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SHORT REPORTS

THE FIXATION EFFECTS OF MAST CELLS IN CHICKEN INTESTINAL MUCOSA

ANDREW CHANG-YOUNG FEI AND YUNG-CHI LEE

Department of Veterinary Medicine, National Taiwan University, Taipei, Taiwan 107, Republic of China

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A. Chang-Young Fei and Yung-Chi Lee (1983) The fixation effects of mast cells in chicken intestinal mucosa. *Bull. Inst. Zool., Academia Sinica* **22**(1): 119-122. In the present study we found that Carnoy fixative is the most satisfactory fixative for the preservation of mast cells in chicken intestinal mucosa as compared to other fixatives tested. The intestinal sections, when stained in 0.5% aqueous solution of astra blue at pH 0.3 for 2 hr and counterstained with eosin for 10 second, displayed a considerably numerous mast cells and goblet cells. The mast cells were confined to the lamina propria and the goblet cells were present between the absorptive cells of intestinal epithelium. Only the mast cells and goblet cells were stained blue while other cells were stained pink-to-red.

It has been demonstrated that the selection of proper fixative is the most important for the preservation of mast cells in gastrointestinal mucosa (Enerback, 1966a). The fixation and staining methods for the mast cells in various mammals have been well documented (Enerback, 1966a; Murry *et al.*, 1968). However, only few investigations dealt with this techniques in chicken mast cells (Enerback, 1966a) were mentioned. This paper describes studies of the preservation of chicken intestinal mast cells by 15 different fixation treatments.

MATERIALS AND METHODS

The present study was performed by using 60 3-week-old white Leghorn cockerels. They were randomly selected and equally divided into 15 groups of 4 chickens in each. They were killed by anesthesia with ether without prior starvation. Three centimeter pieces from the small intestine, 2-5 cm posterior to the yolk sac diverticulum, were sampled. They were dissected out as quickly as possible and fixed in fixatives. The general histological procedure comprised the following standard steps: (1) fixation (Table 1), (2) dehydration, embedding in paraffin and preparation in 5 μ sections, (3) staining, dehydration and mounting in Canada balsam. The tissues from each group were fixed together in each of the fixatives tested, and subjected to the same histological procedures. They were stained on the same slide, in order to minimize random variations in staining and dehydration. The preservative efficiencies were estimated and arbitrarily quantified. The term preservation will only be used to connote preservation of stainability with the adopted staining technique.

Fixatives

The Bouin fixative, Carnoy fixative, 10% formalin, 10% neutral buffered formalin, 10% formalin ethanol, Gomeri 1-2-3 fixatives, Helly fixative, and Zenker's fixative were prepared according to the methods described by Humason (1972). Isotonic Formaldehyde-Acetic Acid mixture (Abbrev.: IFAA) (Enerback, 1966a): 1.5% formalin and 0.5% acetic acid diluted

with McIlvaine buffer, pH 2.9; Basic lead acetate-acetic acid-ethanol (abbrev.: Pb-Ac-EtOH) (Enerback, 1966a): basic lead acetic acetate 1 g, ethanol 50 ml, distilled water 50 ml, acetic acid 0.5 ml; were also tested.

Staining procedure

The sections of the specimens were stained in a 0.5% aqueous solution of astra blue at a pH 0.3 for 2 hr and were counterstained with eosin for 10 second.

RESULTS

All the fixatives tested (Table 1) showed compatible effects on each of the 15 groups of 4 chickens each.

Staining for 2 hr with astra blue generally imparted an intense dark blue color to the mast

TABLE 1
Preservation of intestinal mast cells by different fixation

Fixatives	Fixation time	Mast cells
Carnoy	3 hr	++
Carnoy	6 hr	++++
Carnoy	12 hr	++++
Carnoy	24 hr	++++
IFAA	24 hr	++
Bouin	24 hr	++
Bouin	48 hr	—
10% formalin	24 hr	+
10% neutral formalin	24 hr	±
Gomeri 1-2-3	24 hr	—
Helly	10 hr	—
Absolute ethanol	24 hr	—
10% formalin-ethanol	6 hr	—
Pb-Ac-EtOH	24 hr	+
Zenker	24 hr	—

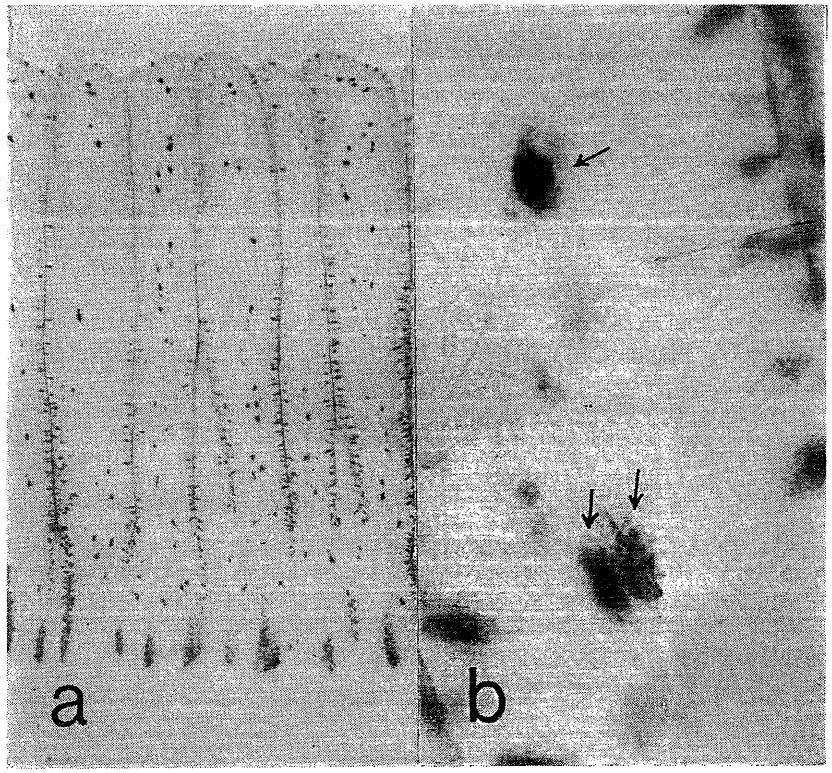

Fig. 1. Photomicrographs of a longitudianl section of chicken jejunum, fixed in Carnoy solution for 12 hr. The section was stained by astra blue and eosin.
a. Mast cells are evident at all levels in the lamina propria and the goblet cells are situated between the absorptive cells of intestinal epithelium (×132).
b. High power photomicrograph of a lower part of a villus in a, showing three mast cells in the lamina propria (arrows) and some goblet cells along sides of the villi (×1320).

cells and goblet cells whereas other cells were stained pink-to-red by the eosin (Fig. 1a). The goblet cells were present between the absorptive cells of intestinal epithelium and could be seen to be covered with a thin layer of some blue materials. The mast cells were generally confined to the lamina propria where they were evenly distributed from the bottom of the glandular crypts to the top of the villi. In addition, mast cells were sometimes observed within the epithelial tissue.

The nuclei of mast cells could seldom be distinguished from the cytoplasm, owing to the dense and heavily stained granulation (Fig. 1b). The best results were achieved by fixation in the Carnoy fixative for 6-24 hr (Table 1). All fixed sections exhibited a large number of mast cells. The results were less good with IFAA, Bouin (fixed for 24 hr), and Carnoy (fixed for 3 hr) fixatives. The mast cells after these fixations were similar in appearance to those in preparations fixed in Carnoy fixative for 6-12 hr but seemed fewer and weakly stained. However, treatments with IFAA and Bouin fixatives yielded sections with very poor tissue fixation, which interfered with assessment. Occasionally, faintly blue staining mast cells could be demonstrated in the intestinal preparations fixed in 10% formalin, either neutral buffered or unbuffered, and in the Pb-Ac-EtOH. No mast cells could be identified after staining in preparations fixed in any of other solutions.

DISCUSSION

The commonly accepted definition of mast cells is based on the metachromasia of their granules towards the cationic basic thiazine dyes (Enerback, 1966a; Schubert and Mamerman, 1956). Toluidine blue is the most frequently used dye. The metachromatic reaction with cationic dyes is mainly caused by the high-molecular polyanionic sulphated mucopolysaccharides, whose sulphated groups maintain their basophilia at low pH (Schubert and Mamerman, 1956). This principle has been utilized by Bloom and Kelly (1960), who demonstrated that mast cells could be stained with the basic copper phthalocyanine dye astra blue at pH 0.2 when all other tissue constituents failed to stain except some epithelial mucins and cartilage. Nowadays, astra blue is very commonly used for the staining of intestinal mast cells. Data from present paper showed that astra blue can stain the mast cells of chicken intestinal mucosa as those of the mammalians (Miller and Jarrett, 1971; Murry *et al.*, 1971). In addition, we found that the specimen, when fixed by Carnoy fixative for 12 hr and stained with toluidine blue by the method of Enerback (1966a), also displayed a considerably numerous metachromatic mast cells as stained with astra blue (data not shown). Thus mast cells which could be demonstrated well with both staining methods agreed with the classical definition (Enerback, 1966a; 1966b).

In addition to the fixative used and the fixation time, we found that the choice of dehydration procedure is also of importance in the preservation of mast cell granules.

Before embedding in paraffin, if the tissue fixed in Carnoy fixative for 12 hr was washed directly with absolute ethanol, a considerable number of mast cells were well demonstrable (Fig. 1). However, if the tissue was washed in 50% ethanol, then dehydrated through a serious of solutions of ethanol in water (70%-80%-95%-100%), almost no mast cells were demonstrated. On the other hand, the specimens fixed in Bouin fixative (fixed for 24 hr) or IFAA and washed in 50% ethanol and then dehydrated through a series of solutions of ethanol in water (70%-80%-95%-100%), still displayed numerous mast cells (Table 1). These data show that the fixation machanisms of IFAA, Bouin, and Carnoy fixatives are different.

In ordinarily used concentration (10%) formalin failed to preserve the stainability of intestinal mast cells (Table 1). It is generally accepted that formalin exerts its effect on proteins partly by combining with basic groups, which would interfere with ionic linkages between acid mucopolysaccharides and proteins and could thus be the reason for a dissolution of stainable material in intestinal mast cell

granules after fixation in high concentrations of formalin. The finding that Bouin fixative in which formalin is in concentration higher than 10%, can preserve the stainability of intestinal mast cells (Table 1) is of some interest. At present, we have not found good reason for this phenomenon. However, Table 1 reveals that all the fixatives tested contained acetic acid can fix the chicken intestinal mast cells. It appears that the presence of acetic acid is critical for the fixation of intestinal mast cells. In conclusion, whatever the machanism is involved, the Carnoy fixative is very satisfactory for the preservation of mast cells in chicken intestinal mucosa.

REFERENCES

BLOOM, G. and J. W. KELLY (1960) The copper phthalocyanine dye "astrablau" and its staining properties, especially the staining of mast cells. *Histochemie* **2**: 48-57. (Cited in Enerback, 1966b)

CARLSON, H. C. and M. A. HACKING (1972) Distribution of mast cells in chicken, turkey, pheasant, and quail, and their differentiation from basophils. *Avian Dis.* **16**: 574-577.

ENERBACK, L. (1966a) Mast cells in rat gastrointestinal mucosa. 1. Effects of fixation. *Acta. Path. Microbiol. Scandinav.* **66**: 289-302.

ENERBACK, L. (1966b) Mast cells in rat gastrointestinal mucosa. 2. Dye-binding and metachromatic properties. *Acta. Path. Microbiol. Scandinav.* **66**: 303-312.

HUMASON, G. L. (1972) *Animal tissue techniques.* (3rd ed.). W. H. Freeman and Company, San Francisco.

MILLER, H. R. P. and W. F. H. JARRETT (1971) Immune reactions in mucous membranes. I. Intestinal mast cell. response during helminth expulsion in the rat. *Immunology* **20**: 277-288.

MURRY, M., W. F. H. JARRETT and F. W. JENNINGS (1971) Mast cells and macromolecular leak in intestinal immunological reactions. The influence of sex of rats infected with *Nippostrongylus brasiliensis*. *Immunology* **21**: 17-31.

MURRY, M., H. R. P. MILLER, and W. F. H. JARRETT (1968) The globule leukocyte and its derivation from the subepithelial mast cell. *Lab. Invest.* **19**: 222-233.

SCHUBERT, M. and D. MAMERMAN (1956) Chemical theory and histochemical use. *J. Histochem. Cytochem.* **4**: 159-189.

雞腸粘膜內肥大細胞之固定效果

費昌勇　李永基

分別以數種不同之固定液及固定時間處理發現 Carnoy 固定液對雞腸粘膜內肥大細胞之固定效果最爲理想。經此液固定之組織切片置 pH 0.3 之 0.5 astra blue 水溶液中染色兩小時。然後以 eosin 對照染色 10 秒可見大量之肥大細胞和杯狀細胞。肥大細胞位於絨毛固有層內，而杯狀細胞則夾雜於絨毛上皮吸收細胞之間。僅肥大細胞和杯狀細胞染成藍色，其他細胞均呈粉紅至紅色。