COMPARISON OF FRACTURE REPAIR IN THE FROG, LIZARD AND RAT

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INTRODUCTION

Though much work has been done on fracture repair in mammals, it was felt that before any satisfactory general theory of fracture repair could be formulated, much more must be known about the process as it occurs in other vertebrates. It was therefore decided to investigate the healing of fractures in the frog, lizard and rat, with the object of separating general features from those peculiar to the species or class.

Very few previous comparative studies of fracture repair have been made. Fujinami (1901) investigated thirteen species of mammal, bird, reptile and amphibian, but only a few animals were used in each series, and no attempt was made to summarize the results or to deduce general principles from them. Wallis (1928) studied certain aspects of repair in the lizard and rat, but his observations lacked objectivity, as they were used chiefly to support Erdheim's (1914) mechanistic theories. The best and most useful account of repair in Amphibia is that of Wurmbach (1927), who worked with newts, salamanders and frogs.

MATERIALS AND METHODS

Twenty-two frogs (*Rana temporaria*), twenty lizards (*Lacerta vivipara*) and forty rats (*Mus norvegicus*) were used. One femur was fractured in each of the frogs, one femur in thirteen of the lizards and both femora in seven, one femur in ten rats and one tibia in the remaining thirty.

Closed fractures were made under ether anaesthesia by bending the limb sharply against the edge of the operating table. No attempt at immobilization of the fracture was made. The animals showed surprisingly little disability afterwards and were soon moving about almost normally.

The frogs and lizards were fed *ad lib.* on flies and fly larvae. The rats had a generous, well-balanced diet. The frogs were housed in a Perspex tank, in running water, the temperature of which could be regulated by means of a thermostatically controlled heating element. Some of the frogs were kept at room temperature (approx. 18° C.), others at 26° C. It was not possible to keep them alive at temperatures much higher than this. The lizards were kept in small cages of which the internal temperature could be controlled by means of electric light bulbs. They were maintained at temperatures of 18, 26 and 32–37° C., and their activity increased markedly with each rise of temperature.

At selected intervals after the fracture had been made the animals were killed with chloroform, or by decapitation, and the damaged segment of the limb was removed, skinned and placed in either Bouin's fluid or absolute alcohol. The former fixative was used when general staining methods were subsequently to be employed, the

latter when the distribution of inorganic salts and alkaline phosphatase was under investigation. The Bouin-fixed specimens were decalcified in 5 % trichloracetic acid. The others were not decalcified.

The majority of the specimens were embedded in paraffin and cut serially at 7–10 μ. A few of the tibial specimens in the rat were embedded in celloidin and cut at 20 μ. In a few of the rats the blood vessels were injected with indian ink before fixation. No special methods for cutting undecalcified specimens were used. In ordinary paraffin sections it was found that the shaft of the old bone was usually fragmented and displaced but the calcified cartilage and the callus cut fairly easily and their relationships to the soft parts were not seriously disturbed in the majority of sections.

Most of the common staining techniques for bone, cartilage and connective tissue were tried, but those found most generally useful were Weigert's iron haematoxylin and van Gieson, for demonstrating bone matrix and collagenous fibres; and 1 % aqueous methylene blue for cartilage. It was found that these stains could be used in combination, provided that the excess methylene blue fixed to the picric acid of the van Gieson stain was removed with absolute acetone. This combination was most useful for studying the general distribution of bone and cartilage in the same section. For more detailed studies, however, it was found best to use the stains separately on alternate sections. The finest collagenous fibres were not shown by van Gieson's stain, but were sharply defined by Wilder's silver diammino-hydroxide technique.

Inorganic phosphates were demonstrated by converting them to cobalt sulphide. The sections were treated for 5 min. with 2 % cobalt nitrate solution, washed thoroughly in distilled water and then placed in dilute ammonium sulphide solution as in the final stages of the Gomori technique for alkaline phosphatase. Inorganic phosphates were thus stained black. Alkaline phosphatase activity was investigated by Gomori's technique. Usually alternate sections were stained for phosphates and phosphatase.

RESULTS

(1) *General features of the repair process*

In all the experiments, examination of the fracture site 1–2 days after operation showed that the bone had been broken cleanly but that the broken ends lay at an angle to each other and in the case of the fractured femora a certain amount of overlap was also present, but in the tibiae this latter displacement was usually slight.

Extravasated blood lay between and around the broken ends of the bones, within the opened medullary cavities and between the muscle fibres, forming a small haematoma in the immediate vicinity of the fracture. The initial muscle damage was slight and few torn or avulsed fibres were seen.

The periosteum was torn from the bone to a varying, but generally slight extent near the fracture. Periosteal detachment was more marked in the rat than in the other animals, and was greatest on the overlapping surfaces of the fragments. Widespread subperiosteal extravasations of blood were not found.

After the fracture, at times varying with the species and the environmental temperature, but always within a few days, cellular multiplication began around the injured area, first in the *undamaged* periosteum (Pl. 2, fig. 16), then in the inter-

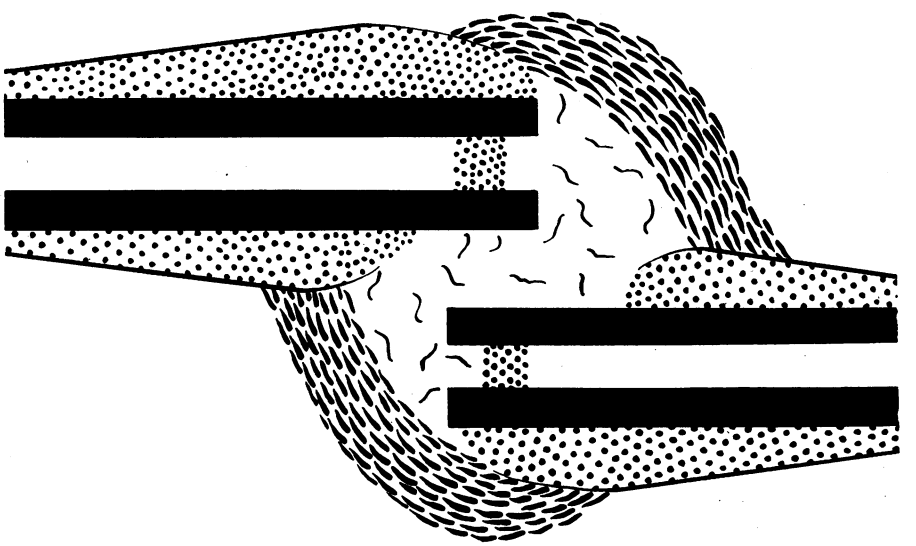
muscular connective tissue and lastly in the medullary cavity. Before long the haematoma was encompassed on all sides by a dense zone of proliferating cells, constituting a regeneration, or reparative, blastema (see Text-fig. 1 and Pl. 1, fig. 1).

The medullary contribution to the blastema, or more shortly, the medullary blastema, was confined to the immediate vicinity of the haematoma. The intermuscular, or parosteal, blastema was more extensive, and involved apparently normal musculature some distance from the fracture, while the periosteal blastema extended for a considerable distance, particularly in the rat, where almost the entire shaft up to the articular extremities was involved. The parosteal blastema was most conspicuous in the rat, less so in the lizard and least in the frog.

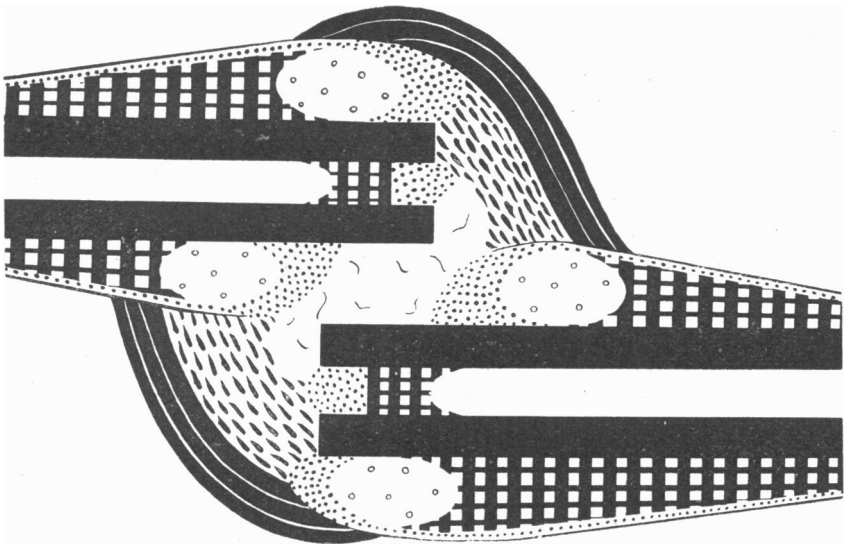
Meanwhile phagocytic cells, polymorphonuclear at first but later almost exclusively mononuclear, invaded the haematoma from around its periphery and the extravasated red blood cells were removed, leaving a network of fibrin strands in place of the original blood clot.

The haematoma was then invaded by cells from the surrounding blastemata. The peripheral part was rapidly replaced by an immature, highly cellular connective tissue, but the central part remained unorganized for a considerable period.

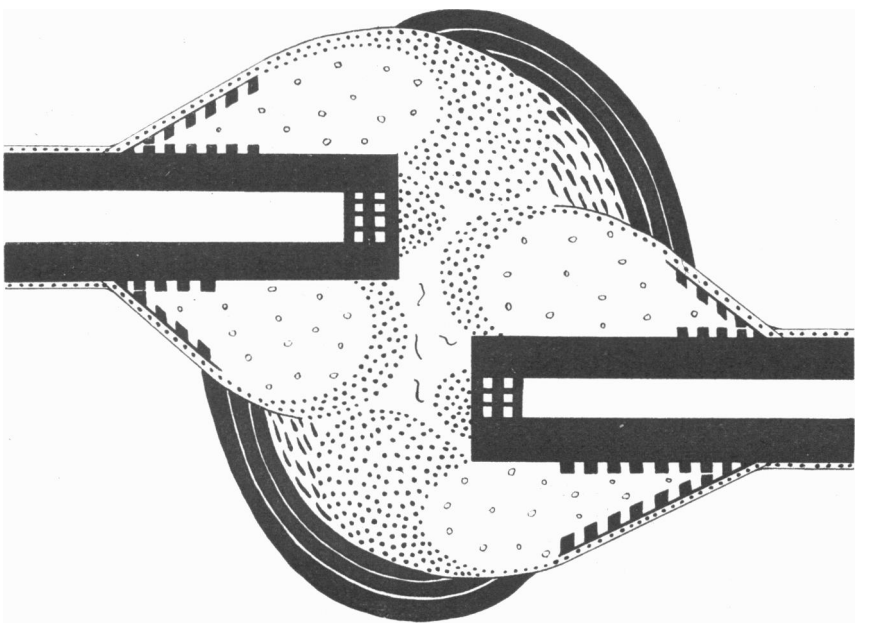
In the frog and lizard the invasion of the haematoma took place predominantly from the periosteal blastema. In the rat parosteal cells were involved to a greater extent, particularly in the overlapping femoral fractures where the majority of the cells replacing the haematoma were derived from the intermuscular connective tissue. The medullary blastema in all species was chiefly concerned in the organization of that part of the haematoma which lay within the medullary cavities, although, at a late stage, it played some part in the organization of the central portion of the haematoma lying between the fragments.



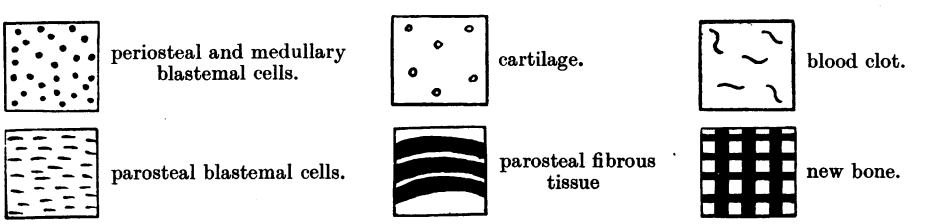
Text-fig. 1. The disposition, in schematic form, of the periosteal, medullary and parosteal blastemata around the fracture haematoma at an early stage. Similar appearances are found in all three species.



Text-fig. 2. The disposition of the new bone, cartilage and fibrous tissue arising in the blastemata of the rat at a later stage than that shown in Text-fig. 1.



Text-fig. 3. A similar stage to that illustrated in Text-fig. 2, but in the frog and lizard.



In the meantime, cellular differentiation and deposition of an intercellular matrix was taking place around the haematoma (see Text-figs. 2, 3). In the thickened periosteum intramembranous ossification occurred in the region remote from the fracture (distal periosteum) while cartilage was laid down near the fracture (proximal periosteum). In the frog and lizard cartilage formation far outweighed that of new bone (Pl. 1, figs. 2, 12); in the rat as much, if not more, bone than cartilage was laid down (Pl. 3, fig. 28; Pl. 4, fig. 29).

Within the medullary cavity, in all species, intramembranous ossification, but not cartilage formation, took place (Pl. 1, fig. 13; Pl. 3, fig. 28). It was also the rule that osteogenesis began later in the medullary cavity than in the periosteum.

The parosteal blastema underwent changes similar to those seen in the maturation of ordinary granulation tissue. The intercellular matrix became increasingly fibrous and this change occurred progressively from without inwards. Bone and cartilage formation did not occur at any stage in the peripheral part of this tissue. The question whether or not the parosteal cells contributed to the formation of cartilage more centrally will be considered at a later stage.

The greater part of the connective tissue which replaced the haematoma was gradually transformed into cartilage, the process beginning as an extension of periosteal chondrification into the fracture gap, and advancing until cartilaginous union between the fragments had been established (Pl. 1, figs. 3, 11; Pl. 2, fig. 22; Pl. 5, figs. 39, 40). Not all the connective tissue differentiated into cartilage, however, for, peripherally, dense fibrous tissue was laid down in the parosteal blastema and this served to repair the gap in the fibrous periosteum, while centrally, strands of fibrous tissue appeared between the advancing masses of cartilage. In the frog and lizard, and in the tibial fractures of the rat this latter formation of fibrous tissue did not prevent cartilaginous union, but in the femoral fractures of the rat fibrous tissue was formed in such amounts that fibrous union resulted. Eventually, however, this fibrous tissue was replaced by cartilage to give a secondary cartilaginous union.

Table 1. *Comparison of repair rates in the four experimental series*

	Days after fracture			
Stages in repair	Rat tibia	Rat femur	Lizard femur (32–37° C.)	Frog femur (26° C.)
Commencement of cellular proliferation in the periosteum	2–3	2–3	4–5	5
First appearance of new bone in the periosteal blastema	3–4	3–4	5	10
First appearance of cartilage in the periosteal blastema	4–5	5	7	10
First appearance of new bone in the medullary blastema	5–7	5–7	7	17
Completion of organization of the haematoma	8	19 (dense fibrous union)	11	17
Commencement of endochondral bone formation	10	10	21	30
Cartilaginous union	10–12 (primary union)	55 (secondary union)	16 (primary union)	36 (primary union)
Bony union	20	Not yet present at 55 days though imminent	31	Not yet present at 70 days. Not much more new bone than at 36 days

The cartilage formed both in the periosteum, and at the site of the original haematoma, was later progressively eroded and replaced by endochondral bone. In the rat and lizard this took place fairly rapidly and led to firm bony union between the fragments (Pl. 1, fig. 5; Pl. 5, fig. 41). In the frog, however, endochondral bone formation was late in starting and progressed at a very slow rate indeed, so that at the conclusion of the experiment (70 days after fracture) most of the cartilage was still uneroded.

(2) *Time-course of repair and the influence of temperature*

The times of appearance of certain critical stages in the repair process in each series are given in Table 1. It will be seen that in the early stages repair in the rat was more rapid than in the lizard which in turn was more rapid than in the frog. The sequence of events, however, was similar in the three species.

In the later stages the repair of the rat's tibia and lizard's femur advanced steadily along parallel courses until bony union had been established on the 20th and 31st days respectively. In the rat's and frog's femora the later stages were greatly delayed, but for different reasons.

In the rat the organization of the haematoma was slow to reach completion, and dense fibrous union resulted instead of the primary cartilaginous union observed in the other cases. This, however, was only a temporary phase, and the fibrous tissue was slowly replaced by cartilage to give a delayed secondary cartilaginous union at the 55th day, when the experiment was completed. Bony union had not then occurred, but appeared to be imminent, for endochondral bone advancing from either side had almost reached the centre of the fracture gap.

In the frog the delay was not in the attainment of cartilaginous union, but in the very slow development of endochondral ossification. When the experiment was completed at the 70th day the greater part of the cartilage originally formed was still present and endochondral bone formation had made little headway in the fracture gap.

In the frog and lizard the rate of repair was markedly influenced by the environmental temperature. In the lizard repair was extremely slow at room temperature (approx. 18° C.), where even after 40 days the centre of the haematoma was still unorganized and no new bone or cartilage had appeared. At 26° C. repair was greatly speeded up, but was still only about half as rapid as at 32–37° C. Thus at 26° C. new bone and cartilage appeared on the 16th day compared with the 5th–7th days at 32–37° C.; and cartilaginous union was incomplete at the 28th day at the lower temperature, although it was already established by the 16th day at the higher. There was little if any difference in the rate of repair in the lizard at 32 and at 37° C.

In the frog repair was considerably faster than in the lizard at room temperature, but at 26° C. this relationship was reversed, in spite of an approximate doubling of the rate in the frog at the higher temperature.

From these results, and from the fact that the lizards remained healthy and active at 37° C. while the frogs were killed by temperatures above 28° C., it would appear that the optimum temperature for repair in the lizard is near the mammalian body temperature, whereas in the frog it is around 26° C. The temperature did not

appear to influence the qualitative aspects of repair, although too few animals were employed at the lower temperatures to establish this with any certainty.

(3) *The periosteal blastema*

In the normal adult frog, lizard and rat, the periosteum consists of a thin layer of mature fibrous tissue containing elongated and flattened cells indistinguishable from ordinary fibrocytes. There is no histological evidence for the existence of a specialized cambial layer of potentially osteogenetic cells beneath the fibrous layer such as is present in the growing animal. For the most part the fibres are orientated parallel to the long axis of the shaft, but some bundles penetrate the shaft obliquely as Sharpey's fibres.

In the early stages of repair the periosteal changes were similar in the three species. They began a few millimetres from the fracture. Mitotic figures appeared and the membrane became progressively thickened, largely as the result of cellular proliferation, but also owing to the occurrence of oedema near the bone. In a short while the thickened periosteum gave the appearance of being divided into an outer and an inner stratum, the former compact and fibrous with fibroblast-like cells between the fibres, and the latter loose and oedematous with ovoid or pyriform cells. A little later the cells nearest the bone became converted into a layer of typical osteoblasts (Pl. 2, figs. 18, 20 and 21). At first sight this appearance suggested that the original periosteum had been passively lifted from the bone by the proliferative activity of a new and independent tissue arising beneath it, and indeed in the literature the formation of the periosteal blastema is often referred to as a 'subperiosteal' reaction. From van Gieson preparations such a conclusion seemed warranted, for the outer layer was chiefly composed of heavily stained, coarse, collagenous fibres similar to those of the resting periosteum, while the inner layer apparently contained few if any collagen fibres. In silver preparations, however, the appearances were very different (Pl. 1, fig. 10), for a rich network of fine fibres showed up in the deeper layer and no sharp dividing line was discernible between the two layers. The fibres merely became finer and more widely separated as one passed inwards, while the original Sharpey's fibres were still present as frayed strands crossing the deeper layer from the outer surface and penetrating the cortex of the bone. While it appeared probable that some of the deeper fibres had been newly formed, the majority had evidently been derived from the stretching and fraying of fibres originally present in the resting periosteum, owing to the accumulation of cells and oedema-fluid between them. The appearances were inconsistent with the view that the original fibrous periosteum had been lifted bodily from the surface of the bone.

There was also no sharp boundary between the cells of the inner and outer strata. The superficially situated fibroblast-like cells, the deeper rounded cells and the definitive osteoblasts lying against the bone formed in reality a continuous series in respect of shape, size, nuclear staining and cytoplasmic basophilia (Pl. 2, fig. 18). Moreover, mitotic figures were most frequent in the outer layer which would not have been the case if this played a passive role while the deeper-lying cells were proliferating. It was therefore concluded that the cells of the periosteal reaction are of similar lineage, the outer layer constituting a reserve of proliferating cells from which

the inner layer of differentiating cells is progressively supplemented. The gradual transition from younger and less differentiated cells in the outer layer to older and more highly differentiated cells in the inner layer naturally follows.

This blastemal reaction was at first confined to the undamaged periosteum a little distance from the fracture; no cellular proliferation was observed at any stage in the avulsed periosteum in the immediate vicinity of the haematoma. As the reaction developed the periosteum was involved at increasing distances from the fracture. The thickened periosteum became wedge-shaped in section, being thickest proximally, i.e. towards the fracture, and tapering distally into normal periosteum away from the fracture. In the rat the transition from thickened to normal periosteum was gradual so that the wedge had a narrow angle, but in the frog and lizard the change was more abrupt, and took place relatively nearer the fracture than in the rat, so that the wedge was of wider angle (c.f. Text-figs. 2, 3 and Pl. 1, fig. 2, with Pl. 3, fig. 28).

In all three species the single layer of osteoblasts lying against the shaft became associated early on with the deposition of a thin lamella of new bone between it and the original bone. In the rat this was rapidly followed by extensive intramembranous ossification throughout the deeper layers of the periosteum in the distal part of the wedge (Pl. 3, fig. 28; Pl. 4, fig. 29), the process being heralded by the differentiation of the periosteal cells into typical osteoblasts. Later the cells in the thick part of the wedge near the fracture differentiated into chondroblasts and cartilage was laid down (Pl. 4, fig. 32; Pl. 5, fig. 39).

In the frog and lizard this extensive formation of new bone in the distal periosteum did not occur, and almost the whole of the periosteal blastema underwent chondrification (Pl. 1, fig. 3). In all species, however, a layer of proliferating cells remained just beneath the fibrous outer layer of the periosteum external to the new bone and cartilage. From these cells were differentiated successive layers of either osteoblasts or chondroblasts which contributed to the increasing thickness of the bone and cartilage. Eventually the new formation of chondroblasts ceased and a thin layer of new bone was deposited between the cartilage and the fibrous periosteum. This outer bony shell was particularly conspicuous in the later stages of repair in the frog and lizard, where it gradually spread towards the fracture (Pl. 1, fig. 3) and eventually, in the lizard, provided the chief bond uniting the fragments after the cartilage had been resorbed (Pl. 1, fig. 5).

With the cessation of osteogenesis and chondrification in the periosteum the outer fibrous layer became the new periosteum. This did not involve any loss of continuity between the various tissues, for in silver preparations it was seen that the fibrous bases of the cartilaginous and bony matrices were continuous both with one another, and with the fibrous tissue of the new periosteum.

There were marked differences in the vascularity of the periosteal blastema in the three species, and these appeared to have an important bearing on the type of matrix deposited. In the rat the distal part of the periosteal blastema was very vascular and the bone deposited here showed an alternation of blood vessels and bony trabeculae similar to that seen in the course of normal intramembranous ossification. The proximal part of the blastema, however, where chondrification took place, was almost entirely avascular.

The transition from typical eosinophil trabecular bone to typical basophil cartilage as one passed towards the fracture line was not abrupt in the rat, for between them there was differentiated a zone of connective tissue having properties intermediate between those of bone and cartilage, and unlike anything seen in the course of normal embryonic development (Pl. 4, fig. 32). The bony trabeculae first became coarser, more cellular and less eosinophil as the blood vessels became less numerous; then the trabecular structure was lost and the cells became intermediate in type between those of cartilage and bone, while the matrix stained faintly with both acid and basic dyes; then some of the cells acquired faintly basophil capsules and finally the tissue merged into frank avascular basophil cartilage.

In the lizard and frog the vascular pattern was quite different. In spite of prolonged search only an occasional blood vessel could be found in the thickened periosteum of the lizard and, in the majority of sections, the tissue appeared to be entirely avascular. In the frog a few blood vessels, widely spaced, were present in the blastema before chondrification began. Later these came to lie within a sparse system of cartilage canals (Pl. 2, fig. 19), where, in some places, a thin layer of bone was deposited around the vessels against the cartilage.

The formation of cartilage in the periosteum was thus associated in all three species with poverty of blood supply, while, on the other hand, bone formation was limited to the neighbourhood of blood vessels. Thus in the rat the extensive formation of new bone in the distal part of the periosteum was associated with an equally extensive system of blood vessels, while nearer the fracture the formation of cartilage took place in an avascular environment. Between the two, as the vascularity diminished, bone merged into cartilage through a series of intermediate stages.

In the lizard and frog the blood supply was poor throughout the blastema and cartilage was formed predominantly. What little new bone was present in the early stages of repair lay either close to the original shaft, where it was near cortical blood vessels; or immediately beneath the fibrous periosteum, where it was not too remote from the blood vessels of the extraperiosteal connective tissue; or on the walls of vascular cartilage canals.

The transition from bone to cartilage in the frog and lizard was more abrupt than in the rat, but on close examination a layer of an intermediate type of tissue could always be distinguished between them.

The modes of differentiation of true bone and cartilage within the periosteal blastema were essentially similar to those seen in the course of normal osteogenesis and chondrogenesis in embryonic life. The early blastema, in its deeper layer, consisted of a relatively homogeneous mass of cells (Pl. 2, figs. 18, 21) embedded in a matrix of fine collagenous fibres. The cells were generally ovoid with a centrally placed, moderately chromatic nucleus and a juxta-nuclear vacuole of moderate size, while the cytoplasm showed a definite affinity for such dyes as thionin, methylene blue and pyronin.

Where bone was to be formed the cells became pyriform with an eccentrically placed, heavily staining nucleus, the juxta-nuclear vacuole increased markedly in size and the cytoplasmic basophilia became greatly intensified. The cells now had the typical appearance of osteoblasts. Between them the collagen fibres became aggregated into coarse strands and a homogeneous cementing substance which

stained intensely with acid dyes was deposited around them. The fibres now were masked and could no longer be seen in van Gieson preparations, though they could still be seen clearly after silver impregnation. At this stage the tissue was readily identifiable as bone matrix, but, as will be shown later, no bone salts had yet been deposited.

The thin trabeculae of new bone bore a constant relationship to the vascular network, lying equidistant from neighbouring vessels, and the osteoblasts became arranged as a pseudo-epithelial surface layer. This early bone therefore had the appearance of typical primary cancellous bone. Later, in some situations, lamellar bone was deposited on the surfaces of the trabeculae, which were correspondingly thickened; in other situations osteoclasts appeared and the primary trabeculae were removed, opening up wide spaces which became filled with haemopoietic marrow (Pl. 5, fig. 41).

Where cartilage was to appear the cells became larger and more nearly spherical and their nuclei stained less heavily with haematoxylin while the cytoplasmic basophilia gradually decreased in intensity. A basophil matrix was then deposited around the cells in the form of thin capsules. At first the cells with their capsules were separated by tracts of fine collagen fibres, but as the deposition of the matrix spread away from the cells these were engulfed and were then no longer visible after ordinary staining methods, though, as in the case of bone, they were still visible in silver preparations.

In the rat and lizard the cartilage formed in this way showed a high ratio of cells to matrix and this hypertrophic condition persisted. In the frog, however, the proportion of matrix gradually increased until the cartilage was of the hyaline variety such as is normally found in the epiphyses of this animal.

Between the areas of normal bone- and cartilage-formation the cells were predominantly large and rounded as in cartilage, but they showed the large juxta-nuclear vacuoles and intense cytoplasmic basophilia typical of osteoblasts. The intercellular matrix was of heterogeneous consistency, with a tendency towards the appearance of thin basophil capsules around the larger cells and an eosinophil matrix elsewhere. The original collagen fibres were incompletely masked by the matrix and many of them remained visible in van Gieson preparations.

(4) *The medullary blastema*

The development of the medullary blastema proceeded along similar lines in the three species. The normal marrow structure disappeared near the haematoma and was replaced by a mass of closely packed proliferating cells of the same general type as those present in the early periosteal blastema. From their first appearance in all species the blastemal cells were associated with a rich network of blood vessels. The origin of the cells remained in doubt, but most probably they were derived from the reticular cells of the marrow. In the normal bone there was no sign of any cambial layer of potentially osteogenetic cells lining the interior of the shaft.

The blastemal cells gradually replaced the haematoma within the marrow cavity up to the fracture line, and also for a short distance into the fracture gap. Their advance was slower than that of the periosteal and parosteal cells, so that they

played a relatively small part in the repair as a whole. The cells nearest the inner aspect of the bone soon differentiated into osteoblasts, and trabeculae of new bone were laid down first of all in their vicinity (Pl. 1, fig. 13). Before long the majority of the cells followed suit and a network of bony trabeculae was formed throughout the blastema (Pl. 3, fig. 28).

The chief result of the activity of the medullary blastema was the formation of a plug of membrane bone sealing off the medullary cavity at the fracture line. This played very little part in effecting bony union between the fragments except in the tibial fractures of the rat where the medullary cavities were in close apposition, and a bridge of new bone was erected between them in the later stages of repair after the medullary blastemata had met across the fracture gap (Pl. 5, fig. 41).

In view of what has already been said about the relationship between vascularity and osteogenesis, it was noteworthy that the medullary blastema, which was always well supplied with blood vessels, did not show cartilage formation in any species.

(5) *The parosteal blastema*

The changes which took place between the muscle fibres near the fracture were similar to those seen around any inflammatory focus. The connective tissue cells proliferated and a wall of young granulation tissue was erected around the haematoma (Pl. 3, fig. 28). During this process many of the muscle fibres included between the strands of proliferating connective tissue became fragmented, while others apparently disappeared. At a later stage, however, regeneration cones were seen sprouting from the ends of many of the fragmented fibres and eventually a more or less complete restoration of the muscles was effected. This observation confirms Murray & Kodicek's (1949) results in experimental fractures of the fibula in guinea-pigs.

As the granulation tissue matured it became increasingly fibrous and more and more restricted to the periphery of the haematoma, while at the sides the line of demarcation between it and the fibrous periosteum was gradually lost. The parosteal blastema thus appeared to be instrumental in restoring the continuity of the fibrous periosteum across the fracture gap. Beneath this fibrous stratum, however, some parosteal cells invaded the haematoma, but discussion of their role in the repair process will be deferred until the organization of the haematoma is dealt with as a whole in the next section.

(6) *The organization of the haematoma*

The haematoma was invaded around its periphery by cells derived from the periosteal, parosteal and medullary blastemata (Text-figs. 1–3). At first the separate invasion paths were clearly distinguishable for they were limited by such natural topographical features as the fibrous outer layer of the periosteum, and the shaft of the fractured bone. As the cells encroached on the more central parts of the haematoma, however, it became increasingly difficult to distinguish them from one another by situation alone and in particular, to distinguish periosteal cells from those of parosteal origin.

There were other criteria by which the cells could be identified, but these became

increasingly difficult to apply as the centre of the haematoma was approached. Thus the periosteal cells, in general, were larger, more closely packed and showed more intense cytoplasmic basophilia than the parosteal cells. The reaction for alkaline phosphatase, however, provided the surest method of distinguishing the cells, for those from the periosteum exhibited intense enzyme activity while the parosteal cells showed little or none. Unfortunately this test could not always be applied for, owing to difficulties inherent in cutting undecalcified material, the most important parts of the sections were often lost.

In spite of these handicaps there was little doubt that in the frog and lizard and in the tibial fractures in the rat, the periosteal cells played the chief role in the organization of the haematoma, while the role of the parosteal cells was largely restricted to the restoration of the fibrous periosteum. On the other hand, in the femoral fractures in the rat, the haematoma was organized very largely by cells of parosteal origin.

To begin with, the haematoma was replaced by a primitive connective tissue containing fusiform cells and a network of very fine collagenous fibres, the majority of which could only be shown after silver impregnation. There were a few blood vessels in the rat and frog, but none in the lizard.

In the frog and lizard and tibial series in the rat the greater part of the primitive connective tissue became directly transformed into cartilage. This was not a localized process, for chondrification advanced in an orderly manner into the fracture gap from the periosteum on either side, and there was direct continuity between the periosteal cartilage and that formed in the gap (Pl. 1, figs. 2, 3). The cartilage also presented similar histological features in both situations. In the frog the ratio of matrix to cells was everywhere high; in the lizard and rat, low.

Few blood vessels accompanied the blastemal cells into the haematoma. In the frog there were a few vessels of capillary size and these later occupied a sparse system of cartilage canals. In the lizard the cartilage was quite avascular. In the rat the blood vessels became surrounded by sheaths of fibrous tissue and these, intersecting the cartilage, gave it a somewhat lobulated appearance.

In all species there was a tendency for the cartilage to degenerate in a few situations. The cells became pyknotic and the matrix was resorbed leaving cyst-like spaces (Pl. 2, fig. 22).

In the femoral series in the rat the conditions were very different. The spread of chondrification into the fracture gap from the periosteum made little headway, and the bulk of the primitive connective tissue became transformed into dense, almost avascular, fibrous tissue interspersed with slit-like cavities resembling bursae. Later, when the periosteal cartilage had all been replaced by endochondral bone, chondrification began anew in the fibrous tissue (Pl. 4, figs. 30, 33), and this led eventually to a secondary cartilaginous union. The secondary cartilage had a different structure from that of the primary cartilage laid down in the periosteum. The latter was of the hyaline variety in which the fibres were masked while the former had the appearance of fibro-cartilage, for the individual cells and their basophil capsules were separated by strands of mature fibrous tissue which stained intensely with van Gieson. The significance of this appearance of two types of cartilage in the course of fracture repair will be discussed later.

(7) *Replacement of cartilage by bone*

The most striking differences between the species in their modes of fracture repair concerned the manner in which the cartilage was resorbed and replaced by endochondral bone.

In the rat resorption began at the junction of the periosteal new bone with the cartilage on either side and advanced towards the centre of the fracture gap (Pl. 5, fig. 40). The invasion front consisted of an advancing line of numerous narrow, closely spaced and parallel erosion bays each of which was occupied by a capillary loop surrounded by blastemal cells. Chondroclasts were present in many of the bays just ahead of the capillary loops. The uneroded cartilage partitions between the erosion bays, left in the rear of the invasion front, became covered with a palisade of osteoblasts and new bone was deposited around them. The resulting trabeculae of endochondral bone retained their cartilaginous cores for some time and the cartilage matrix preserved its ordinary intense basophilia. Endochondral bone formation in the fracture cartilage thus followed very closely the events in the normal epiphysis. The chief difference was that whereas the bulk of normal epiphyseal cartilage is of the small-celled variety and only shows hypertrophic changes in a band just ahead of the invasion front, the fracture cartilage was hypertrophic from the beginning and no further hypertrophy took place.

In the lizard erosion began under the subperiosteal shell of new bone which had previously been formed across the fracture gap (Pl. 1, figs. 4, 7) and not, as in the rat, along linear invasion fronts on each side. Resorption was also a much less orderly process than in the rat and the erosion bays were relatively wide and shallow and irregularly spaced. Each bay was occupied by a centrally placed blood vessel surrounded by blastemal cells, and one or more chondroclasts were usually present. In the rat deposition of endochondral bone followed promptly upon erosion but in the lizard bone formation was delayed. Bone eventually appeared in the form of thin lamellae applied to the surfaces of the uneroded cartilage between the erosion bays (Pl. 2, fig. 17). This residual cartilage formed a network of trabeculae which were much thicker than those of the rat owing to the wider spacing of the erosion bays.

So far, endochondral bone formation at the fracture site in the lizard followed closely that seen at the normal epiphysis in this animal, and the differences between the lizard and the rat at the fracture were paralleled by similar differences at the epiphyseal line; but, in addition, the fracture cartilage in the lizard showed conspicuous changes which were demonstrable only with difficulty at the normal epiphyseal line, and which did not occur at all in the rat. Around the erosion bays the cartilage matrix lost its basophilia and became eosinophil (Pl. 2, fig. 14), and strands of coarse collagenous fibres appeared in it which were easily visible after van Gieson staining (Pl. 2, fig. 15). Moreover, the cells shrank and became more heavily stained until eventually they were indistinguishable from osteocytes. During this process a series of intermediate stages could be identified in a given section between normal basophil cartilage at a distance from blood vessels, and the bone-like tissue described, which lay nearest to blood vessels.

When these appearances were first seen it was thought that possibly the transitional

forms had been present from the beginning, or that the changes were associated with endochondral bone formation in erosion cavities which were sectioned tangentially, but after careful comparison of early with later stages, and of serial sections in the later stages, both these explanations were found to be false. Thus the cartilage before erosion began was homogeneous in its structure and staining properties (Pl. 1, figs. 3, 11), while at its junction with bone only a very narrow band of an intermediate type of tissue was with difficulty distinguishable, whereas after erosion began the intermediate forms were present over wide areas. Then, in serial sections, it was established that the changes were taking place well ahead of the erosion bays in regions where blood vessels and osteoblasts had not yet penetrated. Moreover, the bone laid down on the walls of the erosion bays was of the lamellar type and was covered with a layer of typical osteoblasts, whereas the tissue described above had the honeycomb structure of cartilage although its staining reactions were those of bone and typical osteoblasts were not present (Pl. 2, fig. 17). We were therefore forced to conclude that an actual transformation of cartilage into bone was taking place, involving resorption of the basophil cartilage matrix, substitution of a fibrillar eosinophil matrix, and conversion of the chondrocytes into osteocytes.

In the frog the pattern of endochondral ossification in some respects was like that in the lizard, in other respects it was quite different.

Thus the periosteal cartilage surrounding the ends of the original fragments was eroded in an irregular manner from its external and internal aspects, and wide erosion bays were formed on whose walls lamellae of endochondral bone were deposited, as in the lizard (Pl. 3, figs. 23, 26 and 27). Chondroclasts, however, were very rarely seen. The cartilage was eventually divided up into irregular nodules covered externally by new bone. During the erosion similar changes were observed in the cartilage to those described in the lizard, including loss of basophilia, acquisition of eosinophilia, fibrosis of the matrix and transformation of the cells into osteocytes.

In the cartilage occupying the fracture gap, conditions were quite different. The changes were restricted here to the cartilage canals. Previously these were quite small and were surrounded by homogeneous basophil hyaline cartilage (Pl. 2, fig. 19). Now they became greatly enlarged by the resorption of the cartilage, widening of the blood vessels and increase in the number of the perivascular cells (Pl. 3, fig. 24). During this process some adjacent canals coalesced. The resorption was accompanied by loss of basophilia and acquisition of eosinophilia on the part of the cartilage matrix surrounding the canals. Fibrosis of the matrix, as in the lizard, and in the periosteal cartilage of the frog, was not observed around the canals, and the cells showed little change. The mechanism of erosion was not clear for no chondroclasts were to be seen in any of the canals. The intracanalicular cells nearest the cartilage then became differentiated into osteoblasts, and lamellar bone was formed between them and the cartilage (Pl. 3, fig. 25).

These changes took place very slowly. They first appeared in the canals nearest the ends of the bone on the 30th day, but had not reached the canals at the centre of the fracture gap even after 70 days.

In certain respects the erosion of the fracture cartilage in the frog was similar to that taking place in the epiphyses of the long bones during normal growth. At the junction of the bone marrow with the normal epiphyseal cartilage an invasion front

is normally present where vascular loops and medullary cells advance into wide, shallow erosion bays. No chondroclasts are to be seen and the cartilage is not replaced by endochondral bone. Very significantly, in view of what has been described at the fracture site, the epiphyseal cartilage nearest the marrow cavity is strongly eosinophil in contrast with the greater part of the epiphysis which is strongly basophil, although architecturally the two forms of cartilage appear to be identical. Also the transition from basophilia to eosinophilia is not abrupt but gradually takes place over a fairly wide intermediate zone.

Although evidence of direct transformation of cartilage into bone in the frog could not be found in the epiphyses of the long bones, in the pelvis, where endochondral bone formation does take place, indications of such a process were seen in the cartilage lying between the erosion bays, but on a much smaller scale than that seen at the fracture site.

After a time, in the rat and lizard, the fracture cartilage was entirely replaced by a network of endochondral trabeculae, fine meshed in the rat and coarse meshed in the lizard. The trabeculae in the rat were narrow and consisted of an outer layer of lamellar bone and an inner core of basophil cartilage. In the lizard the trabeculae were much wider and the inner core consisted of a cellular type of bone in which the original cartilage structure, but not its staining properties, was preserved. Later most of the endochondral bone in both lizard and rat was removed in the course of remodelling and replaced by haemopoietic marrow.

In the frog the structure of the endochondral bone was much more like that of the lizard than of the rat. It differed from the lizard chiefly in being more compact.

(8) *Calcification*

Strictly speaking, the available histochemical tests for the mineral salts of bone and cartilage are methods for detecting the presence of insoluble inorganic phosphates. There can be little doubt, however, that these consist predominantly of salts of calcium. By the method used in the present investigation the sites of deposition of these salts are inferred from the locations of deposits of cobalt sulphide in the sections. In order to avoid unnecessary circumlocution the process of deposition of the mineral salts will be referred to as 'calcification' and the salts themselves as 'calcium salts', although the evidence is necessarily circumstantial.

It was found in the case of bone that the intensity of the cobalt sulphide reaction was inversely proportional to the age of the tissue, suggesting that cobalt is able to replace the calcium of recently deposited bone salt relatively easily, but can do so only with difficulty later on. This finding is in agreement with the prevalent view that newly formed bone salt is composed very largely of readily hydrolysable calcium phosphate, but that later this is converted into a complex carbonoapatite in which the calcium is less labile. In cartilage, however, the age of the tissue apparently made no difference to the ease with which its mineral salts were demonstrable.

This ability of the present method to differentiate between recent and older deposits of bone salt is fortunate, for we were naturally most interested in the initial phases of calcification where the reaction was most intense. Another finding was that, whereas in newly formed bone the precipitate of cobalt sulphide was uniformly dense and amorphous, in cartilage it was coarsely crystalline and the overall density

was much less. The reasons for these differences were not clear, but it is tempting to relate them to differences in the fine structure of the organic matrices of the two tissues.

In all three species there was some delay in the calcification of the newly deposited bone matrix. Thus in the trabeculae of membrane bone formed in the periosteal blastema of the rat, the matrix farthest from the old bone, which had been most recently deposited, showed no precipitate of cobalt sulphide (Pl. 5, fig. 35). In the lizard, likewise, the new bone deposited during endochondral ossification at first gave no reaction for calcium salts (Pl. 2, fig. 17). In the frog also an uncalcified layer of bone matrix was found between the calcified bone matrix and the osteoblasts during endochondral bone formation in the walls of the cartilage canals (Pl. 3, fig. 25).

In the case of bone matrix, while the evidence for delay in calcification was quite definite, the actual time lag was probably quite short, and at most was only of the order of a day or two. In the case of cartilage, however, the delay was much greater.

Thus, in the lizard, cartilage appeared in the periosteal blastema on the 7th day, but calcium salts were not deposited until the 16th day, by which time cartilaginous union had been effected. Even then, only the oldest parts of the cartilage farthest from the fracture showed deposits of calcium salts, which were present as coarse crystals in the capsules of the more peripherally placed cells (Pl. 1, fig. 9). From here calcification slowly spread towards the fracture. It did not take place indiscriminately throughout the thickness of the cartilage but only under the fibrous periosteum and on the surface of the old bone. Then as erosion began calcium salts were deposited just in advance of the irrupting blood vessels in the zone where direct transformation of bone into cartilage was taking place. This relationship of calcification to the advance of blood vessels was very definite and probably means that the blood is the source of the mineral salts necessary for calcification. In newly formed endochondral bone the cores of the trabeculae, composed of cartilage which had been transformed into bone, were heavily calcified, but the lamellar bone at the surface of the trabeculae was uncalcified (Pl. 2, fig. 17).

In the rat, cartilage first appeared in the periosteal blastema on the 4th or 5th day, but calcium salts were not in evidence until the 12th day, and even then they were only present in the neighbourhood of the blood vessels (Pl. 5, fig. 37). As in the lizard, this restriction of calcification to the neighbourhood of blood vessels persisted throughout endochondral ossification.

In the frog, that part of the cartilage which was farthest from the fracture became heavily calcified as it was being transformed into bone, but the bulk of the cartilage was completely devoid of calcium salts except for a short distance around the cartilage canals, and even there they were not always present (Pl. 3, fig. 25).

(9) *Phosphatase activity*

In the rat and lizard the osteogenetic and chondrogenetic tissues around the fracture site always gave a strong reaction for the enzyme after a short period of incubation. In the frog, however, only a faint reaction could be elicited even after prolonged incubation, although the specimens from all species were treated similarly prior to incubation. The normal epiphyseal cartilage in the frog, unlike that of the

lizard and rat, also exhibited little or no enzyme activity. The reason for this difference between the species is not known, but possibly the enzyme in the frog is particularly thermo-labile and is almost entirely destroyed in the paraffin oven. On the other hand, it may be that phosphatase activity is in fact very low in the frog and this may be an explanation of the virtual absence of calcification in the fracture cartilage. The observations which follow will therefore be confined to the rat and lizard. In both animals the periosteal and medullary blastemata gave strong reactions, but the parosteal cells showed little or no enzyme activity (Pl. 1, fig. 6; Pl. 4, fig. 31; Pl. 5, fig. 38).

The enzyme appeared as soon as the periosteal and medullary cells began to proliferate, and before bone or cartilage had been formed or calcium salts deposited. At this stage the nuclei, especially the nuclear membranes and nucleoli, and the intercellular collagen fibres gave the strongest reaction, although the cytoplasm generally showed some activity. In the periosteum all the cells, whether of the outer or inner layers, gave the reaction. As bone and cartilage were differentiated the intensity of the reaction waned somewhat in the neighbourhood of the newly deposited matrices, but remained intense in the external zone of proliferating cells. In the region of new bone formation the osteoblasts showed moderately active nuclei and in many cases the Golgi zone was the most active part of the cytoplasm. In the cartilage the activity varied in a patchy manner, some cells showing almost no activity, others having activity concentrated in the capsules, while in other cases the matrix was uniformly and intensely active between the cells. Taken as a whole, however, the concentration of activity in the capsules was the most characteristic feature (Pl. 1, fig. 8; Pl. 5, fig. 36), and it is perhaps significant that the calcium salts deposited at a later stage, had a similar distribution (cf. Pl. 1, fig. 9).

The most striking, and theoretically the most significant, appearances were given at the junction of the periosteal and parosteal blastemata within the fracture gap (Pl. 4, fig. 34). Here the limit of periosteal invasion was marked by an abrupt change from intensely active to almost inactive cells, which paralleled an equally abrupt but less conspicuous change from larger, more closely packed and highly basophil cells to smaller, more loosely packed and less basophil ones. In view of these differences it is difficult to maintain, as Leriche and Policard (1928) and others have done, that the periosteal and parosteal cells are morphologically and functionally equivalent.

In view of the generally accepted thesis that phosphatase activity is an essential accompaniment of normal calcification it was of interest to observe that the appearance of enzyme activity preceded that of calcium salt deposition by a considerable interval. In Pl. 5, figs. 35 and 38, two adjacent sections are illustrated, the former stained for preformed calcium salts, the latter for alkaline phosphatase. It is evident that enzyme activity is well in advance of calcification. In cartilage the interval is much longer, for phosphatase activity is intense throughout the cartilage at the time of cartilaginous union, yet calcium salts are restricted to the peripheral parts near blood vessels. It appears, therefore, that the enzymic capacity for calcification runs ahead of its performance; and also that the presence of the enzyme does not in itself ensure calcification. If, however, it is postulated that the organic phosphates which form the substrate for the enzyme are derived from the blood stream, and do not

diffuse very far into the cartilage, then the restriction of calcification to the neighbourhood of blood vessels would be expected.

In the frog, what little enzyme activity could be demonstrated was limited to the nuclei of the proliferating osteoblasts and the cartilage cells in the immediate neighbourhood of the canals.

CONCLUSIONS AND DISCUSSION

This investigation has shown that, in spite of a number of important differences between the species, and even between the individual specimens from a given species, there exists a fundamental pattern of growth and differentiation during fracture repair which is independent of species and of the type of fracture.

This fundamental pattern seems to involve the following general principles: (1) that repair is due to the combined activities of three blastemal tissues which arise from undamaged periosteal, medullary and intermuscular (parosteal) connective tissues around the fracture site; (2) that the repair process is primarily directed towards the organization of the fracture haematoma from without inwards; (3) that the periosteum possesses a latent, easily-evoked capacity for bone and cartilage production and the medullary tissue for bone production, but the parosteal tissue is primarily fibrogenic although it may give rise to cartilage in certain circumstances; (4) that similar cells are responsible for both bone and cartilage formation in the periosteal blastema; and (5) that the pattern of cellular differentiation and matrix deposition is causally related to the richness or otherwise of the blood supply, and that where abnormal modes of growth and differentiation occur, abnormal conditions of vascularity may be held responsible.

The observations and arguments from which these principles have been derived have already been presented in the earlier part of this paper. A few points, however, need further elaboration, in particular the circumstances in which cartilage is formed, and the role of vascular supply in determining the course of repair.

There was no doubt in our experiments that the bulk of the cartilage arose in the periosteal blastema. The only unequivocal evidence of its formation elsewhere was in the femoral fractures of the rat where it arose in part of the parosteal connective tissue. The deposition of cartilage in the periosteum may seem surprising at first in view of its absence from this situation during normal growth, but the evidence is incontrovertible (see Ham, 1930). The most direct evidence that the periosteum possesses the latent capacity for cartilage production is given by the appearance of both bone and cartilage in cultured explants of periosteum (Fell, 1932, 1933). In bone sarcomata also the periosteum appears to give rise to both types of tissue. Then it must be remembered that in embryonic life the periosteum is derived from the perichondrium of the cartilaginous precursors of the long bones, while throughout the growing period, in the region of the epiphyseal line, periosteum and perichondrium are not sharply distinguishable. It is generally concluded from this evidence that the adult periosteal cells are potentially both osteogenetic and chondrogenetic, and that similar cells may give rise to either osteoblasts or chondroblasts, depending on circumstances external to the cell. Enneking (1948), however, has recently concluded from fracture studies that cartilage and bone are formed from different parts of the periosteum, the former from a specialized cellular cambial layer lying deeply

near the bone, the latter from the fibrous outer layer, but the evidence he adduces is not convincing and is refuted by the occurrence of intermediate types of tissues in which the structure and staining properties of the two tissues overlap.

A more important question is why cartilage is formed in certain situations and bone in others. The evidence has already been presented in favour of the view that bone is formed where the blood supply is rich and cartilage where it is absent or poor. This relationship has been noted by a number of previous workers, and its importance has been stressed by Blaisdell & Cowan (1926) and Ham (1930).

Other workers follow Roux (1895) in emphasizing the role of mechanical factors in determining the course of differentiation, cartilage appearing where the blastema is subjected to pressure and shearing stresses, and bone where the blastema is relatively unstressed. There is much to be said for this point of view, for the deposition of large amounts of cartilage in displaced and poorly immobilized fractures is a common clinical and experimental finding (Rigal & Vignal, 1881; Groves, 1914). Again, Bast, Sullivan & Geist (1925) found that cartilage was formed in much greater amounts in complete than in incomplete saw-cut fractures, while Kapsammer (1898), Wurmbach (1927) and Wallis (1928) agree that it appears in greatest amount on the concave aspect of angulated fractures, where the mechanical stresses are presumably greatest. Conversely, Pritchard (1946) found that cartilage rarely appeared in experimental fractures of the parietal bone where movement and pressure between the fragments is minimal.

In our present experiments, while it is true that cartilage was characteristically formed in the immediate neighbourhood of the ends of the fragments, at first sight supporting the mechanical hypothesis, the constancy and symmetry of the distributions of bone and cartilage despite marked differences in the relative position of the fragments from specimen to specimen, and the intimacy with which bone and cartilage were mixed in certain situations, made it difficult to ascribe cartilage formation to the operation of mechanical determinants, at least in the early part of repair, while the vascular hypothesis satisfactorily explained all the appearances.

On the other hand, the situation of the later-appearing parosteal cartilage in the femoral fractures in the rat did appear to be closely determined by the position of the fragments, and the mechanical hypothesis in this instance received support.

We do not attempt, therefore, to refute the view that fibrous connective tissues can be induced to undergo chondrification in the presence of certain types of mechanical stress, for which indeed there is direct experimental evidence (Glücksmann, 1939); or that this transformation may explain the production of *some* of the cartilage in certain types of fracture, e.g. where immobilization is poor, but our evidence does suggest that the vascular hypothesis, rather than the mechanical one, accounts most satisfactorily for the cartilage which appears at an early stage in the periosteal blastema.

The poverty of periosteal cartilage production in the repair of the skull, and in incomplete saw-cut fractures of the long bones is, in our view, explicable in terms of the reduced volume of blastemal cells which appear in such circumstances, and the consequent ability of new blood-vessel formation to keep pace with the cellular proliferation.

Finally, the vascular and mechanical hypotheses are not necessarily antagonistic,

even as an explanation for the transformation of parosteal fibrous tissue into cartilage in the later stages of repair, for it is quite likely that shearing and pressure stresses acting on connective tissue would tend to inhibit the ingrowth of blood vessels, or destroy those already present, and so predispose towards cartilage formation.

Whatever its origin, however, the adaptational value of cartilage during fracture repair cannot be doubted, for owing to its relative independence of blood supply, it can speedily restore some rigidity to the skeleton before the new bone, dependent on a rich blood supply, has bridged the gap. In this connexion it is significant that in the frog and lizard and in the tibial fractures in the rat, where periosteal cells were chiefly responsible for the organization of the haematoma, primary cartilaginous union always occurred, whereas in the femoral fractures in the rat, where parosteal cells were chiefly involved, fibrous union was the rule. This clear-cut distinction at once indicates the specificity and importance of the periosteal cells, and stresses the inadequacy of the mechanical hypothesis solely to account for the formation of cartilage in fracture repair, unless one makes the *ad hoc* hypothesis that the periosteal cells are much more sensitive to mechanical stresses than ordinary fibroblasts.

Calcification of cartilage has also been ascribed to mechanical influences, and has been given an adaptational significance (Erdheim's (1914) 'calcio-protective law'). Wallis (1928), in his experiments on lizards, describes a long delay in the replacement of fracture cartilage by new bone, the cartilage meanwhile becoming heavily calcified, and following Erdheim he attributes this to the need for strengthening the bond of union between the fragments. We did not find such a delay, however, possibly because of the higher temperature employed, and moreover we found that calcification of cartilage was an accompaniment of endochondral ossification, and did not occur except in the vicinity of the eroding blood vessels, the bulk of the cartilage before ossification began being uncalcified. The reason for this discrepancy in Wallis's and our results is not clear.

Harris (1933) takes a diametrically opposite view to ours as to the relationship of calcification in cartilage to vascularity, for he regards calcification as the result of ischaemia. It must be pointed out, however, that calcified cartilage is found in the growing skeleton in three main situations, viz. at the centre of ossification in the shaft, in the deepest zone of the articular cartilage, and in the hypertrophic cartilage of the epiphyseal plate, in all of which situations it is nearer blood vessels, viz. those of the vascular periosteal bud, of the bony epiphysis and of the metaphysis, than is the uncalcified cartilage.

Specific differences

Generally speaking, the lizard occupied an intermediate position between the rat and the frog in respect of most of the differences found, though, with two exceptions, the lizard was more like the rat than the frog.

The differences were both quantitative and qualitative. The chief quantitative differences were: (1) in the rates of repair, which may be ascribed to such factors as temperature, and specific differences in metabolic rates; (2) in the relative amounts of periosteal and parosteal blastemal tissues produced in the course of repair, which may be ascribed to specific differences in the ease with which these tissues respond to the fracture stimulus; and (3) in the relative amounts of cartilage and bone formed

by the periosteal blastema, which appeared to be correlated with the richness or otherwise of the blood supply although the reasons for the differences in vascularity remain obscure. In this last respect the lizard and the frog agreed in having a poorly vascularized periosteal blastema, whereas in the rat the vascularity was very much greater.

In the frog the extreme slowness of endochondral bone formation, the difficulty with which alkaline phosphatase activity could be demonstrated, and the virtual absence of calcification in the cartilage, separated this animal sharply from the other two and constituted quantitative differences so gross that they almost partook of a qualitative character, making the differences in these respects between the rat and lizard seem trivial by comparison.

The qualitative differences chiefly concerned the methods of cartilage erosion and endochondral replacement, and the behaviour of the cartilage in the neighbourhood of the erosion cavities.

As far as the actual erosion was concerned, the rat and lizard differed chiefly in respect of the regularity and size of the excavations, differences which were paralleled at the normal epiphyseal lines of the two animals. They agreed in that erosion began peripherally and was accompanied by the appearance of numerous multinucleated chondroclasts. In the frog, however, erosion chiefly occurred around existing cartilage canals, and was unaccompanied by chondroclastic activity. In the rat and lizard the cartilage was of the hypertrophic variety and was calcified prior to its resorption; in the frog, on the other hand, the cartilage was of the normal hyaline type and did not become hypertrophic or calcified prior to its removal.

It may here be stated that in all probability these differences in the behaviour of the cartilage are interdependent. It is generally believed that hypertrophic cartilage is more easily calcified than hyaline, and that chondroclasts do not appear during the resorption of cartilage unless it is calcified (Weinmann & Sicher, 1947). The virtual absence of phosphatase from frog's cartilage is perhaps also related to its hyaline character, for it is well known that in mammalian cartilage this enzyme is only found in the hypertrophic variety. It is significant in this connexion that in the long bones of the frog, endochondral ossification does not occur (Haines, 1942) and the epiphyseal cartilage during growth in length is resorbed without hypertrophic changes, calcification, or the appearance of chondroclasts.

The most important difference between the species, however, concerned the behaviour of the cartilage in the neighbourhood of the erosion cavities. Here the frog and the lizard showed several common features which were not found in the rat. These features have already been fully described in the presentation of the results, where the conclusion was drawn that an actual transformation of cartilage into bone was taking place.

The possibility of such a transformation has alternatively been affirmed and denied in the past. Thus on evidence essentially similar to ours, Wurmbach (1927), denies that, in the newt, an actual transformation is taking place and interprets the appearances as accompaniments of calcification, although in the frog, in our experiments, calcification did not occur, and in any case the appearances were too striking to be dismissed so lightly. Ziegler (1899) and Fujinami (1901), however, express

themselves strongly in favour of an actual conversion of cartilage into bone in amphibian fracture repair.

Weidenreich (1930), in mammals, admits that cartilage cells whose capsules have been partially eroded during the course of normal endochondral ossification may become transformed into osteoblasts and begin to deposit new bone matrix within the remaining parts of the capsules, but he does not regard this as evidence of metaplasia. The appearances we described were not of this character, however, but were more like those described by Mjassojedoff (1922) in the course of ossification of the hen's tracheal rings, which he interpreted as a direct transformation of cartilage into bone; and by Ziba (1910), in the foetal petrous temporal bone of man, who interpreted the appearances similarly. Koelliker (1889) states that cartilage is converted directly into bone in the recovery stage of human rickets and his illustrations are strongly reminiscent of our observations on the lizard. Perhaps the best evidence, however, comes from the tissue culture experiments of Fell (1933) and Roulet (1935), who found that the cartilage developing in explants of embryonic bony tissue is unstable, and becomes converted directly into typical bony tissue.

A priori, such a transformation should not be surprising, in view of the evidence given above for the common origin of osteoblasts and chondroblasts in the periosteal blastema, and when it is remembered that the organic matrices of bone and cartilage have a common basis of collagen fibres, and differ only in the type of cementing substance deposited around the fibres, the so-called 'ossein' of bone and 'chondromucin' of cartilage. Leaching out of the chondromucin, such as seems normally to occur at articular surfaces, and at the medullary extremity of the epiphyseal cartilage in the frog, should therefore leave behind a collagenous framework eminently suitable for subsequent impregnation with ossein to give bone matrix, especially if, at the same time, the irruption of blood vessels into the vicinity of the cartilage cells should stimulate their conversion into bone cells.

Finally, it should be pointed out that this apparent conversion of cartilage into bone is not a phenomenon peculiar to fractures in the frog and lizard, but occurs, although not on so extensive a scale, at the normal epiphyseal lines of the lizard's femur, and in the frog's acetabulum.

As a general rule, therefore, we may state that where qualitative differences exist between the species as regards the course of fracture repair, they reflect specific behaviour characteristics of the normal skeletal tissues, and with this in mind we may perhaps characterize fracture repair from a general biological standpoint, as an expression of inherent patterns of growth and differentiation superimposed on a non-specific reaction of the bony tissues to trauma.

SUMMARY

1. The repair of experimental closed fractures has been investigated histologically in the femora of the frog, lizard and rat, and in the tibia of the rat.

2. In each series a reparative blastema was formed in the early stages by the proliferation of periosteal, medullary and intermuscular (parosteal) connective tissue cells around the haematoma at the fracture site. The haematoma was later organized by cells growing in from these three sources.

3. New bone, cartilage and an intermediate type of tissue were early deposited in

the periosteal blastema; later, new bone appeared in the medullary blastema; while the parosteal blastema developed at first like an ordinary granulation tissue. The periosteal blastema was the most widespread, the medullary the least so.

4. In the rat more bone than cartilage developed in the periosteal blastema, but in the frog and lizard cartilage was the dominant tissue.

5. In the lizard and rat the periosteal and medullary blastemata showed intense alkaline phosphatase activity which preceded calcification of the bone matrix by a relatively short interval, but calcification of the cartilage by a considerable interval of time. Phosphatase activity was difficult to demonstrate in the frog.

6. In the frog and lizard, the haematoma was organized chiefly from the periosteum, and cartilaginous union readily occurred in spite of gross angulation and overlapping of the fragments in some cases. In the rat the parosteal blastema played a more important role, particularly in the femoral fractures, where displacement of the fragments was considerable, and fibrous union preceded a long-delayed secondary cartilaginous union. In the tibial fractures displacement was relatively slight, and primary cartilaginous union was the usual outcome.

7. Replacement of cartilage by bone took place in a different manner in each species. In the rat chondroclasts, blood vessels and osteoblasts rapidly advanced into the cartilage from each side in a regular manner as in endochondral ossification at the epiphysial line. In the lizard the invasion occurred less rapidly, and in an irregular manner from all sides. The resulting vascular spaces were wider, and the endochondral bone trabeculae coarser, than in the rat. In the frog the cartilage was eroded very slowly, chiefly from within, by the enlargement of pre-existing vascular canals, on whose walls bone matrix was later deposited. Chondroclasts in the frog were very rarely seen.

8. In the frog and lizard, but not in the rat, the uneroded cartilage appeared to become transformed directly into bone, for its matrix lost its basophilia and became increasingly fibrous and eosinophil, while its cells shrank to the shape and dimensions of osteocytes.

9. In all species there was some lag in the calcification of the bone matrix, for the newly formed trabeculae had uncalcified borders. In the rat and lizard calcification of the cartilage accompanied the ingrowth of blood vessels, and only occurred in their neighbourhood. In the frog the cartilage was virtually uncalcified except to a variable and slight degree in the immediate neighbourhood of the vascular canals. This is possibly attributable to the low phosphatase activity of the cartilage in this animal.

10. From the results it was concluded (*a*) that osteoblasts and chondroblasts are differentiated from similar cells in the periosteal blastema, the former appearing where the blood supply is adequate, the latter where it is deficient, (*b*) that the parosteal connective tissue cells may differentiate into chondroblasts when acted upon by certain types of mechanical stress, but ordinarily they remain as fibroblasts.

11. On the basis of the observed similarities and differences between the species it was concluded that the skeletal tissues during fracture repair exhibit specific modes of growth and differentiation superimposed on a generalized and non-specific reaction to trauma.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Lizard. 5 days. Unresolved haematoma. Early periosteal and parosteal reactions. Weigert's haematoxylin and van Gieson. ×21.

Fig. 2. Lizard. 11 days. Early cartilage formation in periosteal blastema (cf. fig. 12). Weigert's haematoxylin and van Gieson. ×21.

Fig. 3. Lizard. 14 days. Incipient cartilage union, repair of periosteum and formation of an external bony shell (cf. fig. 11). Weigert's haematoxylin and van Gieson. ×21.

Fig. 4. Lizard. 21 days. Cartilage union and early stage of vascular erosion. Weigert's haematoxylin and van Gieson. ×21.

Fig. 5. Lizard. 31 days. Established bony union. Weigert's haematoxylin and van Gieson. ×21.

Fig. 6. Lizard. 7 days. Alkaline phosphatase in periosteal blastema. Gomori. ×112.
Fig. 7. Lizard. 21 days. Vascular erosion of cartilage. Weigert's haematoxylin and van Gieson. ×112.
Fig. 8. Lizard. 7 days. Alkaline phosphatase in young cartilage of periosteal blastema. Gomori. ×490.
Fig. 9. Lizard. 16 days. Calcification in cartilage. Cobalt sulphide. ×490.
Fig. 10. Lizard. 5 days. Collagenous and precollagenous fibres of the periosteal blastema. Wilder. ×490.
Fig. 11. Lizard. 14 days. Incipient cartilage union (cf. fig. 3). Methylene blue and eosin. ×21.
Fig. 12. Lizard. 11 days. Early cartilage formation in periosteal blastema (cf. fig. 2). Weigert's haematoxylin and van Gieson. ×112.
Fig. 13. Lizard. 7 days. Bone formation in the medullary cavity. Haematoxylin and eosin. ×112.

PLATE 2

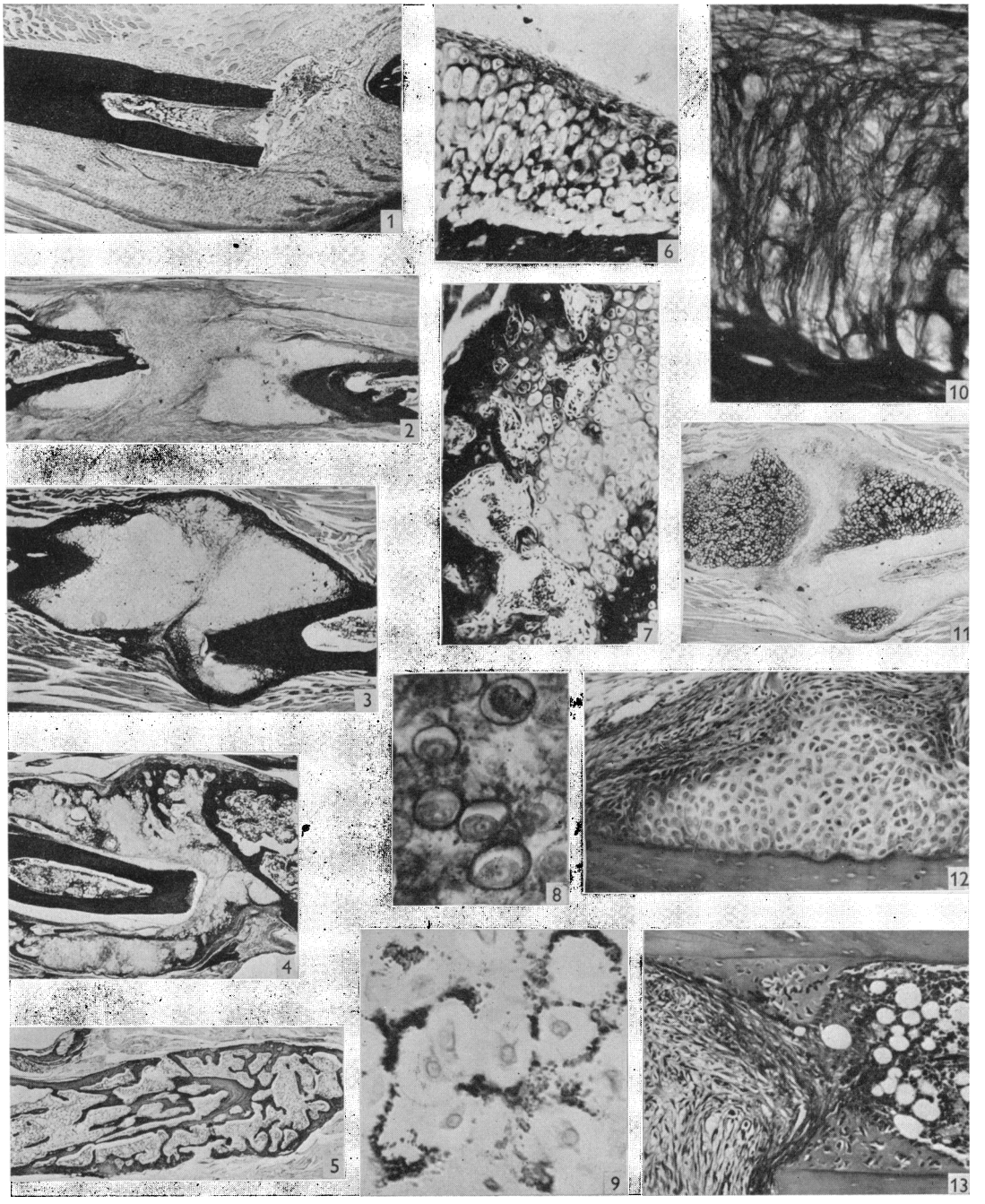
Fig. 14. Lizard. 21 days. Loss of basophilia and appearance of eosinophilia in cartilage near eroding blood vessels. Methylene blue and eosin. ×112.
Fig. 15. Lizard. 21 days. Apparent transformation of cartilage into bone. Weigert's haematoxylin and van Gieson. ×490.
Fig. 16. Frog. 11 days. Early periosteal reaction. Weigert's haematoxylin and van Gieson. ×17·5.
Fig. 17. Lizard. 25 days. Endochondral bone trabeculae. Calcification of central cores of residual cartilage. Uncalcified lamellar bone at surface of trabeculae. Cobalt sulphide. Weigert's haematoxylin and van Gieson. ×112.
Fig. 18. Frog. 11 days. Early periosteal blastema. Weigert's haematoxylin and van Gieson. ×112.
Fig. 19. Frog. 21 days. Vascular canals in cartilage. Methylene blue. ×112.
Fig. 20. Frog. 8 days. Early periosteal blastema. Note osteoblasts on surface of old bone. Pyronin and methyl green. ×490.
Fig. 21. Frog. 11 days. Early periosteal blastema. Weigert's haematoxylin and van Gieson. ×490.
Fig. 22. Frog. 70 days. Cartilage union. Weigert's haematoxylin, van Gieson and methylene blue. ×21.

PLATE 3

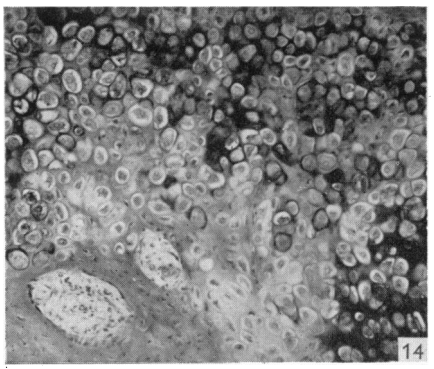
Fig. 23. Frog. 30 days. Bone formation in cartilage canals. Fibrosis of cartilage matrix. Weigert's haematoxylin, van Gieson and methylene blue. ×112.
Fig. 24. Frog. 30 days. Loss of basophilia of cartilage around vascular canals. Early bone formation in walls of canals. Weigert's haematoxylin, van Gieson and methylene blue. ×112.
Fig. 25. Frog. 36 days. Shows changes in and around a cartilage canal. From without inwards note (1) normal basophil cartilage, (2) non-basophil cartilage, (3) highly calcified new bone matrix, (4) uncalcified new bone matrix, (5) osteoblasts, (6) primitive connective tissue. Cobalt sulphide and methylene blue. ×490.
Fig. 26. Frog. 30 days. Shows loss of basophilia preceding fibrosis of cartilage and its apparent transformation into bone. Weigert's haematoxylin, van Gieson and methylene blue. ×112.
Fig. 27. Frog. 70 days. Shows apparent transformation of cartilage into bone between vascular canals, and direct bone deposition in walls of canals. Weigert's haematoxylin, van Gieson and methylene blue. ×112.
Fig. 28. Rat femur. 7 days. Note periosteal new bone and pre-cartilage; medullary new bone; parosteal granulation tissue and the haematoma cavity. Weigert's haematoxylin and van Gieson. ×17·5.

PLATE 4

Fig. 29. Rat femur. 5 days. New bone and pre-cartilage in greatly thickened periosteum. Weigert's haematoxylin and Van Gieson. ×70.
Fig. 30. Rat femur. 24 days. Chondrification and endochondral ossification advancing into dense connective tissue between fragments (cf. fig. 33). Weigert's haematoxylin and van Gieson. ×21.
Fig. 31. Rat tibia. 4 days. Alkaline phosphatase in periosteal blastema. Gomori. ×112.
Fig. 32. Rat femur. 5 days. Shows transitions from bone, through an intermediate tissue to cartilage in the periosteal blastema. Weigert's haematoxylin and van Gieson. ×112.
Fig. 33. Rat femur. 24 days. High power view of part of section shown in fig. 30. Shows endochondra bone, cartilage and fibrous tissue. Methylene blue and eosin. ×112.
Fig. 34. Rat tibia. 4 days. Alkaline phosphatase in fracture gap at junction of periosteal (left) and parosteal (right) blastemata. Gomori. ×112.



PRITCHARD AND RUZICKA—COMPARISON OF FRACTURE REPAIR IN THE FROG, LIZARD AND RAT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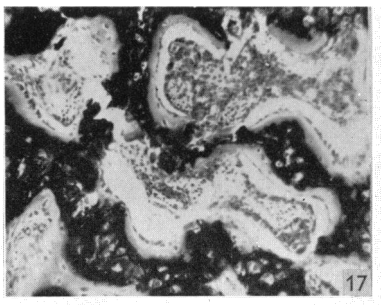
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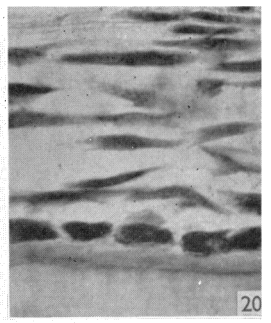
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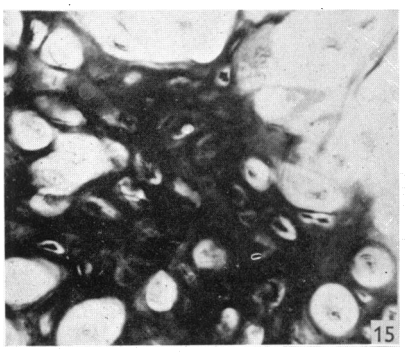
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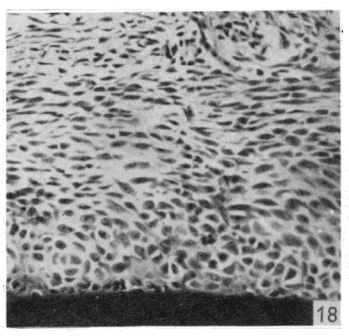
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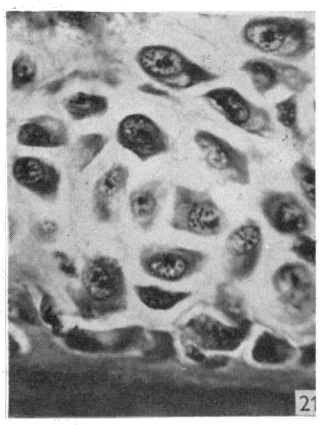
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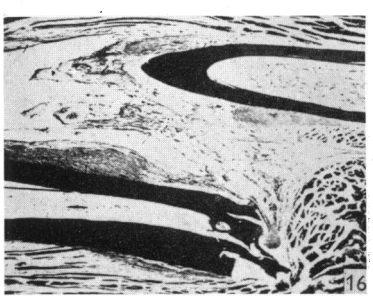
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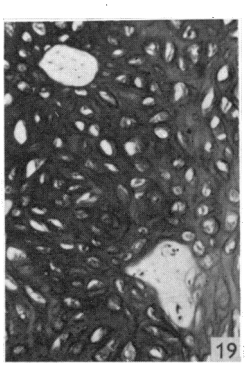
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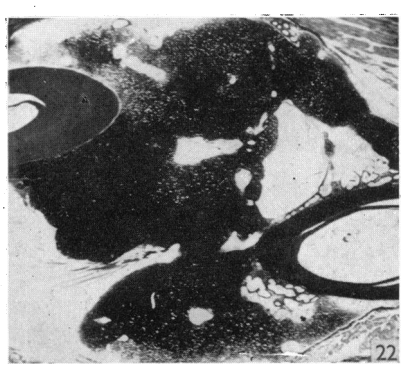
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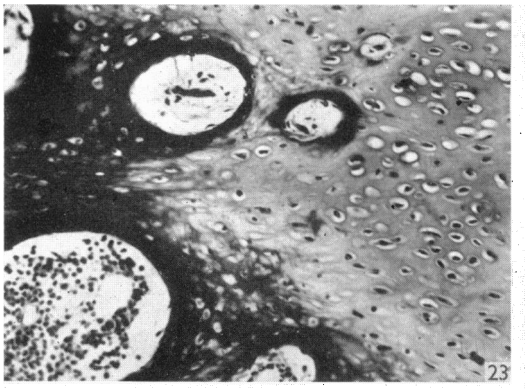

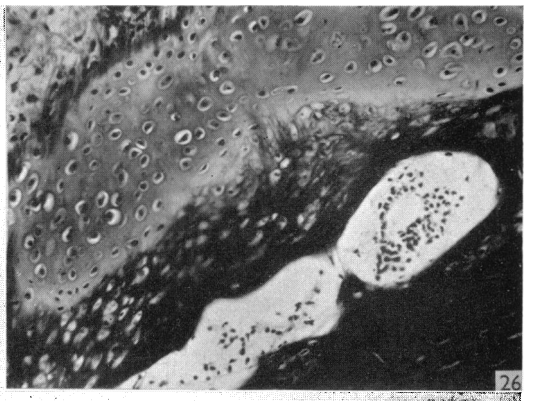

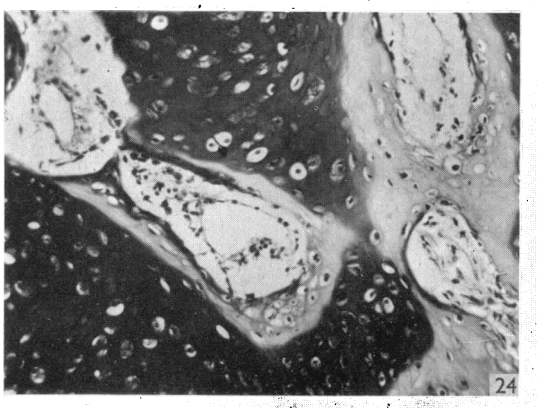

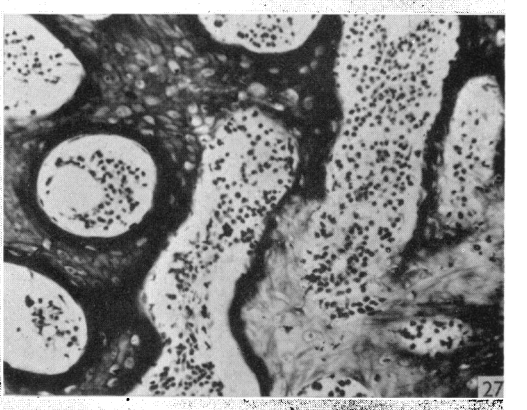

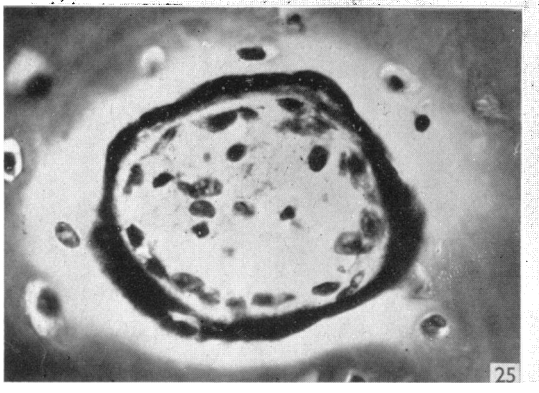

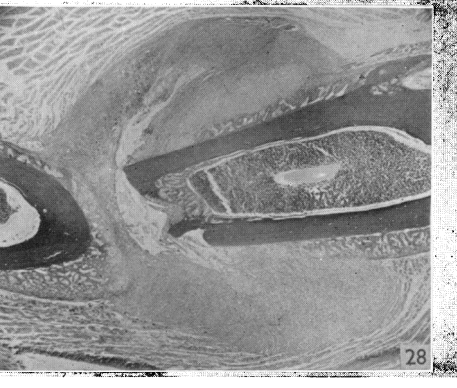

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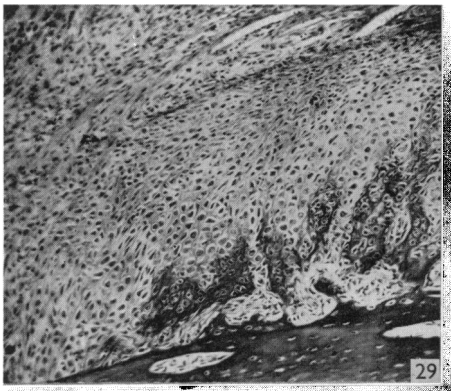
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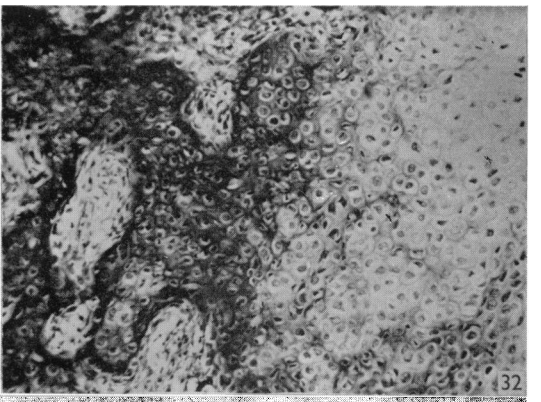
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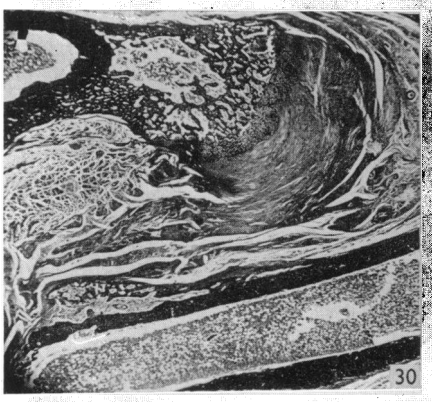
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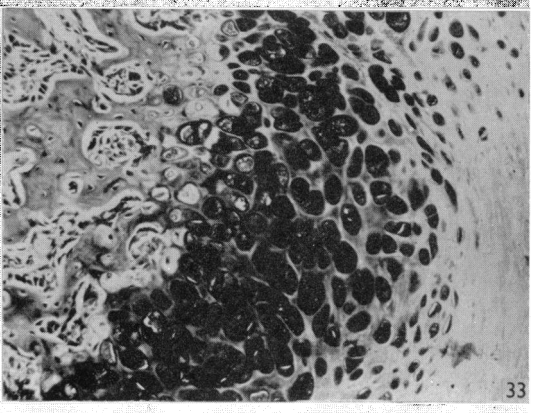
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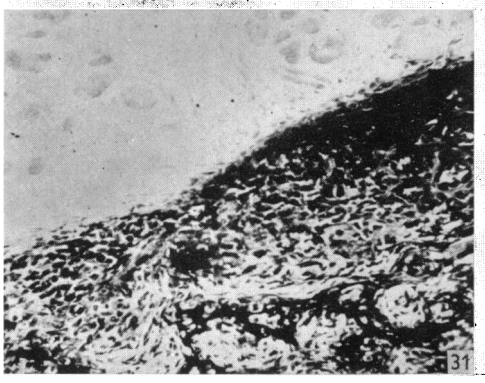
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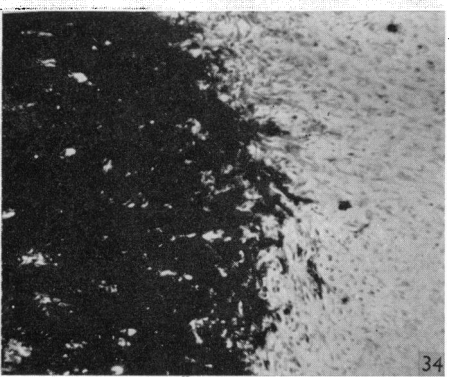
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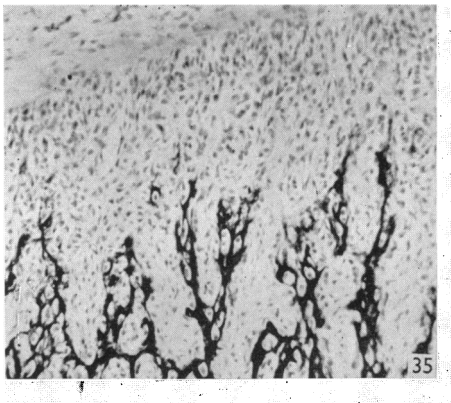

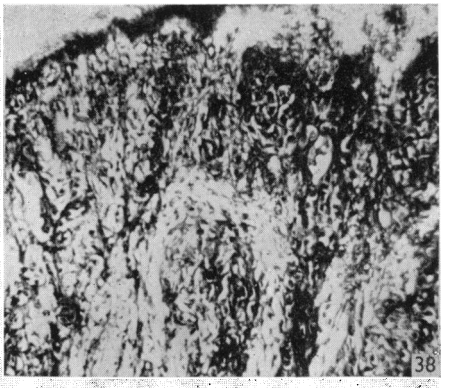

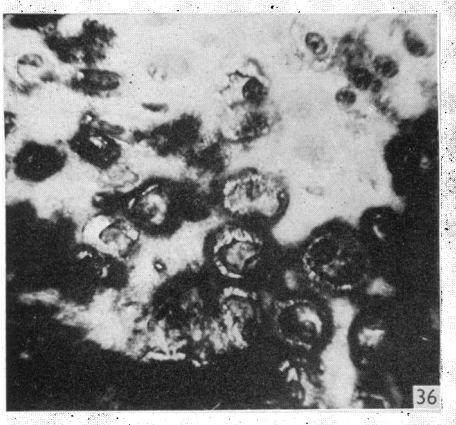

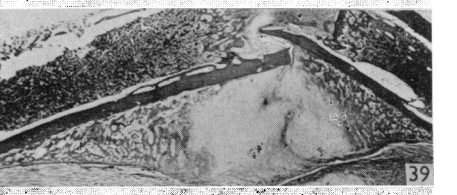

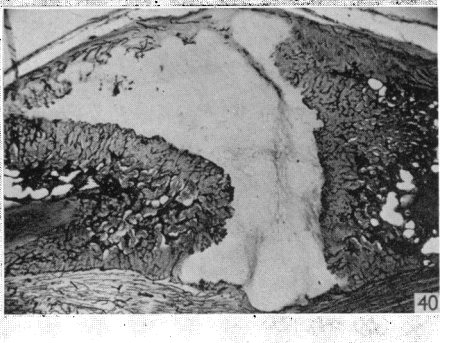

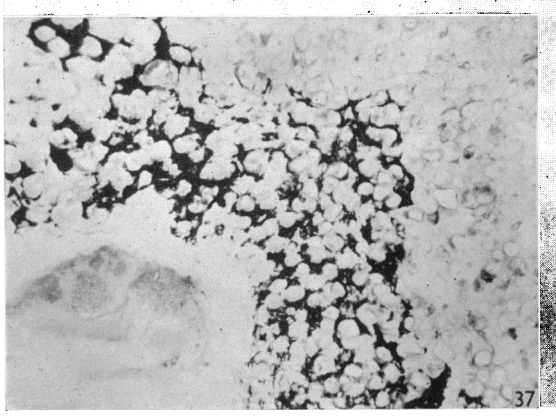

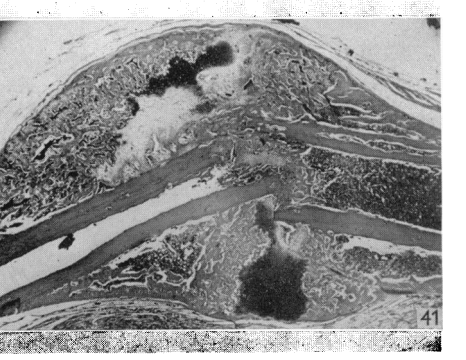

PRITCHARD AND RUZICKA—COMPARISON OF FRACTURE REPAIR IN THE FROG, LIZARD AND RAT

PLATE 5

Fig. 35. Rat tibia. 4 days. Calcified new bone trabeculae in deeper layers of periosteal blastema. Cobalt sulphide (cf. fig. 38). ×112.

Fig. 36. Rat tibia. 4 days. Alkaline phosphatase in newly formed cartilage of periosteal blastema. Gomori. ×490.

Fig. 37. Rat tibia. 12 days. Calcified cartilage around a blood vessel. Cobalt sulphide and eosin. ×112.

Fig. 38. Rat tibia. 4 days. Alkaline phosphatase in periosteal blastema. To be compared with fig. 35 which shows an adjacent section stained for calcium salts. Gomori. ×112.

Fig. 39. Rat tibia. 10 days. Cartilage union. Weigert's haematoxylin and van Gieson. ×17·5.

Fig. 40. Rat tibia. 12 days. Indian ink injected specimen. Cartilage union with endochondral bone formation on either side. Haematoxylin and eosin. ×21.

Fig. 41. Rat tibia. 17 days. Relatively advanced stage in repair. Shows bony and cartilaginous union. Weigert's haematoxylin, van Gieson and methylene blue. ×17·5.