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Dilution Assessment of Cervicovaginal Secretions Obtained by Vaginal Washing for Immunological Assays

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Local immunological defense mechanisms in the cervicovaginal mucosa currently remain incompletely defined, especially from a quantitative point of view. Addition of an inert substance, lithium chloride (LiCl), into the washing buffer used to carry out the vaginal washing for collecting cervicovaginal secretions and measurement of its concentration with a flame absorption spectrophotometer, before and after the specimen is sampled, permits the quantification of the volume of cervicovaginal secretions collected and the approximation of the dilution factor of a soluble component introduced by the washing. Lithium, at a concentration of 10 mM, gives the best precision of measurement and has no effect on the results of the immunoassays. In a population of 27 nonpregnant women (age range, 18 to 45 years), the volume of cervicovaginal secretions collected by vaginal washing with 3 ml of LiCl–phosphate-buffered saline was 12% ± 3.2% (mean ± standard deviation) of the total volume and showed large interindividual variations (range, 5.6 to 18.8%); the mean dilution factor of a soluble component from the vaginal secretions was 9.9% ± 2.8% (range, 6.3 to 18.8%). According to the date of the menstrual cycle, the mean volume of collected cervicovaginal secretions was significantly increased in the luteal phase in comparison with the follicular phase; conversely, the mean dilution factor of a soluble component was more important in the follicular than in the luteal phase. These features strengthen the need to quantify accurately the dilution factor introduced by vaginal washing when studying cervicovaginal immunity.

Mucosal immunity of the female genital tract has recently gained special attention as an important factor that could modulate the transmission of many sexually transmitted diseases, including human immunodeficiency virus (HIV) infection. Furthermore, current concepts in vaccine design against viral infections acquired through sexual portals focus on the potential interest in inducing specific mucosal immunity at the sites of sexual exposure in association with systemic and cellular immune responses. However, local immunological defense mechanisms of the cervicovaginal mucosa currently remain incompletely defined, especially from a quantitative point of view.

Humoral cervicovaginal immunity can be investigated easily in cervicovaginal secretions (CVS) collected by a simple and painless vaginal washing (1). One obvious methodological problem occurring with this sampling method to quantify accurately immunoglobulins or antibodies at the level of the cervicovaginal mucosa is the lack of precise knowledge about the dilution factor introduced by the vaginal washing. This parameter likely depends on numerous factors, including not only the washing procedure itself, but also biological factors, such as the volume of CVS, which is known to be physiologically influenced by the menstrual cycle.

Recently, a new method to assess the dilution of nasopharyngeal secretions obtained with a suction device has been reported (7). It consists of adding an inert substance, lithium chloride (LiCl), to the sample diluent and of measuring its concentration before and after the specimen is added. This procedure permits the calculation of the dilution factor introduced by the sample collection. The aim of the present study was to develop a useful and reproducible method to collect and quantify the CVS obtained by vaginal washing, using lithium in the washing buffer as a marker of dilution, and to analyze its suitability for immunological use.

(Preliminary results from this work have been presented at the 34th Interscience Conference on Antimicrobial Agents and Chemotherapy, Orlando, Fla., 4 to 7 October 1994.)

MATERIALS AND METHODS

Principle of the method and mathematical approach. CVS consist of a solid phase, containing principally exfoliated epithelial cells and, to a lesser extent, leukocytes, polymerized glycoproteins secreted with the cervical mucus, and bacteria, and of a liquid phase which contains electrolytes and various secreted or transudated proteins, such as immunoglobulins, glycoproteins, albumin, and lactoferrin. Therefore, the CVS collected from a vaginal washing can be considered the sum of an essentially cellular solid phase and of a liquid phase, the latter being the most important since it concerns the phase with which local humoral immunity studies are usually carried out.

A volume, v, of CVS is collected by a standardized vaginal washing using a diluent sample containing a well-defined concentration of Li, $[Li]_1$. The collected sample is then centrifuged, the pellet is separated, and the supernatant is stored frozen. The Li concentration after the specimen is added, $[Li]_2$, is measured in the supernatant of the vaginal washing sample.

Because Li does not exist in human secretions, the quantity of Li introduced into the vagina by the washing procedure with a volume, V, of washing buffer at $[Li]_1$ is diluted in a volume $V + v_{Li}$, where v_{Li} is the volume of distribution of the Li cation in CVS. This volume encompasses the volume of the liquid phase (l) and the volume of the diffusible part of the solid phase (s_d), including cervical mucus and dead cells. Li is normally excluded from the volume of living cells (s_c). The volume v of collected CVS is given by:

$$v = l + s_d + s_c = v_{Li} + s_c \quad (1)$$

The equation linking the final concentration ($[Li]_2$) in the vaginal washing sample is the following:

$$[Li]_1 \cdot V = [Li]_2 \cdot (V + v_{Li}) \quad (2)$$

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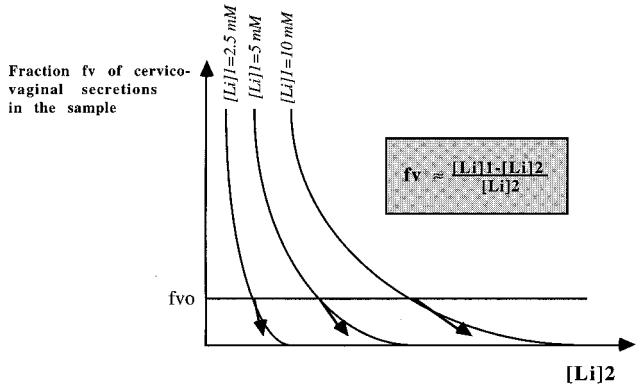

FIG. 1. Theoretical curves representing the fraction f_v of CVS collected by vaginal washing, as expressed by formula 4a and calculated as a function of three initial $[Li]_1$ values, 2.5, 5, and 10 mM, and the $[Li]_2$ value. Note that the slope of each curve is always decreasing, the steepness depending on the inverse of $[Li]_1$. For each curve, the tangent at a given observed fraction (f_{vo}) is traced.

Finally, the fraction of CVS collected by the vaginal washing (f_v; i.e., v/V) is calculated from equations 1 and 2:

$$f_v = \frac{v}{V} = \frac{[Li]_1 - [Li]_2}{[Li]_2} + \frac{s_c}{V} \tag{3}$$

Assessment of the volume of Li distribution in CVS. In a first approach, we verified that the volume v_{Li} is approximately equal to the volume v of the whole collected CVS. To evaluate this assumption, we determined the percentage of living cells in the pellet of vaginal washings from five women with trypan blue vital staining. For these women, the mean percentage of living cells, corresponding mainly to epithelial cells, was 14.3% ± 16% (median, 5%; range, 0.15 to 33%), and the mean volume of living cells in the pellet of vaginal washings (i.e., s_c) was 38.2 ± 42 μl (median, 18.9 μl; range, 5.1 to 99 μl). The ratio s_c/V ranged from 0.002 to 0.033 and can be considered negligible in the calculation of f_v by formula 3.

Estimation of f_v. Since the ratio s_c/V is small, f_v can be approximated by:

$$f_v = \frac{v}{V} \approx \frac{[Li]_1 - [Li]_2}{[Li]_2} \tag{4a}$$

The volume v is accordingly estimated by equation 4b:

$$v \approx \frac{[Li]_1 - [Li]_2}{[Li]_2} \cdot V \tag{4b}$$

Influence of $[Li]_1$ on the precision of the measure. Formula 4a demonstrates that f_v is a function of both $[Li]_1$ and $[Li]_2$. The first-order derivative of f_v is given by formula 5a, and since $[Li]_1$ and $[Li]_2$ are proportional according to formula 2, f_v' can be approximated by formulae 5b and 5c:

$$f_v' = \frac{df_v}{d[Li]_2} = -\frac{[Li]_1}{([Li]_2)^2} \tag{5a}$$

$$f_v' \approx -\frac{k}{[Li]_2} \qquad k > 0 \tag{5b}$$

$$f_v' \approx -\frac{k'}{[Li]_1} \qquad k' > 0 \tag{5c}$$

where k and k' are constants.

Figure 1 shows the theoretical curves representing f_v as a function of $[Li]_2$ for three twofold dilutions of the initial concentration of Li in the washing buffer, $[Li]_1$. According to formula 5c, the slope of each curve is always decreasing, and the abruptness depends on the inverse of $[Li]_1$. For a given variation of $[Li]_2$, the variation of f_v will be more significant if $[Li]_1$ is low. For instance, an error in measuring $[Li]_2$ will entail an error in measuring f_v that will be reduced for elevated values of $[Li]_1$ and raised for low values of $[Li]_1$. Consequently, if one considers that the observed variance of f_v (σ^2_o) is the sum of the variances in the measure of $[Li]_2$ (σ^2_m) and of the intrinsic variance (σ^2_i) of v for each woman, the use of a high value of $[Li]_1$ could be more convenient for evaluating σ^2_i, whereas the use of a low $[Li]_1$ value, which tends to increase σ^2_m, should increase σ^2_o and, as a result, mask σ^2_i. Note that σ^2_i does not depend at all on $[Li]_1$. These theoretical considerations prompted us to evaluate in practice various $[Li]_1$ values.

Evaluation of the dilutions of the whole collected CVS as well as of their solid and liquid phases. The dilution factor of the whole CVS (d_v) in the collected sample is simply given by formula 6:

$$d_v = \frac{1}{f_v} \approx \frac{[Li]_2}{[Li]_1 - [Li]_2} \tag{6}$$

The dilution factor of the solid phase (d_s) can also be estimated easily by the volume of the pellet (v_p) obtained after centrifugation of the diluted sample and of the volume V:

$$d_s = \frac{1}{v_p/V} \tag{7}$$

The dilution factor of the liquid phase of the collected CVS (d_l) corresponds to the inverse of the fraction of the liquid phase of CVS diluted in the collected sample and can be calculated theoretically with formulae 1 and 3 as a function of $[Li]_1$, $[Li]_2$, V, and s_d. However, the precise measurement of s_d is difficult, and the exact expression of d_l is not possible in practice.

Approximation of the dilution factor of the soluble components of CVS collected by vaginal washing. One can suppose that the volume of distribution of the liquid-phase components of CVS is the same as that of the cation Li (i.e., v_{Li}). At equilibrium, a soluble component x at a quantity q_x is at a concentration C_x in the volume $V + v_{Li}$.

According to formula 2, q_x can be expressed by:

$$q_x = C_x \cdot (V + v_{Li}) = C_x \cdot V \cdot \frac{[Li]_1}{[Li]_2} \tag{8}$$

Within the cervicovaginal mucosa, the same quantity is distributed approximately in volume v, at a concentration c_x:

$$q_x \approx c_x \cdot v \tag{9}$$

The evaluation of the dilution factor of a soluble component (d_x) from the liquid phase of the collected CVS is then given as follows, according to formulae 4a, 8, and 9:

$$d_x = \frac{c_x}{C_x} \approx \frac{[Li]_1}{[Li]_1 - [Li]_2} \tag{10}$$

Finally, the evaluation of c_x permits the quantitative approximation of the concentration of the soluble component x within the cervicovaginal mucosa (i.e., c_x) as follows:

$$c_x \approx \frac{[Li]_1}{[Li]_1 - [Li]_2} \cdot C_x \tag{11}$$

Subjects. Fifty-nine healthy women living in Gabon (n = 31) and France (n = 28) were recruited after informed consent was obtained. They did not have evidence of sexually transmitted diseases at genital examination and lacked any vaginal discharge. Women with menses, genital bleeding, or amenorrhea and pregnant women were excluded. For each woman, her age and the day of her menstrual cycle (J1 to J14, follicular phase; J15 to J28, luteal phase) were recorded.

Procedures. (i) Collection, treatment, and storage of CVS. After introduction of a speculum, CVS were collected by a standardized 60-s washing with 3 ml of LiCl–phosphate-buffered saline (PBS; pH 7.2, 150 mmol/liter; BioMérieux, Marcy l'Etoile, France). The sample was then placed immediately in thawing ice for less than 3 h. After centrifugation at 1,000 × g for 5 min, the final supernatant was aliquoted (200 μl) and stored at −20°C. The vaginal washings were performed by two executants (L.B. and C.T.-B.). Three different concentrations ($[Li]_1$) of LiCl (Sigma Chemical Co., St. Louis, Mo.) in the sample diluent, 2.5, 5, and 10 mM, were tested in three groups of nonselected women.

(ii) Lithium concentration in the sample. The Li concentrations in Li-PBS ($[Li]_1$) and in the vaginal washing ($[Li]_2$) were assayed in parallel by a flame atomic absorption spectrophotometer (PHF 102 T; ISA Biologie). The assay rate per sample was 21 s. At every 10th measurement, the zero was calibrated with distilled H_2O, and the calibration for Li was checked with a 1-meq/liter standard. The linearity range of the spectrophotometer, given by the manufacturer, was 0 to 3 meq/liter for Li, its precision was 0.1 meq/liter, its variation coefficient was 2.5%, and its specificity for the Li in presence of the cations Na^+ and Ca^{2+} was less than 0.1 meq/liter.

To save the maximum of the supernatant of vaginal washing for immunological assays, the determination of $[Li]_2$ was usually or preferentially carried out after dilution in a convenient volume of distilled H_2O to maintain the final Li concentration in the linearity range for Li measurements. Then, for $[Li]_1$ values of 10, 5, and 2.5 mM, totals of 125, 125, and 185 μl, respectively, of the supernatant were diluted in 250, 250, 185 μl, respectively, of distilled H_2O to determine the $[Li]_2$ values in the collected sample.

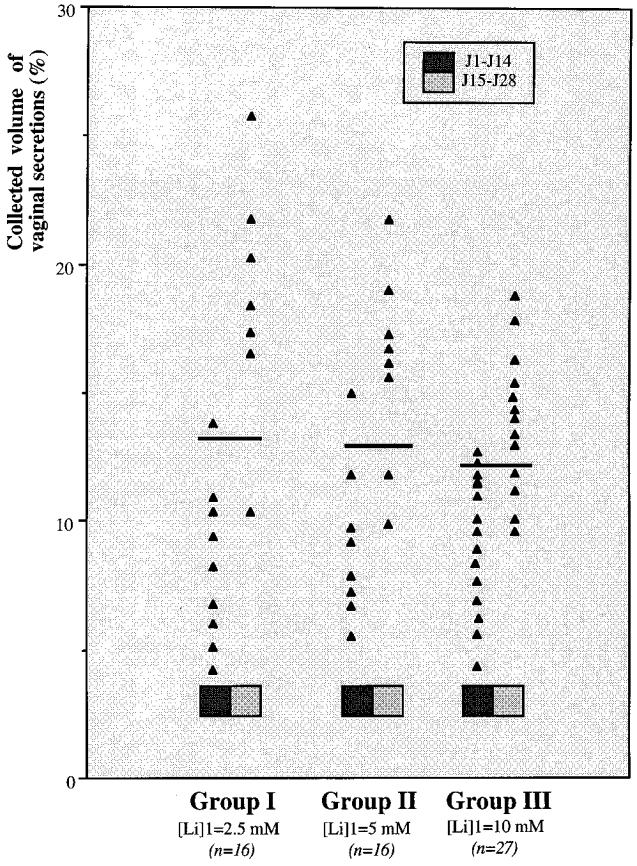

FIG. 2. With the $[Li]_1$ at 2.5 mM in group I, 5 mM in group II, and 10 mM in group III, v values were calculated according to the phase of the menstrual cycle by formula 4a and expressed by fraction f_v as percentages of V (V = 3 ml). The mean v values in groups I (12.7% ± 6.5%), II (12.6% ± 4.9%), and III (12.0% ± 3.2%) did not differ significantly. The variance of the distribution in group III was significantly lower than that in groups I and II. J1 to J14 correspond to the days in the follicular phase, and J15 to J28 corresponds to the days in the luteal phase. Horizontal lines denote the mean value of the distribution.

(iii) Influence of the presence of LiCl (10 mM) in enzyme immunoassays. To test whether LiCl in the sample could affect enzyme immunoassays (EIA), 10 CVS samples from HIV type 1 (HIV-1)-infected women, obtained by vaginal washing with PBS without LiCl, were used in one indirect EIA conceived in our laboratory to detect immunoglobulin G (IgG) to the surface glycoprotein gp160 of HIV-1 in CVS (3) and in two commercially available EIAs, namely, one competitive EIA for anti-gp41 and anti-p24 antibodies (Wellcozyme HIV recombinant; Murex Diagnostics Limited, Dartford, United Kingdom) and one IgG antibody capture EIA (Wellcozyme HIV1+2 Gacelisa; Murex) especially fitted to detect anti-HIV IgG antibody in body fluids (5). Samples were diluted first in PBS at 1:200 for the indirect and competitive EIAs and then at 1:2,500 for the immunocapture assay. These diluted samples were tested in duplicate after a twofold dilution in 20 mM LiCl–PBS and in PBS alone, as a control.

(iv) Statistics. Statistical analyses were carried out with the InStat statistical program (GraphPad Software). Comparisons of variances between groups were investigated by the F test of Fisher; intergroup comparisons of means were established by the Mann-Whitney U test, and intragroup comparisons of means were done by the rank order Wilcoxon test for paired samples; lastly, correlations between the calculated dilution factors and the estimated volumes of collected CVS were performed by Spearman's correlation test. Results are expressed as means ± standard deviations. Dilution factors are given as percentages.

RESULTS

Study population. Group I ($[Li]_1$ = 2.5 mM) consisted of 16 women (age, 31.3 ± 10.1 years), 9 in the follicular phase of the menstrual cycle and 7 in the luteal phase; group II ($[Li]_1$ = 5 mM) consisted of 16 women (age, 25.7 ± 6.2 years), 8 in the follicular phase and 8 in the luteal phase; and group III ($[Li]_1$ = 10 mM) consisted of 27 women (age, 33.4 ± 8.7 years), 14 in the follicular phase and 13 in the luteal phase. The mean age of the included women as well as the number of women in each phase of the menstrual cycle did not differ significantly between the three groups.

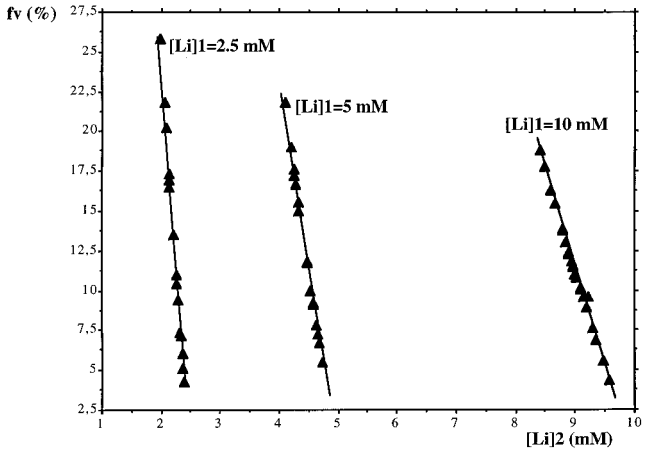

FIG. 3. Experimental curves representing the fractions f_v of collected CVS as a function of the measured $[Li]_2$. The steepness of the slopes was more pronounced at $[Li]_2$ values of 2.5 and 5 mM than at 10 mM.

Optimal $[Li]_1$ for vaginal washing. Figure 2 shows the distribution of the collected fractions (f_v) of CVS obtained after the vaginal washing as a function of $[Li]_1$, calculated by formula 4a and expressed as percentages of the washing volume, V. The means in groups I (i.e., 12.7% ± 6.5%; median, 10.7%; range, 4.2 to 25.8%), II (i.e., 12.6% ± 4.9%; median, 11.8%; range, 5.5 to 21.8%), and III (i.e., 12.0% ± 3.2%; median, 11.6%; range, 4.6 to 18.8%) did not differ significantly. Whatever the $[Li]_1$, the mean v values were similar for both executants (L.B., 12.5% ± 5.7%; C.T.-B., 11.8% ± 3.3%). The observed variance of the collected fractions in group I was fourfold higher than that in group III ($F_{27, 16} = 4.1$; $P < 0.01$) but did not differ from that in group II ($F_{16, 16} = 1.7$; $P > 0.05$). The observed variance in group II was significantly higher (twofold) than that in group III ($F_{27, 16} = 2.3$; $P < 0.05$).

Experimental curves representing the collected fractions (f_v) of CVS as a function of the measured $[Li]_2$ are given in Fig. 3. The slopes were steeper at $[Li]_1$ values of 2.5 and 5 mM than at 10 mM, proving the effectiveness of the theoretical formulae 4a and 5c.

The reproducibility of the method was investigated further by the determination of the intra-assay variation for the three $[Li]_1$ values by 15 serial measurements of f_v in the same cervicovaginal washing. At an $[Li]_1$ of 2.5 mM, the intra-assay variation was 17%; at 5 mM, it was 13%; and at 10 mM, it was 8.5%. These findings demonstrated a better reproducibility at an $[Li]_1$ of 10 mM.

On the basis of these experimental features, the use of an $[Li]_1$ of 10 mM resulted in the minimum observed variance (σ^2_o) in the evaluation of the volumes of collected CVS to be obtained, probably because of the more-precise measurements of $[Li]_2$ at this concentration.

Variations in the v values during the menstrual cycle. Figure 2 shows the distribution of the collected fractions of CVS after vaginal washing at the follicular and luteal phases of the menstrual cycle by $[Li]_1$. For each group, mean v values at the follicular phase were significantly lower than those at the luteal phase (Table 1).

TABLE 1. Variation in v values by phase of menstrual cycle[a]

Subject group	v (% V) measured in:		P
	Follicular phase	Luteal phase	
I	8.2 ± 3[b] (7.4; 4.2–13.5)[c]	18.4 ± 4.8 (17.3; 10.4–25.8)	<0.001
II	9.1 ± 3 (8.5; 5.5–15)	16.2 ± 3.8 (16.9; 9.9–21.8)	0.001
III	10.0 ± 2 (10.9; 4.6–12.4)	14.1 ± 2.9 (13.9; 9.62–18.8)	<0.001

[a] With the $[Li]_1$ at 2.5 mM in group I, 5 mM in group II, and 10 mM in group III, v values were determined according to the phase of the menstrual cycle by formula 4a and are expressed as percentages of V (ratio f_v).
[b] Mean ± standard deviation.
[c] Values in parentheses are medians and ranges of percentages.

In the next part of this study, women in group III ($[Li]_1$ = 10 mM) were chosen for an evaluation of the variations in the fraction f_v, in the volume v_p, and in the estimations of the dilutions d_v and d_x as a function of the day of the menstrual cycle (Fig. 4 and 5).

Although the v_ps measured after centrifugation of the collected CVS from the women of group I (0.374 ± 0.220 ml; median, 0.275 ml; range, 0.1 to 1 ml) showed a tendency to increase between the follicular phase and the luteal phase of the menstrual cycle (Fig. 4), their mean levels did not differ significantly (0.300 ± 0.220 ml versus 0.450 ± 0.210 ml, P = 0.077). Considerable v_p values were determined for four women, all of whom were at the midcycle period (subject A v_p, 0.9 ml; subject B v_p, 0.67 ml; subject C v_p, 1 ml; subject D v_p, 0.6 ml). The f_v and the v_p were weakly correlated (P = 0.048).

Finally, the distribution of the estimated d_v values (calculated by formula 6; 8.9% ± 2.7%; median, 8.6%; range, 5.2 to 17.8%) demonstrated a significant difference between the

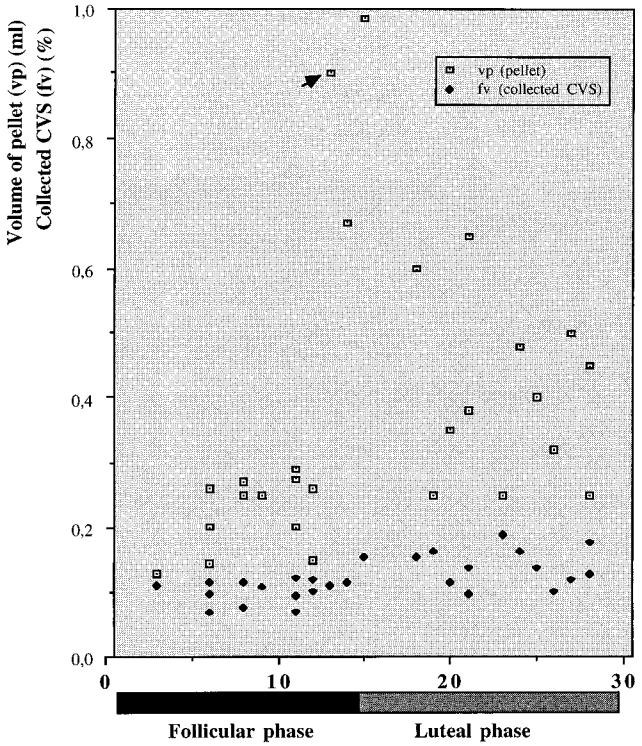

FIG. 4. Volumes of the solid phase, corresponding to the v_p and the f_v, calculated for the women of group III by formula 4a as a function of the day of the menstrual cycle. For instance, for subject A (arrow), the v_p was equal to 0.9 ml; and the f_v was 11% (V = 3 ml); i.e., v was about 330 μl.

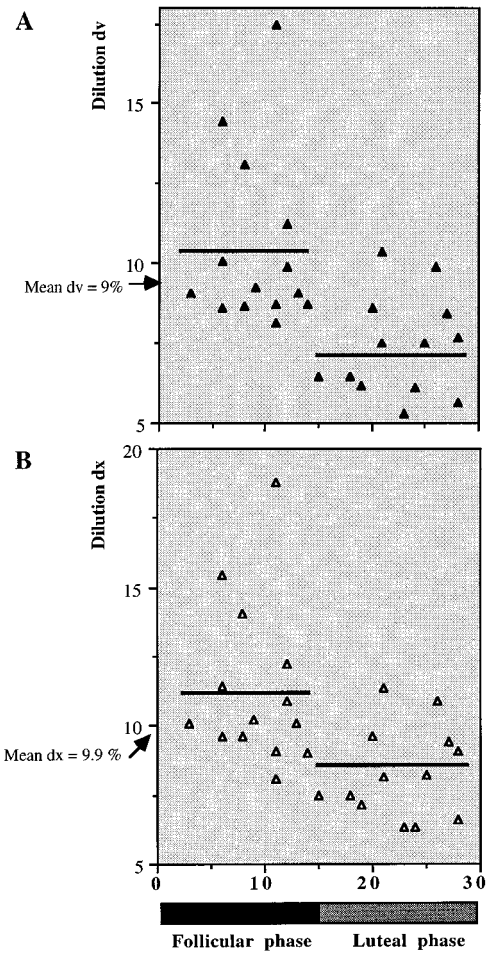

FIG. 5. (A) Distribution of d_v values for the women of group III calculated by formula 6; (B) distribution of d_x values for the women of group III calculated by formula 10 as a function of the day of the menstrual cycle. Horizontal lines indicate the means of dilutions for each phase of the menstrual cycle.

follicular and luteal phases of the menstrual cycle (10.4% ± 2.8% versus 7.4% ± 1.6%, P = 0.002) (Fig. 5A). Similarly, the estimated d_x values (calculated by formula 10; 9.9% ± 2.8%; median, 9.6%; range, 6.3 to 18.8%) were significantly higher in the first phase of the menstrual cycle than in the second phase (11.3% ± 2.9% versus 8.2% ± 1.6%, P = 0.003) (Fig. 5B). Paired d_v and d_x values were strongly correlated ($P < 0.001$). The values of d_x were inversely correlated with the v values ($P < 0.001$) as well as with the volumes of their solid phase estimated by v_p (P = 0.034).

Lithium chloride (10 mM) in enzyme immunoassays. For 10 cervicovaginal washings from HIV-1-infected women, optical densities determined by an indirect EIA used to detect anti-gp160 IgG antibody did not differ in the absence or presence of 10 mM LiCl (1.85 ± 0.25 versus 1.87 ± 0.25). Similar optical density results were obtained with a competitive EIA used to detect anti-gp41 and anti-p24 IgG (0.081 ± 0.003 versus 0.082 ± 0.003) as well as with an IgG immunocapture EIA used to detect total anti-HIV antibody (1.670 ± 0.068 versus 1.659 ± 0.065).

DISCUSSION

We have developed a simple method permitting the quantitative evaluation of the dilution factor introduced in CVS

collected by a vaginal washing. Vaginal washings have been frequently utilized previously to study the total humoral cervicovaginal immunity in humans as well as nonhuman primates (1, 2), but little attention was paid to the quantitative aspects of the local immunological responses, and even less was given to their interindividual fluctuations and cyclic changes.

Our results emphasize that accurate quantification of immunoglobulins or antibodies at the level of the vaginal mucosa would not have been accomplished without precise determination of the dilution factors introduced by the vaginal washing. In the same homogeneous population of women chosen at the same phase of the menstrual cycle, the extreme values of the calculated volumes of collected CVS varied from 1 to 4. Moreover, significant changes in the quantities of collected secretions, and subsequently in the dilution factors introduced by the vaginal washing, were observed to be a function of the cycle period. Thus, the proportions of the total CVS in the collected specimen, including both soluble and solid phases, increased highly from the follicular phase to the luteal phase of the menstrual cycle. This feature is probably related to the cyclic variation affecting the vaginal epithelium. Indeed, the luteal phase of the menstrual cycle is characterized by a physiological and important desquamation of the vaginal epithelium under the control of progesterone, resulting in an increased production of exfoliated epithelial cells. This finding could likely explain the relative increase of whole CVS between the follicular and the luteal cycle periods. Four women close to the ovulatory period of the menstrual cycle had a large quantity of whole collected CVS, mainly characterized by a considerable volume of the solid phase. This observation is also likely reminiscent of the cyclic variation of production of cervical mucus, which becomes abundant at midcycle days. Finally, the mean dilution factor of the soluble components of CVS obtained by vaginal washing in childbearing-aged women can be estimated as 9.9%. Likewise, the dilution factor shows cyclic variations according to the period of the menstrual cycle, with the dilution being more pronounced in the follicular phase than in the luteal phase.

Li is an inert substance which possesses several advantages in the study of cervicovaginal immunity. First, the volume of Li distribution in CVS, corresponding to the volume of diffusion of the Li cation in this heterogeneous body fluid, appears approximately equal to the volume of the whole CVS collected by vaginal washing. Indeed, the volume of living cells, excluding Li, is small and can be neglected in the calculation of the dilution factor of the whole CVS in the collected sample as well as the dilution factor of their soluble components. Second, measuring Li concentration by an atomic absorption spectrophotometer is accurate, sensitive, relatively quick, and inexpensive; flame spectrophotometers are, moreover, usually available in the biochemistry laboratories of most hospitals or research centers. Third, the Li cation is not capable of interaction with immunoglobulins or with immune complexes or of effective chemical binding with plasma proteins (4). Finally, even at concentrations of 20 meq/liter, Li interferes with neither EIA nor the survival of bacteria and viruses (6). Furthermore, we verified that the presence of LiCl at the final concentration of 10 mM did not affect the results of anti-HIV antibody detection in CVS with three EIAs based on different principles.

We recommend a relatively elevated Li concentration in the washing buffer, i.e., 10 meq/liter, when performing a vaginal washing, to allow more accurate assessment of the dilution of CVS for immunological investigations. At this concentration, the error introduced by the measurement of Li concentration on the observed variance is minimal. As a matter of fact, the accuracy of the measurement of Li obtained by a flame spectrophotometer depends on the intrinsic precision of the apparatus and on its specificity in the presence of other cations, such as Na^+. For example, for the apparatus we used, a measurement of Li^+ is given with an accuracy of $\pm(0.1 + 0.1)$ or ± 0.2 meq/liter. At an $[Li]_1$ of 10 meq/liter, a one-third predilution of the collected specimen appears necessary before the measurement of Li to save the maximum of the sample. This dilution causes an additional error in the final value of $\pm 0.2 \times 3/10$, or $\pm 6.7\%$, which is inferior to that occurring at an $[Li]_1$ of 5 meq/liter ($\pm 0.2 \times 2/5 = \pm 8\%$) as well as to that occurring at an $[Li]_1$ of 2.5 meq/liter ($\pm 0.2 \times 2/2.5 = \pm 16\%$). The use of an atomic absorption spectrophotometer could certainly reduce these unavoidable errors of measurement because of its higher precision. However, such spectrophotometers are not readily available, and the cost of the Li concentration determination would become too high.

We conclude that accurate determinations of the dilution factor introduced by vaginal washing is indispensable when quantitatively studying cervicovaginal immunity because of large inter- and intraindividual variations in the production of vaginal fluids. From the perspective of evaluation of possible vaccination methods against sexually transmitted diseases, such features will have to be considered.

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