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RELATIONSHIP OF MAST CELLS TO THE GROUND SUBSTANCE OF CONNECTIVE TISSUE

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According to *Maximov* [18], *Ryzhikh* [24], *Friberg* et al. [8], mast cells are of mesenchymal origin, with distinct ontogeny of their own, as well as with specific morphologic properties. Their function, according to the prevalent opinion, is twofold, viz. (i) Production of the ground substance of connective tissue ; (ii) Heparin production.

Staemmler [26], *Sylven* [27] and *Hansen* [10] concur in crediting the mast cells with the production of the ground substance of connective-tissue. Sylven's investigations into the number, localization and granule content of mast cells in granulating wounds revealed, on the first day of healing, an increase in the number of mast cells around the vessels, giving place at a later stage to the appearance of the ground substance, with a concurrent diminution of the number of mast cells. Accordingly, numerical decrease of mast cells, formation of the metachromic ground substance and proliferation of fibroblasts occur simultaneously at the same site.

The credit for the demonstration of heparin-producing activity in mast cells must go to *Jorpes*, *Holmgren* and *Wilander* (13). The chemical nature of heparin was first determined by *Jorpes* (15), then by *Jorpes* and *Jaques* [14]. It is one of the sulphuric acid esters of mucopolysaccharides, being a polymer of di-and tri-sulphuric acids, which yield, upon hydrolysis, glucosamine and glucuronic acid. Subsequently it was found that several forms of heparin exist, with their biological action and molecular weight varying over a wide range. Marbet and *Winterstein* [17] arrived at the same conclusion and succeeded in isolating a form (β-heparin), which, although chemically it corresponds to chondroitin sulphate, had, on the strength of its biological action, to be assigned to the heparin class.

Holmgren and *Wilander* [11] regard the mast cells as the site of heparin storage and, probably, formation. Heparin is present in the cells in the form of metachromatic granules. Julen, Snellman and *Sylven* [16] found the metachromatic substance in the intergranular microsome fraction and so did *Friberg*, *Graf* and *Åberg* [8]. *Zollinger* [28], on the contrary, considers the granules as mitochondria altered in consequence of heparin storage. They do not consist

of heparin proper, but of a water insoluble, framework capable of swelling in which the hepa in i deposited. While corroborating the presence of heparin in the mast cells, *Horváth* [12] is led by the consideration that the granules unvisible in the native state consist of a toluidine blue-heparin precipitate developing on the action of toluidin blue. *Paff* and *Bloom* [22] observed that explanted mast cells released a metachromatic substance identical with heparin. With the production of heparin the mast cells have been found to degenerate and even perish.

Though the heparin p oduction of mast cells would appear to be a separate process from their production of ground subs ance, some authorities conjecture a connection. *Hansen* [10] believes that the mast cells are responsible for producing the hyaluronic acid precursor, which is identical with, or closely related to, heparin. *Morrione* [21] claims for heparin a share in fibre formation on the evidence that *in vitro* it precipitates fibres in collagen solutions. *Bali* and *Furth* [2] are of the view that heparin is concerned in maintaining the connective tissue in its semiliquid gel state and by so doing promotes cell migration.

Our investigations to be discussed below followed the dual line of inquiry into the variability of the number of mast cells and, concurrently, into the volume and localization of ground substance in the granulation tissue of healing wounds. Four series of experiments were carried out, each on a group of 10 white rats, ranging in weight from 150 to 180 g. By a method described earlier (*Dévényi-Kellner*, 5), a circular wound of 1 sq. cm. was inflicted on the back of each animal. On each of the first 10 days one rat was killed by exsanguination, and the whole of the wound embedded in paraffin after fixation in lead-acetate formaldehyde. Of the maximum diameter we prepared incomplete serial sections 5 μ thick, which were stained with a 0.1 per cent aqueous solution of toluidin blue.

As a control digestion with hyaluronidase (Hyalurase, Kőbánya Pharmaceutical Works, Budapest) was performed in a -pH 4.1 acetate buffer solution containing 15 U hyaluronidase per ml and incubated at 37° C over a period of 18 hours.

Our findings concerning the site of mast cells in the normal rat's skin coincide with *Sylven's* [27] and *Riley's* [23.] The number and site of the mast cells vary with each dermal layer. They are of sporadic occurrence among the collagen fibres of the dermis and in the connective tissue structure of the skin muscle, while in the loose, highly vascular and fat cell-containing suprafascial connective tissue and in the subcutaneous fatty tissue they are present in far greater numbers. (The volume of subcutaneous fatty tissue varies with the nutritional condition of the particular animal). The mast cells in the loose connective tissue and the adipose tissue were found to group themselves distinctly around blood-vessels. The wound base invariably consisted of these two

tissue layers, which we took care to preserve undamaged when excising the wound. Mast-cell counts were performed over the entire wound area and as deep down as the fascia of the dorsal muscles.

The number of mast-cell in the wounds under test varies within a wide range. Discrepancies between counts made at specified intervals were in some cases as great as 100 to 150 per cent. The deviations are traceable partly to individual differences and partly to the variable volume of adipose tissue. The table below shows the arithmetic means of successive mast cell counts, inclusive of the standard deviation. Standard deviation from the mean value :

$$\triangle = \frac{s}{\sqrt{n}}$$

Day	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
Mean value	90,50	91,3	60,3	55,5	73,3	64,0	84,2	97,7	58,9	112,3
Standard dev. from mean	11,8	11,7	7,25	2,86	6,77	3,11	3,57	7,60	3,92	12,47

As can be seen from the table, the high rate of variance makes the mean values uncharacteristic of the series they stand for. The curve plotted upon second-order correction of all the values obtained displayed more than one peak, — an eloquent disproof of any regular change in the mast cell count of the granulation tissue between the lst and 10th days of healing.*

Our findings accordingly contradict *Sylven's* claim who based his argument for the mast cell origin of ground substance on the supposed regularity of changes in the mast cell counts in the granulation tissue.

Many authorities have expressed doubt about the mast cells being productive of the ground substance. According to *Meyer* [20] young and developing fibroblasts produce hyaluronic acid as a prelude to the secretion of chondroitin sulphate and collagen precursor. In certain cases the granules Gersh and *Catchpole* [9] discerned in the plasma of fibroblasts were minute and they incline to interpret this fibroblastic activity as a dominant factor in ground substance formation and take the continual change of the ground substance as granted.Decomposition is by depolymerization and adsorption, while restitution presumably by the secretory activity of fibroblasts. *Bunting* and *White* [4], whose investigations failed to confirm Sylven's views, regard hyaluronidase as another proof

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of the existence of a difference between the metachromasia of the ground substance and that of the mast cells, the latter failing to disappear on treatment with hyaluronidase. As to the mucopolysaccharide production of fibroblasts, or mesenchimal cells, there is no direct evidence. The time of fibroblast proliferat on has been observed to coincide with the appearance of mucopolysaccharides.

In a previous contribution [6] we reported to have observed the appearance of young granulation tissue, in the form of perivascular foci, on the second day of healing.The intercellular ground substance is seen to develop simultaneously with the beginning of fibroblast proliferation, over the area of the incipient granulation foci. Some of these have been found to include mast cells, while others exhibit none. The foci of granulation tissue increase in size, merge, and end by filling up the wound cavity around the 5th or 6th day. The metachromatic substance is invariably present amidst the fibroblasts. With the differentiation of the granulation tissue, there is less and less metachromatic substance in the collagenizing lower layers of the granulation-tissue, while the superficial young layers contain it in an unchanged amount until the wound has been epithelized. No mast cells occur either in the mature or in the young granulation tissue, they being confined, throughout the process, within the wound base as foci of perivascular arrangement.

This different localization of the mast cells from the ground substance in the granulation tissue provides yet another argument against the mast cell origin of the ground substance.

Despite the lack of evidence for the mast cells being directly concerned in ground substance production it may be assumed that their heparin production has a marked influence on the growth of connective tissue. *McLean* [19] and *Alburn* [1] found heparin to exert, even in infinitesimal amounts as *Baserga, de Nicola* and *Vahi* [3] demonstrated by viscosimetric and diffusion methods, an inhibitory action on hyaluronidase. There is alternative evidence in support of this antagonism. *Seiffer* [25] maintains that hyaluronidase administration may lead to rheumatoid complaints and to a recrudescence of latent rheumatic fever. *Donzelot* and *Kaufmann* [7], on the other hand, report improvement in rheumatic conditions as a result of heparin administration. As demonstrated by *Marbet* and *Winterstein* [17], the beneficial effect of heparin in pemphigus is also attributable to antihyaluronidase action.

On the strength of the cited data, we proceeded to study whether heparin is capable of counteracting the granulation tissue depolymerizing effect of hyaluronidase.

First we incubated sections from 1 to 10-day-old skin wounds of rats at 37° C for 18 hours in a pH 4.1 acetate buffer solution containing 16 U of hyaluronidase per ml. (Hyalurase, Kőbánya Pharmaceutical Works, Budapest) and 5 U of heparin per ml. (Heparin, Kőbánya Pharmaceutical Works, Budapest).

The control sections were incubated in hyaluronidase and heparin, respectively, of the above concentration, and in a pH 4,1 acetate buffer solution. Staining was made with a 0,1 per cent pH 7 aqueous toluidin-blue solution.

The sections incubated with hyaluronidase showed no trace of metachromatic stain, except for a reddish discoloration in the granules of the mast cells. In contrast, the metachromatic staining of both the ground substance and the mast cell granules were left unaffected by incubation alike with heparin, with the acetate buffer, and with the hyaluronidase-heparin combination.

Clearly, the ground substance depolymerizing action of hyaluronidase, as reflected in the loss of metachromatic staining,was demonstrably inhibited by heparin at the given degree of concentration.

Our findings seem to favour our assumption that the mast cells play a part, by virtue of their heparin production, in the ontogenesis of granulation tissue, and that heparin protects the granulation tissue ground substance even *in vivo*, assuring its normal maturation by neutralizing the depolymerizing action exerted on it by hyaluronidase.

Summary

(i) Number and localization of tissue mast cells has been studied in healing wounds of rats on each of 10 consecutive days. In the course of healing, no marked variations were observed in the mast cell counts over the wound area.

(ii) Mast cell counts and the different localization of ground substance and mast cells led to the conclusion that mast cells are not responsible for the production of ground substance

(iii) The ability of remarkably low doses of heparin to neutralize *in vitro* the ground substance depolymerizing action of hyaluronidase suggests that the mast cells, in their capacity of heparin producers, act as hyaluronidase inhibitors in the evolution of granulation tissue.

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О СВЯЗИ МЕЖДУ ТУЧНЫМИ КЛЕТКАМИ И ОСНОВНЫМ ВЕЩЕСТВОМ СОЕДИНИТЕЛЬНОЙ ТКАНИ

Л. ХОЛЬЦИНГЕР и И. ДЕВЕНЬИ

Авторы исследовали на крысах изменение количества и локализации тканевых тучных клеток в заживающих ранах в течение 1 до 10 дней. Они установили, что число тучных клеток в области раны в течение периода заживания не проявляет значительных отклонений.

Из поведения тучных клеток, также как и из разницы локализации основного вещества и тучных клеток, авторы пришли к тому заключению, что тучные клетки нельзя рассматривать как клетки, производящие основное вещество.

В своих исследованиях in vitro авторы наблюдали, что гепарин в весьма маленьких дозах отражает вызывающее деполимеризацию основного вещества действие гиалуронидазы. Они предполагают, что тучные клетки при развитии грануляционной ткани, — благодаря их способности производить гепария — играют в организме роль ингибиторов гиалуронидазы.

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