CALCIFICATION IN CALLUS FORMATION AND FRACTURE REPAIR

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Ossification is a local transformation of fibrous tissue into solid bone, the constituents of which are derived from the blood. The mechanism of this local chemical change is not understood and so the problems of normal and pathological calcification have been approached essentially from the systemic standpoint. Obviously, such a procedure limits our understanding of chemical bone pathology. We have seen cases of obvious rickets as well as delayed bone union resist the systemic treatment and so we have initiated some studies on the local chemical processes involved in calcification. A study of the mechanism of the repair of fracture offers another approach to a possible solution of a variety of orthopædic problems which are definitely non-systemic in etiology. Though the formation of callus is not a phenomenon *sui generis* and is a resultant of several factors favoring osteogenesis, it nevertheless offers an experimental method for the evaluation of the nature of the local pathology involved in delayed bone union. This paper is concerned with the relation of callus formation to calcification.

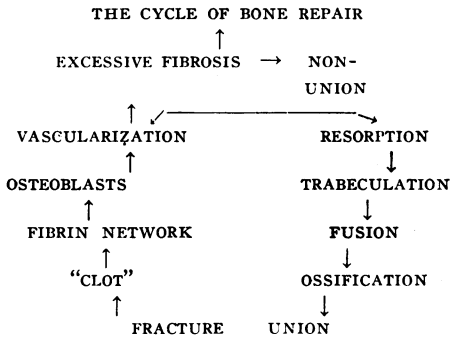
The Mechanism of Bone Repair.—The site of fracture following trauma consists of a blood-clot infiltrating the surrounding tissues. The retraction of the fibrin results in a network around the fragments which rapidly becomes invaded by fibroblasts in several hours. This is followed by a metamorphosis of the fibroblasts into osteoblasts with the formation of a soft or fibrous callus. This connective tissue organization of the clot is accompanied by the formation of penetrating capillary vessels.

The vascularization is a vasomotor reaction following fracture, thus bringing about a softening and resorption of the bone ends, a process preparatory for normal bone union. Any interference with these stages preliminary to osteogenesis alters the ultimate formation of solid callus. Trabeculæ of young bone tissue begin to appear between the periosteum and diaphysis adjacent to the bone in the organized clot, thereby gradually producing bone union.

The bone in its early stages is coarse, soft, and of slighter density than in its adult stages. If too great an amount of fibrous tissue is laid down in the course of callus formation, the tendency is toward non-union and very often in the soft callus or cartilage stage one will be surprised to find a large, diffuse, palpable callus without any apparent calcification as revealed by X-ray. The fracture will feel fairly firm and solid yet one is never justified in concluding that bone union has taken place.

In certain cases re-fracture takes place, either accidentally or during manipulations to improve position, and it appears that new callus is formed with great rapidity. This is due to the fact that the local changes necessary for this function have already been instituted and it is only necessary to initiate the finer changes.

In the light of the local pathology following trauma, a fractured bone is repaired because of hæmorrhage of the soft parts of the actual break in bone and of the paralytic vasodilation. The hæmorrhage results in clot formation. Embryonic connective tissue appears. The vasodilation produces œdema in the soft tissues and resorption of the fractured ends of bone. The calcium salts thus made available locally are absorbed by the fibrin network which with gradually increasing density gives rise to bone.



The Nature of Delayed Union.—Delayed and ununited fractures have a common cause, the difference being a matter of degree. The time required for union varies in the different bones but so long as union is progressing the fracture is regarded as delayed. When organization of the exudate about the fragments is reached so that further osteogenesis is impossible non-union is inevitable. The causes are local rather than constitutional, proved by the fact that local treatment is effective in inducing union in most cases.

Systemic Causes of Non-union	(1)	Focal infection.
	(2)	Tyroid disease.
	(3)	Multiple fractures.
	(4)	Debility.
Local Causes of Non-union	(1)	Inadequate reduction.
	(2)	Inadequate fixation.
	(3)	Circulatory interference.
	(4)	Interposition of muscle.
	(5)	Repeated reduction.

In non-union, condensation of callus takes place upon the ends of the fragments with failure to bridge the defect with solid bone. The callus upon the proximal fragment is usually in excess of that upon the distal end, resulting in the formation of nodular prominence on the proximal fragment with a cavity into which the distal fragment articulates. On the other hand,

there may be no reaction of the bone to form callus with resulting atrophy of the fragments.

Delayed union of bones may be due to either mechanical or to chemical causes. Obviously a variety of conditions may prevent union. The fragments may not be in apposition. There may be inadequate fixation or reduction. Frequently the process is actually interfered with by repeated examinations and finally there may be actual injury to the local blood supply preventing the continuous availability of blood constituents necessary for repair.

Chemical causes locally may be productive of delayed union. Tissue injury at the site of fracture results in hæmorrhage. Decomposition of the hæmatoma yields amino-acid, which decalcifies the bone ends (Henderson). The blood phosphates are made available for bone formation by a hydrolyzing enzyme present in the ossifying cartilage (Robinson). A marked increase in the local hydrogen-ion concentration tends to decrease the effective enzyme activity.

Constitutional treatment for delayed and ununited fractures is of no avail once non-union is imminent. The problem is local, confined to the site of fracture. Attempts at increasing the calcium and phosphorus concentrations of the blood by antirachitic measures have failed to show any effect upon the local condition. The stimulation of callus formation has been attempted by Bier's hyperæmia, maceration of the fragments under anæsthesia, injection of blood, iodine, osmic acid, calcium salts, phosphates, into the point of fracture. And finally, functional use of the fractured extremity, surgical fixation or bone grafts.

Experimental Procedure.—Three groups of rabbits in two separate sets of experiments were studied for the degree and rate of callus formation and calcification. Each rabbit was anæsthetized with ether, blood taken from the heart for chemical analyses and the tibia fractured in mid-shaft. The fracture was immediately put into a plaster-of-Paris case and as soon as this had hardened sufficiently a fenestration was made to allow the introduction of the solution used. These preparations were introduced aseptically through the fenestration directly into the site of fracture. Some of the animals were maintained on the regular diet of hay, oats and leafy greens and the others were put on a special diet. The first group of animals was injected with five cubic centimetres of a 1 per cent. solution of trypsin buffered at 37° C. in the following solution pH 7.4 consisting of 100 cubic centimetres each of:

$NaHCO_3$	0.03 per cent.
NaCl	N/10.
MgSO	0.0016 per cent.
K_2HPO_4	0.009 per cent.

The injections were given at twenty-four- to thirty-six-hour intervals; a long needle was used, being introduced about an inch above the fractured site in healthy tissue so as to preclude as far as possible the danger of infection. The second group of rabbits was treated in exactly the same method with the exception that a tissue extract (fibrinogen) was substituted for the trypsin, one cubic centimetre of the sterile solution being injected immediately upon fracture into the hæmatoma. The third group constituted the control.

These animals were subjected to the same treatment except that no solutions were injected into the site of fracture. The animals were watched carefully for evidence of vascular disturbance which might arise from too snugly fitting plaster bandaging, and in case this occurred new bandages were immediately applied. All animals were X-rayed each week to furnish a basis of comparison in the amount of callus thrown down. Specimens were removed from the animals killed at two- and four-week intervals. These were decalcified *in toto* in a 5 per cent. nitric acid. Longitudinal sections were then made with a very sharp blade for photographs and measurements of the callus. Microscopic sections were finally made from the centre of the callus.

Discussion.—Experimentally induced fractures in animals revealed that, all other conditions remaining constant, the amount of available fibrous

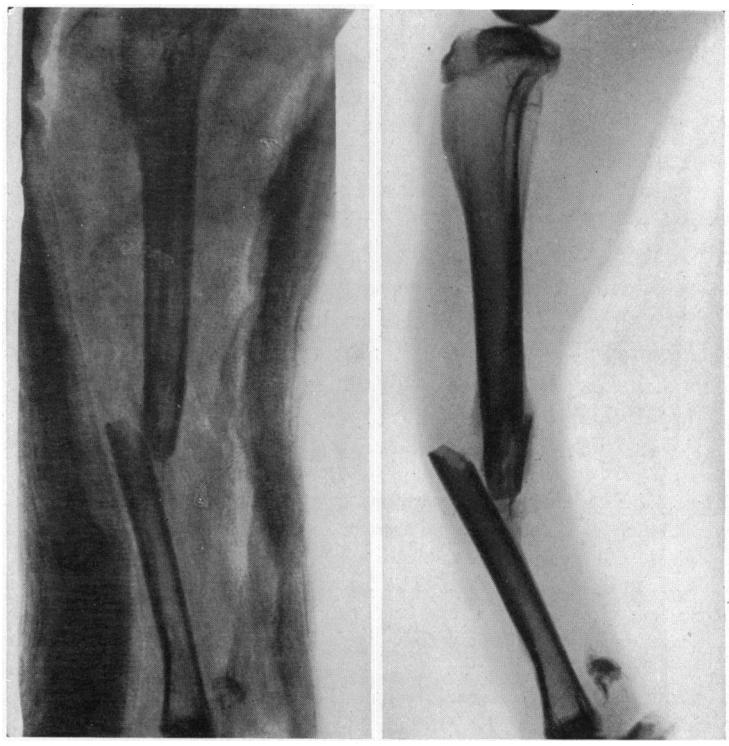


FIG. 1.—"Trypsin" series. First and fourth week. (Slight bony union.)

tissue is directly proportional to the degree and rate of calcification. The dissolution of the fibrous tissue normally formed following fracture, by means of an alkaline trypsin solution, diminished both the quantity and rate of bone formation during the course of bone repair. On the other hand, the local injection, at the site of the fracture, of tissue fibrinogen which in itself contributed to the available fibrous tissue content and simultaneously stimulated the fibrous tissue production, increased both the amount and rate of bone formation. Attempts to alter the local fibrous tissue production by means of high protein diet were ineffective in comparison with results obtained by controls. Histological studies revealed similar results. This

study indicates that the mechanism of bone repair may be altered by the local introduction of substances involved in the process and vary strikingly in comparison with the indefinite results obtained by attempted systemic therapy.

Bone repair involves: (1) the proximation of the fractured ends; (2) their initial union with the network of fibrous tissue; (3) the transformation of this fibrous network by solid calcium salts. Obviously, any interposition of muscle or fascia between the fractured ends of bone will prevent the formation of the fibrous network. Local alterations in tissue may diminish the available production of fibrous tissue and hence result in a fibrous network inadequate to span the fractured ends. Finally, any injury

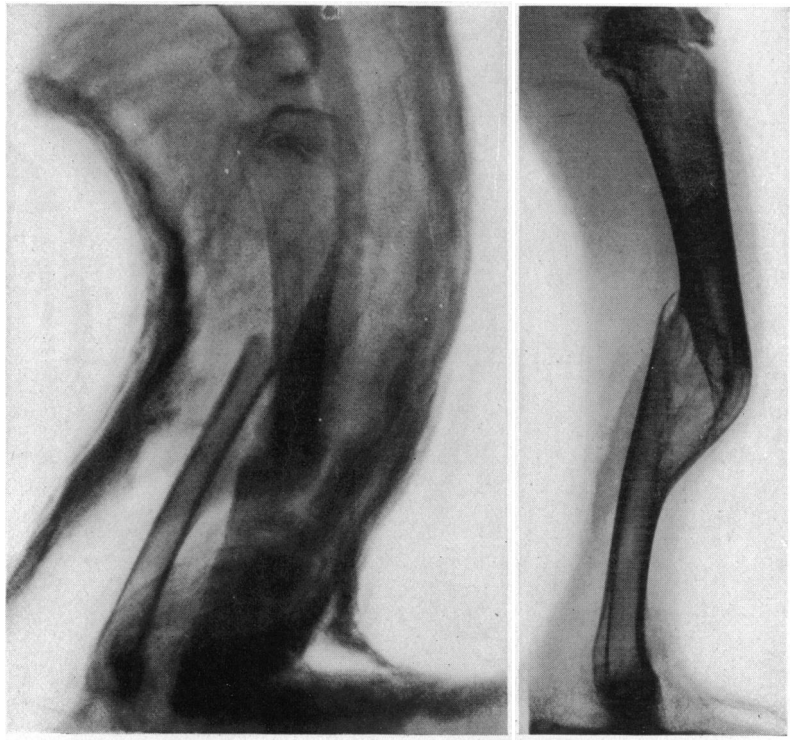


FIG. 2.—"Fibrinogen" series. First and fourth week. (Solid bony union.)

to the circulation will decrease the available supply of blood constituents necessary for the formation of bone tissue.

The trauma which caused the fracture simultaneously produces hæmorrhage. The blood enmeshed in the fractured ends forms the clot of fibrin. Injury to the periosteum favors additional fibrous tissue formation in this network. There is a definite relation between the mechanism of blood coagulation and this subsequent stage of calcification. Freshly formed fibrin has a greater adsorption capacity for calcium salts; hence, the more fibrin or fibrous tissue available, the more rapid will be the bond of union between the two fractured ends. The initial supply of calcium salts available for

adsorption by fibrinogen is obtained by local amino-acid formation which dissolves at least 20 per cent. of the exposed bone ends. The subsequent and continuous supply of calcium salts is obtained from the blood. The spontaneous healing of fractures thus depends upon the local hæmorrhage induced by tissue trauma. There is no providential protection of the unity of the skeletal structure—unless the conditions are favorable, healing will not take place.

Chemical determination of the blood constituents involved in the calcification process revealed certain alterations in the content according to the

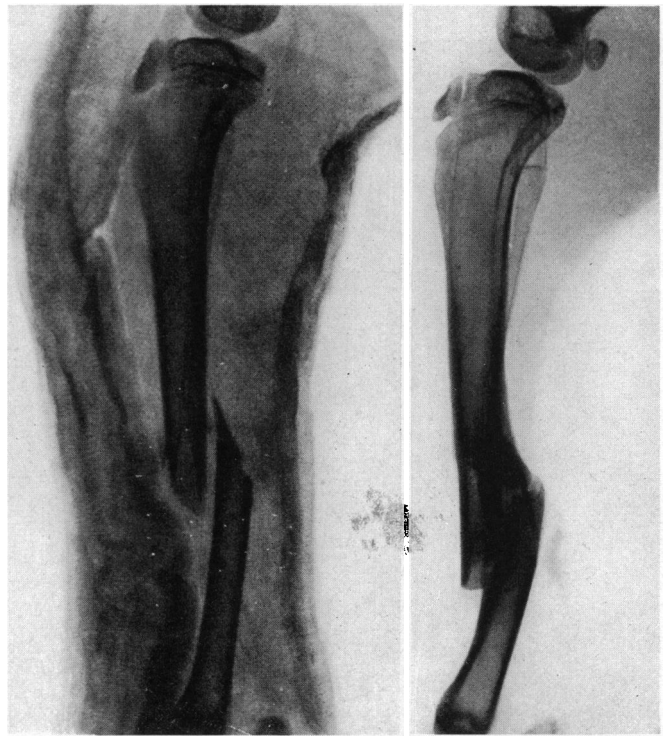


FIG. 3.—"Control" series. First and fourth week. (Good bony union.)

local conditions of the fracture and its treatment. The control series of animals showed that the normal course of fracture repair involves an increase in the calcium level of the blood with a decrease in the phosphorus content. The bone repair therefore appears to be a process of phosphorization rather than of calcification. The injection of tissue fibrinogen at the site of the fracture tended to increase the blood calcium with the simultaneous decrease in the controls. This difference in blood effect produced by the introduction of tissue fibrinogen is explicably on the basis of the relative rates of bone repair. It has been repeatedly shown that the protein content of the blood is related to the total calcium injection. Half of the protein concentration is bonded to blood proteins as calcium proteinate. Fluctuations of the blood

calcium with variations in total protein of the blood have been observed in nephritis and other diseases where the protein metabolism is altered. A high-protein diet favors an elevated blood-calcium level, particularly if the blood-calcium level is below normal. The high-protein diet maintained a higher calcium level than the controls but all were well within the normal range. Protein injected in the form of tissue fibrinogen injected locally showed a similar trend. Diminution of other blood constituents involved directly or indirectly in the process of blood calcification revealed no change with alterations in the treatment of experimentally induced fractures.

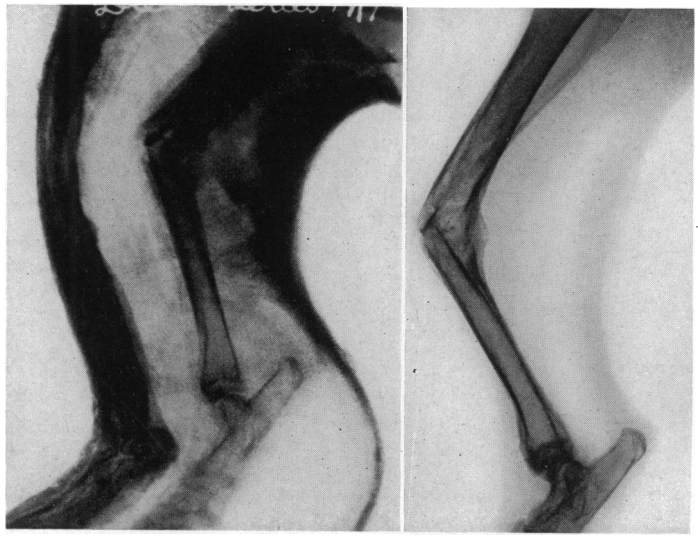


FIG. 4.—"Dietary" series. First and fourth week. (Beginning bony union.)

	Mg. Ca. 100 cc.	Mg. Phos. 100 cc.	Mg. Fibrin 1 cc. pl.	Alb. per cent.	Glob. per cent.
INITIAL BLOOD DETERMINATION					
FIRST SERIES					
(1) Trypsin group	13.0	5.6	.12	5.1	1.4
(2) Control group	13.0	7.8	.14	5.3	1.5
(3) Manipulated fractures	13.0	7.9	.14	4.1	3.6
FINAL BLOOD DETERMINATION					
SECOND SERIES					
(1) Trypsin group	13.1	5.5	.61	4.8	2.6
(2) Fibrinogen group	qns.	qns.	.33	3.9	.67
(3) Control group	9.7	5.4	.39	3.7	2.7
(4) Diet group	14.3	6.7	.25	4.2	3.3
Blood of May 2					
SECOND SERIES					
(1) Trypsin group	13.	4.1	.28	4.0	3.2
(2) Fibrinogen group	14.	3.5	.83	4.0	3.4
(3) Control group	12.	3.5	.24	3.7	3.7

Blood of May 7

THIRD SERIES

(1) Trypsin group	14.	3.6	.23	4.1	2.4
(2) Fibrinogen group	14.	3.8	.56	4.0	2.5

THIRD EXPERIMENTAL SERIES

BLOOD DETERMINATIONS FROM TIME OF FRACTURE TO TIME OF UNION

	Rabbit	Ca.	Phos.	Fib.	Alb.	Glob.
	Blood of April 11					
Trypsin group	1	14.5	6.61	.379	4.43	3.34
	2	13.2	5.99	.465	4.34	1.78
	5	14.8	5.37	.253	4.21	1.41
	6	13.8	7.40	.512	4.70	1.13
Fibrinogen group	7	14.4	7.00	.288	4.34	.57
	8	9.8	9.52	.338	4.29	1.66
	9	8.0	qns.	.669	4.83	2.59
	10	14.2	4.31	.425	4.54	2.21
	Blood of May 2					
Trypsin group	1	12.8	3.37	.219	4.50	2.87
	2	13.8	3.73	.208	4.47	1.79
	5	15.2	4.16	.277	3.47	2.87
	6	14.8	3.51	.221	4.21	2.31
Fibrinogen group	7	14.4	3.75	.487	4.70	2.12
	9	14.4	3.93	.618	3.40	3.09
	10	14.4	3.77	.575	4.05	2.41
	Blood of May 13					
Trypsin group	1	14.1	4.00	.305	3.92	3.07
	2	13.8	4.50	.221	4.17	2.72
	5	16.8	3.80	.229	qns.	..
Fibrinogen group	9	16.0	3.75	.465	4.15	2.46
	10	14.6	3.84	.508	3.90	2.34

CONCLUSIONS

1. The relation between callus formation and the amount and rate of calcification was studied in rabbits with experimentally induced fractures.

2. The greater the local fibrous-tissue formation, the greater the amount and degree of calcification, all other conditions remaining the same.

3. The blood calcium tends to be higher and the phosphate lower in the course of normal bone repair and the more rapid the rate of calcification the more marked is this alteration in the calcium and phosphate content of the blood.

4. Injection at the site of fracture of alkaline trypsin solution to produce fibrous dissolution markedly retarded bony union in comparison with the control series.

5. Injection at the site of fracture of tissue fibrinogen solution to stimulate increased callus formation markedly accelerated bony union in comparison with the control series.