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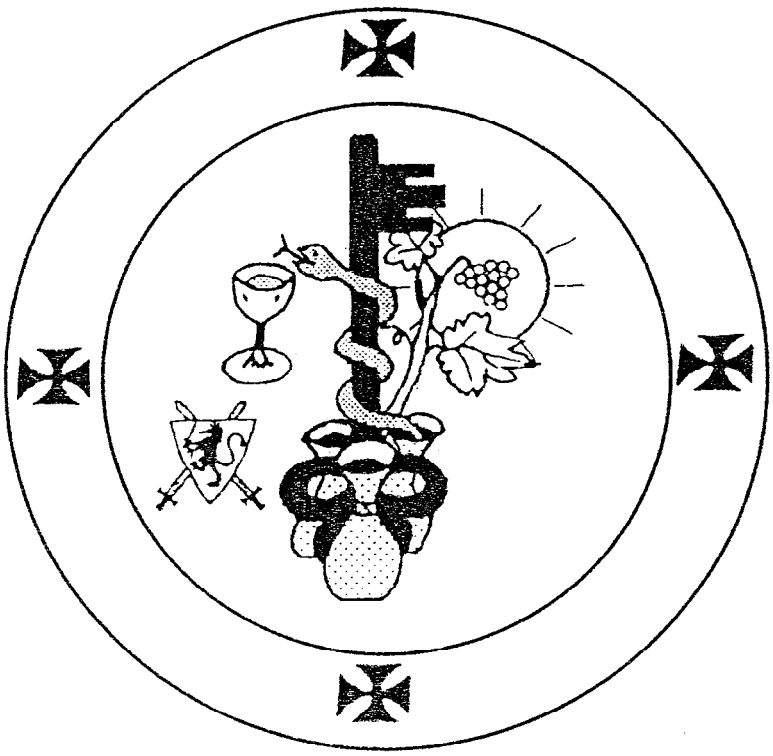

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CONSIGLIO NAZIONALE DELLE RICERCHE

ON THE EVOLUTION OF THE SPINE IN VERTEBRATES

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ABSTRACT

Vertebrates have trilateral riddles to be read, i.e., the mechanism of the evolution, the system of the immunity, and the development of the bone marrow hemopoiesis [1]. Definition of the vertebrate is "the chordates having bony vertebrae with various ossification" [2]. Therefore, definitive organs of vertebrates are the spines, which are composed of the nerve, notochord, cartilage and bone, and the components of the spines are nervous fibers, collagen, cartilage, and hydroxyapatite, respectively [2]. Therefore, if one of definitive components of the four is synthesized, we can read three riddles though artificially sintered components by means of model experiments applying the biomechanics [3]. The author noticed about this fact, therefore started research on developing artificial bone marrow biochamber using synthetic hydroxyapatite (HA) to read the riddle of the cause of the development of bone marrow hemopoiesis in phylogeny [3,4]. Concerning the spine, we have definitive substance and organ for vertebrates, i.e., the osseous tissue and notochord [5]. Therefore, researching on the evolution of the spine we can read the most important riddle of the three, i.e., why the evolution occurs in vertebrates?
The mechanisms of the evolution can be elucidated researching the spine by means of verifying following two theories: the first is the biogenetic law of Haeckel (recapitulation theory) and the second is the use and disuse theory of Lamarck [6]. Using bioceramics of hydroxyapatite, tricalcium phosphate, and titanium biochambers the author developed artificial bone marrow hemopoietic organs [7]. Based on the new research method, he can verify the law of Haeckel as well as Lamarck as action of biomechanical stimuli represented by the gravity during phylogeny and ontogeny [8].

INTRODUCTION

In phylogeny of evolutionary stage from primordial archetypes to mammals, there are four revolutionary states [4]. The evolution of the spine can be investigated through precise observation in processes of the primordial evolution as well as in the second revolution of the terrestrialization, i.e., landing. The author developed trilateral research methods, in which

morphology (phylogeny-ontogeny), physiology (molecular biology), and molecular genetics are integrated by means of biomechanics (in broad sense) i.e., physicochemical stimuli, by which gene expression of mesenchymal cells is triggered. Experimental evolutionary research methods are also developed, applying the trilateral research method to four different stages of animals in phylogeny, i.e., ascidia, Cyclostomata (hagfish), Chondrichthyes (shark), Amphibians (salamander), Aves (chickens), and Mammalia (rats and dogs) [5]. The definitive organ of the vertebrates is the spine, in which the bone marrow hemopoiesis is induced during terrestrialization of the second revolution of the vertebrate. Therefore, the author investigated what is the cause of the development of bone marrow hemopoiesis in the spine during second revolution of the vertebrate, i.e., terrestrialization by means of experimental evolutionary studies [6]. The cause of evolution of bone marrow hemopoiesis is disclosed to be reaction of the animal to the gravity against 1G from 1/6G in seawater by buoyancy. The energies, i.e., substances without mass of the gravity as well as temperature, pressure, electricity, and substances with mass of the air and water, oxygen and nutrition are physicochemical stimuli [6]. Through these physicochemical stimuli gene expressions of the mesenchymal cell s in skeletal organs are triggered [6-8]. As conclusion the author can successfully verify the both laws in evolution inducing hemopoietic nest in spine of the archetype vertebra without osseous inner skeleton by means of artificial bone marrow biochambers [3].

MATERIALS AND METHODS

The Biogenetic Law concerning the Spine - ontogeny and phylogeny of the spine

The Biogenetic Law proposed by Haeckel is represented the dogma "Ontogeny recapitulates phylogeny". Recapitulation means "repetition of the caput (head)". Therefore this law indicate that "In ontogeny the morphology of the viscerocranium repeats just as in phylogeny[1]. This law had been denied until Alberch proposed the concept of heterochrony (1994). It is known in phylogeny that bone marrow hemopoiesis in the spine occurs during second revolution of the vertebrates. Therefore, we can research to find out the cause of the vertebral evolution of the spine, by means of comparative and conjugated studies on the ontogeny-phylogeny. The author investigates ontogeny, then phylogeny, after that he conjugates them by experiments in the spine evolution.

RESULAT

The origin of the spine and phylogeny of the skeletons

The first stage of notochord formation can be seen in degenerated chain salpa, i.e., sequential ascidia. Independent chain salpa constructed with about 40 single ascidia, organizes into unified somite organism with metameric structure, which has real notochord. Archetype somite animal is amphioxus and cyclostomata (Figure 1). The function of the notochord is skeletons for visceral guts. Branchial arches are derived from notochord, which is developed from endodermal mesenchyma. On the other hand perineural spinal bones are somatic skeletons derived from dermal bone differentiated de from exodermal mesenchyma (Figure 2). The first skeletal substance was developed as cartilaginous placoid at the surface of ascidia. At this stage notochord of larvae was made of collagen. After the primordial revolution of vertebrate amphioxus as well as archetype cyclostomata were developed. Placoids as well as

teeth of cartilage developed even though collagenous notochord without cartilage. However, amphioxus and cyclostomata have lost cartilaginous placoids, during the next stage, i.e., the first revolution.

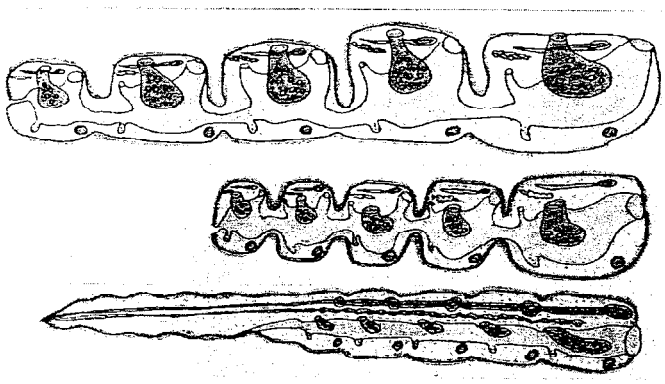

Figure 1. Unified somite organism

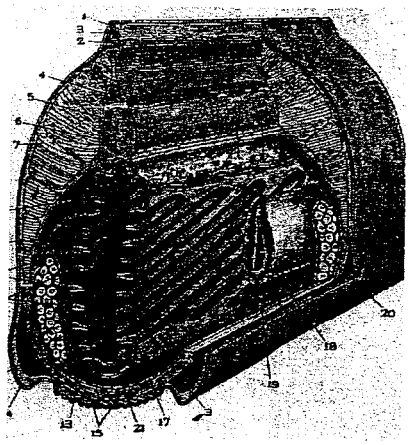

Figure 2. Notochord and perineural bone of the amphioxus

The evolution of the spine

Figure 3 shows metamorphosis of the vertebrae. Inner skeletons of chondrichthyes in Devonian era have changed into ossified tissues with bone marrow. The site of hemopoiesis in the spleen has changed into skeletal bone marrow cavity. Precise changes of the spine in evolution are shown in Figure 4. Visceral skeletons for notochord and somato skeletons from neural tube (the spinal cord and brain) join together. In mammals notochord becomes relict of nucleus pulposus. Establishment of bone marrow hemopoiesis in the spine in phylogeny, hemopoiesis inducement of the spine occurred in second revolution of the terrestrialization in Devonian era [1, 2]. In ontogeny this phenomenon occurs in delivery. In phylogeny as well as ontogeny the cause of hemopoiesis immigration is change of the gravity from 1/6G in seawater or in amniotic fluid to 1G during landing and delivery.

DISCUSSION

Verification of the use and disuse theory of Lamarck during terrestrialization

The essence of the use and disuse theory of Lamarck is the biomechanical stimuli represented by the gravity of the earth which induce gene expression of an organ using or disusing. Lamarck's theory can be interpreted correctly by the author as the gravity corresponding or the biomechanics-responding evolutionary theory [6]. Not only morphology but also hemopoiesis of the skeleton in vertebrates can be controlled by biomechanical stimuli induced by muscle movement of habitual behaviors as well as external mechanical stimuli like the gravity applied to the organism in landing. If these biomechanical stimuli are transmitted to the organisms of next generation through habitual behaviors by soft information like education, the skeletal change can be transmitted by biomechanics even in same genetic character [5]. This is the

correct interpretation proposed by the author (1998) of the use and disuse theory of Lamarck in the age of molecular biology of 20th century [8]. According to the Lamarck's evolutionary theory, hemopoiesis in the spine can be induced by the gravity, against which organisms can make blood pressure higher, by struggle during terrestrialization.

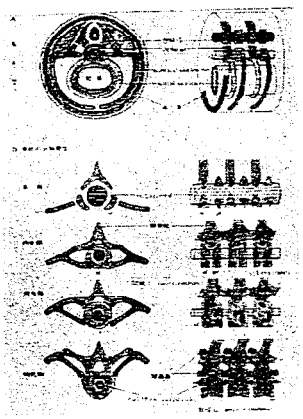

Figure 3. Metamorphosis of the vertebrae

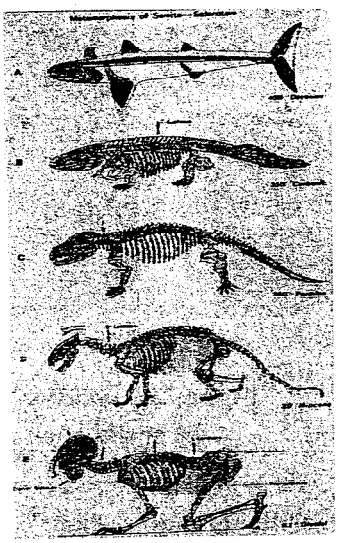

Figure 4. The spine evolution

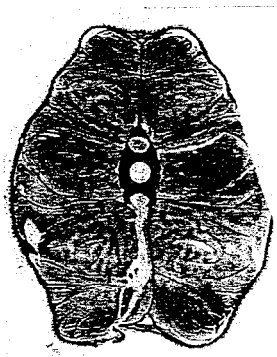

Figure 5. Cross section of shark (for control)

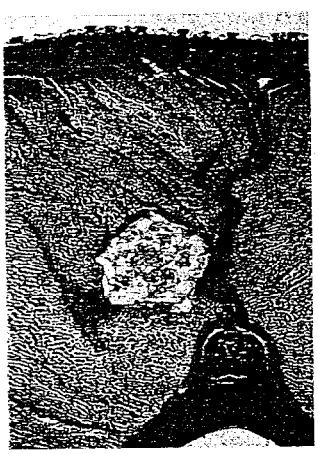

Figure 6. Induced hemopoietic nest by sintered HA

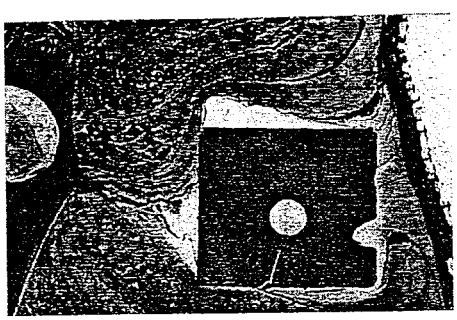

Figure 7. Induced hemopoietic nest by collagen-HA composites

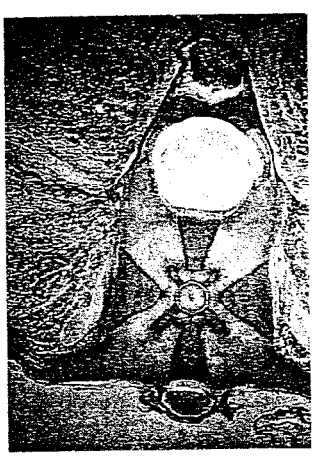

Figure 8. Induced hemopoietic nest by electrode

The hemopoiesis in spine can be induced by the streaming potential which is induced by higher pressure of the blood in organisms after landing than in sea water [7]. The organisms are closed system of fluid and open system against the energy, which is substance without mass. Therefore, If organisms move or are applied with biomechanical stimuli, movement and stimuli are converted to streaming potential. After that the gene expression in mesenchymal cells are excreted by the streaming potential. The author developed artificial bone marrow chambers made of hydroxyapatite bone, artificial cartilage and titanium electrode with 15μA current, which are implanted into dorsal muscles of archetype vertebrate [7]. This hypothesis can be verified if these can induce hemopoietic nest in the spine. Figure 5 shows cross section of shark dorsal muscle with the spine for control has without bone marrow in the spine. Figure 6 shows induced hemopoiesis (arrow) in the spine, of which cartilage changes into osseous tissue, by conventionally sintered hydroxyapatite implanted into the shark dorsal muscle. Figure 7 shows also hemopoiesis (arrow) in the spine normally induced by collagen-hydroxyapatite composite sintered with low temperature high-pressure technique. Used collagen is extracted from adult cow with antigenicity. From this experiment the author discloses that archetype vertebrates of Chondrichthyes have no major histocompatibility antigen (MHC) [6].

Figure 8 shows hemopoietic nest induced in the shark spine by electric artificial bone marrow chamber. Figure 9 shows cross section of the spine of a chicken. Both quite resemble. Applying hydroxyapatite or electrode that are substances brought about during evolution, evolutionary stage can be moved to the system of higher animals in shark dorsal muscle as well as in the spine.

Figure 9. Cross section a chicken

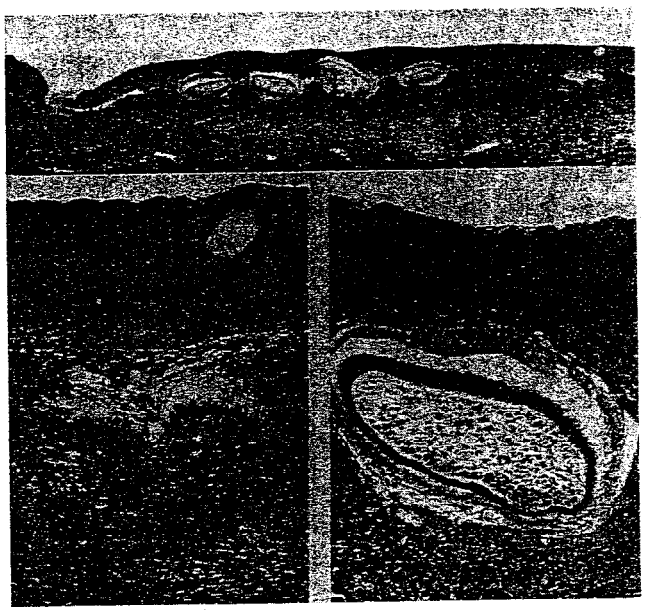

Figure 10. Chimera placoids between shark and xenopus

Integration of the Lamarck's Theory and the Heackel's Law.

The author discovered those archetype animals, e.g., hagfish and sharks have no MHC [6]. This means that they have immunotolerance. All of embryo of higher animals has immunotolerance. As Haeckel discovered the tissue immunity in ontogeny also repeats in phylogeny. Therefore, the author tried to carry out skin graft of xenopus to shark. Dermal transplantation can be successfully carried out. Figure 10 shows chimera placoids of shark with xenopus skin.

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