Department of Pathological Anatomy and Histology of the Medical University, Pécs
(Director : Prof. G. Romhányi)

EFFECT OF CYTOTOXIC AGENTS ON TISSUE MAST CELLS

G. Kelényi

(Received November 13, 1953)

The granules of the mast cells in the loose connective tissue, which stain metachromatically with toluidine blue or thionine, are known to contain heparin *(Holmgren* and *Wilander*, [12], *Horváth*, [13]). The functional rôle which they aresupposed to play in the regulation of blood coagulation seems probable on an account of their heparin content and location around the capillaries. This function is also suggested by the observation *(Jorpes* et al., [14]) that blood will not clot in the anterior chamber of the eye because of an inhibitory release of heparin by the mast cells in the limbus corneae. Likewise the increase in the number of the mast cells might be taken to explain why, in spite of its extremely slow circulation, the blood fails to clot in hibernating frogs. *(Snomalainen* and *Herma*, [22]). To the same cause may be ascribed the fact that in advanced elephantiasis scroti no thrombosis develops despite the very marked local blood stasis *(Ehriech* at al., [8]). In peptone shock the decrease in the number of mast cell granules has been observed to protract the clotting time *(Wilander*, [26]). To the correlation between mast cells and heparin should also be referred *Frik*'s observation ([10]) according to which intraperitoneally injected heparin finds its way into the mast cells. There are several observations to show that, over and above their supposed rôle in the regulation of blood clotting, mast cells may also take part in some functions of the loose connective tissue. They are supposed to play part in the formation of the ground substance *(Asboe—Hansen*, [4]) and in local detoxication processes *(Stuart*, [23], *Turchini* et al., [25]). *Bloom* [(6]) and *Asboe—Hansen* ([3]) described changes in mast cells following administration of cortisone and ACTH. In their opinion these changes reflect the action of the pituitary-adrenal system on the connective tissue. According to *Burkl* and *Sonnenschein* [(7]), the mast cells, owing to their heparin content, show antihyaluronidase activity and form an integral part of the protective mechanism of the organism against noxious influences.

Accordingly, it would appear that in addition to their rôle in the regulation of blood clotting, the mast cells also perform some function in the protective system of the organism, represented by the connective tissue. It is with regard to these manifold and for the greater part as yet unclarified functions that a

detailed study of the altered reactivity in the system of the mast cells has seemed to be of interest.

In a previous study [15] the changes observed in the mast cells after X-ray irradiation were discussed. One of them was the high sensitivity to X-rays of the mast cells in the mesentery and subcutis of the rat. Irradiation was followed by rapid clumping of the mast cell granules and a relatively short process of regeneration.

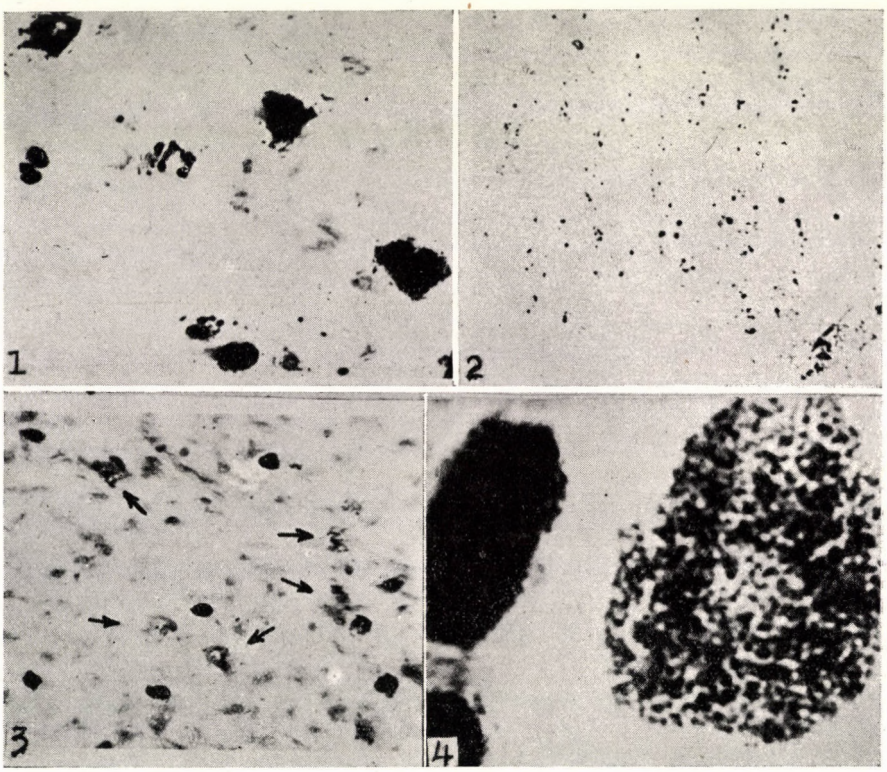

Fig. 1. Membrane preparation from the mesentery. Toluidine blue. 24 hours after injection of 100 γ of colchicine. Clumped granules in the mast cells. (Appr. $\times$300).
Fig. 2. Membrane preparation from the mesentery. Toluidine blue. 24 hours following the injection of 300 γ of colchicine. High degree of polymorphism of mast cells. (Appr. $\times$ 30).
Fig. 3. Membrane preparation from subcutis. Toluidine blue. 72 hours following injection of 100 γ of colchicine. Mast cells with homogenous metachromatic cytoplasm marked by→ (Appr. $\times$100).
Fig. 4. Membrane preparation from the mesentery. Toluidine blue. 24 hours following injection of 60 units of ACTH. Swollen, degranulated mast cells. Seat of the nucleus well discernible. On the left: intact mast cell. (Appr. $\times$1300).

The fact that ionising radiations and certain cytotoxic substances have similar biologic and cytologic effects (*Alexander*, [1]), has also tempted us to study the altered reactivity of the mast cells to cytotoxic agents.

In our experiments, colchicine and nitrogen mustard and, for purposes of comparison, ACTH were administered to 61 male and female albino rats ranging in weight between 100 g and 250 g. Details concerning the number of animals, doses and timing are given in Table 1. The drugs were administered intravenously. The mast cells were studied in membranes prepared from the subcutis and mesentery, and in bone marrow smears stained with 0,1 per cent watery toluidine blue. All animals used in the experiments were kept under identical environmental conditions and fed the same diet.

In the course of our experiments significant changes in the mast cells could be observed. These alterations presented themselves in three morphological forms corresponding also in order of time to three not sharply defined stages.

(i) In Phase I, the mast cell granules of the subcutis and the mesentery, normally varying in size from 0,5 to 3 μ, fused into coarser granules of from 6 to 15 μ within 1 to 4 hours after the injection of 200 to 300 γ of colchicine, and in 4 to 12 hours following the administration of 100 γ. This type of change may generally be observed in the pronounced cases in about 50 to 70 per cent of the mast cells, for from 24 to 36 hours after the administration of the drug (Fig. 1). A remarkably high degree of polymorphism was seen in this phase (Fig. 2). The changes, however, were found to differ in extent even when identical doses were applied. The granules of the mast cells were frequently noticed to be scattered, a fact which might be caused by undue interference during the preparation of the membranes, since the same phenomenon was encountered in mast cells of normal animals.

(ii) In Phase II., the number of mast cells showing coarser granulation gradually decreased and 36 to 96 hours after the application of colchicine a new type of mast cells made their appearance, the plasma of which consisted, in addition to some rough and few normal sized granules, of a homogenous bright metachromatic halo (Fig. 3). Similarly to those which formed in Phase I. by the fusion of small granules into coarser ones, this new type of mast cells was seen in 50 to 70 per cent too. This fact, besides of evidence of direct morphological observation, seems to indicate that this new type with a metachromatic halo develops from mast cells with clumped granules.

(iii) If the animal survived the colchicine injection by 72 to 96 hours, it was found that the number of normal-sized granules in the mast cells of the second-phase type gradually increased and thus seemingly normal mast cells were formed.

Bone marrow mast cells presented no significant change during the experiments.

The applied doses of nitrogen mustard caused in the mast cells lesions of the same character but a lesser degree. The excessive polymorphism usually seen following the administration of colchicine could not be observed after nitrogen mustard injections.

ACTH produced a different morphological picture. 24 hours after its injection the mast cells in the connective tissue of both the subcutis and mesentery were greatly swollen, the number of the granules staining with toluidine blue decreased and the nucleus or its seat became well discernible (Fig 4).

These phenomena appeared within 4 to 36 hours of administration of ACTH. 48 hours after the injection apparently intact mast cells were only present. The changes just described were generally observed in 1 to 5 per cent of mast cells. Morphologically they show a pattern that seemingly corresponds to the slight initial changes of the mast cells seen in the early stages after local X-ray irradiation. The view, that these changes are initial and only of reactive nature, seems to be corroborated by the observations of *Asboe—Hansen* [3], who found following large doses of ACTH, mast cells with clumped granules in the subcutaneous connective tissue.

We have failed to observe any changes in the intensity of the periodic acid-leucofuchsin reaction in connection with the above described changes of the mast cells.

In addition to the alterations in the cytoplasm of the mast cells it was endeavoured to study the behaviour of the nuclei, all the more as *Kellner* and *Matkó* [17] had observed significant nuclear changes in various tissues upon administration of colchicine and nitrogen mustard in doses similar to the ones used in the present study. Isolated staining of the nuclei of the mast cells is, however, hardly feasible, owing to the intensively staining mass of basophilic granules. This is why nuclear conditions in the cells with clumped granules could not be followed, and why it is not possible to form a definite opinion of the nuclear damage in that type of cells. In the swollen, degranulated and homogeneously metachromatic mast cells the nuclei appeared intact.

In examining the functional significance of the changes taking place in the mast cells upon the action of the cytotoxic agents applied in the present experiments, the conditions of blood clotting must be taken into account on the one hand, and the rôle in connective tissue function on the other.

The effect of colchicine on coagulation has not yet been clarified. It is known, however, from data published by *Smith* et al. [21], that on treatment with nitrogen mustard clotting time becomes considerably protracted and also that the protracted clotting time may be restored to normal by means of toluidine blue. The observations made by *Antalóczy*, *Komáromi* and *Selley* [2] also indicate that nitrogen mustard causes heparinaemia. There is no doubt that in the course of the present experiments we encountered large numbers of such mast cells with a homogenously metachromatic cytoplasm which, according to *Lehner* [18], release heparin. Yet careful observation of the sequence of the cytologic changes of the mast cells has convinced us that homogeneously metachromasia should not be interpreted as indicative of heparin release, as the homogeneously metachromatic forms are gradually transformed into normal

mast cells. Consequently they might have a regenerative character, and the heparinaemia demonstrated by the above-mentioned authors on treatment with nitrogen mustard might be correlated with the clumping observed in the early phase.

The question still remains to be answered what relations exist between the cellular changes observed in the mast cells and other functions of the loose connective tissue. Considering the rôle that heparin as an acid mucopolysaccharide is supposed to play in the formation of collagen fibres *(Gross*, [11]), we may safely assume that the changes in the mast cells as observed in our experiments are in some way correlated with the fibrosis described by *Holczinger* and *Kellner* (16) following the chronic administration of colchicine. It should also be taken into consideration that desintegration of tissue components, such as nucleoproteins, may release a compound of basic nature (histone), which combines with the acid mucopolysaccharides of the connective tissue, giving rise to formation of fibres, as suggested by *Beigeböck* and *Sickel* [5]. The large number of mast cells, usually occuring in fibrotic tissues, as in cirrhosis of the liver fibrosarcoma, cirrhotic carcinoma, sclerosed angioma and chronic inflammations *(Morrione*, [20]), supports some reasons to this view too.

TABLE I

Distribution of animals according to doses and time of examination

	Hours							
	1	4	12	24	48	72	96	Total
Colchicine								
300 γ	2	1	3	3	—	—	—	9
200 γ	2	—	3	—	1	—	1	7
100 γ	2	1	1	6	3	3	1	17
Total	6	2	7	9	4	3	2	33
Nitrogen mustard								
0,8 mg	—	1	2	2	—	—	—	5
0,4 mg	2	—	3	3	1	1	—	10
Total	2	1	5	5	1	1	—	15
ACTH								
30 units	—	1	2	1	2	—	—	6
60 units	1	1	1	1	2	1	—	7
Total	1	2	3	2	4	1	—	13
Sum total								61

Doses in the Table are given per 100 g of body weight.

Summary

The effect of colchicine, nitrogen mustard, and ACTH on the mast cells of the subcutaneous tissue and the mesentery was studied in 61 albino rats in the period from the 1st to the 96th hour following administration. Clumping of the mast cell granules took place 4 to 36 hours after treatment with 100 to 300 γ of colchicine or 0,4 to 0,8 mg of nitrogen mustard per 100 g body weight. After 36 to 72 hours, the mast cells with clumped granules decreased in number, and cell forms with a homogenous metachromatic cytoplasm developed. The number of granules subsequently increased in the cytoplasm of the mast cells and apparently normal cells were formed. 24 to 36 hours after treatment with 30 to 60 units of ACTH, swelling of the cells with a decrease in the number of the granules was observed. According to these, the mast cells of the loose connective tissue undergo rapid changes not only following X-ray irradiation but also on treatment with cytotoxic agents. These changes might play a part in blood coagulation as well as in the development of connective tissue alterations.

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ДЕЙСТВИЕ ЦИТОТОКСИЧЕСКИХ ВЕЩЕСТВ НА ТКАНЕВЫЕ ТУЧНЫЕ КЛЕТКИ

Г. Келеньи

Р е з ю м е

Автор исследовал действие колхицина, горчичного азота и адренокортикотропного гормона (АКТГ) на тучные клетки подкожной и брыжеечной клетчатки 61 белой крысы в течение 1—96 часов после подачи упомянутых веществ.

По истечению 4—36 часов после подачи колхицина и горчичного азота (100—300 гамма или же 0,4—0,8 мг на 100 гр. живого веса) наблюдается глыбчатая агглютинация зерен тучных клеток, а через 36—72 часа постепенно уменьшается количество тучных клеток с глыбчатой агглютинацией зерен. В то же самое время возникают клетки, имеющие метахроматическую протоплазму. В дальнейшем в плазме тучных клеток постепенно увеличивается число зерен, и возникают клетки повидимому нормального вида. В течение 24—36 часов после подачи АКТГ (30—60 единиц) возникают набухшие клетки с уменьшенным количеством зерен.

Все вышеупомянутые наблюдения указывают на то, что эти специальные клетки рыхлой волокнистой соединительной ткани проявляют быстро развивающиеся изменения, не только после воздействия рентгеновскими лучами, но и после применения цитотоксических веществ. По всей вероятности данные изменения играют роль в нарушениях свертывания крови и других реакций соединительной ткани.