Morphological Effects of the Secretion of Histamine and Mucopolysaccharides by Mast Cells

Recent studies have proved that granula of mast cells contain histamine as well as heparin. RILEY[1] states that the quantity of tissue-histamine corresponds to the number of mast cells in the tissue or organ concerned. The application of substances liberating histamine results in an extensive degranulation of mast cells[2]. For this reason, degranulation of mast cells is generally considered as a cytological phenomenon connected with the liberation of histamine[3].

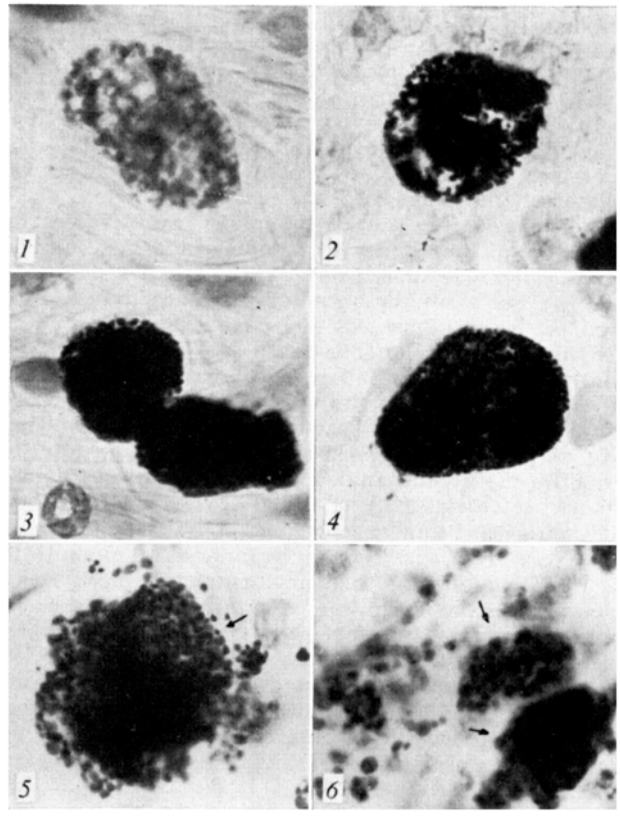
Preliminary experiments, in which we studied the effect of non-specific stress on the cytology of mast cells, made us realize that degranulation is a secondary process which does not commence before the histamine phase has been completed. This fact led us to a revision of the present views on cytological changes coexisting with the secretion of histamine. The results of this revision, which include certain facts concerning ASBOE-HANSEN's[4] views about the relationship of mast cells to mucopolysaccharides of the ground substance, are presented in this report.

The liberation of histamine from the mast cells of the mesentery of rats was achieved by intraperitoneal injection of sterile redistilled water at body temperature, at the rate of 10 ml/100 g of weight. According to FAWCETT[5] and KELLER and BURKARD[6], such interference produces the secretion of a great quantity of histamine into the peritoneal fluid resulting from the disintegration of mast cells. In the control experiment, Tyrode's solution was similarly applied. Specimens of mesenteries were prepared in the following manner. The mesenteries were carefully stretched on glass slides, fixed in methanol for 30 min and stained in 0·01% alcoholic solution of toluidine blue[7]. If necessary, a water solution of toluidine blue of the same degree of concentration was also used, in a 0·1 *M* citrate buffer of pH 4·15, whereupon the metachromatic changes were fixed in ammonium molybdate[8].

Within 5 min after the intraperitoneal injection of redistilled water, symptoms of a severe shock became noticeable: loss of reactiveness to outside stimuli, accelerated surface breathing, motor slowing down. Injection of Tyrode's solution did not result in shock.

Mast cells of the mesentery did not react on the injection of distilled water by osmotic disintegration, but by a change in staining the granules. Immediately after the injection, the granules lose their pronounced dark-violet metachromatical stain, swell, and become less refractive. Many of them display a gradual decolorization which is particularly noticeable in specimens stained in an aqueous solution of toluidine blue (Fig. 1). In the course of the decolorization, the metachromasia of these granules loses its alcohol-resistance. Spots in the cytoplasm of mast cells, where the granules have completely lost their ability of metachromatic staining, make the impression of vacuoles. Hereafter we shall refer to them as to pseudovacuoles. The remaining granules retain their original affinity to toluidine blue, but they are smaller. Their swelling soon abates. Frequently they join and form pedicles and thus border the pseudovacuoles. After these transformations mast cells acquire a somewhat honeycomb structure (Fig. 2).

Cytoplasm condenses about 5 min after the injection so that the pseudovacuoles, i.e. gaps originating from group decolorization of granules, disappear. The whole



Mast cells of the rat mesenteries after intraperitoneal injection of redistilled water. Toluidine blue staining. × 1300.

Fig. 1. – Immediately after injection. Group decolorization of granules in the course of the formation of honeycomb structure (water solution of toluidine blue).

Fig. 2. – 2 min after injection. The honeycomb structure of the mast cell. In cytoplasm there are partly gaps (pseudovacuoles), partly original granulation, no longer displaying signs of swelling.

Fig. 3. – 5 min after injection. The honeycomb structures disappear, cell diminish in size.

Fig. 4. – 15 min after injection. Some granules stain full red, the rest dark-violet. Spots where the red staining granules are deposited appear lighter in the microphotograph.

Fig. 5. – 90 min after injection. In cytoplasm partly a densely accumulated original dark-violet granulation may be seen (in the lower portion of the cell), and partly a lighter, full red granulation (↗). The red granulation is being expelled from the cell.

Fig. 6. – 4 h after injection. Group of macrophages (↗) in whose cytoplasm there is a phagocytized granulation of dark-violet stain. The surrounding area is flooded with swollen expelled granules which stain faintly and are not phagocytized.

[1] J. F. RILEY and G. B. WEST, J. Physiol. *120*, 528 (1953).
[2] J. F. RILEY, J. Path. Bacteriol. *65*, 471 (1953). – D. W. FAWCETT, J. exper. Med. *100*, 217 (1954).
[3] J. F. RILEY, Blood *9*, 1123 (1954).
[4] G. ASBOE-HANSEN, Ann. Rheumatic Dis. *9*, 149 (1950).
[5] D. W. FAWCETT, J. exper. Med. *100*, 217 (1954).
[6] R. KELLER and W. BURKARD, Helv. physiol. Acta *14*, 289 (1956).
[7] H. KRAMER and G. M. WINDRUM, J. Histochem. Cytochem. *3*, 227 (1955).
[8] L. LISON, Acta histochem. *2*, 47 (1956).

body of the cell is again evenly filled with fine granulation (Fig. 3). The size of these restituted cells is on the whole smaller than before commencement of the experiment.

In the succeeding intervals, we could see another, quite different cytological process. It consists in an intracellular change of stainability of the granules, the latter being finally separated from the cytoplasm of the mast cells. The changes of the stainability are in this case different from the above-mentioned decolorization. The deep dark-violet metachromatic tone turns full red. This means that granules do not lose their metachromatic character, but there is only a certain change in their staining properties and/or basophilia, resulting in the reduction of the affinity to toluidine blue. The full red granules at first appear singly here and there (15 min) (Fig. 4), but gradually outnumber the original granulation. At certain stages (30–90 min) mast cells contain two kinds of granules (Fig. 5). In the end, all the dark-violet granules turn full red. In the course of these intracellular changes, the full red granules are being gradually expelled into intercellular spaces. Disintegration of mast cells does not take place before all the granules have succumbed to the above-mentioned changes of stainability (1–2 h); prior to this the granules are just being expelled without disintegration of the cells. The extracellular granules thereupon lose their stainability and regular form; they swell, turn amorphous, and disappear. The ground substance in places abounding particularly in free granules stains metachromatically. During the degranulation, however, some of the granules with unchanged stainability are also expelled. These granules are phagocytized and accumulate as coarse aggregates in the cytoplasm of macrophages. On the other hand, the red granules are not phagocytized (Fig. 6).

The control rats that received Tyrode's solution were not shocked. Even in these cases, we could notice in short intervals after the injection (10 min) single honeycomb mast cells. The degree of changes in single cells and the number of cells reacting in this manner are incomparably smaller than after the injection of the redistilled water. Elimination of the granules from the cytoplasm does not take place.

Coagulativeness of blood remained on the whole normal in the course of the experiment.

Discussion.—From the report we may conclude that an injection of redistilled water causes in the mast cells two independent successive processes: first a group decolorization of granules resulting in the formation of honeycomb structures, and secondly an intracellular change of the stainability of granules and their expulsion from the bodies of the cells. Immediately after the injection, i.e. at the stage of shock, the honeycomb structures are formed. A curve denoting the histamine content in peritoneal fluid, as shown by KELLER and BURKARD[6], reaches its maximum at this time. It is therefore the honeycomb structure of mast cells that is related to the liberation of histamine, and not degranulation, as authors have hitherto assumed[9]. The degranulation take place after the disappearing of the honeycomb structure. At this time, the signs of the shock, i.e. histamine phase, cease. According to WERLE and AMANN[10], histamine is bound up with the heparin component of granula. Considering the fact that the formation of a honeycomb structure is the result of decolorization of granula, we may assume that the liberation of histamine depends on the intracellular depolymerization of the heparin component of the granules.

The honeycomb structure of the histamine phase was followed by the degranulation of mast cells. Prior to degranulation, the mast cells were restituted *ad normam*. Degranulation cannot be looked upon as a mere osmotic disintegration; it appears to be an active expulsion of the granules. According to WEGELIUS and ASBOE-HANSEN[11], the stimulus of such expulsion is most likely the increasing quantity of water in the ground substance of the connective tissue. The intracellular change of stainability of granules may be due to the loss of the sulphate groups of heparin, with regard to the reduced affinity of granules to toluidine blue and to the preserved metachromatic qualities at the same time. The assumed difference in the biochemical constitution of the metachromatic component of the original granules and the altered ones is proved not only by the staining properties but also by their affinity to the macrophages: the original heparin containing granules are phagocytized, while the altered full red ones are not. Normal coagulativeness of blood persists throughout the whole experiment, in spite of a complete degranulation of mast cells, as was pointed out also by others[12]; this is another proof that the granules of the mast cells in this case do not expel heparin but mucopolysaccharide of the hyaluronate type, which is derived from the former. On the basis of the above-mentioned facts, we may conclude that the degranulation of mast cells after intraperitoneal injection of redistilled water is essentially an expression of the secretion of mucopolysaccharide of the hyaluronate type into the ground substance of connective tissue mediated by the expelled granules. This mucopolysaccharide originates through an intracellular process from the heparin component of the granules.

M. HILL

Histologic-Embryological Institute of the Faculty of Medicine, University of Brno (Czechoslovakia), May 21, 1957.

Zusammenfassung

Das morphologische Bild der Sekretion des Histamins und der Mukopolysaccharide vom Hyaluronattypus wird in den Mastzellen festgestellt. Die Histaminsekretion äussert sich im Verlust der metachromatischen Eigenschaften mancher Mastzellgranula und in der dadurch auftretenden Wabenstruktur der Mastzellen selbst, während die Sekretion der Mukopolysaccharide durch die intrazelluläre Änderung der Färbbarkeit der Granula und ihre darauffolgende Ausschüttung aus dem Zytoplasma der Mastzellen charakterisiert ist.

[9] J. F. RILEY, J. Path. Bacteriol. *65*, 471 (1953). – D. W. FAWCETT, J. exper. Med. *100*, 217 (1954). – R. KELLER and W. BURKARD, Helv. physiol. Acta *14*, 289 (1956). – I. MOTA, W. T. BERALDO, and L. C. U. JUNQUEIRA, Proc. Soc. exp. Biol. Med. *83*, 455 (1953).

[10] E. WERLE and R. AMANN, Klin. Wschr. *34*, 624 (1956).

[11] O. WEGELIUS and G. ASBOE-HANSEN, Exp. Cell Res. *11*, 437 (1956).

[12] I. MOTA, W. T. BERALDO, and L. C. U. JUNQUEIRA, Proc. Soc. exp. Biol. Med. *83*, 455 (1953). – R. KELLER, Arch. int. Pharmacodyn. *107*, 382 (1956).