Analysis of Phagocytosis and Birefringence in the Peritoneal Cells of the Rat, with Special Regard to the Mast Cells

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흰쥐의 腹腔內 細胞, 特히 肥滿細胞의 喰作用 및 複屈折性에 관한 分析

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摘 要

1. 10種染料(neutral red, toluidine blue, pyronin, methylene blue, alcian blue, trypan blue, carmine, orange G, aniline blue, Janus green B, India ink)로 處理된 흰쥐의 腹腔內細胞 特히 肥滿細胞의 喰作用 및 複屈折性의 有無를 位相差顯微鏡法 및 偏光顯微鏡法으로 分析하였으며 附隨的으로 cytomorphic change도 觀察하였다.
2. 腹腔內 肥滿細胞는 大部分의 染料에 對하여 아무런 喰作用을 보이지 않았으며 India ink 群에서 輕微한 喰作用을 보였다. 大喰細胞와 各種 白血球는 大體로 旺盛한 喰作用을 나타내었다.
3. 腹腔內 肥滿細胞는 大部分의 染料를 處理하여도 아무런 腹屈折性을 보이지 않았으며 trypan blue와 aniline blue 處理群에서 매우 輕微한 反應을 보였을 뿐이었으며 白血球는 全然 反應을 보이지 않았다.
4. 各種染料에 對한 肥滿細胞의 細胞形態學的 反應은 一定하지 않았으며 大體로 輕微한 變化를 일으켰다. 白血球의 同染料에 對한 反應은 特히 trypan blue, orange G, India ink 群에서 顯著하였다.

INTRODUCTION

Whether mast cells are capable of phagocytosis has been a much debated problem. Of course, it was established that discharged mast cell granules can be phagocytosed by adjacent cells which look like mast cells. Even metachromatic foreign materials can be thus phagocytosed by cells which assume the appearance of mast cells or "Quasi-mast cells". Toeroe (1942) reported that if histamine is applied to the shaved skin of the rat, the regional mast cells are occasionally laden with India ink, and they are closely related to the vascular function. Velican and Velican (1958) reported that iron and carbon particles can be detected in the mast cells of tracheobronchial lymph nodes in man. On the other hand, Kiyono (1914) reported that the peritoneal mast cells of the rat do not phagocytose intravenously injected lithium carmine. Nevertheless, a number of reports on the phagocytic activities of mast cells have been presented: cocci (Tsuda, 1923), erythrophagocytosis (Brinkmann, 1959, 1962 and Ultmann *et al.*, 1964). However, it is clear that phagocytic activities of mast cells to some particular dyes were not yet fully discussed and established in detail.

Normally, neither the metachromatic granules nor the cytoplasm of mast cells are birefringent, but using certain dyes, it is possible to induce anisotropy in the cytoplasm. Horvath (1959, 1960) reported that certain dyes(pyronin G, toluidine blue, neutral red), dissolved

in acid medium, produce anisotropy in the mast cells, and this anisotropy is due to the intergranular substance or circularly arranged protoplasmic structure. Some experimental evidences on existence of catecholamine, 5-hydroxytryptamine, and histamine in the mast cells have also been suggested by Adams-Ray (1964) and Hultén and Ponten (1964) by means of fluorescence microscopic techniques.

However, it is interesting thing to detect the birefringence of the cytoplasm of mast cells of rats for its regularly arranged substances.

This present study was made to observe the phagocytosis and birefringence of the peritoneal cells with special regard to the mast cells, which are closely related to its vascular function and cytoplasmic structure.

MATERIALS AND METHODS

Healthy adult albino rats, weighing about 200gm., were used in this study. Food and water were allowed *ad libitum*. The animals were divided into two groups; the control(intraperitoneal injection of 5ml saline) and the experimental (dye substances, refer to Table 1). Dye substances tested were dissolved in solvents just before use and were injected intraperitoneally into the animals under ether anesthesia. Eight to twenty two hours later, the intraperitoneal fluid was sampled with clean droppers. And the fluid was dropped on the slides and covered with wide cover glasses, and then the phagocytic activities and the birefringence of the mast cells were analyzed by means of phase contrast microscopy and polarizing microscopy, respectively.

In addition to the above characteristics of the mast cells, the cytomorphic changes of mast cells to the dye substances were also analyzed and excepting the mast cells the other cellular elements of the peritoneal fluid were included for phase contrast microscopic observations. Beside these, neutral red and toluidine blue were also dissolved in acid medium for analysis of birefringence. For a reference the osmolarities of the dye substances were measured and compared with each other.

Table 1. The dose, dilution, and osmolarity of dye substances

No. Dye substance	Dose/kg. of body weight	Dilutions (solvents)	Osmolarity (mosm/liter)	Remarks
1. Neutral red	50mg.	1% PSS##	62	#1, acid
2. Toluidine blue	20mg.	0.4% AQS##	49	#2, acid
3. Pyronin	0.2mg.	1,000:1 PSS	285	#3
4. Methylene blue	45mg.	0.3% PSS	225	#4
5. Alcian blue	50mg.	1% AQS	690	#5
6. Trypan blue	50mg.	1% PSS	399	#6
7. Carmine	125mg.	2.5% PSS	36	#7
8. Orange G	45mg.	2,000:1 PSS	50	#8
9. Aniline blue	10mg.	3,000:1 PSS	36	#9
10. Janus green B	50mg.	10,000:1 PSS	276	#10
11. India ink	(1ml. of conc. aq. solution)		87	#11

#1) National Aniline & Chemical Co., Inc., New York, N.Y.,C.I.No. 825. 2) E. Merck AG Darmstadt. 3) E.Merck AG Darmstadt. 4) Kishima Chemical Co., Osaka. 5) National Aniline Division. 6) British Drug Houses, Ltd., for vital staining. 7) Katayama Chemical Co., Ltd., Osaka. 8) Bezene Group Trade Mark., C.I. No. 27. 9) The Coleman & Bell Co., Norwood, O. 10) E.Machlett & Son Co., New York. 11) Made by rubbing an ink stick. PSS-physiological saline, AQS-aqueous solution.

RESULTS

A) Control group: The mast cells of the rat peritoneal fluid are large and round or slightly ovoid in shape. Free floating peritoneal mast cells have a large round nucleus, the cytoplasm is filled with relatively fine metachromatic granules. The intracellular canal was also

observed(Fig. 1). The other cellular elements of the peritoneal fluid including macrophages are generally round in shape, but far smaller than the mast cells in size. The incidence of peritoneal mast cells to all of the peritoneal cell elements was similar to the observations made by Oh *et al.* (1968). Hardly any morphological changes of the peritoneal mast cells were found in the control. The macrophages seldom appeared in the specimens. No phagocytosed granules were observed in the cytoplasm of the macrophages by means of phase contrast microscopy. And no birefringence of the cytoplasm of the mast cell was found. A mumber of white blood cells mainly consisted of lymphocytes were also observed in the control specimens. But neither cytomorphic changes nor birefringences of the white blood cells were observed in the control (Table 2). No ameboid movement could be demonstrated in rat peritoneal mast cells observed with phase contrast mocroscope.

B) Experimental group: The experimental results are summerized in Table 2.

1. Neutral red. The cytomorphic effect of neutral red on mast cells of the rat was little. The birefringence of the cytoplasm was not observed in the mast cells treated with neutral red. Most macrophages revealed active phagocytic activities and their cytoplasms were filled with tiny dark granules. The other white cell elements except neutrophilic leucocytes did not show phagocytosis. Some vacuoles of varying sizes were found in both the mast cells and the macrophages. Binulceated mast cells with distinct nuclear boundaries were noted in this group. Dimorphism was not observed either.

2. Toluidine blue. The effects of toluidine blue on the mast cells were noticeable. Any phagocytic activity of the intraperitoneal mast cells treated with toluidine blue was not observed. But a considerable amount of mast cells were disrupted and degranulated.The solitary mast cell granules discharged from the cells and the other cell debris were sporadically found. An active phagocytic activity of the macrophage was also observed. It was not possible to induce anisotropy in the cytoplasm of mast cells and in the other cell elements.

3. Pyronin. The indistinction of the nuclear contour occurred in the peritoneal mast cells. None of the phagocytosed granules of the mast cells were found, but swelling of granules was easily observed in the mast cells by means of phase contrast microscopy. It was also remarkable that the macrophages showed active phagocytic activities, and clear Brownian movement of the cell inclusions mainly consisted of black particles just like the dye substance was recongnized in this specimen. No birefringence of the cytoplasm of the mast cell was found.

4. Methylene blue. By phase contrast micrescopy, the cytomorphic changes of mast cells were remarkable; indistinct contour of the nuclear boundary, polarity of localization of the nucleus, and disappearance of large sized mast cells. In spite of these abnormal changes, degranulation of the mast cells was not observed in this group. However, the macrophages revealed vividly their proper activities, and the phagocytosed granules were mainly aggregated around the perinulcear region (Fig. 2).

5. Alcian blue. It was noticeable that the mast cells were hardly found in the peritoneal fluid, and some severely affected mast cells with varying sized vacuoles were frequently found, and no phagocytosis of mast cells occurred. The phagocytic activityies of macrophages were similar to the former group. Neither birefringence of the cytoplasm of mast cells nor cytomorphic changes were observed in this group.

6. Trypan blue. By phase contrast microscopy, it was demonstrable that a number of mast cells were provided with varying sized and swelled granules. The cytoplasm of the macrophage was vesiculated due to the existence of vacuoles. By polarizing microscopy, it was possible to induce slight birefringence of the cytoplasm of the mast cell, but this anisortropy was so pale that the distinct difference could not easily be recongnized (Fig. 6). The fine slender cytoplasmic processes extending from the cell body were observed in most of the white blood cells (Fig. 3).

It seemed likely that the white blood cells had been provided with cytoplasmic processes floated freely in the peritoneal fluid and the tips of them were thickened.

7. Carmine. The intraperitoneal mast cells with sligh

tly increased number of cytoplasmic vacuoles were scattered in the fluid, but no phagocytosis of the mast cell was observed. Some phagocytosed particles were frequently found in the white blood cells(lymphocytes). By polarizing microscopy anisotropic characteristics of the mast cells were not found, and it was found that the background was darker than those of the others. Phagocytic activities of macrophages were relatively less typical. The vesicular cytoplasms of the white blood cells were noticeble.

8. Orange G. By phase contrast microscopic observations, no vesicular cytoplasms of mast cells were observed but short and small sized cytoplasmic processes of the lymphocytes or monocytes were recognized. Sporadically distributed black particles were found in the macrophages. As was observed in the alone case no birefringence of the mast cell cytoplasm was observed.

9. Aniline blue. Cytomophic changes of mast cell were hardly found in this group. Few mast cells appeared and this may be due to degranulation of the cells. But macrophages showed active phagocytic activities as in the other cases. Phagocytosed particles were sporadically found in the cytoplasm of the macrophage even in the nuclear area. By polarizing microscopy, very slight birefringence of the cytoplasm ofthe mast cell was observed and was similar to that of the trypan blue group (Fig.6). The ectoplasmic zone corresponding to the peripheral boundary was so bright as those in the other groups.

10. Janus green B. Little cytomorphic and morphological changes of the mast cells were observed; typical contour of the cytoplasm, non-swelling of the metachromatic granules, and central localization of the nucleus. No birefringence of the cytoplasm of the mast cell was also found. But slight phagocytic activities of macrophages were observed in these specimens.

11. India ink. The cytomorphic reaction of the mast cells and white blood cells were evident in this group. Occasionally several large vacuoles with only one metachromatic granule and dark phagocytosed particle-like granules which were deeper than the other ordinary granules were easily found scattered throughout(Figs.4, 5). It was noticeable that short slender protoplasmic processes protruded from the cell surface were observed all around the surface of the mast cells, and depolarity of the nuclear localization was also observed. The macrophages also revealed active phagocytosis, varying sized black particles were observed all over the cytoplasm. These particles seemed to be phagocytosed dye particles of the India ink. Active mobile particles were also found in the protoplasm of the macrophage; this zigzag movement of the particles might be due to the so-called Brownian movement.

Table 2. Analysis of phagocytosis and birefringence of the peritoneal cells exposed to various dyes

No.	Dye substance	Phagocytosis			Birefringence			Dimorphism		
		MC#	WBC#	M#	MC	WBC	M	MC	WBC	M
A)	Control	—	—	+	—	—	—	—	—	—
B)	Experimental									
1.	Neutral red	—	+	+	—	—	—	—	—	—
2.	Toluidine blue	—	—	+	—	—	—	—	—	—
3.	Pyronin	—	+	+	—	—	—	—	—	—
4.	Methylene blue	—	—	+	—	—	—	—	—	—
5.	Alcian blue	—	—	+	—	—	—	—	—	—
6.	Trypan blue	—	—	+	+(?)	—	—	+	+	—
7.	Carmine	—	+	+	—	—	—	+	—	—
8.	Orange G	—	—	+	—	—	—	—	—	—
9.	Aniline blue	—	—	+	+(?)	—	—	—	+	+
10.	Janus green B	—	—	+	—	—	—	—	—	—
11.	India ink	+(?)	—	+	—	—	—	+	+	+

MC: mast cells, WBC: white blood corpuscles, M: macrophages

DISCUSSION

Metchnikoff (1892) and Marchand (1901) have reported that the mast cells are assumed to possess a phagocytic potency. But Kiyono (1914) has reported that the peritoneal mast cells of the rat do not phagocytose intravenously injected lithium carmine and Tsuda (1923) also reported that the injected cocci are occasionally found within mast cells, yet vital staining shows no ingestion of particular dyes. However, though the direct evidences of phagocytic activities of the mast cells were little and limited in certain materials, it is known that discharged mast cell granules can be phagocytosed by abjacent cells which look like mast cells and extensive erythrophagocytosis (Brinkmann, 1959; Ultmann *et al.*, 1964) by mast cells appear to occur in the various patients with anemia. But neither erythophagocytosis of the mast cells nor ingestion of particular dyes were found in the most groups of the present investigation.

Selye (1955) has reported that chronic treatment with carboxymethylcellulose results in the development of numerous mast cells of the adrenal medulla and true mast cells, like these "pseudomastocytes" may develop by phagocytosis of metachromatically-staining material. Brusca (1958) reported that *in vitro* treatment with hemoglobin abolishes the toluidine blue metachromasia of cutaneous and peritoneal mast cells of the rat, and this loss of metachromasia is due to the fixation of hemoglobin on the mast cell granules, and finally assumed that the mast cells play a defensive role in taking up foreign particles from the connective tissue. Selye *et al.* (1963) have reported that polymyxin subcutaneously given with India ink intravenously causes localization of carbon particles particularly in a halo around the polymyxin injection site, the India ink and discharged mast cell granules are found in close association with connective tissue and in the walls of small blood vessels, parhaps because some component of the mast cells fixes blood-borne particles by increased adhesiveness of tissues. This process was designated as mastopexis. Goezsy and Kato (1964) have also reported that if India ink is injected simultaneously with polymyxin B or compound 48/80 intradermally in the rat, the carbon accumulates at the injection site of the histamine liberates where the mast cells are discharged, and the carbon particles are attached to the capillary endothelium and appear also in the pericapillary space, and this is ascribed to the liberation of histamine and 5-hydroxytryptamine.

In the present study, phagocytic potencies of the peritoneal mast cells were not found in most groups,except for the India ink group. Such an ingestion of the carbon by the peritoneal mast cells was so doubtful that large metachromatic granules may be deeply stained with some component of the India ink and not ingested or phagocytosed as those were.

Baglioni (1954) and Smith (1963) have reported that using certain dyes, it is possible to induce anisotropy in the cytoplasm and thereby to detect otherwise indistinguishable details in the intergranular substance. In the present study, only trypan blue and aniline blue produced very weak birefringence in the peritoneal mast cells, and the other dye substances did not produce birefringence even in acid medium (neutral red and toluidine blue). These results are different from those of Horváth (1960) and this loss of anisotropy may be due to the difference in concentration of the acid medium or technical procedures displaying a positive polarization. However, it was noted that the birefringences produced by trypan blue and aniline blue were so pale that the positivity was not recognizable by the ordinary vision.

The cytomorphic effects of various dyes on the peritoneal mast cells were slight and variable in the present study. Padawer (1960, 1966) and Choi *et al.* (1966) have reported that after the intraperitoneal injection of colchicine or colcemid the peritoneal mast cells of the rat show an altered morphology; e.g., elongated cytoplasm, nodal constrictions of the cell, displacement of the nucleus, and extrusions of the ectoplasm. In the present study, such an inconstant morphology was hardly found in most groups. But it was also noted that on the way of the phase contrast microscopic observations, the cytoplasmic processes protruded from the cell surface were frequently found in the mast cells even in the white blood cells. These pseudopodial processes of the peritoneal colls were particularly remarkable in the India ink group, and may be closely related to the

phagocytic activities of the cells. Actually varying sized vacuoles are observed in the normal peritoneal mast cells, but in this study the mast cells with swelling and irregularly shaped vacuoles were respectively classified into the category of dimorphism.

SUMMARY

The phagocytic potencies and birefringences of peritoneal mast cells of rats treated with particular dyes (neutral red, toluidine blue, pyronin, methylene blue, alcian blue, trypan blue, carmine, orange G, aniline blue, Janus green B, and India ink) were analyzed by means of phase contrast microscopy and polarizing microscopy. In addition, cytomorphic effects of the dyes on the peritoneal mast cells were also discussed.

Phagocytic activities or ingestion of the dye particles were not observed in most cases, except for the India ink group. Hardly a macrophage appeared without some dark particles which were ingested or phagocytosed. Trypan blue and aniline blue produced very weak birefringence in the cytoplasm of mast cells but the rest did not produce even in the acid medium (neutral red and toluidine blue).

The short and slender ectoplasmic processes of the mast cells and the leucocytes were also found in certain groups. The cytomorphic effects of the dyes on the mast cell were slight and variable.

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EXPLANATION OF FIGURES

Fig. 1. Rat peritoneal mast cell treated with physiological saline solution. Cytoplasmic granules, intracellular canal, and ectoplasmic halo are visible. Phase contrast. 970X.

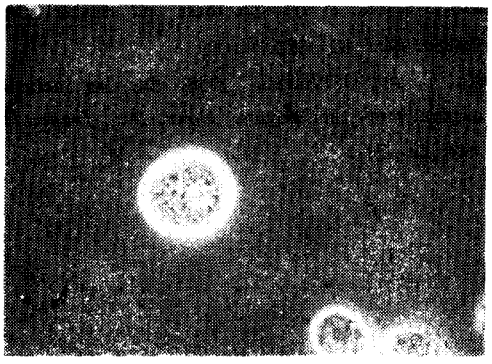
Fig. 2. Rat peritoneal mast cell and macrophage treated with methylene blue. Phagocytic activity of the macrophage and neutrophilic leucocyte are visible. Phase contrast. 970X.

Fig. 3. Rat peritoneal leucocytes treated with trypan blue. Some phagocytosed granules are visible in their narrow cytoplasm. Notice varying lengthed cytoplasmic processes around the cell surfaces. Phase contrast. 970X.

Fig. 4. Rat peritoneal mast cell and leucocytes treated with India ink. A swelled mast cell and phagocytic leucocyte are visible. Notice indistinction of the ectoplasmic halo. Phase contrast. 970X.

Fig. 5. Rat peritoneal mast cell treated with India ink. A mast cell with some vacuoles and two dark particles are visible. Notice two varying sized vacuoles containing "only one cytoplasmic granule". Phase contrast. 970X.

Fig. 6. Rat peritoneal cells treated with aniline blue. Varying sized mast cells showing very slight birefringence are visible. Notice round dim area of the mast cell. Phase contrast. 970X.



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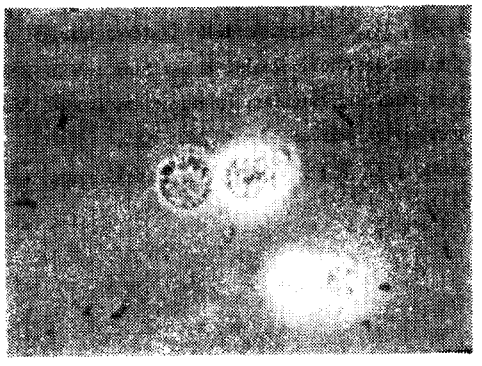

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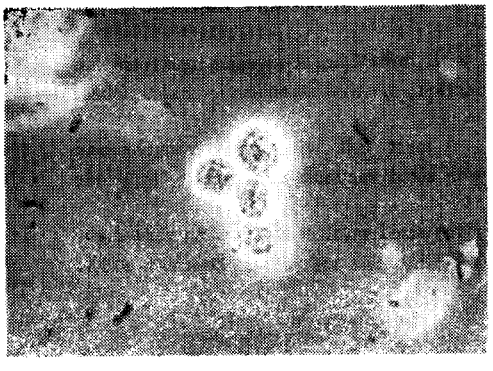

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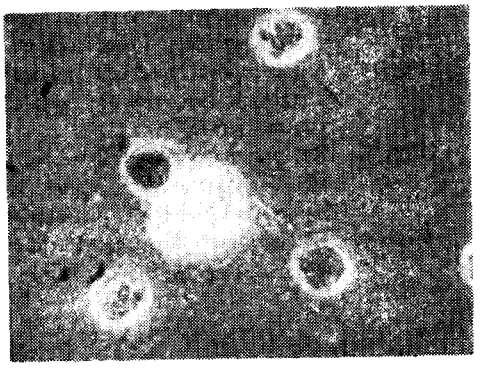

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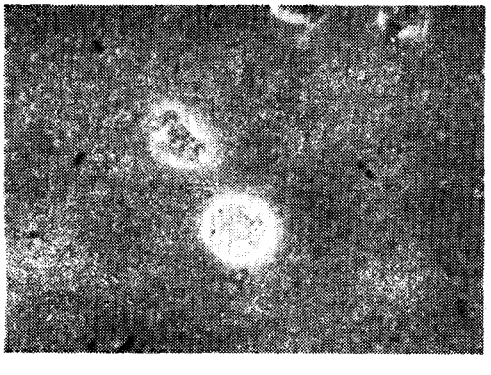

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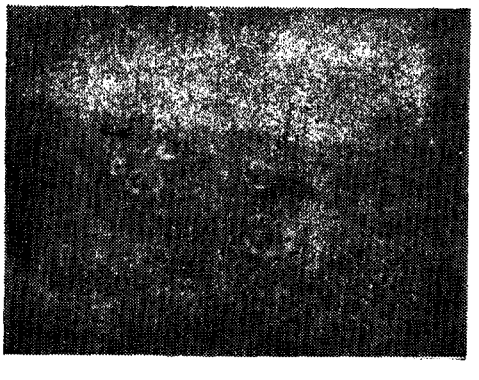

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