Acta orthop. Scandinav. 38, 123–128, 1967

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FRACTURE HEALING AND MAST CELLS

II. Influence of 17-hydroxy-corticosterone

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Received 22.xi.66

The precence and increment in number of mast cells in the periosteal callus cuff of experimental fractures have recently been clearly demonstrated (*Lindholm, Lindholm & Liukko* 1966) in rats fed the ordinary laboratory diet. Mast cells are known to diminish in size and develop vacoules during theatment with cortisone (*Asboe-Hansen* 1950, *Cavallero & Braccini* 1951, *Fulton & Maynard* 1953, *Wegelius & Asboe-Hansen* 1956). New bone formation is delayed under the influence of cortisone (*Koskinen* 1959, *Hulth & Olerud* 1964).

In order to elucidate the relationship between mast cells and the process of fracture healing, the influence of 17-hydroxy-corticosterone on the mast cells in the periosteal callus was investigated.

MATERIAL AND METHOD

The material consisted of 28 white rats of both sexes, weighing on an average 101 g at the start of the experiments. The animals had been reared under the same physical conditions and fed the usual laboratory diet and tap water.

Under ether anaesthesia the right tibia and fibula were manually fractured (Figure 3). 2 mg 17-hydroxycorticosterone (hydrocortisone) [1] was administered subcutaneously every day to all the rats. The animals were sacrificed, and histological specimens were taken on the days: 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 22, 32 after fracturing. The specimens consisted of callus tissue from the external site, and control specimens were removed from the periosteum of the left tibia, the heart, the lung and the stomach. Roentgenograms were taken of both lower extremities. The preparations were fixed in a 4 per cent aqueous solution of basic lead acetate and stained in a 1 per cent toluidine blue aqueous solution as described elsewhere (S. Lindholm 1959). The mast cells were studied in a Leitz binocular

[1] Hydro-Adreson aquosum Organon.

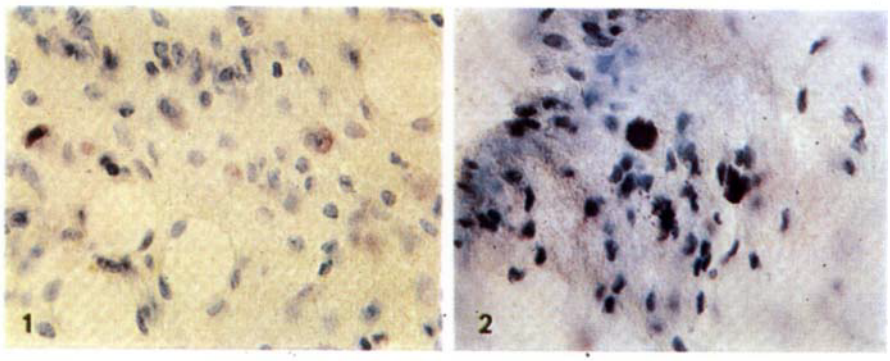

Figure 1. Aggregation of mast cells of medium and small size, some ressembling fibrocytes with a weakly metachromatic and homogenously staining plasma. Nuclei clearly visible. Ground substance almost unstained. Microphoto ×1000 (oil immersion). Six-day-old experimental fracture in a rat under the influence of hydrocortisone.

Figure 2. Large, heavily staining, granulated mast cells near the cartilage zone in the periosteal callus. The cells appear to be in a stage of 'maturation' and degranulation. Ground substance invaded by granules. Microphoto ×1000 (oil immersion). Six-day-old experimental fracture in a rat under the influence of hydrocortisone.

microscope during the process of healing until the 32nd day. An approximate cell count was done, and the number of cells per cu.mm. was calculated according to a technique described elsewhere (Lindholm, Lindholm & Liukko). The cell count was performed in the early (on the 3rd day), middle (on the 10th and 14th day) and late phase (on the 22nd day) of repair.

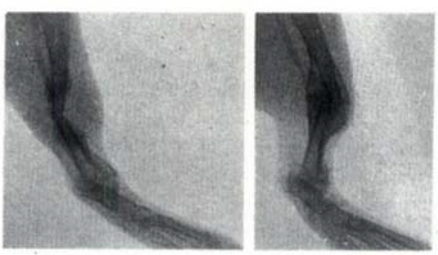

Fig. 3. *Fig. 4.*

Figure 3. Experimental eight-day-old fracture of the right hind leg in a rat under the influence of hydrocortisone. Clearly visible callus.

Figure 4. Experimental twenty-two-day-old fracture of the right hind leg in a rat under the influence of hydrocortisone. Callus cuff completely mineralized and fracture clinically stable.

RESULTS

For technical reasons it was not possible to make any quantitative mast cell count. The impression of a progressive increase in the number of mast cells from the 8th day after trauma was obtained. Between the 10th and 16th days the mast cell count appeared to be high and then decreased until the 32nd day, when no more mast cells could be seen. During the first week of fracture healing only a few and scattered mast cells were seen in the metachromatic ground substance of the early callus tissue. The metachromasia in the ground substance reached its maximum between the 5th and the 6th day and decreased continually after the 10th day. The formation of cartilage took place from the 3–4th day, and bone could be visualized histologically and roentgenologically on the 8th day (Figure 3). The fracture was stable when manipulated on the 22nd day and the callus appeared to be calcified roentgenologically (Figure 4).

The controls showed numerous large mast cells in the periosteum, muscle and subcutis, an abundance of mast cells of smaller size in the stomach, only a few cells in the heart, and none in the lung.

The mast cells seen in the callus were both large and small, the granules stain isometachromatically violet or reddish and varied concerning homogeneity and size. Some cells do not differ very much from surrounding fibrocytes in shape, size and nucleus (Figure 1), others appear like mature, deeply staining and densely packed large granulated mast cells, whose nucleus is entirely blurred by intracellular granules (Figure 2).

The following mast cells counts were obtained: 3rd day 10 per mm^2 = 714 per cu. mm, 10th day 74 per mm^2 = 5284 per cu. mm, 14th day 93 per mm^2 = 6447 per cu. mm and 22nd day 9 per mm^2 = 643 per cu. mm.

DISCUSSION

The previously known effects of cortisone on the morphology of the mast cells could be observed in the callus during the early phases of repair. Our observations support the theory that mast cells are formed *in situ* from fibrocytes (Figure 1). Cortisone causes moderate to extreme retardation of bony union (*Hulth & Olerud* 1964, *Koskinen* 1959). A moderate delay was observed by us. One explanation may be a difference between cortisone and hydrocortisone in their action on bone formation. It may perhaps be concluded that the process of

mineralization is influenced by the presence of mast cells. The formation of cartilage from chondroblasts need not necessarily depend on the function of the mast cells. The delay in healing seems to concern the process of maturation and mineralization of the cartilage rather than the early phases of chondrogenesis.

It is difficult to explain why the morphology of the mast cells shows variations under the influence of cortisone. Retardation of maturation and prolongation of heteroplasia may be valuable hypotheses for further investigations. In any case, our mast cell counts suggest an initial depression, followed by a compensatory increase of cells to a somewhat higher level than in the normal series (*Lindholm, Lindholm & Liukko*). However, the method of counting mast cells must be improved before reliable quantitative values can be obtained.

SUMMARY

A series of 28 rats were given daily injections of 2 mg 17-hydroxycorticosterone (hydrocortisone) subcutaneously after the right tibia and fibula had been fractured.

Histological specimens were stained with toluidine blue and the mast cells were studied.

An increase in number was observed, beginning from the 8th day and reaching a maximum between the 10th and the 16th day. The formation of cartilage and bone was moderately delayed compared with non-steroid—treated animals of earlier series. Despite the delay in callus formation, firm union of the fracture was ultimately obtained.

The mast cells showed greater differences in size, metachromatic properties, and granulation than those in the previous group of untreated animals. Mast cells are probably created through metachromatic transformation of fibrocytes.

RESUME

Il a été administré quotidiennement des injections sous-cutanées de 2 mg de 17-hydroxycorticosterone (hydrocortisone) à une serie de 28 rats après fracturation des tibia et péroné droits.

Des échantillons histologiques ont été colorés au bleu de toluidine et les mastocytes ont été examinés.

Une augmentation de leur nombre a été observée à partir du 8ème jour, atteignant le maximum entre le 10ème et le 16ème jour. La for-

mation de cartilage et d'os a été légèrement retardée par rapport aux animaux de séries antérieures qui n'ont pas été traités aux stéroïdes. Malgré le retard dans la formation du cal, la fracture a fini par se souder parfaitement.

Les mastocytes ont de plus grandes différences quant à la grandeur, aux propriétés métachromatiques et à la granulation que dans le groupe précédent d'animaux non-traités. Les mastocytes se créent probablement par une transformation métachromatique des fibrocytes.

ZUSAMMENFASSUNG

Nach Zerbrechen der rechten Tibia und Fibula wurden 28 Ratten tägliche subcutane Injektionen von 2 mg. 17-Hydroxycorticosterone (Hydrocortisone) gegeben. Histologische Proben wurden mit Toluidineblau gefärbt und die Mastzellen wurden beobachtet. Eine Zunahme der Zahl der Mastzellen wurde vom achten Tage an mit einem Maximum zwischen den 10. und 16. Tage beobachtet.

Im Vergleich mit Tieren in früheren Versuchsserien, die nicht steroidbehandelt worden waren, war die Formation von Knorpel- und Knochengewebe mässig verzögert. Trotz der verzögerten Callusbildung heilten die Knochenbrüche schliesslich gut.

Die Mastzellen zeigten grössere Differenzen in Umfang, metachromatischen Eigenschaften und Granulation als die der früheren Gruppe von nicht behandelten Tieren. Die Mastzellen werden wahrscheinlich durch metachromatische Transformation der Fibrocyten gebildet.

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.

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