proteins and protein fractions in sea urchin eggs and their changes on fertilization. *Exp. Cell Res.* 1: 92-104.

- MONROY, A. (1953a). Biochemical and structural changes at fertilization. In *Proceedings of the symposium on the biochemical and structural basis of morphogenesis*, pp. 18-30. Brill, Leiden.
- MONROY, A. (1953b). Biochemical and structural changes at fertilization. In *Proceedings of the symposium on the biochemical and structural basis of morphogenesis*, pp. 18-30. Brill, Leiden.
- MONROY, A. (1954). Alcuni aspetti biochimici della attivazione dell'uovo. *Scientia* 48: 1-4.
- MONROY, A. (1965a). An analysis of the process of activation of protein synthesis upon fertilization. *Archives de Biologie* 76: 511-522.
- MONROY, A. (1965b). *Chemistry and physiology of fertilization*. Holt, Reinhart and Winston, New York.
- MONROY, A. (1970). Developmental biology and genetics. A plea for cooperation. In *Current topics in developmental biology* (ed. A.A. Moscona and A. Monroy), pp. xvii-xxi. Academic Press, New York.
- MONROY, A. (1974). Recenti acquisizioni sui processi di fecondazione. In *Scienza e Tecnica* 74 (ed. Macorini, E.), pp. 135-142. EST Mondadori, Milano.
- MONROY, A. (1977). Embriologia. In *Enciclopedia del Novecento, v. II*, pp. 442-465. Enciclopedia Italiana, Roma.
- MONROY, A. (1986). A centennial debt of developmental biology to the sea urchin. *Biol. Bull.* 171: 509-519.
- MONROY, A. and AUGUSTI-TOCCO, G. (1975). Gene controlled regulatory processes in the development of marine organisms. In *Biochemical and Biophysical Perspectives in Marine Biology*, vol. 2, pp. 291. Academic Press, London, New York.
- MONROY, A. and BACCETTI, B. (1975). Morphological changes of the surface of the egg of *Xenopus laevis* in the course of development. I. Fertilization and early cleavage. *J. Ultrastruc. Res.* 50: 131-142.
- MONROY, A. and MAGGIO, R. (1964). Biochemical studies on the early development of the sea urchin. *Advances in Morphogenesis* 3: 95-145.
- MONROY, A. and MONTALENTI, G. (1946). Cyclic variations of the submicroscopic structure of the cortical layer of fertilized and parthenogenetic sea urchin eggs. *Nature* 158: 239.
- MONROY, A. and MONTALENTI, G. (1947). Variations on the submicroscopic structure of the cortical layer of fertilized and parthenogenetic sea urchin eggs. *Biol. Bull.* 92: 151-161.
- MONROY, A. and MOSCONA, A.A. (1979). *Introductory concepts in developmental biology*. University of Chicago Press, Chicago.
- MONROY, A. and ODDO, A.M. (1944a). Struttura submicroscopica degli spermatozoi di echinodermi (*Paracentrotus liv.* e *Arbacia pust.*). *Bollettino della Società italiana di Biologia sperimentale* 19: 2 p.
- MONROY, A. and ODDO, A.M. (1944b). Ulteriori osservazioni sulla struttura submicroscopica degli spermatozoi di echinodermi (*Paracentrotus liv.* e *Arbacia pust.*). *Bollettino della Società italiana di Biologia sperimentale* 19: 2 p.
- MONROY, A. and ROSATI, F. (1979). Cell surface differentiations during early embryonic development. *Curr. Top. Dev. Biol.* 13: 45-69.
- MONROY, A. and RUFFO, A. (1947). Hyaluronidase in sea urchin sperm. *Nature* 159: 603.
- MONROY, A. and RUNNSTRÖM, J. (1948). Some experiments pertaining to the chemical changes occurring at the formation of the fertilization membrane of Sea urchin eggs. *Arkiv för zoologi* 40: 1-6.
- MONROY, A. and TOLIS, H. (1964). Uptake of radioactive glucose and amino acids and their utilization for incorporation into proteins during maturation and fertilization of the eggs of *Asterias forbesi* and *Spisula solidissima*. *Biol. Bull.* 126: 456-466.
- MONROY, A. and TOSI, L. (1952). A note on the jelly-coat sperm interaction in sea urchin. *Experientia* 8: 393.
- MONROY, A. and TYLER, A. (1958). Changes in the efflux and influx of potassium upon fertilization in Eggs of *Arbacia punctulata*, measured by use of K42. *Biol. Bull.* 115: 339-340.
- MONROY, A. and TYLER, A. (1963). Formation of active ribosomal aggregates (polysomes) upon fertilization and development of sea urchin eggs. *Arch Biochem. Biophys.* 103: 431-435.
- MONROY, A., CASAZZA, G., DE SANTIS, R., PINTO, M.R. and ROSATI, F. (1986). Sperm-egg interaction and fusion in fertilization. *Progress In Clinical And Biological Research* 217B: 57-68.
- MONROY, A., MAGGIO, R. and RINALDI, A.M. (1965). Experimentally induced activation of the ribosomes of the unfertilized sea urchin egg. *Proc. Natl. Acad. Sci. USA* 54: 107-111.
- MONROY, A., TOSI, L., GIARDINA, G. and MAGGIO, R. (1954). Further investigations on the interactions between sperm and jelly-coat in the fertilization of the sea urchin egg. *Biol. Bull.* 106: 169-177.
- MONTALENTI, G. (1932). Sull'embriogenesi degli ibridi di *Bufo vulgaris* e *Bufo viridis*. *Rendiconti dell'Accademia Nazionale dei Lincei. Classe di scienze fisiche, matematiche e naturali* 15: 994-1000.
- MONTALENTI, G. (1933). L'ontogenesi degli ibridi fra *Bufo vulgaris* e *Bufo viridis*. *Physiological Zoology* 2: 329-395.
- MONTALENTI, G. (1936). *Rendiconti dell'Accademia Nazionale dei Lincei. Classe di scienze fisiche, matematiche e naturali* 7: 191.
- MONTALENTI, G. (1938). L'ibridazione interspecifica degli anfibii anuri. *Attual. Zool.* 4: 157-213.
- MONTALENTI, G. (1939a). *Elementi di Genetica*. L. Cappelli, Bologna.
- MONTALENTI, G. (1939b). I recenti studi sul problema della determinazione del sesso e dei caratteri secondari negli animali. In *Atti della III Riunione della Società Italiana di Genetica ed Eugenia (Bologna, 5-7 settembre 1938-XVI)*. Genus, organo della Società Italiana di Genetica ed Eugenia, pp. 3-23. Tipografia Operaia Romana, Roma.
- MONTALENTI, G. (1945). *Compendio di embriologia*, III ed. 1959. Idelson, Napoli.
- MORGAN, T.H. (1910). Chromosomes and Heredity. *Amer. Naturalist* 44: 449-496.
- MORGAN, T.H. (1934). *Embryology and genetics*. Columbia University Press, New York.
- MORGAN, T.H. (1935). The Relation of Genetics to Physiology and Medicine, Nobel Lecture 1933. In *Nobel Lectures in Molecular Biology*. Elsevier, New York.
- Nakano, E. (1987). Alberto Monroy Remembered. *Bollettino dell'Associazione Biologica Italo-Giapponese* 10: 5-6.
- NAKANO, E. and MONROY, A. (1957). A method for the incorporation of radioactive isotopes in the sea urchin eggs. *Experientia* 13: 416-441.
- NAKANO, E. and MONROY, A. (1958). Incorporation of S35-methionine in the cell fractions of sea urchin egg and embryos. *Exp. Cell Res.* 14: 236-244.
- NAKANO, E., GIUDICE, G. and MONROY, A. (1958). On the incorporation of ³⁵S-methionine in artificially activated sea urchin eggs. *Experientia* 14: 11-22.
- NEEDHAM, J. (1934). Morphology and Biochemistry. *Nature*: 275-276.
- NEEDHAM, J. (1942). *Biochemistry and Morphogenesis*, 1950, 2nd ed. with a new foreword, 1960. Cambridge University Press, Cambridge.
- NEIFAKH, A.A. (1960). A study of nuclear function in the development of the sea urchin *Strongylocentrotus dröbachiensis* by radiational inactivation. *Dokl. Akad. Nauk SSSR* 132: 1458-1461.
- OLBY, R.C. (1986). Structural and Dynamical Explanations in the World of Neglected Dimensions. In *A History of Embryology* (ed. Horder, T.J., J.A. Witkowsky and C.C. Wylie). Cambridge University Press, Cambridge.
- PAULING, L., ITANO, H.A., SINGER, S.J. and WELLS, I.C. (1949). Sickle Cell Anemia, a Molecular Disease. *Science* 110: 543.
- PERLMANN, P. (1953). *Exp. Cell Res.* 5: 394-.
- RANZI, S. (1933). Sui cambiamenti chimico-fisici che accompagnano il differenziamento istologico degli embrioni. *Atti Pontif. Acc. Sc.* 86: 36-.
- RANZI, S. (1939). Ricerche sulle basi fisiologiche della determinazione negli embrioni di Echinodermi. *Arch. zool. Italiano* 26: 427-440 424 figg.
- RANZI, S. (1947). Azione delle sostanze chimiche sullo sviluppo embrionale. *Ricerca Scientifica e Ricostruzione* : 47-55.
- RANZI, S. (1952a). Proteins, protoplasmic structure and determination. In *Proc. Symp. Bioch. Struct. Bas. Morph.*, pp. 92-107.
- RANZI, S. (1952b). Ricerche per uno studio della determinazione embrionale al livello molecolare. *Atti dell'Accademia dei Lincei. Rendiconti* 12: 449-454.
- RANZI, S. (1957). Early determination in development under normal and experimental conditions. In *The Beginning of Embryonic Development*, pp. 291-318. American Association for the Advancement of Science, Washington.
- RANZI, S. and FALKENHEIM, M. (1937). Ricerche sulle basi fisiologiche della determinazione nell'embrione degli Echinodermi. *Pubblicazioni della Stazione Zoologica di Napoli* 16: 436-.

- RAVEN, C.P. (1954). *An outline of developmental physiology*. Pergamon Press, London.
- REVERBERI, G. (1940). Lo sviluppo dei frammenti dell'uovo centrifugato delle Ascidie. *Pubblicazioni della Stazione Zoologica di Napoli* 18: 129-139.
- REVERBERI, G. (1946). La riproduzione delle molecole nel problema dell'origine della vita. *Medicus* 2: 196-206.
- REVERBERI, G. and MINGANTI, A. (1947). La distribuzione delle potenze nel germe di Ascidie allo stadio di otto blastomeri, analizzata mediante le combinazioni e i trapianti di blastomeri. *Pubblicazioni della Stazione Zoologica di Napoli* 21: 1-35.
- RIES, E. (1937). *Pubbl. Staz. Zool. Napoli* 16: 364- .
- RUFFO, A. and MONROY, A. (1945). *Bollettino della Società italiana di Biologia sperimentale* 20: 403-.
- RUNNSTRÖM, J. (1928). *Protoplasma* 4: 388.
- RUNNSTRÖM, J. (1949). Comments on Buzzati-Traverso's lecture. *Pubblicazioni della Stazione Zoologica di Napoli* 21 (Suppl.): 205-206.
- RUNNSTRÖM, J. (1959). A search for insight into the problems of embryonic development. *Wenner-Grens Inst. 1939-1959* : 15-23.
- SANDER, K. (1986). The role of genes in ontogenesis - evolving concepts from 1883 to 1983 as perceived by an insect embryologist. In *A History of Embryology (Symposium of the Brit. Soc. Dev. Biol.)* (ed. Horder, T.J., J.A. Witkowski and C.C. Wylie), pp. 363-395. Cambridge University Press, Cambridge.
- SANDER, K. (1996). On the causation of animal morphogenesis: concepts of German-speaking authors from Theodor Schwann (1839) to Richard Goldschmidt. *Int. J. Dev. Biol.* 40: 7-20.
- SIEKEVITZ, P. (1952). Uptake of radioactive alanine *in vitro* into the proteins of rat liver fractions. *J. Biol. Chem.* 195: 549-565.
- SILVESTRONI, E., BIANCO, I., MONTALENTI, G. and SINISCALCO, M. (1954). Genic equilibrium of Microcythaemia in some Italian districts. *Nature* 173: 357-359.
- SONNEBORN, T.M. (1946). Experimental Control of the Concentration of Cytoplasmic Factors in Paramecium. *Cold Spring Harbor Symp. Quant. Biol.* 11 : 236-255.
- SPEMANN, H. (1938). *Embryonic development and induction*, 2nd ed. 1962, reprint 1st ed. 1988 New York: Garland. Yale University Press, New Haven.
- SPIEGELMAN, S. (1945). The physiology and genetic significance of enzymatic adaptation. *Ann. Mo. Bot. Gdn.* 32: 139-163.
- SPIEGELMAN, S. and KAMEN, M.D. (1946). Genes and nucleoproteins in the synthesis of enzymes. *Science* 104: 581-584.
- STERN, H. and MIRSKY, A.E. (1952). The isolation of wheat germ nuclei and some aspects of their glycolytic metabolism. *Journal of General Physiology* 36: 181-200.
- STERN, H., ALLFREY, V., MIRSKY, A.E. and SAETREN, H. (1952). Some enzymes of isolated nuclei. *Journal of General Physiology* 35: 559-578.
- TYLER, A. (1939). *Proc. Natl. Acad. Sci. USA* 25: 523.
- TYLER, A. (1948a). Fertilization and immunity. *Physiol. Revs.* 28: 180-219.
- TYLER, A. (1948b). On the chemistry of the fertilizin of the sea urchin *Strongylocentrotus purpuratus*. *Anat. Rec.* 101: 8-9.
- TYLER, A. and MONROY, A. (1956). Change in rate of release of K42 upon fertilization in Eggs of *Arbacia punctulata*. *Biol. Bull.* 111: 296.
- VASSEUR, E. (1951). Demonstration of a jelly-splitting enzyme at the surface of the sea urchin. *Exp. Cell Res.* 2: 144-146.
- WARBURG, O. (1908). Beobachtungen über die Oxidations Prozesse von Seeigelei. *Z. Physiol. Chem.* 57 : 1-16.
- WARBURG, O. (1910). Über die Oxidationen in Lebenden Zellen nach versuchen am Seeigelei. *Z. physiol. Chem.* 66 : 305-340.
- WARBURG, O. (1925). Über Eisen, den sauerstoffübertragenden Bestandteil des Atmungsferments . *Ber. Deut. chem. Ges.* 48: 1001-1011.
- WEISMANN, A. (1883). *Über die Vererbung. Ein Vortrag*. Fischer, Jena.
- WEISMANN, A. (1892). *Das Keimplasma. Eine Theorie der Vererbung*. Fischer , Jena.
- WHITMAN, C.O. (1878). The Embryology of Clepsine. *Quart. J. Microsc. Sci.* 18 : 215-315.
- WHITMAN, C.O. (1887). A Contribution to the History of the Germ-layers in Clepsine. *J. Morphol.* 1: 105-182.
- WILSON, E.B. (1925). *The Cell in Development and Heredity*. The Macmillan Co., New York.
- WILT, F.H. and HULTIN, T. (1962). Stimulation of phenylalanine incorporation by polyuridylic acid in homogenates of sea urchin eggs. *Biochemical Biophysical Research Communications* 9: 313-317.
- WRIGHT, S. (1945). Physiological aspects of genetics. *Ann. Rev. Physiol.* 7: 75-106.