STUDIES IN BONE GROWTH

TRIPLE CALCIUM PHOSPHATE AS A STIMULUS TO OSTEOGENESIS

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In presenting the experimental findings included in this paper, the author wishes to express appreciation of the resources in organization and equipment made possible through the generosity of the Surgeon General who permitted the addition of an animal research annex to the Laboratories at U. S. Army General Hospital No. 3. Such equipment and efficient technical assistance allowed the continuation of investigations in bone growth and allied subjects, from the early days of this hospital's establishment.

These animal experimentations were carried on coincidently with our clinical bone work at the hospital, with the purpose of supplementing earlier studies,[1] made about ten years ago at the Cornell Animal Hospital, relative to various phases of bone growth. Those earlier experiments included the fusing of the vertebræ of a dog by means of the inlay graft, as well as investigation of the relative osteogenesis of bone secured from different portions of the anatomy of dogs and rabbits. Other experimental findings emphasized the high osteogenetic potency of the periosteum when removed *completely,* which was quite possible if secured by *scraping* with force the outer surface of the bone with a sharp instrument. Studies were also made which bore out the inadvisability of attempting transplantation of bone from one species into another, such as dog's tissue into a sheep, or sheep's bone into a rabbit.

Among our early animal researches at U. S. Army General Hospital No. 3 have been studies in the etiology of pseudarthrosis,[2] in which attempts were made to produce this condition by use of massive and repeated exposures to the X-ray, by the removal of portions of the shafts of long bones and by various types of splinting. In all of our efforts, however, we were unable to produce a single case of pseudarthrosis, owing to the early and rapid union of the shafts of the long bones. We were able to report, however, that in our experience, no appreciable influence was exerted by the X-ray upon callus formation, there being no difference in

[1] Albee, F. H.: "An Experimental Study of Bone Growth and the Spinal Transplant," J. A. M. A., 60, 1044–49, April 5, 1913.

[2] Albee, F. H., and Morrison, H. F.: "Studies in Bone Growth: an Experimental Attempt to Produce Pseudarthrosis," Am. J. Med. Sc., January, 1920.

length of time required for union in those cases given repeated massive X-ray treatment and in their controls.

A second purpose of our research work at U. S. Army General Hospital No. 3 was to discover, if possible, a reliable artificial stimulus to bone growth. In the aim of increasing the osteogenetic activity of bone in delayed union or in pseudarthrosis, a great variety of substances have been injected into the site of the lesions, or otherwise introduced into the system. An abstract of recent literature shows that the following materials have been used for this purpose: Osmic acid, fibrin, blood, gelatine and lime salts (calcium chloride), zinc chloride, thyroidin, glacial acetic acid, iodine tincture, adrenalin, hypophysis extract, bone marrow, copper sulphate, oil of turpentine, ammonia, lactic acid (50 per cent.), silver nitrate solution, alcohol, carbolic acid (5 per cent. solution), oak bark extract (tannic), vaccines, and sera.

The very number and variety of these methods would indicate that none has proved successful in promoting osteogenesis. The great advantage, however, of such an agent has led one to the search with ever renewed enthusiasm. Early in the history of this hospital, investigations were begun on the value of triple calcium phosphate as a stimulus to bone growth. These studies have been extended over a long period of time, and the authors take pleasure in stating that they believe that an efficient and trustworthy stimulus to osteogenesis has been found in this chemical agent. Due credit should be given to Capt. Richard J. Behan, M. C., U. S. A., for the verbal suggestion of the agent.

Technic.—In all of our research work in bone growth, rabbits were used as experimental subjects, young to middle-aged adults being always chosen. Careful asepsis was invariably observed, the field of operation being treated with a 3½ per cent. tincture of iodine preparation. In our earlier studies in pseudarthrosis,[2] it was usually our method, after fracturing both bones of the foreleg, to apply an external splint for fixation and support. It was later found possible to avoid the necessity of external splinting by fracturing only one of the two bones of the foreleg, relying upon the other bone for fixation and support. We found that the wounds healed successfully when the skin edges were carefully approximated by suture and the line of suture was painted with 3½ per cent. tincture of iodine. This method was accordingly followed in all of our experiments with triple calcium phosphate, and it is of interest to report that in this whole series, numbering over 60, together with their controls, there occurred not one instance of infection.

In our investigation of triple calcium phosphate as a stimulus to bone growth, a classification of cases has been made, according to type of operative treatment. In cases of the first type, fragments of bone were first removed completely from the radius, leaving a gap in the shaft. Into this hiatus between the bone ends was then injected 1 c.c. of 5 per cent. solution of triple calcium phosphate. This was prepared by suspension of 5

per cent. of triple calcium phosphate in distilled water which was then sterilized for three successive days in the Arnold sterilizer, at 60° C. In the second type of experiment, no bone was removed nor was the radius fractured, but an attempt was made, after exposing the radius, to introduce ½ c.c. of 5 per cent. solution of triple calcium phosphate under the periosteum. The animals in all cases were radiographed frequently, and the clinical progress noted. The procedure in each type of operation is given in detail, as follows:

First Type.—After the usual preparation by shaving and iodine technic, an incision was made in the foreleg (usually the right), exposing the radius. A portion of the shaft, about one-quarter inch in length, was then removed with its periosteum intact. The wound was closed with plain catgut No. 1, and after painting the limb with iodine, the animal was returned to its cage without dressing or splint, the other bone serving in the capacity of the latter. Usually on the third day following operation, 1 c.c. of 5 per cent. solution of triple calcium phosphate was injected into the gap between the ends of the bone fragments (see Fig. 1). In a few instances the solution was injected at time of operation, before closing the skin, while in some of our later experiments the injection was made five days after fracture. These various cases will be noted in the report of the detailed experimental findings. In this type of experiment, in order that the controls might be absolutely trustworthy, the radius of the other leg of the same animal was always used, when possible.

Second Type.—In the second class of cases, the foreleg was given the same preliminary preparation as in the preceding group and the radius was exposed. The bone was not broken in these cases, but after injuring it to a slight extent by scratching with the point of the needle, an attempt was made to inject ½ c.c. of 5 per cent. solution of triple calcium phosphate beneath the periosteum of the radius with the hypodermic syringe (see Fig. 19). No controls were considered necessary in this type of experiment, as in no instance was there appreciable stimulation of osteogenesis. It is quite probable that in these cases the solution infiltrated the soft parts, and that very little, if any, actually came in contact with the bone tissues.

A detailed report of the following experiments is given:

EXPERIMENT 10.—Subject: Common hare.

March 11, 1919: Operation (first type) on right radius.

March 12: 1 c.c. of 5 per cent. solution triple calcium phosphate injected into hiatus between ends of bone.

Radiographic Findings.—March 11: X-ray shows gap of ¼ inch, with no free fragments of bone (see Fig. 1).

March 18: The ends of the shaft fragments are already beginning to close in, with some callus formation in the soft parts about the lesion (see Fig. 2).

March 25: Fourteen days after operation. Already there is evidence of union of the fragments, with a large amount of callus formation (see Fig. 3).

April 1: The gap is entirely filled. There is an increased density of callus about the site of the lesion, with more perfect solidification (see Fig. 4).

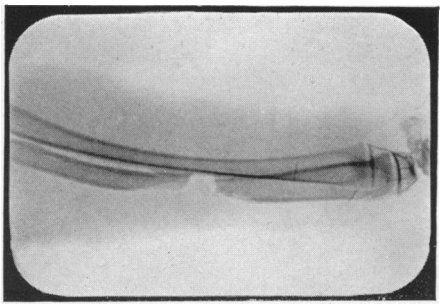

FIG. 1.—Experiment 10. Day of operation, showing gap in shaft of right radius, due to removal of about one-quarter inch of bone.

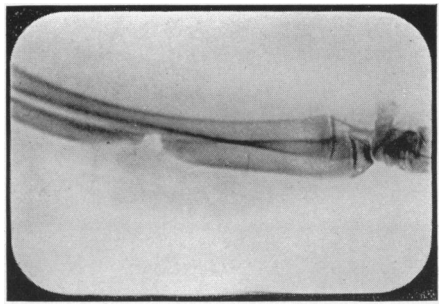

FIG. 2.—Experiment 10. Seven days after operation. Into the hiatus in the shaft was injected one cubic centimetre of five per cent. solution triple calcium phosphate, six days before this radiograph was taken. Note that the ends of the shaft fragments are already beginning to close in. There is some callus formation in the soft parts about the lesion.

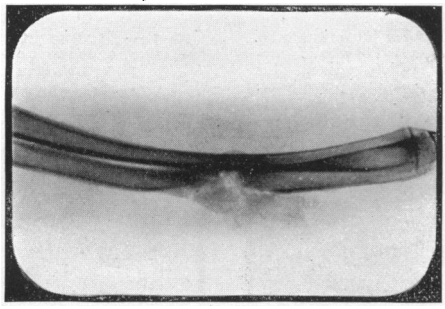

FIG. 3.—Experiment 10. Fourteen days after operation. Union of the radial fragments is evident with a large amount of callus formation.

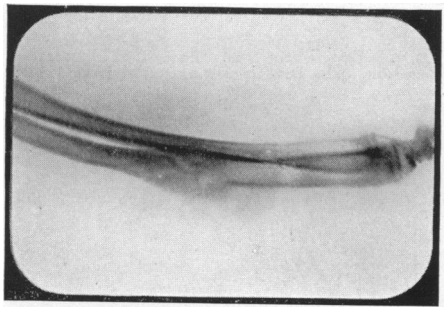

FIG. 4.—Experiment 10. Twenty-one days after operation. Note the increased density of callus formation and the more perfect solidification.

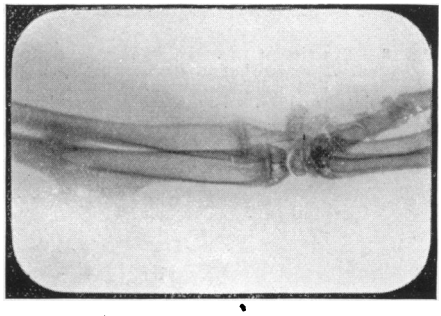

FIG. 5.—Experiment 10. Thirty-one days after operation. The exuberant callus is now beginning to flatten and to disappear in the periphery.

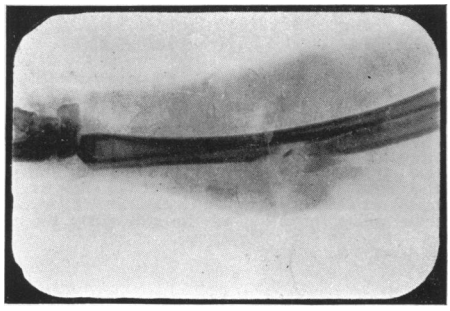

FIG. 6.—Experiment 10*a*. Control of Experiment 10. Day of operation, showing removal of about one-quarter-inch of bone from shaft of left radius. This case was not treated with triple calcium phosphate.

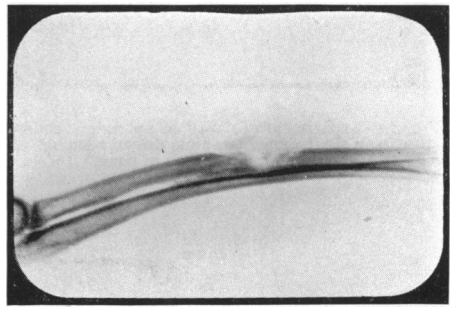

FIG. 7.—Experiment 10*a*. Thirteen days after operation, showing no appreciable change. Compare with Experiment 10 (Fig. 3).

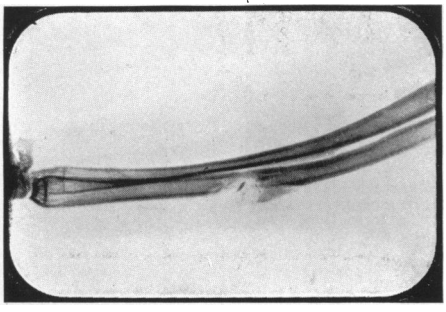

FIG. 8.—Experiment 10*a*. Thirty-one days after operation. The space has not yet been bridged, and there is very little callus formation. Compare with Experiment 10 (Fig. 5), in which triple calcium phosphate was used, and note the striking contrast in amount of bone proliferation in the two cases at the same length of time after operation.

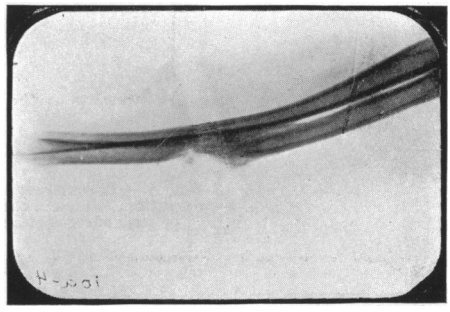

FIG. 9.—Experiment 10*a*. Forty-four days after operation. The bone is now united along one side, leaving a V-shaped defect. There is a small amount of callus.

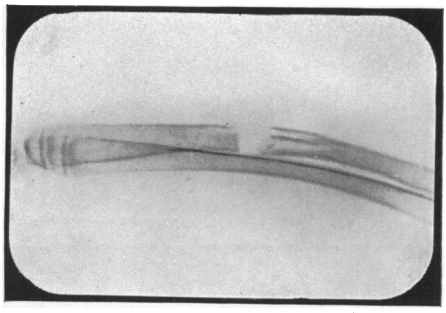

FIG. 10. — Experiment 11. Day of operation, showing gap in shaft of right radius. On the day following operation, one cubic centimetre of five per cent. solution triple calcium phosphate was injected into this hiatus.

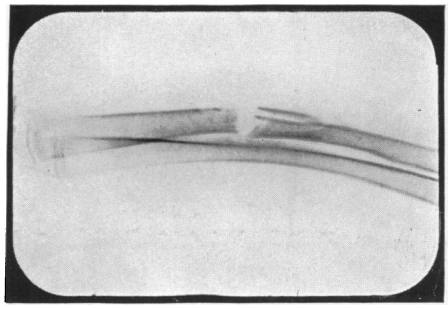

FIG. 11.—Experiment 11. Seven days after operation. There is evidence of some callus formation, and the distance between the ends of the fragments seems slightly decreased.

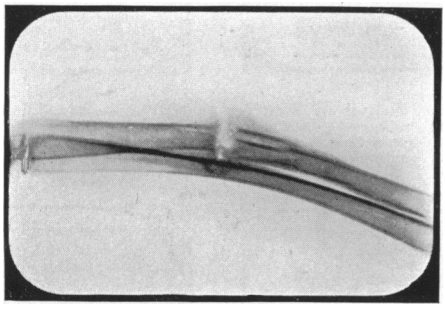

FIG. 12. — Experiment 11. Fourteen days after operation. Already union of the shaft fragments is evident, with marked formation of callus producing a plumber's "wiped joint."

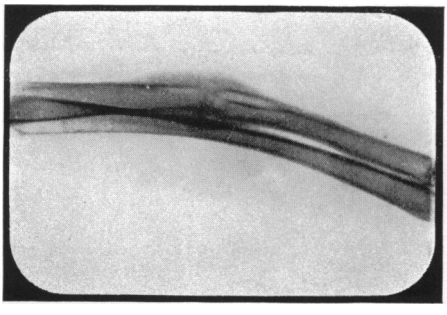

FIG. 13.—Experiment 11. Twenty-one days after operation, showing the hiatus completely filled. There is solid union of the fragments, with a large amount of well-formed callus.

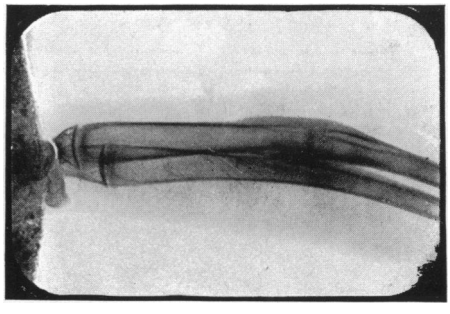

FIG. 14.—Experiment 11. Thirty-one days after operation. Much of the excess callus has now disappeared; the bone is beginning to shape itself.

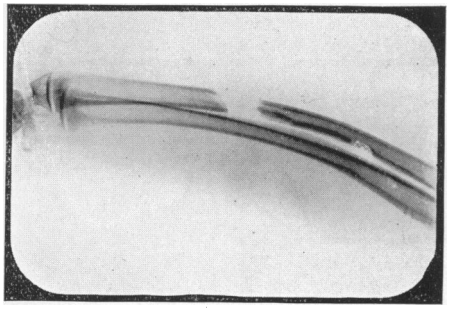

FIG. 15.—Experiment 11*a*. Control of Experiment 11. Day after operation, showing hiatus in shaft of left radius. This case was not treated with triple calcium phosphate.

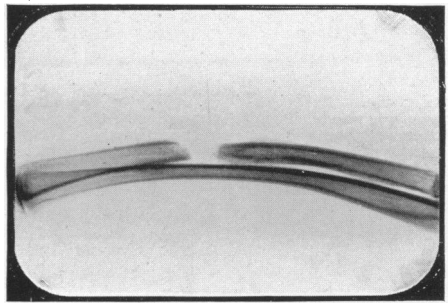

FIG. 16.—Experiment 11*a*. Twenty-nine days after operation. The gap is now about one-third closed with no excess callus formed

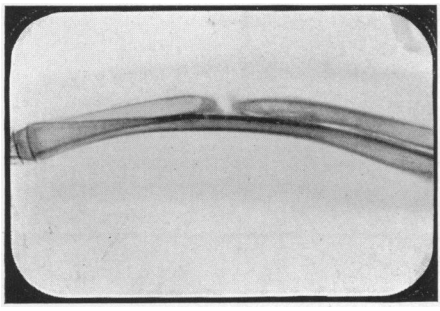

FIG. 17.—Experiment 11*a*. Thirty-seven days after operation. The hiatus is nearly closed. Callus formation is meagre.

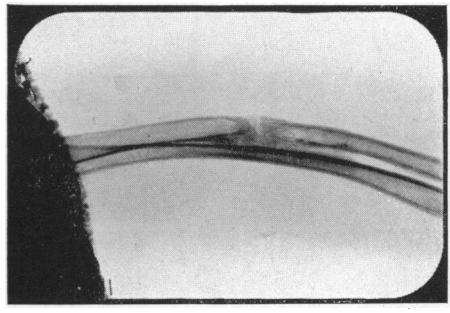

FIG. 18.—Experiment 11*a*. Forty-nine days after operation. Union of the fragments is now complete.

April 11: Thirty-one days after operation. The exuberant callus is beginning to flatten and to disappear in the periphery (see Fig. 5

EXPERIMENT 10a.—Control of Experiment 10.

March 17, 1919: Operation on left radius—fragment of bone removed as in No. 10. This case was not treated with triple calcium phosphate.

Radiographic Findings.—March 17: Day of operation, showing gap in shaft (see Fig. 6).

March 30: Thirteen days after operation. There is no appreciable change, as shown in Fig 7. Compare with Experiment 10 (Fig. 3).

April 17: Thirty-one days after operation (see Fig. 8). The X-ray shows that the space has not been bridged; there is very little callus formation. Compare with Experiment 10 at the same length of time after operation (see Fig. 5).

April 29: Forty-four days after operation. The bone is now united along one side, leaving a V-shaped defect. There is a small amount of callus (see Fig. 9). Note the striking contrast between this case and Experiment 10 (in which triple calcium phosphate was used) in the amount and rapidity of bone proliferation.

EXPERIMENT 11.—Subject: Common hare.

March 11, 1919: Operation (first type) on right radius.

March 12: 1 c.c. of 5 per cent. solution triple calcium phosphate injected into gap between ends of bone.

Radiographic Findings.—March 11: The X-ray shows gap of ¼ inch in right radius, with no free fragments present (see Fig. 10).

March 18: A small amount of callus formation is noted. The distance between ends of bone seems slightly decreased (see Fig. 11).

March 25: Fourteen days after operation. Marked formation of callus is noted, producing a plumber's "wiped joint," extending from both distal and proximal fragments, with evidence of union of the fragments (see Fig. 12).

April 1: Twenty-one days after operation. The gap is now completely filled and there is solid union of the fragments, with a large amount of well-formed callus (see Fig. 13).

April 11: Thirty-one days after operation. Much of the excess callus has disappeared; the bone is beginning to shape itself (see Fig. 14).

EXPERIMENT 11a.—Control of Experiment 11.

March 17, 1919: Operation on left radius—fragment of bone removed, as in No. 11.

Radiographic Findings.—March 18: Day after operation, showing gap in shaft (see Fig. 15).

April 15: The space is about one-third closed, with no excess callus formed (see Fig. 16).

April 23: The hiatus is almost closed, with very little callus formed (see Fig. 17).

May 5: Forty-nine days after operation. Union is complete (see Fig. 18).

EXPERIMENT 12.—Subject: Belgian hare.

March 20, 1919: Operation (first type) on right radius.

March 23: One c.c. of 5 per cent. solution triple calcium phosphate injected into the defect in shaft.

Radiographic Findings.—March 21: X-ray shows gap of ⅛ to ¼ inch. There are no free fragments between ends of the bone.

March 28: There is no change in appearance.

April 4: There is very little change noted.

April 14: Union is almost complete.

April 23: There is solid union.

EXPERIMENT 12a.—Control of Experiment 12.

April 23, 1919: Operation on left radius. Fragment of bone removed as in No. 12.

Radiographic Findings.—April 24: X-ray shows gap of ¼ inch in left radius, with no free fragments.

April 30: No changes are noted.

May 8: There is very little change in appearance of radiogram.

May 16: A small amount of callus formation is noted.

May 21: There is partial union.

May 27: There is solid union.

EXPERIMENT 13.—Subject: White buck.

March 20, 1919: Operation (first type) on right radius.

March 23: One c.c. of 5 per cent. solution triple calcium phosphate injected into gap in shaft of radius.

Radiographic Findings.—March 21: X-ray shows gap of ¼ inch in right radius.

April 4: Partial closure of space is noted, with but meagre callus formation.

April 21: There is solid union, and good alignment, with no excessive formation of callus.

EXPERIMENT 13a.—Control of Experiment 13.

April 23, 1919: Operation on left radius. Fragment of bone removed as in No. 13.

Radiographic Findings.—April 24: X-ray shows a gap of ¼ inch, with no free fragments.

May 10: No changes are noted until May 10th, when the X-ray shows formation of callus, but not in excessive amounts.

May 20: There is solid union, but with no excess of callus.

EXPERIMENT 14.—Subject: Common hare.

April 3, 1919: Operation (first type) on left radius. At time of operation, 1 c.c. of 5 per cent. solution triple calcium phosphate was introduced into the bony defect, and wound was then closed.

Radiographic Findings.—April 4: X-ray shows a bony defect of ⅛ inch in the left radius. A few free fragments of bone are present.

April 12: Very little change is noted.

April 18: Fifteen days after operation, showing evidence of union of the ends of the bone along one border. Compare with Cases 10 and 11, in which union was evident fourteen days after operation (see Figs. 3 and 12). The presence of a few free fragments of bone, as noted above, should also be borne in mind.

April 30: The gap is completely filled, except for a V-shaped space on one border. Very little callus is noted.

EXPERIMENT 14a.—Control of Experiment 14.

February 13, 1919: Operation on right radius. Fragment of bone removed, as in Experiment 14.

Radiographic Findings.—February 13: X-ray shows defect of ¼ inch in right radius.

February 23: No change is noted.

March 8: The gap is about two-thirds closed, with very little proliferation of bone.

March 25: There is solid union. The outlines of the bone are very nearly normal.

EXPERIMENT 15.—Subject: Common hare.

April 3, 1919: Operation (first type) on right radius. At time of operation 1 c.c. of 5 per cent. solution triple calcium phosphate was introduced into gap between the ends of the bone fragments; the wound was then closed.

Radiographic Findings.—April 4: X-ray shows a V-shaped defect of right radius, ¼ inch across, at the open side. A few free fragments are present.

April 12: No change is noted.

April 18: The defect has closed in from each end to about one-fourth of its original extent.

April 30: Twenty-seven days after operation the defect is entirely filled, save for a slight depression on the surface of the bone. Callus formation is very meagre.

May 6: The defect is completely closed.

EXPERIMENT 15a.—Control of Experiment 15.

February 17, 1919: Operation on left radius. Fragment of bone removed as in Experiment 15.

Radiographic Findings.—February 17: X-ray shows a defect of ¼ inch in the left radius, with two small free fragments present.

February 28: Slight proliferation of bone from proximal and distal fragments is noted.

March 7: Eighteen days after operation. New bone bridges the gap, leaving a defect on the outer surface of the radius. The presence of free fragments of bone, originally left in the hiatus, may have a significant bearing on the rapid union in this case.

March 17: Union is complete, with no excess callus.

EXPERIMENTS 16 to 25, inc., which were of the second type, follow Experiment 30 given below.

EXPERIMENT 26[8].—Subject: Common hare.

June 16, 1919: Operation (first type) on right radius.

June 21: Injection of 1 c.c. of 5 per cent. solution triple calcium phosphate between the gap in the shaft fragments.

Radiographic Findings.—June 21; The X-ray shows a bony defect of ¼ inch in the right radius, with no free fragments.

July 5: Fourteen days after injection of triple calcium phosphate, good union is noted, with strong callus formation about the ends of the bone. (Compare with Cases 10 and 11.)

EXPERIMENT 27.—Subject: Common hare.

June 16, 1919: Operation (first type) on right radius.

June 21: One c.c. of 5 per cent. solution triple calcium phosphate injected into gap between the ends of the shaft fragments.

Radiographic Findings.— June 21: X-ray shows a bony defect of ¼ inch in right radius, with one small free fragment.

July 5: The defect is almost closed, with meagre callus formation.

July 10: Nineteen days after injection of triple calcium phosphate there is complete union, with little or no excess callus formed.

EXPERIMENT 28.—Subject: Common hare.

June 16, 1919: Operation (first type) on right radius.

June 21: Injection of 1 c.c. of 5 per cent. solution triple calcium phosphate in hiatus between shaft fragments.

Radiographic Findings.—June 21: X-ray shows absence of ¼ inch of bone from right radius, with no free fragments present.

July 5: Fourteen days after injection of triple calcium phosphate, the defect has been bridged by new bone. About the end of the proximal fragment callus has formed; there is no evidence of callus about the distal portion.

EXPERIMENT 29.—Subject: Common hare.

June 16, 1919: Operation (first type) on right radius.

June 21: One c.c. of 5 per cent. solution triple calcium phosphate injected into gap between ends of bone.

Radiographic Findings.—June 21: The X-ray shows a loss of ¼ inch of substance from right radius. A few free fragments are noted.

July 5: Fourteen days after injection of triple calcium phosphate, the defect is bridged by new bone, but the hiatus is not completely filled. There is no excess of callus formation.

July 12: The gap is completely filled and there is solid union. Note, also, in this case that a few free fragments of bone were originally left in the hiatus between the ends of the shaft fragments.

EXPERIMENT 30.—Subject: Common hare.

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[8] Experiments 26 to 30, inclusive, have no controls.

June 16, 1919: Operation (first type) on right radius.

June 21: Injection of 1 c.c. of 5 per cent. solution triple calcium phosphate into gap beween ends of bone.

Radiographic Findings.—June 21: The X-ray shows a loss of ¼ inch of substance from right radius, with no free fragments present.

July 5: The defect is almost closed, with a large amount of callus formed on the adjacent surface of the ulna.

July 14: Twenty-three days after injection of triple calcium phosphate, there is solid union, with a moderate amount of callus.

EXPERIMENTS 16 to 25, inclusive (Second Type).—These experiments were all of the second type, the first five being done on one litter of rabbits of adult age, using the right radius; the last five being performed likewise on animals of one litter, the left radius being used in these latter instances.

Cases 16 to 20, inclusive, were injected with ½ c.c. of 5 per cent. solution triple calcium phosphate on May 6, 1919. Radiographs taken as late as June 12th (thirty-seven days after operation), failed to show the slightest formation of callus, or of new bone. Illustrative of experiments of this group is Case 17. It was possible to observe this case forty-four days after operation, at which time radiographic findings were negative (see Figs. 19 to 22).

Cases 21 to 25, inclusive, were injected on May 12, 1919. Radiographs taken thirty-one days after operation, on June 12th, were negative.

CONCLUSIONS

1. Cases of fracture, with loss of substance, showed a much more rapid bone growth and union when triple calcium phosphate was injected into the gap between the bone ends than did the controls without its use.

Of the cases treated with this agent, especially remarkable bone formation is shown in Experiments 10 and 11 (see Figs. 1 to 5 and 10 to 14).

2. Callus formation in the cases of fracture treated with triple calcium phosphate extended far into the soft parts, apparently following the penetration of the solution (see Figs. 3, 4, 5, 12 and 13). In some cases, the callus even extended out beneath the skin.

3. For our entire series of experiments, the average length of time for union in cases of fracture treated with triple calcium phosphate was thirty-one days.

The average length of time for union in the controls was forty-two days.

In our total series, therefore, cases of fracture treated with triple calcium phosphate showed union at least eleven days earlier than did the controls; in other words, union in cases stimulated by this agent occurred in 73.81 per cent. of the time which seemed to be required in the controls. For the smaller number of experiments, herewith reported in detail, even more striking relationships may be observed. The average number of days elapsing between date of injection of triple calcium phosphate and the first radiographic evidence of union in these experiments[4] was 19.

[4] In rare instances, it was found by early radiographic examination that one or more free fragments of bone were present in the hiatus between the ends of the shaft. In such cases, already noted in the foregoing detailed reports, an added influence on rapidity of bone growth may have been exerted by these loose fragments.

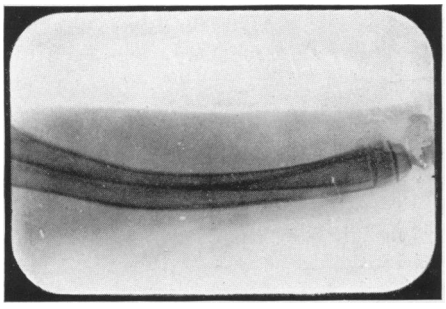

FIG. 19.—Experiment 17. Ten days after operation of the second type. In this case no bone was fractured, but an attempt was made to inject one-half cubic centimetre of five per cent. solution triple calcium phosphate subcutaneously beneath the periosteum of the right radius. Note that no shadow is cast by the solution.

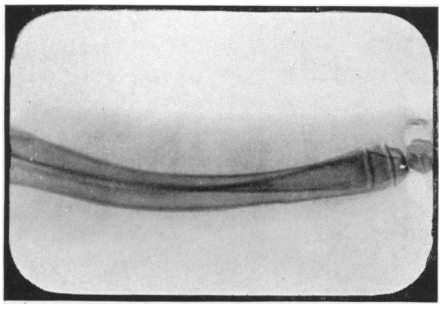

FIG. 20.—Experiment 17. Showing case twenty-four days after injection of triple calcium phosphate. There is no change apparent.

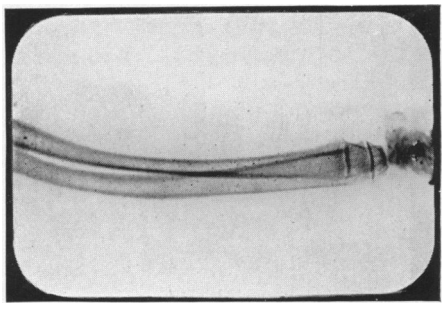

FIG. 21.— Experiment 17. Thirty-seven days after injection of the solution. The radius remains unchanged.

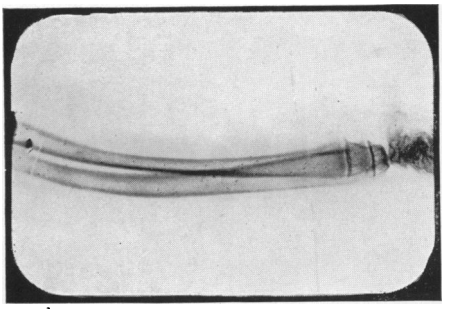

FIG. 22.—Experiment 17. Forty-four days after injection of triple calcium phosphate. Note that in this case there has been no apparent stimulation of bone growth, even to the slightest degree.

Furthermore, of these cases, over 50 per cent. showed union by the fifteenth day after injection of the agent.

In computing the length of time required for union to take place, some error may arise owing to the impossibility of determining the precise time at which union of the fragments occurred. With due allowance for error, however, the decided advantage in favor of the cases treated with triple calcium phosphate is evident.

4. No appreciable bone growth was stimulated by an attempt to inject triple calcium phosphate beneath the periosteum of the radius in cases of the second type, in which the bone was not fractured. Undoubtedly the solution in such cases infiltrated the soft parts overlying the periosteum and did not actually come into contact with the bone-growing tissues.

It is evident that osteogenesis was stimulated by triple calcium phosphate in conjunction with fracture, or, in other words, with trauma of sufficiently great severity to open up those bone surfaces containing active bone-growing cells, namely, the periosteum, compact bone, endosteum and marrow, thereby allowing the intimate contact of the solution with these tissue layers.

5. It was demonstrated in the early portion of those experiments in which an attempt was made to inject triple calcium phosphate subcutaneously beneath the periosteum, that the solution itself did not produce an X-ray shadow, since all the radiographs were entirely negative, thus ruling out any possible error in this respect (see Fig. 19).

Moreover, callus formation in all our series of cases treated with triple calcium phosphate seemed in no degree inhibited by frequent exposures to the X-ray. This had already been clearly demonstrated in our earlier studies in pseudarthrosis.[2]

6. No toxic symptoms were noted in any of the cases treated with triple calcium phosphate. At no time did this agent act as an irritant locally.

7. In our animal experimentations, only one injection of triple calcium phosphate was administered in each case treated. We would offer the suggestion that the stimulating action of this agent might be increased by *repeated* injections at frequent intervals in unfavorable clinical cases of pseudarthrosis, whether or not preceded by a bone graft operation.

8. It is believed that these findings with triple calcium phosphate are of sufficient value to warrant their clinical application. We wish to state that this agent is now being tried upon human subjects, and a report of these results will be subsequently presented.