(FROM THE PATHOLOGICAL-ANATOMICAL INSTITUTE OF THE UNIVERSITY OF COPENHAGEN AND THE BIOLOGICAL LABORATORY OF THE MEDICINALCO LTD.)

EXPERIMEMTAL INVESTIGATIONS INTO THE HEALING OF FRACTURES

BY

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I.

HEALING OF FRACTURES OF THE FEMORAL DIAPHYSIS IN RATS

Introduction.

The healing of fractures has aroused great interest ever since the days of *Galen* and *Hippocrates* and has been the object of numerous investigations. In 1940, according to *Urist* and *Johnson,* there were more than 4000 publications on this subject and this figure has since been considerably increased.

The questions arousing special interest have been the periosteal and endosteal formation of bone, direct and indirect ossification, the factors which might have an influence on the healing of bone, and, during the last few decades, questions concerned with the transplantation of bone and osteosynthesis. Investigations have been based mainly on experimental fractures, clinical and histological studies, and radiography.

Gradually, agreement about the main features of the healing of fractures has been reached, but different investigations still show considerable differences of detail.

Urist and *Johnson*'s work of 1943 contains an excellent survey of the various stages of healing of fractures and a copious bibliography, to which reference may be made.

Briefly, the healing of a fracture in man takes place as follows: Immediately after a bone is fractured a haematoma forms at the site of the fracture: it contains large and small fragments of bone, of bone marrow, and of the surrounding muscles, ligaments, fasciae and, possibly, articular cartilage and capsular tissue. Very soon aseptic inflammatory changes and processes of organisation take place within the haematoma. The resulting swelling at the site of the fracture is termed procallus.

The haematoma is invaded by a highly vascularized granulation tissue from the surrounding tissues and at the same time the periosteum, and to some extent the endosteum, proliferates in the circumference of the fracture, forming, in the case of the periosteum, an annular cancellous thickening of the fracture ends, which become club-shaped. These periosteal formations gradually grow beyond the fractured surfaces, across the more or less organised haematoma, and are seen in sections to form a bridge-like osseous connection between the fragments. The granulation tissue which has replaced the haematoma contains hyaline cartilage, fibrocartilage and fibrous cicatricial connective tissue. Thus, the thickening at the site of the fracture, the true callus, consists at this stage of a disc of connective and cartilaginous tissue lying between the fragments and between the bone formed externally by periosteal and centrally by endosteal bone formation.

The actual consolidation of the fracture by ossification begins early but is completed very late. Like "primary healing" its course is mainly centripetal, the fibrocartilaginous callus disc being gradually replaced by osseous tissue growing in from the external callus. There seems to be no sharp delimitation between osteoid and osseous tissues.

As new bone is formed, loose tissue fragments and the fractured ends are resorbed and demineralized, and for a long time after osseous union is established reorganisation continues, superfluous tissue being removed and the new structure as far as possible adapted to the static and mechanical conditions.

The time required for the healing of a fracture varies widely: it depends on many different factors, such as the type and site of the fracture, the amount of displacement, the mobility and the patient's age. Occasionally a fracture does not unite, and a pseudarthrosis is formed.

In animals the healing of fractures is the same as in man, with the formation of procallus, fibrocartilaginous callus and osseous callus.

The animals most frequently used for experimental studies of the healing of fractures have been rats, rabbits and guinea-pigs. *Hertz* (1936) used 4-week-old rats; he fractured the

fibula by means of a special forceps, constructed by himself, and killed the animals from 4 to 50 days later. From a study of 27 animals treated in this way, he found that the haematoma was completely organised in 4 days and the callus increased in size up to the tenth to fourteenth day and then decreased. After 35 days it could not be demonstrated macroscopically. The mobility at the site of the fracture steadily decreased, and after 22 days only a very slight "springiness" remained. In 27 guinea-pigs treated by the same method he found that the fracture was firm after 26 days, and that the callus was removed in about 50 days.

Urist and *McLean* used over 400 7-week-old rats; they fractured the tibia and examined the callus histologically, in particular the relation of calcification to osteogenesis. The fracture was observed for 24 days; it was not immobilised. No details of the time of union or of the mobility at the fracture site at the different dates are given.

Methods and Assessment.

The present work was designed to show the course of normal healing of fractures in rats, using a particular fracture. Special attention was paid to the time and degree of healing.

We first tried Hertz's method of fracturing the fibula but we abandoned it, partly because of the difficulty of producing the fracture and partly because exact radiographic control was not possible. Instead manual fracture of the femur proved to be fairly easy and to be better suited to radiographic study. White laboratory rats, aged 4-5 months were used; they were kept in single cages on a standard vitamin-containing diet and thrived normally. The femur was fractured manually under ether anaesthesia. Immobilization of the fractures proved impossible, so there was usually considerable deformity. Otherwise, the fracture seemed to cause comparatively slight inconvenience to the animals. After 5 or 6 days they stood on their hind legs and jumped about as they had done before the fracture. In order to make the material more homogeneous,

only fractures of the middle third of the bone were included. Fractures of the lower third appeared to heal far more rapidly. The degree of healing was assessed on the basis of *the mobility, radiographic and histological appearances.*

Material.

The material comprises 271 rats with fracture of the middle third of the left femur. As the object of the examination was to determine the course of the normal healing of fractures under the given conditions, the animals were usually killed at intervals of about a week. A small number of animals was also killed at shorter intervals during the first four weeks with a view to following the processes of healing histologically, while 13 animals were not killed until 8 months after the fracture. In order to obtain both a qualitative and a quantitative expression of the healing, the numbers of animals killed after 14, 21, 28 and 56 days respectively were relatively large. The distribution was as follows:

Days after fracture	Number of animals
2	1
4	2
7	8
9	4
11	5
14	44
16	2
21	38
24	2
28	57
35	12
42	5
49	5
56	51
63	5
64	2
70	5
84	5
94	5
243	13

The larger groups were collected at different seasons and from different litters in order to reduce errors as far as possible.

Healing of the Fractures Assessed According to Degree of Firmness.

The firmness of a fracture is the best criterion of its healing, except in impacted fractures, which are very rare in fractures of the diaphysis. The mobility at the fracture site was tested immediately after the animals had been killed and the soft parts had been removed. One fragment was fixed, and then the range of movement of the other fragment was measured in degrees with a protractor. If the range was greater in one plane than in the other, the greater range was recorded. Ranges of more than 90° are recorded as 120°; if between 5 and 10°, for example, as 10°. Arranged according to the time after fracture the following ranges of movement were found:

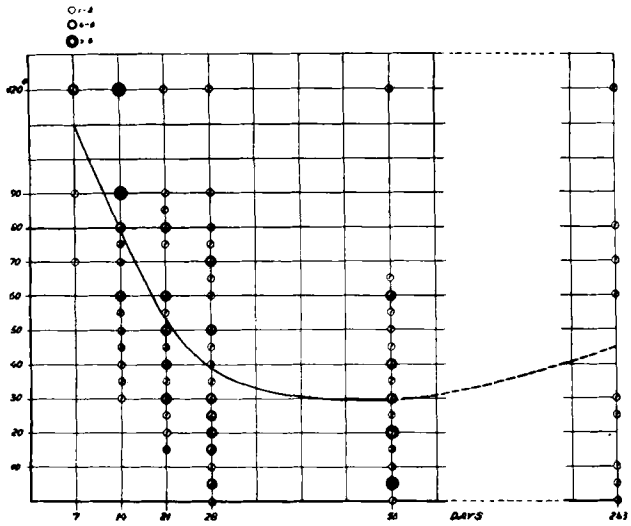


Fig. 1.
Curve showing the degree of firmness in relation to time of healing.

Days after fracture	Number of animals	Mobility min.	Mobility max.	Mobility mean
2	2	120°	120°	(120°)
4	2	120	120	
7	8	70	120	110
9	4	120	120	
11	5	90	120	
14	44	30	120	78
16	2	90	90	
21	38	15	120	53
24	2	45	90	
28	57	0	120	37
35	12	5	120	
42	5	0	75	
49	5	0	120	
56	51	0	120	28
63	5	30	120	
64	2	15	60	
70	5	10	35	
84	5	10	120	
94	5	15	120	
243	13	0	120	(45)

These figures, and figure 1 show that the mobility was complete or very large during the first fortnight, and firm union did not occur before 4 weeks. The mean values of mobility were computed only for the larger groups; they show a fall during the first 8 weeks. After 8 months they were somewhat higher and quite a considerable number of fractures did not become firm during the observation period, presumably due to the formation of a pseudarthrosis. Further, a group of 13 subjects is not large enough for any definite conclusions. For all groups there was a very considerable standard deviation.

Cumulative curves of the larger groups show an initial marked and later a decreasing displacement to the left. The displacement decreases with the age of the fracture (see Fig. 2).

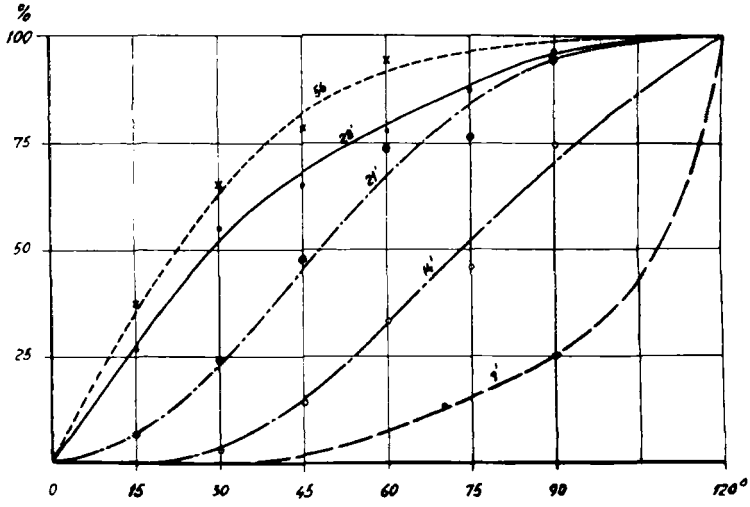


Fig. 2.
Cumulative curves showing degree of firmness after 7, 14, 21, 28 and 56 days.

Healing of Fractures Assessed by Means of X-rays.

Nearly all fractures were radiographed in two planes after removal of the soft parts. In addition, in most cases the range of movement, the shortening and the diastasis were measured, and the nature and position of the fracture were determined. The actual basis of the assessment was, however, the periosteal formation of new bone and the resorption of the fractured ends, assessed at values from 0 to 3. The faintest periosteal reaction, a very narrow, not very dense shadow, was assessed as ½; a narrow, but rather dense shadow as 1; a fairly marked periosteal thickening with perhaps adaption of the fractured ends at these thickenings, as 2; while the maximum value 3 was reserved for the rather few cases in which complete osseous healing occurred.

The assessment of the periosteal reaction gave the following results:

Days	No. of animals	Periosteal reaction min.	max.	mean
2	1	0.0	0.0	
4	2	0.0	0.0	
7	8	0.0	0.5	0.25
9	4	0.0	0.5	
11	5	0.0	0.5	
14	44	0.0	0.5	0.53
16	2	0.5	1.0	
21	38	0.5	1.5	0.84
24	2	1.0	1.0	
28	57	0.5	2.0	1.14
35	12	0.5	2.5	
42	5	1.0	2.0	
49	5	0.5	1.5	
56	51	0.5	3.0	1.63
63	5	0.5	2.0	
64	2	1.0	2.5	
70	5	1.0	1.5	
84	5	0.5	1.0	
94	5	0.5	1.0	
243	13	0.5	2.0	1.20

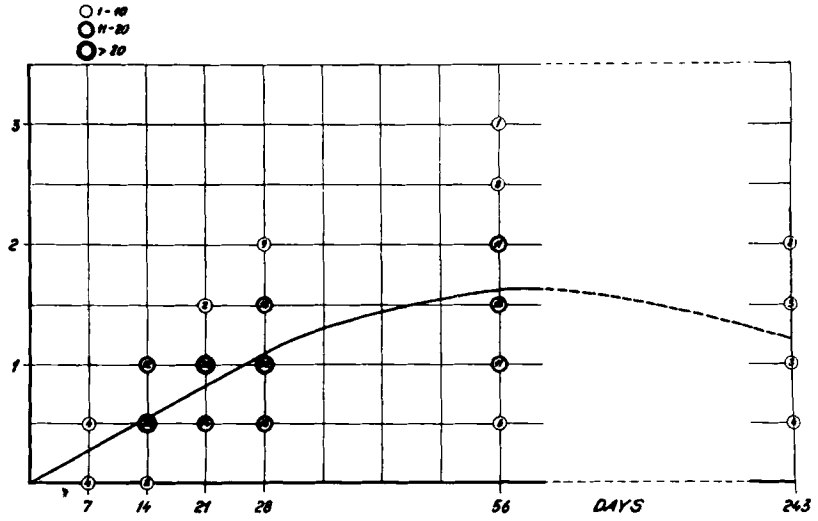


Fig. 3.
Curve showing healing, as judged by the periosteal reaction.

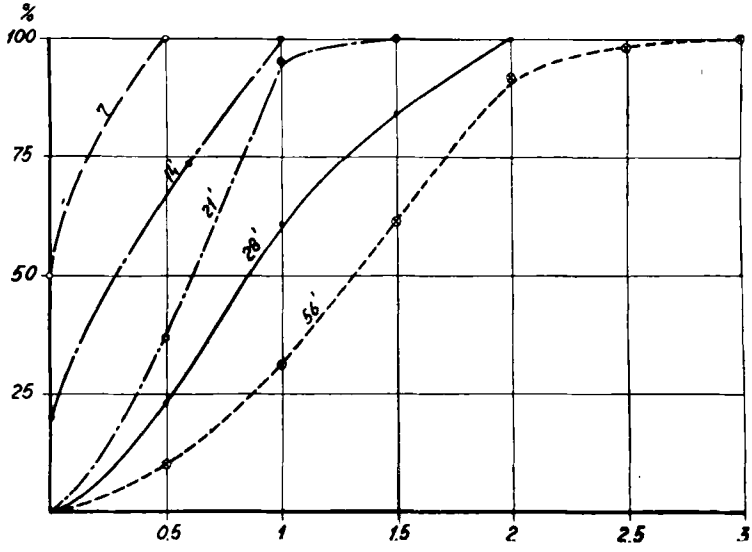


Fig. 4.
Cumulative curves showing the periosteal reaction after 7, 14, 21, 28 and 56 days.

In the larger groups the mean figures show that by the end of a week the reaction is faint and that it then steadily increases. It reaches its maximum of 1.63 after about 8 weeks (see Fig. 2, page 439). After 243 days the figure is lower, presumably because the callus is being remodelled, and, when a pseudarthrosis develops, partly resorbed. The minimum figures show that there was a visible periosteal reaction after 2 weeks in all cases, but that some, irrespective of their duration, had only a very slight tendency to healing.

The cumulative curves show a quite even displacement to the right in the groups examined between the 7th and 56th days. The distance between the curves for 28 and 56 days, as compared with that between the cumulative curves of the other assessments, is relatively large (see Fig. 4).

The resorption was assessed in the following way: slight abrasion and some rarefaction of the fractured ends, 1; a fairly pronounced rarefaction with some blurring of the cortex and rounding of the fractured ends, 2; marked atrophy

of the fractured ends and possible formation of a new articular cavity with pseudarthrosis, 3. The following results were obtained:

Days	No. of animals	Resorption min.	max.	mean
2	1	0.0	0.0	
4	2	0.0	0.0	
7	8	0.0	0.0	0.0
9	4	0.0	0.0	
11	5	0.0	0.5	
14	44	0.0	1.0	0.36
16	2	0.5	0.5	
21	38	0.5	1.0	0.76
24	2	0.5	1.0	
28	57	0.5	2.0	1.14
35	12	0.5	2.0	
42	5	1.5	2.0	
49	5	0.5	2.0	
56	51	0.5	2.5	1.53
63	5	0.5	2.0	
64	2	1.5	2.0	
70	5	1.5	3.0	
84	5	1.0	2.0	
94	5	2.5	3.0	
243	13	1.0	3.0	1.50

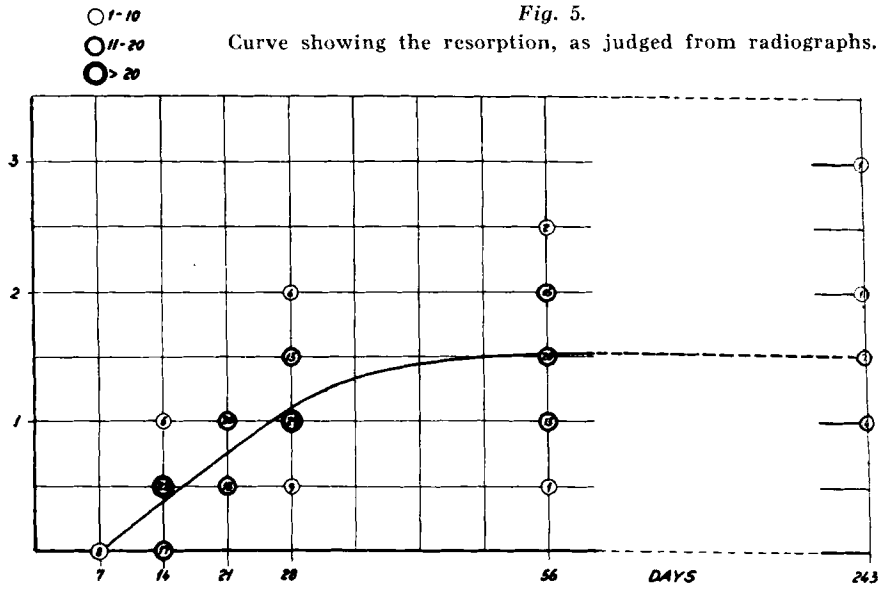


Fig. 5.
Curve showing the resorption, as judged from radiographs.

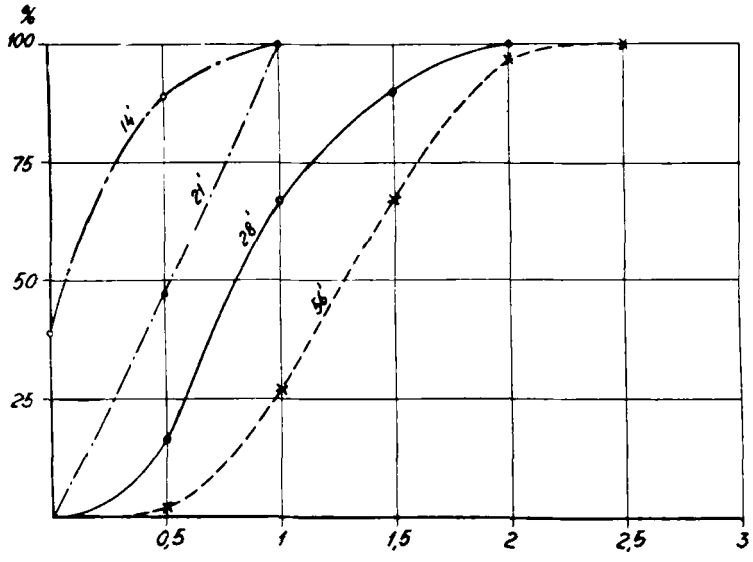


Fig. 6.
Cumulative curves showing the resorption (as judged from radiographs) after 14, 21, 28 and 56 days.

Thus it appears that the resorption begins in the second week, increases steadily until about the seventh week, then remains fairly constant. Roughly speaking, the curve (Fig. 5, page 442) follows that showing the periosteal reaction, but it begins a little later and does not fall towards the end of the period of observation, when the bone becomes more compact. At first, the cumulative curves show some displacement to the left as compared with the curve for the periosteal reaction, but, later, they fall together with it. (Fig. 6).

(Dr. *V. Thayssen* kindly interpreted some of the radiographs).

Histological Examination and Assessment of the Healing of Fractures.

The sections for microscopy were prepared after decalcification (nitric acid, 5 per cent; ammonium ferric sulphate solution, 3 per cent; in the ratio 25:1 c. c.) of the isolated, formalin-fixed bones. These were embedded in paraffin and longitudinal sections were made through the callus. The sections were stained with haematoxylin-eosin and with van Gieson-Hansen's stain. Cutting the sections proved to be technically rather difficult and in some cases numerous sections had to be cut before the correct plane was reached.

These sections do not of course give a complete picture of the processes of healing, as only a small part of the fracture could be observed. We considered the possibility of cutting serial sections but abandoned the idea because of the large number of bones being studied.

A number of details which seemed to have importance for the various stages of the process of healing were selected from the motley histological picture of the fractures; they were assessed, like the radiographs, at values from 0 to 3. Our aim was to obtain a numerical expression of the healing by computing the mean figure of the various assessments. In this way we assessed the following details of the histological picture: haemorrhage, inflammatory infiltration, cavity in the callus, closure of the medullary cavities, periosteal formation of bone and cartilage, other formation of bone and cartilage, the size of the medullary cavity in the newly-formed periosteal tissue and the resorption of bone.

At the time of fracture there is a fairly large *haemorrhage* from the torn vessels; it is, however, quite soon organised and resorbed. Thus, groups of 7 or more subjects showed the following results:

Days	No. of animals	Haemorrhage min.	max.	mean
7	7	1	2.5	1.64
14	15	0	2.0	0.30
21	15	0	1.0	0.27
28	57	0	2.0	0.13
56	14	0	1.0	0.07
243	13	0	0.0	0.00

Thus these figures and also those from the intervening smaller groups show, as might be expected, that the haemorrhage has its maximum immediately after the fracture. It rapidly diminishes, and after 14 days extravasation was seen in only 4 out of 15 cases, and later only few and scattered haemorrhages were observed, usually only in fractures which were fairly mobile. These presumably secondary haemorrhages were probably due to trauma when the animals were removed from the cages. These figures and Fig. 7, page 446, show that the actual fracture haematoma is largely resorbed in the course of 14 days.

Very soon after fracture an aseptic *inflammatory reaction* develops at the site of the fracture, in response to the tissue destruction and haemorrhage. The haematoma is gradually replaced by a granulation tissue rich in cells and vessels and infiltrated with polymorphonuclear leucocytes, lymphocytes, plasma celles and phagocytic cells. Gradually this inflammatory reaction passes into a chronic stage. The fibroblasts in the granulation tissue become more plentiful and finally fibrous scar tissue is formed. The cellular infiltration persists longest in the outer layers of the callus where necrotic muscle fibrils, injured by the fracture, are degenerating.

The quantitative assessment gave the following results for the larger groups:

Days	No. of animals	Inflammation min.	max.	mean
7	7	1	2.0	1.57
14	16	0	2.0	1.13
21	15	0	1.5	0.47
28	57	0	1.0	0.13
56	14	0	2.0	0.21
243	13	0	1.0	0.08

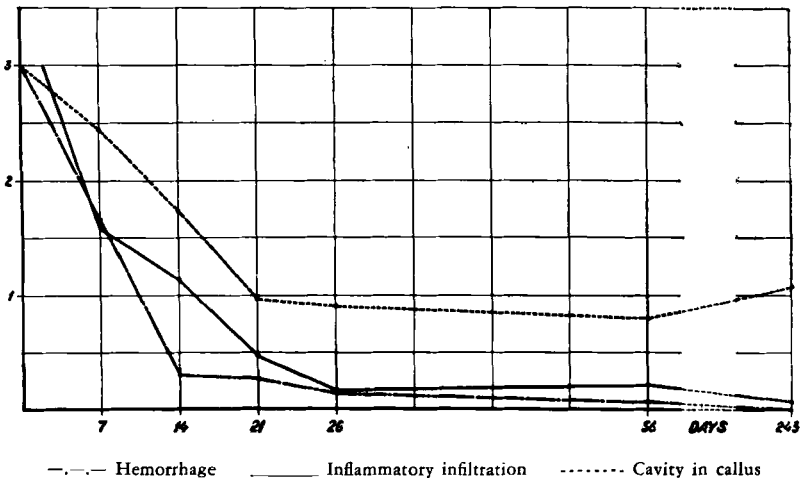


Fig. 7.
Curves showing haemorrhage, inflammatory reaction and cavity in callus.

Thus this material and Fig. 7 show that the inflammatory reaction reaches its maximum on the second to third day after the fracture. It then decreases rapidly during the first week and rather more slowly during the next two weeks, by the end of which time it has largely subsided, though a diffuse, scanty infiltration may still be found many weeks later. Marked inflammatory changes were found in a few fractures after 2 or 3 months; they were presumably due to secondary infection.

The central part of the callus usually contains a cavity which gradually becomes smaller as healing proceeds. At first this cavity is enclosed by the fractured ends of the bones and by loose granulation tissue, often containing deposits of fibrin. Later, it is enclosed by denser fibrous tissue. In the cases where a pseudarthrosis develops the articular cavity is enclosed by cartilage-covered osseous surfaces and connective tissue. In some cases, and particularly in fractures with much

displacement, two smaller cavities are found, one round each bone end. If the fracture is firm, or nearly firm, the cavities are, usually small or completely obliterated. A quantitative assessment of the large groups of the material gave the following results:

Days	No. of animals	Cavity in callus min.	max	mean.
7	7	1	3.0	2.43
14	15	0	3.0	1.73
21	15	0	3.0	0.97
28	55	0	2.0	0.90
56	14	0	2.0	0.79
243	13	0	2.0	1.08

This table and Fig. 7, page 446, show that the cavity becomes fairly steadily smaller during the first 3 weeks; it then remains rather constant at a mean value of about 1 for the rest of the period of observation. By the end of 8 months there even seems to be a slight rise in the curve, presumably with the formation of a pseudarthrosis with larger cavities. Only 2 out of the 13 fractures in this group showed no cavity.

Immediately after the fracture the medullary cavities are open, and usually there is necrosis of the medullary tissue adjoining the fractured surface. *Closure of the medullary cavity,* which is a feature of constant occurrence in the processes of healing, takes place in the following manner. First, the necrotic medullary tissue is resorbed and replaced by a disc of loose connective tissue. This soon becomes more dense, and strands of hyaline fibrils develop and form the basis for the osseous tissue which closes the medullary cavity. This disc of bone varies in thickness; sometimes it forms the starting point of an additional central formation of new bone. In the assessment given below, closure of the connective tissue is assessed as 1, immature osseous tissue as 2, and mature osseous tissue as 3.

Days	No. of animals	Closure of medullary cavity min.	max.	mean
7-9	9	1.0	2.5	2.00
14	9	2.0	3.0	2.89
21	11	2.0	3.0	2.91
28	41	2.5	3.0	2.98
56	11	3.0	3.0	3.00
243	12	3.0	3.0	3.00

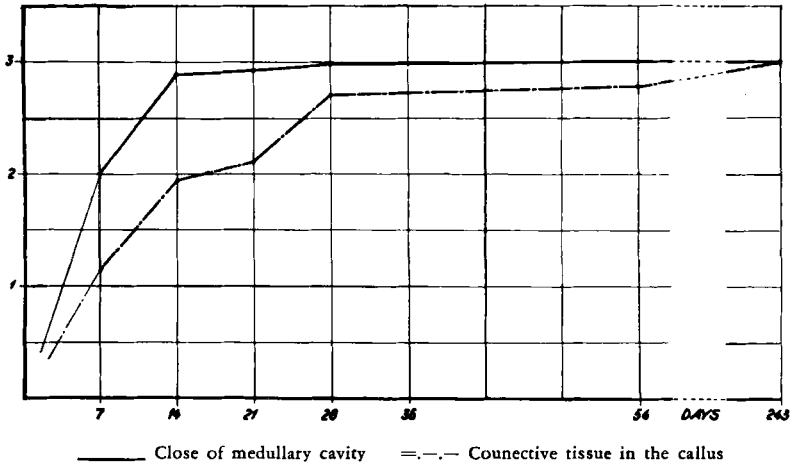


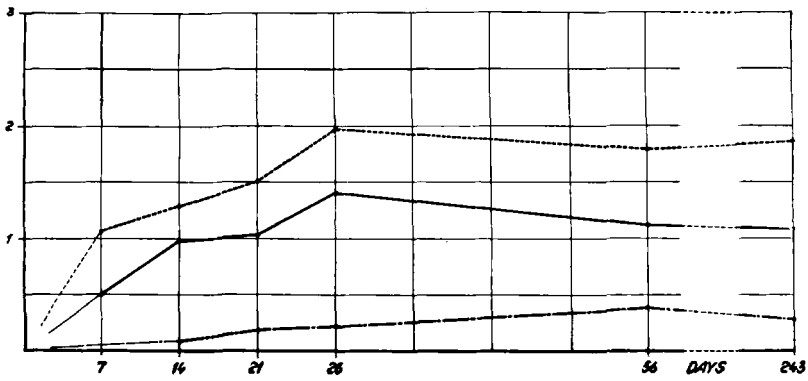
Fig. 8.
Curves showing *closing* of medullary cavity, and maturation of connective tissue in the callus.

This table and Fig. 8 show that the osseous closure of the medullary cavity has begun by the end of one week and is almost complete by the end of two weeks. It seems to be quite independent of the actual course of healing of the fracture. In some cases it is seen to be penetrated by new vessels from the medullary cavity at rather a late stage in the course of healing of the fracture. When union is complete, the disc is presumably resorbed, but this was not observed.

The periosteal reaction, with new bone formation, begins very early. First there is a rapid proliferation of the cells in

the deepest layers of the periosteum. This is followed by the formation of collagenous fibrils between the cells, which are gradually transformed into typical osteoblasts; these are found on the surface of broader strands of collagenous fibrils. The tissue becomes osteoid and is then transformed by calcification into true bone. Usually, the new bone formation extends well beyond the actual callus mass. The thickness of the periosteal new bone varies considerably, not only from fracture to fracture, but also on the two fractured ends and on different aspects of one bone. It is often most vigorous on the side of the lesser angle. between the fractured ends. On section the thickening is found to begin at, or, if there is necrosis of the fractured end, a short distance from the fracture line. Its thickness sharply increases, then steadily decreases with the distance from the seat of the fracture. The assessment of the degree of the reaction is according to the same scale as for assessment on the radiographs.

Days	No. of animals	Periosteal formation of new bone min.	max.	mean
7	7	0.5	1.5	1.07
14	14	0.5	2.0	1.29
21	15	0.5	2.0	1.50
28	57	0.5	3.0	1.96
56	14	0.5	3.0	1.79
243	13	1.0	3.0	1.85



Cartilage from periost —.—.— Cartilage elsewhere ------ Periosteal formation of new bone

Fig. 9.

Curves showing periosteal formation of new bone, formation of cartilage.

This table, the rest of the material and Fig. 9, page 449, show that the periosteal reaction is visible after a few days, shows rather an even increase during the first 3 or 4 weeks, and then remains fairly constant at from 1.5 to 2.0 during the rest of the observation period. Compared with the radiographic results, it appears to begin earlier and to be, on the whole, less. The former is due to the fact that the osteoid tissue does not produce any shadow, the latter due to the fact that the microscopy shows the thickness of the new-formed periosteal tissue in one plane only, whereas more can be seen on the radiographs. Further, the sections did not always include both the fractured ends.

The formation of *medullary cavities* is part of the development of the periosteal osseous tissue; it depends on the age and thickness of the latter. At first there are only small cavities with loose connective tissue and vessels between the osseous trabeculae. Gradually part of the osseous tissue is resorbed (near the original surface of the bone), and the cavities become larger. In addition, the marrow now contains haematogenous tissue. At the stage of full development a larger cavity is seen, and then a more or less successive transition into small cavities in the superficial layers, which are enclosed externally by a more compact layer of bone. The numerical assessment is 3 for a large cavity, 2 for a medium cavity and 1 for small cavities.

Days	No. of animals	Cavities in the periosteal layer min.	max.	mean
7	7	0.0	0.5	0.07
14	16	0.5	3.0	1.91
21	15	1.0	3.0	2.20
28	57	2.0	3.0	2.74
56	14	0.0	3.0	2.18
243	13	0.0	3.0	2.15

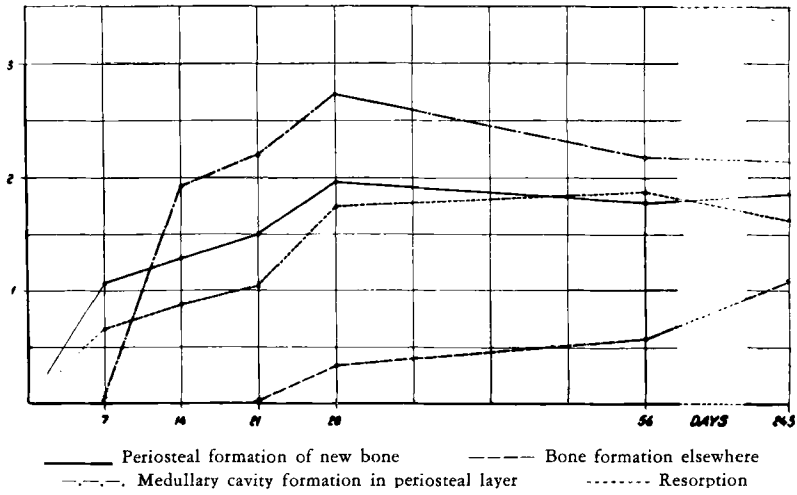


Fig. 10.
Curves showing formation of new bone, resorption, and formation of medullary cavity in the periosteal layer.

These figures and the curve in Fig. 10 show that the formation of the medullary cavities begins in the second week, and reaches its peak in 3 or 4 weeks. It then remains between 2 to 2½ during the rest of the period of observation.

As the healing of the fracture progresses the tissue on the surface of the periosteal thickening of the bone becomes chondroid; it is then transformed into cartilaginous tissue, which forms the basis of further bone formation. The *formation of cartilage from the periosteum* begins at the most prominent part of the thickened bone close to the fracture surface, and this is the origin of the osseous bridge which will gradually unite the two fragments.

The quantity of cartilaginous tissue may vary within a very wide range. In order to find a fairly reliable basis for the quantitative assessment, sections from a number of fractures with an increasing amount of cartilage were collected and fixed in correct sequence on a glass slide; each fracture

was estimated by comparison with this scale, ranging as usual from 0 to 3.

Days	No. of animals	Formation of cartilage from periosteum min.	max.	mean
7	7	0.5	0.5	0.50
14	15	0.5	2.0	0.97
21	15	0.0	2.0	1.03
28	57	0.0	3.0	1.41
56	14	0.0	2.5	1.11
243	13	0.0	2.5	1.08

This table and the figures and Fig. 9, page 449, show that cartilage formation begins after about a week and increases evenly during the weeks that follow. The apex of the curve occurs at 28 days and coincides with the group with the greatest number of individuals—at 28 days. The curve falls a little during the following week and then remains at about 1. In each case the amount of cartilaginous tissue depends on the growth on the external aspect and the transformation of the tissue into bone from the basal part. When complete bony union develops the cartilaginous tissue decreases; its amount is no exact measure of the healing, but the brisker the proliferation of cartilage, the more rapid the healing of the fracture. In very mobile fractures with only a slight tendency to healing there is, as a rule, little or no cartilage.

If the curves representing periosteal new bone formation and proliferation of cartilage respectively (see Fig. 9, page 449) are compared, they are seen to run almost parallel. The curve representing the periosteal formation of new bone is rather higher, but this might be due to the different assessment.

Cartilage formation occurs also in other parts of the callus, though usually only in small quantities. In this connection we do not consider the fibrocartilage in the intermediary callus which can hardly play any decisive role in the actual healing of the fracture. 16 out of 57 cases with cartilage-formation arising elsewhere than from the peri-

osteum occurred in the animals examined on the 28th day. Usually it occurred as a narrow rim of cartilage outside the osseous closing of the medullary cavity. Rarely, cartilage arose from fragments of bone which had remained in continuity with the periosteum. The numerical assessment was made as described above.

Days	No. of animals	Cartilagenformation min.	max.	mean
7	7	0	0.0	0.00
14	13	0	0.5	0.08
21	14	0	0.5	0.18
28	57	0	1.5	0.21
56	13	0	1.0	0.38
243	13	0	1.0	0.27

This table and Fig. 9, page 449, show that the cartilage formation cannot be found before two weeks; it then shows a steady but rather slight increase during the next 6 weeks. After 8 months it is slightly reduced, but the difference is insignificiant. This central cartilage formation cannot be regarded as having great importance for healing of the fracture.

Far greater importance must, on the other hand, be attributed to the *bone formation extending beyond the periosteal layer*. By this is meant incipient or complete osseous union between the fragments. As already mentioned, the cartilage on the surface of the primary periosteal thickening proliferates, forming a matrix for an osseous process or, rather, collar, which protrudes towards the other fragment and, by fusion with this, forms the external callus.

This group also includes a few cases in which bone formation was found to extend from the osseous closing of the medullary cavity, joining the osseous tissue from the periosteum. The assessment was made on a rough estimate and on the same principles as in the periosteal formation of new bone.

The actual process of osseous healing only begins after about 3 weeks (Fig. 10, page 451); it increases rather rapidly

Days	No. of animals	Bone formation min.	max.	mean
7	7	0	0.0	0.00
14	15	0	0.0	0.00
21	15	0	0.5	0.03
28	56	0	2.0	0.33
56	13	0	2.0	0.58
243	13	0	3.0	1.08

during the next week and then fairly steadily during the rest of the period of observation.

It has already been mentioned that after about 4 weeks these fractures may feel completely firm. This is due to a primary fibrous healing. At first the *connective tissue* in the callus is a loose, vascularized granulation tissue, but gradually the aseptic inflammation subsides; the tissue becomes richer in collagenous fibrils and poorer in vessels. At last it is completely cicatricial, and in many cases has been transformed into fibrocartilage. In the more mobile fractures the collagenous fibrils often form almost ligamentous strands which are surrounded by loose connective tissue, and run in various directions. The different phases of development of the connective tissue are seen very distinctly in preparations stained by van Gieson-Hansen's staining method.

Loose granulation was assessed as 1, the fibrous tissue still fairly rich in cells as 2, and the tissue which was poor in cells, cicatricial, fibrous or fibrocartilaginous as 3.

Days	No. of animals	Connective tissue of callus min.	max.	mean
7	7	1.0	1.5	1.40
14	16	1.5	2.0	1.94
21	15	2.0	2.5	2.10
28	56	1.5	3.0	2.70
56	14	2.0	3.0	2.79
243	12	3.0	3.0	3.00

The table and Fig. 8, page 448, shows that after one week the tissue is a granulation tissue, after two weeks it is fibrous

and after four weeks usually cicatricial. In some cases the nature of this tissue determines the firmness of the fracture; thus, fractures may be firm after 4 weeks, though osseous healing must be considered out of the question at this time. On the other hand, it is not a decisive factor, as all the fractures in the last group were assessed at 3, although some of them were still mobile. The maturation of the connective tissue of the callus seems to be mainly determined by the age of the fracture.

Finally, *the resorption of the bone* at the fracture site has also been assessed numerically. If there were only few osteoblasts and lacunae, the resorption was assessed as 1, vigorous resorption, including rarefraction of the cortical layer, as 3, the intermediary stage as 2.

Days	No. of animals	Resorption min.	max.	mean
7	6	0.0	1.0	0.67
14	13	0.5	2.0	0.88
21	13	0.5	1.0	1.04
28	54	0.0	3.0	1.74
56	13	0.5	3.0	1.88
243	13	1.0	3.0	1.62

These figures and the curve in Fig. 10, page 451, are at first a little below those for the periosteal formation of bone, but after the twenty-eighth day they almost coincide with them.

All these numerical assessments are, of course, open to much criticism, and they can hardly represent more than a rough estimate. On the other hand, the detailed analysis gives a better idea of the course of the individual processes of healing and so makes it possible to demonstrate deviations from the usual pictures.

It is striking to see how small a part the endosteal formation of bone plays in the healing of these fractures.

In order to obtain a total exponent of the stage of healing, we have computed the mean figures of all the single assess-

ments in each fracture. The figures of the first three assesments were reserved, e.g. no haemorrhage was assessed as 3, slight haemorrhage as 2 etc., since the extent and amount of haemorrhage, of infiltration, and of cavity decrease with progressing healing.

Days	No. of animals	Total histological assessment min.	max.	mean
2	1	0.00	0.00	0.00
4	2	0.27	0.45	
7	8	0.68	0.89	0.78
9	4	0.95	1.18	
11	5	0.95	1.45	
14	15	1.05	1.64	1.44
16	1	1.00	1.00	
21	15	1.23	2.15	1.65
24	2	1.50	1.56	
28	57	1.40	2.55	1.79
35	11	1.18	2.50	
42	5	1.64	2.27	
49	5	1.50	2.09	
56	14	1.05	2.46	1.97
63	5	1.41	1.82	
64	2	1.50	2.27	
70	5	1.65	2.09	
84	4	1.15	1.94	
94	3	1.40	1.80	
243	13	1.45	2.59	1.98

These figures and the curve, Fig. 11 and 11 a show a rapid rise during the first 4 weeks and then a constant level. As was the case for clinical firmness and the radiographic appearances, the standard deviation was large. The method becomes far more cumbrous and time-wasting in the larger experimental series and, as it is the result of many single assessments, becomes more uncertain. Its greatest value lies in its indication of possible deviations, particularly during the first 4 weeks, from the usual picture of healing.

In the preceding pages we have examined the course of healing of a single form of fracture: a transverse fracture through the middle third of the femur in 271 white rats,

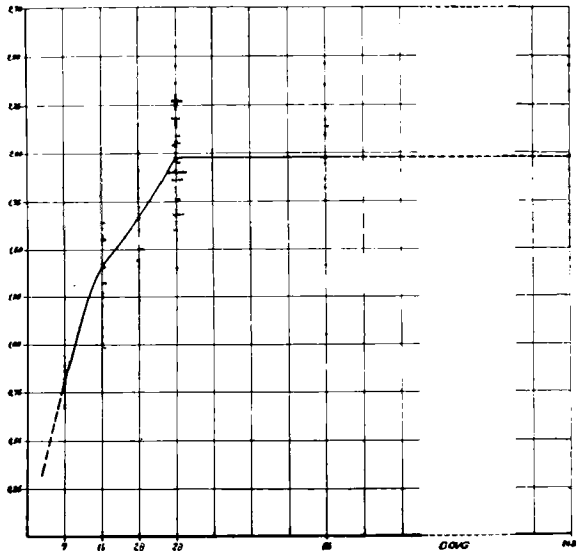


Fig. 11.
Curve showing healing as judged from the histological findings.

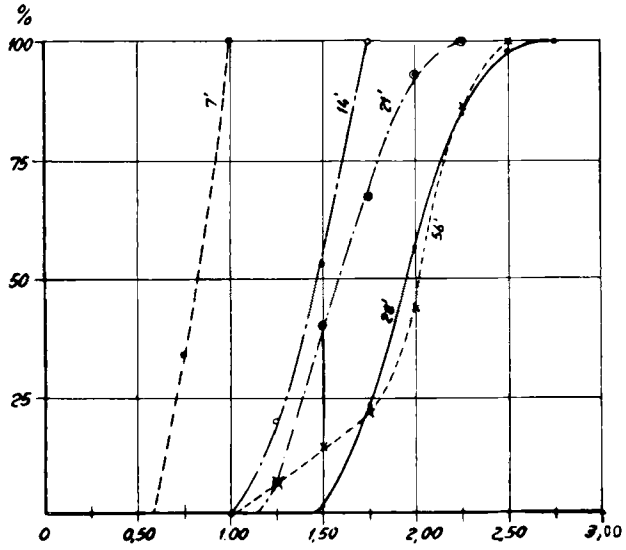


Fig. 11 a.
Cumulative curves showing healing (judged from histological findings) for 7, 14, 21, 28 and 56 days.

aged 4 to 5 months and born at different times of the year. The period of observation varied, the longest being 243 days. The degree of healing was assessed according to the mobility, the radiographic picture and the histological appearances. The mobility is recorded in degrees of movement, and the radiographic picture and histological appearances in grades of 0 to 3. The following mean figures were found:

Days	No. of animals	Mobility	Radiographic appearance		Histology
			periosteal reaction	resorption	
14	44	78°	0.53	0.36	1.44
21	38	53°	0.84	0.76	1.65
28	57	37°	1.14	1.14	1.79
56	51	28°	1.63	1.53	1.97

The figures, which indicate the normal course of healing of these fractures, make it possible to detect any delay or acceleration of healing in any experimental series in which such alterations are sufficiently large.

II.
INVESTIGATIONS INTO THE INFLUENCE OF VARIOUS TISSUES ON THE RATE OF HEALING OF FRACTURES

The time required for the healing of a fracture depends on the nature of the fracture, its site, the vascularization, the age of the animal and the accuracy of the reduction. We know that in humans a fracture at a definite site usually heals after such and such a time when the best possible conditions are provided. Experimental attempts to influence the healing of fractures have included, in addition to osteosynthesis, the administration of calcium, tissue transplantation, vitamins, and some of the sex hormones.

Osseous tissue sometimes forms in the cicatricial tissue

after operations on the bladder (*Straus,* 1914, *Neuhof,* 1917, *Phemister,* 1923). This heterotopic bone-formation was shown by *Huggins* (1930) to be brought about by the epithelium of the urinary tract. The examinations reported below started from these observations; the aim was to ascertain whether the factor in the epithelium of the urinary tract which was responsible for the heterotopic bone-formation might also influence the healing of fractures.

The procedure employed in our experiments differed in principle from that employed by *Polletini* (1923), *Levander* (1935, 1938), *Annersten* (1935-1940), and *Bertelsen* (1944). They injected extracts of various tissues intramuscularly, thus producing heterotopic bone-formation or growth of cartilage. As extracting agent they used different liquids, e.g. benzene, alcohol and acid alcohol. They found that extracts of osseous tissue (*Levander* and *Annersten*) and medullary tissue (*Bertelsen*) contained a substance, presumably a lipoid, which promoted heterotopic bone-formation. With this experimental procedure the lesion of the tissue caused by the extracting agent is undoubtedly of some importance as a basis of the bone-formation.

In addition to the epithelium of the bladder we have also tested a number of other tissues and tissue extracts. All the preparations were tested on femoral fractures in white rats aged from 4 to 5 months. Only fractures through the middle third of the femur were used; fractures at a lower level have a shorter healing time. In the first section of the present publication we described the examinations of the course of healing of this kind of fracture in detail. Usually there was a considerable displacement and some of the fractures never became firm, so that the number of subjects in each series had to be comparatively large before any decisive importance could be attributed to possible deviations.

The time at which the treated and untreated controls were compared varies somewhat. The animals were usually killed when the fractures were 4 weeks old, but in some series they were of 2, 3, 5 or 8 weeks' standing.

The comparison with the controls was based mainly on measurements of mobility at the seat of the fracture. For comparison we used partly the mean figure for the mobility, and partly the number of fractures with a maximum of 15° or, in larger experimental series, cumulative curves. These figures and those for histological healing agree on the whole with the mobility findings, though they show rather a wide range of variation.

In the preliminary series, rather few subjects were used. If there appeared to be deviations from the normal, the results were usually checked by means of larger series which excluded chance variations. The figures from the controls are shown in parentheses.

Epithelium of the Urinary Bladder and Extracts of it.

The first preparation tested was freshly abraded material from a rats' bladder. Half the quantity removed from the bladder was injected into the fracture site of 18 rats at intervals of two days; the animals were killed after 5 to 24 days. 9 animals, however, had to be excluded as the fracture was in the distal half of the femur. The remaining 9 seemed to show a somewhat better healing than the controls.

A further series was injected with freshly-abraded material from rabbit's bladders. The amount obtained from the bladder of one rabbit was approximately equal to that obtained from four rats' bladders. 10 rats each received an injection of 0.1 cc. of abraded material seven times into the fracture site; they were all killed at the end of two weeks. The healing of the fractures showed the following mean figures: Mobility 35° (78°), periosteal reaction 1.00 (0.53), resorption 0.95 (0.36), histological assessment 1.83 (1.44). Though the experimental series was small, the figures suggest an increased rate of healing.

In order to decide whether this improvement was due to a local or a systemic effect, scrapings from rats' bladders

were injected, in the same dose as in the first experiment, three times a week intramuscularly into the femur of the healthy side. The series comprised 52 animals which were killed at intervals of a week. The period of observation varied from 7 to 35 days. The findings were as follows:

2nd Series

Days	Range of Mobility	Periosteal reaction	Resorption	Histological assessment
7	102° (110°)	0.65 (0.25)	0.70 (0.00)	0.90 (0.83)
14	78° (78°)	0.78 (0.53)	0.83 (0.36)	1.46 (1.44)
21	50° (53°)	1.00 (0.84)	1.05 (0.76)	1.70 (1.65)
28	57° (37°)	1.05 (1.14)	1.00 (1.14)	1.78 (1.79)
35	37° (33°)	1.23 (1.40)	1.09 (1.42)	1.89 (1.79)

With regard to mobility, the experiment shows no difference. On the other hand, in the first two groups the periosteal reaction and the resorption are rather greater than in the control groups. The histological assessment shows normal figures. On this occasion all the scrapings for injection were prepared at the same time, at the beginning of the experiment, and it is most reasonable to suppose that the poor effect in the later groups is due to autolysis, though the preparation was stored in a refrigerator. The only conclusion that can be drawn from this experiment is that the active agent does not keep well in a suspension of cells.

As it was difficult to obtain the epithelium of rats' and rabbits' bladders in sufficiently large quantities, a preparation was made from epithelial scrapings from ox bladders. 31 fresh bladders, sent on ice from the slaughter-house, yielded 35 gm. of abraded epithelium. 15 gm. were suspended in 100 cc. of physiological saline. 2 cc. of the suspension was then injected into the fracture site three times a week in 8 rats; the animals were killed at the end of 4 weeks. The following figures were found: Mobility 61° (37°), periosteal reaction 1.00 (1.14), resorption 0.75 (1.14), histological assessment 1.57 (1.79). Thus all the results were less good than in the series of controls.

The remaining 20 gm. of abraded epithelium were extracted for 48 hours in 50 cc. of physiological saline solution and

centrifuged, and the sediment was suspended in 50 cc. of physiological saline solution. The aqueous extract was tested on 8 animals, the sediment on 10; 0.2 cc. being injected twice a week into the fracture site. In 4 weeks the figures were as follows:

Liquid:	Mobil.	84°,	periost.	react.	0.81,	resorp.	0.63,	histol. 1.40
Sediment:	„	38°,	„	„	1.42,	„	1.00,	„ 1.67
Control:	„	37°,	„	„	1.14,	„	1.14,	„ 1.79

The number of animals was small, but the findings suggest that there may be an active agent, which is not soluble in physiological saline solution. All the fractures in the first series had a mobility of more than 15°, while in 4 out of 10 fractures of the second series 15° was the maximum range.

A double dose of the sediment was then injected into the healthy side; the experimental conditions were otherwise the same. The number of subjects was 10; the figures were as follows: Mobility 36° (37°), periosteal reaction 1.55 (1.14), resorption 1.50 (1.14), histological assessment 1.86 (1.79). Thus the result was better than when the injection was made into the fracture site.

In another experimental series 15 gm. of abraded epithelium of ox bladder were extracted for 48 hours with 100 cc. of distilled water in an ice-box, after which sodium chloride was added to give a concentration of 0.9 per cent. After centrifuging 0.2 cc. of the aqueous extract was injected into 7 rats twice a week for 4 weeks. The injection was made into the seat of the fracture itself. The figures for healing were as follows: Mobility 79° (37°), periosteal reaction 1.00 (1.14), resorption 0.86 (1.14), histological assessment 1.37 (1.79), i.e. decidedly poorer than the normal.

The cellular remains were suspended in 100 cc. of physiological saline solution, and tested on 6 rats under the same experimental conditions. The result showed no acceleration of the healing after 28 days. The figures were: Mobility 58° (37°), periosteal reaction 1.33 (1.14), resorption 0.92 (1.14), histological assessment 1.85 (1.79).

Another preparation of epithelium of ox bladders was made by washing the epithelium abraded from 100 bladders three times in physiological saline solution and suspending 30 gm. in 100 cc. of saline solution. This preparation, which was thus twice as concentrated as the former, was kept for a fortnight in sealed ampoules and then tested on 32 rats. 12 animals were given 5 injections of 0.2 cc. into the healthy side and were killed after a fortnight. The figures show improved healing. The following mean figures were obtained: Mobility 54° (78°), periosteal reaction 1.12 (0.53), resorption 1.00 (0.36), histological assessment 1.73 (1.44). 11 subjects were treated for 21 days with 8 injections of 0.2 cc. The figures were as follows: Mobility 53° (53°), periosteal reaction 1.32 (0.84), resorption 1.23 (0.76), histological assessment 1.81 (1.65). After 4 weeks the figures relating to the remaining 9 animals, which had been given 11 injections were as follows: Mobility 50° (37°), periosteal reaction 1.06 (1.14), resorption 1.06 (1.14), histological assessment 1.70 (1.79). These three series show that in the first series the preparation was active 4 weeks after its preparation. In the second series, only the later figures were somewhat increased. In the third series all the figures were lower than those in the controls. Abscesses were found at the site of injection in several cases of this series. There was no bone formation at the site of injection.

In order to ascertain whether the abraded epithelium might possibly keep better in the form of powder, part of it was dessicated at 42° C. immediately after preparation and two months later it was suspended in physiological saline solution. It was tested in 10 animals, 0.2 cc. being injected into the healthy side. After a fortnight the figures were as follows: Mobility 104° (78°), periosteal reaction 1.00 (0.53), resorption 0.95 (0.36), histological assessment 0.67 (1.44). The last figures are certainly higher than the normal, but the firmness was decidedly less.

Another portion of the abraded epithelium was boiled for half an hour and then stored in ampoules. This preparation

was tested in 10 animals with 8 injections of 0.2 cc. injected during 3 weeks into the healthy side. The results correspond with the normal, and were as follows: Mobility 88° (53°), periosteal reaction 0.90 (0.84), resorption 0.85 (0.76), histological assessment 1.64 (1.65). This seems to suggest that the active agent was not thermostable.

The preceding experiments suggest that the epithelial cells of the bladder (in rats, rabbits and oxen) contain a substance which promotes the healing of fractures. This substance does not keep very well in the cells; it is presumably thermolabile and is insoluble in water. In the fresh state it exerts both a local and a resorptive action, and seems to be associated with the solid constituents of the cell body.

In order to avoid the possibility of a local action on the fracture, the preparations were injected intramuscularly into the femur of the healthy side in all the following experiments.

In the following experiment the possibility of extracting the active agent with alcohol was investigated. 25 cc. of fresh epithelium abraded from ox bladders was washed in physiological saline solution, suspended in 100 cc. of 70 per cent alcohol and extracted for one hour while being shaken. After centrifugation the alcoholic phase was mixed with 25 cc. of physiological saline and evaporated in a vacuum to 25 cc. This preparation was tested in 10 rats, 0.3 cc. being injected 9 times in the course of 3 weeks. The sediment from the extraction was also tested in 10 rats, 0.2 cc. being injected 4 times in the course of two weeks. The results obtained with the alcoholic extract were: Mobility 61° (53°), periosteal reaction 1.40 (0.84), resorption 1.35 (0.76), histological assessment 1.81 (1.65). For the residue the figures were as follows: Mobility 85° (78°), periosteal reaction 1.10 (0.53), resorption 0.95 (0.36), histological assessment 1.64 (1.44). In both series the firmness was on the average a little less than the normal, but in the first series the mobility was below 45° in 70 per

cent (normal 47 per cent), and in the second series in 40 per cent (normal 14 per cent). As this experiment, without showing anything definite, pointed in the direction of improved healing after injection of the alcoholic extract, another and more concentrated preparation was made by extracting the abraded epithelium of 25 ox bladders for 48 hours in 400 cc. of 70 per cent alcohol and separating by centrifugation. The alcohol was evaporated in a vacuum at 35° C. to about 75 cc., and this solution was kept in a refrigerator till the next day. During the evaporation, minute precipitations appeared in the liquid, and next day a sediment had collected which was separated by centrifugation and suspended in physiological saline solution up to 75 cc. This preparation was tested in 11 rats, each being given 12 injections of 0.2 cc. After 4 weeks the following results were obtained: Mobility 21° (37°), periosteal reaction 1.73 (1.14), resorption 1.86 (1.14), histological assessment 1.92 (1.79). This result is decidedly better than the normal, particularly considering that the mean figure relating to mobility is normally 28° after 56 days. Only one of the fractures in this experimental series had a mobility exceeding 35°, and in 73 per cent the mobility was less than 15° (26 per cent in the controls).

The clear aqueous phase from the centrifugation was tested in 11 rats with the same arrangement of the experiment. The figures were as follows: Mobility 37° (37°), periosteal reaction 1.27 (1.14), resorption 1.32 (1.14), histological assessment 1.80 (1.79). This result stresses the fact that the active agent had been extracted with alcohol.

The keeping qualities of suspensions of the substance extracted with alcohol were then examined after the preparation had been kept in a refrigerator for 1½ months. 10 rats were given a double dose, and, after 4 weeks, showed the following figures: Mobility 28° (37°), periosteal reaction 1.62 (1.14), resorption 1.46 (1.14), histological assessment 1.88 (1.79). The mobility of half the number of fractures was less than 15° as compared with the normal 26 per cent. Thus there was still some effect, though it was rather less than before.

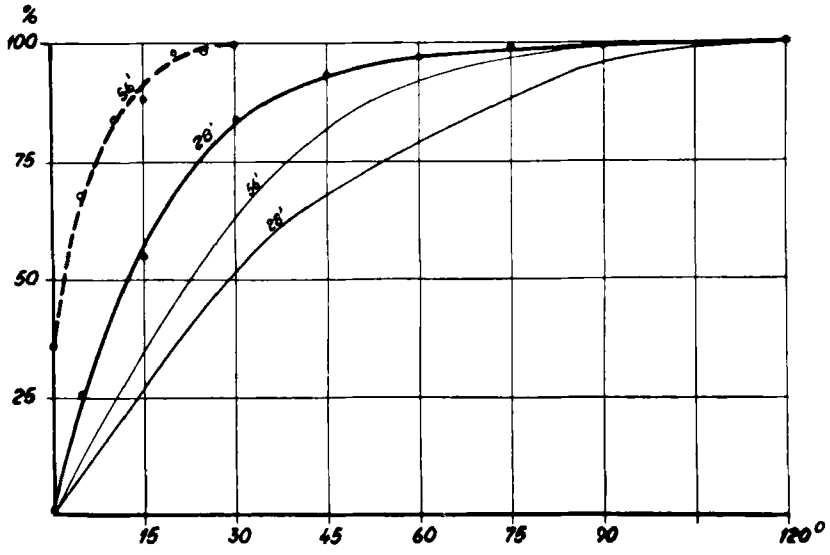


Fig. 12.
Cumulative curves showing the effect of the lecithin fraction from the epithelium of the urinary bladder after 28 and 56 days.
(The thin curves are controls).

After the same period half a dose was tested in 12 subjects. The figures showed no definite deviation from the normal.

The next experiment comprised the testing of four preparations which had been made as follows: Epithelium abraded from ox bladders was washed repeatedly in physiological saline solution. The sediment was extracted with 70 per cent alcohol for about 24 hours, after which the alcohol was evaporated at a temperature below 40° C. The alcohol was extracted with ether and, after admixture of acetone, greasy yellow-brown masses were precipitated; these were suspended before use by shaking with distilled water. A 1 per cent suspension was used in the first experiment and 2 per cent suspensions in the others. The results were as follows:

The mean figures for the last three preparations, which were of the same strength, are as follows with regard to mobility and radiographic assessment: Number of subjects 69;

Prep.	No. of animals	Mobility	Periosteal reaction	Resorption	Hist. asseess-ment	Max. mobility 15°
8/13 (1 %)	12	40°	1.37	1.25	1.92	42 %
7/90 (2 %)	12	24°	1.58	1.37	1.83	58 %
8/24 (2 %)	30	12°	1.30	1.25		67 %
8/32 (2 %)	27	25°	0.83	0.89		41 %
Controls	57	37°	1.14	1.14	1.79	26 %

mobility 19°; periosteal reaction 1.17; resorption 1.13; maximum mobility 15° in 55 per cent. When these figures are compared with the control figures, it is clear that the firmness is greatly increased. On the other hand, the reaction seen in the radiographs is not increased and, consequently, the firmness must be supposed to be due largely to fibrous healing. The cumulative curve for the mobility of the last three specimens showed a marked displacement to the left (Fig. 12, page 466).

As the active agent might exert its effect on the connective tissue only, and perhaps even delay the actual healing, preparation 8/32 was injected in doses as stated above for 4 weeks, and the animals were then left untreated for another 4 weeks. The result was as follows:

Prep.	No. of animals	Mobility	Periosteal reaction	Resorption	% of animals with max. mobility 15°
8/32 (2 %)	50	7°	2.07	1.95	88 %
Controls	43	28°	1.63	1.53	42 %

This result confirmed the previous findings and also showed that the increased fibrous firmness was followed by osseous consolidation. The same appears from the cumulative curves representing periosteal reaction and resorption (Fig. 13, page 468).

The aqueous phase from the alcohol extract from preparation 7/90 was injected into 11 animals in injections of 0.2 cc. 3 times a week for 4 weeks with the following results: Mobility 36° (37°), periosteal reaction 1.27 (1.14), resorp-

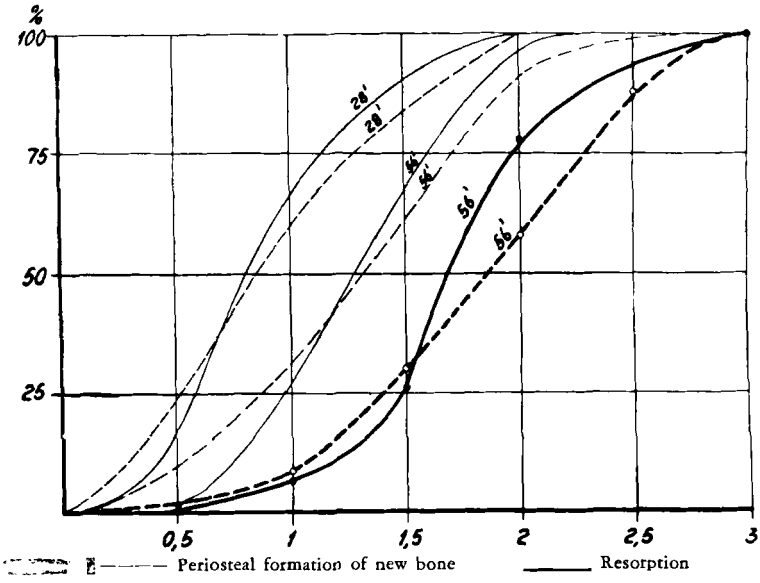


Fig. 13.

The effect of the lecithin fraction from the epithelium of the bladder on the healing of fractures as judged from the radiographs. (The thin curves are controls).

tion 1.41 (1.14), histological assessment 1.92 (1.79). This did not seem to produce any great effect.

The fats which were present in the ether extract but were not precipitated by acetone were tested in two series. The fats were dissolved in oil, and 0.2 cc. was injected three times a week for 4 weeks:

Prep.	No. of animals	Mobility	Periosteal reaction	Resorption	Max. mobility 15°
8/13	24	37°	1.44	1.21	29 %
8/24	30	23°	1.13	1.17	40 %
Controls	57	37°	1.14	1.14	29 %

The greater part of the active agent seems to be precipitated by acetone, and thus accompanies the lecithin fraction.

As an additional control of the extraction experiments stated above, 10 gm. of crude lipoid was extracted from all

the tissues of the bladder. This crude lipoid was fractionated[1] into sphingomyelin (1.48 gm.), cephalin (2.3 gm.), lecithin (2.6 gm.) and a fraction presumably consisting of cerebrosides (0.4 gm.).

	No. of animals	Mobility	Periosteal reaction	Resorption	Max. mobility 15°
Total bladder extract	25	33°	1.36	1.34	44 %
Sphingomyelin	15	34°	1.03	1.07	20 %
Cerebrosides (?)	15	37°	1.20	1.10	27 %
Cephalin	15	51°	1.20	1.17	20 %
Lecithin phase	26	28°	1.27	1.21	54 %
Controls	57	37°	1.14	1.14	26 %

Even though the deviation was less than when preparations made of epithelium of the bladder only were used, the mean figures relating to mobility, and in particular the percentage of fractures with a maximum mobility of 15°, still show that the active factor is present in the lecithin fraction. Radiography did not reveal any great deviation from the normal in these experiments either.

Thus the final result of these investigations into the effect of epithelium of the bladder on the healing of fractures is that the epithelial cells contain a substance of resorptive action which accelerates the healing of fractures (about 100 per cent in the experimental material), and accompanies the lecithin fraction in the course of preparation. The increased firmness seems first to depend on fibrous healing, followed by osseous healing. Thus the substance is not actually osteogenetic. Bone formation was never found at the sites of puncture.

Sperm.

In view of the results of the influence of epithelium of the bladder on the healing of fractures described in the preceding pages, experiments were made with injection of spermatozoa. The preparation employed was a suspension of fresh sperm-

[1] The fractionation was kindly undertaken by Frank Lundquist, civil engineer, M.Sc.

atozoa of bulls (supplied by the Royal Veterinary and Agricultural College); counts showed about 59,000 spermatozoa per cubic millimetre. 0.1 cc. of the suspension was injected into the fracture site twice a week for 3 weeks. This series comprised 10 subjects, and gave the following results: Mobility 84° (53°), periosteal reaction 1.15 (0.84), resorption 1.00 (0.76), histological assessment 1.24 (1.65). Thus there was definitely less firmness, even though the periosteal reaction was increased.

Medullary Tissue.

As previous examinations had shown that the epithelial cells of the bladder contained a substance promoting the healing of fractures, it was natural to examine whether bone marrow, the site of maturation of the cells of the blood, would also influence the healing of fractures. The marrow was aspirated from the bones of 5 rats, and immediately suspended in physiological saline solution. It was injected 5 times in two weeks into the healthy limb of 9 rats. The result was as follows: Mobility 95° (78°), periosteal reaction 1.00 (0.53), resorption 0.89 (0.36), histological assessment 1.46 (1.44). In this case, too, there was less firmness, though both the periosteal reaction and resorption were increased.

Extract of Brain Tissue.

The active agent in the epithelium of the bladder was obtained from the lecithin fraction. A similar preparation of rats' brains was made. It contained 1.50 per cent of lecithin in physiological saline solution, and 0.3 cc. was injected twice a week for 4 weeks into the healthy leg of 12 rats with the following: Mobility 25° (37°), periosteal reaction 1.33 (1.14), resorption 1.25 (1.14), histological assessment 1.96 (1.79); this suggests some effect, though hardly so great as that exerted by preparations of epithelium of the bladder. 33 per cent of the animals treated had a maximum mobility of 15°

(normal 26 per cent) and 83 per cent had a maximum mobility of 45° (normal 65 per cent).

Choline chloride, which is present in lecithin, was also tested, 0.3 cc. of 1.5 per cent solution in physiological saline was injected into 12 rats twice a week for 4 weeks; the following figures were obtained: Mobility 34° (37°), periosteal reaction 1.29 (1.14), resorption 1.33 (1.14), histological assessment 1.98 (1.79); thus there was no change in the healing of the fractures.

Extract of Liver Tissue.

The livers of 25 rats were extracted with alcohol and ether and precipitated with acetone; 0.2 cc. of a 2 per cent suspension of this preparation in physiological saline solution was injected 11 times in the course of 4 weeks into 12 rats. The result showed a distinct improvement of the healing: Mobility 17° (37°), periosteal reaction 1.62 (1.14), resorption 1.50 (1.14). In 54 per cent of the fractures the maximum mobility was 15° (normal 26 per cent). Pig's liver was prepared in the same way and injected in the same doses into 32 rats; the results were: Mobility 28° (37°), periosteal reaction 1.28 (1.14), resorption 1.37 (1.14). 34 per cent had a maximum mobility of 15° (normal 26 per cent). Thus the results were less marked than in the preceding experiment, though the healing had been distinctly accelerated. A mobility of 28° normally corresponds to 8 weeks' healing time.

Extract of Stomach Tissue.

Hog's stomachs, which are otherwise used for the production of ventriculin, were also prepared in the same way, and 0.2 cc. of a 2 per cent suspension in physiological saline was injected 11 times into 22 rats. All the figures were poorer than the normal figures of healing.

In conclusion it may be mentioned that extracts of blood (400 cc.) and striated muscle were also prepared, but in both cases the outcome was minimal.

Comment.

The healing of a fracture is made up of a number of component processes, each of which depends on a number of factors. The healing depends on the nature, localisation and reduction of the fracture. The actual healing process, which consists in the formation of an osseous bridgelike union between the fractured ends of the bone, follows quite a definite course, but is influenced by the different factors mentioned above. In the present work the possibility of influencing the course of healing by injections of tissue or tissue extracts has been investigated.

The healing of one particular type of fracture, fracture of the diaphysis of the femur of white rats, was analysed quantitatively and in detail. It appeared that the course of healing could be assessed numerically by testing the firmness of the fracture and by radiographic and histological examination. The healing is best represented by assessment of the degree of firmness. The radiographic appearances approximately reflect the course of healing, but may not follow it exactly. The histological assessment of the healing is useful during the first four weeks, but a close analysis of the single components showed that it is less accurate than the other two methods.

A number of tissues and tissue extracts were tested. It was found that the epithelium of the bladder contained a substance which was insoluble in water, but was soluble in 70 per cent alcohol and ether; it was precipitated by acetone. This substance had a resorptive action; it did not cause any local irritation. It gave a negative ninhydrin reaction.

It is presumably a lipoid; it accompanies the lecithin fraction and seems to be thermostable. It does not seem to be osteogenetic in the true sense of the word, since an increased firmness of the fractures is found without corresponding radiographic changes, but increased firmness provides better conditions for healing. The effect seems to depend on a primary fibrous healing, and its action seems to be mainly

a more rapid ripening of the connective tissue. That the epithelium of the bladder should contain a factor having a specific osteogenetic effect is hardly probable. It seems more reasonable to suppose that the rapidly growing epithelium of the bladder contains a substance which is of importance to the growth and ripening of cells. Correspondingly, no heterogeneous formation of bone was ever found after injection of this extract. The factor caused the percentage of healing to rise from 26 to 55 (after 4 weeks).

Extracts of other organs were tested by the same technique. Liver extracts gave varying results. An extract of homologous hepatic tissue caused a rise of the percentage of healing from 26 to 54, whereas heterologous liver extracts were of doubtful effect. An extract of homologous brain tissue did not produce any certain improvement in spite of the high lecithin content of the brain. This suggests that the active factor either is only a fraction of lecithin, or accompanies the lecithin fraction. Heterologous stomach extract had no effect, and heterologous extracts of blood and striated muscle were so poor that they were not tested.

SUMMARY

The course of healing of diaphyseal fractures of the femur in 271 rats was examined. It was estimated by assessing the degree of firmness and the radiographic and histological appearances.

Employing this normal material as controls, the influence of a number of tissues and tissue extracts on the healing of fractures was examined. The epithelium of the urinary bladder contains a factor which accompanies the lecithin fraction; this factor has a resorptive effect, and causes the percentage of healing after 4 weeks to rise from 26 to 55. Extracts of liver tissue produced somewhat varying results; extracts of heterologous brain tissue had no certain effect, and extracts of heterologous stomach tissue had no effect at all.

RESUME

Le cours de la guérison de fractures diaphysaires du fémur a été examiné dans 271 cas. Il a été évalué en établissant le degré de fermeté de la fracture et en se basant sur les aspects histologiques et radiographiques.

Utilisant ce matériel normal à titre de contrôle, l'influence d'un certain nombre de tissus et d'extraits de tissus sur la guérison des fractures a été examinée. L'épithélium de la vessie contient un facteur qui accompagne la lécithine ; ce facteur a un effet de résorption et fait monter au bout de quatre semaines le pourcentage de guérison de 26 à 55. L'extrait du tissu du foie produit des résultats tant soit peu durables et les extraits de tissu hétérogène du cerveau n'ont pas d'effet certain, ceux de tissu hétérogène d'estomac absolument aucun effet.

ZUSAMMENFASSUNG

Der Heilungsverlauf von Diaphysenbrüchen des Femurs in 271 Ratten wurde untersucht. Er wurde beurteilt nach dem Grade der Festigkeit des Bruches und den histologischen und røntgenologischen Bildern.

Indem man dieses normale Material zur Kontrolle verwendete, wurde der Einfluss einer Anzahl von Geweben und Gewebsextrakten auf die Bruchheilung untersucht. Das Epithel der Harnblase enthält einen Faktor, der mit der Lecithinfraktion verbunden ist. Dieser Faktor hat eine resorptive Wirkung und verursacht eine Erhörung des Heilungspercentsatzes nach 4 Wochen von 26 zu 55. Lebergewebsextrakte geben einigermassen variierende Resultate, Extrakte von heterologem Gehirngewebe hatten keinen sicheren Effekt und Extrakt von heterologem Magengewebe hatten überhaupt keinen Effekt.

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One of us (C.M.P.) is indebted to the Carlsberg foundation and King Christian the Tenth Fund for a grant held during this work.