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EVIDENCE OF BIOMECHANICS-RESPONDING EVOLUTIONARY THEORY BY USING BIOCERAMICS

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In vertebrates we have trilateral riddles to be read, i.e., mechanisms of the evolution, immune system, and development of hematopoiesis in bone marrow in phylogeny as well as in ontogeny. Definition of the vertebrates is a cordate having bony backbone, with the various degree of ossification. Therefore, if we can synthesize cartilage or bone artificially, we can read the trilateral riddles by means of model experiments using these skeletal substitutes.

The present research aims to prove use and disuse theory by means of newly developed experiments, i.e., trilateral research methods. Lamarck proposed the empirical law of use and disuse theory as results of precise observation of the vertebrates. However, this theory has not been explained by means of molecular biology and molecular genetics. For this reason this theory has been disregarded in evolutionary science.

Trilateral research methods are developed by the author integrating 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.

The author discovers through studies on evolution of hemopoiesis that the morphology of an organism can be changed by vicissitudes of inner or outer stimuli of biomechanics, i.e., environmental factors, which act to the organism, and if these vicissitudes of biomechanical stimuli are transmitted to the next generation morphological changes can be transmitted. Through this discovery the use and disuse theory of Lamarck can be explained biomechanically in molecular genetics.

Experimental evolutionary studies are carried out as follows: developing artificial bone marrow biochamber, the author has implanted them into archetype vertebrates as well as mammals, compared them each other, and analyzed them. Developments of hemopoiesis in bone marrow chambers in phylogeny are evident as the action of the gravity in terrestrialization, which is converted into heightening of blood pressure in chondrichthyes. As conclusion use and disuse theory is evidenced in second revolution of vertebrates for the gravity to trigger genetic expression in mesenchymal cells producing hemopoiesis conjugated with ossification of the cartilage.

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ABSTRACT
Trilateral research methods are developed by the author integrating 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.
The author discovers through studies on evolution of hemopoiesis that the morphology of an organism can be changed by vicissitudes of inner or outer stimuli of biomechanics, i.e., environmental factors, which act to the organism, and if these vicissitudes of biomechanical stimuli are transmitted to the next generation morphological changes can be transmitted. Through this discovery the use and disuse theory of Lamarck can be explained biomechanically in molecular genetics.
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KEYWORDS: Biomechanics, Evolution, Immunity, Hemopoiesis, Biogenetic Law, Use and Disuse Theory, MHC, Hydroxyapatite

INTRODUCTION
In vertebrates we have trilateral riddles to be read, i.e., mechanisms of the evolution[1-3], immune system, and development of hematopoiesis in bone marrow in phylogeny as well as in ontogeny. Definition of the vertebrates is a cordate having bony backbone, with the various degree of ossification. Therefore, if we can synthesize cartilage or bone artificially, we can read the trilateral riddles by means of model experiments using these skeletal substitutes.
The present research aims to prove use and disuse theory by means of newly developed experiments, i.e., trilateral research methods. Lamarck proposed the empirical law of use and disuse theory as results of precise observation of the vertebrates. However, this theory has not been explained by means of molecular biology and molecular genetics. For this reason this theory has been disregarded in evolutionary science.

MATERIALS AND METHODS
The following experiments were carried out.
Each of them is already reported [6,7].

1) Development of several kind of artificial bio-chambers:
   (1) Artificial bone marrow biochambers made of conventionally sintered hydroxyapatite (HAP) by Asahi Optical Co.
   (2) Artificial bone marrow biochambers made of high pressure sintering collagen-HAP composite (by National Inst. for Research in Inorganic Mat.; collagen was extracted from cow skin.)
   (3) Artificial bone marrow biochambers made of titanium (Ti) mesh with a 10μA current by National Inst. for Research in Inorganic Mat.
2) Following experimental evolutionary studies were carried out using newly developed artificial bone marrow biochambers.
   (1) Implantation of the artificial bone marrow biochambers of sintered HAP into vertebrates which represent each stage of phylogeny:
      a) cyclostomata (hogfish),
      b) chondrichthyes (dochishark),
      c) amphibian (xenopus),
      d) aves (chicken),
      e) mammals (dogs and Japanese monkeys).
   (2) Transplantation of artificial bone marrow biochambers (artificial cartilage) into muscles of sharks made of high pressure sintering collagen-HAP composite (collagen from cow skin with antigenicity)(Fig.1, 2).
   (3) Transplantation of Ti bio-chambers with a 10 μA current into muscles of sharks and dogs.
   (4) The following xenotransplantation between archetype and higher vertebrates are carried out.
      a) Shark cartilage and muscle transplantation to two dogs.
      b) Intestine of sharks to that of dogs. .
      c) Cornea of sharks to that of dogs

RESULTS

Artificial induction of hemopoiesis was carried out successfully with each of the HAP chambers in all species, i.e., cyclostomata, chondrichthyes, amphibian are, aves, and mammals. It is show that the leukocyte and lymphocyte are inducted around the Ti bio-chamber with a 10μA current in a dog 4 months after implantation. Leukocyte production induced from undifferentiated mesenchymal cells by the electrical current around the Ti was observed. It was shown that induction 12 months after surgery of hemopoiesis was in conjunction with osteogenesis by the HAP bio-chambers implanted in Japanese monkeys. Osteogenesis as well as hemopoiesis could be observed in the chambers. On the contrary, in dog muscles around the artificial cartilage implanted, marked tissue differentiation that resembled digestive tract formation could be observed. The collagen-hydroxyapatite (artificial cartilage) artificial bone marrow chambers were implanted in shark muscle (Fig. 1). Hemopoiesis and osteoid formation 4 months after surgery were observed around the hydroxyapatite implanted in the shark muscle as well as in vertebral cartilage (Fig.2). On the contrary no bone marrow in the cartilaginous tissue around the spinal cord is evident in control sharks. A cross section of a shark with a Ti bio-chamber with a 10μA current shows the histopathological findings associated with the dorsal cartilage with hemopoietic marrow induction by the dorsal Ti bio-chamber implanted.

All xenotransplantations between organs of sharks and those of dogs are successfully carried out (Fig. 3). Neither rejection nor infection was occurred. Histopathological findings show successful xenotransplantation between dogs and sharks of the intestine (Fig. 4).

Discussion

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. Therefore, the theory of evolutionary mechanisms of the vertebrates can be precisely interpreted into that of metamorphosis in the skeletal 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, electromagnetic, gravity, thermodynamics, hydrodynamics, pressure are almost completely disregarded. Nevertheless, we have two laws of biomechanics concerning the morphology, and evolutionary transformation.
The first, the law of the skeletal system has been known as Wolff's law of functional adaptation since 1892. The second, the use and disuse theory of organs in organisms, proposed by Lamarck has been known since 1809. The former is restricted in the morphology of skeletons within one generation, and the latter theory covers all organs in vertebrates in phylogenetical span through generations. Both laws deal with the other side of same phenomena differing in life span and organs.

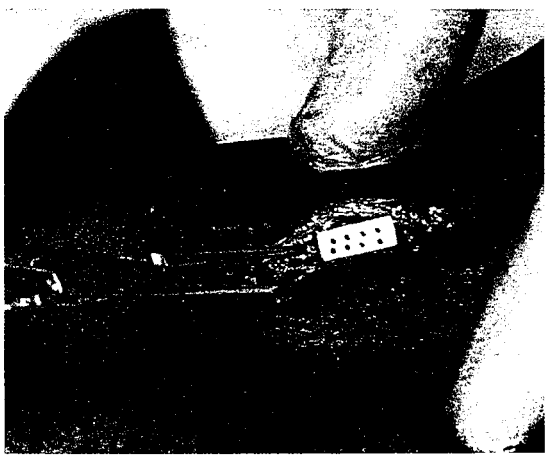

Fig. 1 Implantation of bone marrow chamber made of artificial cartilage

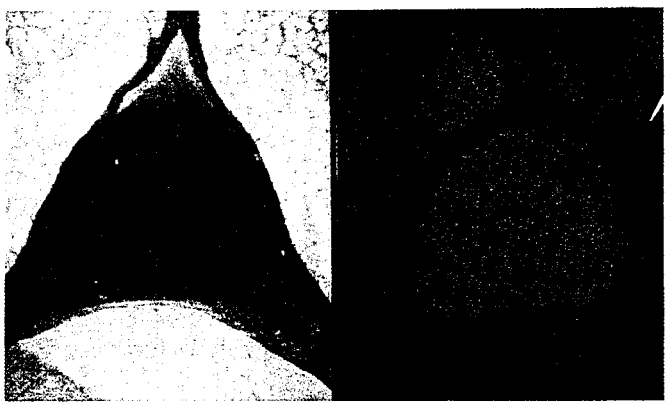

Fig. 2 Hemopoietic induction in shark dorsal vertebral cartilage. (by polarized microscope)
left: Control cartilage
right: Induced hemopoiesis in cartilage

Fig. 3 Successful xenotransplantation between dog and shark

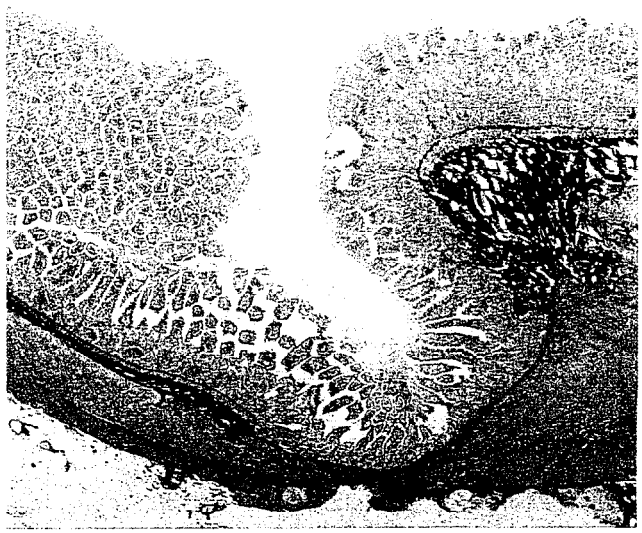

Fig. 4 Histopathological findings of transplanted shark intestine (arrow)

As skeletal substance of the vertebrates is definitive material, we can verify the use and disuse theory through disclosing phylogenetical change in skeletal organ, i.e., mechanism development in bone marrow hemopoiesis during second revolution of the vertebral evolution. Through precise analyzes of the second revolution, two marked changes against organisms are recognized; change of the gravity action of the earth of 1 G from 1/6 G in sea water by buoyancy and change of the solvent of oxygen of the air from sea water. Major differences of environmental factors between in sea water and after terrestrialization, i.e., landing is the first, biomechanical effect of water as well as the gravity, i.e., decrease of water pressure upon surface of the organisms and increased gravity action upon animal bodies and the second, material effect of the air.

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 hydrogen and develop in water. Therefore, all organisms are made of water soluble gel substance with electric electrolyte. In terrestrialization organisms lose water 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 calcified skeletons streaming potential increases according to the blood pressure. By this potential gene expressions of the mesenchymal cells of calcified cartilage are triggered. Streaming potential of sintered porous hydroxyapatite can be easily measured using physiological saline solution with pressure.

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 cow collagen with antigenicity. It is known that sharks have MHC. Therefore sharks are in immuno-tolerance just like embryo of mammals. 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 immune-tolerance of sharks xenotransplantations of shark intestine to that of dogs are successful carried out.

Dynamic cardiomyoplasty has been developed and applied clinically by which striated dorsal muscle can be converted into heart-muscle (cardiac muscle) with electricity by pacemaker. From these facts the skeletal system including muscle, tendon, and cartilage are controlled by electric current, i.e., streaming potential [8]. Therefore, if we can induce hemopoietic nest in sintered HAP, biomechanically or with electric current in mammals heterotopically, Wolff's law can be verified as biomechanics-responding metamorphosis theory. If we can induce hemopoietic nest with HAP chamber or electric chamber in chondrichthyes, we can verify biomechanics-responding evolutionary theory.

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