Acta orthop. Scandinav. 38, 115–122, 1967

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FRACTURE HEALING AND MAST CELLS

I. The Periosteal Callus in Rats

By

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Received 22.xi.66

The rate of fracture healing under normal and pathological conditions, and the mineral metabolism, biophysical properties and reaction of bone to trauma have recently been given considerable attention (*Nilsonne* 1959, *Koskinen* 1959, *Falkenberg* 1961, *Wendeberg* 1961, *Hulth & Olerud* 1964). The elements of callus—blood cells, blood vessels, fibrous tissue, cartilage and bone—are well known (*Collins, Ham & Leeson, Ham & Harris, McLean & Urist*). And yet, there is still controversy as to which of these elements plays the principal role in bone regeneration, and biochemical knowledge of the calcifying mechanism is incomplete.

A striking parallelism between certain features of the specialized histiocytes known as mast cells (*Ehrlich*) and the formation of callus in bone repair may be observed in regard to metachromatic stainability with thiazine dyes and reaction to hormonal stimuli.

In their metabolism, mast cells are biochemically closely related to histamine (*Riley & West* 1953), hyaluronic acid (*Asboe-Hansen* 1950), 5-hydroxytryptamine (*Parrat & West* 1956) and heparin-like mucopolysaccharides (*Jorpes, Holmgren & Wilander* 1937). The granules of the mast cell stain heavily metachromatically. One of the most impressive characteristics of the colloid ground substance in callus tissue, too, is its metachromasia, indicating high-molecular polysaccharides (*Levander* 1964). A close association between mucopolysaccharides and the phase of calcification has been observed. The mast cells diminish and develop vacuoles during treatment with cortisone, and the spread of metachromatic substance decreases (*Asboe-Hansen* 1950, *Cavallero & Braccini* 1951, *Stuart* 1951, *Fulton & Maynard* 1953). If fractures in rats heal under the influence of cortisone, new bone for-

mation is greatly delayed or totally arrested (*Koskinen* 1959). Thyrotropin + somatotropin increase the number of mast cells (*Wegelius & Asboe-Hansen* 1956) and the metachromatic staining of the ground substance in connective tissue. The action of thyrotropin + somatotropin vigorously promotes the formation and maturation of the cells in the repair of experimental fractures in rats (*Koskinen* 1959).

The granules of the mast cell contain alkaline phosphatase (*Noback & Montaga* 1946, *Wislocki & Dempsey* 1946, *Riley & Drennan* 1949) and, in addition, acid phosphatase (*Montaga & Noback* 1947, 1948). Alkaline phosphatase has been traced to the region of the hypertrophied cartilage cells in the growing zone of the epiphyseal plate in the rat tibia (*Morse & Greep* 1951).

The mast cell has attracted little attention as an element in osteogenesis. During the process of wound healing of the skin in rats the number of mast cells increase in the vicinity of the growing tissue (*Wichmann* 1955).

S^{35} studies, however, have revealed the presence of mast cells both inside and outside the bone substance in the growing proximal end of the tibia in rats (*Duthie & Barker* 1955). Deposition of S^{35} in a healing fracture site could not be established.

The purpose of this experimental study of fracture repair has been to discover the location and behaviour of the mast cells in the periosteal callus during the process of endochondral ossification.

MATERIAL AND METHOD

The material consisted of 25 adult white rats of both sexes, weighing on an average 134.6 g (range 120–145 g) at the start of the experiments. The animals had been reared under the same physical conditions and fed usual laboratory diet and tap water.

Under ether anaesthesia the right tibia and fibula were manually fractured. Specimens for histological examination were taken from the periosteal callus of the tibial fracture and control specimens from the periosteum and muscles of the left tibia. Before dissection, roentgenograms were taken of both lower extremities.

Histological specimens were taken on the days: 1, 2, 3, 4, 5, 6, 7, 9, 11, 13, 15, 19, 21 after fracturing. The preparations were fixed in a 4 per cent aqueous solution of basic lead acetate for 48 hours, prepared and cut into 10 μ sections in the manner previously used by one of the authors (*S. Lindholm* 1959). Sections were stained in a 1 per cent toluidine blue aqueous solution for 1–2 minutes. The mast cells in the periostcal callus were studied in a Leitz binocular microscope.

The mast cells were counted in 2 sq.mm (= 30.2 high power (×450) fields). The results were recorded on the average per sq.mm. The number of cells per cu.mm.

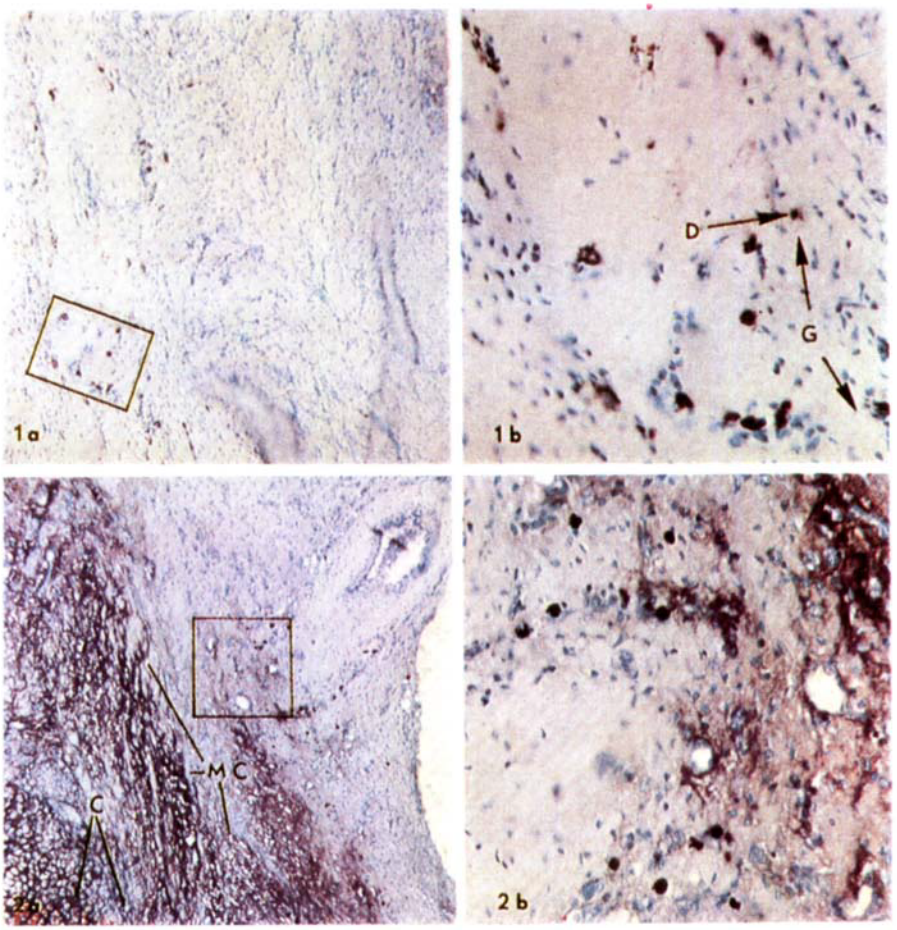

Figure 1a and 1b. Aggregation of strongly metachromatically staining mast cells in young undifferentiated mesenchymal callus. Cells apparently in a state of exhaustive degranulation. Metachromatic granules (G) in the surrounding matrix. Some cells (D) have almost disappeared. Microphotos a) × 100 and b) × 400. Four-day-old experimental fracture in a rat.

Figure 2a and 2b. Mast cells in the vicinity of the metachromatic zone (MZ) of prechondroblasts. Mature cartilage (C) in left lower corner. Microphotos a) × 100 and b) × 400. Six-day-old experimental fracture in a rat.

may be determined from *Floderus'* (1944) formula $n \frac{1000}{a+d-2h}$, where n is the number of nuclei per sq.mm., a the thickness of the section (10 μ), d the diameter of the nucleus in y (5μ) and h the diameter of the smallest nuclear segment found in the tissue (0.5μ).

Unequal and variable localization of the mast cells in the proliferating con-

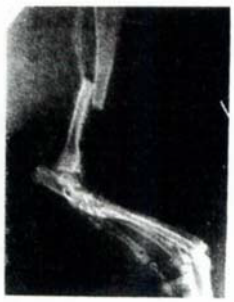

Figure 3. X-rays of fracture from Figure 2. Earliest visible signs of mineralization in the callus cuff.

nective tissue in the vicinity of the newly formed cartilage made any quantitative study uncertain. Therefore, we decided to make only an approximate count of the mast cells in the early (after 24 hours), middle (on the 7th and 13th days) and late phase (on the 19th day) of repair.

RESULTS

We were impressed by the progressive increase in the number of mast cells from the 3rd to the 13th day. Thereafter, the number of cells continually decreased, and on the 19th day the situation was quite the same as on the 1st day after the trauma. In the control material we found a large number of mast cells in the periosteum, subcutis and muscle, but there were no variations in the number of cells during the experiment. In the callus the mast cell usually had a deeply staining nucleus and contained plenty of metachromatic granules. In the controls the nucleus appeared somewhat smaller and contained fewer metachromatic granules. Initially, the increase of metachromasia in the ground substance ran parallel to that of the mast cells, but there was a diminution after the 7th–9th day. Formation of cartilage began on the 2nd–3rd day. The cartilage showed significant metachromatic properties. Macroscopic and palpable callus was demonstrated on the 4th day. The fracture was stable by the 19th day; firm union of the fragments was established clinically and roentgenologically. Macroradiographically (Figure 3) the first signs of mineralization could be visualized on the 5th day, and by the 15th day union in the middle part of the ossifying collars was almost complete.

Multiplication of mast cells was mainly observed in the location where the mesenchymal blastema was undergoing differentiation (Figure 1). The majority of cells were situated in the vicinity of the carti-

lage zone (Figure 2), but not in the cartilage itself, being more scattered at the periphery of the periosteal cuff. No cell divisions were seen in the callus. Perivascular aggregation was not an outstanding feature.

After 24 hours no cartilage could be seen as yet, but there were already areas of slightly metachromatic, proliferating connective blastema. The mast cell counts were as follows: 1st day 20 per sq.mm = 1428 per cu.mm., 7th day 41 per sq.mm. = 2927 per cu.mm., 13th day 58 per sq.mm. = 4141 per cu.mm. and 19th day 18 per sq.mm. = 1285 per cu.mm. fibrous callus tissue.

DISCUSSION

The mast cells proliferate in the callus tissue from the 3rd to the 13th day, probably by invasion; they were not seen to divide within the callus. The function of the mast cells in the callus probably consists in synthesis and secretion of high-molecular substances necessary for the metaplasia of the connective tissue in the process of endochondral ossification.

Closer contact with the growth zone of cartilage and bone formation runs parallel with exhaustive degranulation and transformation or disappearance of the mast cells. Their role as furnishers of alkaline phosphatase, phosphorylase and other enzymes must be taken into account, besides their ability to produce histamine, 5-hydroxytryptamine and heparin.

Delay of callus formation by ACTH and acceleration of fracture healing by STH are possibly secondary results of the suppression and activation of the mast cells in the periosteal callus, a point which requires further investigation.

It appears likely that the mast cells do not initiate the process of ossification but promote it. The inducing mechanism may be part of the regenerative power possessed by specific cells, in this case the osteoblasts in the periosteum and endosteum.

SUMMARY

Typical mast cells aggregate in the mesenchymal part of the periosteal callus of experimental fractures in rats. An increase in number, probably by invasion, was observed from the 3rd to the 13th day of repair. Cells in the periosteum, subcutis and muscle of the unfractured

leg were screened as a control. The number of mast cells in these did not show any variations during healing.

Mast cells in close contact with the metachromatically staining zone in the periosteal callus cuff are seen to degranulate and disappear from the visual field. No mast cells were seen within the areas of newly formed cartilage or bony trabeculae.

The mast cells are obviously intimately involved in the process of bone repair in rats, mainly in the region where preformation of cartilage takes place. It seems likely that the mast cells furnish substances essential for the process of endochondral ossification.

RESUME

Des mastocytes typiques s'accumulent dans la partie mésenchymale du cal périostal des fractures expérimentales chez les rats. Une augmentation de leur nombre, qui se produit probablement par invasion, a été observée entre les 3ème et 13ème jours de la régénération. Les cellules du périoste, du tissu sous-cutané et du muscle de la jambe non fracturée ont été examinées à titre de contrôle. Il n'y avait là aucune variation du nombre des mastocytes pendant le processus de guérison. On a observé que les mastocytes en contact étroit avec la zone colorée des corpuscules métachromatiques du périoste du cal pouvaient perdre leurs granulations et disparaître du champ visuel. Aucun mastocyte n'a été observé là où il y avait formation récente de cartilage ou des trabécules mâles.

Les mastocytes sont intimement liés au processus de régénération des os chez les rats, principalement dans la région où il y a préformation de cartilage. Il semble que les mastocytes fournissent les substances essentielles nécessaires à l'ossification endochondrale.

ZUSAMMENFASSUNG

Im Mesenchymalen Teil des periostalen Callus bei experimentellen Knochenbrüchen bei Ratten ergibt sich eine Anhäufung typischer Mastzellen. Eine Zunahme der Anzahl, die wahrscheinlich durch Invasion zustande kommt, wurde vom 3–13 Tage der Heilung beobachtet. Zellen von Periost, Subcutis und der Muskulatur des nicht gebrochenen Beines wurden als Kontrolle benützt. Die Anzahl der Mastzellen zeigte dort während der Heilung keine Variationen.

Eine Degranulierung und ein Verschwinden aus dem Blickfeld wurde

an denjenigen Mastzellen gesehen, die mit der metachromatischen Farbzone in nahem Kontakt waren. Im Gebiete neugebildeten Knorpels und der Knochentrabekel waren keine Mastzellen zu sehen.

Bei Ratten sind Mastzellen offenbar mit dem Prozess der Knochenheilung verbunden, besonders in Regionen, in denen Praeformation von Knorpelgewebe stattfindet. Wahrscheinlich liefern die Mastzellen Substanzen, die für die enchondrale Ossifikation wichtig sind, ab.

ACKNOWLEDGEMENT

We are indebted to the Department of Pathology (Head: *P. Fortelius.* M. D.), the Department of Radiology (Head: *E. Autio*, M. D.) and the Board of Directors, Vaasa Central Hospital, Vaasa, Finland, for sponsoring this research and for the advice given.

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