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FRACTURE HEALING AND MAST CELLS

III. Action of Combined Growth Hormone and Thyrotropin

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Variations in the number of mast cells in the periosteal callus of experimental fractures in rats, untreated and under the influence of 17-hydroxy-corticosterone, have recently been demonstrated (*Lindholm, R., Lindholm, S. & Liukko 1966, Lindholm, S., Lindholm, R. & Liukko 1967*).

The mast cells increase after thyrotropin medication (*Asboe-Hansen 1951*). In the cheek pouch of the living hamster treated with thyrotropin and somatotropin a large number of well granulated mast cells were seen (*Wegelius & Asboe-Hansen 1956*). Thyrotropin or somatotropin, however, did not influence the number of mast cells in the skin of dwarf mice (*Dyrbye, Ahlqvist & Wegelius 1959*). New bone formation under the influence of growth hormone, especially when combined with thyrotropin, is vigorously stimulated (*Koskinen 1959*). No investigations on mast cells seem to have been carried out during hormonally stimulated regeneration of bone.

MATERIAL AND METHODS

The material consisted of 30 white rats of both sexes, weighing 125-135 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. All animals received daily 0.6 IU (= 30 tibia units) somatotropin¹ and 0.1 IU (= 0.1 U.S.P. units) thyrotropin² subcutaneously. The animals were sacrificed and histological specimens were taken on the days: 1, 2, 3, 4, 5, 6, 8, 9, 12, 15, 16, 18, 19, 22 after fracturing. The specimens consisted of callus tissue from the external site, and control specimens were removed from the periosteum of the left tibia.

¹ Somacton Ferring AB Malmö Sweden.

² Actyron Ferring AB Malmö Sweden.

Roentgenograms were taken of both hind limbs. The mast cells were stained and counted in planes perpendicular to the periphery of the callus cuff according to a technique described previously (*Lindholm, R., Lindholm, S. & Liukko 1966*). An approximate count was made of the mast cells in the early (on the 4th and 6th days), middle (on the 8th and 12th days) and late phase (on the 15th and 16th days) of repair.

RESULTS

Palpable callus was demonstrated on the 4th day, and the fracture was clinically stable on the 15th day. The first signs of mineralization were visualized macroradiographically on the 5th day. By the 12th day the entire callus showed signs of slight mineralization on the roentgenogram.

The mast cell counts showed the following number of cells: 4th day and 6th day 27 per mm² = 1925 per cu. mm., 8th day 58 per mm² = 4135 per cu. mm., 12th day 52 per mm² = 3708 per cu. mm., 15th day 37 per mm² = 2638 per cu. mm. and 16th day 19 per mm² = 1355 per cu. mm. In the controls no variations were observed. The mast cells were situated in the mesenchymal parts of the callus and none could be seen in cartilaginous or osseous areas. Their distribution and localization appeared unequal and variable. The mast cells disappeared from view at the borderline of cartilaginous tissue, where the metachromatic stainability of the matrix generally increases. Morphologically the mast cells did not differ substantially from those in the periosteal callus under normal laboratory conditions.

DISCUSSION

The increase in the number of mast cells in the periosteal blastema undergoing differentiation seems to parallel the process of healing in general. Under the influence of 17-hydroxy-corticosterone there is a delay which almost corresponds to the delay of mineralization and consolidation of the fracture (*Lindholm, S., Lindholm, R. & Liukko 1967*). The action of growth hormone and thyrotropin somewhat accelerates the increase in number, in comparison with the normal series. Despite the delay of healing, hydrocortisone does not decrease the number of mast cells; on the contrary, it causes a relative increase, a phenomenon probably secondary to degranulative or secretory insufficiency of the mast cells. Combined growth hormone and thyrotropin medication did not alter the absolute number of mast cells in any appreciable way as compared with the normal series.

Histochemical and tracer studies may reveal disturbances in the mast cell metabolism of a nature which cannot be visualized by metachromatic staining alone. In view of the constant finding of mast cells and their invariable multiplication in the callus tissue where endochondral ossification takes place, it is anticipated that further studies using agents known to cause disturbances of osteogenesis, *e.g.* the sweet pea (*Robinson & Bast 1934*), will be invaluable in unravelling the mechanisms whereby mast cells are involved in the healing of fractures.

SUMMARY

A series of 30 rats were given daily injections of somatotropin and thyrotropin subcutaneously after the right tibia and fibula had been fractured. Healing was stimulated, and early mineralization of the callus cuff was demonstrated roentgenologically. A typical increase in the number of mast cells in the differentiating blastema was noted, the peak being reached 3–4 days earlier than in untreated rats. The curve obviously parallels that of accelerated mineralization. The absolute number of cells found in some approximate counts was of the same magnitude as in normal rats and somewhat lower than in rats on 17-hydroxycorticosterone medication.

RESUME

Il a été administré à une série de 30 rats des injections quotidiennes sous-cutanées de somatotropine et de thyrotropine, après provocation de fractures du tibia et du péroné droits. La guérison de la fracture s'est trouvée stimulée et à l'examen radiologique il est apparu que le cal se minéralisait précocement. Il a été noté une augmentation typique des mastocytes du blastème qui se différenciaient. La culmination fut atteinte 3–4 jours plus tôt que chez les rats à l'état normal. La courbe est, semble-t-il, parallèle à celle de l'augmentation de la minéralisation. Le nombre absolu des cellules obtenues par numération indicative a été de la même grandeur que chez les animaux normaux et parfois un peu plus bas que chez les animaux auxquels il était administré une médication de 17-hydroxycorticostérone.

ZUSAMMENFASSUNG

Nach Zerschneiden der rechten Tibia und Fibula wurden 30 Ratten täglich subcutane Injektionen vom somatotropin und Thyreotropin ge-

geben. Die Frakturheilung wurde dadurch stimuliert und die Callusmanschette zeigte röntgenologisch eine frühzeitige Mineralisation.

Im Blastem wurde eine typische Zunahme der Zahl der sich differenzierenden Mastzellen gefunden. Die Spitze wurde 3–4 Tage früher, als bei Ratten, die sich in normalen Verhältnissen befanden, erreicht. Die Kurve geht augenscheinlich parallel mit der der Mineralisation. Die absolute Zellzahl, die durch einige Probezählungen erhalten wurde, war gleich der bei normalen Tieren und etwas niedriger als die bei Tieren, die mit 17-hydroxycorticosterone behandelt worden waren.

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