Studies on the Mast Cells in Gingival Tissue

By

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I. Introduction

In the histological application of aniline pigment, Paul Ehrlich in 1877 discovered that the cytoplasmatic granules are dyed deep in basic pigment and this cell which shows so called metachromasia in the connective tissue of a vertebrate, was named later by him mast cell. Since then many precious studies on this tissue mast cells in the internal organs or in the tissues of various kinds of animals have been performed by other workers. In regard to the mast cells in the soft tissues of the oral cavity, however, scarcely any contributions in the research works have been done so far.

Nevertheless, the oral cavity occupying such more frontal position than either digestive system, respiratory apparatus or speech organ, being affected by the outside air, and because of scraps of food constantly lodged inside, is likely to be a regular germbed which gives rise to actual causes or something incentive of various kinds of diseases. Especially the gingival tissue is representative in that, often showing a notable partial sign of the constitutional diseases.

Since Ehrlich discovered the tissue mast cell in 1876, there have been various conjectures and inferences expressed on its substance by many scholars. Recently, however, it was clarified that there is close relationship between this cell and heparin, and that histamine exists constantly in these cells. In recent years while its substance has been clarified, its biological functions have come into the limelight and their importance is being recognized biologically and pathologically as well.

Therefore, the present writer, after having clarified the forms and the distribution of the gingival tissue mast cell of a normal human,
patients of alveolar blennorrhea, and of various kinds of animals considers it very significant to discuss on its functions based on the experimental results.

II. Experimental Methods

The gingiva close to the inside cheeks of a normal healthy man, patients of alveolar blennorrhea, and of a cow, pig, mouse and rats were tested for this study.

First, of 20 normal healthy men from 30 to 40 years of age, apparent healthy parts of the gingival close to their cheeks were examined immediately after their surgical operations of various kinds were carried on them.

In the case of patients of alveolar blennorrhea, 20 cases of men from 30 to 40 years of age who were slightly affected by diseases of inflammatory type were examined on the gingival close to the inside cheeks.

As to animals, 5 full grown healthy cows and pigs each and 5 rabbits (some 100 g. each) and pigs (some 10 g. each), irrespective of sex were clinically tested upon the materials derived from the gingiva close to their inside cheeks.

In order to gather the materials, in the case of man, they were prepared immediately after their oral surgical excisions were carried on. And in the case of cows and pigs, they were prepared immediately after they were killed at a butchery, and those of rats and mice, soon after their neck veins were cut off under chloroform. And their fixation was carried on the moment when they were gathered.

As to their fixation and staining, various kinds of methods which the writer had tried so far were jointly used for this study, but chiefly the following were used:

1. Staining in 1/20 M israbin aqueous solution.
   Fixed in this solution for 24 hours (through which the tissue mast cells can be stained well simultaneously). Immediately they were soaked step by step in a series of alcohol—from 80% alcohol to raised percentaged alcohol, then they were sealed with balsam as usual.

2. Staining in 0.25% toluidine blue solution in which alcohol is solute at the rate of 75%.
   First, they are fixed for twenty-four hours in the fixative solution in which formalin (10 cc), 95% alcohol (90 cc) and calcium acetate
Next, they were washed in 95% alcohol twice every hour. After having stained for one hour under the temperature in the laboratory in the fixative solution in which toluidine blue (0.25 g) 70% alcohol (100 cc), and concentrated hydro-chloric acid (0.5 cc) were solute, then they are washed briefly in 95% alcohol in which 0.5% hydrochloric acid is solute.

Then, after-staining was performed in the 95% alcohol in which 0.01% eosin was solute.

After soaking them gradually in a series of alcohol from 95% alcohol to raised percentaged alcohol. And the spreads are sealed with balsam as usual.

3. Further, in order to take an accurate measurement of their sizes, the materials, having been cut into pieces (15µ) through the process of celloidin embedding, were stained again in toluidine blue as mentioned above.

4. According to Holmgren's and Wilander's method of fixation, they were fixed in 4% basic lead acetate for 12 hours, then stained with 0.1% toluidine blue for 1 hour, and after being soaked in a series of alcohol—from 90% alcohol to raised alcohol, they were sealed with balsam.

5. McManus' (1948) periodic acid Schiff's reaction.

After having fixed in Carnoy's fixative solution for 3 hours, immediately, the materials were removed into absolute alcohol, treated for 20 minutes with 0.5% periodic acid solution, washed carefully in distilled water, stained for 2 hours Schiff's reagent.

Further, this preparation is put into each of the 3 bottles containing sulfurous acid liquid, one after another each for 2 minutes, washed in distilled water for 2 or 5 minutes, stained with haematoxylin for 20 minutes.

Then this preparation, after the process of soaking in a series of alcohol—from 90% to raised alcohol, then was sealed with balsam.

And then, this stained preparation, were examined as minutely as possible microscopically of the sites, numbers, types, state of stainability (esp. about metachromasia) of the gingival tissue mast cell, etc.

Observations

1. The gingival tissue of a normal, healthy man.

As to the number of the tissue mast cells, there were some
regions where none of the tissue mast cells were observed in one field of vision, (×400) and if there existed, there were no more than one or two only; number was less than 1 on the average of several fields of vision.

As to the location of tissue mast cells, they were observed to lie distributed chiefly along the small blood vessels in the depth of the outer edge (tunica propria) of lamina propria mucosae. Namely, many of them were found generally located near the outermost layer of the skin or epithelium, consisting of fibrous loose connective tissue where there are plentiful of blood vessels, but there were none of them found either in the regions of the deeper layer where tough and minute connective tissues are abundant, or inside the epithelium.

As to the size of the gingival tissue mast cells, they were observed to be between 11μ and 20μ, and many of them were about 14μ. Though some were several times as large as red blood corpuscles, almost all others were generally twice as large as red blood corpuscles, and the differences of their size were not much distinct. Especially, those of the cells in the same regions or in its neighborhood could scarcely be observed. As a whole, however, the deeper the cells were located from the upper layer of the subepithelial connective tissues, the larger the cells seemed to become and the size of the nuclei also were observed usually in proportion to those of their cells and the size of the nuclei in both long and short diameter could be generally ascertained to be nearly half those of their cells. The size of the granules, too, is approximately in proportion to those of the cells themselves and of their nuclei.

Generally speaking, the difference of size of the granules could scarcely be recognized, much less so in the same regions. As there were differences in the size of the cells between in the outer layer and in the deeper layer, so the granules in the cells, less in number in comparison to those in the deepest layer.

The forms of the gingival tissue mast cells were round or oval and no tendency of forming a projection was observed. The nuclei were single, and their forms were generally similar to those of their cells. i.e., they were round or oval in shape. Most of them were located in the center or in its neighbor and they were centric. Those which were eccentric could be scarcely observed.

Many of the nuclei contained a nucleolus which was relatively rich in chromatin and the formation of minute chromatin networks were recognized. The granules of the mast cells surrounding the
nuclei nearly so pervaded the protoplasm that some of the granules could hardly be distinguished individually, but the majority of them could be observed.

There were a small portion of the granules which obscured the borderline between the nucleus and the protoplasm and still the least number of the granules which enveloped the nuclei so that they were almost unable to be identified. As a whole, however though there were cased where it was difficult to identify the granules individually, their nuclei which were stained light blue in toluidine blue solution, and yellowish brown in israbin solution, could be observed comparatively clear.

The granules of the gingival tissue mast cells pervaded the protoplasm and were comparatively evenly arranged there. Neither granules of them which made any differences of density in arrangement in protoplasm nor the granules which were dispersed out of their cell bodies were observed. No vacuoles either in the nuclei nor in the protoplasm could be observed. That is, neither remarkable deformity nor degeneration in both regions could be recognized.

As the granules of the protoplasm in these tissue mast cells are basophilic, the color-tone of these cells showed a comparatively beautiful blue purplish color with toluidine blue solution, so they were easily distinguishable from other connective tissue cells, but were deficient in such a brightness as were seen in the granules of eosinoocyte. Besides, their color tone, generally speaking, was deep blue tinged with orthochromasia rather apparent.

As the granules in the protoplasm with israbin solution contain heparin, they were stained dark yellowish brown and could be distinguished at first sight from the surrounding connective tissue cells. As to the density of the stainability of individual granules, the difference in their stainability between with toluidine blue solution and with israbin solution could hardly be recognized.

The granules of the gingival tissue mast cells proved strongly positive to the PAS reaction (periodic acid Schiff's reaction) according to McManus method, some of the granules showed granularity, and the whole protoplasm of some granules assumed reddish purple.

In the above observation little difference in individual cases was observed.

2. The gingiva of patient of alveolar blennorrhea.

The subepithelial gingival tissues of a patient of alveolar blennorrhea were observed to have enlarged and proliferated, the tissue mast
cells were detected not only in the loose connective tissues in the upper layer or around the blood vessels, but also even in the connective tissues composing of comparatively thick fibers in the deepest layer, where there are scarcely blood vessels. Even in the intermediate layer or in the basal layer of the epithelium the cells could be detected. The number of these cells was 9 (the mean number between 6 and 11) within one field of vision in low magnification (×400) and they tend to gather in groups of 3 to 6.

The forms of these cells were observed to have various kinds of shape. Comparing with the gingival tissue cells of normal healthy man, there were scarcely cells found to be normal or nearly so in shape. Most of them were indefinite in shape and had a tendency of forming a projection. The cells themselves were found corpulent and as enlarged as 21 μ on the average of 11 μ and 28 μ.

Thus the size difference between these cells and those of a normal healthy man was so conspicuous. There were many of the cells whose contours were indistinct and whose stainability was insufficient. As a whole, a sign of their degeneration was remarkable, esp., their irregularity in shape was conspicuous. The granules in the protoplasm were observed to have become loose in density, their arrangement also irregular and many of them were indefinite in forms and stainability. The extent of metachromasia shown in these cells, comparing with that of the gingival tissue cells of normal healthy man, was remarkable and there were many of them which were noticed to assume a strongly purplish red color. Among these cells there were some in which granules were delivered and dispersed out of the cell bodies, and in their protoplasm only several granules were observed to remain around the nuclei and some vacuoles were detected there.

There were dispersed some granules near the epithelium and some which already penetrated into the epithelium. Such phenomena could not be found in the gingival tissues of normal healthy man. To the PAS test according to McNamur's method these mast cells showed more strongly positive as compared with those of a normal healthy man. Some were found in the formation of granulation and the whole protoplasm of some cells were red purple.

3. The gingiva of a cow.

The number of the gingival tissue mast cells was more than that of the normal gingival of man, averaging 11 within one field of vision in low magnification (×400). The location of these mast cells was observed nearly the same with that of a normal healthy man. The
size of these cells was larger than that of a normal healthy man, most of them being about 20 μ. The forms of these cells being different from those of a normal healthy man, comparatively many of them were spindle shaped or prolate, comparatively few were round in shape, and only a portion of them was observed to have a projection. As to the nuclei, there were observed often some which were covered with the granules and could hardly be identified, but generally speaking, they were single-cell and their forms were in accord with those of the cell bodies as in the case of a normal healthy man. Many of them being spindle shaped or prolate, and some of them were observed though rarely, simply segmented. The site of the nucleus was generally located in the center or in its neighborhood in the cell body, but often was eccentric. The granules were a little smaller than those in the case of man and their arrangement were looser, too, dyed a beautiful reddish purple, of metachromatic color with toluidine blue solution and deep yellowish brown with israbin solution, and was easily distinguishable from circumferential phagocyte. The basic substance between the granules was observed comparatively clearly to be transparent gap. And the granules in the same cell body did not show any remarkable difference in size, density of stainability, were arranged as evenly in the cytoplasm as in the case of the gingiva of a normal healthy man. The granules of these mast cells were often observed to gather around both ends of the protoplasm, and nearly none of them were observed in its center, and others had no granules, forming so called areola annularis.

These granules showed positive to the periodic acid Schiff’s reaction according to McManus’ method, but not so strongly positive as in the case of the gingiva of man, and the whole of protoplasm appeared reddish purple.

These granules showed positive to the periodic acid Schiff’s reaction according to McManus’ method, but not so strongly positive as in the case of the gingiva of man, and the whole of protoplasm appeared reddish purple.

The above mentioned observation was nearly the same in the case of either individual differences or the regional differences in the cell body. The above mentioned observations about the cow made no remarkable difference in between the cases of the individual cell bodies and those of their parts, in the cell body.

4. The gingiva of a pig:

The number of the gingival tissue mast cells, as compared with
that of a cow's ones, was slightly more than that. It averaged within one field of vision in low magnification (×400). The site of these cells was nearly the same as in the case of a normal healthy man, but some of the cells were found in the deepest regions composing of comparatively compact fibrous connective tissues where blood vessels were scarce. The observation specially worthy of notice was that the pig's cells did not exist in groups and were scattered evenly in the connective tissues. The size of these cells was smaller than those of the cow's cells and that of the gingival tissue of a normal healthy man. They were from 8 μ to 18 μ, and 11 μ of the average. The forms of the cells were, spindle shaped, oval, prolate or indefinite shape and not a little of them were observed to be segmented. Further, they had a strong tendency of forming projections. The shape of the nuclei were observed to tend to correspond to those of their cells in common with the cases of a man and a cow, but as to the site of the cells, few cells of them were centric while many of them were eccentric. The nucleus was dyed light blue with toluidine blue solution and yellowish brown with israbin solution, but the chromatin in the nucleus was hardly identified.

These granules were much smaller than those of a cow and were dyed beautiful red purple, metachromatic color and dark yellowish brown with israbin solution. As to the granules of the cells, there were two different kinds of cells mixed together—the one formed of granules which were comparatively many in number, pervaded the cytoplasm, and were arranged evenly and the basic substance between the granules were not observed clear and the other formed of granules which were comparatively few in number and arranged unevenly and loose. In the case of a pig, phenomena worthy a specific notice were that there were many of the nuclei in which the granules formed closely together while comparatively many of the cells were observed to certain the formation of transparent areole annularis without any granules around their nuclei. These observations were scarcely found in man and only a few in the case of a cow. Excepting these, the other observations were nearly the same as those in cases of the man and a cow. They were weakly positive to the PAS reaction of McManus' method and dyed pink.

The above mentioned observations made no remarkable difference between individual cells difference of animals and the regional difference of the cells in the same way as the cases of the man and the cow.

5. The gingiva of a rat.
The number of the gingival tissue mast cells were far more than that of those animals described above, averaging 24 within field of vision in low magnification (×400).

The site of these cells was nearly the same as in the cases of man, the gingival tissue mast cells and the phenomenon that these cells were found in the deepest regions composing of comparatively compact fibrous connective tissues, where blood vessels were scarce was similar to those of the cow and pig.

The size of those cells averaged 11 μ between 7 μ and 18 μ and their size difference was noticeable. Their forms were spindleshaped, oval, oblong or indefinite shape and tended to form 1 or 2 short projections. The shapes of the nucleus appeared to tend to proportionate to those of the cells. The site of the nuclei bearing similarity to the case of pig was eccentric. The nucleus was stained light red purple of metachromasia with toluidine solution and were stained yellowish brown with israbin solution, but any chromatin in it was not recognized.

There were two kinds of granules in these cells. The granules of one kind was stained a beautiful red purple color of metachromasia and the granules of the other kind were stained deep blue color rather including toward ortochromasia, and with israbin solution, the former was dyed deep yellowish brown color and the other was dyed light yellowish brown, some granules arranged evenly in the cytoplasm and other groups arranged unevenly and loose and mixed together.

The PAS reaction according to McManus method was full of variety and the mast cells were easily recognized in low magnification. In high magnification (or in an oil immersion equipment) some of the granules were formed positive while other granules, contrasting to the other granules, contrasting to the cytoplasm which dyed pale pink, did not stain and looked like bubbles. These mast cells, however, were observed negative in many other preparations.

The above mentioned observations were not remarkable in the differences of individuals as compared with the regional differences.

6. The gingiva of a mouse.

The number of the gingival tissue mast cells was found to average 18 in low magnification (×400) and less than that of a rat. The site of these cells was similar to that of a rat as mentioned above. The size of these cells averaged 9μ between 7μ and 12μ and the size difference was not so noticeable as that of a rat. Their forms were chiefly round or spindle shaped and had a tendency of forming a projection. The shape of the nucleus was nearly proportionate to those of their
cell bodies. The site of the nucleus unlike that of a rat was chiefly centric and resembled that of the gingiva of a normal healthy man. The nucleus was stained light red purple with toluidine blue solution and yellowish brown with israbin solution, but any chromatin in the nucleus could not be observed. The granules of these cells were stained a beautiful red purple color of metachromasia with toluidine blue solution. The granules were generally arranged loose and unevenly unlike those of a rat. To the PAS reaction according to McManus method they were observed nearly the same in the case of a rat.

The above mentioned observations were not remarkable in the difference of individuals as compared with the regional differences.

Discussion

In 1817 Paul Ehrlich discovered in the connective tissues of various kinds of vertebrates some cells containing granules which were stained deep in basic aniline pigment and shows so called metachromasia quite different from the original color tone of the pigment and regarded them the same as the plasma cells in which W. A. L. d e r made a report in 1875, but afterwards (1879) he noticed the singularity of this cell, changed his view and stated that this cell is quite different from the plasma cell and congests at the region where local nutritive condition is improved, such as chronic inflammation, congestion, neoplasm. Considering that this cell arises because of the hypertrophy of connective tissue cell, it was named that mast cell. The information about this cell was all recorded in detail by Westphalin 1880.

Later Michaelis (1902), Maximow (1906), Lehner (1924) and many other scholars' researches and investigations confirmed that this mast cell is quite different from the mast cell found in the peripheral blood and this cell became to be called the tissue mast cell.

However, as to the origin of the tissue mast cell, esp. the substance of the granules showing a peculiar metachromasia, there have been for a long time so various theories among many scholars that they hardly seemed to reach any conclusion, but since Ehrlich's discovery of this tissue mast cell, it passed 60 years when noticing the fact that morbid tissues containing many of these mast cells showed anticoagulability with Jorpe's solution, and after many studies, made clear that the metachromasia of this cell is due to the heparin contained in the granules.

Thus the mystery of the metachromasia of the tissue mast cell,
a long pending question for the histological scholars seemed to have been solved at last. By the way it is a well-known fact that heparin is first produced by Howell in 1918 out of the liver of a sheep and mucotin sulfuric acid chemically which prevents the coagulation of the blood without changing the quality of the blood at all.

Recently Rocha e Silva (1943) discovered that while the sensitized liver of a dog was being perfused, an antigen was injected into the perfusion fluid. There, not only heparin, but also a great quantity of histamin were let loose by this process.

Taking a notice of this phenomenon, Riley (1933), using a white rat as an experimental animal, re-examined this experiment histologically and histo-chemistically and found out that the tissue mast cell contained a great quantity of histamin as well as heparin. Namely, in the first experiment, after the tissue of the animal was injected with the released substance of histamin and anaphylatoxin, it was observed under microscopic examination that a remarkable integration was taking place whereas if antihistamin was applied in advance, even though just the same procedure was repeated, there took place no integration.

Further, in the second experiment, when a normal healthy tissue and a morbid one containing various degrees of the tissue mast cells were examined to measure pharmacologically how much histamin exists in the cell, it was ascertained that there was a close parallel relation between the tissue mast cell and histamin, so he named the tissue mast cell histaminocyte and opined that this cell played the part of a histamin cell and this theory seems to have become final.

On the mother cell of the tissue mast cells, there are various theories and as it may become so complicated to state all of them, let the theories historically most worthy being summarized as follows: ‘The Connective Tissue Cell Theory’, ‘The Blood Vessel Adventitial Coat Theory’, ‘The Blood Vessel Endothelial Cell Theory’, ‘The Reticulo Endothelial Cell Theory’, ‘The Reticulo Endothelium Cell Theory’, etc.

Riley (1953) using a mouse and a rat as experimental animals, confirmed that the tissue mast cells are young cells which have grown out of the undifferentiated mesenchymal cell of the adventitial coat cells of the blood vessels and as they mature, they move off the walls of blood vessels into the tissue, then later lose the granules. Takeeda (1958) after the experiment, utilizing a mouse and a rat, opined that this cell has grown out of the adventitial coat cells and undifferentiated
mesenchymal cell, then later, as it moves into the circumferential connective tissue cells, the heparin contained in the granules are activated and shows metachromatic color.

When Riley and West (1955) repeatedly applied carcinogenic carbohydrate (hydrocarbon) to the skin of mouse, it was observed that the histamin and the tissue mast cells contained in the affected regions increased in quantity. Again Yamazaki and Kawamoto (1955) through the vital staining of toluidine blue proved that when the intravenous injection was performed, the disintegration in the subcutaneous tissue mast cells was taken place.

As mentioned above, as the result of experiments conducted by many scholars, the relationship between the tissue mast cells and histamin was ascertained. In this way, the close relationship of the tissue mast cells, heparin, and histamin gradually became clear.

In spite of the fact that they have been more and more appreciated, the importance of this tissue mast cell it is regrettable to say that the studies on the soft tissue in the oral cavity, esp. of the tissue mast cell has been so scarce that as far as the writer has hunt up, there are only two. One is Shindo’s (1957). The studies on the soft tissue in the oral cavity and the gingival tissue mast cells and the other’s Fujioka’s (1957). The studies on the gingival tissue mast cells of normal healthy men and patients of alveolar blennorrhea. In view of that, the writer’s present study might be the footing for breaking up a new field in the studies of diseases in the soft tissues of the oral cavity.

As a whole, as the tissue mast cells have a tendency to exist generally widely around small blood vessels in the connective tissue, the disintegration of this cell likely to cause histamin to act on the blood vessel system. And it is easily supposed that these two kinds of cells, in opposite way, are likely to be influenced by the change of the blood components.

Consequently, it can be said that the tissue mast cells have a most important signification since the reactions of this mast cell in the patients of inflammation or some other morbid states are regarded as a histological manifestation of the conditions and functions of histamin in the affected parts of body.

In this respect, too, the gingival tissue is an important, as it tends to be influenced physically and chemistrically and assume various kinds of vital reactions.

And the reactions of this cell are significant as an important
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morphological manifestation of histamin in the affected parts of body.

The gingival tissue, when viewed from the point of histological structure, its lamina propria mucosae are composed of the loose connective tissue consisting of minute fibres, blood vessels, lymph vessels, and nervous tissues.

And as the blood vessels constitute the main part of them, the reactions of tissue mast cells as compared with these of the other internal organs should be regarded to have as an important signification as the latter.

Concerning the distribution and forms of these tissue mast cells, Miura (1952), as the result of his minute examination of their origin and the distribution and forms of every internal organ in the phylogeny of various kinds of animals, found out that the distribution and conditions of the tissue mast cells varied according to the kinds of animals and their internal organs.

As to the case of man, since their discovery of Stemmler (1921) their origin has been studied systematically and as a result it was affirmed that they are especially plentiful in thymus, the corium of skin (esp. most plentiful in the capillary stratum of the corium), subcutaneous connective tissue, lymphatic gland, gastrointestinal tract (submucosa) and the wall of urinary bladder. The phenomenon that the tissue mast cells exist more plentifully around small blood vessels having no muscle coat, esp. around the capillary seems to have some important meaning. Itô (1942) opined that pretty many of the tissue mast cells exist in the axillary skin of man and most of them could be found much around the blood vessels. Further, Riley (1953) and Takeeda (1958) observed that in the subcutaneous connective tissue of a mouse and rat, many young and immature tissue mast cells exist much around the small blood vessels having a circular muscle coat. In the gingival tissue of man, too, Niijima (1957) and Fujioka (1957) opined that the tissue mast cells exist much along the blood vessels of submucous coat.

Summarizing these studies, many workers seem to agree in the following.

There exist comparatively many of these mast cells very close to the small blood vessels of the submucous coat (subcutis) where the connective tissue cells are abundant. In this respect, the result of the present writer's microscopic observations on the gingival tissue of a normal man, the patient of alveolar blennorrhea, and various kinds of animals are in accord with them.
Concerning the subject when the tissue mast cells increase or decrease in number, Stea m l e r (1922) opined that when young and immature connective tissue was formed, these cells increase and they disappear at the last stage of sclerosis. Namely, when the connective tissue are formed with the fresh granulation tissues, the connective tissue mast cells are likely to increase. In the case of a tumor, too, when the connective tissues are formed, a great quantity of these mast cells are observed there. Syl v e n (1941) opined that whenever the connective tissue in man is formed, whatever process may be used in its formation, metachromatic substance and the tissue mast cells are observed to increase in number. This phenomenon T a k e d a (1958) proved quantitatively in his observations on the reaction of these tissue mast cells in the inflammation. That is to say, from the time when the inflammation recedes and the fibroblast begin to appear, the tissue mast cells increase in number.

Ehr l i ch affirmed already in 1877 that the tissue mast cells decrease in acute inflammation whereas in a certain kind of chronic inflammation, they increase. Stud e r (1954) opined that it is characteristic of the case of urticaria pigmentosa that there are many tissue mast cells around the capillary of epithelium observed.

According to Fra k en 's (1952) opinion, whenever the eosinocyte of the epithelium increases, it is sure that the tissue mast cells increase, and even in case lymphatic vessels are closed such as elephantiasis, the tissue mast cells are remarkably increased.

Inferring from the theories of these scholars, it is easy to suppose that in the case of the gingival tissue there appears a similar tendency. In the case of the gingival tissue mast cell of the patient of alveolar blennorrhea, too, the similar phenomenon came under this writer's observation. Riley and W e s t (1952) announced that the tissue mast cells generate and separate histamin. Later F a w e t t (1954) and B e n d i t t (1955–1956) affirmed it. Ri l a y and W es t proved that there is remarkable positive interrelation between the quantity of the tissue mast cells in the normal tissue and that of the histamin taken away out of the same tissue. Further Ri l a y (1935), F a w e t t (1954) and B e n d i t t (1955–1956) observed that the tissue mast cells were damaged, they set free histamin of high percentage of density. When Fu ji o k a (1957), injecting histamin into the gingival tissues of a dog and a cat, caused to develope symptoms of illness similar to those of alveolar blennorrhea and them measured the number of histamin and of the tissue mast cells, the increase of histamin twice as such as
that of normal part as well as the increase of the tissue mast cells in number was observed. And it is very interesting indeed to observe that there is an interrelation between the histamin in the gingival tissue of the patient of alveolar blennorrhea and the tissue mast cells in the gingival tissue.

Concerning the forms of the tissue mast cells, many scholars such as Maximow (1906), Pappenheim (1919), Lehner (1924), Kiyono (1925), Nakajo (1927-28), Nakashima (1928), Miura (1932) and Kubota (1944) have researched and written in detail so far. According to their opinions, the forms of the tissue mast cells have a great variety—many are round, oval, spindle or prolate shaped and animals excepting man, have a tendency of forming a projection and many of them are indefinite in shape, prolate, starlike, or octopus shaped. Generally many of the tissue mast cells in the interstitial connective tissues have a spindle shape or a projection and many tissue mast cells in the loose connective tissues are round or oval.

The size of this cell is several times as large as a red blood corpuscule. The nuclei are usually single and many of them are round, oval or spindle shaped. Excepting the case of man, (though they are rare) some of these cells are observed to be kidney shaped or ring shaped and sometimes segmented. Generally speaking, however, most of the forms of the nuclei correspond with those of their cells.

The forms of the tissue mast cells in man and several animals, according to this writer's microscopical examinations, also were round, oval or indefinite in shape and their nuclei were single and corresponded with their cells in shape. Many animals, excepting a normal healthy man and mouse, seemed to have the tendency of forming a projection.

The granules of these tissue mast cells are stained a beautiful metachromatic color in the basic aniline dye. In a physiologic normal condition, the granules in the same cell body are observed irregular in shape, size and stainability. There seems no definite relationship between their sizes and stainability. Usually they pervade the protoplasm and arrange evenly.

In this connection, again, this writer's observation on the gingival tissue mast cells also turned out nearly the same as the preceding statement. However, it was interesting to notice that the gingival tissue mast cells in man as compared with those of a cow, pig, rat and mouse, were stained rich blue-colored purple skin to ortho-
chromatic color. This phenomenon seems to be due to specific character of heparin.

Lison (1935) and Jorpes (1948) opined that heparin was stained metachromatic color with toluidine blue solution, but the extent how much it could be stained purple was in proportion to the quantity of sulphide, esterized heparin monocules. From this point of view, this writer supposes that the gingival tissue mast cells in man were chiefly mono sulphuric acid ester or skin to it whereas those in a cow, pig, mouse and rat are chiefly di- or tri-sulphuric acid ester. Although he has no further ground so far to prove it, it may be explained easily through the PAS reaction. Namely, the phenomenon that the tissue mast cells show positive to periodic acid Schiff's test was opined by Jorpes and Aberg (1948) Compton (1954). However, what is the exact chemical substance which reacts to the periodic acid Schiff's reagent seems not be cleared yet.

However, so as to discriminate various kinds of sulphide, esterized heparin monocules, the result thus gained through this method, i.e. the PAS reaction seems reliable (Riley 1953).

According to Jorpes (1948) and some others, heparin monosulphate is positive to the PAS reaction, but negative to di- and tri-sulphate. It is observed that the less the quantity of the sulfuric acid is, the more the amount of consumption of periodic acid is. When mono sulfuric acid ester is decomposed, 2 hydroxyl groups are obtained and in the case of di- or tri-sulfuric acid ester, 3 or 4 hydroxyl (\(-\text{OH}\)) groups are obtained. When a large quantity of mono-sulfuric acid ester exists, this can be proved histologically (Jorpes and Aberg 1948).

Now, let the gingival tissue mast cells be examined. In the cases of the gingival tissue of normal healthy man, and of patient of alveolar blennorrhea, their tissue mast cells show a strongly positive reaction to periodic acid Schiff's test (PAS reaction), and some of the mast cells assume granulation and some whose whole protoplasm show red purple color and others, when part of which damaged and even when the granules within are diffused and dispersed out of the cell bodies, the granules are positive to the periodic acid Schiff's reaction.

The gingival tissue of a cow and pig was observed nearly the same with those of man, but the former had a remarkable variety of stainability and some of them whose protoplasm were stained pale pink color, but their granules did not dye and looked just like bubbles and others were dyed from orange to red purple and the difference
of size of their granules could be observed.

In the case of a rat and mouse, there were comparatively few of them which were positive to the periodic acid Schiff's reaction.

Judging from the result of the above mentioned experiments it is considered that the tissue mast cells showing strongly positive reaction to the periodic acid Schiff's test contain much of di- or tri-sulfuric acid.

The irregularity or dispersing of the granules in normal healthy conditions are regarded by Maximow (1906) and Lehner (1924) as artificial products. Tsudoa (1923) regarded the granules extricated out of their cell bodies as nothing but an artificial product. The discharge of the granules of these tissue mast cells are opined by Michael (1938) to be an artificial production in breaking up of the cell bodies due to either the microtome or sudden fixation.

According to the writer's experiment, in the case of the granules of the gingival tissue mast cells, the scattering or extricated ones out of their cell bodies were scarcely observed. Considering from this point of the view, the appearance of indefinite shapes of the granules or their diffusion and dispersing, out of the cell bodies are supposed to be mostly somehow by man's technical process in fixation, staining or the use of a microtome, etc.

As for the way of detecting the tissue mast cells, Michaelis (1902), Wolff (1902) Maximow (1906), Arnold (1914) and many other scholars stated that as the tissue mast cell granules are soluble in water, in order to detect them, they should be fixed without fail in absolute alcohol and be examined through celloidin sections. However, as Miura (1932) opined on it, the extent how much are the tissue mast cells soluble differs according to the kinds of animals and of their internal organs. As far as the writer's experiments of men, cows, pigs, rats and mice. either absolute alcohol or the process of celloidin sections was not always necessary for their satisfactory detection.

On this subject of the way of detecting of the tissue mast cells, Ehrlich found out long ago that these mast cells are basophilic. Since then there have been many special methods of staining devised, but the basis upon which every one of the methods depends is the application of basis aniline dye. Namely, but applying such a medium as toluidine blue, thionin, brilliant kresyl blue, gentian violett, godviolet, methylenazur, kresyl violett, R.R. violett, etc. the presence of the tissue mast cells can be proved, since heparin which is con-
tained in the granules of the tissue mast cells assumes sometimes metachromatic color.

According to Seki (1954) the cause of metachromasia is due to the fact that dye molecules suffer strain by a strong electro static action sulfuric ester (R-OSO$_3$H) of high molecular compound polysaccharide, such as heparin, chondroitin sulfuric acid, atoms of dye molecules are transformed, polymer is formed, the phase of vibration of electron is changed and then the selective absorption of light is changed.

Wilander and Holmgren (1937, 1938) recommended 4% basic acetate fixative and 0.05% toluidine blue staining and Ura Kami (1940) and Minami (1955) stated that after fixing in 10% basic acetate solution the cells will dye well with 0.05-0.1% toluidine blue solution combined with acetate buffer solution pH 2.4. It is certain, however, the granules of this tissue mast cells as compared with other cells are substances which are unstable to water and it is doubtful whether these methods of detecting this tissue mast cells can determine them in detail quantitatively. As Lison (1937) pointed out, the temperature, the density of hydrogen ion and the presence of salt, etc. make a change, hence they may hardly become the base of their determining. Further, he stated that toluidine blue of high density is absorbed by other substance more than sulfuric acid ester. Therefore, he advises not to use the solution or high density. Lison (1936) and Sylén (1941) opined that true matachromasia is better shown in that solution very weak or strong acid solution. After scrutinizing these studies, Smith and Atkinson (1956) recommended the staining in 0.25% toluidine blue solution after fixing for 24 hours in the fixative composing of 10cc. formalin, 95% alcohol, and calcium acetate 1g. When this staining fluid is used, the circumferential tissues are dyed light blue and the tissue mast cells, from deep blue purple color to reddish purple. This is because toluidine blue non-specifically bands together with some oxide compound, esp. acid mucous polysaccharide. As the pH. of the staining fluid is low, the pigment is likely to combine fast with acid compound, esp. acid mucous polysaccaride. Of course, the forms of these mast cells are thought to be very good ones to get a good result for the examination of the chemical properties.

After having used this method, the writer further performed the after staining with 1% eosin and made clear of the relation between the circumferential tissues and these tissue mast cells. Kityama (1942)
succeeded to detect these tissue mast cells with trypaflavine. Takeda (1958) using 1/20 M israbin and 1/50 acrinol solutions got a good result in detecting them, too. This is because a reaction of israbin, acrinol, and various kinds of sulfuric acid ester in vitro and some kind of sulfuric acid ester through these substances produces some insoluble sediment in alcohol and its sedimentary reaction is of a remarkably fixed quantitative. This phenomenon, however, is not metachromasia so this method is sometimes not satisfactory, according to the kinds of studies on the tissue mast cells, but it is a good method nevertheless, for it is very simple and does not produce any artificial product. Moreover, this mast cell can be detected without discoloring for many years.

In this experiment, this writer, using this method in the study of the forms of the tissue mast cells, and of their granules, that is, in the morphological study as it is called, met with a satisfactory result.

As to the method which Holmgren and Wilander extol, its process is very simple and its staining solution is so easily obtainable that some researchers prefer to that. Indeed, through this method, the tissue mast cell granules show very beautiful reddish purple color, and their discrimination is quite easy, but it has drawbacks, too, as the staining of oxyphilic substances is not sufficient, slightly though it is, and it is now and then apt to discolor within several weeks.

Therefore, the present writer concludes that in the study of the tissue mast cells without distribution of either the kinds of animals or of their organs, the joint use of 1/20 M israbin staining in combination with Smith and Atkinson's method is the best possible method.

III. Conclusion

In order to know about the distribution and forms of the gingival tissue mast cells and discuss their functions, the present writer, after staining with 0.25% toluidine blue solution in which 70% alcohol was solute (according to Smith and Atkinson's method) imparted after staining in 95% alcohol in which 0.01% eosin was solute.

Then, according to Holmgren's and Wilander's method of fixation, fixing in 4% lead acetate and staining in 0.05% toluidine blue, then applied the periodic acid oxidation Schiff's test (PAS reaction) and the following data were collected.

1. The number of tissue mast cells of normal gingiva of man was 1 or less than 1 in one field of vision in low magnification (×400).
They were observed to lie chiefly distributed along the small blood vessels composing of loose connective tissues consisting of fine fibers in lamina propria mucosa. Their size was observed to be about 14 \( \mu \) on the average. Their forms were round or oval and no tendency of forming a projection was observed. The nuclei were single-celled and their forms were generally similar to those of their cells and centric. The nucleus was relatively rich in chromatin. The colortone of these tissue mast cell showed a beautiful blue purplish color, rather skin to orthochromasia with toluidine blue solution and with israbin solution they were stained deep yellowish brown. They proved strongly positive to the PAS reaction.

2. The tissue mast cells of the gingiva of the patients of alveolar blenorhrea were detected not only in the loose connective tissues in the upper layer of lamina propria mucosae or around the blood vessels, but also even in the connective tissues composing of relatively thick fibers in the deeper layer, where there are scarcely blood vessels. Even in the intermediate layer or in the basal layer of the epithelium, the tissue mast cells could be detected. Most of the tissue mast cells were indefinite in shape and were corpulent and enlarged. Some granules were delivered and dispersed out of the cell bodies. Their contours became indistinct and their stain ability insufficient. As a whole, a sign of their degeneration was recognized. With toluidine blue solution, they showed a beautiful reddish purple of metachromasia and with israbin solution, they were stained yellowish brown and strongly positive to the PAS reaction.

3. As to gingival tissue mast cells of a cow, pig, mouse and rat, as in the case of those of man, many of them were observed to lie distributed chiefly along the small blood vessels of lamina propria mucosae and there were some of them observed in the regions composed of comparatively compact fibrous connective tissues where blood vessels were scarce. Their forms being different from in shape and some those of a normal healthy man, there were comparatively few which were round, but many were spindle shaped, prolate, or indefinite were observed segmented. They have a tendency of forming a projection, the characteristic of which was observed specially strong in the case of a pig. With toluidine blue solution these tissue mast cells showed a beautiful reddish purple, metachromatic color and with israbin solution, yellowish brown and their PAS reaction was less positive than that of the gingival tissue mast cells of man. Not all of the tissue mast cells of a rat and mouse were
positive, but many of them were found to be negative.

4. The gingival tissue, when viewed from the standpoint of histological structure, its lamina propria mucosae where there exist the tissue mast cells are composed of the loose connective tissue consisting of minute fibers, blood vessels, lymph vessels, and nervous tissues, especially, the blood vessels constituting the main part of them. The gingival tissue mast cells, too, in the same way as those of other internal organs, have a tendency of lying around the blood vessels, hence the integration of these tissue mast cells immediately is likely to impart the action of histamin upon the blood vessel system and in the opposite way, it is easy to suppose that those tissue mast cells themselves are likely to be influenced by the change of the blood components.

Consequently, the gingival tissue mast cells have an important signification since the reactions of these tissue mast cells in the patients of inflammation or some other morbid states, are regarded as a histological manifestation of the conditions and functions of histamin in the affected parts of body. Therefore, these studies of the cells will give a new direction in the future study of alveolar blennorrhea which shows chronic inflammation in the gingiva.

References

Studies on the Mast Cells in the Gingival Tissue

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Explanation of Figures

Fig. 1. The gingival tissue mast cells of a normal healthy man. Along the small blood vessels in the subcutaneous connective tissues only one of the tissue mast cells, which is round in shape dyed densely is observed.
(with 1/20 M israbin staining. ×150)

Fig. 2. The gingival tissue mast cells of the patient of alveolar blennorrhea. The blood vessels in the subcutaneous connective tissue has become corplent and enlarged and 5 of the tissue mast cells lie in a group around the blood vessels, their corpuscle and the abnormality of the shapes are recognized vacuoles in the cytoplasm are seen. The nuclei which lost their original shapes are enveloped with coarse granules, and the largeness and uneveness of the granules are conspicuous. Some granules delivered and dispersed
out of the cell bodies and some are observed to have been degraded.
(with 1/20 M israbin staining. ×1500)

Fig. 3. The gingival tissue mast cells of the patient of alveolar blennorrhea. The tissue mast cells being positive to the PAS reaction according to Mc Manus method, the whole of the protoplasm is stained reddish purple. The tissue mast cells around the blood vessels show conspicuously positive reaction.
(periodic acid Schiff's staining. ×600)

Fig. 4. The gingival tissue mast cells of a cow. The tissue mast cells are positive to the PAS reaction according to Mc Manus' method but not so strong as that of man. The whole of protoplasm is dyed reddish purple.
(with periodic acid Schiff's staining ×1500)

Fig. 5. The gingival tissue mast cells of a pig. Some granules pervade the cytoplasm are arranged evenly while others are comparatively few in number and arranged loosely and unevenly in the protoplasm. They are observed to lie in the mixture of the two types granules in this diagram.
(with toluidine blue staining. ×1500).

Fig. 6. The gingival tissue mast cells of a pig. They are weakly positive to the PAS reaction according to McManus' method. Their protoplasms are dyed pink.
(with periodic acid Schiff's staining ×1500)

Fig. 7. The gingival tissue mast cells of a rat. Along the small blood vessels of the subcutaneous connective tissue, the tissue mast cells which are spindle-shaped, prolate, or indefinite in shape are observed, and the tendency of forming one or two projections, also are recognized.
(with toluidine blue staining ×400)

Fig. 8. The gingival tissue mast cells of a mouse. The tissue mast cells which are located in the depth of the layer composing of comparatively compact fibrous connective tissues are chiefly indefinite in shape and some of them are observed simply segmented. The cells in the upper layer are larger than those in the deeper layer.
(with toluidine blue staining ×400)
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Plate II.