were a product of the Minnesota Mining and Manufacturing Company ('Superbrite', Type 130-5005, 0.1 mm diameter) and prior to use, were treated with hot cleaning solution, washed extensively with water and dried. 15 g of dried cells, 250 g of beads and 250 ml of distilled water, all precooled in ice, were put into a 400 ml stainless steel mixing chamber which was also precooled to 0°. The chamber was then transferred into a freezing mixture, which was kept at a temperature of about −5°. It was attached to the homogenizer, and mixing at top speed was carried out for 50 min. This period of time was sufficient for virtually complete breaking of the cells, as judged by the release into solution of non-sedimentable (10,000 × *g*, 20 min) ultraviolet absorbing material (260 mμ). The temperature inside the mixing chamber was 3–4° throughout the operation. At the end of the mixing time, the supernatant was transferred into a 2 l beaker kept in ice. The beads were washed by decantation with 5 × 200 ml of ice-cold water. The supernatant and washings were combined, and centrifuged for 15 min at 3000 RPM in the large (GSA) rotor of the Servall RC-2 refrigerated centrifuge. The precipitate, which contains some unbroken cells and cell membranes, as well as small metal particles (from abrasion of the chamber and stirrer), was discarded. The supernatant was centrifuged at 9000 RPM for 15 min to precipitate the cell walls. The supernatant containing cytoplasmic material was discarded. The top white layer of the precipitate was suspended in 500 ml of water, transferred into clean centrifuge bottles and centrifuged at 9000 RPM. This procedure, resulting in further removal of contaminating cytoplasmic material from the sedimenting cell walls, was repeated twice. Another centrifugation at 3000 RPM was carried out after the last wash to remove additional impurities. This was followed by centrifugation at 9000 RPM for 15 min. The packed cell walls, which at this stage are white, were then suspended in 250 ml of water, and heated for 20 min at 100° to destroy lytic enzymes[6]. They were then treated with trypsin[7] and washed with the aid of the centrifuge three more times with water. Finally, they were lyophilized. The yield from 15 g of cells amounts to 2.0 g of walls.

The preparation obtained is homogeneous as seen in the electron microscope. The walls are rendered completely soluble by lysozyme, are free of ultraviolet absorbing material, and do not contain significant amounts of amino acids other than those usually present in *M. lysodeikticus* walls[8]. The same procedure can also be used for the disruption of small amounts of bacterial cells, using the smaller chambers of the Omni-Mixer, and the same proportions of cells, beads and water. The mixing time can, however, be shortened, since disruption of 1 g of *M. lysodeikticus* cells in the 50 ml chamber is complete within 10 min.

Résumé. Une méthode est décrite, qui permet la rupture de la paroi de bactéries en utilisant des quantités relativement larges à chaque essai, ainsi qu'un équipement de coût modique. La préparation de parois de *M. lysodeikticus* à partir d'une prise de 15 g de bactéries sèches est rapportée en exemple.

N. SHARON[9] and R. W. JEANLOZ

Laboratory for Carbohydrate Research, Departments of Biological Chemistry and Medicine, Harvard Medical School and the Massachusetts General Hospital, Boston (USA), August 15, 1963.

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Distribution of Mast Cells in the Mucous Membrane of the Human Nasopharynx

The distribution of mast cells in the various animal and human tissues is well documented in the literature[1–5]. Under normal conditions, certain organs show high density of mast cells while others contain fewer, depending on the amount of connective tissue present[6]. However, a notable increase in the mast cell population is also seen in chronic inflammatory reaction[7], epithelial metaplasia[8], certain hormonal influences[9], precancerous lesions and in tissues to which carcinogenic chemicals have been applied[10–13].

According to HLAVÁČEK and LOJDA[7], the human respiratory tract is rich in mast cells which are increased in chronic inflammatory conditions. Carcinoma of the nasopharynx being the commonest malignant neoplasm amongst the Chinese[14,15], it is intended to study the changes in mast cell population in premalignant lesions of the nasopharyngeal mucosa. The purpose of this preliminary report is to provide data on the normal distribution of mast cells in the mucosa of the human nasopharynx. The multiracial population in the state of Singapore made it possible to compare the findings in specimens obtained from four ethnic groups.

Material and Methods. The tissues for this study were obtained from sixty medico-legal necropsies of apparently healthy male adults who died as a result of accidents, homicide or suicide. The persons necropsied belonged to four racial groups: 20 Chinese, 20 Indians, 10 Malays and 10 others (6 English, 2 Japanese, 1 Javanese and 1

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Eurasian). In each case the whole of the nasopharyngeal mucosa was obtained en bloc. Six sections from each specimen were taken from identical areas in the anterior, posterior and lateral walls.

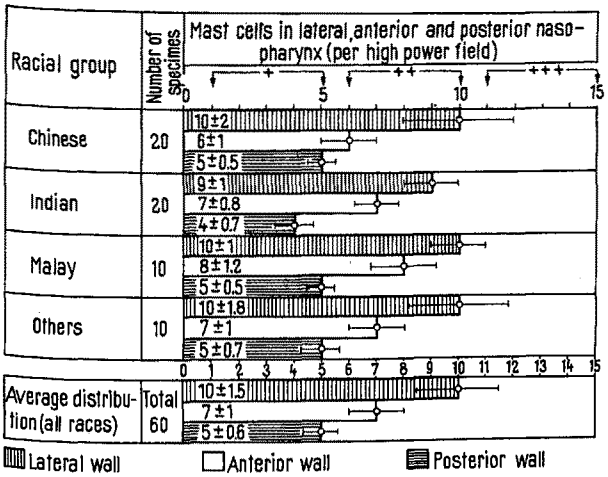

Fig. 1. Histogram showing the distribution of mast cells in the lateral, anterior and posterior walls of the human naso-pharynx. The pattern of distribution is shown for four racial groups and an average for the total number is given.

show large numbers of mast cells (++) that is, ranging from 9 ± 1/hpf to 10 ± 2/hpf. The anterior wall, next in order, falls into the same group (++) but averaging 7 ± 1/hpf. The posterior wall contains no more than 5 mast cells per high power field. The average for all the sixty specimens is shown at the bottom of Figure 1. It is noteworthy that there seems to be no racial difference in the distribution of mast cells (Figure 1).

An even distribution of mast cells is seen in areas covered by ciliated epithelium (Figure 2A). However, patchy areas of squamous metaplasia in the lateral walls are accompanied by an increase in the number of mast cells (Figure 2B). Although the posterior wall is mostly covered by squamous epithelium, the mast cell count is low, rarely above 5/hpf. Whenever lymphoid follicles are present in the tunica propria, the mast cells seem to detour along the lower border of the follicle and merge into the underlying connective tissue and muscles.

Discussion. Despite the voluminous literature, little is known about the origin and function of mast cells[18–20]. According to SIMPSON[21], there is no common pattern of mast cell changes with age. NOZAKA and SIMPSON[5] found no difference in distribution between the male and female. The present study was limited to the regional distribution of mast cells in four racial groups and the factors of age and sex were not taken into account.

Zusammenfassung. Die Mastzellen sind in der Schleimhaut des Naso-Pharynx-Gebiets ubiquitär, und ihre Zahl

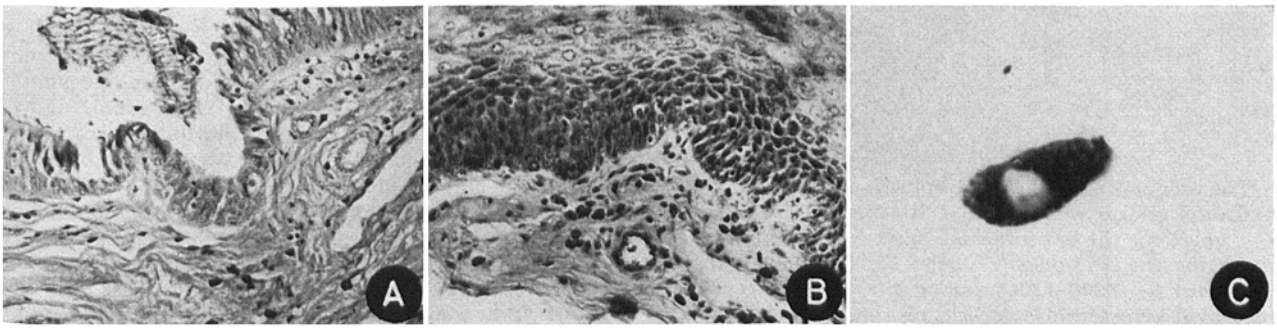

Fig. 2. 2A = Section from lateral wall of naso-pharynx covered by ciliated epithelium. The loose connective tissue of the tunica propria contains evenly distributed mast cells. Modified Dominici stain. 130 ×. 2B = An area of squamous metaplasia in which denser aggregates of mast cells are shown. Modified Dominici stain. 130 ×. 2C = Appearance, under oil, of a mast cell stained by the modified Dominici stain. 1200 ×.

The sections, alcohol- and formalin-fixed, were stained by a modified Dominici stain adopted by LITT[16]. The total number of 360 sections were examined under a Leitz Ortholux microscope (objective 45:1, N.A. 0.65, and periplan × 8 eyepieces). The metachromatically stained mast cells were counted in the tunica propria per high power field (hpf) and at least twenty readings taken in each section. The resultant figures were tabulated and the standard deviation calculated for each region using the method recommended by BRADFORD HILL[17]. Furthermore, a grading system similar to that given by DUNN and MONTGOMERY[8] was introduced. The number of mast cells per high power field (hpf) was represented as follows: 0; 1–5/hpf = +; 6–10/hpf = ++; 11–15/hpf = +++.

Results. The results are summarized in Figure 1. The lateral walls, particularly around the Eustachian tube opening and areas of squamous metaplasia, consistently

ist in den seitlichen Wänden erhöht. Es gibt aber keine Verteilungsunterschiede in den vier untersuchten Rassengruppen.

M. Y. ALI

Department of Pathology, University of Singapore, General Hospital, Singapore (Malaysia),
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