MYOMETRIAL RESPONSES TO THE MENSTRUAL
PLAIN-MUSCLE STIMULANT

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SUMMARY

The guinea-pig uterus and strips of human myometrium are stimulated in vitro by ether extracts of menstrual fluid; the guinea-pig uterus is also stimulated in vivo by intravenous or intracardiac injections of the same extracts. The amount of the active material recovered from menstrual fluid probably varies from subject to subject, and in one instance was shown to decrease steadily from the beginning to the end of the menstrual period. The uterine responses in vitro take several forms, all excitatory; they are compared with the contractions of the human uterus in vivo during normal menstruation and dysmenorrhoea.

Among the lipids of the human endometrium are a group of slowly acting stimulants of plain muscle. There is some evidence that these or similar substances circulate in the systemic blood during menstruation, in concentrations great enough to stimulate the myometrium; and such an action has been suggested as the main physiological function of these stimulants [Pickles, 1957, 1959; Chambers & Pickles, 1958]. The present paper describes the uterine responses in detail, and gives some account of the variations in the amounts of the stimulants recovered from the menstrual fluid. The substances concerned are not yet identified and are referred to collectively as the 'menstrual stimulant'. The term 'menstrual hormone' is reserved for any similar but not necessarily identical substance circulating in the blood during menstruation.

METHODS

Menstrual extracts

Crude lipid extracts of menstrual fluid were first made in the way described previously for 'total lipids' [Chambers & Pickles, 1958], except that the elution with petroleum ether was omitted. The crude extracts were taken up as completely as possible in approx. 1 ml. diethyl ether (B.D.H. Laboratory Reagent, not Na-dried) to each 5 mg lipid; the solutions were filtered and the ether evaporated, and the lipid dissolved or suspended in a concentration of 1 mg/ml. in physiological saline adjusted to a pH of about 7·5.

Since substances with the pharmacological properties of acetylcholine, histamine and possibly 5-hydroxytryptamine had been found in the crude extracts, the possibility that traces of these rapidly acting stimulants might pass into the ether re-extracts was tested as follows: 120 mg of mixed menstrual lipid was divided into two equal parts, and to one part was added 0·6 mg each of acetylcholine hydrochloride, histamine acid phosphate and 5-hydroxytryptamine creatinine sulphate. Each lot of lipid was then taken up in 12 ml. ether, from which aqueous solution-suspensions
were made as usual. These were tested on preparations of guinea-pig duodenum and terminal ileum, without addition of atropine etc. There was no significant difference in the responses to the two extracts. The sensitivity of the ileum was such that had only 1/1000 of each of the three rapidly acting stimulants passed into the ether solution, the ileum would have shown a definite ‘rapid’ phase of contraction in addition to the characteristic ‘slow’ contraction caused by, for example, a 0·2 mg dose of the lipid. It is thus unlikely that significant quantities of acetylcholine, histamine or 5-hydroxytryptamine pass into the ether re-extracts of menstrual lipids.

The ether-insoluble parts of the crude lipid extracts contained only about one-twentieth of the total amount of the characteristic slowly acting stimulant, resistant to atropine, mepyramine and bromolysergic acid; they were therefore rejected. The ether-soluble part could be divided chromatographically into active and inactive fractions, but such fractionation was not thought necessary for the present purpose.

Isolated plain-muscle preparations

Strips of human myometrium were prepared and suspended as described previously [Chambers & Pickles, 1958]. Eighteen of the twenty myometria in this series were from non-pregnant patients aged from 34 to 49; the other two were from patients aged 54 and 66. The uteri had been removed for various clinical reasons but in every instance the main mass of the myometrium, from which the preparation was made, was macroscopically normal.

Guinea-pig uterine horns, opened lengthwise, were usually suspended in a low-Ca solution containing NaCl 0·82%, KCl 0·052%, CaCl₂ 0·008%, NaHCO₃ 0·047%, MgSO₄ 0·015%, glucose 0·1%. The guinea-pig duodenum, suspended in the same solution, was frequently used for comparison of the extracts, since it was more sensitive than the uterus and gave better base-lines than did the terminal ileum in this solution. (Solutions with a high Mg content, such as Tyrode, decrease the sensitivity of plain muscle to the menstrual stimulant). Although it had been shown that acetylcholine, histamine and 5-hydroxytryptamine were unlikely to pass into the ether extracts in significant amounts, atropine sulphate, mepyramine maleate and bromolysergic acid diethylamide bitartrate (‘BOL’) (2 × 10⁻⁸ g/ml. each) were added as an additional precaution in most experiments. The few experiments in which these inhibitors were not added gave the same types of result as the others.

Guinea-pig preparations in vivo

Female guinea-pigs, weighing 700–900 g, were anaesthetized with pentobarbitone sodium (‘Nembutal’), the lungs were ventilated mechanically, the abdomen and thorax were opened in a bath of oxygenated Locke’s solution at 37° C and the bleeding points were ligated. Ether extracts of menstrual lipids were injected either intravenously or into the right or left ventricle and the effects on the viscera were watched.
RESULTS

Individual variations between menstrual extracts

Thirty single specimens from a total of ten subjects were extracted individually and the potencies of the ether-soluble fractions compared. Each extract was tested on both the guinea-pig uterus and the duodenum; and within the rather wide limits of experimental error the two tissues gave the same results in all cases. The duodenum was always the more sensitive of the two preparations.

The concentrations of ether-soluble material that caused a moderate contraction of the duodenum (10–15\% shortening) within 3 min, and a small but definite response of the guinea-pig uterus, ranged from 10 to > 100 \( \mu g/ml \). The majority of the individual extracts however lay within a narrower range of potency, giving moderate responses in concentrations of 20–40 \( \mu g/ml \). Some of this variation may possibly have resulted from differences in the way the specimens were collected by the subjects. One subject, however, a multipara who commonly experienced mild dysmenorrhoea, produced lipid of relatively high potency in successive months.

Another subject collected the whole of the loss during the first 3 days and recorded the duration of flow represented by each specimen. The amount of ether-soluble material extracted averaged 4-0 mg/hr of flow, but it was 9-6 mg/hr for the first specimen. Equal concentrations (20 \( \mu g/ml \)) of ether-soluble material from the different specimens showed rather irregular differences in potency; but samples representing equal periods of flow contained progressively less and less activity.

Incomplete results from the other subjects suggested that the rate of 4 mg of ether-soluble material per hr was representative of the whole group. The loss during the whole of a normal menstrual period was thus of the order of 300 mg.

Types of uterine response

Guinea-pig uterus in vitro

The following four types of response were seen, singly or in combination, each type being seen several times in the course of twenty consecutive experiments.

1. An increase in the extent of the pre-existing rhythmical contractions, as judged by the excursions of the recording lever, with complete or almost complete relaxation between contractions.

2. An increase in the extent of the contractions, but with poor relaxations intervening, so that the amplitude of the contractions decreased.

A single preparation might show responses of either type (1) or type (2) according to the quantity of menstrual extract applied, the larger quantities tending to cause responses of type (2). An example is shown in Fig. 1, which also shows the tachyphylaxis occurring when tests are repeated too frequently on this preparation.

3. An increase in the frequency of the rhythmical contractions; an example is shown in Fig. 2.

4. When the preparation was already contracting in an irregular or incoordinated way, the addition of a menstrual extract was sometimes followed by well-coordinated and powerful contractions, which might persist for as long as 20 min after the extract had been washed out (see Fig. 3).

Uteri from guinea-pigs pretreated with stilboestrol, or which appeared to be in
natural oestrus, were not more, and possibly less, sensitive to the menstrual stimulant than were other guinea-pig uteri, notwithstanding their sensitivity to oxytocin or vasopressin.

*Human myometrial preparations in vitro*

The human myometrial preparations showed all the types of response described above for the guinea-pig, but with greater variability. Four possible causes of such variability are as follows:

(A) Two preparations made identically from adjacent pieces of the same uterus.

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Fig. 1. Responses of guinea-pig uterine horn to different quantities of extract of menstrual fluid; for further description see text, p. 152. In this and the later figures the quantities of ether-soluble material shown were added to the 10 ml. organ bath as indicated by the arrows and washed out at the ends of the horizontal lines; washings are also shown by dots on the tracings. Time marker 1 min.

Fig. 2. Increase in frequency of the rhythmical contractions of a guinea-pig uterine horn on addition of menstrual stimulant.
sometimes differed markedly in their patterns of spontaneous activity and in their responses to the menstrual stimulant.

(B) Some preparations showed little spontaneous activity or responsiveness to either menstrual or neurohypophysial extracts, and within an hour or two became completely inactive. This is not surprising since the uteri were inevitably ischaemic for at least a few minutes during the hysterectomy, and there was always a further unavoidable delay before the preparation could be cut and suspended. A few uteri felt particularly tough to cut, and these were relatively inactive. Drugs used in pre-medication of the patient may possibly also affect the myometrium.

Fig. 3. The irregular pattern of the contractions of a guinea-pig uterine horn, as shown during the first 16 min of the part of the record reproduced, had been present for at least ¾ hr previously. Addition of menstrual stimulant was followed by regular coordinated contractions, which persisted for 20 min after the stimulant was washed out.

Fig. 4. A human myometrial preparation, at first showing uncoordinated contractions. Addition of 0.2 mg ether-soluble menstrual lipid caused first a spasm and then well-coordinated rhythmical contractions.

(C) The two myometria from the older patients (aged 54 and 66) were almost inactive and unresponsive; otherwise there was no correlation between responsiveness and age, within the rather narrow age-range of the majority of the specimens.

(D) In some cases, when the patient’s menstrual history was known and a histological report on the endometrium was available, the phase of the cycle at hysterectomy could be assessed. These cases suggested that the myometrium was most
sensitive to the menstrual stimulant near to the time of menstruation, and least sensitive about mid-cycle. The most sensitive preparation in this series was from a uterus removed for incapacitating dysmenorrhea in a patient aged 44, and it was in the late secretory phase.

If a preparation gave a good immediate response to the menstrual stimulant, the response might persist for long periods such as 1 hr while the stimulant remained in the organ bath.

![Fig. 5. Another human myometrial preparation. A larger quantity (0.6 mg) of the same extract as that used in the experiment of Fig. 4 caused a maximal contraction.](image)

![Fig. 6. A preparation from a human myometrium removed 11 days after beginning of the last menstrual period. Even the very large quantity of menstrual stimulant used had only a minimal effect.](image)

Examples of human myometrial response are shown in Figs. 4–6. Fig. 4 shows the production of co-ordinated rhythmical contractions in a preparation that previously showed only unco-ordinated activity. By contrast, Fig. 5 shows (in another preparation) a powerful single contraction caused by a larger amount of menstrual stimulant applied for a short time. Fig. 6 is an example of a poor response to even a very large dose of menstrual stimulant; this uterus had been removed shortly before mid-cycle.
Guinea-pig uterus in vivo

Although the finer details of uterine motility could not be studied in these preparations, there was no doubt that injection of about 2 mg of ether-soluble menstrual lipid (2-8 mg/kg body weight) into the circulating blood caused strong and persistent uterine contractions, probably an increase in gastro-intestinal movements, and no other obvious effect. In one experiment the uterine contractions were recorded by means of a thread attached to the mid-point of one horn; the tracing is shown in Fig. 7. In this instance 3 mg lipid was injected.

![Fig. 7. Response of a guinea-pig uterine horn in vivo to 3 mg ether-soluble menstrual lipid injected into the left ventricle, at the time indicated by the arrow. The break in the record represents a mechanical disturbance due to the injection.](image)

DISCUSSION

The human uterus expels the menstrual fluid by rhythmical contractions, which occur every 1–1½ min at the beginning of the menstrual period [Hinselmann, 1925]. Schröder [1927] confirmed this observation and found that during dysmenorrhoea the contractions have a greater frequency. Both the normal contractions and those that underlie dysmenorrhoea may be hypothetically explained as the myometrial responses to a substance with the properties of the menstrual stimulant, passing from the endometrium to the myometrium either by some local route or in the general circulating blood; provided that the human uterus in vivo responds as do the myometrial preparations in vitro and that the stimulant is not inactivated. The co-ordinated contractions shown in Fig. 4 are of the same frequency as the normal menstrual contractions described by Hinselmann and by Schröder, and it can readily be imagined that if the whole corpus uteri contracted in this way the menstrual fluid would be effectively expelled. The circulating blood of one subject has been found to contain substances with similar properties to those of the menstrual stimulant during the menstrual period, but not 8 days later [Pickles, 1959]; if this finding is confirmed in more subjects, it will seem likely that the menstrual contractions are in fact due to the liberation of ‘menstrual stimulant’ into the circulating blood.

Very powerful contractions such as that shown in Fig. 5 would seem likely to cause a sensation of uterine colic, and if relaxation between contractions were poor (as in
some of the responses of the guinea-pig uterus shown in Fig. 1) they might well be less effective in expelling the menstrual fluid than more moderate contractions would be. The uterine contractions of dysmenorrhoea may thus hypothetically be explained either by excessive production of menstrual stimulant or by excessive myometrial sensitivity to it.

The fact that the guinea-pig uteri gave the same kinds of response as did the human myometrial preparations makes it unlikely that the responses of the latter were artifacts, arising only from the possibly ‘unphysiological’ state of the tissues. The differences that were sometimes seen between two similar preparations from the same human uterus, however, emphasize that the myometrium is not simply a uniform mass of plain muscle, and that the responses of the whole human uterus in vivo are not necessarily identical with those of the isolated tissue preparations.

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