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A Contribution to the Bone-regeneration: with Observations upon the Transplantation of Periosteum and Bone-marrow.

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I. Introduction.

Our knowledge of bone regeneration began about the eighteenth century and since that time there have been many reports about the experimental and clinical sides of the subject. Bone transplantation is now becoming an important branch of surgery, however, no definite conclusions as to the real origin of the bone regeneration have been reached. Generally, in the primary ossification in the embryo is divided into two parts:

1. Intramembranous, direct, or metaplastic ossification,—this development comes from the embryonal connective tissue,—the mesenchym.

2. Intracartilaginous, indirect, or neoplastic ossification,—hyaline cartilage, which is also derived from the embryonal connective tissue.

In both types, the actual processes of ossification are essentially the same and the bone is completed by the cooperation of the periosteum. It is hitherto believed that the bone regeneration is effected by osteoblasts which give rise to new bone. These osteoblasts in children and adolescents may apparently be derived either from the marrow or from the periosteum, but in adults cheifly, if not wholly, from the periosteum. This periosteum is a connective tissue membrane covering the external surface of all the bone except the cartilage in the joint and it consists of two layers: an outer layer of dense connective tissue, rich in blood vessels and containing also lymphatic vessels and nerves, and an inner layer which poor in blood vessels but has an abundance of elastic fibers. The cells of the inner layer of the periosteum are spindle shaped or flattened connective tissue cells, together with the more cuboidal osteoblasts which rest against the bone. In young bone there are so numerous as to form a third layer of the periosteum. In adults they are few in number, but are capable of proliferation, and together with those in the endosteum, they are the source of new bone after destruction of its osseous tissue.

It is quite difficult to distinguish microscopically on section, the boundary line of the periosteum and the real bone, also the outer layer of the periosteum blends with the surrounding looser connective tissue and in places with fasciae and tendons. Whether the periosteum as well as the endosteum is the main agent in bone regeneration or not, is a matter of discussion. I briefly quote some literature as to the subject:—

II. Literature.

Duhamel[1] 1739. He placed a silver ring under the periosteum of a long bone of young animal and after a while, he found that it had become covered by the bone and so he believed that the bone was derived from periosteum.

Virchow[2] 1858. He claimed that the bone cell was derivered from the

periosteum by the process of metaplasia.

Gegenbauer[3] gave the term "Osteoblast" and he agreed with the above view.

Ollier[4] 1867. His theory is that all transplanted bone with the periosteum, retains its inherent activity and the bone denuded its periosteum, undergoes necrosis and is completely absorbed.

Moschcowitz[5] 1895. He pointed out, based upon the study of calcification and ossification of the ovary that osteoblast is simply an endothelial cell from the lining of the blood vessel. He also said that ossification does not occur without preliminary calcification and calcification occurs only in dead tissue.

Macewen[6] 1912. He said that the periosteum is of great use in limiting, within specific boundaries, the distribution of the osteoblasts, and preventing them during their evolutionary period from being scattered into the soft tissue, where their presence would be prejudicial to the function of these parts. In the loose areolar tissue existing between the periosteum and the bone, the osteoblasts find nutrient for their growth and also space to generate, free from undue pressure. While not under-estimating the periosteum is a limiting and protection membrane, of great use in physiological and pathological conditions, there are no data to indicate that it can, of itself, secrete or reproduce bone. He claimed that in bone growth, diaphyseal osteoblasts are generated from the nuclei of the diaphyseal cartilage cells. This diaphyseal cartilage is but a phase in the evolution of the bone cells. When the nuclei of the cartilage cells proliferate, the cartilaginous envelopes become less distinct and finally disappear, the space being occupied by osteoblasts. These osteoblasts, once formed, have the power of direct and vigorous proliferation, showing great vegetative capacity; are capable of dissemination, of growing in the midst of the soft tissue, or of being carried by the blood stream and deposited in the blood clot, where they proliferate after the matrix has been supplied with new formed blood vessels. As long as the bone cell remains embryonic, it exhibits the power of proliferation; but when it reaches maturity it assumes the fixed tissue type and becomes stationary. This period is coincident with calcareous depostion and with it the cessation of active regeneration,

though its proliferating potentiality still remains. The diaphyseal bone is reproduced by the proliferation of osteoblasts derived from pre-existing osseous tissue and its regeneration takes place independently of the periosteum.

Gedden[7] 1912. He agreed that the periosteum takes no part in the work of bone formation. He, however, opposed that the osteoblasts was derived from the cartilage cell, that cartilage was always present potentially in bone even when mature and that when repair took place the development of cartilage preceeded the formation of bone. He believed that the osteoblasts are ectodermal in origin. He gave two definite reasons for his opinion.

1. If cartilage, in which ossification is just commencing, is sectioned and stained with Sabin's modification of Mallory's stain, the osteoblasts which lay down the peripheral rind of bone, the cells which erupt, and the osteoblasts forming the endochondral bone all stained to the same bright red tint, which differs markedly from the blue-red tint of the cartilage cells. This makes it extremely improbable that the endochondral osteoblasts are different things from the subperiosteal, and it also makes it extremely improbable that they have any direct genetic relation with the cartilage cells; for it is rule, that cells of a common lineage have, for a time similar staining reaction.
2. If the tissue examined is serially sectioned and no sections are missed, it is always possible to trace a continuous chain of osteoblasts and definite cell paths from one or the numerous points of irruption to the most advanced osteoblasts in the cartilage.

Paterson[8] 1912. He upheld the fact that the cause of ossification appeared to be essentially irritation. He thought that the agent was an embryonic connective tissue cell and that the process was a chemical one which occurred in situ, and that the ossification was extravascular.

Wm Wright[9] 1912. He pointed out that there must be other ways of forming bone than from cartilage, as evidenced in the formation of the membrane bones of the cranium, and he doubted whether it was possible to remove the periosteum from a healthy bone without fundamentally affecting the periosteum; that its future behaviors could scarecely be regard-

ed as the normal behavior of intact periosteum. He further believed that the evidence of comperative anatomy was in support of Gedden's contention that bone was ectodermal in origin.

Douglas-Crowford[10] 1912. They accepted Macewen's view of formation and growth of bone.

Mayer and Wehner[11] 1914. They opposed the Macewen's opinion and both agreed on the point that periosteum transplanted in the muscle of a young dog and periosteum after subperiosteal bone resection on the rib of a young rabbit produce new bone. There was, however, no bone formation in the "capsule test" by covering the bone with steel or glass, where the periosteum was removed. They also observed that in the bone transplantation, the bone cell does not show its activity, but the periosteum, as well as the endosteum has the function of bone formation.

Gallie and Robertson[12] 1914. They viewed that the periosteum is not osteogenetic, and the presence or abscence of the periosteum in autogeneous bone transplants has no decided influence on the activity of the subperiosteal osteoblasts, however, it is of great importance, because of its control of the circulation throughout living bone. When a piece of bone has been cut free from its circulation and transplanted into other part in the same animal, if its surface is freely bathed in lymph, those cells which are present on the surface and in the open mouths of the Haversian's canals will live and undergo rapid proliferation. The cells in the lacunae or those deep in the Haversian's canal die, and in the course of two or three weeks disappear. The proliferating cells on the surfaces, whether derived from the graft or from the neighboring living bone immediately commence the absorption of the graft, and do not cease their activity until every vestige of the old bone has disappeared. Bone transplanted from one animal to another of the same species may act similarly to autogeneous transplants. It probably always does so in dogs and cats, but in man there is reasonable doubt as to whether the cells from all bones will live in the fluid of all recipient.

Minoura[13] 1914. He transplanted the metatarsophalangeal joints, either intact or split longitudinally, of two months old rabbits, into subcutaneous tissue of the back, into the liver, or in abdominal cavity of the same or

different animals. The joint cartilage remained intact for a considerable time and always showed a cartilage cell regeneration on the periphery. The marrow degenerated and was substituted by fibrous tissue and later by fat. The trabeculae of the epiphysis degenerated and later was substituted by new formed bone, which however was not permanent. The epiphyseal cartilage line underwent necrosis and absorption, only the parts of the proliferating zone near the periphery showed multiplication of the cartilage cells. The periosteum always showed new bone formation in the inner layers, but the new formed tissue finally underwent absorption.

Nakahara[14] 1904. He injected the periosteum of rabbit, which was smashed to pieces, in the subcutaneous—and muscle—tissue and he observed from the transplanted periosteum, the newly formed bone, cartilage and also a transitinal type from osteoid to real bone tissue.

Goto[15] 1914, and Tongu[16] 1915. They also proved that the transplantation of an emulsion of periosteum can form new bone.

Yokoi[17] 1912. He described the like result as above one. He added that when it is injected an emulsion of periosteum with fresh blood, he could not prove any special increased bone formation, but when one per cent solution of fibrin is substituted for blood there is apparently increased proliferation on new bone.

Tsunoda[18] 1912. He observed that an autotransplanted periosteum in the muscle tissue of a young rabbit has an activity to grow new bone. He succeeded the above experiments in six from 44 cases (15%) within two weeks after transplantation, however, failed of the transplantation of the so-called periosteal osteogenetic cells, which were romoved by scrubbing the inner surface of the periosteum.

Goto[15] 1914. He said that the transplantation of an emulsion of so-called subperiosteal osteogenetic layer in normal saline solution failed to grow new bone, on the contrary, the direct transplantation of the above one without the saline solution, following Pochhammer, can grow new bone.

Murphy[19] 1912. He concluded that the periosteum fully detached from bone and (1) transplanted into a fatty or muscle tissue bed in the same individual, if he be young, may produce a lasting bone deposit; (2) transplanted into another individual or animal of the same species under the

same condition, it rarely produce a permanent bone deposit; (3) transplanted into another species it never produce a permanent bone deposit. Bone with its periosteum transplanted into muscle, fat, etc., in the same individual, and free from bony contact, practically always dies and is absorbed, except in the case of very young children or infants. Transplanted into another species it is always absorbed. Bone transplanted without the periosteum into the muscle or cellular tissue always dies and ultimately is absorbed. Bone with or without periosteum transplanted in the same individual and coming in contact with other living osteogenetic bone at one end or both ends of the transplanted fragment always becomes united to the living fragment and acts as a scaffolding for the reproduction of new bone of the same size and same shape as the transplanted fragment, if asepsis is obtained.

Albee[20] 1913. He considered that bone from which the periosteum had been removed proved equally satisfactory to bone grafts on which the periosteum had been retained and also it seemed that it is largely a question of definition of what the periosteum is and what it includes as to whether it is to be actively osteogenetic or not. If by chance the cleavage is deep as when the periosteum is removed with a sharp elevater and the bone scrapped, the periosteum is very sure to be actively osteogenetic. On the other hand, if the periosteum is stripped off or remooved with a blunt instrument, the cleavage is not likely to be deep enough to include the osteogenetic layer of cells on the periphry of the compact bone. In that instance the periosteum would constitute a connective limiting membrane only and slight or no osteogenesis would occur. It is believed that the periosteum and the marrow substance on the bone graft, play an important rôle in aiding to establish an early and more abundant blood supply from recipient bone to the transplant.

Graves[21] 1914. His theory is that the periosteum is the product, and not the mother of bone. All the osteogenetic properties of the periosteum, whether in the repair of fracture or in grafting, are due to the more or less, accidental presence of the outer layer of bone cells adherent to its deeper surface. Living bone is the chief source and origin of callus, which grows mainly from its outer or periosteal surfaces, and to a less extent from its

deep or medullary surfaces and its cut ends.

Fujimori[22] 1915. He viewed from his experiment that the fate of the periosteum, whether it is auto-, homo-, or hetero-transplant, or it is either old or young, when it is transplanted in the subcutaneous or muscle tissue and quite free separated from the bone system, always undergoes to the regressive degeneration. From his 40 cases of experiments he could find none of bone formation but a case of cartilaginous tissue.

Tomita[23] 1908. He noticed that the bone cell has no activity in bone formation, but it grows always from the periosteum and marrow cells. He also proved that a piece of bone transplanted into the abdominal cavity kept its vitality for 95 days.

Ryerson[24] 1913. He illustrated by X-ray examination that the bone regeneration appeared in two cases in which the fracture occured in infancy, and the X-ray showed a shadow a quarter of an inch wide beneath the stripped periosteum of the femur and extending well into the osteo-periosteal angle. This fact is explained because at this age a rapid growth of bone occurs from the large number of osteoblasts which can be demonstrated histologically beneath the periosteum. If periosteum were osteogenetic, then new bone would be produces beneath it over the area where it is stripped from the bone and the osteo-periosteal angle would be one of the first parts to be filled. That this is not the case is manifested by the plates which show that the osteo-periosteal angle is actually the last part of the space to be filled with a shadow, indicating new bone formation. From this, therefore, the natural inference is that the periosteum is not osteogenetic in character.

Bancroft[25] 1914. From his experiments, two types of repair of bone have been observed.

1. Where the periosteum has been well stripped off the bone for a distance of several centimeters it is separated from it by serosanguinous fluid, here the bone has apparently been repaired from the medullary cavity by a framework of connective tissue upon which calcium salts have been deposited, forming osteoid tissue; there has also been a reparative process from the connective tissue of the Haversian's canals at the sides of the bone incision.

2. Where the bone has repaired under a definite fibrous connective tissue capsule, it is continuous with periosteum and also adherent to the surrounding muscles. This connective tissue is apparently not different from other connective tissue, except that in immediate approximation to the newly forming bone, there is a single layer of cells which are larger in shape and have nuclei that take a heavy stain. In places there is a metaplasia from the connective tissue cells to these osteogenetic cells. The periosteum acts as a limiting membrane to the bone, tends to conserve its shape and to furnish its blood supply.

Davison and Smith[26] 1916. They summarized that the periosteum has no osteogenetic properties. Beneath the periosteum is found very cellular structure composes of many small cells and is a part of bone propers and not of the overlying fibrous tissue capsules. This cellular layer is called the "periosseous osteogetic layer" since by virtue of its cellular constituents it can, when stimulated to proliferation, produce bone. This osteogenetic layer is present before the surrounding mesenchym undergoes a condensation into a staut membrane.....the periosteum..... . Regeneration of bone from transplanted periosteum cannot be accomplished without the presence of some of the cellular components of the periosseous osteogenetic layer. They also noticed that osteogenesis is dependent upon the maturation of connective tissue into bone cells, capable of differentiation into osseous tissue, are scattered throughout the entire structure of the bone, i. e., in the periosseous osteogenetic layers, especially surrounding the bone and to some extent in the layers about the medullary canal. The most that can be expected in the transplantation of an osseous graft, large enough for practical purposes, is the continued developement of the implanted osteoblasts, together with such stimnlus as may be obtained from the osteoblasts of the host and the retention of vitality in some of the implanted bone cells with their corresponding intercellular calcium matrix.

Ely[27] 1919. He described that neither periosteum nor marrow is necessary for bone formation, and that neither of them "formes bone," in the proper meaning of the word. He also said that the fibrous tissue of the periosteum is the same as other fibrous tissue if there is osteoblasts under it, they will form bone, as well as any other osteoblasts. The osteoblast

is the bone building cell, if the present theory of bone formation, the neoplastic theory, is correct. The function of bone needs three important conditions as follows:

1. Blood vessels.
2. Either a loose meshed fibrous tissue, or a homogeneous (cartilage matrix) or a granulus or a necrotic material.
3. A stimulus, physiologically or pathologically as the case may be.

Hyde[28] 1920. He classified his conclusion as follows:—1, that periosteum, as such has no independent osteogenetic power, 2, that the power of regeneration resides chiefly in the outer layer of the cortical bone.

Bonome[29] 1885. He viewed that the bone cells in the transplant do not loss their vitality but multiply.

Bart[30] 1921. He stated that bone cells are not senile, functionless osteoblasts but observations indicate that they are active cells which are in some way related to bone production.

III. Methods and Materials.

First of all, it was investigated that whether the periosteum has the activity to proliferate new bone or not. It is as above mentioned, very difficult to determine with certainty the boundary of the periosteum and the bone. In the embryo, the osteogenetic structure is present before the periosteum is formed and so they must not be considered as the same. To remove the periosteum, a sharp chisel was used and made a rectangle cut on the femur. By pinching, pulling the cut-edge with forceps, and scrubbing lightly the surface of the bone with a sharp knife, a delicate flap of the periosteum is stripped off. Even great delicacy of technic is made, some amount of cortices of the femur underneath the periosteum is sometimes detached and therefore, from a portion of thus obtained periosteum a microscopic examination must be always required. Mostly young rabbit and some cat and dog were used for these experiments and they were all operated on under ether anesthesia.

The tissues were fixed in Zenker's fluid or 10 per cent solution of

formaldehyde, decalcified in 5 per cent solution of nitric acid if necessary, then washed thoroughly in one per cent solution of sodium carbonate after removal from the nitric acid solution and then embedded in paraffin or celloidin. Stained in Mallory's eosin methylene blue or alum hematoxylin eosin.

IV. Experiments.

Experiment 1.

The autotransplantation of periosteum obtained as above mentioned was made into the muscle tissue of back or thigh of rabbit or cat. The outcomes are as follows:—

Table 1.

No.	Animal No.	Species	Age	Sex	Weight (gm.)	Duration	Result	Remarks
1.	1B	rabbit	young	F	2625 a 2625 b	45 days	negative	killed
2.	2B	„	„	M	1575 a 1677 b	7 weeks	„	„
3.	3B	„	„	F	1387 a 1920 b	7 „	„	„
4.	4B	„	„	M	1875 a 2100 b	7 „	„	„
5.	7B	„	4 months	F	1200 a 2250 b	10 „	„	„
6.	8B	„	„	M	1125 a 1725 b	10 „	„	„
7.	27B	cat	1 month	F	735 a 1560 b	13 „	„	„
8.	49B	rabbit	2 months	M	712 a 1612 b	10 „	„	„
9.	55B	„	young	M	1780 a 3000 b	10 „	„	„
10.	56B	„	„	M	2130 a 3630 b	80 days	„	„

'a' signifies the weight at the time of transplantation.
'b' signifies the weight at the time of examination.

The transplanted periosteum disappeared in a short time and some amount of fatty tissue substituted the locality. Some underwent necrosis, later, was ultimately absorbed and none of bone formation could be found.

Appendix of Experiment 1.

On Nos. 19C and 21C, ca. 1 c.m. by 0.2 c.m. flap of periosteum was stripped off, with attaching one end to the femur and this pedunculated periosteum was sutured to an adjacent muscle tissue. After 5 weeks and 32 days respectively, it was observed that thus pedunculated periosteum has changed into a bony spicule and at its top, a silk-gut suture previously used was discovered. This experiment is quite different from that of Experiment 1; viz., the former, the periosteum is entirely detached and transplanted freely into muscle tissue where the bone does not physiologically exist and the latter, it is a pedunculated periosteum attached at one end to the femur and so it is a continuity from the thigh bone.

Experiment 2.

A composition of magnesium phosphate, calcium carbonate, and calcium phosphate as following proportions was prepared and an emulsion in normal saline solution was made, then transplanted into the muscle tissue of rabbit.

Composition of Human bone.

$Mg_3(PO_4)_2$ 1.75
$CaCO_3$ 9.2
$Ca_3(PO_4)_2$ .....87.8 by Carnot.[31]

Microscopic Examination:

No. 26B 5 weeks after transplantation.

There are several particles of artificial bone calcium in various sizes in the transplanted bed and the smaller one was fused with so-called foreign-body giant cells and the bigger one is capsulated with some layers of newly formed connective tissue cells and its inside was invaded with the same cell-groups. Several newly formed blood vessels also occupied the locality. In some place, the calcium particles nearly disappeared, having left a group of giant cells.

The artificial bone calcium was also transplanted in the subcutaneous tissue of dog, rat and mouse and examined in various periods. Still, the injection of the bone calcium emulsion as above mentioned, was made once every day continuously for 25 days into the muscle tissue of dog and rab-

bit. Still more, to give continuous stimulation in the injected portion, the artificial bone calcium emulsion in two per cent solution of formaldehyde was used. This 2 per cent solution of formaldehyde gives good result on tissue for continuous stimulant but does not destroy the tissue. All the tests that were attempted failed for bone formation, and the results were practically similar to that of No. 26B on the main points.

Experiment 3.

The bone dust was substituted for the above artificial bone calcium, and the bone dust was made as follows:—

Material: rabbit's thigh bone. A thigh bone obtained from a young rabbit was preserved into 10 per cent solution of formaldehyde for a few days and then boiled in water for an hour. Both the periosteum and the marrow were entirely removed, then the minute bone dust was made and sterilized. It will be summarized the results of 7 cases in rabbits and cat, extended from 9 to 131 days. The bone dust was transplanted into subcutaneous or muscle tissue as previous cases, however, all these tests failed to form new bone. The transplanted portions were generally occupied with fibrous and fatty tissues and there formed many so-called foreign body giant cells around the bony particles. In later period, the bony particles have become nearly homogeneous or disappeared, having left several groups of giant cells. In early period, there were found some unaffected bone dust and some extend of hemorrhage in the transplanted bed.

Experiment 4.

By Experiment 1, it is affirmed that the periosteum itself transplanted freely into the muscle tissue has no activity to proliferate new bone, Here, the transplantation of the periosteum intermingled with above bone dust was attemped.

Method:—A flap of periosteum removed, with above mentioned-attention, from the femur of rabbit was intermingled with the bone dust and transplanted in the muscle tissue of the loin or the thigh of the same animal.

The results are shown in Table 2.

Table 2.

No.	Animal No.	Species	Age	Sex	Weight	Duration	Result	Remarks
1.	2A	rabbit	young	M	1575 a 1680 b	7 weeks	negative	killed
2.	3A	„	„	M	1380 a 1830 b	7 „	„	„
3.	4A	„	„	M	1924 a 2100 b	7 „	„	„
4.	7A	„	4 months		1237 a 2205 b	10 „	„	„
5.	8A	„	4 „	M	1125 a 1875 b	10 „	positive	„
6.	17A	„	4 „	F	1575 a 2505 b	10 „	negative	„
7.	18A	„	3 months	M	1080 a 1575 b	5 „	„	„
8.	22A	„	young	F	1125 a 2925 b	20 „	„	„
9.	23A	„	„	M	1500 a 2205 b	12 „	positive	„
10.	24A	„	3 months	M	1110 a 1680 b	15 „	negative	„
11.	25A	„	3 „	M	1605 a 2100 b	9 „	positive	died, unknown
12.	32	„	1 month	M	510	9 days	negative	died, coccidium
13.	33	„	1 „	M	487	11 „	„	died, ditto
14.	35	„	1 „	M	262	4 „	„	„ , „
15.	36	„	1 „	F	322	18 hours	„	„ , „
16.	55A	„	young	M	1780 a 3000 b	10 weeks	positive	killed
17.	46	„	8 weeks	F	600 a 1012 b	7 „	negative	„
18.	46	„	8 „	F	525 a 1125 b	10 „	positive	„
19.	48	„	8 „	M	525 a 420 b	40 days	negative	died, diarrhea
20.	49A	„	2 months	M	721 a 1695 b	10 weeks	„	killed
21.	75B	„	1 year	F	2100 a 2250 b	31 days	„	„
22.	77B	„	1 „	F	2325 a	19 „	„	died, peritonitis
23.	79B	„	15 months	M	2962 a 2737 b	30 „	„	killed, abscess
24.	80B	„	15 „	F	2925 a 2737 b	30 „	„	killed, ditto
25.	81A	„	3 „	M	1327 a 1540 b	4 weeks	±	killed
26.	82A	„	3 „	M	1110 a 1390 b	4 „	negative	„

Notice: Homo-transplantation was tried in Nos. 21 to 25, viz., a flap of periosteum was obtained from a young rabbit and transplanted in the muscle tissue of another young rabbit of same species.

There could be obtained 5 positive results from 26 cases and one osteoid. However, all of the positive cases but one showed very poor development of new bone. No. 55A only showed an excellent result and will be here described in detail.

No. 55A. 10 weeks after transplantation.

Gross finding:—

All the wounds operated on were completely healed. Nothing remarkable appeared in the transplanted portion and the black silk suture was only a guidance in finding the locality where it was transplanted. A hard lump was palpated on the spot.

Microscopic examination:—

The transplanted bed was occupied with abundant fibrous tissue and some fatty tissue. In the connective tissue, there was an area of newly formed bony tissue which appeared macroscopically as an oval shaped and a grain sized mass. This young bony tissue possessed many well stained and somewhat flattened bone cells and large young osteoblasts lined the whole bone. There also appeared some Haversian's canals which contained many red blood corpuscles and fibrous cells, somewhat smaller than osteoblasts, and its inside was also lined with osteoblasts. This new bone was surrounded with several layers consisted of many flattened cells, i. e., the periosteum. There were also many foreign body giant cells in various sizes around the bone dust. These particles of bone dust were mostly homogeneous and some nearly absorbed, having left a group of giant cells. No connections between the new bone and the giant cells seemed to be existed.

On negative cases, generally the newly formed connective tissue and some fatty tissue occupied the spot and giant cells fused the bone dust particles in various sizes could be seen in situ. These newly formed connective tissue cells seemed to have come from that of the host's muscle tissue.

For comparison, one half of the removed periosteum used in this experiment was always transplanted without bone dust in the muscle tissue of the opposite side and none of bone formation could be found.

Experiment 5.

Periosteum sprincled with artificial bone calcium was transplanted in the muscle tissue of young rabbit. The Table 3 illustrates it.

Table 3.

No.	Animal No.	Species	Age	Sex	Weight	Duration	Result	Remarks
1.	17B	rabbit	4 months	F	1575 a 2250 b	10 weeks	negative	killed
2.	18B	„	3 „	M	1087 a 1575 b	5 „	„	„
3.	22B	„	3 „	F	1125 a 2925 b	20 „	„	„
4.	23B	„	3 „	M	1500 a 2205 b	12 „	„	„
5.	24B	„	3 „	M	1119 a 1680 b	15 „	„	„
6.	25B	„	3 „	M	1605 a 2100 b	9 „	„	died, unknown
7.	27A	„	1 month	F	735 a 1560 b	13 „	positive	killed

There showed new bone formation one in 7 cases, and the size, shape, and ist appearance of the new bone is quite similar to that of No. 55A. It was situated in the connective tissue of the host's muscle bed and no fatty tissue was existed. No particle of artificial bone calcium nor giant cells could be found.

Experiment 6.

A piece of bone removed from the femur of a rabbit was completely denuded its periosteum, subperiosteal layers and endosteum and transplanted into muscle tissue of the same animal. On the other hand, a piece of bone which possesses completely its periosteum as well as endosteum was removed from the femur of a rabbit and transplanted into muscle tissue of the same animal.

Microscopic Examination:—

The former transplant, at the seventh week, the bone cells of No. 6 poorly stained and some disappeared. The periphery of the transplant was irregularly and extremely eroded and was surrounded by newly formed large cuboidal cells at the adjacent, and still by slight flattened cells. There

was no newly formed bony tissue found. At the tenth week, in No. 5 case, the regressive process still further advanced and no more stained bone cells could be found. Groups of fibrous and fatty tissues were only left on the transplanted portion.

The latter transplants Nos. 15 and 16, showed both at the fifth and tenth weeks, their bone cells stained fairly well, and moreover, present newly formed bony tissue which stained light pink more than the old bone. However, the proliferative activity in both cases has been suspended.

Furthermore a patella was buried into muscle tissue of the another rabbit in two cases, Nos. 55C and 56C. After 10 weeks and 80 days respectively, in both cases, it was discovered that the bone cells had either disappeared or strongly atrophied but the cartilage cells situated at the junction where the bone part transfers to the tendon have stained fairly well. Some of the Haversian's canals were filled with vascularized fibrous tissue, the other contained many polymorphonuclear cells and red blood corpuscles or the latter only. Still more, the red blood corpuscles were nearly homogeneous and some showed fibrin formation in several places.

Besides these cases, transplantation of tail-bone of rat and mouse was attempted and this result will be reported later by another paper.

Experiment 7.

In this test, the surface of the femur of a young rabbit was strongly curretted and accordingly the periosteum thus obtained unquestionably contains some amount of subperiosteal layer and cortices of femur. Such a flap of periosteum was transplanted into muscle tissue of the same animal and the result is shown in Table 4.

(Table 4 s. next page.)

Microscopic Examination: —

By this examination, 3 in 12 cases showed newly formed bone tissue which was surrounded with large cuboidal or somewhat flattened osteoblasts. In No. 31, there are seen some particles of dead bone which have lost their bone cells. Several groups of giant cells are found around these dead bone particles and some of them were nearly homogeneous. These groups

Table 4.

No.	Animal No.	Species	Age	Sex	Weight	Duration	Result	Remarks
1.	30	rabbit	25 days	F	300 a 712 b	10 weeks	positive	killed
2.	31	„	25 „	F	280 a 280 b	2 „	„	died, unknown
3.	34	„	1 month	M	430 a	8 days	negative	died, diarrhea
4.	37	„	1 „	M	318 a	12 hours	„	died, ditto
5.	38	„	1 „	M	300 a	4 days	„	„ , „
6.	52	cat	3 months	M	1387 a 1687 b	5 weeks	„	killed
7.	59	rabbit	4 weeks	F	225 a 825 b	7 „	„	„
8.	69B	„	3 months	M	1237 a 1612 b	3 „	„	„
9.	70B	„	3 „	M	1200 a 1612 b	4 „	„	„
10.	71B	„	3 „	M	1237 a 1650 b	4 „	„	„
11.	81B	„	3 „	M	1327 a 1540 b	4 „	positive	„
12.	82B	„	3 „	M	1110 a 1390 b	4 „	negative	„

of active and non-active bony tissues are surrounded, for a whole, by several layers of connective tissue cells in the muscle bed of the host. No. 81B showed two groups of new-bone-islands which were thickly surrounded with fibrous tissue. In addition, there are seen several groups of giant cells in the fibrous tissue.

Appendix: —

After removed a flap of periosteum from the femur of a young rabbit, the inner surface of thus removed periosteum and the denuded femur were slightly curretted and a coagulated bloody-like mass was collected; namely, it is so-called subperiosteal osteogenetic layer. This mass was transplanted into muscle tissue of the same animal. This was tried only in one case No. 49C and after 10. wooks, no trace could be found in situ.

Experiment 8.

On this test, the transplant, similar to that of previous case was in-

termingled with bone dust, as above mentioned, namely, the mixtures of periosteum, subperiosteal layer, some amount of cortices of the femur and bone dust were autotransplanted into muscle tissue of young rabbit.

The result shows on Table 5.

Table 5.

No.	Animal No.	Species	Age	Sex	Weight	Duration	Result	Remarks
1.	28	rab it	25 days	M	262 a 412 b	30 days	negative	died, diarrhea
2.	29	„	25 „	F	262 a 720 b	10 weeks	„	killed
3.	52B	cat	3 months	M	1387 a 1687 b	5 „	positive	„
4.	56A	rabbit	young	M	2130 a 3630 b	80 days	„	„

2 in 4 cases showed positive results, and especially, No. 56A showed an excellent proliferative activity.

No. 56A. 80 days after transplantation.

Gross finding:—

The denuded portion on the femur was slightly protruded and a hole which had been made on it could be found except there is seen black silk suture on the spot.

Microscopic Examination:—

In the section, there appeared newly formed bony tissue in two groups which can be discovered by naked eye. They were situated in fatty tissue, having stemmed with connective tissue which have arised from the host's muscle tissue and these two groups of bone were also connected each other with connective tissue band. In both groups of the bone, some bone marrow has been formed and they consisted of red-blood-corpuscles and well stained cuboidal cells, viz., the marrow cells. No bone dust could be found in this case.

Experiment 9.

It is well known that the endosteum has the same function with the periosteum and therefore, it was here investigated whether the endosteum has the activity to proliferate new bone or not. It is more difficult to

determine, with certainty, the boundary of the endosteum and the bone. It is practically impossible to remove the endosteum alone and when it is removed, not only the endosteum but the bone marrow always accompany to the endosteum. Furthermore, the inner surface of the bone in contact with the endosteum is rather softer in hardness than that of the bone in contact with the periosteum and this fact stillmore increases the above difficulty.

On this test, the transplant, therefore, consists of endosteum, subendosteal layer, some amount of bone part and bone marrow. The result is shown in Table 6.

Table 6.

No.	Animal No.	Species	Age	Sex	Weight	Duration	Result	Remarks
1.	9B	rabbit	4 months	M	1012 a 1050 b	7 weeks	positive	died, diarrhea
2.	10B	„	4 „	M	1088 a 1072 b	10 „	„	killed
3.	11B	„	4 „	M	1312 a 1725 b	10 „	„	„
4.	12B	„	4 „	M	1200 a 1350 b	5 „	negative	„
5.	13B	„	4 „	F	1275 a	15 days	positive	died, diarrhea
6.	14B	„	4 „	M	1350 a 1860 b	5 weeks	„	killed
7.	19B	„	3 „	F	1425 a 1720 b	5 „	„	„
8.	20B	„	3 „	M	1312 a	10 „	„	„

As the table shows, the percentage of the bone regeneration is quite large and the proliferative activity is very marked in all positive cases. Several cases will be here described in detail.

No. 19B. 5 weeks after transplantation.

Microscopic Examination:—

In the fatty tissue of the transplanted bed of the host, there are seen two groups of newly formed bony tissues and their appearances are just like a coral reef in the sea of fatty tissue. These circled islands of new bone hold some amount of marrow rich in red-blood-corpuscles and their outer layer is surrounded with fibrous tissue. The bone cells as well as

the osteoblasts are quite larger than those of surrounded connective tissue.

Generally, at fifth to seventh week after transplantation, the proliferative activity is in its maximum and later it ts gradually going to regressive degeneration though the newly formed bone still retains its vitality at tenth or twelfth week. At tenth week, No. 10B showed a small mass of new bone in the transplanted bed, having surrounded with connective tissue, and No. 20B also showed the similar condition in the fatty tissue of the host. Commonly, the coral reef appearance of newly formed bone seems to be a special feature in this experiment.

Appendix :—

Furthermore, the transplant as previous case, with either bone dust or artificial bone calcium was used but so satisfactory result was not obtained by adding bone dust or artificial bone calcium. The former showed one positive in 4 cases and the latter, 2 in 3 cases. Some of the positive cases will be stated.

Microscopic Examination :—

No. 14A. 5 weeks after transplantation.

This appearance is quite similar to that of previous one No. 19B, except there are several small groups of giant cells which have fused the homogeneous bone dust particles.

No. 19A. 5 weeks after transplantation.

A fairly large area of newly formed bony tissue which is very irregularly constructed a lattice was situated in the fatty tissue. No bone marrow could be found. The particles of artificial bone calcium nearly disappeared.

No. 20A. 10 weeks after transplantation.

There are two small areas of newly formed bone which are surrounded with fibrous tissue in the fatty tissue and its proliferative activity is very faint, compared with the previous one. Several groups of giant cells fused the particles of artificial bone calcium which are nearly disappearing were also found in the fatty tissue.

Experiment 10.

If the fact that the endosteum has the same function and is similar

tissue with the periosteum is consented, then the result of Experiment 9 from where the bone marrow was excluded must be revealed to compare with Experiment 7. However, it is quite impossible to exclude the bone marrow from the Experiment 9, as above mentioned, and so the bone marrow was added to the Experiment 7, viz., this test consists of periosteal layer, some amount of cortices of femur and bone marrow. It revealed the excellent result and having compared with Experiment 9, both the proliferative activity were almost the same. Table 7 is illustrated.

Table 7.

No.	Animal No.	Species	Age	Sex	Weight	Duration	Result	Remarks
1.	53B	rabbit	6 months	M	2250 a 2137 b	5 weeks	positive	killed
2.	57A	„	4 weeks	M	375 a	15 days	negative	killed, abscess
3.	58A	„	4 „	M	406 a	2 weeks	positive	killed
4.	60A	„	3 months	M	1200 a 1781 b	5 „	„	„
5.	61A	„	3 „	M	1162 a 2500 b	12 „	„	„
6.	72A	„	3 „	M	1237 a 1387 b	3 „	negative	„
7.	73A	„	3 „	M	1237 a 1340 b	4 „	„	killed. abscess
8.	74A	„	3 „	F	1200 a 1345 b	4 „	positive	killed

No. 60A especially displayed a great deal of newly formed bone tissue.

Microscopic Examination:—

No. 60A. 5 weeks after transplantation.

A quite large area of newly formed bony tissue occupied the transplanted muscle bed of the host and it was lined with large cuboidal cells; —osteoblasts—and was still surrounded with several layers of large spindle shaped cells. No fatty tissue around the new bone could be found. The sign of "Halisteresis" was observed in some spots. A few particles of homogeneous bone dust was obtained in the fibrous tissue.

Appendix:—

The Experiment 8 showed positive results and here the similar transplant as in Experiment 8 was mixed with bone marrow which obtained

from the femur of the same animal. The transplant, therefore, consists of periosteum, subperiosteal layer, some amount of bone part, bone dust and bone marrow. No. 53A revealed a positive result at the fifth week after transplantation. The new bone was situated in the fatty tissue of the host. Several groups of foreign body giant cells which consist of homogeneous bone dust were also observed.

Experiment 11.

The bone marrow was selected from two parts of a femur of young rabbit and transplanted in the muscle tissue of the same animal; the one was obtained from the central part of the femur and the other, from the periphery to come in contact with the endosteum of the same femur, and the results between the two were vice versa. Table 8, A and B show the result. The former result is shown in Table 8 A and the latter, in Table 8 B.

Table 8 A.

No.	Animal No.	Species	Age	Sex	Weight	Duration	Result	Remarks
1.	83A	rabbit	15 months	M	2340 a	3 days	negative	killed
2.	84A	„	15 „	M	2310 a 2030 b	4 weeks	„	„
3.	57B	„	4 weeks	M	375 a	15 days	„	„
4.	73B	„	3 months	M	1237 a 1340 b	4 weeks	„	„
5.	74B	„	3 „	F	1200 a 1345 b	4 „	„	„
6.	21B	„	3 „	M	1200 a 1138 b	4 „	„	„

Table 8 B.

1.	58B	rabbit	4 weeks	M	406 a	2 weeks	negative	killed
2.	60B	„	3 months	M	1200 a 1781 b	5 „	positive	„
3.	61B	„	3 „	M	1162 a 2500 b	12 „	„	„
4.	72B	„	3 „	M	1237 a 1387 b	3 „	negative	„
5.	83B	„	15 „	M	2340 a	3 days	„	died, abscess
6.	84B	„	15 „	M	2310 a 2030 b	4 weeks	positive	killed

The former was investigated in six cases and failed to form new bone in all, but the latter revealed three positive ones from six cases and its appearance was quite similar to that of Experiment 9, that is, the new bone was distinguished along the endosteum and the bone marrow also exhibited their proliferative activity.

Experiment 12.

Either the bone dust or artificial bone calcium was mixed with the bone marrow of young rabbit and buried into muscle tissue of the same animal and examined after 5 and 12 weeks respectively. However, no newly formed bony tissue could be found and the locality was always occupied with fatty tissue. Foreign body giant cells also fused the particles of either bone dust or artificial bone calcium.

Experiment 13.

In this test, the bone marrow was added to that of Experiment 4; viz., the transplantation of periosteum, bone dust and bone marrow was attempted and one positive result was obtained from four cases in which the bone marrow was homotransplant in two cases.

No. 62A. 5 weeks after transplantation.

The microscopic appearace was quite similar to that of No. 19A and so will not here describe it.

Furthermore, the transplantation which consists of periosteum, artificial bone calcium and bone marrow of the same animal was tried and was be able to form new bone.

No. 68A. 10 weeks after transplantation.

There is an irregular, nearly homogeneous group of artificial bone calcium in the fatty tissue of the host and it is capsulated with fibrous tissue. On this spot, a semicircular new bone was situated, having been mediated with large and somewhat spindle shaped cells. The free surface was covered with several layers of flattened spindle shaped cells.

Experiment 14.

The spleen has a resemble function with the bone marrow and so in

this test, the spleen emulsion in fresh was substituted for the bone marrow of Experiment 10. An emulsion was prepared from a piece of spleen which was aseptically obtained from a young rabbit immediately after it was sacrificed. A piece of periosteum removed by strongly curretting the femur of a young rabbit was transplanted into muscle tissue of the same animal, having mixed with the above prepared spleen emulsion. The negative result was obtained.

Experiment 15.

The spleen emulsion was also substituted for the bone marrow in Experiment 13. The transplant, therefore, consists of periosteum, bone dust and spleen-emulsion. The result was also negative.

Both the above two experiments were limited to a few cases and so must be further more investigated and stillmore must be noticed that the spleen emulsion in this occasion, was a homotransplant.

V. Summary.

Summarizing the above experiments, the following table shows the all results at a glance.

Nos.	Kind of transplant.	Result.	Total Nos.	Positive Nos.
1.	Periosteum (free)	negative	10.	0.
2.	Periosteum (pedunculated)	positive	2.	2.
3.	Artificial bone calcium, ...	negative	12.	0.
4.	Bone dust, ...	„	7.	0.
5.	Periosteum plus bone dust, ...	positive	26.	6.
6.	Periosteum plus artificial bone calcium,...	„	7.	1.
7.	Denuded bone, ...	negative	2.	0.
8.	Complete bone, ...	positive	2.	2.
9.	Periosteum, subperiosteal layer, some amount of bone-part, ...	„	12.	3.
10.	So-called subperiosteal osteogenetic layer,	„	1.	0.
11.	Periosteum, subperiosteal layer, some a-			

Nos.	Kind of transplant.	Result.	Total Nos.	Positive Nos.
	mount of bone-part plus bone dust, ...	positive	4.	2.
12.	Endosteum, subendosteal layer, some amount of bone-part, bone marrow, ...	„	8.	7.
13.	Endosteum, subendosteal layer, some amount of bone-part bone marrow plus bone dust, ...	„	4.	1.
14.	Endosteum, subendosteal layer, some amount of bone-part, bone marrow plus artificial bone calcium, ...	„	3.	2.
15.	Periosteum, subperiosteal layer, some amount of bone-part plus bone marrow,	„	8.	5.
16.	Periosteum, subperiosteal layer, some amount of bone-part, bone marrow plus bone dust, ...	„	1.	1.
17.	Bone marrow (central part)	negative	6.	0.
18.	Bone marrow (periphery)...	positive	6.	3.
19.	Bone marrow plus bone dust, ...	negative	2.	0.
20.	Bone marrow plus artificial bone calcium,	„	2.	0.
21.	Periosteum, bone dust plus bone marrow,	positive	4.	1.
22.	Periosteum, artificial bone calcium plus bone marrow, ...	„	1.	1.
23.	Periosteum, subperiosteal layer, some amount of bone-part plus spleen, ...	negative	3.	0.
24.	Periosteum, bone dust plus spleen, ...	„	2.	0.

VI. Appendix.

For an appendix, a few lines on bone formation which occurs without any relations with bone-system will be here added. There are reported bone-formation in almost all the organs and tissues and such instances are not so infrequent. Several instances which hitherto have been experimentally proved will be here stated. So-called myositis ossificans which grows

after operation or trauma in abdominal muscle tissue and etc., where there is physiologically no bone tissue existed is well known. The periosteal type must be excluded from this instance and it must come from the metaplasia of connective tissue of muscle (Goto[15], Nakayama[38], etc.). Bone formation in the artery, especially in tunica media of full grown rabbit with a 3 per cent solution of silver nitrate or a 2 per cent solution of cupric sulphate- application was proved by Harvey[39], etc. By ligation of renal blood vessels on rabbit, some bone tissue can form in the affected kidney (Liek[40], Koga[33], Asami[32], etc.). Furthermore, bone formation in the tonsil, thyroid, ovary, lung, liver, heart, eye, etc., are hitherto, frequently reported. As above mentioned, the bone can be formed independently in such a place where there is absolutely no bone-system existed, and consequently no periosteum nor bone marrow concerns on bone formation.

An experiment on renal vessel ligation of a rabbit was attempted.

Animal No. Extra 1. Species rabbit.

Weight 2625 gm. Sex male Age 1 year old.

Method :—

Opened the right side of abdomen and ligated the renal vessels with silk gut after the suprarenal capsule was stripped off. Closed the wound. Sacrificed after 10 weeks. Weighted 2362 gm. on examination.

Gross finding : —

The right kidney has been buried in the fatty tissue and a part was strongly adhered to the liver. The shape was well kept but its size was nearly three times smaller than that of healthy side, and its consistence was just like cartilage. The cut-surface showed that it is occupied with white calcified masses, radiated from pyramid to cortex.

Microscopic Examination :—

Mostly the cortical portion and some medullary portion are occupied with calcified masses, stained homogeneously in pink, which radiated, and arranged to the directions of tubules. Malpighians bodies and all tubules-apparatuses disappeared. Some amount of fibrous tissue existed among the above calcified masses and the surface of cortex is also surrounded with vascularized connective tissue. The medullary portion mostly consisted of fibrous tissue and there were many groups of somewhat large spindle shaped

cells which stained in deeper blue than that of fibrous tissue ground. The pelvis also consisted of fibrous tissue and a bone tissue which possesses some bone marrow rich in red-blood corpuscles appeared in situ. The activity of this newly formed bony tissue was very poor and the large cuboidal bone cells were poorly stained.

VII. Discussion.

There are many reports that the periosteum transplanted freely into muscle tissue regenerates bone. However, it is very difficult to remove the periosteum alone as previously described and in such a positive case, it is very doubtful that whether the periosteum alone was really removed or not. This is the point of discussion. Even if an attention is paid, the so-called subperiosteal osteogenetic layer and still some amount of bone part are sometimes shaved off with the periosteum on its removal. Furthermore, the cortices of the bone do not contain so much calcium than that of the deeper part and accordingly the periphery of the bone is rather soft in hardness, and especially it is still softer at young age. These matters increase the above difficulties on removal of the real periosteum. In the Experiment 1, none of positive cases could be given on the transplantation of the periosteum. However, if the above attention is unpaid and when such a periosteum as it is removed by strong currettment is transplanted, relatively a majority of positive cases will be proved as Experiment 7 shows. This is the reason why there are many opinions pro and con. Yokoi[17] stated that an auto- or homo-transplantation of periosteum can be proved new bone. He still, added that the bone pieces which were shaven off at the same time on removal of the periosteum has lost its vitality within a few days after transplantation and so the bone cells disappear, having emptied their lacunae, etc. This fact relates obviously that he is transplanted not only the periosteum but the so-called subperiosteal osteogenetic layer and still some amount of bone part. With regard to the appendix of Experiment 1, the fact that the pedunculated periosteum altered into a bony spicule looks as if the periosteum itself has activity to form new bone. However, it can

not be adopted. By removal of periosteum, the so-called subperiosteal osteogenetic layer which was removed its protector the "periosteum" and the exposed bone part cooperate with the periosteum, thus it fulfils all conditions required as that of Experiment 7. Moreover, the continuity of the pedunculated periosteum to the femur can be supplied sufficient nutrient; the blood vessels, from the femur. This matter is exceedingly beneficial to bone regeneration and the periosteum may, on this ocassion, rather play an important rôle as a guide.

The transplantation of either the artificial bone calcium or the bone dust failed to form new bone. Asami[32] and Dock injected calcium salts consisting of calcium chloride, calcium carbonate and sodium phosphate, subcutaneously or intramuscularly on rabbit, but no evidence of bone formation was observed as late as 50 days after injection. This experiment, however, must be furthermore continued as far as the metaplasia is generally consented. In fact, Koga[33] proved new bone formation by repeated injection of one per cent solution of calcium chloride into subcutaneous- or muscle-tissue, or scrotum. Goto[15] also tried the same experiment and he could be observed only an osteoid tissue. By Experiment 1, it was affirmed that the periosteum itself has no activity to form bone, but the Experiment 4, viz., the periosteum combined with bone dust was favourable to the bone formation in a minority. The following things are great essentials to form new bone.

1. Early and sufficient establishment of blood supply.
2. Suitable stimulation.
3. Non-pressure.

This is out of question that not only in the transplant but all the organs and tissues require early and sufficient blood supply; the nutrient. The transplant, therefore, must not be inserted into cicatrical or nonvascular tissue which furnishes an unfavorable environment for it. Referring to the stimulation, the muscle motion is an important agent for this purpose. The locations of the transplant, therefore, cannot be disregarded and two places were practically selected for comparison; the one is the muscle tissue of both sides of lumbar column for the most quiet place and the other, the muscle tissue of the thigh for the most movable place. However, no

significance could be found between the two. It must be borne in mind that animals (rabbit in this occasion) in the laboratory cages usually do not allow free excercise and so it may have no influence upon the locality. As to the favourable stimulant it will be described in the following pages. It may be considered that in such a place where there is no stimulant the transplant will undergo to atrophia, later, necrosis, following the absorption. Non-pressure toword the transplant is also one of requisite conditions. The fact that the lasting pressure of the breast aneuryam on the sternum upon which it impringes, at last destroys the sternum bone eloquently relates that even a delicate pressure, if it works very long, even such a hard bone cannot bear the pressure. At first, a long gap along the muscle fiber of the host was made and inserted a transplant in it. It was, however, great failure and the transplant in such a bed is easily pressed out to the subcutane by the motion, pressure of the muscle and was discovered it had changed into a necrotic mass on examination. It was, afterward, obtained a good result by making a cavern in the muscle bed and put a transplant in it. The quantity of bone bust and the size of its particles are another requisite conditions. If bone dust is too much and too large it will rather work for foreign body having become inflamed reactively to the locality and will interfere to form new bone. Both the periosteum and the bone dust must be ripen and assimilate to the location at the same time to cooperate on bone formation, and if either one or the other is delayed to be ripened, one cannot wait the other and so it will undergo necrosis or absorption. The smaller the particle of the bone dust, the better the result will be obtained. The proportion of the periosteum and the bone dust must be the similar as they are in normal bone. The age and nourishment of animal, still a predominal cause also concern for the bone production. It must be considered that transplantation rises freely in such a place where it physiologically does not allow to exist, without any connection to the osteo-system, and this matter makes the regeneration hesitated. Long exposure to air and drying of the transplant on account of the operation time being prolonged are also subsides the regenerative process. As above enumerations, the bone regeneration on this ocassion, requires many things and it is very difficult to obtain the all requisits.

This is the reason why the positive cases are in quite small number. Whether the deposit of bone dust will work upon the proliferation as it stands or after it dissolves is the problem, but the latter will probably be adopted. b Koga[33] viewed that on the ocassion of bone formation by injecting one per cent solution of calcium chloride, an insoluble calcium which had deposited in the host becomes soluble and acts to the germ cells transformed from the connective tissue, thus the bone is produced. On the transplantation of the periosteum with artificial bone calcium, one in 7 cases obtained positive result. The positive percentage was smaller than that of previous one. This tells that there must be something differences between the bone dust and the artificial bone calcium, though the chemical compositions of the two are the similar.

The denuded bone piece on Experiment 6, no doubt, has vitality at the onset when it is removed from the femur of a young rabbit and transplanted into muscle tissue of the same animal. However, during its process, it is supposed to undergo regressive degeneration, because there is no periosteum as well as endosteum or anything necessary on regeneration and therefore, this transplant, at its later period is likewise as that of Experiment 3, viz., bone dust transplantation. At the seventh week, the denuded bone still has bone cells stained though it is poor, but at the tenth week, no more stained bone cells could be found. On the contrary, the transplantation of complete bone piece is to be considered that it is the combination of Experiments 7 and 9, viz., it contains both the periosteum and the endosteum and all layers of bone tissue. It is obvious that this result is successful to form new bone, as both the Experiments 7 and 9 showed. From the above two results, it is ascertained that the periosetum as well as the endosteum is essential for bone formation. Albee[20] pointed that the graft on bone transplantation should be autogeneous, consisting of all four bone layers; namely periosteum, endosteum, complete thickness of cortex and marrow. Considering from the results of above experiments, it is also obvious that Experiment 7 is to be certainly accomplished a satisfactory result, namely, the transplant obtained by strong currettment from the femur which naturally consists of periosteum, subperiosteal layer and some amount of bone part. The positive percentage on this experiment is supe-

rior to that of Experiment 4, viz., the transplantation of periosteum with bone dust. These things that the bone part accompanies to the periosteum is an auto-transplant and if Bart's[30] view that bone cells are not senile functionless osteoblasts but active cells which are in some way related to bone production is acknowledged, this bone part is more beneficial, moreover, that the subperiosteal layer assists this process are a great advantage on Experiment 7 and a disadvantage on Experiment 4 is that the bone dust is a homo-transplant, non-active and also that no subperiosteal layer to help the regeneration is existed. No new bone could be found in the transplant of so-called subperiosteal osteogenetic layer. This experiment must be furthermore extended. Goto[15] obtained positive result in the similar experiment. The bone dust in Experiment 8; namely, the bone dust in addition to the previous one is superfluous when the transplant furnishes a fovourable environment to form new bone as previous one. When the bone dust is too much, it surely disturbs for the bone regeneration as hitherto mentioned. However, when the bone part coexists with the removed periosteum is insufficient, the bone dust substitutes for the former and will be, with benefit, adopted to the regenerative process.

The Experiment 9 was subjected from the standpoint that the endosteum has the same function with the periosteum. It is practically impossible to remove endosteum alone as it is illustrated at Experiment 9. The Experiment 9 consists of endosteum, subendosteal layer, some amount of bone part and stillmore the bone marrow. The regenerative activity on this experiment was exceedingly beneficial. If the fact which is hitherto written in the text-book that the endosteum has the same function and similar tissue with the periosetum is consented, the result of the Experiment 7, namely, the transplantation of periosteum, subperiosteal layer and some amount of bone part and an assumed result of Experiment 9 where the bone marrow is excluded must be considered to be the same. Only the differences between the Experiments 7 and 9 as to whether the bone marrow exists or not, resulted a great difference on bone regeneration of the two. In fact, the bone marrow cannot be excluded from the Experiment 9 and therefore, for comparison, the Experiment 10 was performed. This test consists of Experiment 8 plus bone marrow; viz., periosteum,

subperiosteal layer, some amount of bone part and bone marrow, and the result revealed that the proliferative activity of both the Experiments 9 and 10 are almost the same. The positive results on Experiment 9 plus either bone dust or artificial bone calcium are out of question. The bone dust or the artificial bone calcium on this occasion, is also superfluous as the bone dust in Experiment 8. Moreover, it is obvious that the Experiment 10 plus bone dust can form new bone, having considered from the above results.

Judging from above experiments, it must be borne in mind that the existence of bone marrow plays an important rôle to the bone formation and consequently, it must dissolve the question how the bone marrow itself will be able to proliferate new bone or not and the Experiment 11 reveals this question.

Some literature on the transplantation of bone marrow will be here added. Saltykow[34] proved for the first time, that the bone marrow transplanted in the subcutaneous tissue of an animal mostly dies, however, what was retained its vitality can regenerate marrow cells and from the endosteum coexists with the transplanted marrow, new bone is formed. Frangenheim[35] also obtained a positive result on homo-transplantation of bone marrow. Stillmore, Miyauchi[36] investigated that new bone was formed from the endosteum autotransplanted with bone marrow in the liver of the same animal. Tongu[16] removed bone marrow and made an emulsion which he injected subcutaneously or muscularly. The experiment lasted for 14 to 31 days. In other cases, the bone marrow was transplanted in toto from 8 to 129 days. He found that the osteogenetic activity of the bone marrow was very much decreased in the former. This is due partly to the loss of the regenerative power on account of the crushing of osteoblastic elements, partly to the insufficient volume of the marrow as a matrix of new bone formation, and to the increase in the surface of the surrounding tissues for absorption. Where the marrow was transplanted entire, there was a ring of new bone formed arround it. The marrow especially the central part of it generally underwent necrosis. Ueda[37] observed an auto-transplantation of bone marrow in the subcutane or in the liver of rabbit, for 3 days to 4 months and as to the bone regeneration, he viewed that the

osteoblasts in the endosteum which associate with the marrow form an osseous tissue.

Experiment 11 showed two quite different results. An attention must be drawn on removal of the real bone marrow. At least, on rabbit, the bone marrow is in inseparable condition with the endosteum at their boundary and so the bone marrow on this transplanting purpose must be selected from central portion of a long bone, apart from the periphery of the bone. On the other hand, the bone marrow in contact with the periphery of the bone always associates with the endosteum and accordingly this brings a different result. In fact, the former failed to form new bone and the latter, on the contrary, showed newly formed bone. Even the bone part coexists with the endosteum is quite small amount, having considered that the calcium composition in bone marrow are similar to those of normal bone, this calcium will be applied for its requirement, and thus the calcium in bone marrow may probably take part in the action. The fact of Miyauchi's[36] experiment that the new bone was formed from the endosteum transplanted in the liver must be considered as a product of cooperation of, at least, bone marrow and endosteum, and neither from endosteum alone nor bone marrow alone. His experiment does not mean the real bone marrow transplantation as there is some endosteum existed. Ueda[37] and the others' experiments are also quite similar to above one. This is point of discussion and as to the fact of many positive results, hitherto, described, it is very doubtful whether the real bone marrow has been removed or not. From the views of Experiments 10, 11, and 9 it may be considered that the bone marrow itself has entirely no activity to form new bone, but it is excellently serviceable for a stimulant and nutrient to the bone regeneration. The question next occurs is that if the blood coagulum is substituted for the bone marrow, how it will be resulted, but the blood coagulum have always associated with all the transplants above mentioned, for their nutrient, however, no particurality could be found in any cases. Yokoi[17] also pointed the same view. He added that one per cent of fibrin apparently increases proliferation. The transplantation of bone marrow combined with either of bone dust or artificial bone calcium failed to form new bone. However, this experiment must be furthermore investigated as that of Ex-

periment 2 as far as the bone marrow is an urgent stimulant and also it must be considered that this transplant ought to have the possibility on bone regeneration more than that of Experiment 2. The positive result on the transplantation of periosteum with either of bone dust or articial bone calcium (Experiments 4 and 5) was quite in a minority and therefore, on Experiment 13, a stimulant, namely, the bone marrow was added to Experiments 4 or 5 and the result was beneficial, thus it can be definitely concluded that the bone marrow is great essential for a stimulant and nutrient to accomplish bone regeneration. The number of Experiment 14 and 15 was too small and cannot definitely tell the result and must wait further investigation. However, at least as far as the experiments 14 and 15 showed, the spleen was not served, with benefit, for bone formation.

By appendix, it is well understand that no periosteum nor bone marrow is absolutely essential for bone formation, and it may say without hesitation that bone can be formed in such a place where there is absolutely no periosteum nor endosteum existed.

VIII. Conclusions.

1. Periosteum as well as endosteum itself has entirely no activity to form new bone.
2. However the cooperation of periosteum (endosteum) and bone calcium, when it furnishes a favourable environment, can be accomplished bone regeneration.
3. Bone marrow has also no activity to form new bone but it plays a role as a stimulant and nutrient for bone regeneration.
4. Periosteum (endosteum) is not always necessarily on bone formation.

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X. Description of Plates.

Figure 1. No. 55 A. Transplantation of periosteum plus bone dust.
A quite large area of newly formed bone which possesses bone marrow is seen in this preparation. Several groups of foreign body giant cells are also seen at the lower part of the field.

Figure 2. No. 47 A. Transplantation of periosteum plus bone dust.
Some foreign body giant cells in the fatty tissue are seen at the left side of the field. A new bone occupies the central part.

Figure 3. No. 56 A. Transplantation of periosteum, subperiosteal layer, some amount of bone part and bone dust.
There are two groups of newly formed bone which possess some bone marrow are shown.

Figure 4. No. 81 B. Transplantation of periosteum, subperiosteal layer, some amount of bone part.
Between the two insels of newly formed bone, there are seen several groups of foreign body giant cells.

Figure 5. No. 19 B. Transplantation of endosteum, subendosteal layer, some amount of bone part and bone marrow.
A ring formed new bone tissue is seen in the fatty tissue. Some bone marrow rich in red blood-corpuscles is also seen at one side of the ring.

Figure 6. No. 84 B. Transplantation of bone marrow (periphery).
The appearance is quite similar to that of previous case.

Figure 7. No. 60 A. Transplantation of periosteum, subperiosteal layer, some amount of bone part and bone marrow.
The field is occupied with a quite large area of newly formed bone. No bone marrow nor giant cells can be seen in this preparate.

Figure 8. No. 74 A. Transplantation of periosteum, subperiosteal layer, some amount of bone part and bone marrow.
A small rounded area of newly formed bone is seen in the fibrous tissue. Besides this, a great deal of giant cells-groups occupies nearly all the transplanted bed.

Figure 9. No. 53 A. Transplantation of periosteum, subperiosteal layer, some amount of bone part, bone dust and bone marrow.
New bone groups are exhibited in the field and some foreign body giant cells are located at the lower part of the field.

Figure 10. No. 62 A. Transplantation of periosteum, bone dust and bone marrow.
A newly formed bone occupies the central area of the field and some degenerated marrow is scarecely seen at the lower part of the field.

Figure 11. No. 68 A. Transplantation of periosteum, artificial bone calcium and bone marrow.
A semicircled new bone is situated in the area which consists of artificial bone calcium. This calcium group is nearly homogeneous.

Figure 12. No. 19 A. Transplantation of endosteum, subendosteal layer, some amount of bone part, bone marrow and artificial bone calcium.
A quite large area of newly formed bone is seen and no artificial bone calcium can be found.

Fig. 1.

Fig. 2.

Fig. 3.

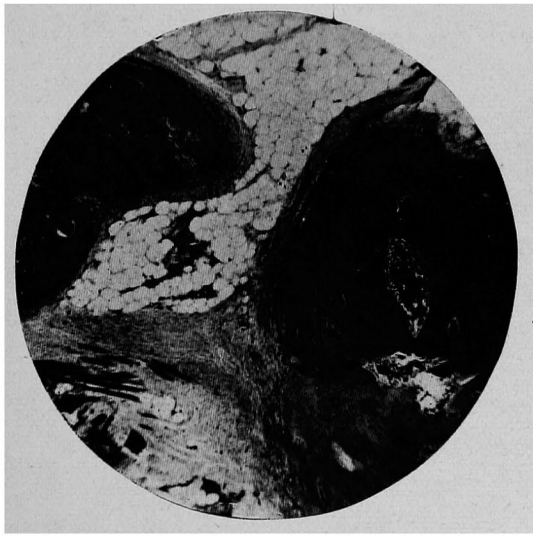

Fig. 4.

Fig. 5.

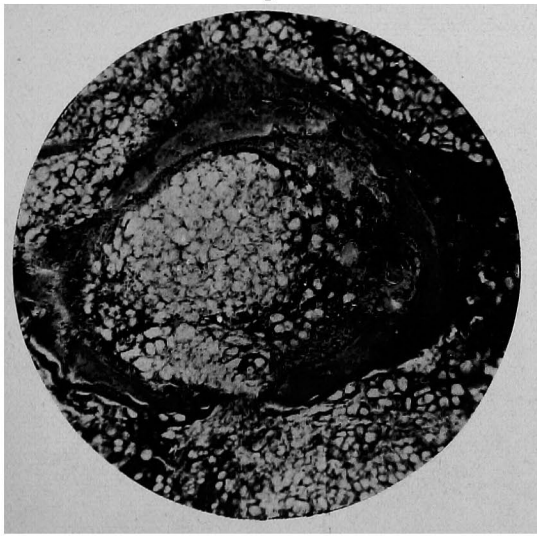

Fig. 6.

Fig. 7.

Fig. 8.

Fig. 9.

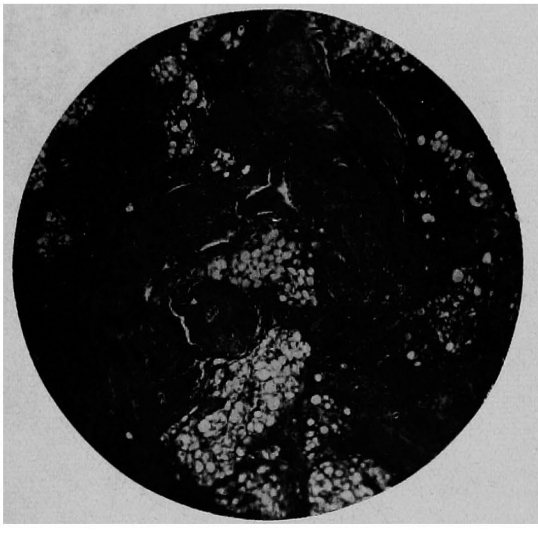

Fig. 10.

Fig. 11.

Fig. 12.