PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The Role of the Various Elements in the Development and Regeneration of Bone

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VI. *The* Rôle *of the Various Elements in the Development and Regeneration of Bone.*

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The present inquiry has been undertaken with the view of obtaining data, chiefly by direct experiment, as to the *rôle* which the various elements play in the development and reproduction of bone. The periosteum has long been regarded as the chief factor in the reproduction of bone. What is its potentiality in this respect ?

Preservation of Periosteum and Removal of Bone.—Result.

If a portion of the shaft of a growing long bone, including its entire circumference, were removed, leaving its periosteum intact, would restoration of osseous continuity ensue ?

This would depend on the length of the shaft removed and its state of osseous development and, as far as the periosteum was concerned, whether it had already received from the bone a supply of osteoblasts to meet the emergency or whether it was in contact with proliferating osteoblasts on either side of the gap, to which it might afford shelter and abundant pabulum for their development. The osseous tissue in the growing shaft would certainly throw out ossific material and make an attempt to bridge the interspace, but if this space was great it would not accomplish it.

Even in young animals, when the bone development is most vigorous, the periosteum fails to supply the defect, as the following experiment shows :—

Dog K. *Preservation of Periosteum and Removal of Bone.*

Shaft of radius removed sub-periosteally to the extent of $1\frac{3}{4}$ inches, the periosteum being carefully preserved and being left *in situ*.

There was no attempt made to detach plaques of bone which might have adhered to the periosteum during its separation. At the upper extremity of the bone shaft an irregular spike-like process of bone was left. The wound was then closed, the soft tissues being allowed to coalesce. A case of plaster of Paris was applied loosely to the limb to prevent pressure from without.

No bleeding. Aseptic wound healing resulted, leaving no visible cicatrix, and no adherent scar. After removal of plaster, four weeks subsequently, there was no

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apparent union, a gap being detected between the extremities of the radius. At the end of six weeks, the gap was quite marked and the ulna was still bending.

Examination of Specimen 10 weeks after.—The right ulna had markedly bent, and this bending had lessened greatly the interval which would otherwise have existed between the two extremities of the un-united radius. At the part from which the bone had been removed and the periosteum had been left *intact*, there was a gap, void of osseous formation, but filled with dense connective tissue. When this was turned aside, the proximal portion of the bone was seen to be flattened laterally, a new formation of bone continuous with the shaft projecting toward the gap.

A somewhat similar formation had taken place below. So that with the exception of the flattened osseous new bone produced from the growth of the shaft there was a complete gap between the ends of the bone at the part where the periosteum was left intact.

FIG. 1.—Preservation of periosteum and removal of portion of shaft of bone. The specimen shows result 10 weeks after. The portion of the shaft which was removed sub-periosteally is seen between the two larger specimens. The figure on the right is the radius and ulna of the normal limb for comparison.

Transplantation of Periosteum.—If periosteum has osteogenic power, it ought to show it on transplantation. Before transplanting the periosteum, any osseous plaques which may have been torn from the growing bone and may be adherent to the periosteum ought to be removed.

If this be done, no osseous production results after transplantation. As an illustration, take Experiment N, in which two portions of periosteum were transplanted and seven weeks after there was not only no bone production from either transplant, but the periosteum had become absorbed.

Duhamel's Silver Ring Experiments.

Duhamel placed (1739) a silver ring under the periosteum in a living animal and found, some time after, that the ring had become covered by bone. He inferred from this that the periosteum secreted bone. This experiment is often quoted as a proof of the osteogenic power of the periosteum.

There is no doubt about the fact that when a metallic ring which closely fits the shaft of a growing bone is placed under the periosteum, new bone will in course of time surround the ring. The deduction drawn therefrom, that the periosteum must, therefore, be the source of the new bone, does not necessarily follow. Irritation of a bone excites proliferation of the bone cells and the osteoblasts are poured out from the Haversian Canals on to the surface of the bone and fill the interstices of the soft tissues surrounding the shaft, such as the loose areolar tissue existing between the bone and the periosteum. In this way, layers of new bone emanating from the bone itself are deposited peripherally and may soon cover a foreign body of limited size surrounding the bone or lying on its surface.

If this be so, would bone, denuded of its periosteum, be capable of throwing out sufficient ossific matter to clothe a metallic ring placed upon its surface?

It is obvious that the naked bone, being bereft of its periosteal blood supply, would lose to that extent its osteoblastic regenerative power, though the abundant inosculations of the numerous branches of the nutrient vessels in the interior of the bone might suffice to modify the nutrient defect of the periphery of the shaft.

In order to test this, the following experiments were performed :—

Dog B. *A Circle of Periosteum removed from the Entire Circumference of the Shaft of Right Radius and a Silver Ring placed upon the Denuded Bone.*

Dog B had a circle of periosteum comprising the whole circumference of the right radius, and measuring half an inch in breadth, raised from about the middle of the shaft. A flattened silver ring was made to encircle the denuded bone at this part. The operation was bloodless, the wound healed aseptically, without visible scar and without adherent cicatrix. The bones of the left limb are preserved for comparison.

Description of the Specimen as seen 12 *weeks after.*—The right radius was covered—at the part that had been denuded of the periosteum—with a new-formed connective tissue which was more firmly attached to the bone than that of the normal periosteum on the shaft above and below this part. After denuding the bone from this new-formed connective tissue and from the periosteal covering, there was no trace of the silver ring to be seen. The shaft of the bone was smooth all over and if the silver ring still existed, it must have become enveloped in the bone. There was, however, a thickening of the shaft at one part, and it was considered probable that the silver ring lay underneath. The bone was scraped through in a vertical direction and after penetrating the bone for about one-eighth of an inch the silver ring was exposed, completely buried in firm osseous tissue.

Three other apertures were scraped through at different parts of the circumference of the shaft so as to expose the silver ring at each.

The thickness of the new bone covering the ring in front was fully one-eighth of an inch and it was a little less behind. Thus the silver ring placed upon the bone denuded of periosteum had in three months become completely enveloped in newly-formed bone one-eighth of an inch in thickness.

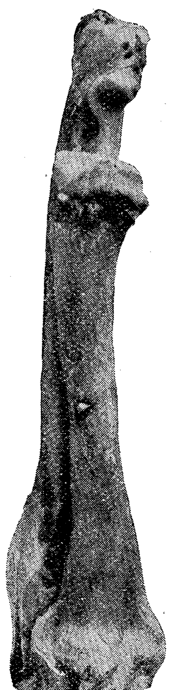


FIG. 2.—Silver ring experiment No. 1. Specimen shows the silver ring exposed by removal of enveloping portion of bone.

The shaft operated upon is smaller in circumference than its fellow in left limb, the diminution in bulk being apparent at the part which was denuded of periosteum, but the new bone at this part is denser than that on the other parts of the shaft which were not operated on.

Though the results attained in this experiment pointed in the direction that the bone produced the osseous tissue which surrounded the silver ring, it was not conclusive, since, owing to the small circle of periosteum removed, measuring only half an inch in diameter, it might be said, by way of criticism, that the periosteum at either side might have grown quickly over the gap and then produced the osseous tissue which covered the silver ring. In order to meet this criticism two other experiments were made.

Second Silver Ring Experiment.

Two inches of the diaphysis of the right radius, comprising the whole shaft with the exception of half an inch from either epiphyseal line, were denuded of periosteum which was entirely removed along with the superficial osseous layer of the shaft. Two silver rings were then placed upon the centre of the shaft about half an inch apart. During the necessary manipulation the connective tissue covering of one of the adjacent muscles was encroached on so as to expose the muscular bundles, and these naked muscular fasciculi came into direct contact with the denuded diaphysis.

Aseptic wound healing without visible cutaneous scar resulted.

Description of Specimen as seen 7 *weeks after* (6 weeks and 6 days after).—There was no skin scar visible to the unaided eye. The periosteum at the epiphyseal ends was normal and could be separated from the underlying bone readily. Toward the centre of the shaft, over the whole extent previously denuded of periosteum, the soft parts were closely adherent to the bone. In part of the circumference of the shaft the longitudinal muscular bundles were adherent to the shaft and the connective tissue surrounding them was also partly infiltrated with osseous matter. At other parts the shaft was covered with dense connective tissue infiltrated with fibro-cartilage and bone nodules—detectable by the unaided sight and also by touch.

Microscopic examination further confirmed this. Had the animal lived a few weeks longer, this tissue would have been converted into well-formed bone.

On exposure of the bone, the shaft was seen to be uniform in outline. The silver rings were nowhere visible, the new bone completely covering them. After thinning the bone at one part, a small portion of one of the silver rings was shown shining through a superimposed layer of bone. The second ring is left buried in the bone without being exposed.

Comment.—Here were two silver rings placed upon a denuded shaft bereft of its periosteum, which, seven weeks afterwards, were entirely covered over with newly-formed bone. The newly-deposited bone had in some parts of its circumference a covering of new-formed connective tissue enclosing fibro-cartilage and islands of bone; while in other parts the shaft was covered by adherent muscles infiltrated with osseous matter from the growing bone. But where the bone was covered by adherent muscle and where it was covered by connective tissue, the silver rings were equally covered over with osseous deposit. Presumably no one will suggest that at the part of the circumference covered by muscle the muscle deposited the osseous matter covering the ring.

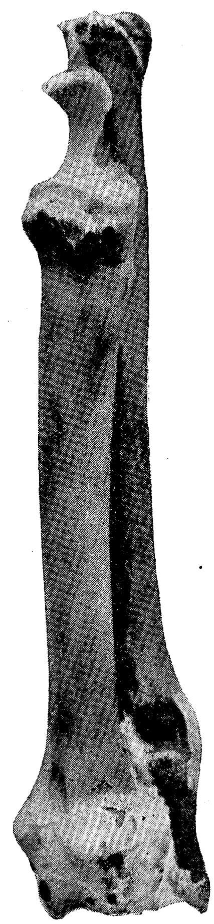


FIG. 3.—Silver ring experiment No. 2. Both silver rings are invisible, being completely covered with bone.

Third Silver Ring Experiment.

A ring of periosteum, measuring an inch in breadth, was removed from the entire circumference of the shaft of the right radius. The superficial osseus tissues were taken away in order to ensure the removal of any remnants of periosteum. In the centre of this denuded shaft a silver ring was made to encircle the bone so as to adhere closely to the shaft for two-thirds of its circumference, while it bulged beyond the circumference of the shaft for the remaining third.

The gap existing between the bone and the ring, at the part where it bulged, measured from one thirty-second to one-sixteenth of an inch, the soft tissues being kept apart from the bone at this point to fully that extent, taking the thickness of the silver ring into consideration. There was thus a space left between the silver ring and the shaft which extended for one-third the circumference of the shaft. Aseptic healing without visible scar ensued.

Examination of the Specimen which was obtained over 12 *weeks after* (12 weeks 5 days after).—There was no visible cutaneous scar. A layer of connective tissue continuous with the periosteum covered the previously denuded shaft of the bone. The silver ring was covered by new bone, and was completely hidden from view for over two-thirds of the circumference. At the part of the ring which had been made to bulge beyond the bone, the surface of the ring was still exposed, though the interval previously existing between the bone and the silver ring had been filled up by new osseous tissue which had also partly covered the thickness of the silver ring. The surface of the exposed silver ring was adherent to and closely invested by the connective tissue layer, which was continuous with the periosteum above and below, and yet there was no bone formed between the outside of the ring and the newly-formed connective tissue which adhered to it. This connective tissue layer covering the ring had been left *in situ* in the specimen. There is no osseous growth in this connective tissue, and it is quite smooth and polished where it covers the ring.

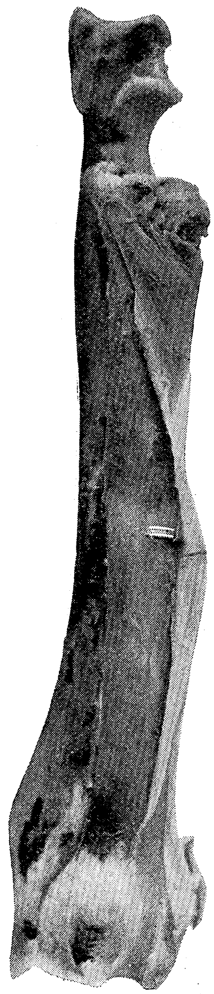


FIG. 4.—Silver ring experiment No. 3, showing gap between bone and silver ring filled with new osseous tissue from the denuded bone.

Comment.—The osteoblasts emanating from the denuded bone covered the portion of the silver ring which was applied closely to two-thirds of the circumference of the bone, and also filled the gap which existed between the bone and the bulged portion of the ring over the remaining third. Probably, had longer time been given, the osteoblasts emanating from the bone would have covered the remaining portion of the silver ring. It is to be noted that the connective tissue covering the bulged part of the ring, though closely applied to it, had not produced any ossific material to cover the silver ring. If it did not do so here, is there any evidence to show that it does so at any other part?

Comment on the Results obtained in the Silver Ring Experiments.—When in the canine species the periosteum is entirely removed from the shaft of a long bone, and a silver ring is made to encircle the circumference of the denuded bone, the osteoblasts emanating from the exposed shaft are poured out peripherally, where they become ossified and increase the thickness of the bone, and at the same time cover the silver ring with fresh osseous tissue.

Under suitable conditions the osteoblasts emanating from the bone penetrate the adjacent soft parts. This is seen in the ossific penetration of the newly-formed connective tissue and the exposed muscular bundles which thereby become ossified and adherent to the shaft. At the parts of the circumference where the exposed muscular bundles are placed in contact with the denuded bone, the peripheral increase of new bone to the shaft takes place as uniformly as it does at other parts which have been surrounded by newly-formed connective tissue continuous with the periosteum at the proximal and distal portions of the shaft.

The Regeneration of Bone from Proliferation of Osseous Tissue.

The Periosteum as a Limiting Membrane to the Osteoblasts.

The periosteum acts as a limiting membrane to the osteoblasts issuing from the interior of the bone. This is well illustrated in fractures. When a fracture takes place without rupture of the periosteum, the union of bone occurs so perfectly that it is difficult after a time to discern the seat of fracture, and at no time is there much if any provisional callus. This obtains likewise in fractures occurring in lower animals when the periosteum remains intact.

When the periosteum and bone are simply incised, as in osteotomy, and the cut osseous tissues are at once accurately co-opted and kept at perfect rest, ossification ensues with little or no callus, and in a short time it is difficult to detect the seat of the lesion.

If, on the other hand, the fracture has been attended by tearing of the periosteum, and the limb has been subjected to much movement subsequent to the production of the osseous lesion, then the osteoblasts are poured out from the fractured surfaces into the gap between the bones, from which they overflow into the surrounding soft tissues. The limiting membrane of the periosteum having been ruptured, the free movements to which the limb is subjected will express the osteoblasts and blood accumulated in the osseous gap, and these osteoblasts will form fresh osseous deposits outside the bone.

Again, in malposition of the fragments the ossific matter is often poured out in great abundance, but if there be on any side a continuity of the periosteum it will limit the osseous deposit at that part. Frequently, in fractures, the ossific material poured out from the bone lesion covers in the periosteum. In such a case the overlying callus is only loosely attached to the outside of the periosteum covering the bone and may be easily peeled off unless the periosteum becomes absorbed, when intimate organic adhesion between bone and callus takes place.

The potency of the periosteum as a limiting membrane is seen when in cases of fracture it is torn up and stretched across the fractured surface of one of the fragments. It here forms an effective barrier against osseous union, the ossific formation being absolutely limited by the periosteum, and fibrous union results.

In compound fractures many opportunities have been afforded of demonstrating how frequently the periosteum is torn from one fragment and laid over one or other of the fractured surfaces, where, had it been allowed to remain, non-union would have occurred.

On several occasions during operation for un-united fracture, the interposition of the periosteum between the fragments has been found by me to have been the sole cause of non-osseous union. Were it not for the fact that the periosteum is normally so tightly stretched over the bone, delayed and non-union would be more frequently met with after fractures due to the interposition of the periosteum between the fragments.

Is the Production of Callus inherently greater in the Lower Animals than in Man?

Fracture in the Lower Animals.—It is believed by many that the lower animals are inherently prone to excessive formation of callus after fracture, and especially does it seem great in them when compared with the amount which is poured out after fractures in man.

Abundant formation of callus is found after fracture in the lower animals, but instead of the large amount being inherent to them, may it not be incidental to the single factor of excessive and prolonged movement allowing the dispersal of the osteoblasts through the rupture of the periosteum? In the lower animals the periosteum must generally be torn after fracture, owing to the violent movement ensuing. In the following instance, however, it seemed to have remained intact:—

Fracture in a Red Deer's Leg—with Periosteum intact.—A deer sustained a broken leg on attempting to get through a wire fence; the leg was seen afterwards dangling and the animal remained standing for a long time, apparently unfit to move. By and by it was able to feed, and it was allowed to remain on the low ground until it was capable of using freely its damaged leg.

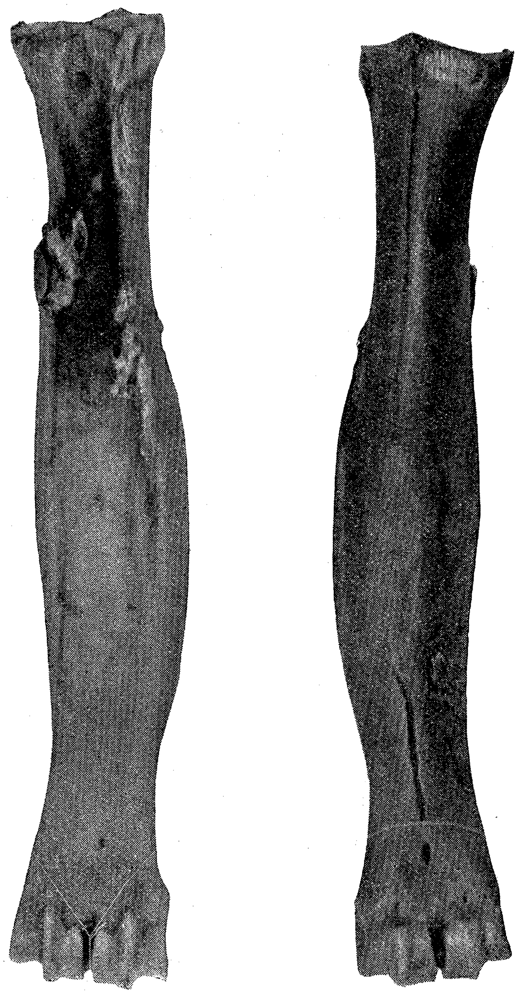
About 18 months subsequently it was shot on the hill and the specimen was sent to me. The periosteum had remained intact and the fracture was so perfectly healed that there was no evidence of external callus except a small spicule at the back.

Many human bones are not so compactly united after fracture. Here, however, the periosteum—the limiting membrane—had remained intact, and the animal had refrained from subjecting the broken part to much movement.

If a human limb be subjected to excessive and prolonged movement after fracture, with tearing of the periosteum, a great amount of callus is thrown out, quite in the same way as is so often seen in the lower animals.

Result of Free Movement after Fracture in Man.—The result of free and continuous movement after fracture in man is well illustrated in the case of a seaman who was shipwrecked in a storm in the Bristol Channel. He had his femur fractured in its upper third, and as he was unable to jump into the boats or to swim, he was tied to a spar which lay on deck.

He lay there, in a helpless condition, for 48 hours, his fractured leg being washed to and fro by every sea which broke over the wreck. He was then rescued in a semi-insensible condition, and taken on shore to a hospital, where he lay for many months. A formidable osseous mass grew in the muscles of the thigh, all round the fracture, and owing to its bulk and rapid increase, and also to the fact that the



FIGS. 5A and B.—Healing of fracture under the periosteum in the red deer.

bone remained un-united, the question arose in the minds of the surgeons who were treating him as to whether it was a tumour formation.

He was ultimately sent to me (1887). He was then unable to walk, from malposition and non-union of the fragments with great shortening of the limb, and also from the tumour-like mass of callus, which caused a large swelling in the middle and upper thirds of the thigh, and interfered with muscular movement.

On operation, it was found that the osseous tissue had infiltrated the torn muscular

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bundles, and was in such masses, many inches thick, that an extensive quarrying with chisel and mallet was required before the normal shaft was exposed.

The bone was placed in proper position and sutured, and rapidly healed, with only an inch of shortening. The torn muscles from which the osseous masses had to be detached were in many places so short that they were only brought together by plastic operation.

He was ultimately able to walk with slight halt, and to work for his living.

Here the amount of callus and bone formation in the soft tissues surrounding the fracture was as great as in any specimen seen by me taken from the lower animals.

Can a Bone, deprived for the most part of its Periosteum, continue to grow?

Doubtless, the deprivation of the periosteal blood supply would limit the peripheral increase of bone, just as the cutting off of the nutriment of any other part would

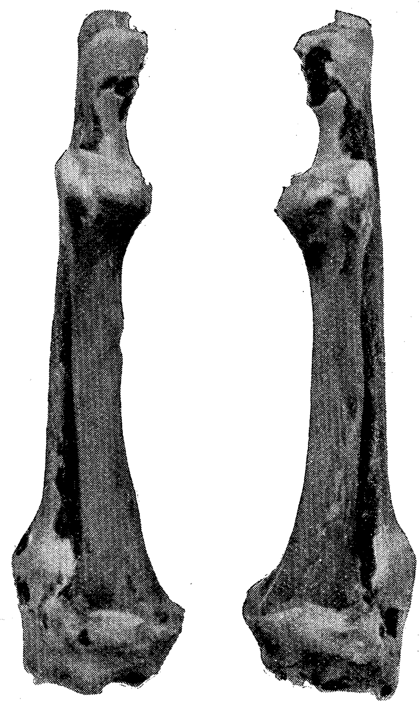

Fig. 6.—Showing that a shaft of bone, deprived for the most part of its periosteum, can continue to grow. Its fellow on the normal side is shown for comparison.

deduct from its development. Some believe that a portion of bone deprived of its periosteum must die. This does not necessarily follow, as many clinical facts show and the following experiment proves :—

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The Periosteum removed from the Entire Circumference of the Diaphysis of the Right Radius, the bone remaining in situ.

Dog A.—Had the periosteum removed from the entire shaft of the right radius, leaving only a quarter of an inch of periosteum on the diaphyseal side of the epiphyseal lines.

The operation was practically bloodless, aseptic healing ensued without visible scar or adherent cicatrix. Along with this specimen there is the corresponding left radius for comparison.

Description of Specimen as seen about 12 *weeks afterwards.*—The shaft of the bone was found to be entirely covered with a layer of newly-formed connective tissue, closely investing the bone and adhering to it much more firmly than periosteal tissue. It was found more difficult to detach this fibrous layer from the bone than normal periosteum. The bone was quite healthy, and had acquired an abundant new blood supply. It had, however, not increased circumferentially quite to the same extent as its fellow on the left side, but the difference in circumference did not amount to more than one thirty-second of an inch in some parts, and in others the two shafts were nearly alike.

Mosaic Work of Bone. Skull.

Can a flat bone, such as those of the cranial vault, continue to grow and its elements proliferate after it has been deprived of its periosteum and has been re-implanted? There have been many opportunities of testing this, from which the following observation may be cited :—

A weak, ill-fed boy, æt. 9 years, was admitted into Ward 29, Glasgow Royal Infirmary, in January, 1884, suffering from a compound comminuted fracture of the skull, with penetration of the brain substance, received about two hours previously by the fall of *débris* from a chimney. The brain symptoms are not referred to here.

There was a wound situated over the left side of the head of a somewhat crescentic shape, and extending from above the middle of the left eyebrow to an inch behind the auriculobregmatic line. The scalp was torn into several pieces, some of which lay over the ear. All of them were much bruised and lacerated. The skull was found shattered from an inch above the middle of the left eyebrow to a point half an inch behind the auriculobregmatic line. The depressed portion was somewhat elliptical, with very irregular margins. It measured, at its broadest part, $2\frac{1}{2}$ inches. All of these portions of bone were depressed below the level of the skull, most of them having penetrated the brain membranes into the brain tissue. These portions of bone were all elevated. It was found that they consisted of 11 pieces, the periosteum having been scraped by the injury from all of these, with the exception of the most posterior one, which was only partially denuded. Many of them were infiltrated with lime *débris*, brick-dust, etc.

These pieces, as they were elevated, were placed in an aseptic solution. They were then pared with a chisel in order to remove the *débris.* This was especially necessary over the external surface, where they had been scraped and ingrained with dirt. They were afterwards thoroughly washed in an aseptic solution, divided into fragments, and replaced. In this way a mosaic work of 14 pieces of bone was formed. It was difficult to retain these in position owing to four things. First, to the extent of the osseous defect; second, to the fact that the dura mater had been so extensively lacerated and torn that it formed a very irregular floor to rest the fragments upon; third, to the great bruising and crushing of the scalp, which rendered it difficult to bring the several pieces into apposition, and made sloughing of a part of it almost certain; and fourthly, to the force of the cerebral impulses, which caused a distinct movement of the fragments, producing crepitation by the one rubbing against the other. It was feared that, owing to these four circumstances, some of the fragments would be shed.

It is to be borne in mind that the periosteum had been by the injury entirely removed from all these fragments, except the most posterior one, and that most of them had to have their external surface pared with a chisel. The soft tissues were brought together as well as possible, and the wound was dressed.

On the sixth day after the operation the wound was examined. A portion of the anterior aspect of the flap, which was lacerated and contused, had sloughed, and already the process of separation from the living part had commenced. On the tenth day the wound was re-examined, and this portion of the slough was removed. It was then seen that four fragments of bone were exposed, two of which lay side by side, and presented a striking contrast. The one was suffused with the pinkish blush of life, the other with the pallor of death.

The condition of the remaining exposed fragments was doubtful, one of them, however, being very pale. On the 21st day, at the next dressing, two pieces of bone were found to have shed, while all the remainder had lived. The wound was all but healed. At the termination of a month it was firm.

Had that large osseous defect, about $2\frac{1}{2}$ inches in greatest breadth, extending from the middle of the left eyebrow to the auriculobregmatic line been left without this mosaic work of re-implanted bone, the cranial periosteum, if any of it was left at that part, would have failed to have covered the defect with bone, and a permanent fibrous covering transmitting the cerebral impulse would have marked the seat of injury. Yet, here we re-implant the osseous fragments, and the majority of them live, grow, and throw out ossific matter sufficient to unite them individually to one another and to the rest of the uninjured cranium.

Ten years after this operation the lad was examined. He was then 19 years old, strong and robust. The skull was firm all over, the bones over the site of prior injury had grown in proportion with the rest of the skull.

Growth of Bone in Muscle.

In the foregoing (and in some of the subsequent) observations attention has been called to the fact that muscles lying alongside of bone, which had been bereft of its periosteum, were apt to have the connective tissue surrounding their contiguous muscular fibres infiltrated with osteoblasts, and hence they became adherent to the fresh osseous layers forming on the outside of the shaft.

This was more marked where the nude bone was introduced in the shape of small shavings and the muscles were made to intimately surround them, and more especially where the connective tissue covering of the muscle fibres was lacerated. At such places the invasion of the osteoblasts was greater.

An instance of the growth of bone in the midst of torn muscle, at a distance from the source of the osteoblastic supply, may be here given, as its history is known, and it may throw light upon other cases of a similar kind.

It occurred in a strong healthy man aged 34 years, who fell from a short distance, the outside of his thigh striking against the edge of a steel plate. The femur was injured at its upper third, an indenture in the bone ensuing with the separation of a small portion of the shaft, but without destroying the continuity of the femur as a whole. The deeper layers of the intervening soft tissues on the outside of the thigh were severely injured and infiltrated with blood, which caused a swelling of the thigh, increasing slowly during the first 24 hours. This compelled him to maintain the recumbent position for some weeks, during which he had massage applied firstly by himself, and when it was seen that the swelling was not being reduced sufficiently quickly to satisfy the patient, the massage was undertaken by some of his vigorous fellow workmen, who had had "first-aid" lectures, and who continued it every night for some weeks.

Under this treatment the swelling subsided, and as it did so a hard lump was felt on the outside of the thigh—as they thought—not far from the skin. The patient was the first to notice the hard lump, and is positive that there was no such hard mass there before the injury. This was shown to the doctor who first examined the man after his accident, who at once detected it and said there was no such hard swelling there when he had first examined the limb before the thigh was swollen.

He examined the man fortnightly afterwards, and each time he found the osseous nodule increased in size. On the third occasion a second nodule was found on the upper part of the first nodule, and it also gradually increased in bulk. The man, however, though impeded in his movements, was able to work, and continued to do so until about nine months after the accident, when the bone mass, which had been growing all the time, had attained such a size that it greatly impeded him in walking and in his work. He was then sent by his medical attendant to me.

When examined by me there was on the outside of the thigh, in the situation of the vastus externus and apparently under the fascia lata, a hard tumour of bony consistence, which seemed to extend from below the trochanter major to the lower third of the thigh. The tumour was movable on the underlying structures, though somewhat attached to the fascia lata. Although it now seemed to form a continuous tumour, the upper part moved on the lower, and the patient showed that when he sat the upper mass was in a different relation to the lower mass than that which it occupied when he stood up. It seemed flexed when he sat, and straight when he stood erect.

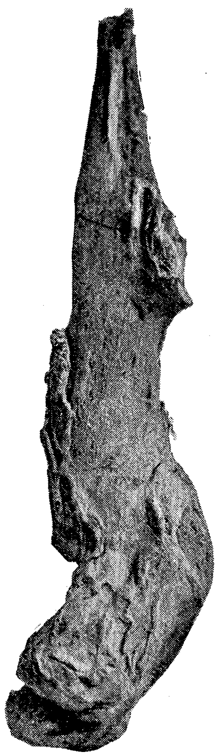

FIG. 7.—Mass of bone, 7 inches long, growing in the injured muscular tissues of the thigh, from osteoblasts poured out from the femur. Showing tendons enveloped at its apical part.

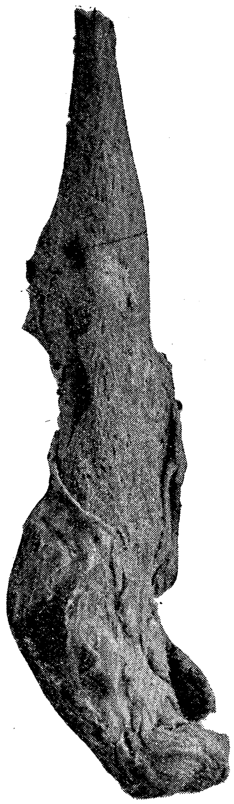

FIG. 8.—Another view of same specimen, showing apertures in the new bone through one of which a nerve, and through another of which a tendon, passed.

FIG. 9.—Upper portion of bone growing in muscles of thigh and united to lower by a new-formed joint.

During the operation it was seen that the osseous masses lay principally in the vastus externus under the fascia lata, and extended from a couple of inches below the trochanter major to within $3\frac{1}{2}$ inches from the condyles of the femur. They lay abutting each other, the upper measuring $3\frac{1}{2}$ inches in length and the lower one was 7 inches long—together they measured 10 inches in length. The lower fragment was $1\frac{1}{2}$ inches at its greatest breadth, while the upper was $1\frac{1}{4}$ inches.

They were both under the fascia lata and the tensor vaginæ femoris; some,

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however, of the fibres of the former fascia were caught in the interior of the bone; one elongated portion of the fascia was completely surrounded by bone.

Many muscular bundles of the vastus externus were included within the grasp of this ossific formation which impeded their movement, while one passed through a tunnel in the bone through which it worked, and the sides of which were polished as if by the continual gliding muscular movement.

The structure was, as a whole, very vascular. There were several vessels of large calibre—one fully that of the radial in its upper third, which passed through apertures in the bone as if the osteoblasts had formed round these vessels, making an archway for their accommodation. Though these vessels contributed to the vascular supply of the new bone, they were distributed mainly to the soft parts, and one of them came directly from the vicinity of the femur.

The upper bone mass lay near the notch in the femur, a portion of connective tissue separating the upper fragment from the bone. The lower mass was separated from the femur by muscular tissue, and lay nearer to the fascia lata.

It was interesting to see at the part where the two newly-formed bones came in contact that the surfaces fitted each other, and were polished and felt almost as smooth as if covered with cartilage. They were at this part surrounded by a capsule of fibrous tissue formed in strong bands, in one of which there was an osseous nodule somewhat like a sesamoid bone, flattened like the patella in shape. The fibrous capsule, when opened, was seen to contain a thin serum, which, though not of the consistence of synovial fluid, still aided in lubricating the polished osseous surfaces as they played over one another. Here were the elements of a joint formation between these two newly-formed bones which had grown in the midst of the muscles.*

Remarks.—Here was a case of limited injury to the bone causing an indentation in the shaft with the detachment of a small portion of the femur. This was accompanied by laceration of the muscles under the fascia lata, and by extravasation of blood. The fascia lata here acted as a limiting membrane. The osteoblasts poured out from the osseous hiatus, and the small fragment of the femur escaped into the surrounding tissues, where they probably would have formed a nodule of bone, cementing the detached fragment to the femur. The osteoblasts were not allowed to remain at rest in their normal position, but were scattered broadcast among the injured tissues by the vigorous massage of the "first-aid" taught workmen. The extravasated blood, while holding the injured tissues apart, afforded easy penetrability to the osteoblasts, and supplied temporary pabulum, which was augmented by the contact with the blood-vessels of the part.

If a strip of periosteum, while retaining its blood supply, be partially detached

* A somewhat similar instance was seen by Dr. P. PATERSON, Surgeon, Glasgow Royal Infirmary, in which the specimen, though much smaller than the above, was found in the muscles of the thigh after injury to the femur, though the new bone in Dr. PATERSON's case was attached in two places to the femur.

from the bone and carry osteoblasts or bone plaques with it, bone nodules may form from the proliferation of such detached osteoblasts; but it would be wrong to deduce therefrom that the periosteum was the generator of those osteoblasts.

Tendons which are directly inserted into bone, without the intermediary of the periosteum, are, under exceptional circumstances, liable to osseous infiltration. When such tendons are subject to violent strain, this is transmitted to the bone in which they are inserted. The traumatic irritation thus produced causes a proliferation of osteoblasts, which penetrate the partially ruptured, loosened, and retracted fibres of the tendon, and set up ossification in their midst. Some of the fibres of the tendon, which were in contact with the bone, may retract into the tendon, carrying osteoblasts from their point of insertion. Riders bone results in this way.

Is it possible that, after injury or other cause of osteoblastic proliferation, the osteoblasts, under very exceptional circumstances, may gain access to the lymph or blood-vessels and be carried to, and deposited in, distant parts where ossification may be established from proliferation of the cells? It is probable that some forms of myositis ossificans may be thus produced. Many cases of myositis ossificans occur subsequently to an injury in which the bones are involved.

Can Shavings of Nude Bone continue to grow after being placed between the Muscles, and can they unite together and form again the continuity of the Shaft?

Shaving Experiments. Dog N.

The shaft of the radius was removed, with the exception of half an inch on the diaphyseal side of the epiphysis. The periosteum was then stripped off from the portion of the shaft taken away.

Two portions of the removed periosteum, about half an inch in length and a quarter of an inch in breadth, were transplanted. One was rolled upon itself and placed under the cellular tissue of the back of the head behind the left ear. The other was placed on the aponeurosis covering the cranium near to the right ear. This latter portion was spread out over the aponeurosis.

The shaft of the bone removed, destitute of its periosteum, was then cut into very fine shavings, and these shavings were placed between the muscles bulging into the gap in the bone left by the removal of the shaft. The neighbouring muscles were then stitched over the bone shavings in order to keep the shavings in position, and especially to prevent them being extruded from the wound.

The wound healed aseptically, without visible scar. The rolled-up portion of periosteum could be detected under the skin behind the left ear. Three weeks after the operation it was thought to have diminished, and this diminution continued till the end of the fifth week, when the roll of periosteum could no longer be felt externally.

Three weeks after the operation there was apparent union between the bone shavings and the shaft, and a thickening was forming at the part where they had been introduced. This thickening continued to increase in bulk until the end of the seventh week, when the experiment ceased, sufficient time having been allowed to show the result. The animal had grown considerably during the seven weeks.

Examination of the Specimen obtained seven weeks after operation.

There was no naked eye trace of external wound in the skin. The continuity of the shaft was entirely restored. There was a marked increase in the diameter of the shaft opposite the part where the shavings had been inserted.

The muscles over the new bone had become adherent to it and, at the parts where they were contiguous to the bone, their fibres were infiltrated with osseous tissue. So that the longitudinal fibres of the muscle had become imbedded in new bone, and these muscular bundles formed the only periosteum which the bone had at these places. At other parts of the circumference the new bone was surrounded by newly-formed connective tissue.

When the bone was exposed, the part where the re-implantation of shavings had taken place was represented by a marked circumferential increase in bulk of the shaft—almost tumour-like in proportion—with irregular surface, where the various ends of the shavings presented externally. All the component parts had become fused into one another and to both ends of the shaft. The actual measurements are :—

Shavings, No. 1.

Right Radius.

Length of shaft—$4\frac{14}{16}$—nearly 5 inches.

Greatest diameter of nodule antero-posteriorly, $\frac{15}{16}$, almost 1 inch.

Greatest diameter of nodule transversely, $\frac{14}{16}$ inch.

Diameter of shaft below expansion ($\frac{1}{2}$ inch), $\frac{8}{16}$ inch.

Left Radius.

Length of shaft, $5\frac{2}{16}$ inches.

Greatest diameter in centre of shaft, $\frac{7}{16}$ inch (not quite $\frac{1}{2}$ inch).

So that the antero-posterior diameter of the nodule was nearly double that of the normal diameter of the shaft and exactly double that of the transverse diameter.

Dog M. Shavings and Marrow, and what could be expressed from the Bone, No. 2.

Right Radius.

Length of shaft, $5\frac{3}{16}$ inches.
Greatest diameter of nodule antero-posteriorly, $1\frac{2}{16}$ inches.
Transversely, $1\frac{4}{16}$ inches.

Left Radius.

Length of shaft, $5\frac{9}{16}$ inches.
Greatest diameter of shaft antero-posteriorly, $\frac{6}{16}$ inch.
Transversely, $\frac{9}{16}$ inch.

So that the antero-posterior diameter of the nodule was three times greater than the corresponding diameter of the normal shaft and more than a-half greater than the transverse diameter of the normal shaft.

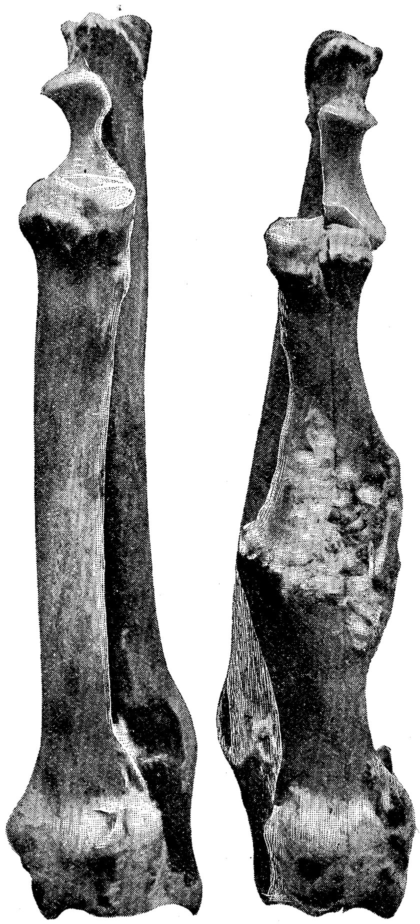


FIG. 10.—Shaving experiment. Marked increase in circumference of shaft, almost tumour-like in bulk, from proliferation of osseous tissue growing from bone-shavings. The corresponding normal bones are given for comparison.

FIG. 11.—Shaving experiment. The same in reverse, showing that the increase in osseous bulk has assumed aggressive character, causing pressure and absorption of neighbouring bone.

This mass of bone had so far outgrown its normal proportions as to have exercised pressure upon, and caused a marked flattening and indentation of, the ulna. Just as one sees atrophy and deformity arising in neighbouring bones from pressure effects due to sarcomatous tumours originating in another bone.

In making a longitudinal section of the shaft, one sees a number of ossifying centres surrounded by cartilage occurring in the interior of the part where the osseous shavings were inserted, and these are somewhat demarcated by thin layers of cartilage from the growing shaft on either side.

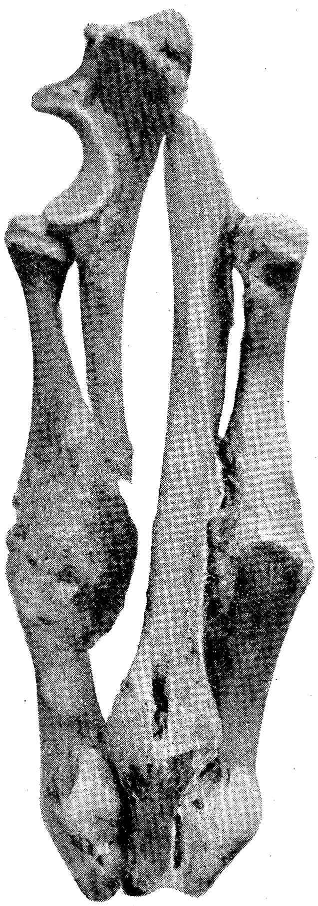


Fig. 12.—Shaving experiment. The specimen in section, posterior view. Contrast the proximal and distal portions of the shaft with the increase in bulk of the grafted shavings.

Fig. 13.—Shaving experiment. Front view of specimen in section showing ossification proceeding from many centres and all becoming fused together, and to the end of shaft.

Comment.—Each of these living osseous particles introduced into the soft parts and between the bones would quickly become bathed with abundance of serous pabulum, which would be sufficient for their immediate wants.

This would be followed by the rapid proliferation of new blood-vessels, not only from the surrounding soft parts, but also from the cut ends of the existing shaft, which later would doubtless be followed by osteoblasts which would aid in forming the bulk and in soldering the adjacent bone spiculæ to the shaft at both ends. The bone cells in the spiculæ, once their vitality was restored, would proliferate and, being set free in the periphery, would quickly form increase in osseous bulk, each spicula supplying its little quota to filling up the interspace, and collectively to the formation of the bulk.

How much of the great increase in bulk is to be attributed to the growth from the cut ends of the shaft, and how much is due to the proliferation of the individual osseous shavings, would be impossible to apportion. The vegetative capacity of the bone cell is as great as that of the epithelial cell, and if one grants not only the viability of the transplanted epithelium, but also its power of extensive proliferation, then, judging by analogy, the bone cell ought to show, as it has done in this instance, equal capability of living and growing when transplanted. The smaller the graft the greater the proliferation.

Bone Growing in Sponge.

Is there any direct evidence to show that transplanted living bone actually grows and proliferates instead of forming, like blood-clot, a passive framework for the granulation tissue to penetrate, and which framework will then be absorbed?

In this relation an interesting fact was observed, where a minute portion of bone broken off from the deeper layers of a new osseous growth, far removed from any periosteal connection, was found growing in the midst of a sponge filled with granulation tissue.

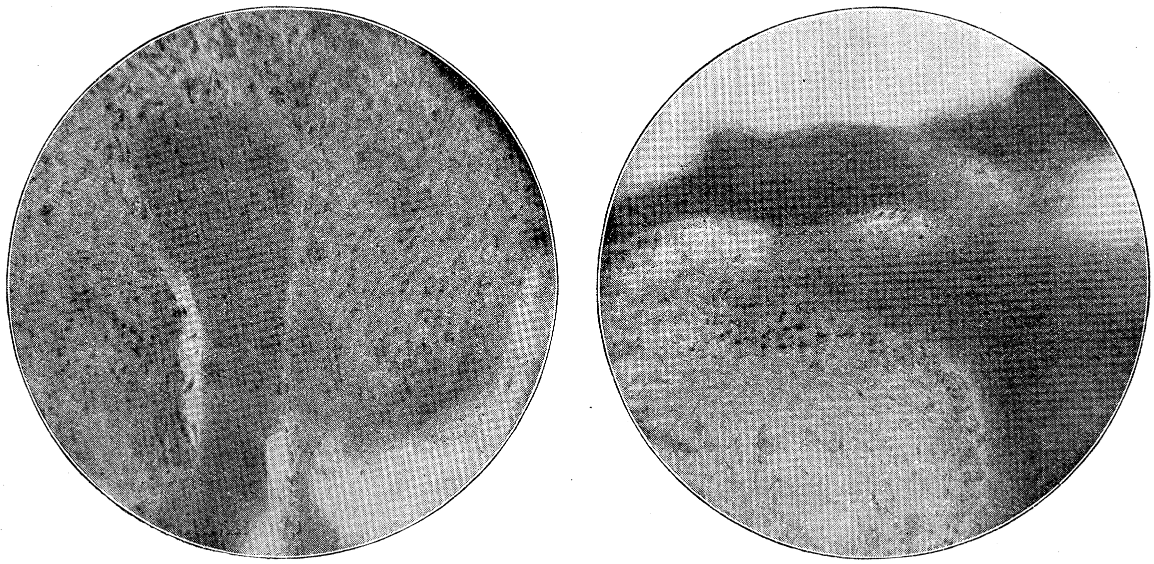
After removal from the fibula of a central sequestrum, measuring 3 inches in length, a hollow cylinder of new osseous tissue was left. Into this hollow cylinder a piece of decalcified, aseptic, specially prepared sponge (Professor Hamilton's method) was being introduced, when it became entangled on an osseous spicule projecting from the central aspect of the new bone into the lumen of the hollow cylinder.

This spicule became embedded in the sponge so firmly that it was detached from its osseous connections, when the sponge carrying the spicule entangled in its meshes was introduced fully an inch further into the lumen of the osseous tube and left there.

At the end of 11 weeks, when for certain reasons the sponge was removed, it was found filled with granulation tissue, in the midst of which the spicule of bone was seen, and from the extremity of the spicule, which was most deeply buried in the sponge, a knob of what appeared like ossifying cartilage projected.

The sponge and its contents were carefully examined throughout, but there were no other evidences of the presence of bone or ossifying cartilage. The whole spicule was three-sixteenths of an inch in length, and the ossifying knob, which projected from its extremity, was almost one-eighth of an inch in greatest diameter. When this specimen was decalcified and microscopic sections were made, it was seen that the knob consisted of new bone growing from the osseous spicule into the meshes of the sponge framework, and granulation tissue with islets of new bone which were being increased in bulk by peripheral osteoblastic augmentations. There was no evidence of prior cartilage formation, the ossification seeming to form directly from the osteoblasts. At one part toward the periphery, however, there were traces of cartilage cells in process of devolution, followed at a short distance by bone formation.

Toward the base of the spicule, that portion nearest the bone comprising the osseous cylinder, the bone spicule presented lacunar defects filled with leucocytes. The portion of bone nearest the periphery and, therefore, perhaps, subject to friction occasioned by the arterial pulsations intensified in a confined space, was undergoing absorption, while its opposite and most central extremity was throwing out ossifying cartilage from the osseous spicule.



Bone grown inside of a decalcified sponge filled with granulation tissue.

FIG. 14.—50 diameters. Showing new osseous spicule with bone cells and osteoblasts in midst of granulation tissue.

FIG. 15.—150 diameters. Showing bone islands with osteoblasts at periphery in process of bone formation.

This growth of bone took place under adverse circumstances at a distance from immediate contact with other bone, subject to continual pulsating movements, and in the midst of a sponge filled with granulation tissue.

There was here no possibility of periosteal connection; there was no bone marrow, as the central sequestrum had just been removed and the whole cavity had been thoroughly washed with a free stream of watery solution of carbolic acid, prior to the introduction of the sponge.

The bone formation, though actively occurring in the sponge, was scattered and

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less purposive than usual, as if the osteoblasts, while attempting to accommodate themselves to their adverse conditions, had been somewhat confused and indefinite in design.

Bone Growing in Glass Tube.

Glass Tube Experiment.

In order to test the osteogenic power of the bone cells constituting the shaft of a long bone, the following experiment was made :—

A portion of the entire circumference of the shaft of the canine radius, measuring $1\frac{1}{8}$ inches, was removed along with its periosteum. Into the gap thus left a glass tube was inserted in such a manner that the distal extremity was firmly fixed into the centre of the osseous tissue of the shaft, while the proximal part abutted against the centre of the shaft above. Some days after, the proximal portion of the tube slipped from its connection with the shaft and lay against the muscles. This relation persisted, though the lower two-thirds of the tube was rapidly surrounded by an osseous mass growing from the shaft, which held it firmly in position, while the free end could be felt under the skin with a portion of muscle intervening. The healing was aseptic, without visible scar.

The specimen was examined eight weeks and five days after.

The glass tube was found firmly imbedded in new-formed osseous tissue, except half an inch which projected beyond the newly-formed bone and abutted on the overlying muscles. Between the projecting portion of the tube and the muscles there was an intervening connective tissue layer which covered the upper portion of the tube and its mouth like a living capsule. Within this capsule, which was translucent, there was a straw-coloured serous fluid which communicated with a drop of fluid within the distal extremity of the tube. The small quantity of serous fluid in the tube bathed the solid-looking contents within.

On splitting the living capsule, a few drops of serous fluid escaped. The surface of the inside of the capsule—that which was next the glass—was smooth and almost polished. This connective tissue which formed the capsule was continuous with the connective tissue which covered the new bone over the shaft, and yet there was no trace of osseous formation in the capsule. When the capsule was opened, it was found that the glass tube was almost filled with a greyish-red, firm, resisting substance which, when touched by the probe, conveyed the sensation of a somewhat cartilagenous structure.

The entire lumen of the exposed portion of the tube was filled with this dense material, with the exception of its most distal aspect, where there was sufficient space left to accommodate the drop of serous fluid which occupied it, when the capsule was opened, and which of course communicated with the fluid inside the capsule.

The shaft of the radius was then divided into two in order to expose the relations of the tube to the deeper parts. It was seen that the greater portion of the tube was firmly imbedded in the new bone, and that the distal extremity of the glass tube was fixed in the very centre of the diameter of the shaft of the radius at a part where the regeneration of bone was vigorous. The ossific material from the centre of the shaft grew directly into the glass tube and filled it. The material inside the tube could only receive its pabulum and nutriment from the centre of the shaft, as, once inside the tube, the sides of the glass prevented it from coming into contact with the living tissues of the neighbourhood.

Yet, with this constricted area for blood supply, the ossific material grew inside and filled the tube, and thus reached a level beyond the extent of the growth of bone outside of the glass tube.

It is possible that the relief of pressure of the soft tissues aided the ossific material to form more quickly inside of the tube than outside, and the capillarity of the tube might have facilitated physically the entrance of the osteoblasts.

Microscopic Examination.—A portion of the solid contents were removed from the distal part of the glass tube, the section being made by introducing a knife within the glass tube which, after some difficulty, owing to the resistance offered, severed the tissue.

On viewing the cross-section of the portion thus removed, it seemed to consist of an inner and an outer zone of tissue, differing in appearance the one from the other. The inner zone was reddish-grey and somewhat softer than the outer zone. The outer was firm and had a greyish-white colour.

Under the microscope, the centre was seen to be made up of highly vascular connective tissue, with here and there traces of osseous formation. The peripheral zone presented a loose areolar reticulum, enclosing many blood capillaries interspersed by numerous islands of bone in process of formation. Most of these bone islands were placed on the external zone of the section with thin walled blood-vessels of large size on their central aspect and minute blood capillaries on their periphery. Some of the bone islands, however, abutted on the rim of the glass tube with blood-vessels on their central aspect only. In these cases the osseous tissue lay against the lumen of the glass tube with no other tissue intervening.

When the sections from the more proximal portion of the tissue in the tube were examined, it was seen that the islands of bone became more numerous and encroached on the centre of the specimen—while the peripheral portions of osseous tissue were better formed than those at the more distal part. The proximal part of the tube was filled with well-matured bone, continuous with the centre of the shaft. This part was left *in situ* and not submitted to microscopic section.

Comment.—In this instance the bone grew into the glass tube from the osseous tissue in the very centre of the shaft, to the exclusion of all other sources. Neither periosteum nor the surrounding soft tissues could have aided in the production, as access by them to the interior of the tube had not occurred. One sees that the

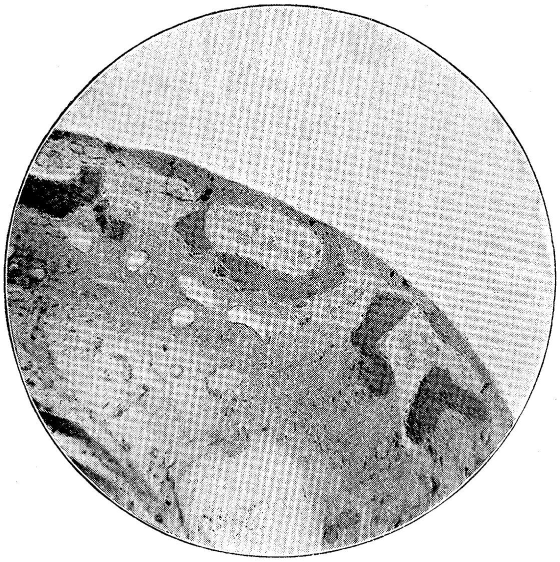

FIG. 16.—Microphotographs of bone growing inside of glass tube, 50 diameters. Section taken from most distal part of new growth, showing peripheral bone formation abutting on the glass of the tube, while the central portion of the contents consists of loose areolar tissue—containing many thin-walled blood channels.

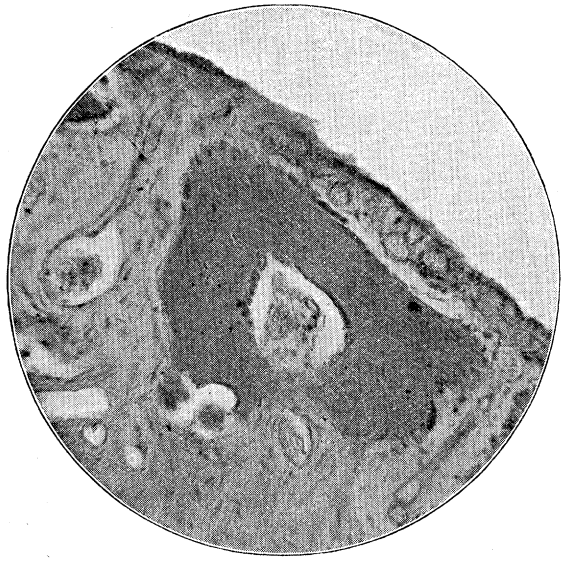

FIG. 17.—Bone growing in glass tube, 150 diameters, showing well-formed bone island with osteoblasts in periphery and in centre, and capillary blood-vessels intervening between glass and bone island.

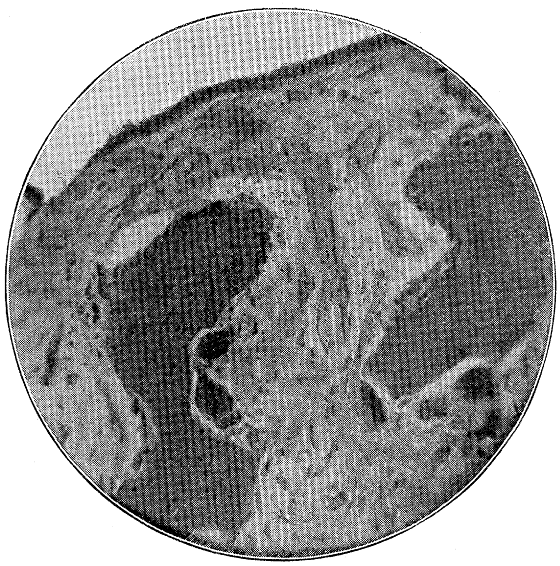

FIG. 18.—Bone grown in glass tube, 150 diameters, showing islands of bone and osteoblasts in process of bone formation and several giant cells.

growth of bone enclosed in the glass tube has taken place first in the periphery, and secondly centripetally. In this case no increments could be deposited on the outside after the first deposition, as the glass tube limited them in that direction.

It was also obvious that the bone cells were deposited abundantly where the

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capillaries were numerous; and where blood-vessels with thick walls alone existed, the osteoblastic development was restricted or absent. One large vessel with thick walls, which formed the nutrient artery of the bone in the glass tube, was not surrounded intimately by bone, though doubtless it supplied the nutriment for osseous development which flowed through the capillaries.

Intra-Human Transplantation of Bone.

Case and Result 28 *Years after.*

A paper on intra-human transplantation of bone was read by me before the Royal Society on May 3, 1881, and published in the 'Proceedings' of the Society in the same year (May 19, 1881, p. 232). Academy of Science, Paris, same year. Glasgow Royal Infirmary, 1878.

It is now 28 years since the humeral shaft was rebuilt, and during the greater part of this period the man has depended upon his physical exertions for the earning of his livelihood. He worked as a joiner for many years, and now is an engineer's pattern maker. His arm has increased in length, but not proportionate to the increase of his left arm—the sound one.

Measurements.—The grafted humerus measures, from tip of the acromion to tip of internal condyle, 10 inches, but following the curve in the bone, it is 11 inches long. The sound humerus from same points measures 14 inches—3 inches longer than the other.

Photos: X-ray.—A skiograph* shows that the increase in length of the affected arm has taken place almost entirely from the proximal epiphysis, as the new bone has been interposed between the proximal epiphysis and that portion of bone which grew from the transplantation. All but a minute portion of the distal epiphyseal cartilage was destroyed at the time of the osteomyelitis and, consequently, little growth in length would be expected from this extremity.

The increase in length of the diaphysis, which has occurred from the proximal epiphyseal cartilage, may be taken as an index of the amount of growth which usually occurs from the proximal humeral epiphysis.

Data bearing on the Increase in Length and Breadth of Bone.

These facts corroborate and supplement some of the deductions made by John Hunter from experiments performed by him on the lower animals. He bored two holes in the tibia of a pig, one near the upper end and the other near the lower; the space between the holes was exactly 2 inches. A small leaden shot was inserted into each hole. When the bone had increased in length by the growth of the animal the pig was killed, and the space between the shot was exactly

* Kindly taken by Dr. Macintyre, Glasgow.

2 inches. He inferred that "bones are not elongated by new matter being interposed in the interstices of the old."

Probably what is meant is that new additions of bone will be made to the length of the diaphysis from either epiphysis. To that extent the case detailed here agrees and shows that the diaphyseal increase in length occurs in the same way in man.

In this human case, however, the distal epiphyseal cartilage had been rendered in great measure functionless by disease, and the grafted portion, which was at first contiguous with the distal epiphysis, has remained nearer that end, while the increase in length has occurred mainly from the proximal epiphysis and, consequently, the new bone has, for the most part, formed between this epiphysis and the grafts. At the same time it is interesting to observe that, though the distal epiphyseal cartilage was, for the most part, destroyed, the epiphysis has increased greatly in bulk, though it is probably not quite of normal size. The grafted portion of tissue, which is easily recognised from the rest of the shaft by form and contour, has increased markedly in thickness and also somewhat in length, so that there has been here interstitial osseous increase.

It is presumed by some that the increase in length of the diaphysis comes mainly from the epiphyseal line toward which the nutrient vessel runs. In the present case the length of the humerus from the tip of the acromion to the internal condyle is 10 inches, and from the same points on the sound limb the measurement is 14 inches. If the measurement is taken following the humeral curve on the grafted humerus, then the length is fully 11 inches and, perhaps, this is the fairer measurement, if the increase in bone has to be considered.

After the $4\frac{1}{4}$ inches of bone had been added to the limb by grafting, 28 years ago, the length of the humerus then measured fully 6 inches. If the measurement following the curve of the bone as it is now, be taken, then the length at present is 5 inches more than formerly, namely, 11 inches. The greater portion of this increase in length has come from the proximal diaphysis, but still not the whole, as there has been an interstitial growth between the fragments of the transplant, as their original form has been altered, and the irregularities constituting the various parts have been separated from one another by interposition of new osseous tissue. Though there are no positive measurements to go on relatively to the increase of interstitial growth in length of the part which had been grafted, still 1 inch would be a rough estimate of that increase—the part that had been grafted would now measure about $5\frac{1}{4}$ inches in length instead of $4\frac{1}{4}$ inches as formerly. This leaves 4 inches of new growth to have come mainly from the proximal diaphysis. It also leaves 3 inches of shortening between the length of the sound and that of the grafted arm. Could this 3 inches have been made up were the distal epiphysis in normal condition? If so, the increase in length from the proximal epiphysis (if it were normal in this case) would only have been 1 inch less than that of the distal.

Conclusion.

From the foregoing observations and experiments it may be deduced that bone is reproduced by the proliferation of osteoblasts, derived from pre-existing osseous tissue, and that its regeneration takes place independently of the periosteum. While not underestimating the periosteum as a limiting and protecting membrane of great use in physiological and pathological conditions, there are no data to indicate that it can of itself secrete or reproduce bone.

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IG. 1.—Preservation of periosteum and removal of portion of shaft of bone. The specimen shows result 10 weeks after. The portion of the shaft which was removed sub-periosteally is seen between the two larger specimens. The figure on the right is the radius and ulna of the normal limb for comparison.

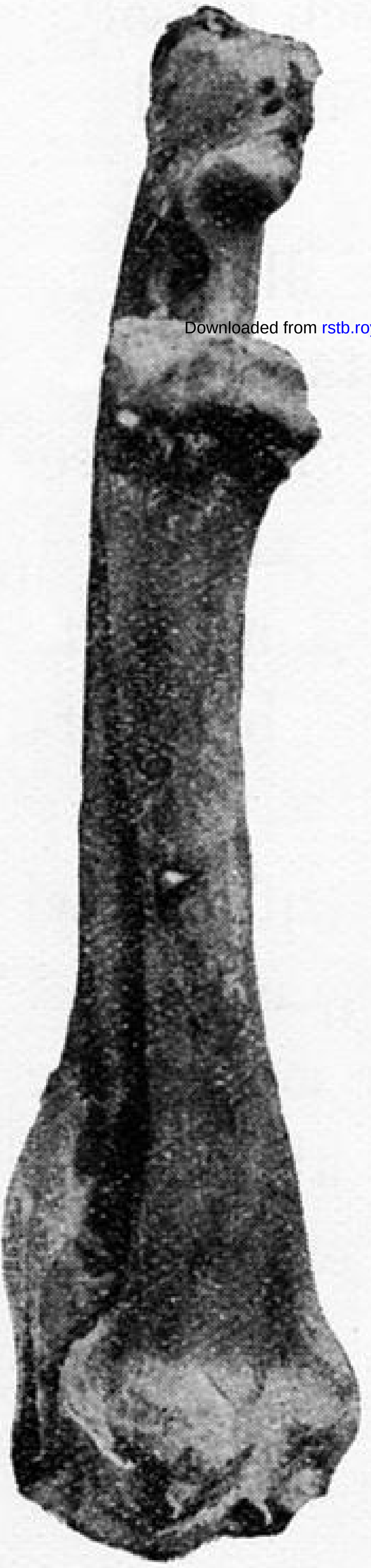

FIG. 2.—Silver ring experiment No. 1. Specimen shows the silver ring exposed by removal of enveloping portion of bone.

FIG. 3.—Silver ring experiment No. 2. Both silver rings are invisible, being completely covered with bone.

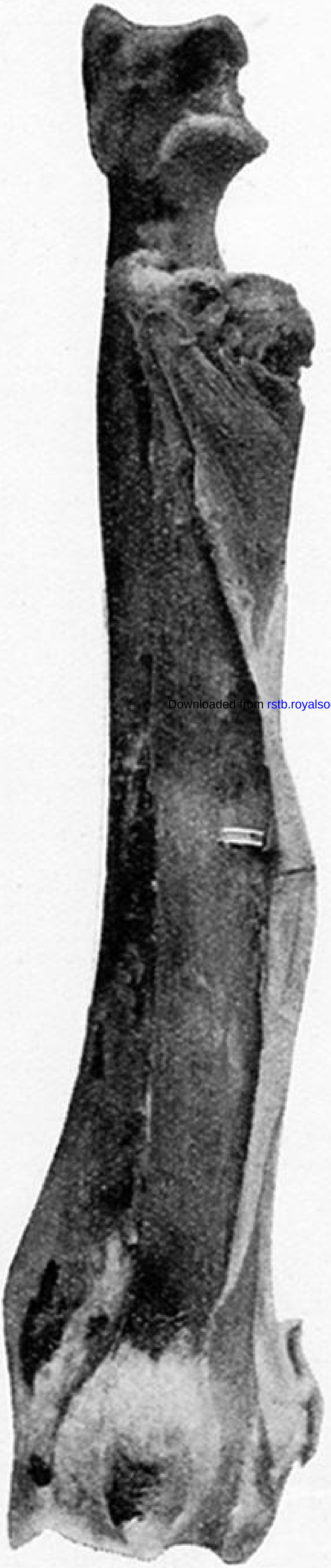

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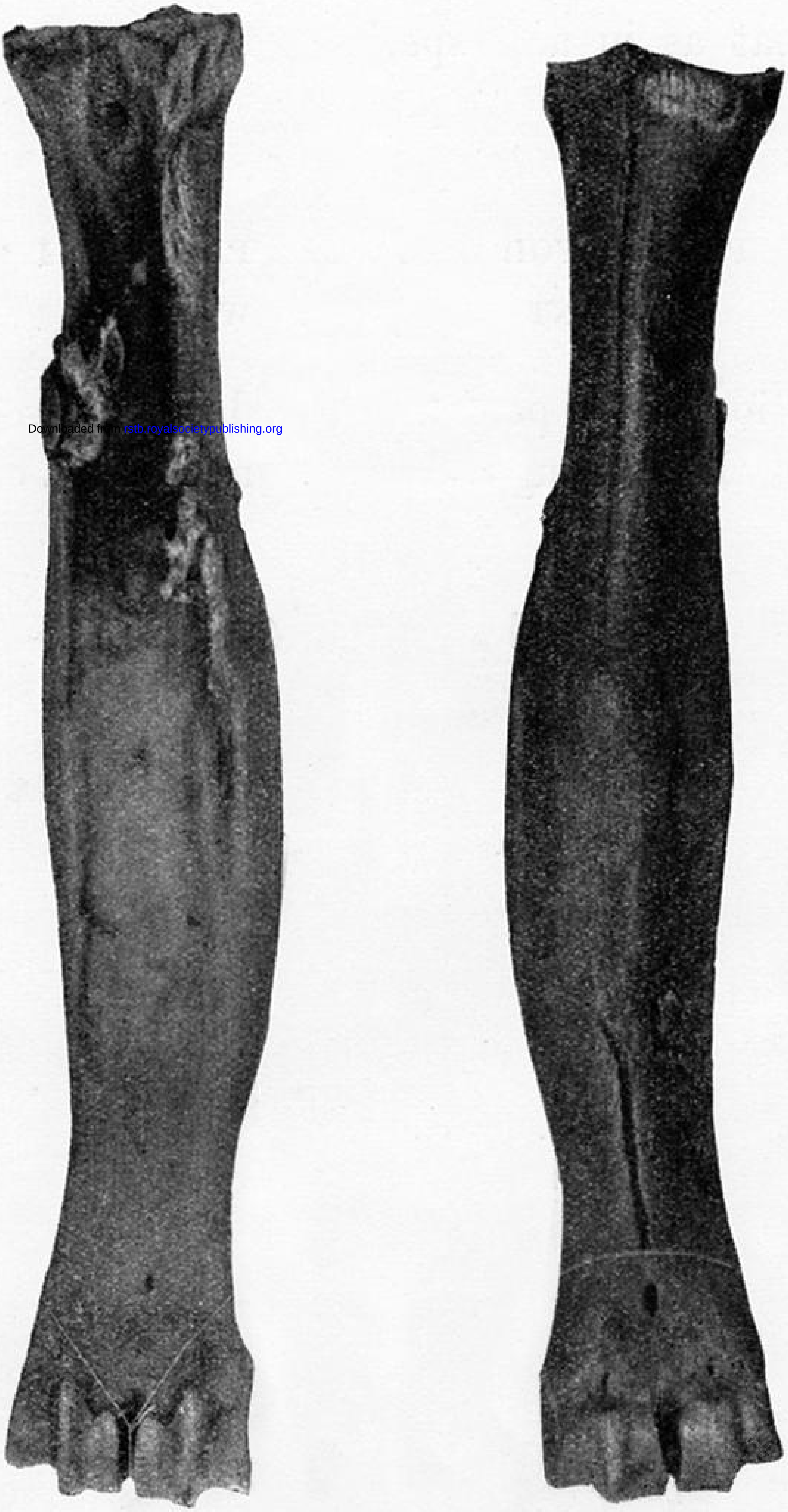
FIG. 4.—Silver ring experiment No. 3, showing gap between bone and silver ring filled with new osseous tissue from the denuded bone.



[F]IGS. 5A and B.—Healing of fracture under the periosteum in the red deer.

IG. 6.—Showing that a shaft of bone, deprived for the most part of its periosteum, can continue to grow. Its fellow on the normal side is shown for comparison.

FIG. 7.—Mass of bone, 7 inches long, growing in the injured muscular tissues of the thigh, from osteoblasts poured out from the femur. Showing tendons enveloped at its apical part.

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FIG. 8.—Another view of same specimen, showing apertures in the new bone through one of which a nerve, and through another of which a tendon, passed.

[F]IG. 9.—Upper portion of bone growing in muscles of thigh and united to lower by a new-formed joint.

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IG. 10.—Shaving experiment. Marked increase in circumference of shaft, almost tumour-like in bulk, from proliferation of osseous tissue growing from bone-shavings. The corresponding normal bones are given for comparison.

FIG. 11.—Shaving experiment. The same in reverse, showing that the increase in osseous bulk has assumed aggressive character, causing pressure and absorption of neighbouring bone.

FIG. 12.—Shaving experiment. The specimen in section, posterior view. Contrast the proximal and distal portions of the shaft with the increase in bulk of the grafted shavings.

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G. 13.—Shaving experiment. Front view of specimen in section showing ossification proceeding from many centres and all becoming fused together, and to the end of shaft.

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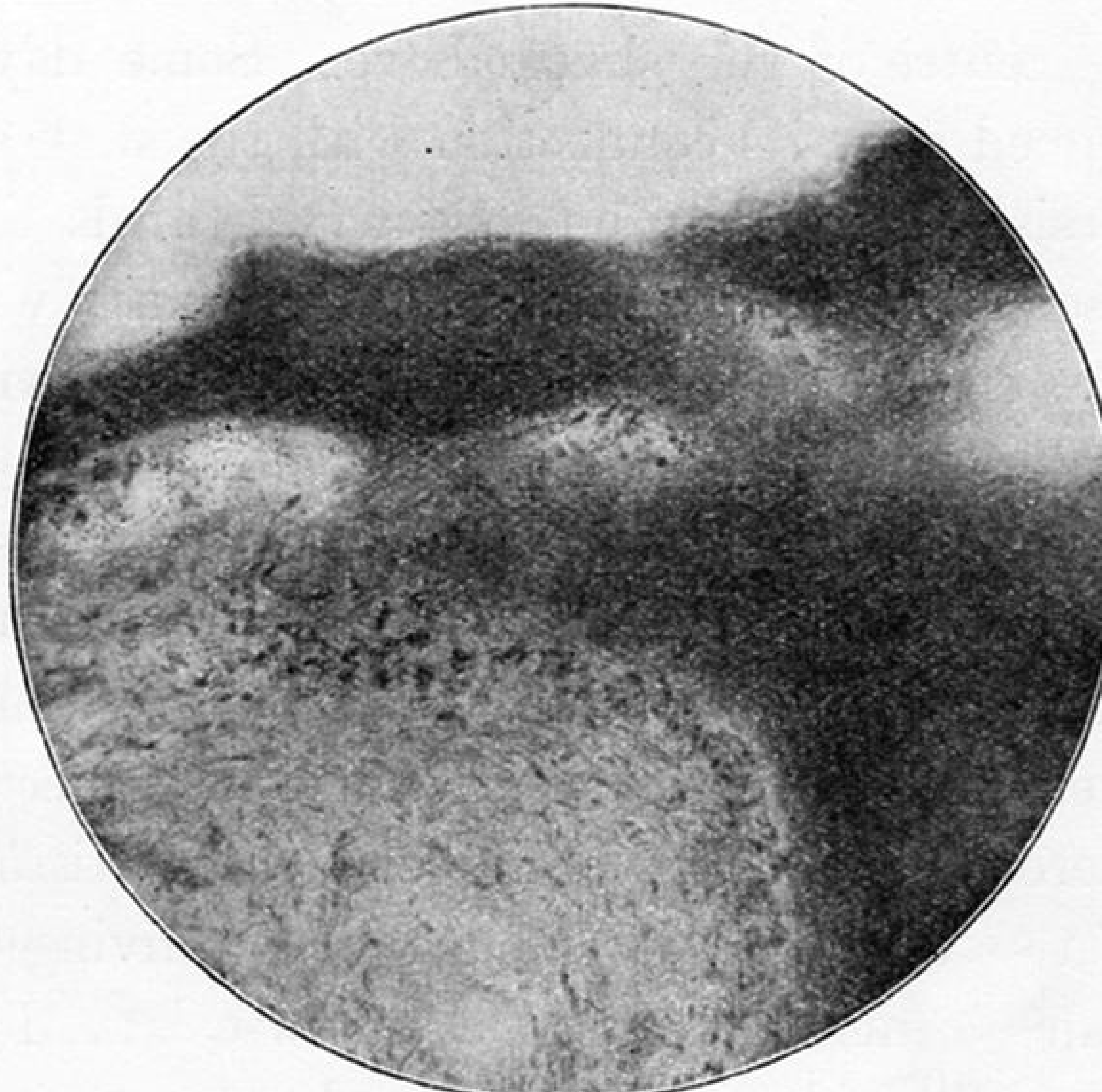

Bone grown inside of a decalcified sponge filled with granulation tissue.

FIG. 14.—50 diameters. Showing new osseous spicule with bone cells and osteoblasts in midst of granulation tissue.

FIG. 15.—150 diameters. Showing bone islands with osteoblasts at periphery in process of bone formation.

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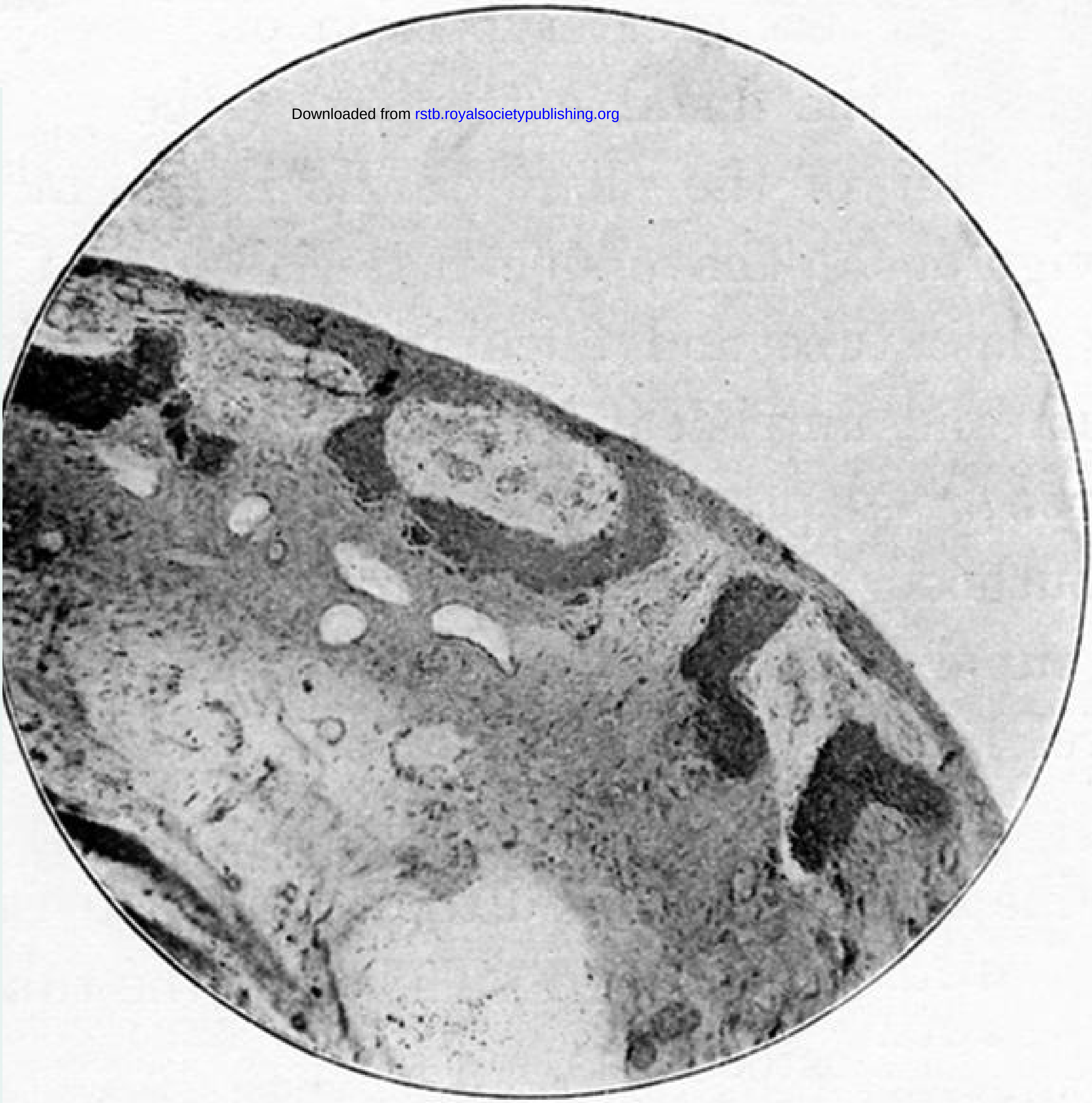

FIG. 16.—Microphotographs of bone growing inside of glass tube, 50 diameters. Section taken from most distal part of new growth, showing peripheral bone formation abutting on the glass of the tube, while the central portion of the contents consists of loose areolar tissue—containing many thin-walled blood channels.

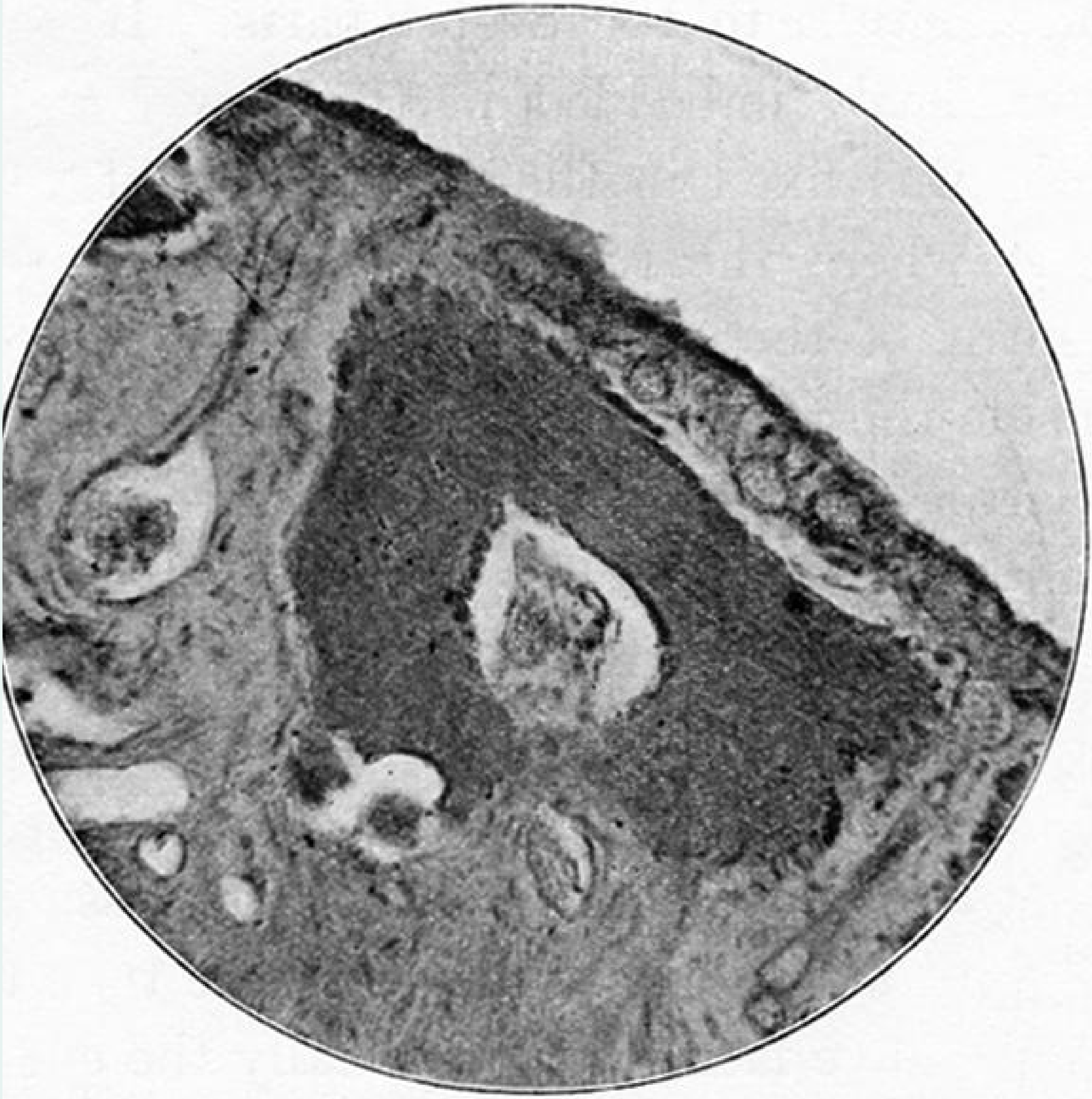

Fig. 17.—Bone growing in glass tube, 150 diameters, showing well-formed bone island with osteoblasts in periphery and in centre, and capillary blood-vessels intervening between glass and bone island.

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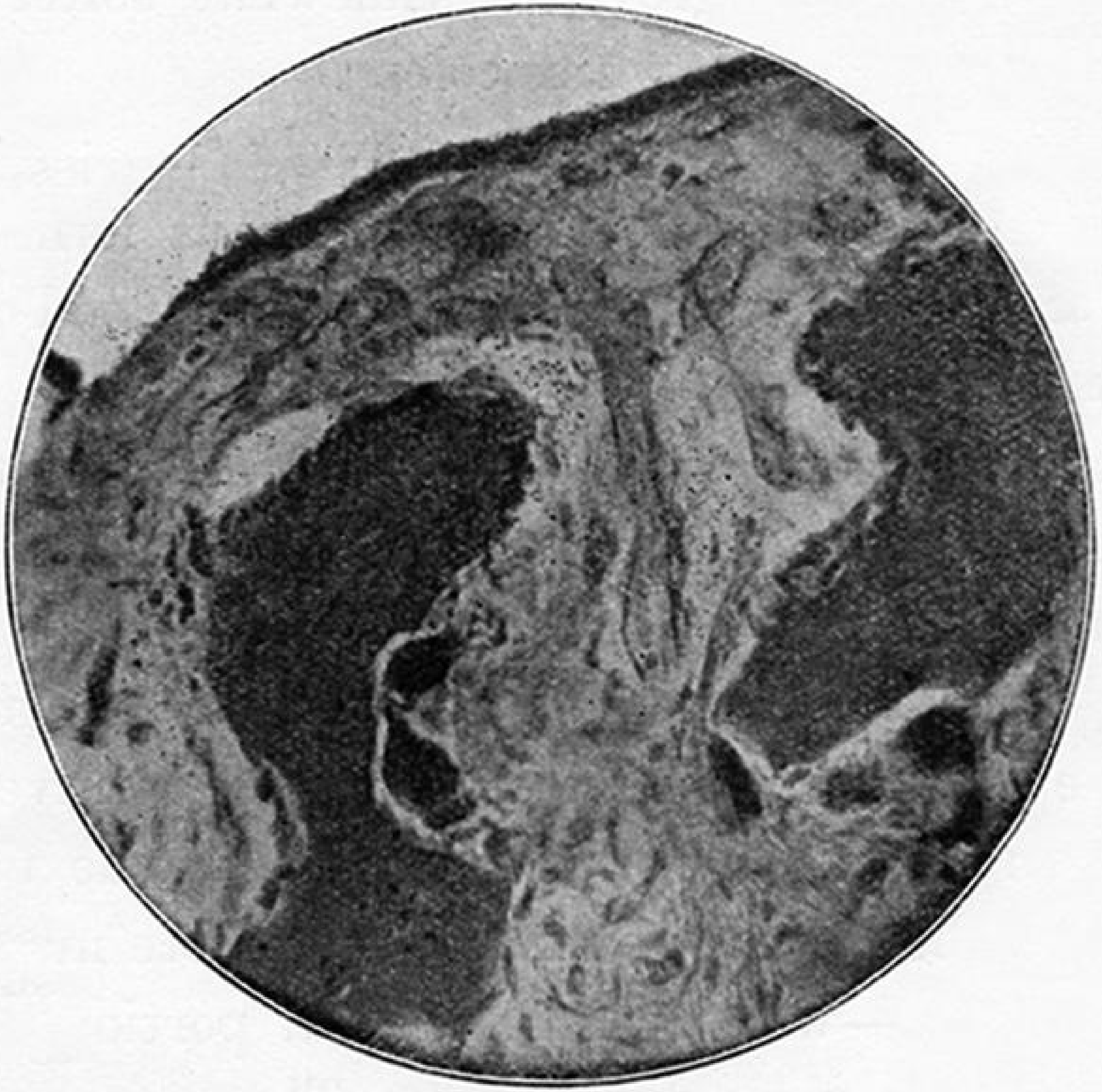

IG. 18.—Bone grown in glass tube, 150 diameters, showing islands of bone and osteoblasts in process of bone formation and several giant cells.

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