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Mast cells in the normal uterus and in dysfunctional uterine bleeding

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Summary

Mast cells in the human uterus were examined following fixation in 10% formalin and staining with Azure B. Mast cells were present in all parts of the corpus uteri. Cyclical changes were observed by light microscopy for mast cell numbers/mm^2 in the functional endometrium, basal endometrium and the endometrial/myometrial border throughout the menstrual cycle. No significant differences were found for mast cell numbers in the menstrual, proliferative or secretory phases of the menstrual cycle in dysfunctional uterine bleeding (DUB). No correlation was found between mast cell numbers in the uterine wall in the secretory phase of the menstrual cycle and average menstrual blood loss for patients with DUB.

Mast cell; Uterus; Uterine bleeding, dysfunctional

Introduction

The presence of mast cells in the endometrium of the human uterus was first reported by D'Erchia in 1897 [1]. Although these cells contain highly active biological substances, their physiological role in the human uterus remains unknown. Heparin and histamine are present in mast cells and the amount of these substances in tissues correlates with the number of mast cells [2,3]. Heparin-like activity has been found in uterine fluid, with an increase during the menstrual cycle and a decrease during menstruation [4]. Histamine causes vasodilation, hyperaemia and increased capillary permeability [5–7]. Mast cells may therefore be involved in the process of menstruation [8–12]. We have investigated mast-cell numbers and their location in the uterine wall throughout the normal menstrual cycle and in patients with dysfunctional uterine bleeding (DUB).

Patients, Materials and Methods

Patients

The control group consisted of 28 patients with normal menstrual loss on whom a vaginal hysterectomy was carried out for utero-vaginal prolapse (Group 1, Table I). The group with dysfunctional uterine bleeding consisted of 37 patients who had a hysterectomy (Group 2, Tables II and III). The menstrual blood loss was measured in two consecutive menses prior to hysterectomy in 11 of the patients in Group 2. All of the patients

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Table I

Summary of patients with normal menstrual cycles having hysterectomy for utero-vaginal prolapse (Group 1, $n = 28$)

Stage of menstrual cycle	No. of patients	Mean age (years)	Age range (years)	Mean ± SD Duration of vaginal bleeding (range) (days)	Mean ± SD Duration of menstrual cycle (range) (days)	Mean No. of pregnancies (range)
Menstrual	5	46	41–50	5.16 ± 1.04 (4–6)	28.83 ± 0.76 (28–29.5)	5.5 (4–7)
Early proliferative	4	37	29–44	4.50 ± 0.68 (4–5.5)	27.66 ± 0.57 (27–28)	3.0 (0–6)
Mid proliferative	2	45	42–49	4.25 ± 1.76 (3–5.5)	26.00 ± 4.94 (22.5–29.5)	3.5 (3–4)
Late proliferative	3	45	41–49	6.66 ± 1.52 (5–8)	24.50 ± 3.27 (21–27.5)	7.0 (6–9)
Early secretory	5	43	37–49	4.12 ± 1.10 (3–5.5)	27.12 ± 4.21 (21–30.5)	3.4 (2–6)
Mid secretory	3	36	31–40	5.50 ± 0.70 (5–6)	29.75 ± 2.47 (28–31.5)	2.6 (0–5)
Later secretory	6	40	36–45	5.60 ± 1.63 (3.5–7)	28.50 ± 0.86 (28–30)	4.8 (1–10)

in Group 2 complained of excessive menstrual bleeding, and the diagnosis of dysfunctional uterine bleeding was based on the absence of any other abnormality in the endometrium at diagnostic curettage and the subsequent histological examination of the uterus. For each patient tissue sections were stained with haematoxylin and eosin for histological dating of the menstrual cycle according to Noyes et al. [13] modified by Fox [14]. All patients dated as secretory phase were post-ovulatory; patients in the menstrual or proliferative phase were ovulatory in the cycles prior to the cycle studied.

Tissue processing and staining

Uterine biopsies of the anterior wall of the upper uterine body from the endometrium to the serosal surface were obtained immediately following hysterectomy and placed in unbuffered 10% (v/v) formalin. 10% formalin has been shown to be a suitable fixative for human mast cells [15]. As staining of mast cell granules was the criterion for identification of mast cells, non-granular mast cells could not be positively identified. After fixation, biopsies were embedded in paraffin and 4-μm thick sections were cut. The sections were stained with 1% Azure B for 2 min, using the uranyl

TABLE II

Summary of patients having hysterectomy for dysfunctional uterine bleeding (Group 2, $n = 37$)

Stage of menstrual cycle	No. of patients	Mean age (years)	Age range (years)	Mean ± SD Duration of vaginal bleeding (range) (days)	Mean ± SD Duration of menstrual cycle (range) (days)	Mean No. of pregnancies (range)
Menstrual	6	43	34–51	7.33 ± 2.67 (3.5–10)	27.80 ± 4.96 (20–33)	5.6 (2–11)
Early proliferative	2	40	32–48	5.50 ± 0.70 (5–6)	– *	2.5 (2–3)
Mid proliferative	2	45	40–51	9.50 ± 6.36 (5–14)	31.50 ± 4.94 (28–35)	4.0 (3–5)
Late proliferative	3	49	47–51	6.75 ± 1.06 (6–7.5)	26.75 ± 3.18 (24.5–29)	3.0 (0–5)
Early secretory	6	40	33–46	8.30 ± 4.86 (4.5–15)	28.62 ± 4.02 (26–34.5)	3.6 (0–6)
Mid secretory	8	40	36–46	8.12 ± 2.50 (4.5–12)	29.18 ± 6.91 (21–45)	3.6 (1–6)
Late secretory	10	39	32–47	6.70 ± 2.03 (4–11)	26.20 ± 2.54 (21–29.5)	3.9 (3–6)

* data not available

TABLE III

Summary of patients having hysterectomy for dysfunctional uterine bleeding with known menstrual blood loss (Group 2, $n = 11$). These patients are included in those listed in Table II.

Stage of menstrual cycle	No. of patients	Mean ± SD Menstrual blood loss (mls)	Mean age (years)	Age range (years)	Mean ± SD Duration of vaginal bleeding (range) (days)	Mean ± SD Duration of menstrual cycle (range) (days)	Mean No. of pregnancies (range)
Early secretory	4	233 ± 144	40	33–44	8.3 ± 5.7 (5–15)	26	4.6 (4–6)
Mid secretory	4	227 ± 141	38	36–45	8.25 ± 3.2 (4.5–12)	25.00 ± 3.6 (21–28)	2.2 (1–3)
Late secretory	3	244 ± 69	40	37–47	8.00 ± 3.6 (4–11)	26.66 ± 1.52 (25–28)	4.3 (3–6)

nitrate metachromatic method of Hughesdon [16]. For staining with Alcian Blue, sections were taken to water and stained with 0.1% Alcian Blue in 0.7 N HCl followed by distilled water, stained for 30 s in 0.5% eosin, rinsed in distilled water, dehydrated and mounted in DPX (modified from Bloom and Kelly [17]). Staining of mast cells with Azure blue was used in the uterine biopsies as staining with Alcian blue proved unreliable for mast cells in the endometrium.

Mast cell quantitation

Mast cell numbers/mm^2 were determined separately in four areas: functional endometrium, basal endometrium, myometrial side of the endometrial/myometrial border and the myometrium. Total counts of mast cells in a total cross-section of the uterus were not undertaken due to the variations in size observed for the uterus. A 10 mm^2 eyepiece graticule was orientated along the section. Mast cells in the endometrium and endometrial/myometrial border were counted at magnification ×625 and mast cells in the myometrium at magnification ×312 (light microscope). All mast cell counts were performed on coded slides and sections used were not consecutive. Care was taken to count in evenly distributed areas of tissue as problems were encountered with oedematous changes in the endometrium commonly associated with oestrogen secretion. Due to the loss of tissue during menstrual shedding, no attempt was made to quantitate mast cells in the functional endometrium in the present study. For each patient 2 sections and 20 areas, as outlined by the grid of the eyepiece graticule in each of the above-defined anatomical areas, were counted and the mean mast cell count per grid area calculated. Results were expressed as number of mast cells per mm^2.

Menstrual blood loss measurement

Menstrual blood loss was measured in two consecutive menstrual cycles using the method of

TABLE IV

The mean number of mast cells/mm^2 and the range of mast cell numbers in the uterine wall for Groups 1 and 2

	Endometrium		Myometrium	
	Functional endometrium	Basal endometrium	Endometrial myometrial border	Myometrium
Group 1 (Control)	$n = 23$ 0.94 ± 2.25 (0–25)	$n = 28$ 5.57 ± 6.13 (0–56.25)	$n = 28$ 10.20 ± 7.80 (0–75)	$n = 28$ 31.52 ± 7.93 (0–100)
Group 2 (DUB)	$n = 31$ 0.86 ± 1.18 (0–25)	$n = 37$ 7.11 ± 5.41 (0–56.25)	$n = 37$ 10.76 ± 6.40 (0–112.5)	$n = 36$ 32.20 ± 9.01 (0–121.87)

TABLE V

The mean number of mast cells/mm^2 and the range in the uterine wall of patients in Group 2 with known menstrual blood loss

Area of uterine wall	*n*	Phase of menstrual cycle		
		Early secretory ($n = 4$)	Mid secretory ($n = 4$)	Late secretory ($n = 3$)
Functional endometrium	11	1.28 ± 1.49 (0–18.75)	0.50 ± 0.41 (0–12.50)	1.53 ± 1.50 (0–12.50)
Basal endometrium	11	7.73 ± 5.57 (0–25.00)	3.27 ± 1.48 (0–25.00)	4.47 ± 2.16 (0–18.75)
Endometrial/myometrial border	11	10.63 ± 6.06 (0–50.00)	5.77 ± 1.20 (0–31.25)	9.63 ± 4.49 (0–37.50)
Myometrium	11	28.44 ± 8.32 (3.12–78.12)	29.64 ± 9.68 (0–75.00)	29.54 ± 13.80 (6.25–71.87)

Hallberg et al. [18] as modified by Shaw et al. [19]. The error of recovery ranged from 0.1 to 13.0% with an average of 4.9% [20].

Statistical analysis

All results were analysed by a DEC-20 (Digital Equipment Corporation, Series 20) using the stat-

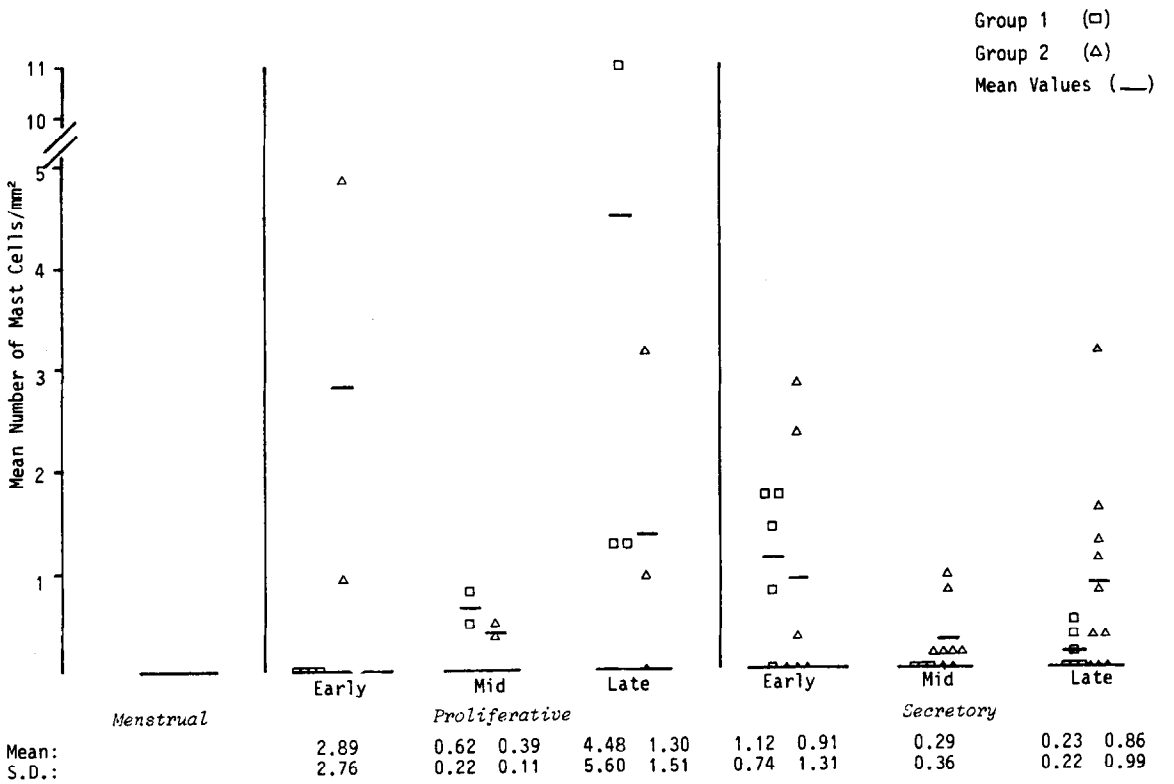

Fig. 1. Relation between the mean number of mast cells/mm^2 in the functional endometrium and phases of the menstrual cycle for individuals in Groups 1 and 2. Group 1 (□), Group 2 (△), Mean values (–).

istical package SPSSX (Statistical Package for the Social Sciences). The statistical tests used included a Spearman's rank correlation test, a two-way analysis of variance and a Mann-Whitney U Wilcoxan rank sum W test.

Results

Location of mast cells

Mast cells were identified in the four areas of the corpi uteri. In the stroma of the functional endometrium mast cells showed a poorer staining quality than those in the myometrium. In the endometrium densely stained mast cells predominated in the basal regions which interdigitate with the myometrium. Endometrial mast cells were normally seen in the stroma close to epithelial cells in glandular tissue and were absent in the glandular epithelium. In some parts of the basal endometrium mast cells were found in clusters. A large number of mast cells was usually present in areas of dense stroma in the basal endometrium. In the myometrium, mast cells were found in and between muscle bundles and around blood vessels. Mast cells were sometimes seen in the endometrium near small blood vessels or lymphatics.

Mast cell numbers in the uterine wall

Mast cells were found to have a skewed distribution in the four areas of the uterine wall (Table IV). Overall highly significant correlations ($P < 0.001$) were found between the four uterine areas except functional endometrium versus endometrial/myometrial border ($P < 0.05$) and functional endometrium versus myometrium ($P > 0.1$).

Results for correlation of mast cell numbers with age differed for DUB Group 2 in that correlation of functional endometrium with age was insignificant and correlation of basal endometrium with age was less significant ($P < 0.05$, as

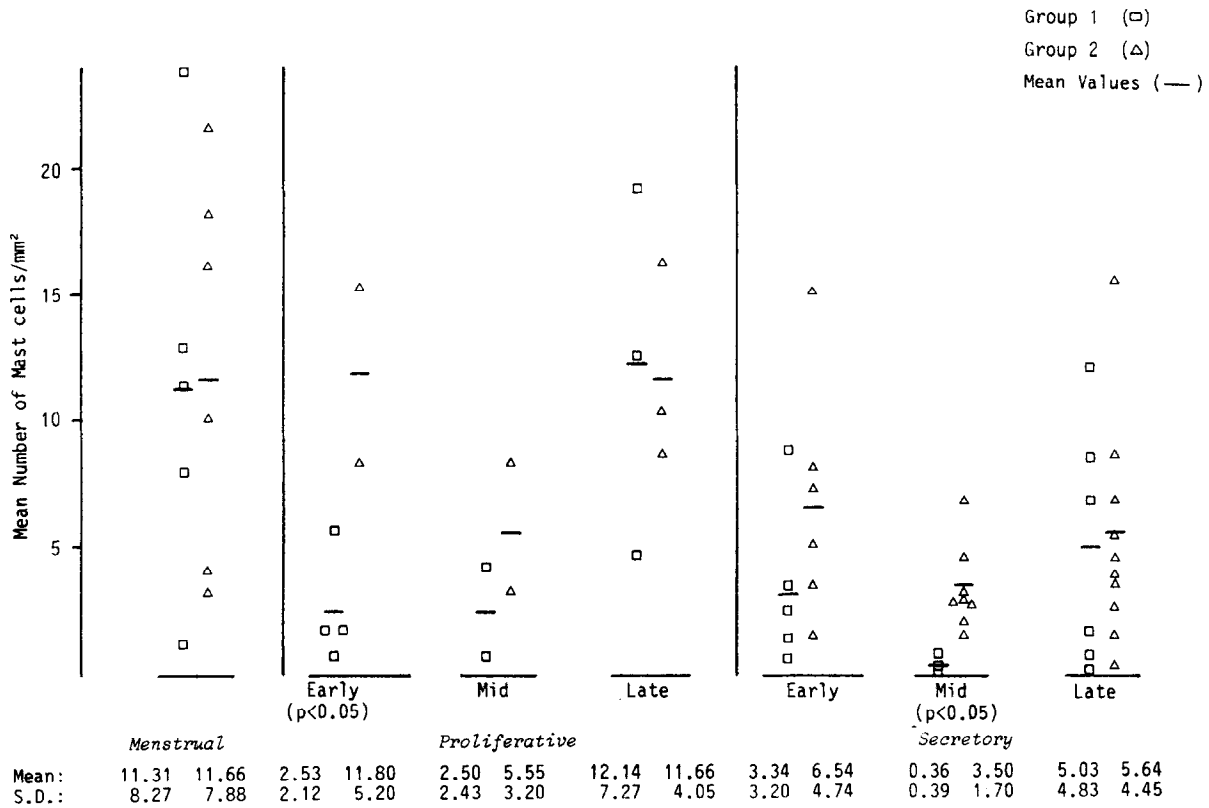

Fig. 2. Relation between the mean number of mast cells/mm² in the basal endometrium and phases of the menstrual cycle for individuals in Groups 1 and 2. Group 1 (□), Group 2 (△), Mean values (–).

compared with $P < 0.01$). Correlation of values for both groups for the myometrium with the number of pregnancies was insignificant; however, correlation of values for both functional and basal endometrium with the number of pregnancies for Control Group 1 were significant ($P < 0.05$).

Mast cell numbers for phases of the menstrual cycle

In view of the skewed distribution data were expressed as $\log_{10}$ for comparing phases within and between groups using a two-way analysis of variance. A significant change in the mean number of mast cells/mm^2 was seen for the functional endometrium ($P < 0.005$) and the basal endometrium ($P < 0.01$) throughout the menstrual cycle for both groups. Mast cell counts in the basal endometrium in the early proliferative and mid-secretory phases of the menstrual cycle were significantly lower in Control Group 1 than DUB group 2 ($P < 0.05$) (Figs. 1–4).

Mast cell numbers and menstrual blood loss

No correlation was found between menstrual blood loss and mean mast cell numbers/mm^2 for the four uterine areas quantitated in biopsies from patients in Group 2b.

Discussion

In the present study mast cells were identified in all areas of the anterior wall of the corpus uteri, which were stained with the uranyl nitrate metachromatic method following fixation in 10% formalin.

Previous studies have been based on subjective impressions of mast cell numbers in the uterus unlike the present study where mast cell numbers

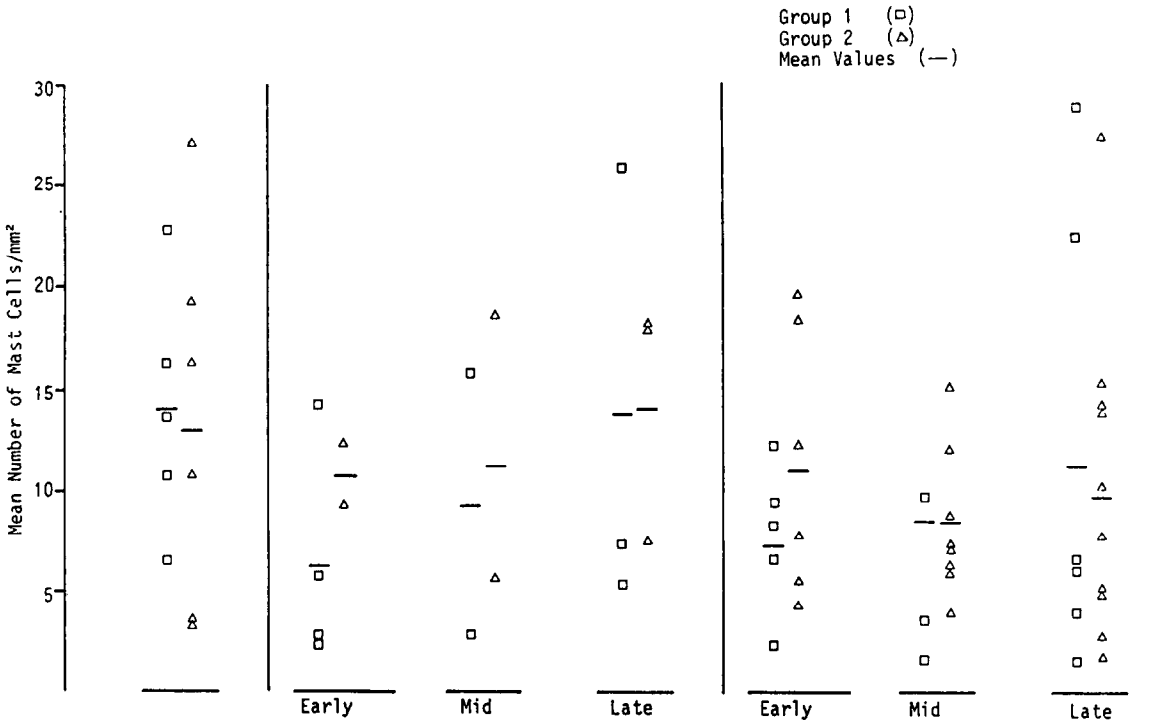

Fig. 3. Relation between the mean number of mast cells in the endometrial/myometrial border and phases of the menstrual cycle for individuals in Groups 1 and 2. Group 1 (□), Group 2 (△), Mean values (–).

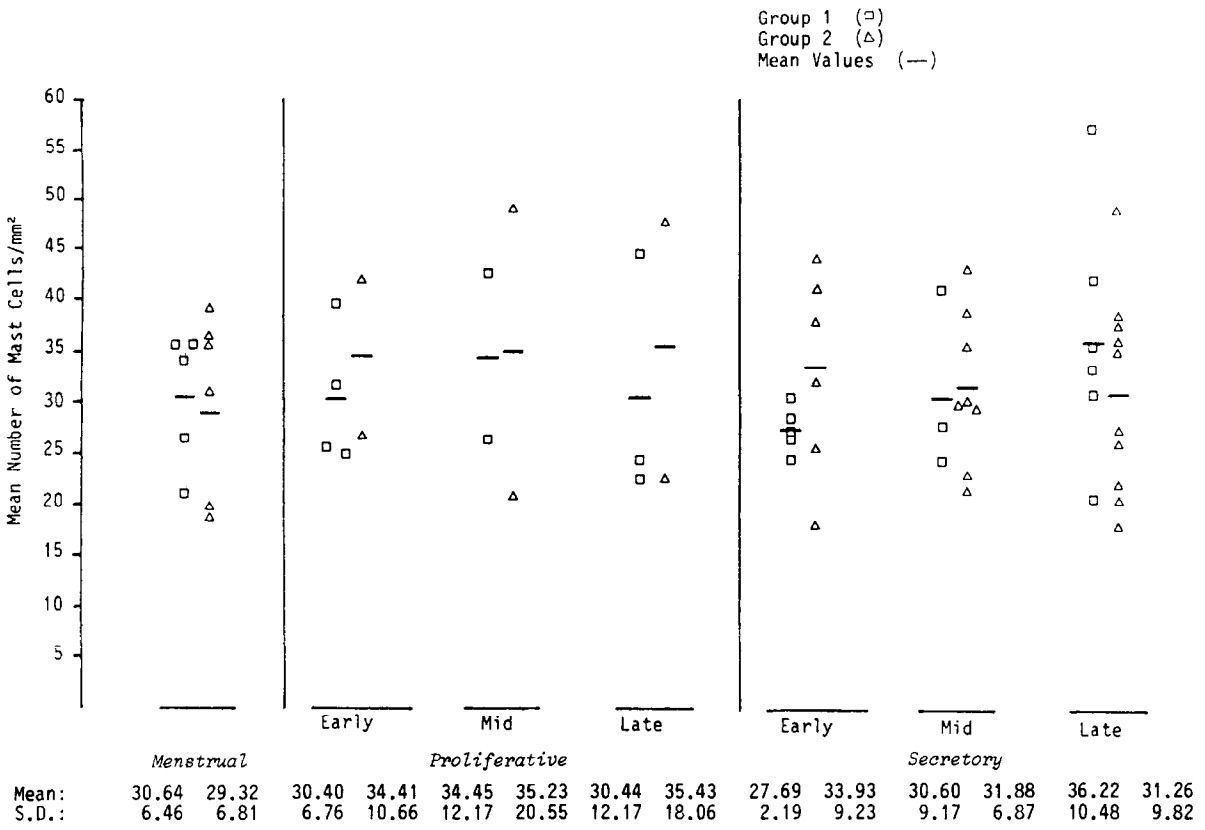

Fig. 4. Relation between the mean number of mast cells/mm^2 in the myometrium and phases of the menstrual cycle for individuals in Groups 1 and 2. Group 1 (□), Group (△), Mean values (–).

were expressed per mm^2 to establish a yardstick by which variations from the norm may be detected. Division of the uterine wall into specific areas for quantitation helped to reduce the standard deviation of the mean number of mast cells/mm^2 in the uterine wall. The presence of more mast cells in the myometrium than in the endometrium has also been shown in mammals, e.g., the mouse [21], rat [22] and the Syrian hamster [23]. Bergman et al. [21] observed an increase in mast cells in the uterine wall of the mouse at each pregnancy; an increase that persisted for at least 3 months following delivery. In the present study significant correlations with the number of pregnancies, were observed only in Control Group 1 in mean number of mast cells/mm^2 in the functional and basal endometrium. However, due to both the observation of cyclical changes in mast cell numbers and patient variability, a larger group of patients with normal menstrual cycles would be necessary before overall variations from the mean could be detected.

Earlier workers reported the absence of mast cells in the endometrium [24–28]. This is in contrast to the results presented here as, although mast cells were not observed in each patient in the functional endometrium (Fig. 1), they were present in the basal endometrium in all cases (Fig. 2). Unlike previous studies [29–31], although overall marginally higher values were observed for both groups for the proliferative phase when compared with the secretory phase, the difference was not very obvious when individual values in the cycle were taken into consideration. Although Mehra et al. [10] observed an increase in mast-cell number in the endometrium of patients with IUDs who complained of menorrhagia, this was only observed in the bleeding phase and controls were not

matched for phase of menstrual cycle. In the present study high numbers of mast cells were seen in both groups in the menstrual phase when compared to other phases of the menstrual cycle.

Abnormal bleeding tendencies related to an excess of circulating anticoagulant, most likely heparin or a heparin-like substance, was described by Allen et al. [32]; an increase of this anticoagulant during menstruation and in certain cases of menorrhagia has been observed by Elghammer et al. [33]. Foley et al. [4] showed that heparin-like activity in uterine fluid increased throughout the menstrual cycle and decreased during menstruation. They found no correlation between the numbers of endometrial or myometrial mast cells and heparin-like activity in uterine fluid. The present study demonstrates the necessity of studying mast cells in different areas of the uterine wall before conclusions can be drawn about their function in normal and pathological states.

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