STUDIES ON SALIVA IN MENSTRUATING, PREGNANT AND POST-MENOPAUSAL WOMEN

By

S. Kullander and B. Sonesson

ABSTRACT

Saliva from normally menstruating, pregnant and post-menopausal women was collected by catheterizing the excretory duct of the submandibular gland. The saliva was studied for rate of secretion, water content and the crystallization pattern. In addition the spontaneous secretion of saliva as well as the secretion stimulated by histamine and by pilocarpine were studied.

The observations can be summarized as follows:

Histamine had no effect on the rate of secretion, while pilocarpine increased the secretion considerably.

In fertile women, the rate of spontaneous and histamine-stimulated secretion was somewhat higher during the secretion phase of the menstrual cycle than during the proliferation phase. No significant difference was found between the water content of the saliva in the two phases.

In pregnant women there was a decrease in the rate of secretion as compared with normally menstruating women. In the post-menopausal women the decrease in secretion of saliva was still more marked.

In pregnant women the crystallization pattern of the saliva was coarser than in menstruating women. After the menopause the crystallization pattern was still coarser and in one case no crystallization occurred at all.

No constant differences were observed between the patterns during the proliferation and secretion phases, respectively.

Papanicolaou (1946) observed that when cervical mucus, collected at ovulation while the cervix is under the influence of oestrogens, was allowed to dry on a glass slide, it crystallized into a particular pattern (arborization). At the time of ovulation the cervical mucus changed in composition, i.e. the water
content was increased and it was less viscous. Arborization disappeared during the secretion phase of the menstrual cycle, i.e. when the cervix is under the influence of progesterone. Several other body fluids, such as follicular fluid and nasal mucus, also tend to arborize and form palm-leaf-like crystals on evaporation (Zondek 1954, 1956; Abon-Shabanah & Plotz 1957; Davis & Abon-Shabanah 1958; and others). This phenomenon is linked to the presence of protein and electrolytes, particularly sodium chloride. Whether the arborization phenomenon of body fluids other than cervical mucus is under hormonal oestrogenic control and is inhibited by progesterone is debatable. Henderson (1956), who studied 2 women, found changes in the nasal secretion comparable to those in the cervical mucus during the menstrual cycle, and Andreoli & Della Porta (1957) stated that this also applies to the saliva. Zondek (1959), however, found that the saliva gave a positive reaction – arborization – during the whole cycle, showing no difference in crystallization pattern between the proliferation and secretion phase. Saliva from pregnant women or women in the menopause also showed this reaction. In contrast, cervical mucus collected from women in the menopause or during pregnancy, shows no arborization. Zondek (1959) concluded that the hormonal balance is not reflected in the saliva in the same way as in the cervical mucus.

The purpose of the present investigation, which is a link in a series of studies (Kullander & Sonesson 1959) on the possible effect of sex hormones on saliva in women, was to find out whether the secretion and water content of saliva or the crystallization pattern vary with the phase of the menstrual cycle, during pregnancy or after the menopause. A search of the literature failed to reveal any investigations of the variation, if any, of the rate of secretion and water content of the saliva in women.

**MATERIAL AND METHODS**

Saliva was collected by catheterization of the excretory duct of the submandibular gland. Collected in this way, the saliva is not contaminated by cells or food debris from the oral cavity. All the subjects were healthy women, some with normal menstrual cycles, some pregnant and some at the menopause. The samples of saliva were first collected in the morning before breakfast. The basal temperature charts and menstruations, if any, were recorded.

Five minutes after a plastic catheter (inner diameter of 0.5 mm and an outer diameter of 1 mm) had been introduced about 5 mm into the excretory duct of the submandibular gland, »spontaneous secretion« was measured, the saliva secreted being collected for a period of 10 minutes.

The secretion of saliva was then stimulated by histamine and pilocarpine. Pilocarpine, particularly, is known to be a strong stimulant of secretion of saliva (Rauch 1959). 0.2 ml of 0.1% histamine hydrochloride was injected subcutaneously. »Histamine-stimulated saliva« was then collected for a further 10 minutes. Finally, after 10 minutes pause, the subjects were given a subcutaneously injection of 0.2 ml of 2%
pilocarpine and the »pilocarpine-stimulated saliva« was collected for 10 minutes. The water content of the various samples was determined by depositing a drop of saliva on a glass slide of known weight, which was weighed and placed in a sulphuric acid desiccator for 24 hours. The slides were then weighed again with the dried saliva. Crystallization was studied by allowing a small amount of saliva of roughly the same volume to evaporate at room temperature on a slide. Photographs were taken of the crystallization pattern of all samples, always of an area of roughly uniform size equally near the margin of the dried drop.

The investigation was carried out on 8 healthy normally menstruating women, 19 pregnant women (4 of these had symptoms of emesis), and 7 healthy post-menopausal women (>42 years of age). In the group of menstruating women, the saliva was examined, during the proliferation phase, and secretion phase, as judged by the basal temperature curve. For each of the normally menstruating women, 6 values were thus obtained, namely, on two occasions during the menstrual cycle (proliferation and secretion phase) 1 for spontaneous secretion, 1 for histamine-stimulated secretion and 1 for pilocarpine-stimulated secretion.

The pregnant women were subdivided into three groups according to the month of pregnancy (see Table 2). From each pregnant woman saliva was collected on only one occasion, and three values were obtained (cf. above). In this group we wanted to know whether the increased histamine concentration during pregnancy had any inhibitory action on the effect of histamine.

**RESULTS**

**Rate of secretion.** Table 1 shows that secretion of saliva, stimulated by pilocarpine, is about 3 times as large as the spontaneous secretion, while histamine did not produce an increase in secretion. Statistically the total amount of saliva, produced spontaneously and after stimulation with histamine was somewhat larger during the secretion phase than during the proliferation phase ($P = 0.05$). The amount of pilocarpine-stimulated saliva was not found to vary with the cyclic phase. But here the inter-individual variations were larger and might have masked any phasic difference.

The pregnant women were studied on only 1 occasion, and then before and after the delivery.

**Table 1.**

<table>
<thead>
<tr>
<th></th>
<th>Spontaneous secretion</th>
<th>Histamine-stimulated</th>
<th>Pilocarpine-stimulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proliferation phase</td>
<td>1.59</td>
<td>1.50</td>
<td>4.46</td>
</tr>
<tr>
<td>Secretion phase</td>
<td>1.75</td>
<td>1.62</td>
<td>4.14</td>
</tr>
</tbody>
</table>

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Table 2.
Secretion of saliva by submandibular gland during pregnancy (ml/10 min).

<table>
<thead>
<tr>
<th>Month of pregnancy</th>
<th>No. of cases</th>
<th>Spontaneous secretion</th>
<th>Histamine-stimulated</th>
<th>Pilocarpine-stimulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>- IV</td>
<td>4</td>
<td>1.23</td>
<td>1.13</td>
<td>4.28</td>
</tr>
<tr>
<td>V - VII</td>
<td>4</td>
<td>1.13</td>
<td>1.00</td>
<td>4.08</td>
</tr>
<tr>
<td>VIII - X</td>
<td>7</td>
<td>0.84</td>
<td>1.01</td>
<td>2.61</td>
</tr>
<tr>
<td>- IV with vomiting of pregnancy</td>
<td>4</td>
<td>1.40</td>
<td>2.20</td>
<td>5.95</td>
</tr>
</tbody>
</table>

after stimulation with histamine and pilocarpine, respectively. The secretion noted for the different months of pregnancy are given in Table 2.

It is clear from the table that histamine had no demonstrable effect on secretion during pregnancy. If the values for spontaneous secretion and histamine secretion are taken together (which appears justifiable because no statistical difference in secretion was demonstrable) the probability of a true decrease in the secretion of saliva with advancing pregnancy, is about 20 %, the corresponding probability for a decrease in pilocarpine-stimulated secretion with advancing pregnancy being between 5 and 10 %.

It is remarkable that in the pregnant women with vomiting the spontaneous, histamine- and pilocarpine-stimulated secretion was increased, suggesting a higher sensitivity of the salivary gland in these cases.

The spontaneous secretion of saliva in postmenopausal women was, in the average, 0.37 ml/10 min. After injection of histamine the value was 0.30 ml/10 min. Thus no difference was found between the rate of spontaneous secretion after histamine in the menopausal group. After pilocarpine the secretion was 2.07 ml/10 min.

The difference between the rate of secretion of saliva in menstruating and postmenopausal women was statistically significant (see Table 3). After the menopause the secretion decreases markedly.

The mean secretion of saliva under various conditions is summarized in Table 3.

Water content of saliva. The water content of the saliva was usually 98–99 %. The water content of spontaneous secretion from normally menstruating women was found to be higher than that of histamine-stimulated secretion. The difference was 2.2 times the mean error. The water content of pilocarpine-stimulated secretion was higher than that of spontaneous secretion and hista-
Table 3.

Secretion of saliva from submandibular gland (ml/10 min) in healthy women.

<table>
<thead>
<tr>
<th></th>
<th>Normally menstruating</th>
<th>Pregnant</th>
<th>Post-menopausal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous and histamine-</td>
<td>1.53 ± 0.22</td>
<td>1.06 ± 0.17</td>
<td>0.34 ± 0.06</td>
</tr>
<tr>
<td>stimulated secretion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secretion after stimulation</td>
<td>4.44 ± 0.47</td>
<td>3.65 ± 0.50</td>
<td>2.07 ± 0.26</td>
</tr>
<tr>
<td>with pilocarpine</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

mine-stimulated secretion. The difference was about twice the mean error. No significant difference was found between the water content of saliva from the proliferation and secretion phase or between saliva from pregnant and non-pregnant and menopausal women.

Crystallization pattern. Saliva from all the normally menstruating women showed a characteristic palm-leaf-like crystallization pattern – arborization (Fig. 1a). Saliva collected during the proliferation phase of the menstrual cycle showed the most distinct and delicate crystallization pattern while that of saliva collected during the secretion phase was sometimes somewhat sparse and occasionally crude.

In the group of pregnant women, the crystallization pattern tended to be coarser with advancing pregnancy (Fig. 1b). Thus in the very »advanced group« (9 months), the arborization was often very coarse with thick, plump frond-like crystals.

With the exception of 1 case, in which no crystallization occurred, distinct though rather coarse pattern was seen in the group of menopausal women (Fig. 1c).

DISCUSSION

The salivary secretion is said to be increased by histamine injection (Krantz & Carr 1958) but the effect is probably not constant (Mac Kay 1927; Emmelin et al. 1954). In our experiments, injection of 0.2 ml histamine, caused a typical skin reaction with local erythema and occasionally a moderate flushing of the face of the patient but no increase in the secretion of saliva from the submandibular gland was observed. This was in contrast to the effect of pilocarpine, which in all cases produced a striking increase in the rate of secretion.

It is of interest that in the group of females suffering from vomiting during the early stages of pregnancy, there was an increase in the secretion of saliva after stimulation with histamine (cf. Table 2). From this it might be con-
Fig. 1.

Typical crystallization patterns of saliva collected in
a. proliferating phase of normally menstruating woman;
b. mid-pregnancy;
c. post-menopausal woman.
Magnification in all pictures 140 times.
cluded, that the wellknown increased histaminase concentration during pregnancy did not inactivate the histamine in these cases.

In the pregnant groups there was a decrease in the rate of secretion with advancing pregnancy. This is in contrast to the results reported by Bernstine & Friedman (1957) who, in the majority of cases studied, found that the salivation continued and even increased as pregnancy progressed.

The hypothesis of an increase in dental decay during pregnancy has, however, been seriously questioned (Ziskin & Hotelling 1937; Rauramo et al. 1962). In our pregnant group the frequency of caries was observed and no tendency to increase dental decay was noticed with advancing pregnancy.

The decrease which we found in the rate of saliva secretion during pregnancy may, however, play a role in the frequent gingival diseases during pregnancy.

The marked decrease in the rate of secretion in the post-menopausal women is interesting and may be a causal factor in sialosis and other oral diseases which increase in frequency at this period. It may be due to an absence of hormonal stimuli (oestrogens) and/or age atrophy of the glands. In animals (mice) it is well known that the histological picture of the salivary gland changes after oophorectomy.

The crystallization pattern showed a typical arborization phenomenon in the normally menstruating woman. Our observation that there is sometimes a cruder pattern during the secretion phase might be explained by the effect of progesterone; a similar phenomenon is familiar in the cervical mucus. The same progestogenic hormonal mechanism may be responsible for the crystallization changes in the saliva found by us during pregnancy.

The water content of the saliva from the submandibular gland was the same as that reported by Rauch (1959) for the total saliva collected from all the salivary glands. The tendency of a decrease in water content of histamine-stimulated saliva which was found is in accordance with our observation that the latter saliva was more mucous.

REFERENCES

Mac Kay M. E.: Amer. J. Physiol. 82 (1927) 546.

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