THE RESPONSE TO MECHANICAL DISTENSION OF THE NON-PREGNANT HUMAN UTERUS IN VIVO

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I Lalos O and Joelsson I:
Determination of tonicity in the non-pregnant human uterus in vivo. Accepted for publication in Acta Obstet Gynecol Scand.

II Lalos O:
Uterine tonicity in the proliferative and secretory phases of the menstrual cycle in relation to estradiol and progesterone in serum. Accepted for publication in Acta Obstet Gynecol Scand.

III Lalos O and Rosén J:
A hysterometric study of the effect of inhibitors of prostaglandin synthesis in primary dysmenorrhea. Submitted for publication in Int J Gynecol Obstet.

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ABSTRACT

Lalos, Othon: The response to mechanical distension of the non-pregnant human uterus in vivo.

The ability of the uterus to contract in response to mechanical distension has been utilized in the development of a method, hysterometry, for the quantification of hormonal and pharmacological effects on the human myometrium in vivo. The method has been built up with due consideration for laws from mechanics of materials and hydrodynamics. By applying mathematical theorems and accepting certain approximations, the basic results of recordings of intrauterine pressure are transformed into wall tension, allowing for the size of the uterus. The equivalent of an elasticity modulus in the myometrium has been used as a synthetic discriminator (without any real physiologic correlation) for the evaluation of hormonal and pharmacological effects.

Hysterometry has been used for the evaluation of uterine tonicity during the menstrual cycle. It was demonstrated that uterine tonicity is higher in the proliferative than in the secretory phase. The uterine tonicity correlated well with the concentrations in serum of estradiol and progesterone at the time of the examination.

Hysterometry has also been used for the evaluation of effects of inhibitors of prostaglandin synthesis (Naproxen Sodium and Naproxen acid) and of a selective beta-2-receptor stimulator (Salbutamol) upon the uterus in women with primary dysmenorrhea. It was shown that uterine tonicity was high on the first day of menstruation in untreated or placebo-treated dysmenorrheic women. Administration of inhibitors of prostaglandin synthesis or beta-2-receptor stimulating agents markedly decreased the uterine tonicity and relieved the menstrual pain.
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INTRODUCTION

Since 1872 many investigators have been interested in the contractility of the human uterus. Various techniques have been used, including rubber balloons of various sizes, filled with fluid or air, open-end and sometimes sponge-tipped catheters, microtransducer catheters, hysterosalpingographies and even bimanual palpation. The functional characteristics of the pregnant myometrium are now relatively well defined, after much controversy, but those of the non-pregnant human myometrium are still being debated. The difficulties in characterizing the same organ in two physiological states lie in the hydrodynamic differences. The pregnant uterus is a fluid-filled, closed muscular spheroid, readily lending itself to reliable intrauterine pressure measurement, whereas the non-pregnant uterus is a hollow ellipsoid, open and containing an everchanging mass of endometrial tissue, cellular debris and blood. These structural and hydrodynamic differences make it necessary to modify the techniques used for intrauterine recordings. Intrauterine pressure is affected by the tension within the muscular wall, the size of the uterus and the thickness of its wall. Furthermore, the spontaneous activity in the non-pregnant uterus is disturbed by the pressure-recording instrument. Failure to take all these factors into account is one reason why the results of research often differ.

The contractility of the non-pregnant human uterus changes during the menstrual cycle. Most authors who have studied uterine activity by recording the intrauterine pressure agree about the characteristic contractility patterns in the different menstrual phases (5, 14, 16, 52). In the proliferative phase the contractions have a comparatively high frequency, small amplitude and short duration. In the secretory phase there is a gradual change to contractions of lower frequency, higher amplitude and longer duration. These contractions are most conspicuous around the onset menstruation. A reversal to the pattern of the proliferative phase begins on the 2th to 3rd
menstrual day.

Increased uterine contractility in women with primary dysmenorrhea was described by Moir in 1936 and since then many other authors have studied uterine contractility by measuring changes in intrauterine pressure. Some investigators have found that contractions with extremely high intrauterine pressures or "dysrhythmic" contractions are typical of these conditions (45, 46, 79). Others have reported that elevated uterine tonus is associated with menstrual pain (24, 80). Åkerlund suggested that low abdominal pain in primary dysmenorrhea is a result of uterine ischemia caused by uterine contractions with a high amplitude, long duration or no intervening periods of relaxation (80).

The role of prostaglandins in primary dysmenorrhea has been emphasized during the last decade. Since 1965 many authors have found that patients suffering from dysmenorrheic cramps have higher PGF$_{2\alpha}$ concentrations in the endometrium and in the menstrual fluid than non dysmenorrheic women (27, 28, 59, 77). In vivo experiments have shown that administration of PGF$_{2\alpha}$ has a marked stimulatory effect on the nonpregnant as well as the pregnant myometrium (43, 48, 63).

Since Vane and co-workers demonstrated in 1971 that aspirin-like drugs inhibit prostaglandin synthesis, many authors have conducted clinical studies in women with primary dysmenorrhea using various inhibitors of prostaglandin synthesis, such as flufenamic acid, indomethacin and Naproxen Sodium (8, 19, 42, 44, 66).

Beta-receptor stimulating agents have been found to inhibit uterine contractions in the pregnant uterus and they have been used in obstetrics, e.g. to arrest premature labour. They also been reported to inhibit myometrial activity, increase the uterine blood flow and relieve the pain in women with primary dysmenorrhea (80).
Hysterometry has been found suitable for the study of human uterine tonicity in vivo. This method utilizes the fact that mechanical distension of smooth muscle induces contraction. By applying mathematical theorems and accepting certain approximations, the basic results are normalized with regard to uterine size and wall thickness. Consequently the response to a standardized excitation can be expressed numerically in a way which allows comparison between individuals. As the development of tension within the uterine wall is dependent upon the basic hormonal environment and upon the availability of stimulating or relaxing agents, hysterometry might be a valuable method for the study of uterine tonicity during the phases of the menstrual cycle. It might also be suitable for studying the effects of pharmacologically active agents on the myometrium in women with primary dysmenorrhea.
AIMS OF THE PRESENT STUDY

The aims of the present study have been to:

1. Apply a new method for the study of uterine tonicity in the non-pregnant human uterus in vivo.

2. Study the cyclic changes of uterine tonicity and relate these changes to serum estradiol and progesterone concentrations.

3. Study the uterine tonicity and establish the effect of inhibitors of prostaglandin synthesis in the treatment of primary dysmenorrhea.

4. Investigate the effect of a β₂-receptor stimulator on uterine tonicity in women with primary dysmenorrhea.
MATERIAL

The clinical material comprises 22 women, nulli-gravidae, with regular menstrual periods and normal gynecological examination findings. The mean and median ages of the women were 25 years, range 18 to 31. Their mean weight was 59 kg, range 53 to 74. The women were allotted to the following groups.

a. Six volunteers were studied during consecutive phases of the menstrual cycle. Two had severe dysmenorrhea, two moderate dysmenorrhea and two pain-free menstruations. Hysterometry was performed at the mid-proliferative phase (6th - 8th day), the ovulatory phase (ovulation ± 2 days), the mid-secretory phase (ovulation + 7 days) and the first day of the next menstruation. To detect the day of ovulation, the total cervical mucus amount, spinnbarkeit and fern crystallisation were studied. Moreover, blood samples were drawn daily from the 8th day of the menstrual cycle until two days after the estimated day of ovulation for determination of LH, serum estradiol and progesterone. Immediately before every hysterometry, blood samples were drawn for determination of the current concentrations in serum of estradiol and progesterone.

b. Eleven volunteers with primary dysmenorrhea participated in a double-blind crossover trial using Naproxen and placebo. Hysterometry was performed on the first day of each of two consecutive menstruations and the tonometric indices were evaluated. Blood samples were drawn immediately before hysterometry for determination of estradiol and progesterone in serum.

c. Five volunteers with severe primary dysmenorrhea were investigated with hysterometry on the first day of a pain-ful menstruation. Two recordings were performed, the first without any drug and the second during intravenous infusion of Salbutamol in a dose of 10 μg/min.
Hysterometry: A technique for in vivo determination of tonicity in the myometrium of the non-pregnant human uterus

Hysterometry is a method which utilizes the fact that smooth muscle has the ability to contract in response to mechanical stimulation. This method has been used before for the quantification of drug effect, especially on the pregnant human uterus. The method has been built up with due consideration to laws from the mechanics of materials and hydrodynamics. It yields information about the development of tension in the myometrium as a contractile response to distension, i.e. the stress and strain relationship in the myometrium is determined. The mechanical distension during hysterometry is enforced sinusoidally, in the frequency range 0.1 - 4.0 Hz, by filling and emptying thin-walled rubber balloon introduced into the uterine cavity after gentle dilatation of the cervical canal (6 mm). In order to analyse the development of tension within the muscular wall, it has been necessary to use the intrauterine pressure as a primary datum. By applying mathematical theorems and accepting certain approximations, the basic results of the intrauterine pressure recordings are transformed into wall tension, with allowance for organ size and wall thickness. The uterine dimensions are determined in each case by measuring the corpus and cervix compartments with a probe. The thickness of the uterine wall is defined as 1.25 cm, a measure arrived at empirically.

Definitions and equations from mechanics of materials have been applied in order to arrive at an equation for calculating the stresses within the muscular wall which correspond to a given intrauterine pressure. For this purpose the uterine cavity is considered to represent a hollow ellipsoid: furthermore, in order to simplify the mathematical calculations, an imagined section through this body at its symmetrical rotation radius is considered to form part of a cylinder. The uterine
wall is considered to be incompressible during the hystero-
meteric recordings and the distension provoked by the balloon is
assumed to be distributed uniformly over the entire myometrium.

In the analysis of the recordings, the contractile modulus
(m), the stress difference in tangential ($\Delta \sigma_t$) and axial ($\Delta \sigma_x$)
directions and the differences in tangential strain ($\Delta \varepsilon_t$) were
calculated. The stress differences in radial direction ($\Delta \sigma_r$)
are equal to the intrauterine pressure changes but with nega-
tive sign ($-\Delta P$). Finally, using Hook's law, an expression for a
tonometric index (E) of the myometrium was obtained as the
quotient between the components of stress and strain:

$$ E = \frac{\Delta \sigma_t - (1/m) \Delta \sigma_x - (1/m) \Delta \sigma_r}{\Delta \varepsilon_t} $$

Even though several approximations are involved in the calcu-
lation of the tonometric index, hysterometry has been found
suitable for determining the stress-strain relationship in
myometrium. As the development of tension is dependent upon
both the basic hormonal environment and the availability of
stimulating or relaxing substances, it is suggested that the
method can be useful for the quantification of drug effect on
the human uterus in vivo as well as for the determination of
uterine tonicity during the phases of menstrual cycle.

**Hormone analyses**

The hormone assays were carried out at the hormone laboratory,
Sabbatsberg Hospital, Stockholm. **Serum levels of LH** were deter-
mined by radioimmunoassay using DASP(R) separation (Organon,
Oss, Holland). Anti-LH (rabbit) and purified LH for 125I
labelling were obtained from KABI AB, Stockholm, Sweden. The
values are expressed as units per litre of Human Pituitary LH
or ICSH 68/40. **Serum levels of estradiol-17\(^\beta\)** were determined
after ether extraction by radioimmunoassay using anti-estradiol
-17\(^\beta\)-6 (carboxymethyl oxime) bovine serum albumin (sheep). This
antibody crossreacts to 11% with estrone (41). **Serum levels of
progesterone** were determined radioimmunologically after extrac-
tion with n-hexane (70). Anti-progesterone-11α-hemisuccinate-bovine serum albumin (sheep) was obtained from the Royal Veterinary College, Swedish University of Agricultural Sciences, Uppsala, Sweden.

The intra- and inter-assay variations of the methods were: for LH 8.7 and 9.4%, for estradiol-17β 13.1 and 11.7%, and for progesterone 11.6 and 11.5%.

**Determination of Naproxen in serum**

Naproxen in serum was determined by high-performance, reversed phase liquid chromatography (74, 75). Naproxen is extracted from acidified serum, after the addition of an internal standard, to a mixture of diethylether and hexane. The organic phase is evaporated, the residue is dissolved in a small amount of mobile phase and an aliquot is injected on the chromatographic column. LiChrosorb RP8 is used as support and the mobile phase is a mixture of phosphate buffer pH 7 and methanol. The chromatographic step takes about 3 minutes and detection is made by an UV-detector. The precision is in the range 1.0 - 1.7% at a concentration level of 10 µg/ml with recoveries of 98 - 104% (74).

**Statistical methods**

The equation of linear regression of tonometric indices (along the y-axis) on the logarithm of frequency of stimulation (along the x-axis), $y = a + bx$, has been calculated using the following formulas:

$$b = \frac{\Sigma xy - \Sigma x \Sigma y}{\Sigma x^2 - (\Sigma x)^2/n}$$

for the regression coefficient, slope, and

$$a = \frac{\Sigma y}{n} - b \frac{\Sigma x}{n}$$

for the intercept.
The coefficient of correlation between x and y has been calculated using the following equation:

\[ r = \frac{\sum xy - \frac{\sum x \sum y}{n}}{\sqrt{\left(\frac{\sum x^2 - \left(\frac{\sum x}{n}\right)^2}{n}\right)\left(\frac{\sum y^2 - \left(\frac{\sum y}{n}\right)^2}{n}\right)}} \]

Differences were analysed on the basis of changes in intercepts and regression coefficients between well defined situations in the same individual, i.e. intra-individual differences were used for the statistical evaluations. This was made possible because, for example, all women with dysmenorrhea were examined both without and with the administration of active drug. Likewise, all women studied during normal menstrual cycles were recorded both during the proliferative and secretory phases. Evidently, however, group regression lines could also be formed, each line representing one specific situation. Such a group line had the equation:

\[ y = \bar{a}_i + \bar{b}_i x \]

in which \( \bar{b}_i \) is the arithmetic mean of the separate \( b_i \)-values, while \( \bar{a}_i = \bar{y}_i - \bar{b}_i \bar{x}_i \); \( \bar{y}_i \) and \( \bar{x}_i \) being the total mean values of y and x for the entire material.

As no overlap of individuals occurred between comparable groups, group equations could be used for the calculation of differences in intercepts and regression coefficients, as well. The evaluation of statistical significance was made using the following expression, where \( n_1 \) and \( n_2 \) designate the number of separate individuals in each group:

\[ t = (b_1 - b_2) \sqrt{\frac{(n_1 + n_2 - 2)(n_1 n_2)}{(n_1 + n_2) \left[ \frac{\sum b_1^2}{n_1} - \frac{(\Sigma b_1)^2}{n_1} \right]} + \left[ \frac{\sum b_2^2}{n_2} - \frac{(\Sigma b_2)^2}{n_2} \right]} \]
The t:s are normally distributed with \( n_1 + n_2 - 2 \) degrees of freedom. Provided no statistical difference between the b:s was obtained, the difference between the intercepts (a:s) was tested using the corresponding formula.

Intra-individual differences in estradiol, progesterone and the quotient \( E_2/P \) between the proliferative and secretory phases of the menstrual cycle were evaluated using the formula:

\[
\bar{d}_{E_2} = \frac{\sum (E_{2 \text{ prol}} - E_{2 \text{ secr}})}{n} ; \quad t = \frac{\bar{d}_{E_2}}{SE_{\bar{d}_{E_2}}}
\]

\( \bar{d}_{E_2} \) exemplifying the mean of differences. t is regarded normally distributed with \( n - 1 \) degrees of freedom.

The statistical significance of the differences were evaluated within the following ranges:

- **Non significant:** \( 0.05 < p \)
- **Almost significant:** \( 0.01 < p \leq 0.05 \)
- **Significant:** \( 0.001 < p \leq 0.01 \)
- **Highly significant:** \( p \leq 0.001 \)

In these formulas p is the probability of obtaining a chance difference which is at least as large as the observed difference.

Système International d'Unités has been used, although not in all instances. In this system the pressure 1 mm Hg at 0°C equals 133.332 pascal (Pa). One Pa is the pressure or the tension which is produced by the force 1 Newton upon an area of 1 square meter (56).
RESULTS

Uterine tonicity in the proliferative and secretory phases of the menstrual cycle in relation to serum estradiol and progesterone

The cyclic changes in myometrial activity and tonicity have attracted the interest of many investigators during the last hundred years. Normal function of the endometrium and myometrium is necessary for reproduction. Furthermore, knowledge of these functions would contribute to an understanding of pathological conditions in the menstrual cycle. The cyclic histologic functional changes in the human endometrium are now relatively well-known. The study of myometrial activity and tonicity has been undertaken with more than a dozen recording methods: the results have varied greatly and given rise to controversy. Besides the extremely varied techniques, part of the explanation lies in a failure to allow for all the factors which can influence the result of recordings. The gonadal hormones seem to play a prominent role in myometrial activity. The recent development of protein-binding and radioimmunoassay techniques afforded new possibilities for comparative studies of cyclic myometrial and hormonal changes.

Hysterometry appears to be a suitable method for the study of cyclic changes in uterine tonicity. For this purpose use was made of the modification of muscle response to mechanical distension - the response is considered to depend in part on the prevailing hormonal environment.

The study comprised six volunteers with normal menstrual periods and normal gynecological examination findings. Hysterometry was performed in the mid-proliferative, ovulatory and mid-secretory phases and on the first day of menstruation. The serum concentrations of estradiol and progesterone immediately before hysterometry were determined. As the tonometric indices are bound to the frequency of stimulation, the results for each
individual were calculated by means of regression analysis. All regression equations were based on tonometric indices in the frequency range 0.1 - 4.0 Hz. Statistical analyses were made on intra-individual differences in intercepts between the different menstrual phases. The correlation between the intercepts of regression equations in the proliferative and the secretory phases and the quotient estradiol/progesterone (E₂/P) was also calculated and the correlation coefficient was evaluated. Statistically significant decreases in intercepts were obtained in the secretory phase compared to the proliferative phase. These changes corresponded to statistically significant changes in the quotient E₂/P between the proliferative and secretory phases. The values of tonicity, expressed as intercepts of the regression equation, obtained around ovulation (LH peak ± 2 days) as well as those obtained during menstruation showed wide variability and did not correlate to estradiol, progesterone or their quotient E₂/P. A more detailed account is given in paper II.

The effect of Naproxen on the non-pregnant uterus in women with primary dysmenorrhea

The role of prostaglandins in primary dysmenorrhea has been emphasised during the last decade. It has been found that women suffering from dysmenorrheic cramps have higher prostaglandin F₂α (PGF₂α) concentrations in the endometrium and in menstrual blood than non-dysmenorrheic women. (27, 28, 59, 77). PGF₂α has a marked stimulatory effect on both the pregnant and the non-pregnant uterus (43, 48, 63).

Since aspirin-like drugs were found to inhibit prostaglandin synthesis, many investigators have used them in various clinical trials in women with primary dysmenorrhea. Hysteroscopy has proved suitable for quantification of their effect on uterine tonicity.

Naproxen Sodium, an inhibitor of prostaglandin synthesis, has been found to decrease uterine tonus as well as the ampli-
tude and frequency of uterine contractions, and to relieve the pain in women with primary dysmenorrhea (19, 44). Naproxen acid has the same pharmacological effects as Naproxen Sodium, although the latter is absorbed more rapidly (67). The clinical study involved 11 volunteers with primary dysmenorrhea, who participated in a double blind, crossover trial using Naproxen Sodium (5 subjects), Naproxen acid (6 subjects) and placebo. The purpose of testing both the salt and the acid of the same substance was to determine whether they differ regarding the effect on the myometrium or in the treatment of primary dysmenorrhea.

Hysterometry was performed on the first day of each of two consecutive menstrual periods and the uterine tonicity was evaluated. Uterine tonicity on the first menstrual day was found to be high in all women during the placebo-treated menstruation. Naproxen Sodium significantly decreased the uterine tonicity and relieved the pain in all five women. Significant pain relief and decrease of uterine tonicity also occurred in the five of the six women receiving Naproxen acid; the sixth woman showed no decrease of uterine tonicity, neither did she report any relief of pain.

The difference between intercepts of regression equations representing the placebo situation and the Naproxen-treatment situation were statistically significant, 0.001 < p < 0.01. These findings indicate that high uterine tonicity, provoked by prostaglandins, is a pathogenetic mechanism in primary dysmenorrhea. Naproxen decreases the uterine tonicity and relieves the pain in women suffering from dysmenorrheic cramps.

The reader is referred to paper III for a more detailed account of the effect of Naproxen upon uterine tonicity in women with primary dysmenorrhea.
The effect of Salbutamol on the non-pregnant uterus in women with primary dysmenorrhea.

Increased uterine contractility as a pathogenetic mechanism in primary dysmenorrhea has been considered since 1936 (53). Several authors who have studied uterine contractility disagree concerning its pattern (24, 46, 79, 80). Beta-receptor stimulating agents have been used as uterine relaxants to inhibit premature labour or to relieve the pain in primary dysmenorrhea but concomitant palpitation, tachycardia and, at least transient, hypotension have discouraged a wide acceptance of these drugs.

The action of Salbutamol on receptors mediating bronchial and uterine relaxation (β₂) was found to be considerably greater than its cardiac effect (β₁). Hysterometry has now been used for quantifying the effect of Salbutamol upon the myometrium in women with primary dysmenorrhea. For this purpose use was made of the modification of muscle response to mechanical distension - the response is considered to depend on factors such as the prevailing hormonal environment and the presence of pharmaco-logically active agents. The hormonal conditions were defined in this study and the influence of variations was avoided by using intra-individual comparisons.

The study concerned 5 women with severe primary dysmenorrhea. Salbutamol was given in intravenous infusion in a dose of 10 µg/min. Hysterometry was performed during basic conditions and during intravenous Salbutamol infusion and the tonometric indices were evaluated. As tonometric indices are bound to the frequency of stimulation, the experimental results are evaluated in terms of coefficients of regression equations of the indices upon the logarithm of frequency. Regression equations were calculated for each individual, using the results during basic conditions and during infusion of Salbutamol respectively.

A statistically significant decrease in intercept was ob-
tained with Salbutamol. All patients reported pain relief after 2 - 3 minutes of Salbutamol infusion and a maximum effect after 5 - 10 minutes. The side effects were a moderate increase in heart rate and slight palpitations. No significant changes in blood pressure were observed. These findings indicate that Salbutamol administered intravenously in a dose of 10 µg/min decreases uterine tonicity, relieves menstrual pain and has only slight side effects.

The reader is referred to paper IV for a more detailed description of the effect of Salbutamol upon uterine tonicity in primary dysmenorrhea.

GENERAL DISCUSSION

The main disadvantages in previous studies on uterine contractility and tonicity are represented by the inability to normalize the results and, in part, the application of a variety of techniques. In order to elucidate the problems concerning the recording of intrauterine pressure with open-end and balloon-tipped catheters, respectively, Braaksma and co-workers tested and compared the two systems in vivo and in vitro (10). They found that open-end catheters accurately reflected true manometric pressures, whereas closed catheters did not. The open-end catheters have, however, other disadvantages, the chief one being that the intrauterine end of the catheter is apt to be obstructed by mucus, blood or endometrial tissue fragments. Bengtsson reduced the risk of occlusion of the open-end catheter by surrounding its distal end with a synthetic wash-sponge (6). Åkerlund overcame most of these disadvantages by using a microtransducer catheter which has both the sensor and the pressure transducer located in its tip and does not contain any fluid (81). Before that, in 1964, Hendricks had another technique, using the smallest possible intrauterine catheter, thus minimizing the influence of uterine irritation, but at the same time he showed that the intrauterine pressure as recorded by an open-end catheter depends on how deeply this is inserted in the uterine cavity (32).
In order to overcome the disadvantages and achieve a quantitative evaluation of results, hysterometry was utilized as a method for studying the tonicity of the non-pregnant uterus. As the development of tension within the myometrium is dependent on the basic hormonal environment and on the availability of stimulating or relaxing agents, uterine tonicity was examined during the phases of the menstrual cycle and during the first day of menstruation in dysmenorrheic women with the administration of Naproxen or Salbutamol.

Uterine tonicity was found to be markedly higher during the proliferative than during the secretory phase and correlated well to the concentrations in serum of estradiol and progesterone at the time of examination. The great variations of uterine tonicity around the day of ovulation and the onset of menstruation were not unexpected findings. It is known that very rapid changes in the hormonal environment take place during the so called ovulatory phase and that the responsiveness to pharmacologically active agents alters (48, 49, 50). During menstruation, on the other hand, the concentrations of gonadal hormones are markedly decreased and the released prostaglandins interfere with uterine tonicity. It is also well known that dysmenorrheic women have an increased content of PGF$_2\alpha$ in the endometrium and in the case of registrations during the first day of menstruation, the variation of uterine tonicity reflected the fact that the subjects had different degrees of dysmenorrhea.

Uterine tonicity was found to be high in all women with severe primary dysmenorrhea. Naproxen and Salbutamol markedly decreased uterine tonicity and relieved the pain in all but one of the women investigated.

The excitation-contraction coupling mechanism in smooth muscle has a bearing on the findings from the present study. The calcium ion is considered to play a key role in muscle contraction-relaxation. Uterine muscle, like other muscles, requires calcium for contraction of myometrial fibres. Con-
traction results from interaction of actin-myosin, adenosine-triphosphate (ATP) and the ions surrounding the contractile proteins. The contractile elements are activated when the intracellular free calcium level increases above $10^{-7}$ M; activation is maximal at $10^{-6}$ M. It is suggested that hormones as well as pharmacologically active agents exert their effects upon the myometrium through different mediators (nucleotides). Cyclic 3',5'-adenosine-monophosphate (cAMP) can decrease the intracellular concentration of free calcium by promoting active extrusion of calcium or by increasing the binding of calcium to intracellular structures such as sarcoplasmic reticulum and/or mitochondria. The concentration of cAMP can be increased by an enzyme, adenyl cyclase, which catalyses the formation of cAMP from ATP, and can be decreased by another enzyme, phosphodiesterase, which promotes the formation of AMP from cAMP. Cyclic 3',5'-guanosine-monophosphate (cGMP) is a possible mediator of events that are antagonistic to those mediated by cyclic AMP. It is suggested, for example, that cGMP counteracts the effect of cAMP on calcium binding (25).

The action of estradiol upon the myometrium seems to be associated with the rise of cGMP (39). Progesterone, on the other hand, prevents the estradiol-induced increase in cGMP, when estradiol and progesterone are administered simultaneously (39). Furthermore, progesterone increases the ATP-dependent calcium binding and promotes the storage of calcium in sarcoplasmic reticulum (13). Regarding prostaglandins, it is suggested that PGE$_2$ increases cyclic AMP and that PGF$_{2\alpha}$ increases cyclic GMP (38).

All the aforementioned findings concerning the excitation-contraction coupling mechanism in smooth muscle promote an understanding of and have implications for the findings of the present study. Thus it is suggested that the increased uterine tonicity during the proliferative phase depends on the action of estrogen upon the myometrium, probably mediated by cGMP. Progesterone inhibits the estrogenic effect during the secretory phase and decreases the intracellular calcium ion con-
centration, thus resulting in decreased uterine tonicity.

The uterine tonicity on the first day of menstruation was found to be increased in women suffering from primary dysmenorrhea. The high content of PGF$_{2\alpha}$ in endometrial tissue in these women is a possible etiological factor, as PGF$_{2\alpha}$ has been found to increase cGMP, which in turn augments intracellular free calcium ions with a resultant heightening of tonicity. The administration of Naproxen inhibits prostaglandin synthesis, which results in decreased uterine tonicity and pain relief.

Finally, Salbutamol decreases the high uterine tonicity in women with severe primary dysmenorrhea and relieves the pain. This effect is mediated by cAMP, which decreases the intracellular concentration of free calcium, thereby promoting relaxation.
CONCLUSION

Hysterometry is a new method, which has been applied in a study of uterine tonicity in the non-pregnant uterus in vivo. The method has been built up with due consideration to hydrodynamic conditions. Mathematical theorems from mechanics of materials have been applied to transform measured intrauterine pressure differences to wall tension of the organ. The equivalent of an elasticity modulus in the myometrium has been used as a synthetic discriminator (without any real physiologic correlation) for the evaluation of hormonal and pharmacological effects.

In a study during normal menstrual cycles, hysterometry demonstrated that uterine tonicity is higher in the proliferative than in the secretory phase. The uterine tonicity correlated well with the concentrations in serum of estradiol and progesterone at the time of examination.

Hysterometry has furthermore been used in a study of primary dysmenorrhea. It was shown that uterine tonicity was high on the first day of menstruation in women suffering from primary dysmenorrhea. Inhibitors of prostaglandin synthesis (Naproxen Sodium and Naproxen acid) and a selective β₂-receptor stimulator (Salbutamol) markedly decreased the uterine tonicity and relieved the pain.
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