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Invited Lecture

VERIFICATION OF THE GRAVITY ACTION IN THE DEVELOPMENT OF BONE MARROW HEMOPOIESIS DURING TERRESTRIALIZATION

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In the second revolution of the vertebrates, the author hypothesizes that increased gravity action of the earth after landing affects the blood pressure of chondrichthyes through intensive movement to escape suffocation toward water, then by elevated blood pressure, streaming potential increases and the increased currents trigger gene expression of chondrocytes to develop the bone marrow hemopoiesis as well as the major histocompatibility antigens (MHC) . To verify these phenomena the author carries out following experiments. 1)As preliminary experiments, artificial bone marrow chambers of three kinds of sintered porous hydroxyapatite, of collagen composed hydroxyapatite of adult cattle with antigenicity, and of Ti electrode with 10 μ A current are implanted into dorsal muscle of chondrichthyes and adult dogs. 2)As experimental evolutionary research to verify the theory of Lamarck, artificial landing experiment of dog shark, Triaxis, and Mexican salamander are carried out. After that morphological change of them are studied. 3) To verify close correlation between ontogeny and phylogeny, i.e., the law of Haeckel xenotransplantation of organs of chondrichthyes into respective organs of mammals are carried out. As results, developments of bone marrow hemopoiesis are successfully observed in all chambers and xenotransplantation are successfully carried out in all cases. Development of bone marrow hemopoiesis and HLA (Human Leukocyte Antigen) can be verified as the correspondence of animals against gravity action, and cells of adult chondrichthyes are just the same as embryonic stem cells of the mammals.

1. INTRODUCTION

In this paper the biogenetic law and the use and disuse theory are verified at the first time in the world, respectively, as the fact of "ontogeny recapitutates phylogeny", and the metaplasia of cells, of which processes are taken place by triggering the expression of the genes by not increased blood pressure but various physico-chemical stimuli. In vertebrates we have three riddles to be read, i.e., mechanisms of the evolution, the immune system, and development of hematopoiesis in bone marrow in phylogeny as well as in ontogeny.

Trilateral research methods are developed by the author integrating

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morphology, molecular biology (physiology and biochemistry), and molecular genetics of remodeling by means of biomechanics. Through this research method the author develops artificial bone marrow chamber using bioceramics.[1)~6)] The author discovers theoretically through studied on evolution of hemopoiesis that the morphology of organism can be changed by vicissitudes of inner or outer stimuli of biomechanics, i.e., environmental factors, which act to the organisms, and if these vicissitudes of biomechanical stimuli are transmitted to the next generation, morphological changes can be transmitted without changes of the genetic codes. Through this hypothesis the use and disuse theory of Lamarck can be explained biomechanically in molecular genetics.[7)~10)]

If tissue immune system, i.e., the induction of MHC (HLA) occurs during terrestrialization in phylogeny, concomitant with the development of bone marrow hemopoietic system, organism without bone marrow hemopoiesis has no tissue immunity just like embryo of mammals. To verify this hypothesis xenotransplantation of various organs of chondrichthyes, i.e., the skin, muscle, cartilage, cornea, nerves, a part of brains, and intestine into respective organs of rats and dogs are carried out. Through these successful xenotransplantation, the close correlation between ontogeny and phylogeny i.e. the biogenetic law of Haeckel in tissue immunity can be evidenced.[7)~10),12)]

2. MATERIALS AND METHODS

2.1.As preliminary study following experiments are carried out. Each of them is already reported.[3)7)10)]

2.1.1. Implantation of the artificial bone marrow chambers of sintered HAP into vertebrates which represent each stages of phylogeny:

a) cyclostomata (hogfish),
b) chondrichthyes (dochishark),
c) amphibian (xenopus),
d) aves (chicken),
e) mammals (dogs and Japanese monkeys).

2.1.2. Transplantation of artificial bone marrow chambers (artificial cartilage) into muscles of sharks and dogs (Shepherd) made of high pressure sintering collagen-HAP composite (collagen from cow skin with antigenicity).

2.1.3. Transplantation of Ti bio-chambers with a 10 μA current into muscles of sharks and dogs.

2.2.As experimental evolutionary research to verify the use and disuse theory of Lamarck by molecular genetics, artificial landing experiment of dog shark, (Heterodontas japonicus) dochishark (Triakis) , and neoteny-type

Mexican salamanders (axolotl) are carried out. Morphology in ontogeny of the rats are studied using the atlas *The House Mouse.*[11] Neoteny-type axolotls are artificially landed for months with moisture without water pool. After that they are dissected. Specimens are prepared and morphological studies as well as histological observations are carried out.

2.2.1.For experimental evolutionary researches 50 cm length chondrichthyes Triakis and Heterodantus are used. Comparative anatomy between them is carried out. Two Triakis and Heterodantus are artificially landed for one hour a day during 10 days. After that they are dissected, observed and compared the pericardial sac around the heart. The bronchial arch system, and the collarbone are observed and compared between them. The development of the diaphragm, lung and morphology of nostrils of chondrichthyes are studied and compared with that of rat embryos.

2.2.2.Fifteen Mexican Salamander (axolotl) of neoteny-type 20 cm length are artificially terrestrialized by reducing water levels continuously through 2 months. Each 3 of the 15 salamanders are recovered for morphological observation one month, 2 months, 3 months, 4 months and 5 months after landing, after that histological specimens are prepared.

2.3.To verify close correlation between ontogeny and phylogeny, i.e., evidencing the biogenetic law of Haeckel in the tissue immune system by molecular biology following xenotransplantation of several organs of chondrichthyes (Triakis) into respective organs of mammals are carried out.

a) Skin graft to five rats.

b) A part of brain into those of five rats.

c) Nerve transplantation (femoral nerves) into those respective organs of five rats.

d) Intestine of sharks to that of two dogs.

e) Cornea of sharks to that of two dogs.

3. RESULTS

3.1.Preliminary Study

Artificial development of hemopoiesis in muscle is carried out successfully with each of the HAP chambers in all species, i.e., cyclostomata, chondrichthyes, amphibian, aves, and mammals. It is shown that the leukocytes and lymphocytes are developed around the Ti bio-chamber with a 10μA current in a dog a month after implantation. Leukocyte differentiation from undifferentiated mesenchymal cells by the electrical current around the Ti is observed. It is shown that induction 12 months after surgery of hemopoiesis is in conjunction with osteogenesis by the HAP bio-chambers

implanted in Japanese monkeys. Osteogenesis as well as hemopoiesis could be observed in the chambers. A cross section of a shark with a Ti chamber with a 10μA current shows the histopathological findings associated with the dorsal cartilage with hemopoietic marrow induction by the dorsal Ti chamber implanted. The collagen-hydroxyapatite (artificial cartilage) artificial bone marrow chambers are implanted in shark muscle. Hemopoiesis and osteoid formation 4 months after surgery are observed around the hydroxyapatite implanted, in the shark muscle as well as in vertebral cartilage. The hemopoietic nests as well as osteoid formation are quite resembled with those of conventionally sintered HAP. On the other hand, in dog muscles around the artificial cartilage implanted, marked tissue differentiation with atypical cells that resembled epithelium of the digestive tract can be observed, which are thought to be cytological digestion of the collagen delivered from proteins delivery system of the sintered HAP.

3.2.Experimental evolutionary study

Experiments using chondrichthyes observing development of the diaphragm, heart and lung system

Artificially landed Triakis and Heterodantus are dissected. In Triakis

Fig1.A.
Developed air cells (arrow) in pericardiac sac of Heterodontus
Right side is larger than left just like mammalian lung.

Fig1.B.
After artificial landing of Heterodontus, two air cells resembling lungs in pericardiac cavity develop.

connective tissues with numerous air cells are observed between chest fins and pericardial sac. If numerous enlarged air cells disrupt and fuse with gill apparatus (hemopoietic nest) the lungs resembling amphibian appear.
In Heterodantus two air cells are observed between inner and outer membrane of pericardial sac (Fig.1.), of which latter becomes to be diaphragm after the lungs developed. If these two cells join with gill apparatus, the lungs resembling mammals appear. In the rat atlas in ontogeny in early developmental stage the lung develop and invade into center of the primordial heart, therefore diaphragm develops from bottom pericardial sac of caudal side. At the caudal side of pericardial sac of artificially terrestrialized dog sharks, two air sacs resembling air bladder are observed between inside and outside of the serous pericardial membrane, of which the one on the right side is larger than the other on the left. On the contrary, control Heterodantus japonicus without landing has no air sac between either inside or outside of pericardial sac. Triakis after landing has intact pericardial sac, but marked formation of air sacs are observed between fin muscles and pericardial sac of the heart, which resemble the lungs of the amphibians and reptiles.

3.3.Experiments using axolotl observing development of the collarbone system

3.3.1.The origin of collarbone is found in cyclostomata as pericardial cartilage, which is a part of peri-branchial cartilage covering hemopoietic organs of gill grand. The cartilage of cyclostomata is succeeded to the chondrichthyes as fused cartilage in ventral side of pericardial cavity, which are joined with fins and of which movement can promote function of the heart. This cartilage is succeeded to amphibians and reptiles as 2 plate-type collarbones, which are in ventral side of pericardial sac and are join to each foreleg. To mammalian the collarbones are succeeded from chondrichthyes as the clavicle and scapula, which are at the apex of the lungs. This deference between amphibian, reptiles as well as aves and mammals are dependent upon different development of the lungs and diaphragm.[16] Triaks, amphibians as well as reptiles have fixed diaphragm without respiratory movement at the caudal side of the heart.

3.3.2.From experiments verifying the biogenetic law by means of xenotransplantation, ancestor shark of mammals can be disclosed. All xenotransplantations between organs of sharks and those of dogs are successfully carried out (Figs.3.4.5.).

Neither rejection nor infection was occurred. Histopathological findings show successful xenotransplantation between dogs and sharks of the intestine. From these results the author discloses cells of sharks organs are in immuno tolerance just like embryo of mammals.

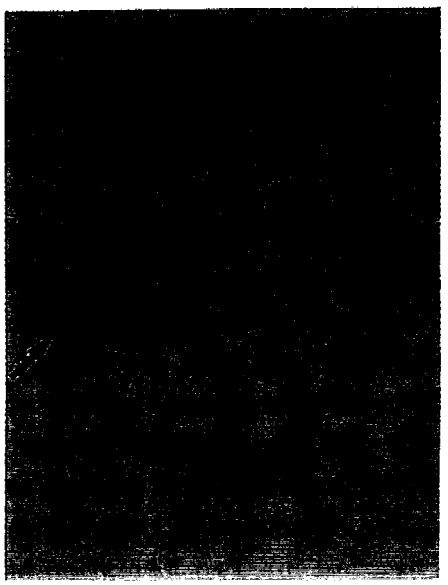

Fig.2.A. Shark eye
The cornea (arrow) is transplanted into that of dog.

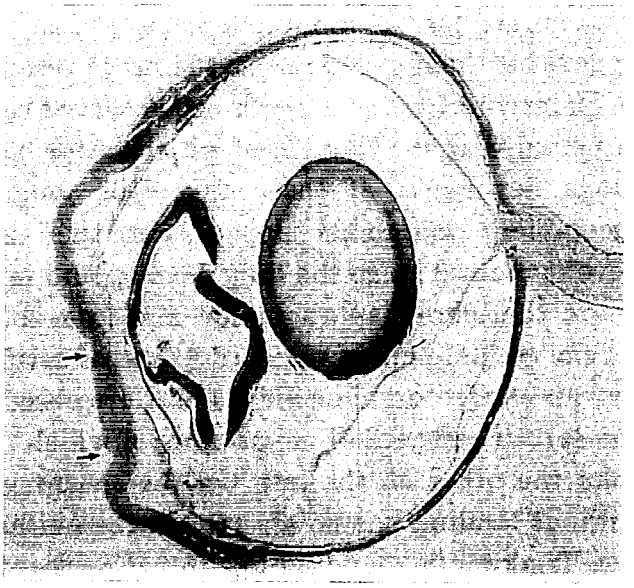

Fig.2.B. Dog eye
Thin cornea transplanted turns into thicken one (arrows) in dog.

Fig.3.A. Fig.3.B. Fig.3.C.
A: Transplanted shark intestine (arrows) into that of dog.(2 months post operation). In shark intestine numerous chalice cells exist but not in that of dog.
B: Original mucosa of the intestine of dog with few chalice cells (goblet cells)
C: In mucosa of dog intestine of transplanted part, quite resembling to the intestine of shark, numerous chalice cells can be observed.

Fig.4.
Successfully transplanted shark brain (arrow) into that of a rat. Rat can be alive normally 6 months, until recovering for specimens.

4. DISCUSSION

4.1.Collagen composed HAP sintered by high pressure low temperature technique is considered some kind of materials-delivery system. So, it can be supposed that even collagen with antigenicity can be composed into HAP without antigenicity or with weak antigenicity. Prior to sinter HAP-collagen composite with high pressure gas technique following two working hypothesis are set; 1) Collagen-composed HAP is digested in vivo slowly, therefore, no antigenicity exert in mammals and chondrichthyes. 2) Collagen-composed HAP is digested in vivo slowly, however, weak reaction against low antigenicity exert in mammals and chondrichthyes which are masked by slow material-delivery system of HAP.

After that the artificial bone marrow chambers of collagen-composed HAP are implanted into dorsal muscle of mammals (dogs) and chondrichthyes (sharks). In the dog muscles around the chambers weak reactions against low antigenicity are observed. In shark muscles, on the contrary no reaction against antigenicity is observed. From experiments of successful inducement of hemopoiesis with ossification of cartilage by collagen-HAP composite (artificial cartilage) implanted into dorsal muscle of sharks, it is disclosed that archetype vertebrates of chondrichthyes, i.e., sharks have no tissue immunity against cattle collagen with antigenicity.

It is known that sharks have MHC. Namely primitive vertebrate chondrichthyes are known to possess genomes of major histocompatibility antigen complex (MHC), but the authors have disclosed that they have no tissue immunity. This means that they are in immuno-tolerance just as the embryo of higher animals such as the reptiles, aves, and mammals. Therefore

archetype vertebrates are in immuno-tolerance just like embryo of higher animals. These facts coincide with the biogenetic low of Haeckel in tissue immunity. In phylogeny, after terrestrialization of chondrichthyes not only hemopoietic nest immigration from the gut system to bone marrow occurred but tissue immunity by MHC was generated through reactions of sharks against increased gravity. To verify immuno-tolerance of sharks xenotransplantation of xenopus and cyclostomata derma as well as rat skin to that of sharks are carried out for preliminary experiment. Successful skin graft with chimera placoids formation between Heterodontas and xenopus is fulfilled. After that xenotransplantations of shark brains, cornea (Fig.2.), muscle, and intestine (Fig.3.) to those of rats and dogs are successfully performed.

Science of forms of organisms, the morphology was defined by W. Goethe, the originator of the morphology, that besides naming of each part of the organisms, the ultimate aim of this science is elucidation of the theory of metamorphosis of the organisms in natural history. Metamorphoses of the vertebrates in phylogeny are called the evolution. The definition of the vertebrates is a chordate having a bony backbone with the various degrees of ossification, characteristic organs are the gut respiratory system, i.e., the gills and lungs. Phylogenetic tree of the vertebrates can be studied exactly by investigating not only the skeletal system but the respiratory system. Therefore, the theory of evolutionary mechanisms of the vertebrates can be precisely interpreted into that of metamorphosis not only in the skeletal system but is the gut respiration system during generations in phylogeny. Conventionally, no methodology to elucidate evolutionary theory has been established, because precise evolutionary changes of vertebrates has not been analyzed and understood. In conventional life science, especially in embryology and in phylogenetic development, the energy of mechanics, electromagnetics, gravity, thermodynamics, hydrodynamics, pressure are almost completely disregarded. Nevertheless, we have two laws of biomechanics concerning the morphology, and evolutionary transformation.

The first, the use and disuse theory of organs in organisms, proposed by Lamarck has been known since 1809. The second, the law of the skeletal system has been known as Wolff's law of functional adaptation since 1892.[13] The latter is restricted in the morphology of skeletons within one generation, and the former theory covers all organs in vertebrates in phylogenetical span through generations. Both laws deal with the other side of same phenomena differing in organs and life span.

Animals correspond to increased gravity action with increased blood

pressure by wriggling from suffocation through gill respiration with the air. After that they can survive by chance. One of major substantial changes of the air from seawater influences upon increased tissue respiration in organisms because of solvent of great increased oxygen content. The other influences upon organisms with dangerous dryness, because all organisms can be constructed with oxidized hydrogen and develop in water. Therefore, all organisms are made of water soluble gel substance with electrolyte. In terrestrialization organisms lose water and mineral (especially calcium) supply through gill respiration. After landing of animals nitrogen metabolism, i.e., urinary system has to separate from the gill system, in which the renal and adrenal system exist in archetype vertebrates of chondrichthyes.

What happened in animals with increased blood pressure during terrestrialization? Increased blood pressure, i.e., hypertension induces increased streaming potential in blood vessels. Especially in cartilaginous skeletons streaming potential increases according to the blood pressure. By this potential gene expressions of the mesenchymal cells of calcification are triggered. Streaming potential of sintered porous hydroxyapatite can be easily measured using stream of physiological saline solution with pressure, which is converted to the velocity.

Metamorphosis of the endoskeleton by biomechanical stimuli is known as functional adaptation named Wolff's Law. The Wolff's Law is the system of metamorphosis in accordance with use and disuse theory of Lamarck precisely restricted in the morphology of skeletal organs, which occurs within one generation.

The evolutionary research can be carried out not only biomechanically as use and disuse theory of Lamarck, but morphologically as biogenetic law of Haeckel, i.e., close morphological correlation between the ontogeny and phylogeny. In this paper comparative anatomy concerning skeletons, .i. e., cartilage and bones of the amphibian, reptiles, and the mammals is carried out. Skeletal morphology of the vertebrates depends not only upon modality of repeated movements of skeletal organs (inner factors by Lamarck) but biomechanical stimuli influencing outer side upon organisms (outer factors by Lamarck, i.e., environmental factors). Therefore, if metamorphosis can be observed among same animal kind of the same phylogenic stage, there should have been differences of inner or outer factors during evolution of these animals. The formation of the diaphragm and the lungs correlation with pericardial sack as well as collarbone are investigated and the period of divergence of the mammals and the amphibian, reptiles is evidenced.

In this paper the author studies evolutionary metamorphosis during the

second revolution, i.e., terrestrialization experimentally using dog sharks (Heterodantus japonicus) and axolotls (Mexican salamanders) with experiments applying biomechanical stimuli, and comparing these phylogenetic metamorphosis with ontogenic processes of mammals (rats). The author calls these experimental studies as practical phylogenetics. Therefore, applying biomechanical as well as physico-chemical stimuli (energies) in broad sense including substance with mass (oxygen, nutrition, and minerals), evolutionary stage can be changed in present animals experimentally, the author develops the experimental evolutionary research methods. Using these methods, phylogenetic researches can be carried out effectively by skeletal development.

Prior to the development of the artificial bone marrow chambers, the author develops in vivo artificial root of the gomphotic type, which induces the cementum around it and represents the mammalian tooth system, using sintered hydroxyapatite. These are hybrid-type artificial organs, which induce highly differentiated cells hetero-topically. On artificial roots surface cementoblasts are induced hybridly from mesenchymal cells of periostium by biomechanical stimuli. In artificial bone marrow chambers highly differentiated hemopoietic cells are induced hybridly by biomechanical stimuli as well. In phylogeny the bone marrow hemopoiesis had developed during the second revolution of the vertebrates i.e., terrestrialization and the gomphotic tooth system had developed during the third revolution of the vertebrates i.e., the mammalian birth. In terrestrialization 1/6 G (gravity) in water turned into 1G on land. At the same time 0.7% content of oxygen in water turned into 21% in air. It is considered that during gill respiration in air content of oxygen in blood became 50~100 times higher in landing. Around the heart surplus oxygen in blood is discharged to form air bladder. When air bladders break through to the sixth gill apparatus (gland), the gills become the airway and the bladders become the lungs. This metamorphosis occurs by metaplasia of cells through physicochemical stimuli.

Conventional phylogenetics tells us that the reptiles evolve into the mammals. The vertebrates evolve by biomechanical stimuli, which influence the organisms throughout phylogenic time span. However in this research through comparative studies on ontogeny of mammals (rats) and amphibian (salamanders), the development of the lung system between them is observed completely different. The morphological changes in second revolution occurred through billion years can be observed by neoteny-type axolotls through 5 months, which are artificially landed by reducing water. Drastic changes of the skeletons, heart, skins, and gill system can be seen to form the movable tongue.

From this study the formation of the lungs from branchial glands is disclosed quite different between reptiles and mammals. From developmental studies on mammalian embryo, it is known that the lung buds extend into primordial pericardial sac of the embryo. On the contrary, those of reptiles extend across the esophagus dorsally to the iliac bone. The differences of the lung development between reptiles and mammals are known by comparative anatomy of landed axolotls with developing embryo of rats. From these research it is known that, divergence between reptiles and mammals had started before the stage of terrestrialization.

As consequence, from experiments of collagen composed HAP the authors discovers chondrichthyes have no HLA even though they have MHC. From the experiments of successful xenotransplantation, the major function of MHC (HLA) is found to be the cytological digestion system mainly functioning for tissue remodeling in de novo synthesis of cells of organism's own organs, and partly to digest parasites as well as transplanted tissue. From these experiments new biomaterials substituting the allograft of various organs are developed. Through comparative anatomy it is evidenced that origins of mammals and reptiles are different at the stage of archetype vertebrates before terrestrialization, i.e., chondrichthyes. Not only skeletons in phylogeny but also the evolutionary processes of the lungs as well as the tongue are quite different of the amphibian as well as reptiles and the mammals. By newly developed experimental research methods, the authors can evidence Heterodantus to be the ancestor of the mammals. From studies of developing artificial bone marrow chambers, experimental evolutionary study, and experiments of xenotrausplantation, the author evidenced that the evolution of the hemopoiesis in endoskeletons as well as tissue immunity and morphology of organs are dependent upon correspondence of organisms against the increased gravity during terrestrialization. After that the author establishes the Gravity (corresponding) Evolutionary Theory in vertebral phylogeny.

5. CONCLUSION

Through multiple experiments followings are disclosed.

5.1.1.Implanting various artificial bone marrow chambers into chondrichthyes, it is disclosed that bone marrow hemopoiesis as well as tissue immune system of leukocytes are developed by correspondent action of organisms against increased gravity through the landing of chondrichthyes.

5.1.2.By artificial landing experiment, correspondence to the gravity action of the vertebrates is evidenced to be in accordance with the use and disuse

theory of Lamarck.

5.1.3.By successful experiments of xenotransplantation between shark organs and these of mammals the biogenetic law of Haeckel is verified.

In conclusion the author establishes The Gravity Evolutionary Theory.

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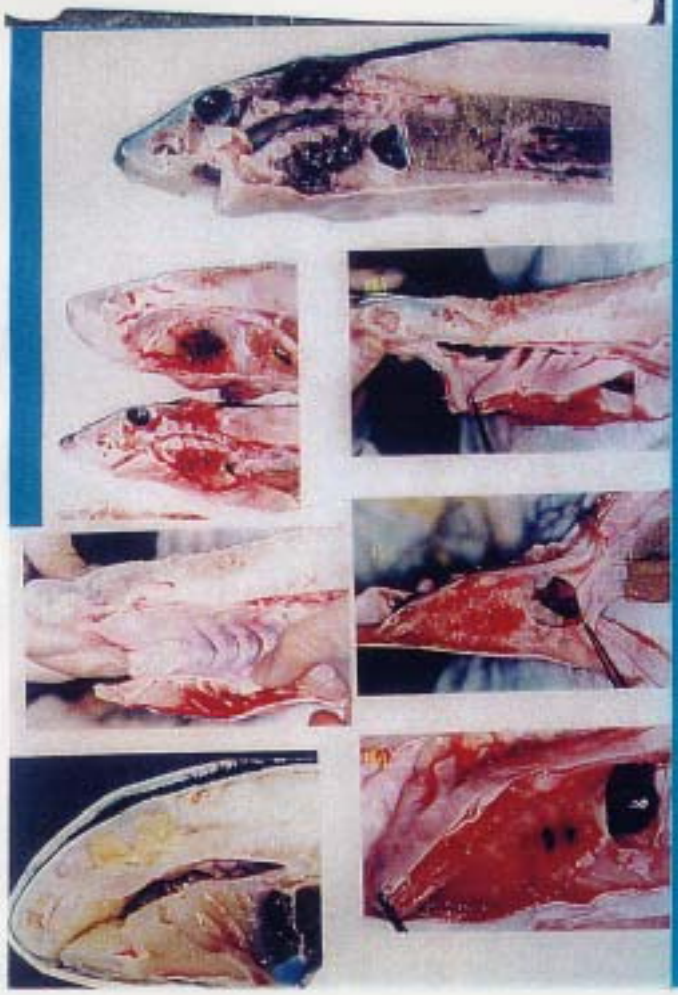
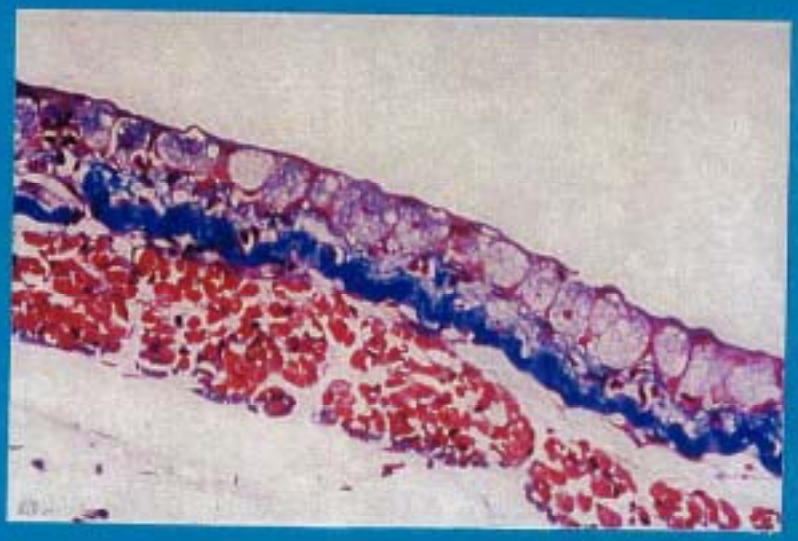
As experimental evolutionary research to verify the use and disuse theory of Lamarck in developing the lungs from branchial glands by molecular genetics, artificial landing experiment of dog shark(Heterodontas japonicus) dochishark (Triakis) , and neoteny-type Mexican salamanders (axolotl) are carried out.



Experiment of artificial landing of shark and axolotol disclose the development of the respiration system of lung and diaphragm.

Inducement of skin respiration can be observed is subcutaneous tissue in which hemopoiesis occure direct by mesonchymal cells throught stimulation of increased oxygen of 21%.

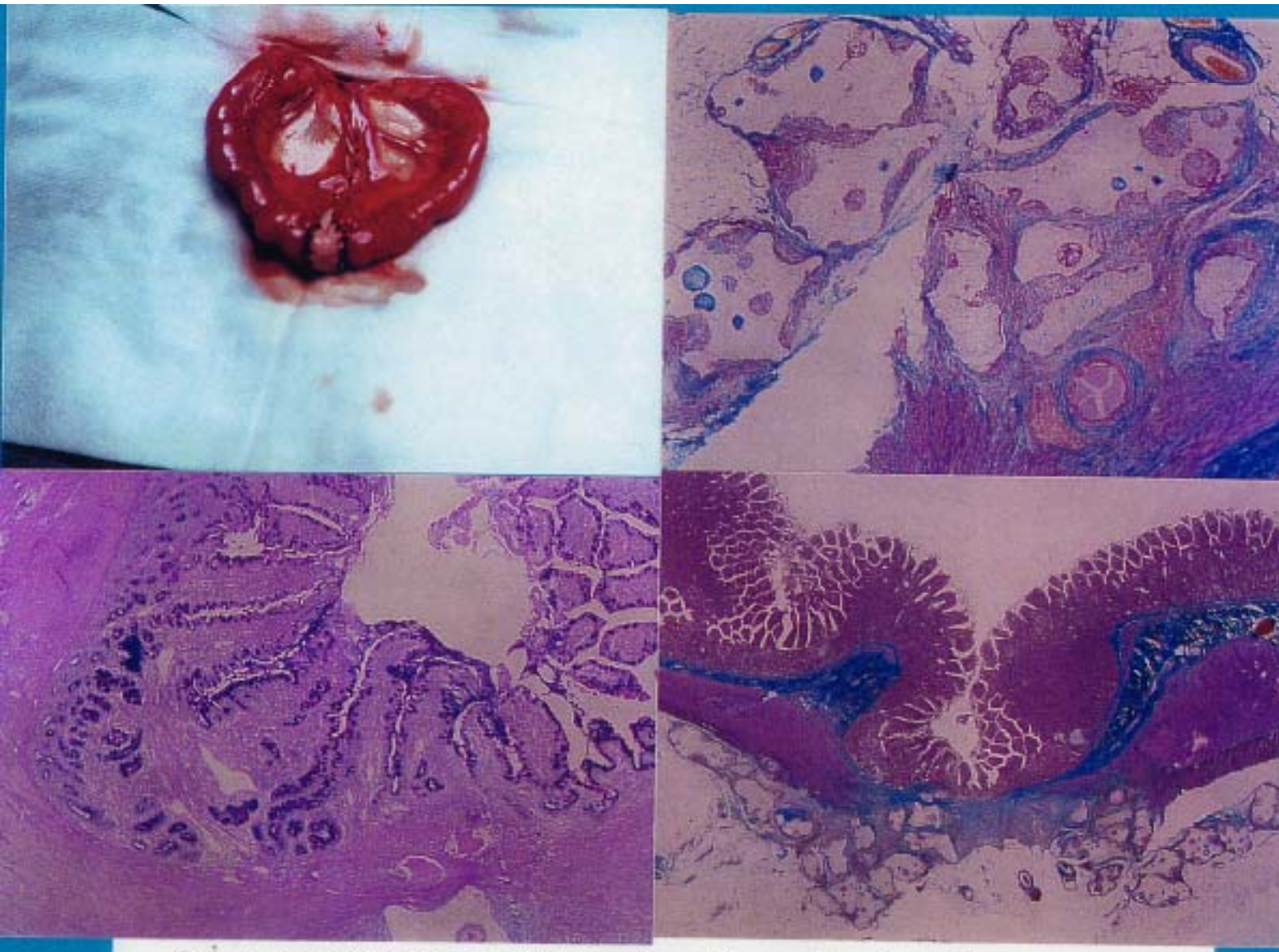


Shark eye

The cornea (arrow) is transplanted into that of dog.

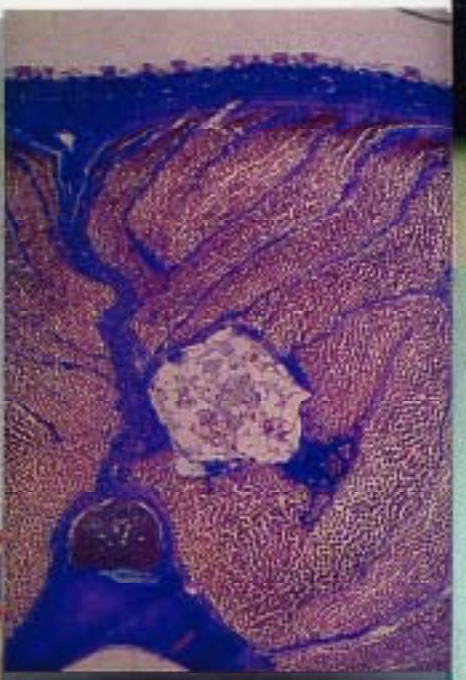
Dog eye

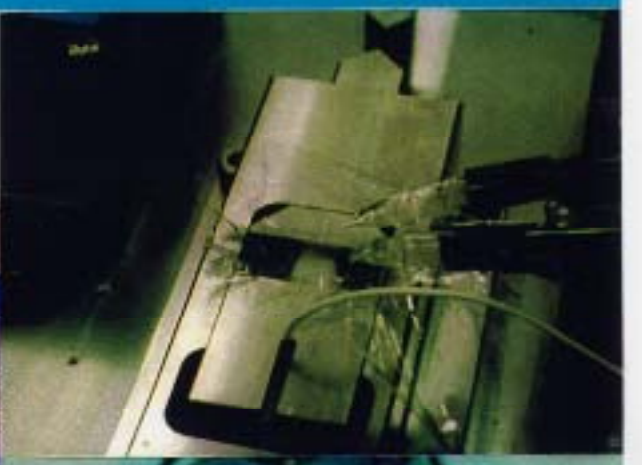
Thin cornea transplanted turns into thicken one (arrows) in dog.

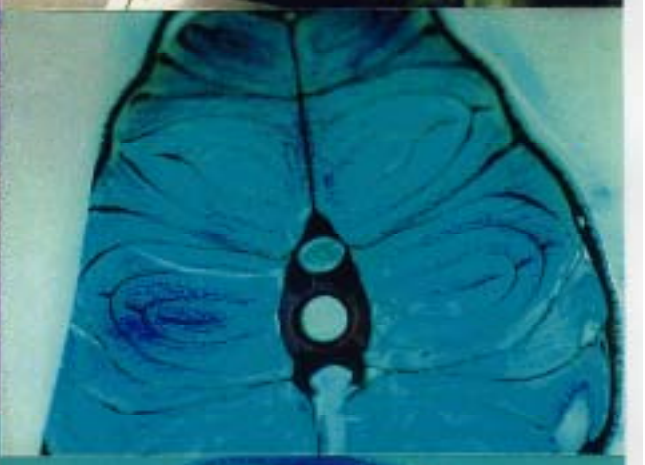


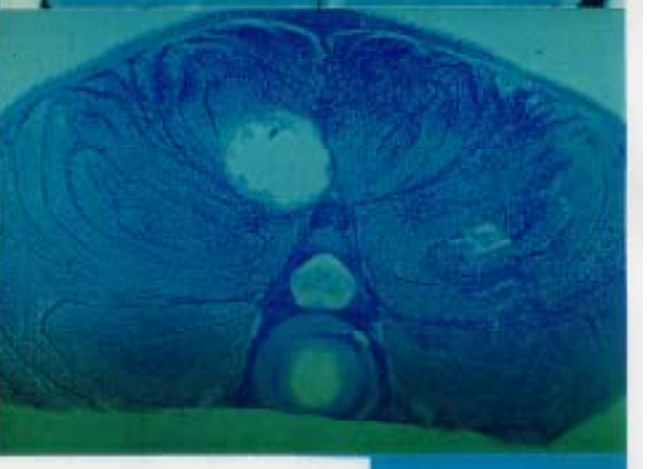
From experiments verifying the biogenetic law by means of xenotransplantation, ancestor shark of mammals can be disclosed. All xenotransplantations between organs of sharks and those of dogs are successfully carried out.

Development of artificial bone marrow chambers discloses the cause of the evolution of skeleton as biomechanical stimuli of flow dynamics which are converted into streaming potential.









Concerning experiments of artificial bone marrow chamber made of hydroxyapatite ,Ti electrode, collagen composed HA. as well as streaming potential evoking gene expression of mesenchymal cells were already reported.