ON THE BIOLOGICAL FUNCTIONS OF TISSUE MAST CELLS IN THE SALIVARY GLAND

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The biological significance of the tissue mast cells has attracted much interest of many investigators, since *Ehrlich* in 1889 first described the cells, which occur in the connective tissues and tend to gather around the regions affected by chronic inflammation, congestion or tumor, and called them "tissue mast cells".

Jorpes, *Holmgren* and *Wilander* (1937) demonstrated that the granules of the tissue mast cells have properties of heparin. *Riley* and *West* (1953) further claim that they contain not only heparin but also histamine in a relatively high concentration. The fact that these cells contain granules of such chemical properties has been recognized as having a great significance in their biological functions.

Thus the biological signification of these tissue mast cells are gradually being clarified. Hence, such problems as: "What significance has heparin physiologically as an anti-coagulative agent?"; "What part does it play as an inhibitor to the growth of tumor?"; and "What physiological functions has it in producing the ground substance of the connective tissue or the hyaluronic acid, or in inhibiting the arteriosclerotic change of the wall of blood vessels?"; and the relationship between histamine in the tissue mast cells and inflammation have become to be investigated on a large scale.

There seems to have been, however, no written record referring to the biological relationship between saliva and the tissue mast cells. Therefore, the present writer, in order to know the biological functions of the tissue mast cells of the salivary gland, started investigations of testing the changes taken place upon the salivary functions according to varied ways of dieting, or when the two autonomic nervous systems are stimulated or checked and when histamine is applied since it is well-known fact that salivary glands are controlled by the autonomic nervous system which constitutes the sympathetic nervous system and parasympathetic one and it is now recognized somewhat biologically and pharmacologically that histamine acts more quickly upon the salivary glands, though not so much as it does upon gastric glands and pancreatic juice. Further, according to *Fujie* and others (1954, 1955) histamine can be decomposed in the saliva.

Therefore, the present writer performed the following experiments so as to know first how the tissue mast cells react when pilocarpine, a stimulant to par-

asympathetic system, adrenalin, a stimulant to sympathetic nervous system, histamine and atropine, depressants to parasympathetic system are applied respectively. Namely, when the functional activities of salivary glands are considered to have highly increased or decreased, the reactions of the tissue mast cells in the three large salivary glands, that is, parotid, submaxillary, and sublingual glands were examined so as to know the biological functions of these cells.

MATERIALS AND METHODS

Each of the rats full grown (about 100 g) as experimental animals was fed with an artificial diet according to *Mc Collum*'s method once a day at a regular time. Then one hour later, if any scraps of the feed remained, they were discarded. In this way, they were fed about a week. In consequence, the rats, as soon as the feed was thrown in, became to eat it all at once and without a break.

Before the experiment started, so as not to prey one another, each of them was segregated, and was fed with nothing but water for 48 hours to him fast, and then was used for the experiment.

Control: 5 rats which had been fed according to *Mc Collum*'s method of artificial dieting until the day of experiment.

The 1st group of experiment: 5 rats which had been fed with nothing but water for 48 hours.

The 2nd group of experiment: 5 rats which had been fed with rice-bran hardened with water.

The 3rd group of experiment: 5 rats which had been fed with vinegar diluted as twice by adding distilled water.

The 4th group of experiment: 5 rats of the 1st group of experiment were given hypodermic injection of 1% pilocarpine 0.3 cc. (3 mg), a stimulant to sympathetic nervous system.

The 5th group of experiment: 5 rats of the 1st group of experiment were given an injection of 0.1% adrenalin 0.1 cc. (0.1 mg) a stimulant to sympathetic nervous system, into the caudal vein.

The 6 th group of experiment: 5 rats of the 1st group of experiment were given a hypodermic injection of 1% atropine sulfate 0.1 cc, (1 mg), an anesthetic to parasympathetic system.

The 7th group of experiment: 5 rats of the 1st group of experiment were given a hypodermic injection of 1% histamine hydrochloride 0.3 cc. (3 mg).

As to the rats of the 2nd group of experiment which were given rice-bran, 30 minutes later when it was considered the time of hyperfunction: as to the rats of the 4th and 5th groups of experiments, when the effect of the stimulants given to them reached the climax respectively: namely, when the effect of pilocarpine caused the rats to flow tears and saliva highly and the effect of atropine caused

the secretion of their saliva to decrease and have a cough: and in regards to the others which had been applied other drugs, too, 30 minutes after their injections: all the rats were thus killed under chloroform their necks being cut off, and their submaxillary, parotid, and sublingual glands were removed. Then immediately they were fixed in Carnoy's solution and a solution composing of formalin 10cc., 95% alcohol 90cc., calcarea acetata 1g. Then they were treated according to rule. The materials fixed according to Carnoy's solution, were cut into paraffin sections (5μ) and stained in periodic acid *Schiff*'s reagent, and with the preparation fixed in the mixed solution of formalin, 95% alcohol, and calcarea acetata 1g, they were also cut into paraffin thin sections, and stained again in hematoxylin and eosin, and after having stained in the solution of 0.25% toluidine blue and 70% alcohol, they were imparted after staining in 95% alcohol in which 0.01% eosin was dissolved. Further, in order to make an accurate measurement in size, at every cases of the experiments the preparations were fixed in absolute alcohol, embedded in celloidin cut into 15μ sections, and then stained in toluidine blue solution above mentioned.

Control: Although the submaxillary and parotid glands of the rats are considered to be serous glands and the sublingual gland, the mixed glands, their main constituents are really mucilaginous cells. (*Eurston*, 1953) The serous cell recorded in literature shows a form like the basket cell in contrast to the glandular cells. The chief morphological changes taken place at the time when the feed was given or the nervous system were stimulated, were in the mucous cells. Close to the outer side of the basement membrane of the glandular cells, there were generally one or two of serous cells observed. These serous cells were semilunar and had an oval nucleus each.

As to the tissue mast cells, the density of their distribution was nearly the same in number in the submaxillary and parotid glands, averaging 3 to 10 in one field of vision in medium magnification ($\times$ 400) observed. Especially in the fibrous connective tissues and in the lymphatic glands around both glands there were many of them observed. In the submaxillary and parotid glands there were observed three or four in groups around the ducts and the blood vessels in the interlobular connective tissues and in the regions where the ducts and blood vessels were scarce there were one or two of them scattered and there were also some scattered observed close to the base of the striated tubule. In the lymphatic glands there were observed two or three chiefly around the marginal sinuses and the efferent vessels and sometimes in the medullary substance, too, some were observed.

The density of distribution of the tissue mast cells in the sublingual glands was 2 or 4 on the average in one field of vision and they were observed around the ducts and blood vessels in the interlobular connective tissue in the same way as in the parotid gland and submaxillary gland, though there were far less in num-

ber as compared with them both.

The size of these cells in the submaxillary and parotid glands varied from 6μ to 20μ or more and indefinite, but many of them were about 10μ and there was little difference in size between those in the interlobular connective tissues and those in the lymphatic node.

The size of the tissue mast cells in the sublingual gland was from 6μ to 15μ and indefinite, but many of them were about 11μ and somewhat larger than the tissue mast cells in the submaxillary and parotid glands. As to the forms, without distinction of either the sublingual gland, parotid gland, or sublingual gland, they were round, prolate, oval, spindle-shaped, indefinite form or a shape like that of Ranvier's clasmatocyte. However, those in the interlobular connective tissues and the fibrous connective tissues around the glands were prolate in shape. They were positive to the PAS reaction and were stained bluish purple, metachromatic color. The granules of the tissue mast cells were round in shape and gathered in minute forms of nearly equal size, arranged compactly in the protoplasms, and there were very few granules dispersed out of the cell-bodies, whereas most of them were not dispersed.

Most of the nuclei were dyed light color though there were some covered with the granules and their location indistinct. The nuclei were single and round, oval, spindle-shaped, or indefinite in shape and sometimes prolate, too, generally corresponding with their cells in shape, and their location was generally in the central part, that is, centric, but some of them were found deviated from the center, that is, eccentric. The chromatin of the nuclei was observed clear. Some of the tissue mast cells were positive to periodic acid *Schiff*'s test (PAS) after *Mc Manus*' method, but others were negative. Even the cells positive had comparatively variety of staining, namely, the cytoplasms were dyed reddish purple, and with the oil-immersed equipment the granules of some were distinct and the cytoplasms of others were dyed pale pink and they looked like vacuoles. Generally speaking, however, those located around the blood vessels were scarce, and had a tendency of staining pink.

THE FIRST GROUP OF EXPERIMENT

The glandular cells of the parotid gland were comparatively bright and around the ducts there were plenty of ground substance of connective tissues and in the glandular cavity a formless substance was observed. The substances positive to periodic acid *Schiff*'s test after *Mc Manus*' method were found comparatively many clustering in the center in minute granulation. The cavities of the secretory ducts were narrow and many of them were empty. The ducts in the sublingual gland contained a little quantity of substances which were positive to the PAS reaction.

The density of distribution of the tissue mast cells in the submaxillary and parotid glands was nearly the same in number on the average from 3 to 7 in a field of vision in medium magnification (× 400), usually from 2 to 4 located in groups in the ducts in the interlobular connective tissues, and in the connective tissue around the glands and some were found to be less than 1 in number.

In the sublingual gland the density of distribution of the tissue mast cells was from 1 to 2 in one field of vision in medium magnification (× 400) and in the excretory ducts of the interlobular connective tissues or in the connective tissues around the blood vessels mast cells were in the same way as in the submaxillary gland and parotid gland. In either of the submaxillary gland, parotid gland, or sublingual gland, the density of distribution of the mast cells was less than that in the control. In lymphatic glands only one or two were observed around the marginal sinuses and the efferent vessels and there was no one in the medullary substance. As to the size of the mast cells, it was nearly the same in the submaxillary gland and parotid gland, that is, from 8 to 12 and there were many which were about 9.

In the sublingual gland it was about 11. None of them had a tendency of having a projection. Their shapes were round or spindle-shaped and none of them were indefinite or prolate in shape. The contours of the tissue mast cells were clear and they were positive to the PAS reaction and dyed bluish purple, metachromatic color with toluidine blue staining. All the granules of the tissue mast cells in any one of the salivary gland were round in shape and clustering in minute forms of equal size, they were arranged densely in the cytoplasm. On account of that, the whole cells looked as if they had been tightened. There were none of them dispersed out of the cell bodies, as most of the nuclei were covered with granules. Generally speaking, many of them were not distinct and a few of them were observed in colored light. The nuclei clearly seen were single and round or spindle-shaped and their shapes generally accorded with those of the cells, as it is so in the control. The site of most of the nuclei were centric and there seemed to be only a few which were eccentric. The chromatin in the nuclei was indistinct, probably because they were covered with the granules. Most of them were negative to periodic acid *Schiff*'s test (PAS) after *Mc Manus*' method and there were only a few which proved positive. The cytoplasms were stained reddish purple and the granules were observed clearly with an oil-immersion equipment.

The 2 nd group of Experiment

The size of the glandular cells in the submaxillary glands enlarged as compared with that of the 1 st group of experiment and interlobular connective tissues of the gland were widened. The remarkable changes taken place in this group

of experiment were the presence of vacuoles in the protoplasm of the glandular cells. The substances positive to the PAS reaction when compared with the 1 st group of the experiment made some difference and were dyed permeably, but here and there there were some which were unevenly distributed. The ducts were enlarged and within these ducts there appeared a great quantity of formless substances proved positive to the PAS reaction and of secretory granules.

The glandular cells in the parotid gland were found enlarged. In the protoplasm in the glandular cell there were observed distinctly to have appeared vacuoles of various sizes, large or small. In the protoplasm in the center of glandular cells here and there were observed some small bacillary or small globular substances which were positive to the PAS reaction.

The cavities of striated tubule were enlarged and in the blood vessels the formless substances were somewhat increased and close to the ducts there appeared many of minute granules positive to the PAS reaction, none of which were observed in the 1 st group of experiment.

The size of the glandular cells in the sublingual gland was enlarged. The interlobular connective tissues were somewhat narrowed. The substance in the glandular cells positive to the PAS reaction increased. At the inside of the striated tubule a small quantity of substance positive to the PAS reaction observed.

As to the density of distribution of the tissue mast cells between the maxillary gland and the parotid gland there was little difference and nearly the same in number, generally from 3 to 5 scattered in the interlobular connective tissue where striated tubule were scarce. There were some cells located very close to the basal part of the striated tubule, but there were more in number than in the control. This is a phenomenon which could not be seen in the 1 st group of experiment.

In the fibrous connective tissue and in the lymphatic gland around the maxillary and parotid glands there were mostly around marginal sinuses and the efferent ducts, and sometimes in the medullary substance, too, there existed here and there. Namely, wherever there were connective tissues in both glands, the tissue mast cells were observed in the same way as in the control, but the number was larger than the latter.

In the sublingual gland, most of them were observed in the circumferential connective tissues and around the ducts only 1 or 2 observed. The size of the tissue mast cells in the maxillary and carotid glands were from 6 to 22 μ or more, the differences of the sizes being conspicuous compared with the control, but generally speaking, there were many which were from about 10 to 12 μ and were a little more corpulent than the control. The mast cells in the sublingual glands were nearly the same in size. They were about 11 μ. This phenomenon is different from the control, but it is quite similar to that of the 1 st group of the experiment.

Generally, those in the interlobular connective tissue are smaller than those in the fibrous connective tissues around the glands, but the fact is not so in the control.

Their forms were round, oval, spindle-shaped indefinite or similar to these of Ranvier's clasmacyte. They exist, mixing together. They tended to have a projection just like the control, but they seemed more likely than the latter. Further, the tissue mast cells located around the ducts and blood vessels and close the base of the striated tubule were spindleshaped and those in the interlobular connective tissues were generally prolate just like in the control and the images of direct fissions that were seen here a phenomenon which could not be found either in the control or in the 1 st group of the experiment. They were positive to the PAS reaction and dyed bluish purple, metachromatic color with toluidin blue. The granules of the tissue mast cells in the connective tissues around the blood vessels were round and gathered together in minute forms of nearly equal size, closely arranged in the protoplasms, but those in the fibrous connective tissues around the gland and ducts were round, somewhat large, and not of equal size and were arranged loosely in the protoplasms. Hence, though the contours of the cells were rather distinct, many of those which were located near the ducts had their granules dispersed out of the cell-bodies. Those located in the connective tissues surrounding the blood vessels had none of their granules dispersed out of the cellbodies. The nuclei being covered with the granules were not recognizable, but most of them were generally dyed metachromatic color. The nuclei were single, their shapes corresponding with those of the cells. Most of them were eccentric, but some of them were centric. The chromatin of the nuclei were generally observed clear.

About two thirds of the cells proved positive to periodic acid *Schiff*'s test (PAS reaction) according to *Mc Manus*' method. Those located in the connective tissues around the blood vessels were dyed reddish purple and strongly positive to the PAS reaction, but those in the other connective tissues and lymphatic glands were weak positive. Most of those which were strongly positive with oil immersion equipment were observed to contain the granules. And there were some whose protoplasms were dyed pale pink and the granules therein, looking like bubbles observed here and there.

THE 3RD GROUP OF THE EXPERIMENT

The glandular cells in the submaxillary glands became more enlarged than those in the 1 st group of the experiment and nearly as large as the control. In the protoplasm there were minute granules observed. Further, the forming of vacuoles, too, was recognized. The forming of vacuoles, comparing with the control, was rather less than the control. In the glandular cells the substances which were positive to the PAS reaction were distributed permeaby in the proto plasms

of the glandular cells, but a little less than that of the control.

The size of the glandular cells in the parotid gland was comparing with the 1st group of the experiment enlarged somewhat as in the case of the control. Some granules in the center of the glandular cells and in the cytoplasms around the nuclei were observed positive to the PAS reaction, though in less degrees than in the contrast.

In the striated tubule, the enlargement of the cavity was somewhat noticeable and at the inside of the striated tubule there were comparatively many of minute granules positive to the PAS reaction observed.

The enlargement of the glandular cells in the sublingual gland was not much, but the substances in the cytoplasms of glandular cells showing positive to the PAS reaction, comparing with those in the 1 st group of the experiment, increased.

The serous cells showed nearly the same change as in the control. The margin of the cavity of duct was dyed positive color to the PAS reaction whereas the duct contains formless substance positive to the PAS reaction.

As for the tissue mast cells, the density of their distribution in the submaxillary gland and parotid gland, there was little difference and nearly the same in number. That is from 6 to 31 on the average in one field of vision in medium magnification ($\times$ 400). Around the excretory ducts, above all, the mast cells from 7 to 14 clustered and in the fibrous connective tissues around both the glands and in the interlobular connective tissues these were scattered everywhere more strongly than those in the 2 nd group of the experiment. This phenomenon was specially noticeable. Some of the mast cells which exist close to the base of the striated tubule of the cells were more than either in the control or those in the 2nd group of the experiment.

In the lymphatic glands those found around the marginal sinuses and the excretory ducts were similar to those of the above mentioned experiment, but were much more, that is, from 5 to 7, especially in the medullary substance 2 or 3 of them were observed here and there. The density of distribution of the tissue mast cells was from 2 to 4 on the average and similar to that of the control. The sizes of the mast cells in the submaxillary and parotid glands varied from 8 to 20 μ and indefinite. But, generally speaking, there were many which were 13 on the average, enlarged more than the control or the 1 st group of the experiment and slightly more than the 2nd group of the experiment.

As to those in the sublingual glands, their sizes were nearly definite and there were many of them which were 11 or so just like those in the control, the 1 st group and 2nd group of the experiment mentioned above.

The forms of the tissue mast cells in both the submaxillary and parotid glands were prolate or indefinite like the shape of Ravier's clasmacyte. The prolate mast cells being constricted halfway or lobulated showed clearly the direct cellular

fission. There were some which tended to have one or two projections just like those in the 2nd group of the experiment.

The tissue mast cells in the sublingal glands were round or spindle-shaped. They were positive to the PAS reaction and there were not many which dyed nearly blue, orthochromatic color whereas most of them were dyed a beautiful metachromatic color. The granules of these mast cells were generally round in shape and rather large, but not equal size and were loosely arranged. Pretty of the granules were found dispersed but of the cell bodies. Hence the contours of the cells can be said to have been indistinct. Their nuclei were clearly recognized as they were dyed light metachromatic color. The nuclei were single and their shapes corresponded with those of the cells. Most of them were eccentric whereas a few of them were centric. The chromatin of the nuclei was clear.

Few of the tissue mast cells were found positive to periodic acid schiff's test (PAS) according to Mc Manus' method. Only a few of them around the blood vessels were dyed reddish purple. With the oil-immersion equipment any granules could not be observed in most of the cells. Only the protoplasms were stained light pink and their granules looking like bubbles that were observed here and there.

The 4th Group of the Experiment.

The sizes of the glandular cells in the submaxillary glands were nearly the same as those in the 2nd group of the experiment. In the protoplasms of the glandular cells minute granules could be recognized clearly, and the forming of vacuoles was the most distinct as compared with the groups of the experiments aboved mentioned. The substances positive to the PAS reaction exist permeably in the protoplams of the glandular cells, and there were some which were dyed locally. The images of secretion in the cells of the striated tubule were similar to those in the 1 st group of the experiment and very weak. In the secretory ducts grey brown secretion was observed. In the parotid gland the enlargement of the glandular cells was conspicuous and every glandular cells, too, according to its location, or its individual cell, showed various colorful coloration. The granules positive to the PAS reaction were observed clear in the center of the glandular cells or around the nuclei. Around the excretory duct in the cell of the striated tubule a few clusterings of minute granules were observed here and there. In the excretory ducts there was a lot of formless substance which was weakly positive to the PAS reaction.

The size of the glandular cells in the sublingual gland was smaller than that in the lst group of the experiment, and the interlobular connective tissue of the gland was very plentiful. The degree of positiveness to the PAS reaction makes little difference between that in the lst group of the experiment.

In regard to the tissue mast cells, the density of their distribution was nearly the same in the submaxillary and parotid glands from 12 to 38 on the average in ono field of vision in medium magnification (× 400), increasing in number than the contrast or those in the above mentioned experiments. They were chiefly scattered in the circumferential fibrous connective tissues and in the circumferential lymphatic glands of both glands, and especially had a tendency of gathering in groups from 6 to 8 around the blood vessels, and everywhere in the interlobular connective tissues in the submaxillary and parotid glands the tissue mast cells exist just like those in the 3rd group of the experiment. The density of distribution of the tissue mast cells in the sublingual glands was 2 or 3 which is nearly the same with that of these experiments above mentioned. The sizes of some cells in the submaxillary and parotid glands varied from 6 to 25μ or more, but there were many which were some 13μ, and the tissue mast cells in the connective tissues tended to enlarge somewhat more than those in the lymphatic glands. On the whole, they were observed to have more enlarged than those in the above experiments. In the sublingual glands there were many whose sizes were about 11μ and smaller than those in the submaxillary and parotid glands. As to the sizes of the mast cells, there were generally few which were round and spindle-shaped whereas many of them were prolate, indefinite or the shape of Ranvier's clasmatocyte. They had a tendency of having a projection just like those in the 3rd group of the experiment. They were positive to the PAS reaction and dyed bluish purple metachromatic color. Those in the neighborhood of the blood vessels and excretory ducts, howeser, were dyed rather inclining to dark blue orthochromatic color, and these in the connective tissues around the glands were dyed a beautiful metachromatic color. The contour of the cells was pretty clear. The granules of the tissue mast cells around the blood vessels and the excretory ducts were round in shape and gathered together in very minute forms of equal size, arranged thickly in the protoplasms while many of coase granules in the circumferential fibrous connective tissues were irregularly arranged in the protoplasms. There were some whose granules were dispersed out of the cell bodies. Some of the nuclei were covered with the granules, so their location was not clear, but most of the nuclei were dyed metachromatic color. The nuclei were single and their forms corresponded with those of the cells. As for their location, some were a mixture of centric and eccentric, but most of them were eccentric. The chromatin of the nuclei was indistinct. There were some which proved negative to periodic acid Schiff's test (PAS) according to Mc Manus' method, but the others around the excretory ducts and blood vessels showed positive to it. The cytoplasms were stained reddish purple and the granules could be seen somewhat clearly with the oil-immersion equipment. While the others positive to the PAS reaction had only, their cytoplasms stained pink.

THE 5TH GROUP OF THE EXPERIMENT

The size of the glandular cells in the submaxillary glands was similar to that in the 2nd group of experiment. The forming of the vacuoles in the glandular cells was recognized, but more slightly than that in the 2nd group of experiment. In the cytoplasms of the glandular cells the substances positive to the PAS reaction were recognized chiefly in the circumscribed upper part of the nuclei of the glandular cells. The marked change taken place in the tissue mast cells was found on the cells of striated tubule, and in the cytoplasms the secretory granules increased. The location of the nucleus in the protoplasm of the cells of striated tubule was not regular, some being in the upper part of the cell, some in the center, and others were located in the basal part of the cell.

The enlargement of the glandular cells in the parotid glands was not conspicuous. The protoplasms of the cells were of reticular structure and the forming of vacuoles was not conspicuous. The striated structure in the striated tubule was observed distinctly. The glandular cells in the sublingual glands enlarged distinctly, far more than those in the experiments mentioned above.

The basophil substance in the protoplasms of the glandular cells remarkably increased. The positiveness to the PAS reaction was distinct. The serous glandular cells flattened were observed distinctly. The excretory ducts contained a great quantity of formless substance positive to the PAS reaction.

As to the tissue mast cells, the density of their distribution was a little more than that in the control from 3 to 8, but in the parotid glands they were nearly the same in number as those in the control. In the sublingual glands there were the most of all the groups of the above experiment, that is, from 7 to 15. The state of distribution of the tissue mast cells in the submaxillary glands and parotid glands was nearly the same as that in the control. In the sublingual glands, however, they were dispersed the circumferential fibrous connective tissues and the circumferential lymphatic glands, and the fact that they exist in groups from 5 to 10 was a phenomenon that could not be seen in the above mentioned experiment.

The sizes of the mast cells in the submaxillary and parotid glands were irregular from 7 to 22μ, but most of them were about 11 μ, generally nearly the same as that in the control, but smaller than that in the 4th group of experiment. In the sublingual gland their sizes were from 8 to 11 μ, and most of them were generally about 12μ and englarged. The tissue mast cells in the lymphatic gland were smaller than those in the connective tissues. Their forms in the submaxillary and parotid glands were chiefly round or spindle-shaped, but sometimes there were a few of them which were prolate. In the sublingual gland there were a mixture of one type of the tissue mast cells which were round or spindle shaped and the others which were prolate or indefinite in shape, tending to have

a projection. They were positive to the PAS reaction and with toluidin blue staining the tissue mast cells in the submaxillary and parotid glands showed the same color as those in the control, but in the sublingual gland the tissue mast cell granules were round in shape and were a mixture of two types. One type gathered in minute forms of equal size and were thickly arranged in the protoplasms and the other type which comprised of forms of unequal sizes were loosely arranged in the protoplasms. The former type showed deep blue colour inclining to orthochromatic colour whereas the latter showed a beautiful metachromatic colour.

There were a considerable amount of whose granules were developed and dispersed out of the cell bodies observed. The nuclei were single and their forms nearly corresponded with these of the cells just as in the above experiments. As to their location, in the submaxillary and parotid glands many of them were centric whereas in the sublingual gland many of them were eccentric. The chromatin of the nuclei was indistinct.

The tissue mast cells in the submaxillary and parotid glands mostly proved negative to periodic acid *Schiff*'s test (PAS) according to Mc Manus' method, but in the sublingual gland the tissue mast cells were a mixture of those of positive and negative. Their protoplasms were dyed reddish purple and with the oilimmersion equipment their granules were distinctly observed. The cytoplasms were stained pale pink. Granules which looked like bubbles were observed scattered.

THE 6TH GROUP OF EXPERIMENT

The interlobular connective tissue of the glandular cells in the submaxillary gland was narrowed. A comparatively large quantity of the substance, more positive to the PAS reaction than those in the 1st group of experiment, clustering in minute granulation in the protoplasms in the glandular cells was observed. The ducts of the excretory ducts were narrower and empty. The glandular cells appeared comparatively light and the connective tissues around the excretory ducts were somewhat plentiful. In the excretory ducts in the sublingual glands the substance positive to the PAS reaction was few.

As to the tissue mast cells, the density of their distribution in the submaxillary and parotid glands was nearly the same in number, from 3 to 8 on the average in one field of vision in medium magnification ($\times$ 400), which was less than the control. Most of them exist in the fibrous connective tissues around both glands and in the circumferential lymphatic glands, but they were less in quantity than those in the 4th group of experiment. The tissue mast cells mostly exist in groups from 3 to 6 in the connective tissues around the excretory ducts and blood vessels. Whereas in the interlobular connective tissues apart from the ducts or the blood vessels they were scattered in groups of 2 to 4 only. There were some which existed close to the basal part of the cells of the striated tubule. In the fibrous

connective tissues and lymphatic glands around the submaxillary and parotid glands they were mostly found dispersed around the marginal sinuses and the efferent vessels and sometimes in the medullary substances. In the sublingual glands they were found chiefly in the circumferential connective tissues and there were only 2 or 3 found around the excretory ducts. The sizes of the tissue mast cells in the submaxillary and parotid glands were from 6 to 12μ or more and their irregularity was pretty conspicuous comparing with the control, but generally speaking, many of them were about 9μ. On the whole, those in the interlobular connective tissues were larger than those around the glands. Those in the sublingual glands were nearly the same sizes; many of them were about from 9 to 15μ and above all those of 9μ or so far more. As to the tissue mast cells in the submaxillary and parotid glands, their forms were round, oval, spindle-shaped, or prolate and had no tendency of having a projection. The cell bodies gave us as much impression of compactness as those in the 1 st group of experiment. There were none which were prolate, lobed or of images of direct fission observed. In the sublingual glands the forms of the mast cells were mostly round, oval or spindleshaped, having no tendency to have a projection or images of direct fissions, which phenomena were very similar to those of the above experiments but the 5th group of experiment. They were positive to the PAS reaction and dyed bluish purple metachromatic color. The tissue mast cell granules were mostly round, clustering in minute forms of equal size and were thickly arranged in the protoplasms. There were some granules, very few though, which were round and somewhat larger forms of unequal size loosely arranged in the protoplasms. There were very few granule though, which existed in the protoplasms. Around the blood vessels and the excretory ducts granules of these three types were observed, but most of the mast cells in the circumferential connective tissues were round in shape, having minute granules compactly arranged in the protoplasm.

The minute granules were more minute than those of either the 1 st group or the 4th group of experiment. Further some granules were sometimes observed to have stuck to the nuclei. The granules attached to the nuclei were loose and coarse ones, which were dispersed out of the cell bodies. The contours of the cells were pretty clear, but many of those close to the excretory ducts were dispersed, but in the fibrous connective tissues around the blood vessels and the glands none of the tissue mast cells were observed whose granules have dispersed out of the cell bodies. Though there were some nuclei which, being covered with granules, could not be recognized, most of them were dyed metachromatic colour. The nuclei were single and their forms corresponded with those of the cells. Most of them were centric, though some of them eccentric. The chromatin of the nuclei was generally clear. Some of the tissue mast cells showed positive to periodic acid *Schiff*'s test (PAS) according to Mc Manus' method while others negative. These located around the excretory ducts and blood vessels were dyed reddish

purple whereas those in the connective and the lymphatic glands were dyed purple. With the oil-immersion equipment most of the granules could be identified. Further there were some cells observed scattering whose protoplasms were dyed pale pink and their granules looked like bubbles.

THE 7TH GROUP OF EXPERIMENT

The blood vessels in all the submaxillary, parotid, and sublingual glands without exception enlarged and were congested. The excretory ducts in the sublingual glands were full of substances whereas those ducts in the parotid glands had their excretory ducts narrowed and many of them empty, which was of nearly the same as in the 1st group of experiment. In the sublingual glands the glandular ducts containd substances positive to the PAS reaction.

As to the tissue mast cells, in the submaxillary and parotid glands the density of their distribution was observed to be from 5 to 13 on the average in one field of vision in medium magnification (×400), which was a little more than the control. In the sublingual glands the density of their distribution was found to be from 5 to 7 on the average in one field of vision in medium magnification, which means an increase in number more than the control, but less in number than that in the 5th group of experiment. The tissue mast cells in the sublingual gland, when compared with those in the submaxillary and parotid glands, were observed to have increased in number just as in the 5th group of experiment. In this experiment it was specially noticeable that there were much more of the cells observed in the connective tissues around the glands than in the circumferential connective tissues of the excretory ducts and blood vessels, but there were scarcely seen in the lymphatic glands. On the other hand, in the interlobular connective tissues, comparing with those in the other experiments there were many of the cells observed and some times there were in the medullary substance, too.

The sizes of the tissue mast cells in the submaxillary and parotid glands were from 7 μ to 25 μ or so and irregular, but most of them were about 14 μ. In the sublingual gland their size was from 6 to 12 and irregular, too, but most of them were about 11 μ.

Their forms were various round, oval, spindle-shaped, prolate or something like the shape of Ranvier's clasmatocyte, etc., those in the interlobular connective tissues and the connective tissues around the glands were mostly prolate, corresponding with the long axes in the connective tissues. They were positive to the PAS reaction dyed bluish purple metachromatic colour with toluidine blue staining. The granules of these cells, which were round and somewhat larger were loosely arranged in the protoplasms. There were some of the cells which had their granules dispersed out of the cell-bodies and others only very few of whose granules

remained in the protoplasms. The nuclei were single and round, oval, indefinite in shape, spindle-shaped, and sometimes prolate in shape, generally corresponding with the forms of the cells, just like the other experiments. As to the site of the nuclei, there were very few which were centric, but most of them eccentric. The chromatin of the nuclei was generally observed clear.

As to the reaction to periodic acid *Schiff*'s test (PAS) according to *Mc Manus*' method, there were some of them positive and others negative, but the ratio of the mast cells showing positive reaction was about two thirds of them. Further those positive, too, were comparatively full of variety in staining. Namely, their protoplasms were dyed reddish purple and with the oil-immersion equipment there were some whose granules were observed clearly and others whose protoplasms were dyed pale pink and their granules looked like bubbles. Generally speaking, those located around the blood vessels were dyed reddish purple and their granules could be recognized whereas those in the connective tissues where the blood vessels were scarce were observed tending to be stained pink.

Discussion

When we consider from the results of the above experiments, we understand that according to the changes of the functions of the salivery gland, tissue mast cells give rise to changes not only upon the number, the state of their distribution, and their forms, but also upon their functions. Especially when the salivatory functions were in top condition as in the case of the 2nd group of experiment and when the salivatory functions were on the wane as in the case of the 1st group of experiment or when the parasympathetic system and sympathetic nervous system were stimulated and when the sympathetic nervous system was suppressed a great difference was observed to have been made between them. Namely, in the experiments where the salivatory functions decreased such as the 1st group of experiment and the 6th group of experiment, the contours of the cells were clear and the cells themselves showed somewhat tightened appearance. Their forms were round or spindleshaped and those in the interlobular connective tissues and the circumferential connective tissues of the glands were prolate in shape and none of the indefinite shapes were observed. Further, the granules in any of the mast cells were round and small forms of equal size, filling the protoplasms were evenly arranged therein. The granules were eccentric and neither attached to the nuclei nor dispersed out of the cell-bodies, most of the nclei were covered with the granules so that their presence was indistinct, but the chromatin of the clear nuclei of the tissue mast cells was distinct. None of the nuclei nor of their chromatin showed any metachromasia with toluidine blue solution.

In the experiments where the salivery functional activities increased such as the 2nd group and the 3rd group of experiments; in the 4th group of experiment

where the parasympathetic system was stimulated; in the 5th group of experiment (in the sublingual glands) where the sympathetic nervous system was stimulated; in the 1st group and the 6th group of experiments where the salivery functional activities were waning; the fact that the state of the distribution, the quantity of the tissue mast cells and the state of their granules, comparing with those in the above described experiments, changed is quite clear. Namely, when the salivery function greatly increased, the tissue mast cells chiefly existing around the ducts and blood vessels in the interlobular connective tissues had a tendency of letting themselves free wandering and dispersing in the connective tissues for distant, and the tissue mast cells in the fibrous connective tissues around the glands do not gather in clusters, either, but were observed to begin to wander evenly.
And the granules of the tissue mast cells became unequal in size, tending to diffuse and to attach to the nuclei, and the granules began to disperse put of the cell bodies. The mast cells having irregular forms containd nuclei which had a tendency of being eccentric. *Ito and Kubota* (1944) opined that the indefiniteness of the site of nuclei is due to the amebaism of these cells. The phenomenon that the nuclei and nucleus chromatin began to show a metachromatic color was observed.

Between the 4th group of experiment and the 6th group of experiment there took place changes similar to those taken place between the 1st group of experiment and the 2nd group of experiment as a result of their different dieting, and in the 7th group of experiment there took place nearly the same change as in the 3rd group of experiment. Namely the reactions of the tissue mast cells in the 4th group of experiment were similar to those in the 2nd group of experiment and those in the 6th group of experiment were just similar to those in the 1st group of experiment, but the difference between the reactions of those cells in the 4th group of experiment and those in the 6th group of experiment was more conspicuous than the difference between those in the 2nd group and the 1st group of experiments, as a result of the different dieting.

As the result of the above mentioned experiments, it was found that tissue mast cells of salvery gland make various changes according to some conditions which control the salivatory functions. Hence, the present writer oppose to *Maxinmow*'s opinion (1906) that the irregularity of forms of the tissue mast cells and the unequal sizes of their granules are artificial products, and *Lehner*'s opinion (1924) and *Tsuda*'s opinion (1923) that the dispersion of the granules out of the cell-bodies are artificial and mechanical products, and *Michels*'s opinion (1938) that they are artificial products of microtome's knife and of their sudden fixation.
And what these opinions are wrong were proved clearly by the experiments of *Takeda* (1958) on the reactions of the tissue mast cells on various fixatives. These phenomena therefore, are to be interpreted as biological reactions in a broad sense.

The phenomena of the physiological changes of the tissue mast cells and the changes in the state of their distribution, forms, number and functions taken place

on account of various stimulations can be indorsed by the following scholars' experiments, that is *Camble*'s observation (1952) in injecting toluidine blue into a rat, *Drennan*'s observation (1952) in injecting ovmucoid in the case of urticaria pigmentosa, *Cavallero* and *Braccini*'s (1952), *Asboe Hansen*'s (1952), *Bloom*'s (1942), and *Stuart*'s (1951) cortisone experiments on the tissue mast cells, *Stuart*'s (1951) anaphylaxis experiment by means of egg-albumen, *Cavallero* and *Braccini*'s and *Asboe-Hansen*'s observations of the decreasing of hyaluronic acid, *Padwer* and *Gordon*'s (1956) observation of thyroid hormone, *Mota* and *Vugman*'s (1955) studies on compound 48/80, *Baetzly* and others' (1949), *Avoy*'s (1955) oestrogen experiments.

The above mentioned experiments indorse the present writer's opinion that the changes of the granules themselves indicate the changes of the physiological functional conditions of the tissue mast cells themselves.

When the changes of the granules of the tissue mast cells are understood to point to the changes of their physiological functions, it is very interesting to notice *Starmmler* (1921) and *Arnold*'s (1914) opinions that the tissue mast cell is a kind of organ which is single-celled and has a glandular property, because the glandular organ changes the forms of the cells and the property of the secretion according to the conditions of the secretory functions.

Well, if the condition were the secretory function of the salivary gland highly increased is considered as the condition of its activity, the tissue mast cell during this period can be said to be in the condition of its activity.

If the period when the secretory function receded is considered, as the conditions of standstill the tissue mast cell during this period can be said in the condition of standstill. Namely, in the condition of standstill of the function of the tissue mast cells, their contours were clear and minute granules of equal size filling the protoplasms were evenly arranged. None of them were dispersed out of the cell-bodies nor attached to their nuclei and the nuclei and their chromatins were dyed orthochromatic color with toluidine blue. On the other hand, in the condition of their functional activity, the contours of the tissue mast cells were indistinct and somewhat larger glanules of unequal size were loosely arranged, the granules sticking to the nuclei or being dispersed out of the cell-bodies and the nuclei became to show metachromatic color with toluidine blue. Namely the tissue mast cells of salivary gland change from static juvenile cells to active ones and have mutual relation with the secretory function of the salivery gland.

Riley and *West* (1953) and *Takeda* (1958) stated that juvenile tissue mast cells which were generated out of the adventitia are rendered active in proportion as they move toward the circumferential connective tissues. This statement as well as what the present writer researched above must be no other than the manifestation of the biological function of the tissue mast cells.

Here is a subject worth consideration. Although *Baudnits* (1912) and *Harris* (1900) and others opined that the tissue mast cells in the condition of their activity

are in the state of mucous degeneration. It is a question whether their opinion is appropriate or not. The tissue mast cells in the condition of activity might be interpreted to be in the process of degeneration in a broad sense, but there are some of the cells which are in the similar process of degeneration just as in the other kinds of cells. That is to say, the nuclei are in the process of pyknosis or karyolysis, and their contours become indistinct, the arrangement of the granules becomes irregular, the difference of the stainability is conspicuous and the difference of their sizes, too, is remarkable. Sometimes clasmatosis taken place, reducing to minute powder, some others develop and enlarge excessively till they are dessolved. Hence, the tissue mast cells in the state of activity might be interpreted to be in the state of degeneration in a broad sense, but it seems improper to the writer to understand them as in the state of degeneration in its original sense. Concerning with this subject, *Miura* (1932), as a result of his dieting experiment and the experiments of atropine and pilocarpine injectiors into the tissue mast cells of duodenum, expressed nearly the same opinion.

On the other hand, considering of the fact that while there are the tissue mast cells in the state of activity, there are others in the state of standstill, we can hardly agree with *Raudnits* (1921) and *Harris* (1900) when they say that the whole tissue mast cells are cells in the process of degeneration. Thus it is understood that the tissue mast cells of the salivery gland, according to the increasing or decreasing of the functional activities of salivery gland or in other words, according to the quantity of salivation, give rise to some changes in the density of their distribution, number, forms, and functions. Whereas, the cell, according to the different dieting, stimulation or control upon the autonomic nerve system, not only greatly increases or decreases its secretion, but also makes some changes in its chemical composition, which was proved by *Bazter* (1933) by means of the experiment upon dog and by *Yoshimura* (1954) by means of his experiment upon man. Pilocarpine and histamine accerate the serous secretion whereas atropine decreases it and adrenalin accerates the mucous secretion.

The salivery gland tissue mast cells also make differences in chemical composition according to the conditions of the functions of the salivery gland as it is proved by the changes of the PAS staining according to *Mc Manus*' method.

It is a matter of common knowledge that the PAS staining is used to detect mucous polysaccharide. Hence the phenomenon that there exist plenty of the substances positive to the PAS reaction shows that there are plenty of mucous polysaccharide in the cells. Now let the salivery gland be summarized that the granules positive to the PAS reaction in any groups of the above experiments appear more numerous than those in the 1 st group. The extent of their appearing is different as a matter of course according to the respective groups of the experiments or to the kinds of the salivery glands. The differences in the submaxillary and parotid glands, however, are generally common in their reactions,

but in the sublingual gland diametrically opposite (*Matsubara*, 1957).

In the writer's experiment, too, in the submaxillary and parotid glands the appearance of those granules was conspicuous in the 2nd, the 3rd, and the 4th groups of the experimants, and in the 5th group, their presence was not conspicuous. On the other hand, in the sublingual gland their presence was distinct in the 5th group of the experiment whereas in the other groups it was indistinct.

Now as to the tissue mast cells, their granules after being periodic acetic-acidified show positive to periodic acid *Schiff*'s test as it was already stated by Jorpes, *Aberg* (1948) and *Compton* (1952). It means that in the tissue mast cell granules 1 or 2 glycols and α-amino alcohol exist in some forms. This meaning of the tissue mast cells was indorsed by *Jorpes*, *Holmgren* and *Wilander*'s (1937) experiments. They separated heparin from the wall of sheep's aorta where there are plenty of the tissue mast cells, and *Oliver*, *Blocm* and *Mangier* (1947) also separated heparin of highly possible unit from the tissue mast cell tumor, making clear the relationship between heparin and the tissue mast cells and opined that the granules of the tissue mast cells contain heparin. *Howell* and *Hoet* (1919), after separating a new anti-blood coagulating factor from the liver of a sheep, called it "heparin". Since then there have been various studies on the structure of heparin. Namely heparin which has properties of polysaccharide (*Chareles* and *Scott*, 1933) is said to be a mixture of die- or tri-sulfuric acid esters and as a result of the chemical analysis of its Ba. salt, it was found as a polysaccharide containing glucosamine, and its sulphuric acid esters are easily separable. The less it contains sulphuric acid radicals, the sooner they will be separated. This phenomenon can be plainly proved by periodic acid Schiff's test, too. That is, when it contains plenty of mono-sulfuric acid esters, it assumes positive to Schiff's test. (*Jorpes* and *Cardell*, 1948) and the less it contains sulfuric acid radicals, the more the consumption of periodic acid will be. For instance, mono-sulfuric acid esters in the chemical analysis obtained 2 of OH whereas die- or tri-sulfuric esters did 3 or 4 of OH. Hence if heparin contains plenty of monosulphuric acid ester, it could be proved histologically (*Jorpes* and *Aberg*, 1948).

In the present experiment, too, the tissue mast cells plainly showed positive to *Schiff*'s test, but their aspects varied according to their experiments. The reason why they showed positive to *Schiff*'s test considered to contain plenty of mono-sulfuric acid esters in their granules. When the granules are considered to be the mixture of esters of unequal quantity, i.e. of mono-, die-, tri-sulfuric acid ester, the ratio of three esters, as *Kubota* and *Inoue* (1954) stated, different according to the kinds of the animals and even among the same kinds of animals the tissue mast cells are different in their own ways, some of them show strongly positive to *Schiff*'s test while others do negative to the test. That is what the tissue mast cells show positive to *Schiff*'s test means that their granules contain heparin of mono-sulfuric acid whereas what the cells show negative means that their granules

contain plenty of die- or tri-sulfuric acid. Further, what their stainability is varied is considered to mean that they contain migrating types of mono-, die-, tri-sulfuric acid.

Hereupon, what the tissue mast cells around the blood vessels and the excretory ducts in the interlobular connective tissues show mostly positive to periodic acid-*Schiff*'s test means that they contain heparin of mono-sulphuric acid. The fact that those in the connective tissues where blood vessels and secretory ducts are scarce show mostly negative to *Schiff*'s test means that they contain heparin of either die- or tri- sulphuric acid or a mixture of both. Further, when heparin is stained metachromatic colour with toluidine blue solution, to what extent it is dyed purple is in proportion to the number of the esterized heparin or sulfide (*Lison*, 1935; *Jorpes*, 1946).

Thereupon, it is interpreted that the tissue mast cells which are dyed orthochromatic color contain mono-sulphuric esters whereas those which are dyed, metachromatic color contain die- or tri-sulphuric acid esters or a mixture of both.

Namely the tissue mast cells, starting from cells of orthochromasia, in other word, the cells containing heparin monosulphuric acid, grow and mature into cells of metachromasia in other word, vitalized heparin die- or tri-sulphuric acid or mixture of both, which phenomenon was observed by *Paff* and others (1947), and *Paff* and *Bloom* (1949) in their tissue culture and by the present writer (1948) in the first part of this study. The heparin matured in this way is understood to be delivered, diffused toward the circumferential tissues where it is let into the blood vessel and act as a strong anti-coagulation agent.

Well, what turning point in the process of the maturing heparin gives rise to such vital reaction. According to *Fujie*'s (1954) theory that saliva decomposes histamine, when the functional activities of salivery gland are increased in the experiment that is, when much of saliva is excereted, probably the saliva acts like a kind of enzyme upon this tissue mast cells, decomposing and getting free histamine contained in the cells. Hence the granules of these cells will be dispersed out of the cell-bodies, taking place various changes in their forms and the phenomena of which will easily be inferred through the experiments of the above mentioned scholars and the seven groups of experiments of the present writer.

According to *Ogata* (1955), when saliva, especially saliva of parotid gland, after being secreted out of the glandular cells passes through the excretory ducts, most of it are absorbed into the blood while the remainder is discharged through the ducts into the oral cavity. This is supposed to be saliva. As to the phenomenon of highly increasing flow of saliva, *Ogata*'s theory (1955) interprets that when saliva secreted out of the glandular cells passed through the excretory duct and was absorbed into the blood, the remainder increased and the increase of the reaminder means the quantity of saliva absorbed into the blood increased, too. This theory could be indorsed by *Fujie*'s studies (1947) on the functional movements of the

minute structures of cells. In the cells of the parotid gland after a diet the decrease of secretory vacuoles was conspicuous. *Ogata*, however, does not make clear why the saliva has to be increased in the blood when the salivary functions are in prime conditions. *Fujie* (1954) stated that it was noticed in the experiment where the increase of saliva was expected that the progress of decreasing of histamine was faster than in the experimant where the increase of saliva was not expected and at the time of fact histamine decreased more than what was deserved.

Considering upon this fact, it is supposed that there is some interrelation between increase or decrease of histamine and saliva absorbed into the blood, in other words, that histamine in the blood is decomposed by saliva in the blood. Further *Kawai* (1957) stated his view that when he tried to remain histamine in the body more than needed he found the secretory function of the glandular cells in the parotid gland (that is, new-growth and discharging of secretion) became prime regardless of a diet, hence saliva can be a mediator to histmine in the blood. Once when *Fujie*'s and *Kawai*'s theories (1954 and 1957) are affirmed, it follows that when saliva is excreted a great deal, histamine in the blood is decreased, and the salivery gland tissue mast cell is considered to have a part of replenishing the histamine decreased in the blood, because the tissue mast cells in the experiment where the salivation is in prime condition develop, diffuse, and disperse their granules out of the cell-bodies, separating histamine.

Summary

In order to investigate the biological functions of the tissue mast cells of the salivatory gland, rats weighing about 100 g, irrespective of the sex, were chosen and devided into seven groups upon which experiments were performed by means of various dieting, applying stimulants to the parasympathetic system and the sympathetic nervous system or paralysing the parasympathetic system with drugs or giving them histamine hydrochloride. During the period of highly increasing flow of saliva, or of decreasing flow of it, or the period of taking effect of drugs the state, number, forms, sizes and the functional conditions, stainability with toluidine blue, of the tissue mast cells and the states and functions of their granules in the three prominent glands were observed and these data were viewed from many angles. In consequence, it was made clear that the tissue mast cells of salivatory gland according to change of the function of salivation give rise to change not only in the number, state of distribution, and forms, but also upon their functions.

1. The observation of the tissue mast cells in all the experiments demonstrated that these cells are numerous in the submaxillary and parotid glands and the circumferential lymphatic glands but few in the sublingual glands. In the submaxillary and parotid glands they had a tendency of gathering around the ex-

cretory ducts and the blood vessels. Some of them existed close to the basal part of the striated tubule, in the lymphatic glands around the efferent vessels and sometimes dispersed in the medullary substances, too. As to their forms, there were remarkable differences in respective experiments. The forms of the nuclei generally corresponded with those of the cells.

2. In the 1st and the 6th group of experiments of the salivatory functions on the wane, the tissue mast cells decreased in number, their contours were clear. Most of them were located in or around the excretory ducts in the interlobular connective tissues and their forms were round, spindle-shaped, oval or prolate. The granules in each of the cells were round, small and of equal size, filling the protoplasms and arranged evenly therein. There was none of them eccentric apart from the nuclei nor dispersed out of the cell-bodies. The nuclei were centric. They were dyed reddish purple, metachromatic color. Most of them were negative to periodic acid *Schiff*'s test (PAS) while some of them were positive, but their stainability was not rich in varieties, that is, was in the process of standstill.

3. In the 2nd, 3rd, 4th and 7th groups of experiments of secretion of the saliva in prime conditions, the tissue mast cells in the submaxillary and parotid glands increased in number and those which existed around the excretory ducts and blood vessels in the interlobular connective tissues tended to wander away and dispersed into the connective tissues far apart and those located in the fibrous connective tissues around the glands, too, began to wander evenly without gathering themselves. At this period of time, the granules became unequal in size, some of the granules had a tendency of departing from their own site toward the nuclei and attaching to them and their granules began to disperse out of the cell-bodies, assuming forms round, oval, prolate, indefinite in shape or that of *Banvier*'s clasmacyte. Some of the mast cells were observed to assume direct cellular fission. Their nuclei were mostly eccentric. With toluidine blue staining there were observed two types of them mixed together. One type of them was dyed strong blue color rather inclining to orthochromatic color while the other were dyed reddish purple metachromatic color. To periodic acid Schiff's test, relatively speaking, they were mostly positive, though there did exist a mixture of those which were strongly positive, weakly positive, and negative and the stainability was rich in varieties, that is, was in the process of activity.

In the 5th group of experiment in the sublingual gland, the images were similar to those above mentioned.

4. There are some interrelationship between the salivatory functions and those of the tissue mast cells. Namely, during the period of activity in the salivary gland the tissue mast cells, too, showed the activity whereas during the period of standstill in the salivary glands, the tissue mast cells also showed the condition of their standstill. These phenomena are due to the biological functions of the

tissue mast cells of salivary gland. When Ogata's Salivary Hormones Theory and the theory of Fujie's and others that saliva decomposes histamine are taken into consideration there are various suggestions given concerning the interrelation between the tissue mast cells and saliva and in the future a great interest will be in store for our research.

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EXPLANATION OF FIGURES

Fig. 1. Control: The tissue mast cells of the parotid gland stained with toluidine blue and eosin. ×400

Fig. 2. Control: The tissue mast cells in the fibrous connective tissue around the submaxillary gland stained with toluidine blue and eosin. ×400

Fig. 3. The tissue mast cells in the sublingual gland in the fast experiment stained with toluidine blue and eosin. ×400

Fig. 4. The tissue mast cells in the submaxillary gland in the fast experiment stained with toluidine blue and eosin. ×400

Fig. 5. The tissue mast cells in the fibrous connective tissue around the parotid gland stained with PAS staining. ×400

Fig. 6. The tissue mast cells in the fibrous connective tissue around the submaxillary gland in the experiment of feeding with rice bran stained with PAS staining. ×400.

Fig. 7. The tissue mast cells in the parotid gland in the experiment of feeding with rice bran stained with toluidine blue and eosin. ×400

Fig. 8. The tissue mast cells in the submaxillary gland in the experiment of feeding with vinegar stained with toluidine blue and eosin. ×400.

Fig. 9. The tissne mast cells in the submaxillary gland in the experiment of pilocarpine injection stained with PAS staining. ×400

Fig. 10. The tissue mast cells in the parotid gland and in the circumferential lymphatic tissues in the experiment of pilocarpine injection stained with toluidine blue and eosin. ×400

Fig. 11. The tissue mast cells in the submaxillary gland in the experiment of adrenaline injection stained with toluidine blue and eosin. ×400.

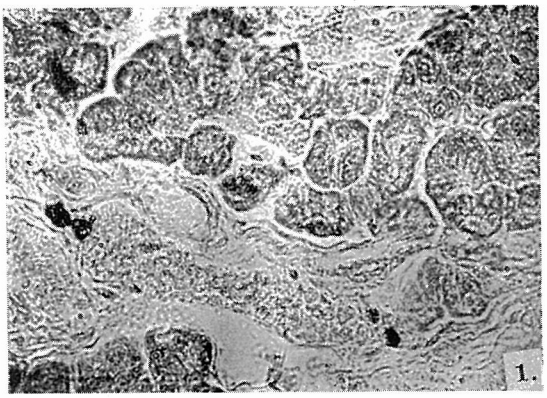
Fig. 12. The tissue mast cells in the submaxillary gland in the experiment of atropine injection stained with PAS staining. ×400

Fig. 13. The tissue mast cells in the parotid gland in the experiment of atropine injection stained with toluidine blue and eosin. ×400.

Fig 14. The tissue mast cells in the lymphatic tissues around the submaxillary gland in the experiment of atropine injection stained with toluidine blue and eosin. ×400

Fig. 15. The tissue mast cells in the fibrous connective tissues around the submaxillary gland in the experiment of histamine injection stained with PAS staining. ×400

Fig. 16. The tissue mast cells in the parotid gland in the experiment of histamine injection stained with toluidine blue. ×400



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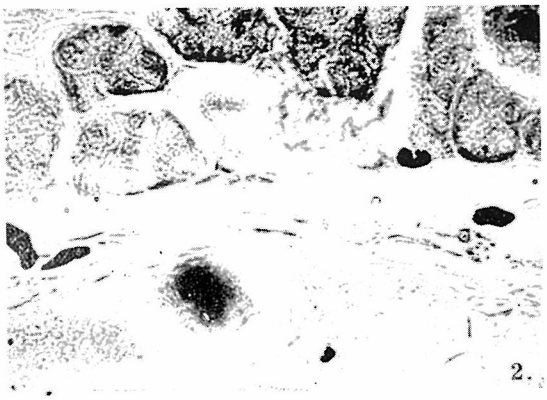

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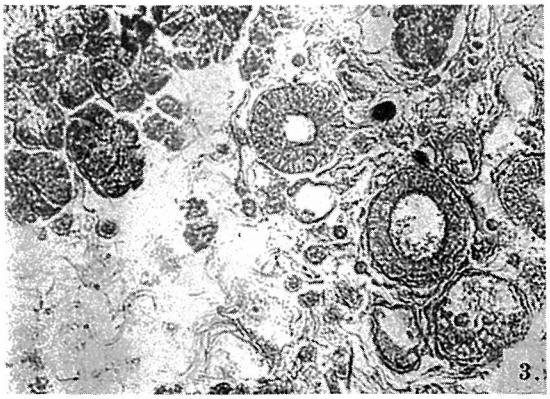

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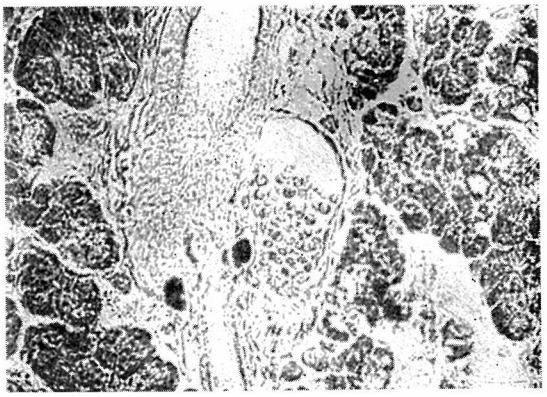

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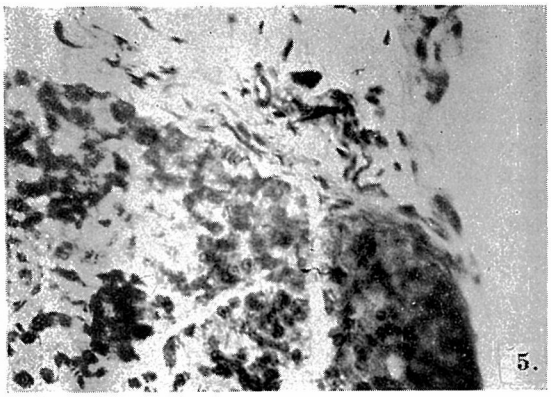

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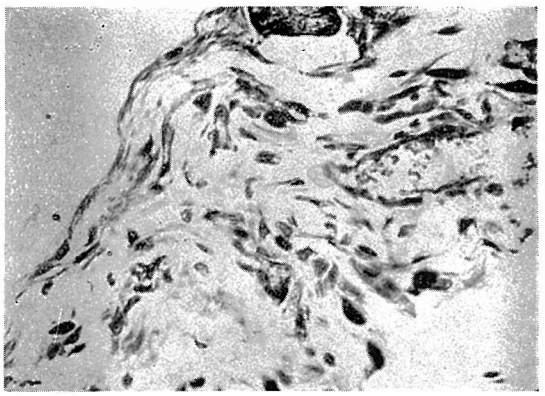

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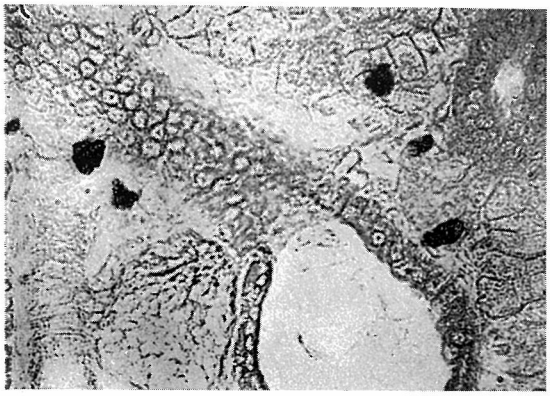

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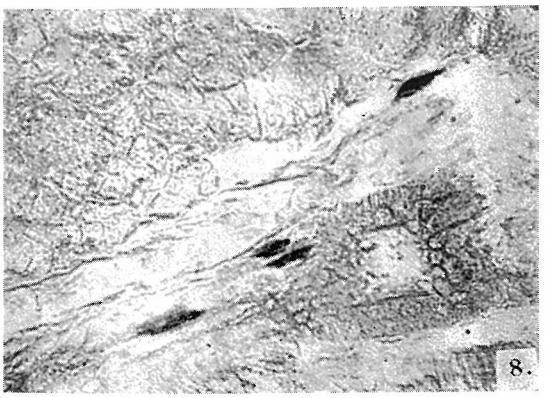

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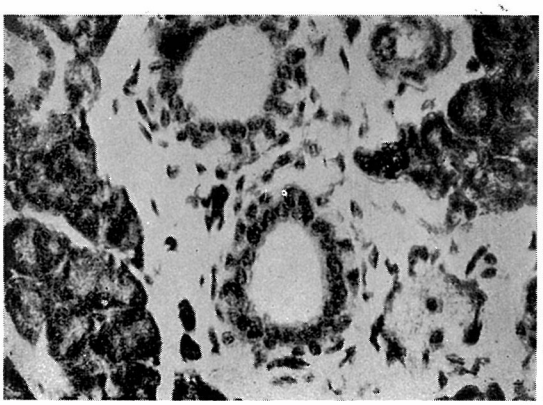

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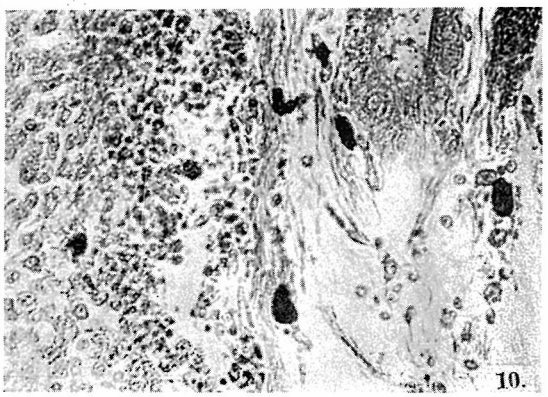

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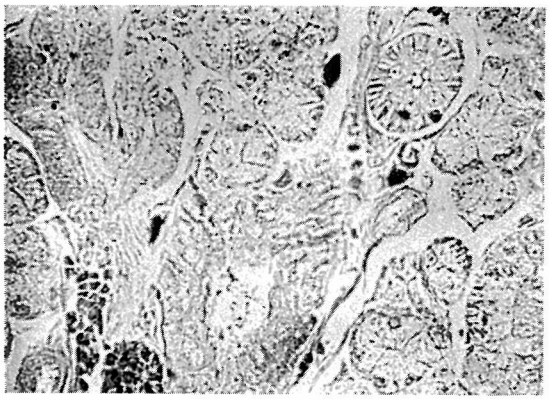

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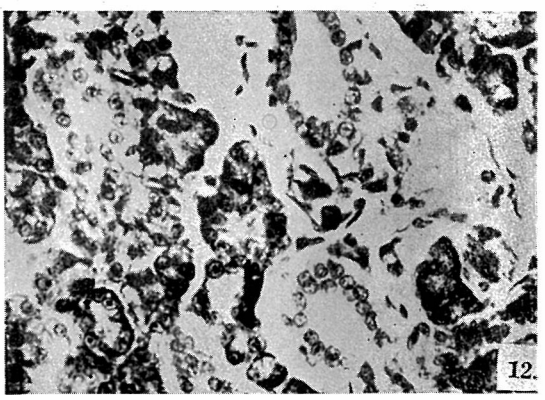

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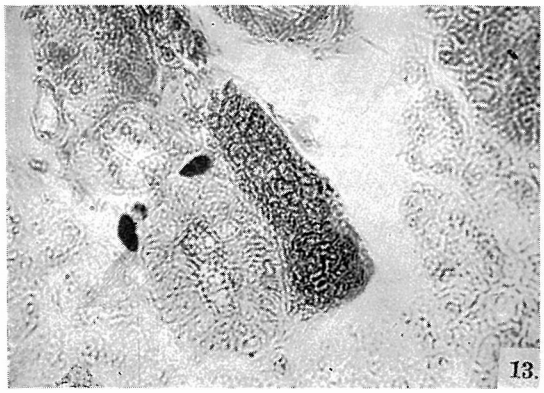

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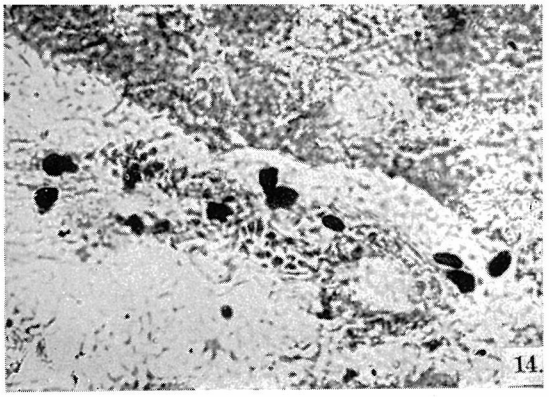

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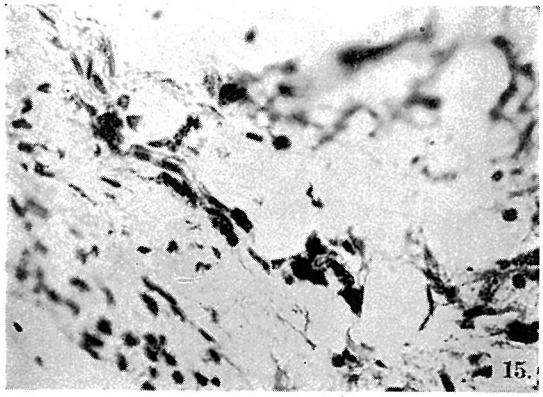

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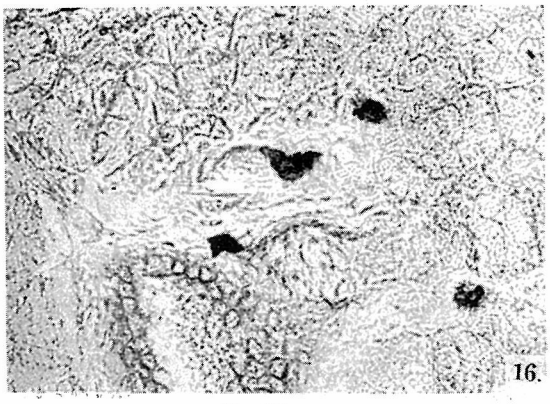

Fig. 16