ESTIMATION OF

CONTENT OF

BONE ASH

PROJECT SUBMITTED BY

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12 D

ROLL NO:

Department of Chemistry

Bonafide Certificate

This is to certify that the Chemistry project ___________________________

____ in Chemistry had been

Submitted by the candidate __________________________________

With roll number __________________ for the class XII practical examination of

the Central Board of Secondary Education in the year 2009.

It is further certified that this project is the individual work of the

Candidate.

Signature: Date:

ACKNOWLEDGEMENT:

I thank the staff of the Chemistry department for the help

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Resources of the school for completing the project.

Name of the Candidate:

Roll Number:

Date:

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Abstract

This project introduces some knowledge about the basics involved in finding the constituents of bone. This Project deals with the principle of qualitative analysis of cation and anion.

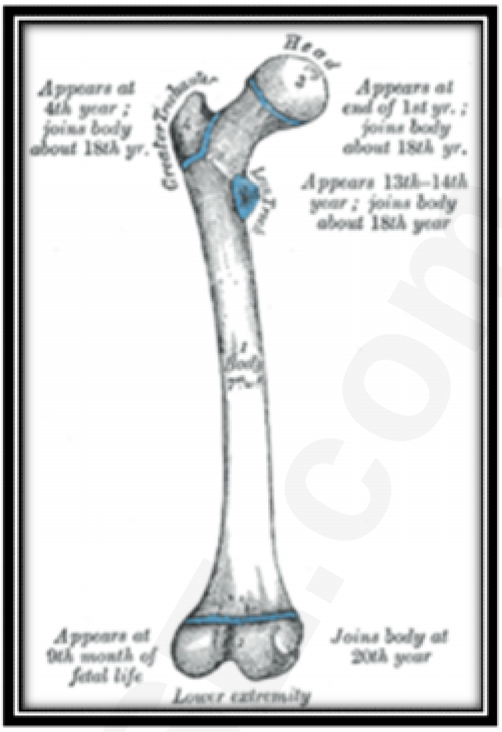
Skeletal system plays an integral part of most of the animals **what is that it makes it form an integral part?** The solution of this can be understood more clearly from this project.

__Significance of project__:

- **Deals with analysis of bone ash**
- **Provides all basic ideas about concentration of various salts present**
- **Provides information about ion exchange reaction**

This project indeed would be a revolution in the world where there is increasing worry about problems of bone like osteoporosis and in this industrial age amount of calcium content in bone is also reducing; this project would indeed be a very good solution.

Bone



Bones are rigid organs that form part of the endoskeleton of vertebrates. They function to move, support, and protect the various organs of the body, produce red and white blood cells and store minerals. Bone tissue is a type of dense connective tissue. Because bones come in a variety of shapes and have a complex internal and external structure they are lightweight, yet strong and hard, in addition to fulfilling their many other functions. One of the types of tissue that makes up bone is the mineralized osseous tissue, also called bone tissue, that gives it rigidity and a honeycomb-like three-dimensional internal structure. Other types of tissue found in bones include marrow, endosteum and periosteum, nerves, blood vessels and cartilage. There are 206 bones in the adult human body and 270 in an infant

Functions

Bones have eleven main functions:

Mechanical

- Protection — Bones can serve to protect internal organs, such as the skull protecting the brain or the ribs protecting the heart and lungs.
- Shape — Bones provide a frame to keep the body supported.
- Movement — Bones, skeletal muscles, tendons, ligaments and joints function together to generate and transfer forces so that individual body parts or the whole body can be manipulated in three-dimensional space. The interaction between bone and muscle is studied in biomechanics.
- Sound transduction — Bones are important in the mechanical aspect of overshadowed hearing.

Synthetic

- Blood production — The marrow, located within the medullary cavity of long bones and interstices of cancellous bone, produces blood cells in a process called haematopoiesis.

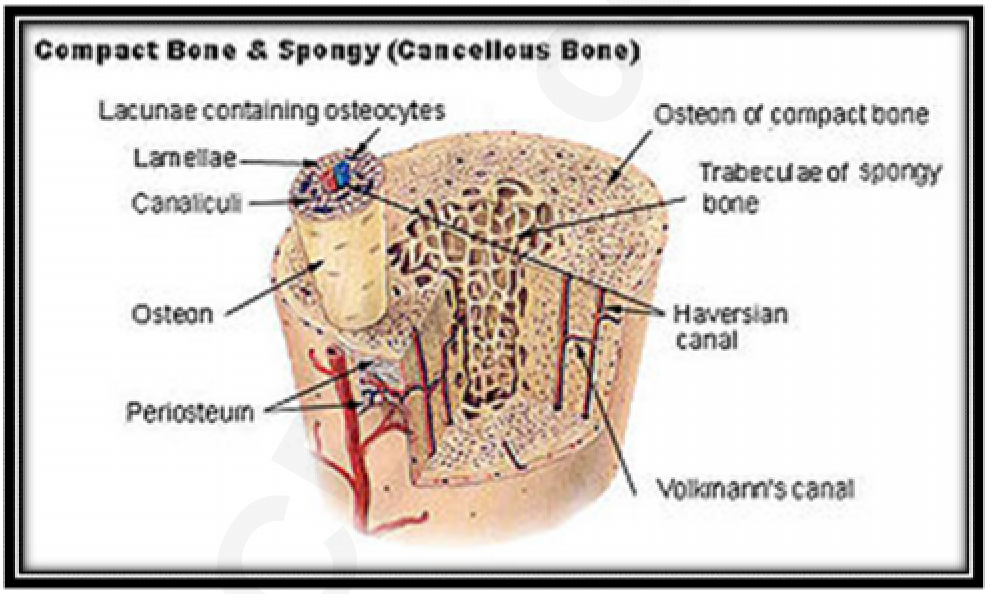
Metabolic

- Mineral storage — Bones act as reserves of minerals important for the body, most notably calcium and phosphorus.
- Growth factor storage — Mineralized bone matrix stores important growth factors such as insulin-like growth factors, transforming growth factor, bone morphogenetic proteins and others.
- Fat Storage — The yellow bone marrow acts as a storage reserve of fatty acids.
- Acid-base balance — Bone buffers the blood against excessive pH changes by absorbing or releasing alkaline salts.
- Detoxification — Bone tissues can also store heavy metals and other foreign elements, removing them from the blood and reducing their effects on other tissues. These can later be gradually released for excretion.
- Endocrine organ - Bone controls phosphate metabolism by releasing fibroblast growth factor - 23 (FGF-23), which acts on kidneys to reduce phosphate re absorption.

Characteristics

The primary tissue of bone, osseous tissue, is a relatively hard and lightweight composite material, formed mostly of calcium phosphate in the chemical arrangement termed calcium hydroxylapatite (this is the osseous tissue that gives bones their rigidity). It has relatively high compressive strength but poor tensile strength of 104-121 MPa, meaning it resists pushing forces well, but not pulling forces. While bone is essentially brittle, it does have a significant degree of elasticity, contributed chiefly by collagen. All bones consist of living and dead cells embedded in the mineralized organic *matrix* that makes up the osseous tissue.

Individual bone structure



Bone is not a uniformly solid material, but rather has some spaces between its hard elements.

Compact bone or (Cortical bone)

The hard outer layer of bones is composed of compact bone tissue, so-called due to its minimal gaps and spaces. This tissue gives bones their smooth, white, and solid appearance, and accounts for 80% of the total bone mass of an adult skeleton. Compact bone may also be referred to as dense bone.

Trabecular bone

Filling the interior of the bone is the trabecular bone tissue (an open cell porousnetwork also called cancellous or spongy bone), which is composed of a network of rod- and plate-like elements that make the overall organ lighter and allowing room for blood vessels and marrow. Trabecular bone accounts for the remaining 20% of total bone mass but has nearly ten times the surface area of compact bone. If for any reason there is an alteration in the strain to which the cancellous subjected there is a rearrangement of the trabeculae. Although adult bone exists in both cancellous and compact forms, there is no microscopic difference between the two.

Cellular structure

There are several types of cells constituting the bone;

- Osteoblasts are mononucleate bone-forming cells that descend from osteoprogenitor cells. They are located on the surface of osteoid seams and make a protein mixture known as osteoid, which mineralizes to become bone. The osteiod seam is a narrow region of newly formed organic matrix, not yet mineralized, located on the surface of a bone. Osteoid is primarily composed of Type I collagen. Osteoblasts also manufacture hormones, such as prostaglandins, to act on the bone itself. They robustly produce alkaline phosphatase, anenzyme that has a role in the mineralisation of bone, as well as many matrix proteins. Osteoblasts are the immature bone cells.

- Bone lining cells are essentially inactive osteoblasts. They cover all of the available bone surface and function as a barrier for certain ions.
- Osteocytes originate from osteoblasts that have migrated into and become trapped and surrounded by bone matrix that they themselves produce. The spaces they occupy are known as lacunae. Osteocytes have many processes that reach out to meet osteoblasts and other osteocytes probably for the purposes of communication. Their functions include to varying degrees: formation of bone, matrix maintenance and calcium homeostasis. They have also been shown to act as mechano-sensory receptors — regulating the bone's response to stress and mechanical load. They are mature bone cells.

- Osteoclasts are the cells responsible for bone resorption (remodeling of bone to reduce its volume). Osteoclasts are large, multinucleated cells located on bone surfaces in what are called Howship's lacunae or resorption pits. These lacunae, or resorption pits, are left behind after the breakdown of the bone surface. Because the osteoclasts are derived from a monocyte stem-cell lineage, they are equipped withphagocytic-like mechanisms similar to circulating macrophages. Osteoclasts mature and/or migrate to discrete bone surfaces. Upon arrival, active enzymes, such as tartrate resistant acid phosphatase, are secreted against the mineral substrate.

Molecular structure

Matrix

The majority of bone is made of the bone matrix. It has inorganic and organic parts. Bone is formed by the hardening of this matrix entrapping the cells. When these cells become entrapped from osteoblasts they become osteocytes.

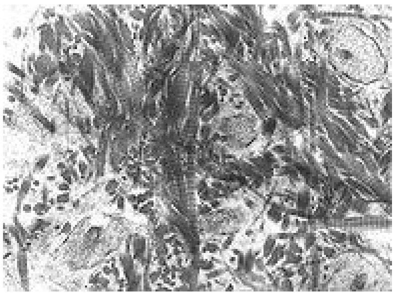
Inorganic

The inorganic is mainly crystalline mineral salts and calcium, which is present in the form of hydroxyapatite. The matrix is initially laid down as unmineralised osteoid (manufactured by osteoblasts). Mineralisation involves osteoblasts secreting vesicles containing alkaline phosphatase. This cleaves the phosphate groups and acts as the foci for calcium and phosphate deposition. The vesicles then rupture and act as a centre for crystals to grow on.

Organic

The organic part of matrix is mainly composed of Type I collagen. This is synthesised intracellularly as tropocollagen and then exported, forming fibrils. The organic part is also composed of various growth factors, the functions of which are not fully known. Factors present includeglycosaminoglycans, osteocalcin, osteonectin, bone sialo protein, osteopontin and Cell Attachment Factor. One of the main things that distinguishes the matrix of a bone from that of another cell is that the matrix in bone is hard.

Woven or lamellar



Two types of bone can be identified microscopically according to the pattern of collagen forming the osteoid (collagenous support tissue of type I collagen embedded in glycosaminoglycan gel

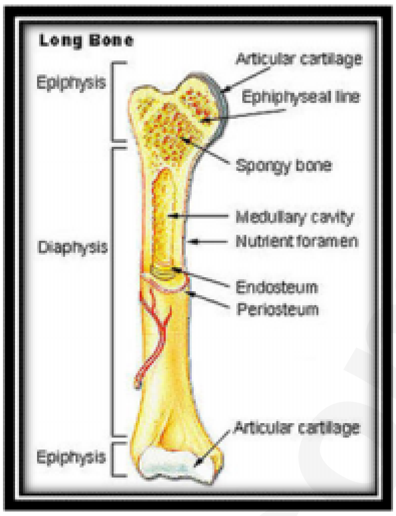
1) woven bone characterised by haphazard organisation of collagen fibers and is mechanically weak, and

2) lamellar bone which has a regular parallel alignment of collagen into sheets (lamellae) and is mechanically strong.

Woven bone is produced when osteoblasts produce osteoid rapidly which occurs initially in all fetalbones (but is later replaced by more resilient lamellar bone). In adults woven bone is created afterfractures or in Paget's disease. Woven bone is weaker, with a smaller number of randomly oriented collagen fibers, but forms quickly; it is for this appearance of the fibrous matrix that the bone is termed*woven*. It is soon replaced by lamellar bone, which is highly organized in concentric sheets with a much lower proportion of osteocytes to surrounding tissue. Lamellar bone, which makes its first appearance in the fetus during the third trimester,[3] is stronger and filled with many collagen fibers parallel to other fibers in the same layer (these parallel columns are called osteons). In cross-section, the fibers run in opposite directions in alternating layers, much like in plywood, assisting in the bone's ability to resist torsion forces. After a fracture, woven bone forms initially and is gradually replaced by lamellar bone during a process known as "bony substitution."

These terms are histologic, in that a microscope is necessary to differentiate between the two.

Types



There are five types of bones in the human body: long, short, flat, irregular and sesamoid.

- Long bones are characterized by a shaft, the diaphysis, that is much greater in length than width. They are comprised mostly of compact bone and lesser amounts of marrow, which is located within the medullary cavity, and spongy bone. Most bones of the limbs, including those of the fingers and toes, are long bones. The exceptions are those of the wrist, ankleand kneecap.
- Short bones are roughly cube-shaped, and have only a thin layer of compact bone surrounding a spongy interior. The bones of the wrist and ankle are short bones, as are thesesamoid bones.
- Flat bones are thin and generally curved, with two parallel layers of compact bones sandwiching a layer of spongy bone. Most of the bones of the skull are flat bones, as is thesternum.
- Irregular bones do not fit into the above categories. They consist of thin layers of compact bone surrounding a spongy interior. As implied by the name, their shapes are irregular and complicated. The bones of the spine and hips are irregular bones.
- Sesamoid bones are bones embedded in tendons. Since they act to hold the tendon further away from the joint, the angle of the tendon is increased and thus the leverage of the muscle is increased. Examples of sesamoid bones are the patella and the pisiform.Compared to woven bone , lamellar bone formation takes place more slowly. The orderly deposition of collagen fibers restricts the formation of osteoid to about 1 to 2 μm per day.

Lamellar bone requires a relatively flat surface to lay the collagen fibers in parallel or concentric layers.

Formation

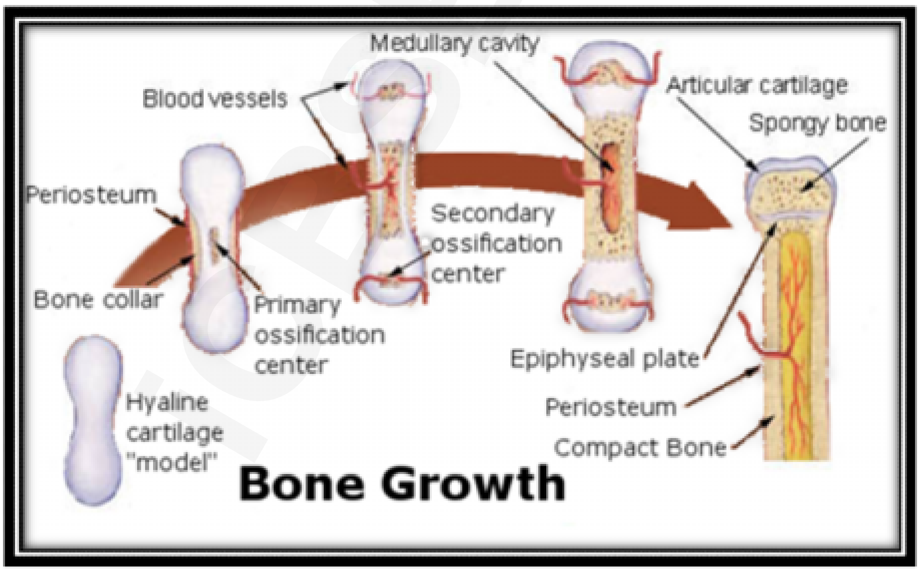
The formation of bone during the fetal stage of development occurs by two processes: Intramembranous ossification and endochondral ossification.

Intramembranous ossification

Intramembranous ossification mainly occurs during formation of the flat bones of the skull; the bone is formed from mesenchyme tissue. The steps in intramembranous ossification are:

1. Development of ossification center
2. Calcification
3. Formation of trabeculae
4. Development of periosteum

Endochondral ossification



Endochondral ossification, on the other hand, occurs in long bones, such as limbs; the bone is formed from cartilage. The steps in endochondral ossification are:

1. Development of cartilage model
2. Growth of cartilage model
3. Development of the primary ossification center
4. Development of the secondary ossification center
5. Formation of articular cartilage and epiphyseal plate

Endochondral ossification begins with points in the cartilage called "primary ossification centers." They mostly appear during fetal development, though a few short bones begin their primary ossification after birth. They are responsible for the formation of the diaphyses of long bones, short bones and certain parts of irregular bones. Secondary ossification occurs after birth, and forms the epiphyses of long bones and the extremities of irregular and flat bones. The diaphysis and both epiphyses of a long bone are separated by a growing zone of cartilage (the epiphyseal plate). When the child reaches skeletal maturity (18 to 25 years of age), all of the cartilage is replaced by bone, fusing the diaphysis and both epiphyses together (epiphyseal closure).

Bone marrow

Bone marrow can be found in almost any bone that holds cancellous tissue. In newborns, all such bones are filled exclusively with red marrow, but as the child ages it is mostly replaced by yellow, or *fatty* marrow. In adults, red marrow is mostly found in the marrow bones of the femur, the ribs, the vertebrae and pelvic bones.

Remodeling

Remodeling or *bone turnover* is the process of resorption followed by replacement of bone with little change in shape and occurs throughout a person's life. Osteoblasts and osteoclasts, coupled together via paracrine cell signalling, are referred to as bone remodeling units.

Purpose

The purpose of remodeling is to regulate calcium homeostasis, repair micro-damaged bones (from everyday stress) but also to shape and sculpture the skeleton during growth.

Calcium balance

The process of bone resorption by the osteoclasts releases stored calcium into the systemic circulation and is an important process in regulating calcium balance. As bone formation actively *fixes* circulating calcium in its mineral form, removing it from the bloodstream, resorption actively *unfixes* it thereby increasing circulating calcium levels. These processes occur in tandem at site-specific locations.

Repair

Repeated stress, such as weight-bearing exercise or bone healing, results in the bone thickening at the points of maximum stress (Wolff's law). It has been hypothesized that this is a result of bone's piezoelectric properties, which cause bone to generate small electrical potentials under stress.[4]

Paracrine cell signalling

The action of osteoblasts and osteoclasts are controlled by a number of chemical factors which either promote or inhibit the activity of the bone remodelling cells, controlling the rate at which bone is made, destroyed or changed in shape. The cells also use paracrine signalling to control the activity of each other.

Osteoblast stimulation

Osteoblasts can be stimulated to increase bone mass through increased secretion of osteoid and by inhibiting the ability of osteoclasts to break down osseous tissue.

Bone building through increased secretion of osteoid is stimulated by the secretion of growth hormone by the pituitary, thyroid hormone and the sex hormones (estrogens and androgens). These hormones also promote increased secretion of osteoprotegerin.[5] Osteoblasts can also be induced to secrete a number of cytokines that promote reabsorbtion of bone by stimulating osteoclast activity and differentiation from progenitor cells. Vitamin D, parathyroid hormone and stimulation from osteocytes induce osteoblasts to increase secretion of RANK-ligand and interleukin 6, which cytokines then stimulate increased reabsorbtion of bone by osteoclasts. These same compounds also increase secretion ofmacrophage colony-stimulating factor by osteoblasts, which promotes the differentiation of progenitor cells into osteoclasts, and decrease secretion of osteoprotegerin.

Osteoclast inhibition

The rate at which osteoclasts resorb bone is inhibited by calcitonin and osteoprotegerin. Calcitonin is produced by parafollicular cells in thethyroid gland, and can bind to receptors on osteoclasts to directly inhibit osteoclast activity. Osteoprotegerin is secreted by osteoblasts and is able to bind RANK-L, inhibiting osteoclast stimulation.

Experimental Analysis

Materials Required

PARTICULARS	QUANTITY
Rib Bone	2 Pieces
Beaker	150 ml
Test Tube	7 nos
Evaporating Dish	1 no
Ring Stand	1 no
Bunsen Burner	1 no
Test Tube Holder	2 nos
Filter Paper	-
PH Paper	-
Dil. Nitric Acid	200 ml
1% Ammonium Hydroxide	100 ml
1% Silver Nitrate	25 ml
1% Ammonium Chloride	50 ml
Acetic Acid	100 ml
1% Potassium Thiocyanate	25 ml
Distilled Water	As Reqd

Report of Project

EXPERIMENT	**OBSERVATION**
• A strip of bone was burnt in evaporating dish	***Yellowish white precipitate was obtained***
• 2 gms of bone as was weighed • To it dilute nitric acid was added • It was diluted with water and the ash was completely dissolved • The above solution was filtered and the residue (left on the filter paper) was discarded • Ammonium hydroxide was added to the filtrate (left on the beaker) • The solution was made basic. The basicity was checked with the help of pH paper • The solution was filtered and the residue was isolated	***On adding Nitric acid the ash sparingly dissolved*** ***The pH was made to <u>8.6</u>*** ***Whitish brown precipitate of <u>Magnesium ammonium phosphate</u> was obtained***
• The filtrate was separated into two test tubes • Silver nitrate was added to one of the test tubes • To the other test tube ammonium chloride and ammonium carbonate was added simultaneously and boiled	***White precipitate of <u>Silver chloride</u> was obtained*** ***White residue of calcium*** ***Carbonate was obtained***
• To the solution left, dilute HCL was added followed by Potassium thiocyanate	***Red colour solution marking the presence of <u>Iron</u> was obtained***

Result

Extrapolation from the above observations

Constituents of bone ash identified were:

I. **Calcium**
II. **Phosphate**
III. **Chloride**
IV. **Magnesium**
V. **Iron**

Apart from this Calcium and Phosphate which is found maximum in bone was estimated from the precipitate got. This was done by weighing the precipitate

• Weight of Calcium carbonate:-	1.7 g
• Weight of Mg (NH_4) PO_4 :-	1.1 g
• **Weight of Ca in 2g of sample:-**	**0.68 g**
• **Weight of Phosphorous:-**	**0.24 g**
• **% of Ca:-**	**34%**
• **% of Phosphorous:-**	**12%**

Disorders

There are many disorders of the skeleton. One of the more prominent is osteoporosis.

Osteoporosis

Osteoporosis is a disease of bone, leading to an increased risk of fracture. In osteoporosis, the bone mineral density (BMD) is reduced, bone microarchitecture is disrupted, and the amount and variety of non-collagenous proteins in bone is altered. Osteoporosis is defined by the World Health Organization (WHO) in women as a bone mineral density 2.5 standard deviations below peak bone mass (20-year-old sex-matched healthy person average) as measured by DXA; the term "established osteoporosis" includes the presence of a fragility fracture.[6] Osteoporosis is most common in women after the menopause, when it is called **postmenopausal osteoporosis**, but may develop in men and premenopausal women in the presence of particular hormonal disorders and other chronic diseases or as a result of smoking and medications, specifically glucocorticoids, when the disease is called **steroid-** or **glucocorticoid-induced osteoporosis** (SIOP or GIOP).

Osteoporosis can be prevented with lifestyle advice and medication, and preventing falls in people with known or suspected osteoporosis is an established way to prevent fractures. Osteoporosis can be treated with bisphosphonates and various other medical treatments.

Other

Other disorders of bone include:

- Bone fracture
- Osteomyelitis
- Osteosarcoma
- Osteogenesis imperfecta
- Osteochondritis Dissecans
- Bone Metastases
- Neurofibromatosis type I

Osteology

The study of bones and teeth is referred to as osteology. It is frequently used in anthropology, archeology and forensic science for a variety of tasks. This can include determining the nutritional, health, age or injury status of the individual the bones were taken from. Preparing fleshed bones for these types of studies can involve maceration - boiling fleshed bones to remove large particles, then hand-cleaning.

Typically anthropologists and archeologists study bone tools made by *Homo sapiens* and *Homo neanderthalensis*. Bones can serve a number of uses such as projectile points or artistic pigments, and can be made from endoskeletal or external bones such as antler or tusk.

Alternatives to bony endoskeletons

There are several evolutionary alternatives to mammillary bone; though they have some similar functions, they are not completely functionally analogous to bone.

- Exoskeletons offer support, protection and levers for movement similar to endoskeletal bone. Different types of exoskeletons include shells,carapaces (consisting of calcium compounds or silica) and chitinous exoskeletons.
- A true endoskeleton (that is, protective tissue derived from mesoderm) is also present in Echinoderms. Porifera (sponges) possess simple endoskeletons that consist of calcareous or siliceous spicules and a spongin fiber network.

Exposed bone

Bone penetrating the skin and being exposed to the outside can be both a natural process in some animals, and due to injury:

- A deer's antlers are composed of bone.
- Instead of teeth, the extinct predatory fish *Dunkleosteus* had sharp edges of hard exposed bone along its jaws.
- A compound fracture occurs when the edges of a broken bone puncture the skin.
- Though not strictly speaking exposed, a bird's beak is primarily bone covered in a layer of keratin over a vascular layer containing blood vessels and nerve endings.

Terminology

Several terms are used to refer to features and components of bones throughout the body:

Bone feature	Definition
articular process	A projection that contacts an adjacent bone.
articulation	The region where adjacent bones contact each other — a joint.
canal	A long, tunnel-like foramen, usually a passage for notable nerves or blood vessels.
condyle	A large, rounded articular process.
crest	A prominent ridge.
eminence	A relatively small projection or bump.
epicondyle	A projection near to a condyle but not part of the joint.
facet	A small, flattened articular surface.
foramen	An opening through a bone.
fossa	A broad, shallow depressed area.
fovea	A small pit on the head of a bone.

Labyrinth	A cavity within a bone.
line	A long, thin projection, often with a rough surface. Also known as a *ridge*.
malleolus	One of two specific protuberances of bones in the ankle.
meatus	A short canal.
process	A relatively large projection or prominent bump.(gen.)
ramus	An arm-like branch off the body of a bone.
sinus	A cavity within a cranial bone.
spine	A relatively long, thin projection or bump.
suture	Articulation between cranial bones.
trochanter	One of two specific tuberosities located on the femur.
tubercle	A projection or bump with a roughened surface, generally smaller than a tuberosity.
tuberosity	A projection or bump with a roughened surface.

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