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# Verification of use and disuse theory of Lamarck in vertebrates using biomaterials

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**Abstract**—The material foundations moulding the vertebrate are skeletal substances, which are collagen, cartilage and bone. If any of them can be synthesized intact artificially, the causes of chondrification of collagen and ossification of cartilage that occur in the process of evolution, will be clarified. The reason is that in the process of the evolution of this phylum, changes from collagen to cartilage and from cartilage to bone alone are observed in terms of materials. Vertebrates pose three riddles to be solved: the development of evolution, of the immune system, and of bone marrow hemopoiesis. These riddles can be investigated all at once by developing artificial bone marrow chambers made of synthetic hydroxyapatite (HA). The development of bone marrow hemopoiesis occurs in the second revolution of evolution. The bone marrow system is the equipment for inducing wandering mesenchymal cells or blood corpuscles, which are responsible for digestion, respiration, metabolism, and remodeling at the cellular level. Thus, the hemopoietic system is the focus of immune capability. Accordingly, the clarification of the mechanism of development of bone marrow hemopoiesis leads to the solution of the three riddles. I have developed an artificial bone marrow using a hybrid method by applying biomechanical stimuli to sintered apatite, which took on the characteristics of bone marrow hemopoiesis peculiar to higher vertebrates after their emergence from water onto land. I have also developed a hybrid-type artificial dental root that took on the characteristics of the gompholic tooth peculiar to mammals. In this way, I have clarified that evolution occurs according to the mechanical functions of the animal in response to gravitation. In order to elucidate the law of evolution, I have developed Trilateral Research that integrates morphology, including embryology and phylogeny, functional study of molecular biology, and molecular genetics concerning remodeling, with biomechanics. I have also devised an Experimental Evolutionary Study method that applies Trilateral Research to work of every phylogenic stage representing phylogeny. From these innovative studies I have tried to reinterpret Wolff's Law, the Use and Disuse Theory, and moreover Haeckel's Biogenetic Law, with the current level of science using biomechanics and molecular biology. Using biomaterials made from synthetic apatite and an artificial bone marrow organ with Ti-electrodes, I moreover verified Lamarck's Use and Disuse Theory using an artificial dental root inducing the cementum by biomechanics.

From these studies, I have clarified the mechanism of the evolution of vertebrates. Furthermore, I have developed a method to induce artificial organs by applying biomechanical loads to biomaterials.

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## 1. CHARACTERISTIC ORGANS AND MATERIALS OF VERTEBRATES

We may start by asking what defines a vertebrate, in terms of material composition and organ specificity. The definition of a vertebrate is that it is a chordate having a bony backbone, in differing degrees of ossification [1, 2]. Therefore, we may define those bony structural materials as being a definitive characteristic of vertebrates. In the evolution of vertebrates, it is possible to observe a substantial change in both outer and external skeletal matter (i.e. placoids and spine), with collagen changing to cartilage, which in turn changes to osseous bone (Fig. 1A–H). A re-definition of vertebrates, therefore, is those animals that possess a chordal skeleton, consisting of any of collagen, cartilage or osseous bone. Conventional evolutionary studies are currently at an impasse, as there have been no observations made on the effect of time, space, material and energy that are essential for reaction and subsequent change, for movement and mechanical reaction, and for the metabolism of the evolving animals.

The structures and organs that we may define as being characteristic of vertebrates are the spine, and the respiratory system using the gut, that is, the gill and lung systems. Invertebrates, for the most part, utilize the dermal respiratory system. This vertebral development of the gut respiratory system has unified the absorption of nutrition and oxygen into the alimentary canal. The change from gill-based to lung-based systems occurred in a phylogenetical stage of the vertebrate evolutionary process, which may be described as the second revolution of this process [3–6].

## 2. RESEARCH ON VERTEBRATE EVOLUTIONARY MECHANISMS

The study of evolutionary mechanisms is the science of researching laws of metamorphosis of the forms and structure of organisms over a multi-generational period. We can use the following approaches to study the causes of evolutionary change.

### *2.1. Haekel's biogenetic law*

According to the famous words of Haeckel's biogenetic law, 'ontogeny recapitulates phylogeny'; we can therefore divide our research on the biogenetic law into the fields of ontogeny and phylogeny [7, 8].

*2.1.1. Ontogenetic studies approached from the viewpoint of molecular genetics* [9] *and biomechanics* [7]. A sequence of the ontogenic processes can be summarized as follows: fertilized ovum, cleavage, blastula, gastrula and neurula, followed by the development of the pharyngula. That is, a fertilized ovum develops into a prochordate-type organism during the gastrula stage, without exhibiting a metameric structure. This is followed by the development of somite structures in the embryo in the neurula stage. The genetic expression of the genome of the

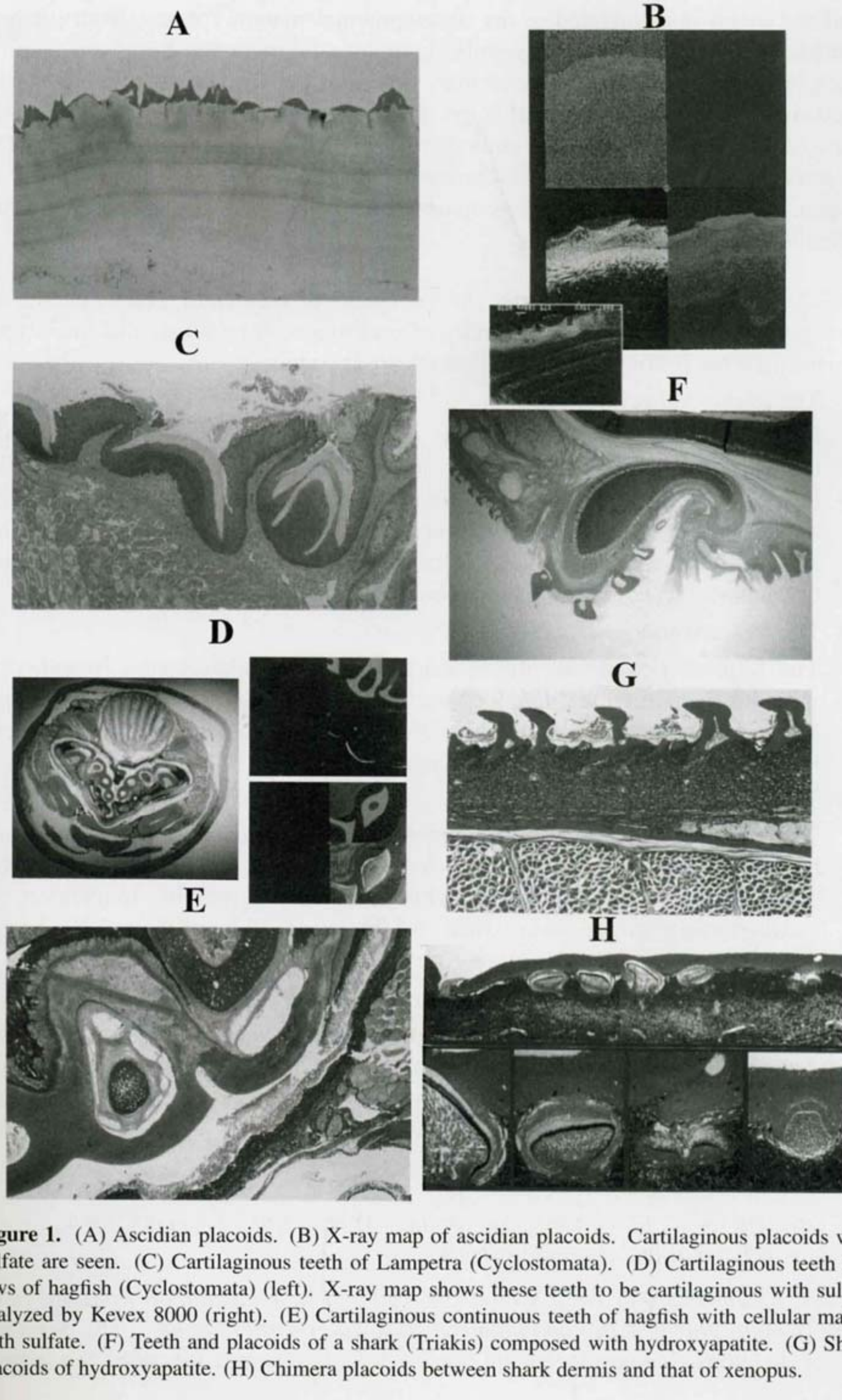


**Figure 1.** (A) Ascidian placoids. (B) X-ray map of ascidian placoids. Cartilaginous placoids with sulfate are seen. (C) Cartilaginous teeth of Lampetra (Cyclostomata). (D) Cartilaginous teeth and jaws of hagfish (Cyclostomata) (left). X-ray map shows these teeth to be cartilaginous with sulfate analyzed by Kevex 8000 (right). (E) Cartilaginous continuous teeth of hagfish with cellular matrix with sulfate. (F) Teeth and placoids of a shark (Triakis) composed with hydroxyapatite. (G) Shark placoids of hydroxyapatite. (H) Chimera placoids between shark dermis and that of xenopus.

fertilized ovum is manifested in the developmental metamorphoses from the prochordate to the neurula and pharyngula. In embryonic metamorphoses, the respiratory, circulatory, metabolic and excretory functions consistently exist, either intra- or extra-cellularly. At an original stage, the genome of a fertilized ovum and that of a somatic cell in a metameric embryo following development are the same. The metamorphoses in ontogenic development occur as a result of the heterochrony of the genome, i.e. the genetic expression of the genome site changes according to the different developmental phases.

*2.1.2. Phylogenetic studies from the viewpoint of molecular genetics.* In the phylogeny of the evolutionary hierarchy of vertebrates, from primordial archetypes to mammals, the following four revolutions are recognized:

(i) The primordial revolution

In this stage, metameric organisms developed through a process of genome duplication from the ascidian, i.e. urochordate mono-somite organisms. Relicts of this primordial revolutionary stage are the amphioxus and cyclostomata. After metameric organisms (vertebrates with a homeobox), metamorphic changes have occurred as the result of biochemical stimuli, with no alteration to the basic structure of the homeobox genome.

(ii) The first revolution

The Silurian period saw the appearance of spiny sharks (the Acanthodii), with jaws, calcified teeth and a carapace of hydroxyapatite acquired through forward movement. Descendants of this are to be found today as cartilaginous fishes (the Chondrichthyes), i.e. sharks and rays.

(iii) The second revolution of vertebrates; terrestrialization in the Devonian period

Two dramatic changes were undergone in the change from water to air as a habitat: the change in gravitational force (from 1/6G, due to buoyancy, to 1G), as well as the change from branchial to pulmonary respiration. In addition, the cartilaginous endoskeleton ossified. Salamanders and lungfish are relics of this change.

(iv) The third revolution; the appearance of mammals

The most important characteristic of mammals is the development of the lactation system, used for feeding the young, after which the sucking organ becomes the masticatory organ. This change in energy metabolism, allowing the maintenance of a constant body temperature, was made possible by the efficient mastication of foodstuffs.

### *2.2. Law and theory of metamorphic change in organisms corresponding to biomechanical and dynamic stimuli*

*2.2.1. Examination of Wolff's Law* [10] *of functional adaptation from the viewpoint of molecular genetics and biomechanics with regard to bone.* The morphology of skeletons changes dependant upon a definite repeated function. Since there is no locomotive system in bone itself, when we discuss the law of functional adaptation, we must discuss it with reference to the forces applied on skeletons, both internal (muscle movements) and external forces, which can both change skeletal morphology. In the skeleton, all forces external to the skeleton, including muscular contractions, are converted into hydrodynamic forces, which are then converted into streaming potential. The mesenchymal cells are then subject to genetic action, differentiating into chondrocytes, hematopoietic cells, tendons, or osteocytes. Wolff's Law accordingly postulates a skeletal metamorphosis as the result of biochemical stimuli in the course of one generation as the result of the remodeling of mesenchymal cells through gene expression.

*2.2.2. Examination of Lamarck's Use and Disuse Theory from the viewpoint of molecular genetics and biomechanics with regard to organs.* This theory, that acquired characteristics are inherited through generations, has been misinterpreted in the twentieth century. However, Lamarck did not mention the acquired characteristics but he proposed a theory of biomechanically induced metamorphosis, from empirical observation. The Use and Disuse Theory may be applied to all organs and structures in organisms, including skeletons.

If biomechanical stimuli (energies) are transmitted in some way, e.g. by a teaching and learning process, to the next generation, transformation can be handed down with the same genetic characteristics (genotype). This is the correct interpretation of the Use and Disuse Theory of Lamarck, when viewed from the standpoint of molecular genetics and biomechanics.

Wolff's Law is concerned with a definite skeletal transformation, based on the Use and Disuse Theory within one generation. Therefore, the mechanisms of the Use and Disuse Theory in molecular genetics are the same as those in Wolff's Law. If we view this from the standpoint of the Use and Disuse Theory, the metamorphosis of vertebrates, i.e. evolution, can be interpreted as a response to the force of gravity, in a molecular biological and biomechanical sense.

## 3. METAMORPHOSIS CORRESPONDING TO ENVIRONMENTAL CHANGES DURING PHYLOGENY

### *3.1. Research on the correlation between ontogeny and phylogeny from the viewpoint of molecular genetics*

As a result of internal and external factors, i.e. behavioral and environmental changes, morphological changes in organisms can be induced at a phylogenetic level without genetic changes. As an example, let us take the case of ascidia, an urochordate of a single-somite structure. Through several duplications of the genome of the single-somite archetypal ascidia, metameric organisms like the cyclosalpa, the amphioxus or the cyclostomate came into existence. In the same way, ontogeny may be said to repeat phylogeny, in that the larva, that is, the form in the initial stage of development of an amphioxus, has initially a single somite. Following this stage, a larval amphioxus has several somites, whose morphology resembles that of a larval ascidia. In the imago stage of the amphioxus, multiple gills with bulbi, i.e. small hearts with the metameric structure associated with the respiratory system, are developed. At the urochordate stage (ascidian), cells having the functions of such organs as the liver, pancreas and spleen are distributed throughout the gut membranes. Respiratory and renal secretory cells are scattered throughout the branchial gut, as well as on the surface of the organism. It can therefore be said that in phylogeny, no organs actually come into existence. Development and evolution of organs are a matter of assembly.

Figure 1C shows the cartilaginous teeth of a member of the Cyclostomata which do not possess hydroxyapatite skeletons. In phylogeny, collagen skeletons develop into cartilaginous skeletons, and subsequently cartilage changes to osseous tissue in the external but not in the internal skeletons. The mechanism of the change from cartilaginous outer skeletons to osseous tissue can be explained as the result of reaction to the metabolism, to movement, and to the mechanical force of water pressure. However, what are the mechanisms for change from cartilaginous to osseous tissue in internal skeletons during the period when we have characterized as the second evolutionary revolution? One is the change in gravitational force (from 1/6G in seawater to 1G on land) and the other is the change in the medium in which oxygen is contained, from water (0.7%) to air (21%).

At the terrestrialization stage, when vertebrates inhabited land or tideland at ebbtide or in drought, organisms without pulmonary respiration responded to the increased gravitational force, by being obliged to raise blood pressure intensely by muscular exertion to escape suffocation. After this acquisition of hypertension, organisms with aqueous gills started breathing through air-using gills, using surface seawater. After a long period on land, these gills developed gradually from water-compatible systems into air-compatible systems. One part of the gill system developed into the air chambers of the inner ear, and the other parts developed into the lungs. The hypertension associated with air respiration can support the large demands on oxygen required by the metabolism through increased movement.

## 4. VERTEBRATE EVOLUTIONARY PHENOMENA FROM THE VIEWPOINT OF MATERIAL COMPOSITION, MOLECULAR GENETICS, BIOMECHANICS AND MORPHOLOGY

Here we examine the evolution of vertebrates, from the viewpoint of material composition, organs, molecular biology, and physical and chemical factors. The constituents of active living organisms, cells, need time in order to remodel themselves. This remodeling of cells over time is termed 'regeneration of cells', and the remodeling of the organisms over generations is called 'inheritance'. When cells are remodeling, it is essential that genes are duplicated, and this duplication takes a certain amount of time. While cells are being remodeled by physical and chemical stimuli, including energy acting on the organism, the effects on the genes of the regenerating cells reflect the effect of these stimuli, and subsequently the function or form of the cell will change as a result of the stimuli, proportional to the strength and duration of the stimuli. From this, it can be seen that the morphology and the function of organs in a vertebrate can change, as well as the form of the animal itself, even with the same genetic characteristics. This mechanism of change in the skeletal organs as a result of biomechanical stimuli within one generation is known as Wolff's Law of functional adaptation. The mechanism of change in the morphology and function of vertebrates over a number of generations (phylogeny) is termed evolution.

Therefore evolution (metamorphosis) can occur as the result of physical and chemical stimuli that act on the remodeling of vertebrate cells with the same genetic characteristics. A mutation in a 'germ cell' may occur only once in several million times during the process of duplication, and so the genetic codes of these germ cells change slowly as far as molecular evolution is concerned, after metamorphoses take place as a result of stimuli on vertebrates. This is an evolutionary law of vertebrates corresponding to biomechanics.

### *4.1. Interpretation of Lamarck's Use and Disuse Theory into an evolutionary law corresponding to biomechanics* [3]

The biomechanical interpretation of Use and Disuse Theory is that animals in the course of development can change the shape or function of their organs and structures (e.g. skeletons) as the result of repeated biomechanical stimuli which influence these organs and structures. The theory presented here can therefore be interpreted as an evolutionary theory which takes responses to biomechanical (gravitational) stimuli into account as the Use and Disuse of organs and structures. These responses are strictly defined as the results of the effects of biomechanical energy that trigger the genetic expression regarding the function of the cells in the organ. From a biomechanical standpoint, the major evolutionary changes during the second vertebrate revolution in evolution (terrestrialization) are bone marrow hemopoiesis moved from the spleen, transformation of gills into lungs, thymus, endocrine and lymphadenoid tissue, and carapace with placoids into dermis with hairs. These changes can be interpreted as transformation as the result of the response to stimuli provoking use and disuse: the increase of gravity on dry land, and the move to a dry environment from water. As an experiment, we can apply these stimuli to neoteny-type Mexican salamanders, which are in water, i.e. adult salamanders having a larval form, which was influenced by these stimuli during the second revolution of vertebrate (terrestrialization). If the water surrounding the salamander is slowly dried up over a period of some three months, the larval-form imago of the Mexican salamander can be changed from an amphibian to a morphology resembling that of a reptile. Here we see the oxygen-carrying medium change from water to air, and the gravitational force change to 1G. By the application of these two stimuli, gravity and dryness, this transformation can be achieved.

### *4.2. Transformation of Haeckel's Recapitulation Theory into a genuine biogenetic law*

Through morphological comparisons between ontogeny and phylogeny, Haeckel proposed his Recapitulation Theory as a biogenetic law: 'ontogeny recapitulates phylogeny'. In Latin, *caput* means head; therefore, Haeckel expressed that the shape of the head is a morphological reproduction in ontogeny of the phylogenetic metamorphic change. The author discovered that this reproduction is to be observed not only in the shape of organisms, but in that of all organs, in tissue immunity, in the expression of the genetic code, in the development of bone marrow hemopoiesis, in metabolism and in respiration (placental gills to lungs). As a result of the repetition of these seven phylogenetic processes in ontogeny, the author proposes the recapitulation theory as the Genuine Biogenetic Law (Nishihara–Haeckel).

### *4.3. Development of the trilateral research method and experimental evolutionary study*

Neither Wolff's Law, nor the theory of use and disuse, can be verified using conventional research methods, as morphological changes that accompany functional changes take place only as a result of the remodeling of mesenchymal cells. This remodeling takes place as a result of cellular genetic expression triggered by biomechanical stimuli. In order to verify Wolff's Law and Use and Disuse Theory in molecular genetics, the author has developed a trilateral research method that integrates morphology, physiology, metabolism, and molecular biology (including molecular genetics) using biomechanics. The author has also developed research methods for evolution designated as an Experimental Evolutionary Study, using this Trilateral Research on vertebrates at every phylogenetic stage of evolution. From these innovative experiments, it is now possible to establish a solution to the problem of bone marrow hemopoiesis in evolution. In addition, a new concept regarding the immune system, and a new evolutionary theory which depends on response to biomechanical stimuli, are proposed.

## 5. VERIFICATION OF THE EVOLUTIONARY SYSTEM

### *5.1. Experimental evolutionary studies to verify evidence of the biomechanics-corresponding law by means of bioceramics and biomechanics*

Induction of heterotopic hemopoiesis in mammalian muscle [7, 8], conjugated with ossification using an artificial bone marrow chamber composed of sintered hydroxyapatite (Fig. 2) [13–20].

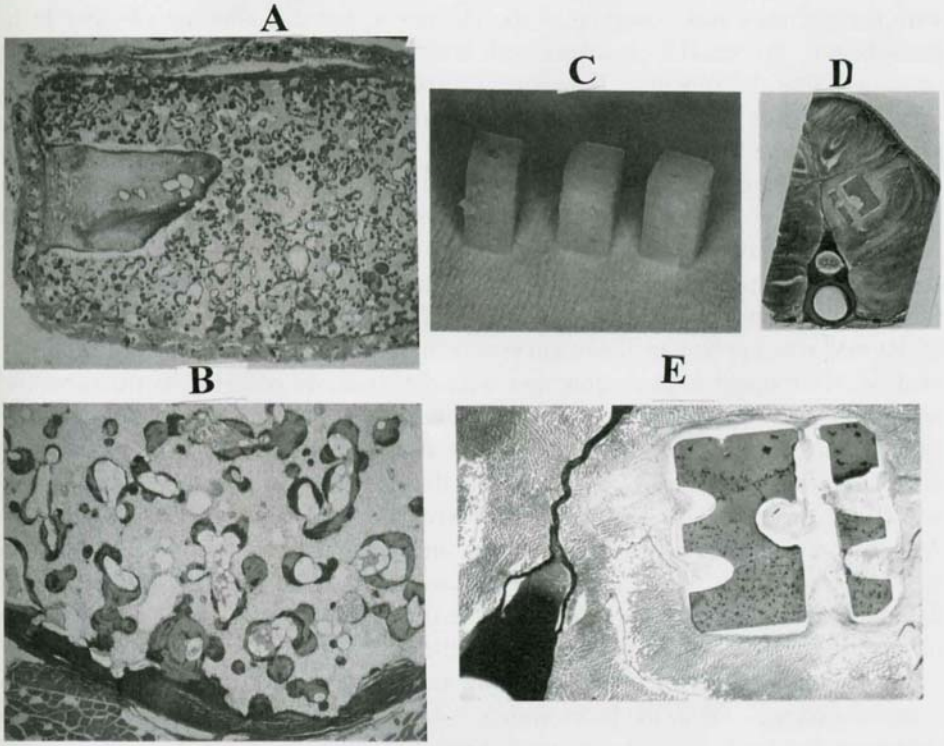


**Figure 2.** (A) Porous sintered HAP implanted into the subcutaneous tissue of an adult dog with BMP Marked bone formation conjugated with bone marrow hemopoiesis can be seen just like the implanted figure in the muscle tissue. (B) Bone marrow hemopoiesis conjugated with osteogenesis in the artificial hemopoietic chamber of sintered porous HAP implanted into the dorsal muscle of an adult dog. (C) Chamber of HAP-collagen composite sintered by high pressure technique. (D) Accepted chamber of HPA-collagen composite implanted in a shark. (E) Collagen with antigenicity is accepted by shark.

The development of bone marrow hemopoiesis during the second revolution was induced by an intense rise in blood pressure of the Chondrichthyes, which was converted into streaming potential in the organism [21]. The author's hypothesis is that cartilage loaded with the streaming potential changes to osseous hydroxyapatite tissue, together with bone marrow hemopoiesis by gene expression of mesenchymal cells. The following experiments were carried out in order to verify this theory.

*5.1.1. Experiments using mammals.*

*Artificial bone marrow chambers.* Artificial bone marrow chambers made of sintered porous hydroxyapatite (HA) were implanted into the muscle as well as into the subcutis of dogs. In both types of tissue, the blood flow from the heart was the same. However, the lymphoid fluid flow was quite different between the subcutis and muscles while the animal was moving. In muscles, hemopoiesis in conjunction with osteogenesis was observed in the chambers, but this was not observable in the subcutis. Porous HA chambers with bone morphogenetic protein (BMP) were implanted into the subcutis. Subsequent to this, hemopoiesis in conjunction with osteogenesis was observed, even in the subcutis.

The difference between hemopoiesis in the muscle and that in the subcutis may lie in the streaming potential, which is converted from lymphatic fluid flow during the movement of the animals. To determine the streaming potential of the sintered HA, the potential was measured with a physiological saline solution having the same pressure as the blood in animals. 10 mV was obtained as the value of the streaming potential of HA under 100 cm water pressure. Instead of BMP, a potential of 10 mV was applied to HA chambers which were implanted into the subcutis of dogs. Hemopoiesis in conjunction with osteogenesis occurred in the subcutis, resembling the case of an HA chamber with BMP. Ti chambers with 5–10 mV current were developed and inserted into the subcutis and the spleens of dogs. After 4 months, the spleen with chambers and the chambers in the subcutis, together with the surrounding tissue, were recovered and the specimens were observed. A significant development of leukocytes and lymphocytes was observed in the regions surrounding the Ti chambers implanted into the subcutis. In addition, a definite transformation into collagen-bundle tissue resembling tendon was observed in specimens of the spleen with a Ti electric chamber.

From clinical experiments in dynamic cardiomyoplasty, muscle development from striated muscles to the heart muscle is known to be controlled proportional to the electricity used. From these experiments it may be verified that skeletal organs and structures, e.g. tendon, muscle, osseous tissue, bone marrow hemopoiesis, and hematopoietic organs are induced by electricity, triggering genetic expression from mesenchymal cells.

*Developing gompholic artificial dental root by means of biomechanical stimuli.* Vertebrate evolution of morphology and function occurs by biomechanical stimuli with the same genetic characteristics. Therefore, artificial roots of mammalian gomphosis or reptilian ankylosis can be easily developed with or without biomechanical stimuli. Both mammalian gompholic roots with cementum and ankylotic reptilian roots with sintered compact hydroxyapatite developed in mammalian jawbones within the Experimental Evolutionary Study using biomechanical stimuli (Fig. 3).

Artificial roots of a corrugated cone type, made from sintered compact hydroxyapatite were developed and implanted into the jawbones of adult dogs and Japanese monkeys. Artificial roots in gomphotic and ankylotic conditions were easily obtained from biomechanical stimuli, i.e. with or without applying masticatory loading by soft or hard diets after the operation.

For investigation of the histological differences between gompholic and ankylotic teeth, animal experiments with functional and nonfunctional groups were carried out. For investigation of the biomechanical differences, stress analyses with conditions modeled to approximate those in the animal experiments were also carried out. Following this, the results were compared. Fibrous tissue and the cementum around the roots with alveolar bone proper were observed in specimens of the functional group (Fig. 3A, B, C). Ankylosis of the artificial roots to the surrounding bone was observed in specimens without occlusal function (Fig. 3I). Severe bone destruction was observed in the cortical bone around the ankylotic artificial root, on which the masticatory loading was applied for 2 years after a postoperative lapse of one year (Fig. 3B, J). The pattern of bone destruction in experimental subjects with the usual occlusal function and the finite element analysis (FEA) pattern showed a close correlation (Fig. 3J). The fibrous junction system around the bioceramic implants plays the most important role, by which the stress is mitigated and dispersed, and the principal stress trajectories are converted into two components — parallel and orthogonal vectors. It was proved that the effective conversion of the principal stress trajectories depends upon an undulating morphology of the bioceramic artificial root.

*5.1.2. Experiments using the Chondrichthyes.* The Chondrichthyes have no HA endoskeleton with bone marrow, but instead possess HA placoids, i.e. exoskeletons. The following three kinds of artificial bone marrow chambers were used for experiments: sintered porous HA chambers, Ti electric chambers with 10 mV and chambers made of HA collagen composite (artificial cartilage). The collagen was extracted from cattle and possesses antigenicity. These chambers were implanted into the dorsal muscles of sharks as well as those of dogs. After 4 months, the specimens were recovered, and the histological specimens were compared.

Observations of hemopoietic site formation together with osteoid formations, resembling those of mammals, were made around the HA chamber as well as the Ti electric chamber. The newly formed hemopoietic nests were induced at the upper part of the spine, which is characteristic of vertebrates. The induced nests in these sharks resembled those observed in chickens.

Around artificial bone marrow HA-collagen composite, hemopoietic nests resembling those of conventionally sintered HA were observed. Also, newly formed hemopoietic nests were induced at the upper site of the spine, resembling the hemopoietic nests of chickens. By comparison, the histological findings of specimens recovered from dogs in which HA-collagen composite chambers had been implanted, showed marked reactive cells around the HA-collagen composite resembling findings of a malignant tumor. These experiments demonstrate sharks possess immuno tolerance to the histocompatibility antigen of mammals.

*5.1.3. Evidence of the Genuine Biogenetic Law as practical phylogeny: verification of the seven kinds of phenotypes in ontogeny and phylogeny.* Haeckel's Biogenetic Law (the recapitulation theory represented by the doctrine that 'ontogeny recapitulates phylogeny') was revived in 1994, when Père Alberch reinterpreted the theory from the standpoint of molecular genetics and interpreted it as the heterochrony of the vertebrate genome. However, this theory had been disregarded from 1900 to 1994, as the primordial revolution of vertebrate evolution could not be eluci-

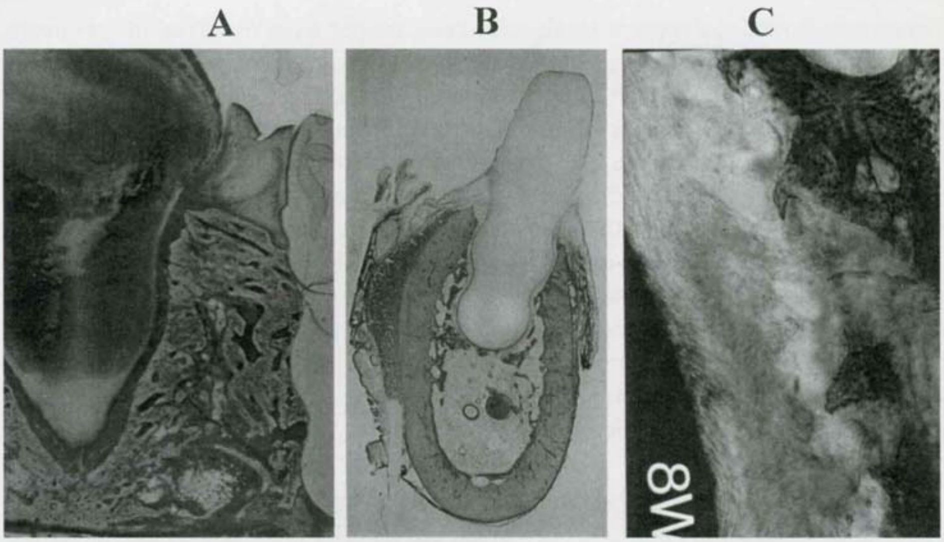


**Figure 3.** (A) Gompholic original tooth (left) and ankylotic artificial root (right). (B) Artificial root implanted in jawbone of an adult dog with occlusal function immediately 2 months after operation. (C) Fibrous tissue attachment can be seen with specimen B. (D) Calcified tissue can be observed at the surface of artificial root of mirror polished specimen, at 11 months post-op. (E) Calcified cementum induced by biomechanical stimuli on the surface of artificial root. (F) X-ray map of cementum analyzed by Kevex 8000. (G) Cementoblasts with fibrous tissue (SEM). (H) Ankylotic tooth of Mexican salamander. (I) Ankylotic artificial root induced by complete rest by shielding occlusal stress with sprint. Ankylotic bone around the root disappears. (J) Ankylotic root with occlusal function induces severe destructive remodeling of alveolar cortex bone without inflammation.

dated using this theory. I regard the primordial revolution as several genomal duplications of primeval urochordates, i.e. archaeo-ascidians. In other words, primeval vertebrates with a metameric structure (somites) appeared following several genome duplications of bryozoans or archaeo-ascidians ascidia (mono-somite animals). In fact, in ontogeny, at the initial stage of development, several somites appear from a mono-metameric structure in gastrula, after which the neulura appears with a multi-metameric structure. After the appearance of primeval vertebrates, morphological transformations occurred as a result of biomechanical stimuli (taken in the broadest sense, i.e. physical and chemical stimuli with the genetic character remaining the same over thousands of generations). However, the genetic codes of vertebrates have also changed quite slowly as the result of mutations in the genomes of gametes after metamorphosis caused by biomechanical forces. The original meaning of the recapitulation theory was that in ontogeny, the metamorphoses of vertebrates, which are observed in phylogeny, are repeated. Haeckel himself paid attention only to metamorphoses of the viscerocranium (*caput*). I found in a series of evolutionary studies, that the following transformations, not only the viscerocranium but seven kinds of phenotypes, are repeated in ontogeny, just as in the major phylogenetic stages as follows: 1) viscerocranial morphology; 2) morphology and function of

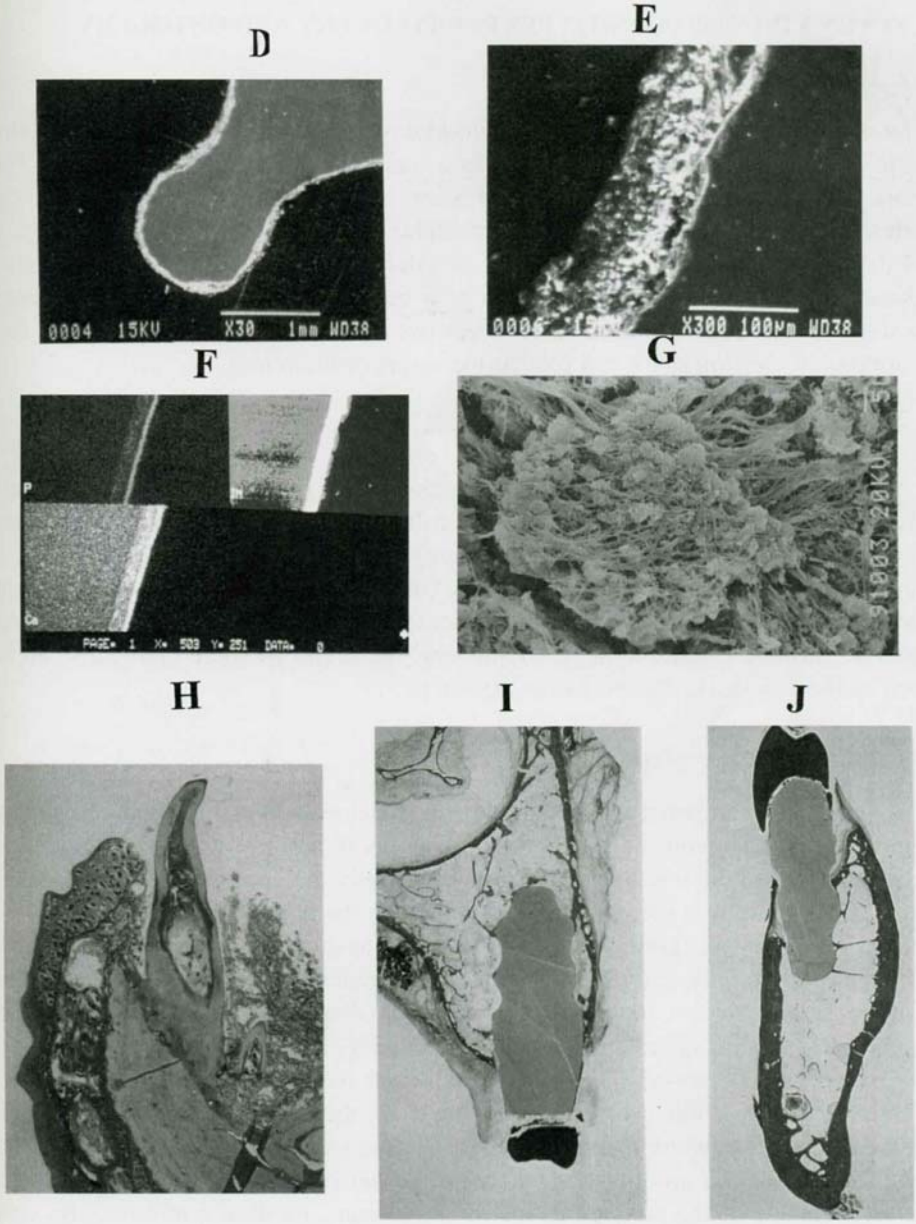


**Figure 3.** (Continued).

the teeth, and development of artificial root; 3) the morphology of the gill and pulmonary respiratory systems; 4) development of the bone marrow hemopoiesis system; 5) the tissue immune system; 6) metabolism of sulfur, phosphate, and nitrogen; and 7) heterochrony of genomic expression.

## 6. SEVEN KINDS OF PHENOTYPES IN PHYLOGENY AND ONTOGENY

### *6.1. Viscerocranial morphology*

I have discovered the dog shark (*Heterodontus japonicus*) to be an animal from which mammals evolved. It devoped into mammals after terrestrialization. The shark *Heterodontus japonicus* can be seen as the origin of mammals before terrestrialization. I have compared the morphological features of human embryos, 34 days after fertilization, with that of an adult dog shark. In this comparison, I found that parts of the viscerocranium of a human embryo correspond precisely to those of an adult *Heterodontus japonicus*. From the point of view of this morphology, the dog shark can seen as the origin of mammals.

### *6.2. The morphology and function of teeth*

*6.2.1.* The *Heterodontus* can be thought of as the origin of the mammal. Mammalian teeth are sited according to their function, and this characteristic feature of the teeth in mammals is called heterodonty. I found that the shark *Heterodontus japonicus* has three kinds of teeth: incisors, canine and plate-like molars. As already mentioned, the morphology of its viscerocranium corresponds to that of a human embryo. From the standpoint of odontology, the origin of some mammals can be seen as the dog shark, *Heterodontus japonicus*.

### *6.3. The morphology of the respiratory system*

The author has compared the developmental transformation of the respiratory system in mammalian embryo (mice and humans) with those of amphibians, reptiles, and birds. After this, it has been discovered that only in mammalian embryos do the sixth gills extend into the pericardial sac, as with the heterodontia chondrichthyes, during the embryonic period in ontogeny as well as during terrestrialization in phylogeny. After that, the caudal bottom of the pericardial sac becomes the diaphragm. In contrast, amphibians, reptiles and birds have no diaphragm. The histological findings of cross- and sagittal sections of the Mexican salamander show that the sixth gill extends to the dorsal site of the chest across the esophagus and forms lungs. Therefore, in the chest near the lungs, the liver, the esophagus and the stomach, or the intestinal omentum of mesenchymal tissue can be seen in the chest cavity. In a sagittal section around the heart with the pericardial sac, the esophagus can be seen outside of the pericardial sac in amphibians, reptiles, and birds. By contrast, in mammals, the lungs are seen in the pericardial sac around the heart, and the esophagus can be seen at the dorsal side of the visceral cavity.

### *6.4. The system of bone marrow hemopoiesis*

As already mentioned above, the author has verified, using bioceramics, that the cause of emigration of hemopoietic sites from the spleen to the bone marrow is gravitational action, to which vertebrates respond by raising their blood pressure with muscle movements.

### *6.5. The tissue immune system*

It is known that the Chondrichthyes, the primitive vertebrates, possess a major histocompatibility antigen complex (MHC) genome. However I have discovered that they have no tissue immunity. This means that they exhibit immuno-tolerance just as the embryo of higher animals such as reptiles, birds, and mammals. I have successfully carried out skin grafts between different kinds of sharks, from *Heterodontus japonicus* (dog-shark) to *Triakis scyllia* (dochi shark). Skin grafts were also carried out successfully from cyclostomates (hagfish) to rats, and the corneas of sharks were successfully transplanted to the eyes of dogs. In addition, the intestine of sharks was successfully transplanted into that of dogs. From these successful xenotransplantations, the major function of MHC (HLA) is found to be a cytological digestion system mainly for tissue remodeling in the organism's own organs, and partly for digesting parasites and transplanted imported tissues.

### *6.6. Metabolism of sulfur, phosphate, and nitrogen*

Metabolism of these elements indicates energy metabolism, i.e. glycolysis and respiration, amino-acid metabolism, and nucleic acid metabolism. These repeat in ontogeny just as in phylogeny.

### *6.7. Heterochrony of expression of the genome*

Alberch proposed that the recapitulation theory could be explained in the embryonic stage by means of the genome expressions, controlled by chrono-biology, i.e. at the time of cell-division following cleavage (blastula, gastrula, neurula, pharyngula, embryo and fetus). After delivery, when the fetus emerges from the amniotic fluid by rupture of the sac, respiration using the lungs starts, and the blood pressure rises as a result of the action of the carotid sinus which derives from the gill system, i.e. the third and fourth bronchial apparatus.

Following that, in the mesenchymal cells of the somatic and visceral systems, the trigger of gene-expressions of adult protein begins to be pulled and the gene-expressions to produce embryonic protein stop as a result of hypertension. Thus, genome expressions are controlled by cell-division chronology from the early stage of fetal development until birth. After birth, genome expressions are triggered by gravitational action, which causes a rise in blood pressure, as well as by chrono-biology.

## 7. CONCLUSION

Through this research the mechanism of vertebrate evolution has been elucidated. Hybrid-type artificial organs using biomaterials have been newly developed by applying biomechanical stimuli, which can induce gene expression in recipient organisms.

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## REFERENCES

1. T. W. Torrey, *Morphogenesis of the Vertebrates*. John Wiley & Sons, New York (1987).
2. E. Nelsen, in: *Comparative Embryology of the Vertebrates*, 1st edn, pp. 733–736. The Blakiston Company Inc., New York (1953).
3. K. Nishihara, Evolution of mechanical supportive organ system and medical application of biomaterials, *J. Jpn. Soc. Biomaterials* **11** (5), 31–40 (1993).
4. K. Nishihara, Phylogeny and biomechanics — Biomechanics concerning vertebrate evolution, *J. S. Mech. Engin.* **940-5**, 84–85 (1994).
5. K. Nishihara, What is the viscerocranium from the standpoint of vertebrate evolution?, *J. Oromax. Biomech.* **1** (1), 73–78 (1995).
6. K. Nishihara, The basic construction of vertebrates, structural defects in the human body and a new concept of the immune system, *J. Oromax. Biomech.* **1** (1), 79–86 (1995).
7. P. Alberch, Heterochrony; Pattern or process?, Biodiversity and evolution, in: *The 10th Inter Symp. on Biology in Conjunction with the Awarding of the International Prize of Biology*, pp. 26–27 (1994).
8. W. Roux, *Gesammelte Abhandlungen der Entwickungsmechanik der Organismus*, Leipzig (1895).
9. M. Delbrük, A physicist looks at biology, *The Transaction of the Connecticut Academy of Arts and Science* **38**, 173–190 (1949).
10. J. Wolff, Ueber die innere Arechitectur der Knochen und ihre Bedeutung für die Frage vom Knochenwachsthum, *Archiv für pathologische Anatomie und Physiologie und für Klinishe Medizin, Virchövs Archiv* **50**, 389–453 (1870).
11. S. Miki, Über die genetische Beziehung der Milz zu den Geäsen des Magens besonders zu seinen sekundäen Venen beim Riesensalamander, *Acta Anat. Nipp.* **38**, 140–155 (1963).
12. A. Hartmann, Die Entwicklung der Milz vom ersten Autreten der Anlage bis zu Differenzierung zum fertigen Organ, *I Amphibien (Pleurodeles) Z mikr. anat. Forsch* **34**, 553–655 (1933).
13. K. Nishihara, T. Tange, H. Tokumaru, *et al.*, Study on developing artificial bone marrow made of sintered hydroxyapatite chamber, *Bioceramics* **5**, 131–138 (1992).
14. K. Nishihara, T. Tange, K. Hirota, *et al.*, Development of hybrid type artificial bone marrow using sintered hydroxyapatite, *Bio-Medical Materials and Engineering* **4** (1), 61–65 (1994).
15. K. Nishihara and J. Tanaka, Successful inducement of hybrid type artificial bone marrow using bioceramics in various vertebrates, *Bioceramics* **9**, 69–72 (1996).

16. K. Nishihara, J. Tanaka and K. Hirota, Study on mechanism of biomechanics-inducing evolution in vertebrates by means of experimental evolutionary research method, *Jpn. J. Oral Diag./Oral Med.* **9** (2), 232–249 (1996).
17. K. Nishihara, T. Tange, R. Matsuda, *et al.*, Successful inducement of artificial bone marrow and the new concept of immune system, *Jpn. J. Oral Diag./Oral Med.* **9** (2), 217–231 (1996).
18. K. Nishihara, T. Tange and K. Hirota, Relation between gravity and cell differentiation in vertebrates — A new concept of immunology, *J. Oromax. Biomech.* **2** (1), 19–22 (1996).
19. K. Hirota, K. Nishihara and H. Tanaka, Pressure sintering of appetite-collagen composite, *Bio-Medical Materials and Engineering* **3** (3), 147–151 (1993).
20. K. Nishihara, Successful inducement of hemopoietic nest in artificial bone marrow chamber, Abstracts of *10th World Congress of the International Society for Artificial Organs in Conjunction with International Faculty for Artificial Organs*, pp. 143 (1995).
21. S. R. Pollack, N. Petrov, R. Salzstein, *et al.*, An anatomical model for streaming potentials in osteons, *J. Biomechanics* **17** (8), 628–637 (1984).