INDUCTION PHENOMENA IN TISSUE REGENERATION

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By

GUSTAV LEVANDER

ALMQVIST & WIKSELL
STOCKHOLM

THE WILLIAMS & WILKINS COMPANY
BALTIMORE

PRINTED IN SWEDEN BY

Almqvist & Wiksells

BOKTRYCKERI AKTIEBOLAG

UPPSALA 1964

Preface

Collaboration between the theoretical and the practical disciplines, so important to the development of medicine, has always been difficult to attain in spite of a genuine desire among the representatives of both sides to realize this aim. For the practising physician there is always a need not only during his years of training but also thenceforth to keep informed of the advances in basic medical research. Such contact, however, is often very difficult to maintain. It is seldom that a hospital clinician, for example, is able to devote a great deal of time over a long period to theoretical research.

It was in the light of these considerations that I attempted to obtain an experimental laboratory that was completely associated with the surgical clinic of Köping Hospital, where in 1935 I became chief surgeon. This experimental department was to be for the exclusive use of the doctors practising at the clinic. In this way it would be possible, without great delay, to carry out planned experiments. The doctor could perform his experiments in parallel with his practical work, and thus utilize many "blank hours" during the day's routine. These ideas were received with great understanding by the hospital governors, and the space required for both a laboratory and an animal house was placed at our disposal. Only the simpler types of experiment could of course be performed in a laboratory such as this, but even they often provided valuable information when the investigations were based on a correct formulation of the problems. It was mainly histological and histochemical investigations, simple chemical fractionations, respiration experiments, tissue cultures, isotope experiments etc. that were carried out. The majority of the studies that are discussed in this book were performed in this department. In this connection I extend my sincere thanks to all my collaborators, who more or less independently participated in the experimental work. I would like to mention in particular Doctors N. Grefberg, G. Jonson, I. Fernström, P. Edholm, R. Valerian, P. Normann, E. Trelde, U. Brunius, H. Andreae and J. Modée. My wife took a very active part in the work of this department, and I would like to express here my special gratitude. The results obtained have been discussed on different occasions at informal symposia, in which representatives of different branches of theoretical basic research have also taken part.

When the resources of the laboratory were insufficient we were able to turn for help to different theoretical institutions both of the Karolinska Institute in Stockholm and the University of Uppsala. It gives me great pleasure to acknowledge their understanding and ever ready willingness to help us in our efforts. There are in particular two institutes, however, with which during the years I have had closest contact, i.e. the Wenner-Gren Institute for Experimental Biology at the University of Stockholm, and the Institute of Physiology at the University of Uppsala. It is a privilege for me to extend to the Heads of these institutes, Professors John Runnström and Torsten Teorell, my warmest thanks. Their elucidation of basic problems within our field has been especially profitable and stimulating.

Since my retirement from Köping Hospital, working facilities have been provided for me at the Anatomical Institute of Uppsala University by the kind courtesy of Professor B. Rexed and the head of the department of electron microscopy, Prosector V. Hanzon.

Finally I would like to express my thanks to Mrs. M. Marsden for the translation of my manuscript into English. In doing this she has not only rendered the exact meaning but has also captured something of the spirit of my discussion.

The investigations have been supported by financial grants from the Swedish Medical Research Council, King Gustaf V's Jubilee Fund and the Västmanland County Council.

Uppsala, April 1964

Gustav Levander

Department of Anatomy,
University of Uppsala,
Uppsala, Sweden

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INTRODUCTION

My interest in the treatment of fractures arose at an early stage of my surgical training. It was soon clear that rational treatment required detailed knowledge of the mechanism of bone-healing. In the literature of the 1920's there were two predominant theories regarding bone-healing—the specific osteoblast concept and the doctrine of metaplasia. Neither of them, however, was satisfactorily proved experimentally or complete from the causative aspect. I therefore carried out some experiments of my own. What I primarily sought in this connection was a mechanism common to both foetal and post-foetal growth. It has always been a principal idea of mine that regeneration of a tissue is a repetition of its embryonic development. At the time of my first experiments the results of the "Entwicklungsmechanik" were little known outside certain professional circles. SPEMANN's epoch-making induction experiments at the turn of the century on the development of the eye were hardly mentioned in the current medical textbooks. On the other hand I found analogies with the classical question regarding embryonic organogenesis —preformation or epigenesis—which had acquired a new significance with ROUX's elucidating concept of embryonic differentiation.

The specific osteoblast concept corresponded in many respects with the theory of embryonic preformation. In certain cell units in the skeleton the specificity required for bone formation is deposited in certain bone cells—osteoblasts—before osseous regeneration occurs. Independent of external stimuli these pre-existing osteoblasts, because of their inherent determination, are only able to develop into bone tissue. These specific bone cells are found mainly in the soft tissue surrounding the skeleton. The so-called periosteal doctrine has played an important role especially in surgical literature. From a causative point of view this theory is complete. In more critical experimental tests, however, it lacks conclusive evidence.

The doctrine of metaplasia, on the other hand, teaches that bone may arise from ordinary connective tissue without the support of any pre-existing bone cells, the connective tissue undergoing a series of typical transitional processes. This theory thus follows an epigenetic line and gives a clear picture of the histogenesis as regards many details of bone formation. On the other hand it is incomplete as regards the primary causation, since it is unable to point out those factors which initiate this typical chain of development in a milieu non-specific from origin. "Quelles sont les causes de l'évolution ostéoblastique des fibroblastes? Personne ne nous le dit." (LERICHE and POLICARD 1926.)

At an early stage in my experiments with implantations of different bony layers into soft tissues it was evident that bone formation could not be the result of the outgrowth of specific bone cells from the implanted material itself. In agreement with the doctrine of metaplasia, it was possible to follow the formation of new bone in the blastemal tissue surrounding the implanted material. Since, as far as could be judged, in spite of the absence of a cellular "outgrowth", there must be some connection between implanted and newly formed bone tissue, I considered the possibility of this being brought about by humoral factors. This process was also in essential agreement with the theory of embryonic *induction*. With the degeneration of osseous tissue a specific factor is released, probably in the form of a substance which spreads into the surrounding areas and stimulates non-specific blastemal tissue to bone formation. This factor, which is thus found in completely developed living osseous tissue and which represents the tissue specificity, corresponds to the inductor active during the embryonic development of bone. The induction theory, which like the doctrine of metaplasia provides an explanation for the histogenesis in bone formation, is thus also complete as regards the causative aspect and in this way combines preformation and epigenesis.

If the induction phenomenon occurs in osseous regeneration, it seems not improbable that similar reactions might be demonstrated also in the regeneration of other tissues. There is no reason to assume that different tissues regenerate in different ways. On the contrary it seems worth while to search for a mechanism common to all tissues. With this aim in view some other tissues representing the different classical "germ-layers" have also been studied. None of them has been dealt with exhaustively. On the other hand, according to the characteristic features of each individual tissue, they have been treated more or less from different aspects—historical, clinical, embryological, causative etc. The main theme common to all, however, is the histogenesis. The experience thus gathered contributes towards a more complete picture of the complexity of problems in the regeneration of tissue.

NICHOLSON, in his book "Studies on Tumor Formation", points out the importance of a study of pathological growth in obtaining better understanding of normal conditions. He emphasizes, as did GOETHE, that nature often reveals its secrets by abnormalities. Apart from the pure malformations it is well known that many tissues, while retaining their normal structures and benign character, occur outside their normal physiological milieu. The way in which these ectopic tissues are spread in the organism should contribute to an understanding of their growth mechanisms.

Pathological growth also includes the malignant variety. As is well known, attempts have always been made to associate this with embryogenesis and regeneration. The final chapter of this book describes another study in this direction.

The introductory chapter gives a short review of some of the data in the field of embryology which may be regarded as of especial significance in regeneration. A region is entered here where success does not depend so much on any one newly-discovered reaction but more on an alteration of the line of thought, which only gradually changes character with experience gathered from several fields. It has therefore seemed of interest to me to attempt to show, by means of a summarizing review, how the concepts prevailing today appear in the light of the historical background. Most of the questions concerning growth are still connected in one way or another with the classical problem—preformation and/or epigenesis.

T. H. Morgan—the eminent geneticist—has been said to have remarked in a lighter moment that since he was unable to solve the problem of regeneration, which had previously been of great interest to him, he decided to devote himself to something easier, such as the problem of heredity. Bonner, in his book "Morphogenesis" (Princeton 1952), maintains that the reason for which Morgan left regeneration was that he was unable to find any "micro theory", which he so successfully produced in the gene theory of heredity. It is possible that the epigenetic-inductive development may also lead to a micro theory in the case of regeneration.

CHAPTER 1

EPIGENESIS AND PREFORMATION DURING EMBRYONIC DEVELOPMENT

1. Short historical review

While ARISTOTLE adopted a somewhat static view of the physical laws of the universe, his interpretations as regards questions of development were on the contrary more dynamic. It was the Greeks too who first became aware that *change* was something essential to life. By following the daily development of birds' eggs, among other things, ARISTOTLE observed the growth of structures that had not been present previously (*epigenesis*). In contrast to the static picture of the universe, which in spite of its crystal-clear formulation was later to be crushed like crystal when serious analysis of movement was commenced as a physical concept, the theory of dynamic epigenesis was to live longer. It met with opposition, however, already in classical times. Before ARISTOTLE, HIPPOCRATES had expressed the opinion that all organs and tissues were already in existence in the egg from the beginning, and that development thus consisted only of growth of the separate small parts—the *preformation* theory. The authority and views of ARISTOTLE, however, predominated throughout the Middle Ages.

It was not until the Renaissance period that new experience was gained. Direct observations were now made with systematic serial investigations of the foetuses of different animals, and were described in detail with reference to illustrations—FALLOPIO (1523–1562), ALDROVANDI (1522–1605), inter alia. The studies of FABRICIUS (1537–1619) attracted especial attention by the good quality of the abundant illustrative material. By defining the different developmental stages in this way his work acquired a static character, which to some extent obscured the dynamic aspects, and this impression was not corrected by the scholastic and unclear text. This, together with the fact that FABRICIUS assigned the described development stage to a phase later than that which it actually represented, made his work fruitful ground for the preformation theory.

One scientist, however, who did not yield to a solely static line of thought, although for a time he was a pupil of FABRICIUS, was HARVEY (1578–1657). With his mobile ingenuity he attempted primarily to survey the dynamic development itself. His numerous observations of different embryonic birds and mammals convinced him that the different organs were not formed in one stage, but successively. He gave a detailed description of an epigenetic development, and also attempted to trace the development backwards to an original unit. In these studies he was searching for the egg, which he did not find, however, with the simple lenses which were the only magnifying equipment at his disposal; for him this only existed as a metaphysical concept. HARVEY was not unfamiliar, however, with ideas of preformation, and considered that "the form ariseth ex potentia materiae pre-existentis".

Great significance was attached to the description made by MALPHIGI (1628–1694) of the appearance of the first foetal stages as seen in the microscope, such as the optic cup, somites etc. It has been suggested that the eggs examined by MALPHIGI had been subjected to solar heat, and could therefore have been more developed than he believed. Even if MALPHIGI was cautious himself in drawing conclusions from his observations and did not mention the word preformation in his text, his work was nevertheless of much importance to his immediate successors. A great number of authors at this time agreed with the preformation theory, especially as it was now considered that there was good reason to connect metaphysical rational concepts with definite biological observations. Some daring conclusions were also made on the basis of the preformation theory. It was said that not only the entire human race but also all of its parasites had at one time been found in Eve's ovary. An attempt was even made to calculate the size of the original rabbit, the progenitor of all its descendents.

It has been said that these strong exaggerations fell under their own weight. It was mainly C. F. WOLFF (1733–1794) who opposed the preformation theory, both with philosophical arguments and by direct observations on foetal material. WOLFF maintained that the preformation concept was to a certain extent self-contradictory. "Qui igitur systemata praedelineationis tradunt, generationem non explicant, sed, eam non dari, affirmant" (cited from OPPENHEIMER). For WOLFF, development was a future and generative process. He liked to compare the plant and animal kingdoms. He found that during their metamorphoses the leaves and flowers of plants emanated from an originally uniform region. This led to his basic thesis that in both the plant and animal kingdoms development occurred by means of a gradual differentiation from an originally homogeneous material. The first instance of this epigenetic development was the demonstration that blood vessels were not found in the chicken blastoderm from the beginning. In his study of organic development he found, further, that many organs started as homogeneous layers and were later transformed to tube-shaped formations—the intestinal and nervous systems. With his tendency to make comparisons between plant and animal life he named these layers "germ-layers", and thus laid the basis of the so-called germ-layer theory.

The epigenetic line was further followed by v. Baer (1792–1876), well known for his many contributions regarding the different stages of development of almost all of the organs. But his name is best known for the discovery of the mammalian egg, which he found in 1827 after a systematic search. PANDER (1794–1865) had somewhat earlier (1817) described in detail the three germ-layers, in an extensive study. It was some time, however, before these were given their present names. The terms ecto- and entoderm were introduced in 1853 by ALLMANN, and mesoderm in 1871 by HUXLEY. The epigenetic concept had thus led to many valuable and definite observations. It was possible to trace the development backwards to an original unit, and there were detailed descriptions of typical structural changes during the developmental process, though no extensive conclusions could be drawn solely