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Examination of Histochemical Techniques for Demonstrating Histamine in Mast Cells*

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ROCHA e SILVA (1952) suggested that the cells containing histamine are the same as those containing heparin from the facts that heparin and histamine are released simultaneously in the pepton shock of the dog. RILEY and WEST (1953) and RILEY (1953 a, b) observed degranulation of mast cells and simultaneous histamine discharge in the rat treated with histamine liberators. It was also noted that there exists an apparent parallelism among the histamine, heparin and mast cell content in various tissues of the rat, ox, sheep, dog, cat and cow. RILEY and WEST (1952, 1953) further found that mast cell tumors in man are relatively rich in histamine, and, therefore, they concluded that the mast cells may contain histamine. BENDITT, ARASE and ROEPER (1956) calculated the histamine content of isolated peritoneal mast cells. It is thus generally accepted that mammalian mast cells contain histamine. FUJITA and his associates (FUJITA and TAKAYA, 1968; TAKAYA, FUJITA and ENDO, 1967; TAKAYA, 1968, 1969) investigated by bioassay and histochemistry the mast cell histamine in fish, amphibia, reptiles and birds, and concluded that histamine may not exist in the mast cells below the level of amphibia in phylogeny.

Though the chemical properties of histamine, which is a small, freely diffusible and water-soluble compound, have made its histochemical demonstration difficult, the following methods have been proposed for staining tissue histamine.

1. Prolonged immersion of tissue in Reinecke salt solution as proposed by SCHAUER and WERLE (1959)

2. Diazotized parabromoaniline staining or fluorescence microscopic observation after formaldehyde vapor-fixation as reported by LAGUNOFF, PHILLIPS and BENDITT (1961)

3. o-Phthalaldehyde stainings as reported by JUHLIN and SHELLEY (1966), EHINGER and THUNBERG (1967) and SHELLEY, ÖHMAN and PARNES (1968).

In the present study the authors intend to examine the specificity of these three techniques for demonstrating mast cell histamine using various tissues of the rat, mouse, hamster and guinea pig. Examination of histamine in mast cells is also attempted in the *Triturus pyrrhogaster* (Boie), whose brain has been found by the present authors to contain strange-shaped mast cells (INOUE, AKITA and SHIMAI, 1968).

Materials and Methods

As materials use was made of: the mesentery, tongue, kidney, liver and submandibular gland of the rat; the mesentery, tongue, kidney and liver of the mouse and

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hamster; the mesentery, tongue, lung, uterus, kidney and liver of the guinea pig; the brain and mesentery of the *Triturus pyrrhogaster* (Boie) (in summer season) and the mesentery of the *Rana catesbiana* (Shaw) (in summer season).

The following methods were applied to these materials:

1. Paraffin 7μ sections were made after Carnoy fixation. (The mesentery was used as a spread preparation.) They were stained with 0.1% toluidine blue solution (pH 2.8).

2. Reinecke salt method by SCHAUER and WERLE (1959).

- a. The mesentery of the various species mentioned above was immersed for 24 hrs in Reinecke salt solution which was proposed by SCHAUER and WERLE (1959), and then spread on a slide glass.

- b. Frozen sections 10μ thick of the tongue, kidney, liver, uterus, submandibular gland and brain were cut in a cryostat and immersed for 6 to 24 hrs in the same Reinecke salt solution.

After prolonged exposure to Reinecke salt solution, the mesentery and the frozen sections were washed out sufficiently and dried. Then they were observed after mounting in glycerin or without it.

3. Formaldehyde vapor-fixation method using paraformaldehyde by LAGUNOFF, PHILLIPS and BENDITT (1961).

The mesentery was stretched and pinned to a cork ring and dried as soon as possible, and then fixed with formalin vapor for 24 hrs which was kept at 56°C in a desiccator jar containing paraformaldehyde and calcium chloride. Frozen sections 10μ thick of the tongue, kidney, liver, brain, uterus and submandibular gland were cut and treated as the mesentery. The spreads and sections were observed by either of the following procedures.

- a. Observation under a Tiyoda fluorescence microscope FM 200A (Osram mercury lamp HBO 200 W, UV filter, No. 1 ultraviolet absorbing filter) with a $365\text{m}\mu$ transmission peak.

- b. Diazotized parabromoaniline staining after the original method by LAGUNOFF, PHILLIPS and BENDITT (1961).

4. o-Phthalaldehyde staining by JUHLIN and SHELLEY (1966)

- a. The mesentery was spread and the fresh tissue of the tongue, kidney and submandibular gland were sliced 150μ thick. Then they were treated with 1% o-phthalaldehyde xylol solution as JUHLIN and SHELLEY proposed for observation under a fluorescence microscope.

- b. The kidney, liver, uterus and salivary gland of the rat, mouse, hamster and guinea pig, and brain of the *Triturus* were frozen in isopentane cooled by ethyl alcohol-dry ice (-70°C), and were sectioned $10-20\mu$ thick in a cryostat at -20°C . Sections were stained with chilled 1% o-phthalaldehyde xylol solution in a cryostat after the original method. They were also observed under a fluorescence microscope.

After histochemical examinations, all specimens were stained with 0.1% toluidine blue solution (pH 2.8) to identify the mast cells.

5. Experimental study by injection of Polymyxin B in the rat and hamster.

- a. Rat: Polymyxin B $100\mu\text{g}$ in normal saline was injected intraperitoneally and 3 hrs later $200\mu\text{g}$ Polymyxin B was added. Rats were killed one hr after injecting

the last dose. The mesentery was prepared for histological and histochemical studies by means of the techniques described above. As controls, rats were given normal saline intraperitoneally and its mesentery was used.

b. Hamster: Polymyxin B 100 μ g in Tyrode solution was injected into the peritoneal cavity of hamsters, and 3 hrs later 200 μ g Polymyxin B was injected. Hamsters were killed one hr after the second dose and the mesentery was treated for observation with the same procedures as the mesentery of the rat. The control group of hamsters was given Tyrode solution without polymyxin B intraperitoneally.

Results

1. Reinecke salt method (SCHAUER and WERLE, 1959)

Remarkable precipitation of coarse crystals was observed in the cytoplasm of the mast cells in the mesentery of the rat, mouse and hamster (Fig. 1). However, the mast cells in the guinea pig, *Triturus* and *Rana* did not have any precipitates in the cytoplasm. Following observation of the frozen section preparations, precipitation in the cytoplasm of the mast cells in the tongue of other tissues of the rat, mouse and hamster was recognized. In the cytoplasm of the mast cells in the guinea pig, however, no precipitates were found in spite of the occurrence of many mast cells in the tongue and uterus. No crystalline precipitates were found in the cytoplasm of the mast cells in the brain of the *Triturus* by means of frozen sections.

2. Formaldehyde vapor-fixation method using paraformaldehyde

(LAGUNOFF, PHILLIPS and BENDITT, 1961)

a. Observation by fluorescence microscopy

A bright yellow-green fluorescence was observed to be emitted from fine cytoplasmic granules which filled the mast cells in the dried mesentery spreads of the rat and mouse. However, no fluorescence was emitted from the mast cells in the mesentery of the hamster, guinea pig, *Triturus* and *Rana*. In frozen sections no mast cells with fluorescent cytoplasm could be found in any species, including the rat and mouse.

b. Diazotized parabromoaniline staining

The cytoplasmic granules of the mast cells were stained red-brown to yellow-brown in the dried mesentery spreads of the rat (Fig. 2), mouse and hamster (Fig. 3). However, mast cells in the mesentery of the guinea pig, *Triturus* and *Rana* were not stained. Red blood cells were also stained strongly red-brown. In the frozen sections the mast cells in none of the tissues were stained.

3. o-Phthalaldehyde staining (JUHLEN and SHELLEY, 1966)

In the fresh preparations of the mesentery and tongue of the rat (Fig. 4), mouse (Fig. 5) and hamster (Fig. 6), a bright yellow fluorescence was seen in the finely granulated cytoplasm of the mast cells. A bright yellow fluorescence was emitted from the circular muscle cells in the wall of the small blood vessels besides the mast cells (Fig. 4, 5, 6). On the other hand, by application of this method to the fresh specimens of the submandibular gland of the rat, a yellow fluorescence was observed emitting from the cytoplasmic granules of the serous cells. Red blood cells and polymorphonuclear leucocytes (Fig. 6) fluoresced yellow to orange-yellow in the fresh preparations of the mouse and hamster. In the fresh specimens of the kidney of the rat and hamster, glomeruli and some convoluted tubules fluoresced yellow.

- Fig. 1.** Reinecke salt method. Three mast cells (*m*) are found in the mesentery of the hamster. The coarse crystals observed to be precipitated in the cytoplasm.
- Fig. 2.** Diazotized parabromoaniline staining. The mast cells (*m*) are stained brownish orange in the rat mesentery, and red blood cells (*rb*) also show a positive reaction.
- Fig. 3.** Diazotized parabromoaniline staining. The mast cell (*m*) shows a positive reaction to diazotized parabromoaniline as well as the red blood cells (*rb*) in the mesentery of the hamster.

- Fig. 4.** o-Phthalaldehyde staining. The mast cells (*m*) are stained bright yellow in the fresh specimens of the rat tongue, and the circular muscle cells in the small blood vessel (*bv*) also show a bright yellow fluorescence in the same hue as the mast cells.
- Fig. 5.** o-Phthalaldehyde staining. The mast cells (*m*) as well as the circular muscle cells in the small blood vessels (*bv*) in the fresh preparation of the mouse tongue are emitting a bright yellow fluorescence.

The mast cells in the mesentery of the guinea pig were observed to be filled with fine granules with a yellow fluorescence (Fig. 7). However, this fluorescence was weaker than in the mast cells of the rat, mouse and hamster. The capillaries and small blood vessels were also found displaying a bright yellow to orange-yellow fluorescence (Fig. 7). As the connective tissue cells, e.g. polymorphonuclear leucocytes, other than the mast cells of the guinea pig showed a weak white-yellow fluorescence, occasionally there was some difficulty in identifying the mast cells under the fluorescence microscope.

The mast cells in the mesentery of the *Triturus* and *Rana*, which show a characteristic shape, were not stained with o-phthalaldehyde, while the nuclei of some other kinds of cells in the mesentery showed a bright white-yellow fluorescence.

Following the adaptation of o-phthalaldehyde staining to the frozen sections of the tongue of the rat, mouse and hamster, the mast cells of these species were observed to be filled with dark yellow fluorescent granules in the cytoplasm. On the other hand, some epithelial cells of the convoluted tubules of the rat kidney were found to contain cytoplasmic granules with a yellow fluorescence. Besides, in the interlobular connective tissue of the submandibular gland of the rat the mast cells emitted a dark yellow fluorescence, while the cytoplasmic granules of the serous cells simultaneously showed a yellow fluorescence. Small blood vessels and capillary walls, displaying a bright yellow fluorescence in the fresh specimens, were unstained in the frozen sections. Though there were a lot of mast cells in the tongue, uterus and lung of the guinea pig, they showed no yellow fluorescence in the frozen sections of these tissues. Also, with this method fluorescence was not found in the cytoplasm of the mast cells in the frozen sections of the brain of the *Triturus*.

4. Experimental study by injection of Polymyxin B in the rat and hamster

a. Rat

The findings in the mesentery of the rat after the injection of Polymyxin B were as follows.

Toluidine blue staining (pH. 2.8) after Carnoy fixation revealed metachromatic granules of the mast cells discharged and scattered outside the cell body (Fig. 8). Some of the mast cells were found to retain a few metachromatic granules in the swollen cytoplasm.

After the Reinecke salt method crystalline precipitates were observed scattered around the nucleus and presumably partly outside the cell body of the mast cell (Fig. 10). This finding seemed to correspond to the release of the metachromatic granules.

In the fluorescence microscopy of the formaldehyde vapor-fixed materials the cytoplasmic granules of the mast cells did not show a greenish yellow fluorescence.

Diazotized parabromoaniline staining revealed no mast cells with cytoplasmic granules showing positive reaction to diazotized p-bromoaniline.

The o-phthalaldehyde staining caused no fluorescence in the mast cells of the rat

Fig. 6. o-Phthalaldehyde staining. The mast cells (*m*) are stained bright yellow in the fresh mesentery of the hamster, and the polymorphonuclear leucocyte (*wb*) shows a weak white-yellow fluorescence. The blood vessel walls (*bv*) are also stained yellow to orange-yellow.

Fig. 7. o-Phthalaldehyde staining. The mast cells (*m*) are emitting a bright yellow fluorescence in the mesentery of the guinea pig. The blood vessel wall (*bv*) also is stained yellow to orange-yellow.

injected intraperitoneally with Polymyxin B, while a bright yellow fluorescence was emitted from the mast cells of the rats which were treated only with normal saline as a control. The connective tissue cells emitted a weak yellow fluorescence and the bright yellow fluorescence of small blood vessel walls was unchanged even after the injection of Polymyxin B.

All the specimens treated with Reinecke salt, formalin vapor and o-phthalaldehyde in this experiment were stained with toluidine blue after investigation with histochemical procedures in order to confirm the mast cells. The mast cells were found to release their metachromatic granules remarkably, and some of them were conspicuously swollen with only a few metachromatic granules in the cytoplasm. However, the mast cells and the discharged granules never showed any positive response to histochemical stainings for histamine except with the Reinecke salt method.

b. *Hamster*

The findings in the mesentery of the hamster treated with Polymyxin B were as follows.

Fig. 8. Toluidine blue staining. The mast cells (*m*) in the rat mesentery show a remarkable release of metachromatic granules due to treatment with Polymyxin B.

Fig. 9. Toluidine blue staining. The mast cells (*m*) in the mesentery of the hamster injected with Polymyxin B show varying stages of the metachromatic granules. The upper left (*m*) shows the almost intact shape of the cell. The bottom (*m*) indicates a disruption and complete degranulation of the mast cell.

Fig. 10. Reinecke salt method. The mast cells in the mesentery of the rat treated with Polymyxin B are found to have crystalline precipitates scattered around the cell body similar to degranulation.

Fig. 11. Reinecke salt method. The mast cells are found to respond in various degrees to Polymyxin B in the mesentery of the hamster. The left upper (*m*) shows a mast cell which has the crystalline precipitates remarkably scattered around the cell body. The right upper mast cell appears to be almost intact.

Toluidine blue staining (pH 2.8) after Carnoy fixation showed various stages of changes in the mast cells of the mesentery, ranging from the almost normal form of the cells through a slight cell swelling and degranulation to the complete disruption and degranulation of the cells whose outline was no longer distinguishable (Fig. 9).

With the Reinecke salt method the distribution of the crystalline precipitates in the cytoplasm of the mast cells was almost similar to that of the metachromatic granules stained with toluidine blue (Fig. 11).

After formaldehyde vapor-fixation the mast cells were unstained with diazotized parabromoaniline staining.

The mast cells in the mesentery showed as a rule four types of response to *o*-phthalaldehyde (Fig. 12, 13). The first type of mast cells contained bright yellow fluorescent, fine granules diffusely in the cytoplasm, and their cytoplasmic granules were too fine to be differentiated from each other. This type was not distinguishable from the normal mast cells in the control specimens. The second type was the same in size and shape as the intact mast cell though some were swollen. However, the cytoplasmic granules of this type, emitting a bright yellow fluorescence, were coarser and distributed more sparsely than in the intact ones. The third type was demonstrated to emit a weak yellow fluorescence from the coarse cytoplasmic granules, which were scattered inside or around the swollen cells. The fourth type did not have any fluorescent granules. They were only identified as discharged metachromatic granules by the restaining of the specimens with toluidine blue (Fig. 13).

Fig. 12 and 13. The mast cells in the mesentery of the hamster injected with polymyxin B are stained with *o*-phthalaldehyde (Fig. 12) and the same specimen is counterstained with toluidine blue to identify the mast cells (Fig. 13). The letters from "a" to "e" show the same mast cells respectively in both figures. These mast cells appear to be in the varying stages of releasing histamine. The mast cell "a" seems to be almost intact, and the metachromatic granules "f" do not fluoresce in Figure 12.

Besides the mast cells, a number of leucocytes distributed diffusely in the mesentery were found to be stained simultaneously yellow or orange-yellow. Connective tissue cells also emitted a white-yellow fluorescence. Small blood vessels did not reveal any change in their bright yellow to orange-yellow fluorescence after the treatment with Polymyxin B.

Discussion

PARRATT and WEST (1957a) pointed out a striking parallelism between the mast cell population and the histamine content in various tissues, and concluded that the mast cells of the rat, mouse, hamster and guinea pig contain histamine. Using these four kinds of rodents, the authors have tried to examine three types of histochemical techniques for investigating histamine. There were found variations in the manner of histochemical response in relation to the species and methods used in the examinations.

By means of the Reinecke salt method (SCHAUER and WERLE, 1959) and diazotized p-bromoaniline staining (LAGUNOFF, PHILLIPS and BENDITT, 1961) the mast cells in a fresh mesentery spread of the rat, mouse and hamster revealed a positive reaction. However, in the guinea pig the mast cells did not show any positive response to either method described above.

When stained with o-phthalaldehyde (JUHLIN and SHELLEY 1966), the mast cells of the rat, mouse and hamster emitted a positive fluorescence in both the fresh specimens and the frozen sections. On the contrary, the mast cells of the guinea pig revealed a positive reaction only in the fresh specimens whereas in the frozen sections the mast cells did not fluoresce. Therefore, in the case of the guinea pig the mast cells showed positive response only to o-phthalaldehyde staining adapted to the fresh specimens.

When stained with toluidine blue, the metachromatic cytoplasm of the mast cells in the guinea pig was very finely granulated and looked somewhat homogenous, differing in appearance from the mast cells of the other rodents which were densely filled with coarse metachromatic granules. UVNÄS (1958) described that the mast cells in the guinea pig did not show any response to the histamine liberator, compound 48/80. Therefore, it is considered that the manner of occurrence of the histamine components in the cytoplasm of mast cells may be different between the guinea pig and other rodents.

LAGUNOFF, PHILLIPS and BENDITT (1961) noted that the mast cells of the dog were unstained with diazotized p-bromoaniline staining as the epithelial cells of the gastrointestinal tract which contain histamine. And they considered that the nature of the binding of histamine in the cells might vary the histochemical responses.

JUHLIN and SHELLEY (1966), using o-phthalaldehyde staining on the fresh specimens, demonstrated a fluorescence in the epithelium of the stomach which may be due to histamine. However, they further described that fluorescence was not found in cells other than the mast cells when the frozen sections were stained with the o-phthalaldehyde technique.

According to our findings and the reports described above, therefore, it is thought that the histochemical reactions of histamine may be influenced by the nature of the existence of histamine in the cells as well as the amount of the histamine content.

LAGUNOFF, PHILLIPS and BENDITT (1961) described that after treatment with formaldehyde vapor the mast cells were demonstrable due to the histamine-formaldehyde condensation product, which showed a yellow-green fluorescence. However, in our experiment the mast cells fluoresced only in the rat and mouse and not in the guinea pig and hamster. From this result it is concluded along with PADAWER (1966) that this type of fluorescence corresponds to serotonin rather than histamine.

PARRATT and WEST (1957b) administered Polymyxin B intraperitoneally to the rat and hamster, and observed a striking disruption of the mast cells and a rapid decrease in the histamine content of various organs under the characteristic signs of histamine shock. The authors tried a similar examination to observe the changes of histamine in the mast cells which would be revealed by histochemical techniques.

After treatment with Polymyxin B the mast cells in the mesentery of the rat and hamster showed a remarkable disruption and degranulation. Of the three histochemical techniques the o-phthalaldehyde and diazotized p-bromoaniline methods showed the response corresponding to the histamine content decreased by the histamine liberator. The mast cells in the hamster stained with o-phthalaldehyde seemed to show particularly convincingly how histamine was released as it was visualized by the changes of the fluorescent component in the cells. The findings obtained suggested that the fine granules containing histamine are fused together to form the coarse granules and then discharged around the cell; a histamine release which is shown by the disappearance of the fluorescence finally occurs from the discharged granules.

On the other hand, the Reinecke salt method seemed to react with some other substances than histamine because the precipitates in this reaction occurred in the manner corresponding to the release of the metachromatic substance of the granules.

Histamine in the mast cells appeared to be best demonstrated by o-phthalaldehyde staining applied to the fresh specimens, which was proposed by JUHLIN and SHELLEY (1966). However, besides the mast cells, the red blood cells and leucocytes of the mouse, hamster and guinea pig showed a yellow fluorescence, and in the rat the serous cells of the salivary gland, glomeruli and some convoluted tubules of the kidney, small blood vessel walls, and capillaries also showed a yellow fluorescence, which sometimes closely resembled the fluorescence emitting from the mast cells. Many connective tissue cells other than the mast cells of the mouse and guinea pig showed a weaker yellow fluorescence.

Since it is unlikely that the yellow fluorescence except in the mast cells was always due to histamine, the specificity of the histochemical techniques for histamine has to be tested still more. Regarding the diazotized p-bromoaniline staining red blood cells were also stained remarkably positive presumably due to the presence of histidine, as LAGUNOFF, PHILLIPS and BENDITT (1961) described.

Taking into consideration the specificity of these histochemical responses to histamine mentioned above, the authors performed histochemical examinations of the mast cells in the brain and mesentery of the *Triturus* and in the mesentery of the *Rana*. However, the mast cells never revealed a positive reaction with any histochemical techniques. This result corresponds to the view of FUJITA and his associates (TAKAYA, FUJITA and ENDO, 1967; FUJITA and TAKAYA, 1968; TAKAYA, 1968, 1969) that the mast cells are free of histamine below the level of the amphibia. The present authors also doubt the existence of histamine in the mast cells of the *Triturus pyrrhogaster* (Boie) from their own histochemical results.

Summary

The specificity in the detection of tissue histamine was examined in three previously known histochemical methods: 1) prolonged immersion of tissue in Reinecke salt solution (SCHAUER and WERLE, 1959), 2) diazotized parabromoaniline staining or

fluorescence microscopy after formaldehyde vapor-fixation (LAGUNOFF, PHILLIPS and BENDITT, 1961), 3) fluorescence microscopy after o-phthalaldehyde staining (JUHLIN and SHELLEY, 1968). Besides the normal mast cells of the rat, mouse, hamster and guinea pig, their changes caused by a histamine liberator, Polymyxin B were observed. The occurrence of histamine in the strange-shaped mast cells in the triton brain (INOUE, AKITA and SHIMAI, 1968) was also examined.

1. The o-phthalaldehyde staining applied to the fresh specimens was found to be the best of the three methods examined for the demonstration of histamine in the mast cells.

2. However, as some cells other than the mast cells, e.g. the serous cells of the salivary gland, the small blood vessel walls and part of the epithelium of the convoluted tubules of the kidney of the rat, and red and white blood cells and the small blood vessels of the mouse, hamster and guinea pig, emit simultaneously a bright yellow fluorescence, the presence and non-presence of histamine should be decided carefully by taking into consideration the species of animals examined, the histochemical procedures used and the possible variety in the manner of the binding of histamine in the cytoplasm.

3. The process of histamine release could be revealed by o-phthalaldehyde staining applied to the mast cells of the hamster which was injected with Polymyxin B.

4. Histamine was not demonstrated in the mast cells in the mesentery and brain of the *Triturus pyrrhogaster* (Boie) and in the mesentery of the *Rana catesbiana* (Shaw).

肥満細胞ヒスタミンの組織化学的検出方法の検討（内容自抄）

ヒスタミンの検出方法として知られている（１）SCHAUER と WERLE（1959）による Reinecke 塩水溶液を用いる方法，（２）LAGUNOFF, PHILLIPS と BENDITT（1961）による フォルムアルデヒド ガスで固定後，螢光を観察するか diazotized parabromoaniline で染色する方法，（３）JUHLIN と SHELLEY（1966）による o-phthalaldehyde で染色後 螢光を観察する方法 について，組織内のヒスタミン検出の特異性を比較した．著者らはヒスタミンの含有が証明されている ラット，マウス，ハムスター，モルモットなどの正常肥満細胞における比較検討のほか，さらにヒスタミン遊離剤 Polymyxin B 注射実験による ヒスタミン放出の状態をも追求した．一方 著者らが先に報告した アカハライモリ 脳の特異な形態の肥満細胞についても ヒスタミン含有の有無を検討した．結果は次の通りである．

1. 上記3方法の中で，新鮮標本を o-phthalaldehyde で染色する場合が 肥満細胞のヒスタミン検出には最良の方法であつた．

2. この螢光法で 肥満細胞以外にも同じ色調の螢光をだすものが認められる（ラットの顎下線の漿液細胞，小血管壁と腎の一部の曲尿管，マウス，ハムスター，モルモットの小血管壁，赤血球，分葉核白血球など）．したがって 動物の種類，組織化学的方法の差異，およびヒスタミンが形質内で異った結合方式をとる可能性などを考慮して，ヒスタミンの存否の判定を慎重に行なわねばならない．

3. Polymyxin B を注射したハムスターでは，o-phthalaldehyde で染色された肥満細胞

胞が ヒスタミン放出の過程を示すと考えられる螢光の変化を示した。

4. アカハライモリの脳と腸間膜, ウシガエルの腸間膜の肥満細胞には, ヒスタミンの反応は認められなかった。

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