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From:

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SALIVARY SECRETION IN PREGNANCY  
A LONGITUDINAL STUDY OF FLOW RATE, TOTAL PROTEIN,  
SODIUM, POTASSIUM AND CALCIUM CONCENTRATION  
IN PAROTID SALIVA FROM PREGNANT WOMEN

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The total protein content; the sodium, potassium and calcium concentration and the flow rate of secretion of parotid saliva were studied in 26 women during pregnancy and 8 weeks after parturition. All the women were examined in the 12th, 18th, 24th, 30th, 34th and 38th weeks of pregnancy as well as 1 to 3 days and 8 weeks after delivery. During pregnancy there was a decrease in the flow rate, but an increase in the total protein concentration, of both resting and citric acid stimulated parotid saliva. No changes could be demonstrated in the sodium concentration of resting saliva. In stimulated saliva the sodium concentration decreased during pregnancy. There was a positive correlation between sodium concentration and flow rate. The potassium and the calcium concentrations of both resting and stimulated parotid saliva increased during pregnancy and decreased after delivery. There was no correlation between potassium concentration and flow rate of resting or stimulated saliva. In resting saliva there was a negative correlation between the calcium concentration and flow rate.

Salivary secretion has been the subject of several quantitative and qualitative investigations, but the results obtained have been discordant. This lack of agreement may be explained by differences in measuring methods and in experimental design as well as by the effect of a wide variety of both mental and physical factors on the rate of production and composition of the saliva (*Afonsky, 1961; Bergen & Emelin, 1961*). The rate of secretion and the chemical composition of saliva found in a given investigation depend on the method of measurement (*Bertram, 1967*), the duration of sampling (*Kerr, 1961*), the hour of sampling (*Holmes, 1964*), the interval since last meal (*Krasnogorski, 1931*), the age, sex, and mental state of the donor (*Nekrasow & Ghranilowa, 1933*), as well as certain systemic disorders (*Bertram, 1967; Ericson, 1968*). It is therefore difficult to determine what may be regarded as the normal rate of secretion and the normal composition of saliva.

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In investigations on the mouse and the rat the function of the salivary glands has been found to be influenced by hormones, such as those produced by the adenohypophysis (*Lacassangue & Chamorro, 1940, Shafer & Muhler, 1955*). *Baker, Clapp & Light (1964)* have found that an adequate supply of somatotropin, tyroxin and corticosterone is important for maintenance of the normal structure and function of the acini of the parotid glands. Only few studies have been made on the effect of sex hormones on the secretion and chemical composition of saliva. The crystallisation pattern of saliva have been studied with conflicting results (*Anderoli & Bella Porta 1957; Zondek, 1959*). According to *Zondek (1959)*, the crystallisation pattern of saliva varies with the phases of the menstrual cycle, though not so strikingly as cervical secretion.

In an investigation of the rate of secretion, water content and crystallisation pattern of submandibular secretion *Kullander and Sonesson (1965)* found that the rate of flow of stimulated saliva was highest during the secretory phase of the menstrual cycle. The rate of secretion of saliva is lower during pregnancy than otherwise. It is also substantially lower after the menopause than during reproductive life (*Kullander & Sonesson 1965*).

As no longitudinal study of salivary secretion in pregnancy has been reported earlier, the purpose of the present investigation was to follow the rate of secretion of parotid saliva, its total protein content and its concentration of sodium, potassium and calcium during pregnancy, during the puerperium, and 8 weeks later.

#### MATERIAL AND METHODS

The clinical series consisted of 26 women, 19 to 30 years of age (Table I).

Six of the women were examined before the eighth week of pregnancy and the remaining 20 in the 12th. The first oral examination was always made at the patient's first visit. Thus, all the women were examined in the 12th, 18th, 24th, 30th, and 38th weeks of pregnancy as well as 1 to 3 days and 8 weeks after delivery. The women received no dental treatment before this final examination.

At the first examination measurements were made of:

- a) rate of secretion of resting saliva
- b) rate of secretion of stimulated saliva,
- c) total protein concentration in resting and in stimulated saliva, and
- d) concentration of sodium, potassium and calcium in resting and in stimulated saliva.

Table I.  
*Age distribution of the patients*

Age in years	19	20	21	22	23	24	25	26	27	28	29	30
Number of patients	1	1	2	2	2	3	3	5	3	1	0	3

Only saliva from the parotid glands was studied. Measurements were made at different times of the day, but never earlier than 1 hour after a meal. The subjects were instructed not to smoke or brush their teeth or rinse their mouth within at least 1 hour before the examination. The saliva was collected with the patient sitting with the sagittal plane of the head vertical.

#### *Parotid saliva flow rate*

*Resting saliva.* After the subject had rinsed her mouth with water and then rested for about 5 minutes a combined suction and collecting cup was placed over the right and left orifices of the parotid gland ducts. Saliva from both sides was then collected for 5 minutes, via 20 cm polyethene tubes, in a test tube. During this time the subjects were asked not to move the tongue or lips. The suction cups were then removed, and the tubes were emptied of all saliva with the aid of a compressed-air syringe.

*Stimulated saliva.* After the subject had rinsed her mouth with water and then rested for about 5 minutes, the cups were again placed over the orifices of the ducts of the parotid glands. Two drops of 2 % citric acid were then allowed to fall on the midline of the anterior third of the superior surfaces of the tongue, after which parotid saliva was collected. The patient distributed the acid solution by gentle movement of the tongue. This application of acid was repeated every half minute for 5 minutes. The cups and tubes were emptied of saliva.

All saliva was collected in tubes of low alkali glass (Pyrex), which had been washed in deionised water. During saliva collection the tubes were cooled in an ice bath. Immediately after resting or stimulated saliva had been collected, the amounts of saliva were determined by weighing and the tubes with the saliva were immediately placed in a refrigerator ( $-80^{\circ}\text{C}$ ). All the tubes were sealed with rubber stoppers.

*Concentration of protein.* The total protein concentration of each sample was determined by the method of *Daughaday et al.* (1952) with bovine serum albumin as standard. The extension was read at 540 nm in a Hitachi spectrophotometer using a standard 1 cm cuvette. Duplicate determinations were

made on each sample and the mean was taken as a measure of the total amount of protein ( $\mu\text{g/ml}$ ) at the time of the examination.

*Concentrations of sodium, potassium and calcium.* After dilution of the saliva with double distilled water in a quartz apparatus the concentrations (mEq/l) of sodium, potassium and calcium were determined by flame emission in a Unicam SP90 (Unicam Instruments Ltd) flame emission spectrometer. Determinations were made in both resting saliva and stimulated saliva. Owing to insufficient production of saliva such determinations were not always possible.

*Statistical methods.* Median values and 95 % confidence intervals for the median were determined for protein-, sodium-, potassium- and calcium-concentrations in parotid saliva as well as for the saliva flow rate.

The values found at the eight examinations of each of the subjects were ranked. For each examination the mean of the persons' ranks was calculated. Saliva flow rate, the concentrations of total protein, of sodium, of potassium

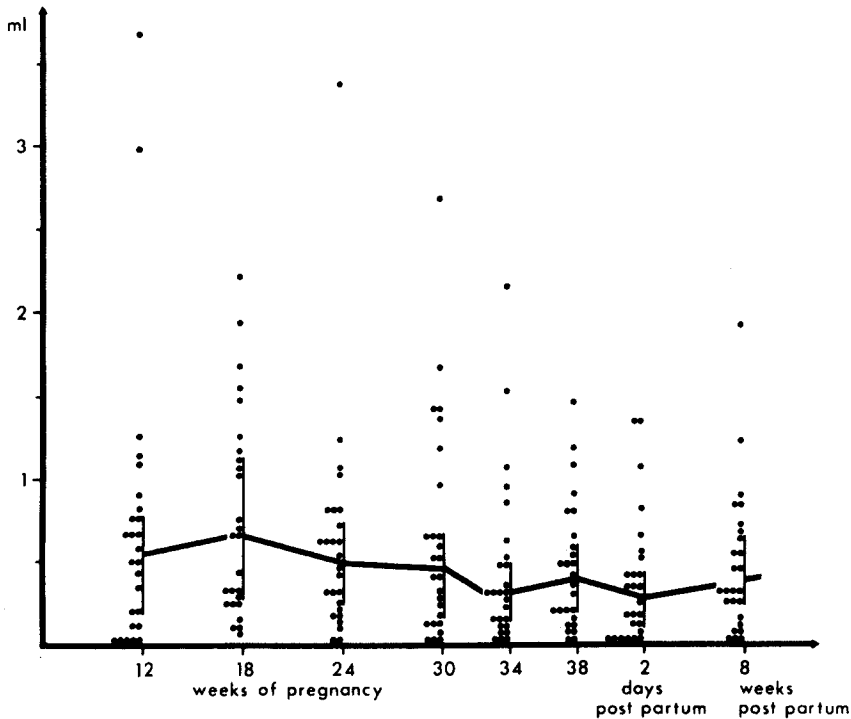


Fig. 1. Original and median values surrounded by 95 % confidence intervals for parotid saliva flow rate during 5 minutes of collection at the various weeks of observation. Resting saliva.

and of calcium in parotid saliva were then studied for any variation with time by Friedman's rank test.

When the variation in time was significant, a multivariate regression was performed of the ranks for protein concentration and sodium, potassium and calcium concentrations on the ranks for saliva flow rate. Finally, after correction for the regression (corrected mean rank), the residuals for total protein, sodium, potassium and calcium, respectively, were studied with Friedman's rank test for any variation with time.

## RESULTS

*Parotid saliva flow rate*

*Resting saliva.* The results are summarized in Fig. 1 and Table II. The median flow rate in the 12th week of pregnancy in the 26 women examined was 0.5. During pregnancy the salivary flow decreased successively to a

Table II.

*Median values and mean rank ( $\bar{rank}$ ) values for parotid resting saliva flow rate (ml/5 min), total protein ( $\mu\text{g/ml}$ ), sodium (mEq/l), potassium (mEq/l) and calcium (mEq/l) concentration during pregnancy and 8 weeks after delivery. n = number of observations*

Weeks of pregnancy	Flow rate n = 26		Total protein n = 21		Sodium n = 16		Potassium n = 15		Calcium n = 17	
	median	$\bar{rank}$	median	$\bar{rank}$	median	$\bar{rank}$	median	$\bar{rank}$	median	$\bar{rank}$
12	0.5	5.15	1633	3.74	0.8	4.19	22.5	3.44	1.7	3.41
18	0.6	5.92	1366	3.42	0.8	4.06	22.0	3.25	1.7	3.12
24	0.5	5.04	1700	4.63	0.7	3.94	24.5	4.19	1.9	3.71
30	0.4	5.04	1682	3.63	0.9	4.13	23.0	4.44	2.1	4.53
34	0.3	3.54	2086	5.47	0.8	4.13	26.5	4.31	1.9	3.59
38	0.4	4.27	2266	5.42	1.1	5.25	28.5	5.31	2.2	5.06
2 days post partum	0.2	3.35	2333	5.42	1.2	5.06	29.5	6.50	3.0	6.82
8	0.3	3.69	1782	4.26	0.9	4.25	24.0	4.56	2.4	5.76
$\chi^2$ $\bar{rank}$		25.97		16.53		6.21		20.00		33.63
		Sign. 0.1 %		Sign. 5 %				Sign. 1 %		Sign. 0.1 %
Regression Coefficient			-0.10 ± 0.18		-0.03 ± 0.20		0.13 ± 0.20			-0.26 ± 0.20
95 % interval										Sign.
$\chi^2$ corrected $\bar{rank}$				13.80				16.56		24.82
								Sign. 5 %		Sign. 0.1 %

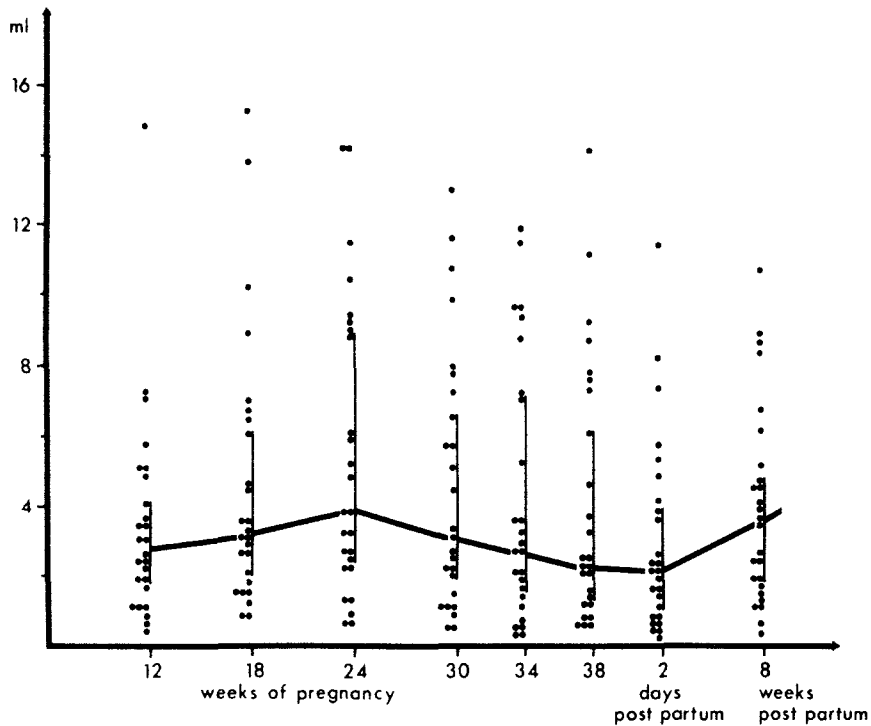


Fig. 2. Original and median values surrounded by 95 % confidence intervals for parotid saliva flow rate during 5 minutes of collection at the various weeks of observation. Citric acid stimulated saliva.

medium value of 0.2 2 days after delivery. During the following 8 weeks the flow increased again. The decrease in the salivary flow during the latter part of pregnancy was significant ( $0.001 > p$ ).

*Stimulated saliva.* The results are summarized in Fig. 2 and Table III. The median flow rate was 2.9 in the 12th week of pregnancy and 3.9 in the 24th. From that time on the flow rate decreased ( $0.001 > p$ ), and 2 days after delivery the median flow rate was 2.0. By eight weeks after delivery it had increased to 3.5.

#### *Parotid saliva total protein concentration*

The results are summarized for resting saliva in Fig. 3 and Table II and for stimulated saliva in Fig. 4 and Table III.

In the 12th week of pregnancy the median protein concentration in resting saliva was 1633 and in stimulated saliva 2328. During pregnancy the total

Table III.

Median values and mean rank (rank) values for parotid stimulated saliva flow rate (ml/5 min), total protein ( $\mu\text{g/ml}$ ), sodium (mEq/l), potassium (mEq/l) and calcium (mEq/l) concentration during pregnancy and 8 weeks after delivery n = number of observations

Weeks of pregnancy	Flow rate n = 26		Total protein n = 24		Sodium n = 22		Potassium n = 23		Calcium n = 25	
	median	rank	median	rank	median	rank	median	rank	median	rank
12	2.9	4.27	2328	3.42	3.7	4.27	20.2	3.04	1.2	2.84
18	3.2	5.15	2133	3.58	16.3	6.05	21.8	3.78	1.3	3.00
24	3.9	6.27	2200	4.35	12.6	5.18	24.2	4.74	2.0	4.80
30	3.0	4.77	1933	3.58	7.1	5.09	23.2	5.17	2.1	5.00
34	2.8	4.38	2700	5.08	2.9	3.45	24.5	5.35	2.1	5.32
38	2.2	3.73	2666	5.65	2.8	3.18	24.3	4.96	2.0	4.64
2 days post partum	2.0	2.88	3233	5.88	6.6	3.83	24.6	5.83	2.1	5.80
8	3.5	4.54	2433	4.46	12.9	4.95	23.0	4.39	1.8	4.60
$\bar{x}^2$ rank	29.80		28.04		24.77		31.74		32.24	
	Sign. 0.1 %		Sign. 0.1 %		Sign. 0.1 %		Sign. 0.1 %		Sign. 0.1 %	
Regression Coefficient			$-0.08 \pm 0.16$		$0.50 \pm 0.17$		$0.00 \pm 0.17$		$0.03 \pm 0.16$	
95 % interval					Sign.					
$\bar{x}^2$ corrected rank			25.37		12.25		31.66		32.66	
			Sign. 0.1 %				Sign. 0.1 %		Sign. 0.1 %	

protein concentration in both resting and stimulated saliva increased ( $0.05 < p < 0.01$  resp.  $0.001 > p$ ) and 2 days post partum the median protein concentration was 2333 for resting saliva and 3233 for the stimulated saliva. After delivery the median protein concentration decreased to 1782 in resting saliva and to 2433 in stimulated saliva. There was no variation with time in resting saliva for the residuals of total protein concentration after correction for the flow rate. In stimulated saliva, however, the corrected mean ranks varied with time ( $0.01 < p < 0.001$ ). There was no regression of total protein concentration on flow rate in resting or stimulated saliva.

#### Parotid saliva sodium, potassium and calcium concentration

*Sodium.* The results are summarized for resting saliva in Fig. 5 and Table II and stimulated saliva in Fig. 6 and Table III.

No changes could be demonstrated in the sodium concentration of resting saliva during pregnancy or after delivery. Several determinations deviating

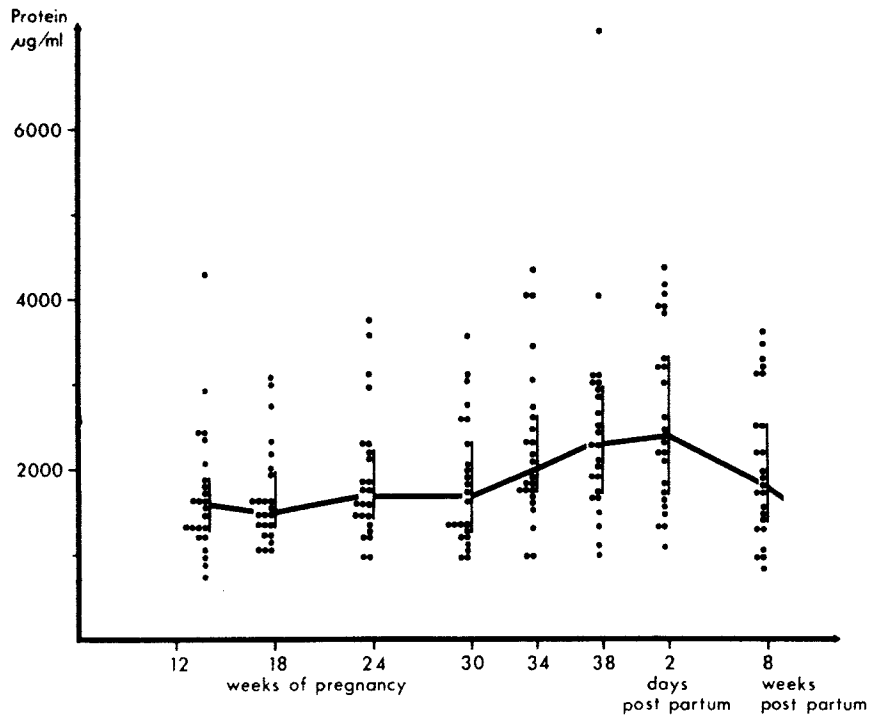


Fig. 3. Original and median values surrounded by 95 % confidence intervals for the concentration of parotid saliva total protein at the various weeks of observation. Resting saliva.

markedly from the median were recorded. There was no regression of sodium concentration in resting saliva on flow rate.

The sodium concentration in stimulated saliva showed characteristic changes. In the 12th and 24th week of pregnancy the median sodium concentrations were 3.7 and 12.6, respectively. From that time on the median sodium concentration decreased ( $0.001 > p$ ) to 2.8 in the 38th week of pregnancy. Eight weeks after delivery the median sodium concentration was 12.9. There was a positive regression of sodium concentration in stimulated saliva on flow rate. There was no variation with time for corrected mean rank of sodium concentration either concerning resting nor stimulated saliva.

*Potassium.* The results obtained with for resting saliva are summarized in Fig. 7 and Table II, and with stimulated saliva in Fig. 8 and Table III.

The median potassium concentration in the 12th week of pregnancy was 22.5 in resting and 20.2 in stimulated saliva. From the 18th week of pregnancy the median potassium concentration increased in both resting ( $0.01 < p <$

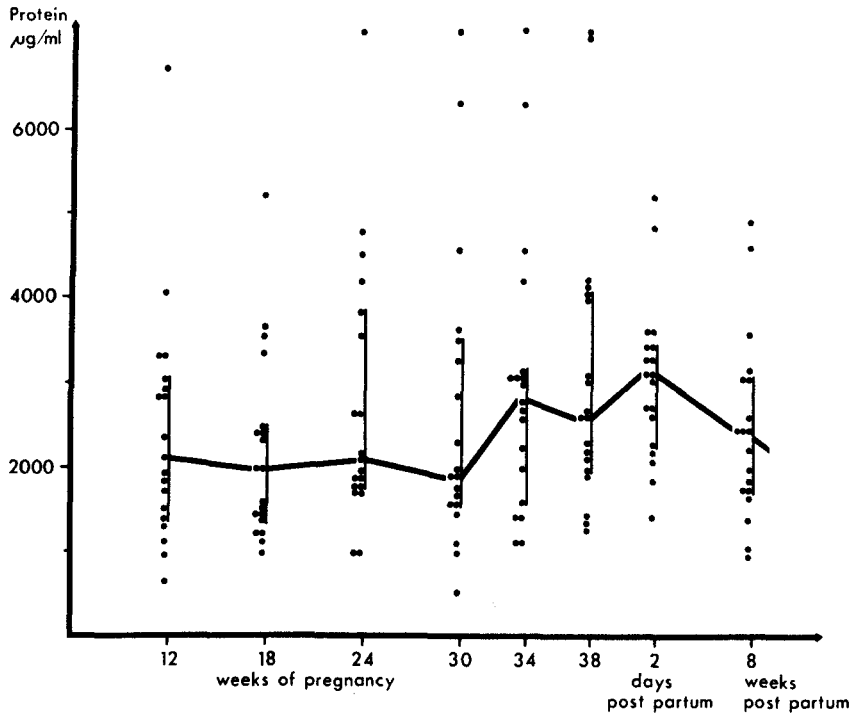


Fig. 4. Original and median values surrounded by 95 % confidence intervals for the concentration of parotid saliva total protein at the various weeks of observation. Citric acid stimulated saliva.

0.001) and stimulated saliva ( $0.001 > p$ ). 2 days post partum the median potassium concentration was 29.5 in resting and 24.6 in stimulated saliva. Eight weeks after delivery the median potassium concentration was found to be 24.0 for resting and 23.0 for stimulated saliva. There was no regression of potassium concentration on parotid saliva flow rate in resting or stimulated saliva. The corrected mean rank for the potassium concentration in both resting and stimulated saliva varied with time ( $0.5 < p < 0.01$  resp.  $0.001 > p$ ).

*Calcium.* The results are summarized in Fig. 9 and Table II for resting saliva and in Fig. 10 and Table III for stimulated saliva.

In the 12th week of pregnancy the median calcium concentration was 1.7 for resting saliva and 1.2 for stimulated saliva. During pregnancy the median calcium concentration increased and reached a maximum of 3.0 ( $0.001 > p$ ) in resting saliva and of 2.1 ( $0.001 > p$ ) in stimulated saliva 2 days post partum. After parturition there was a reduction in calcium concentration in both resting and stimulated saliva and 8 weeks post partum the median potassium

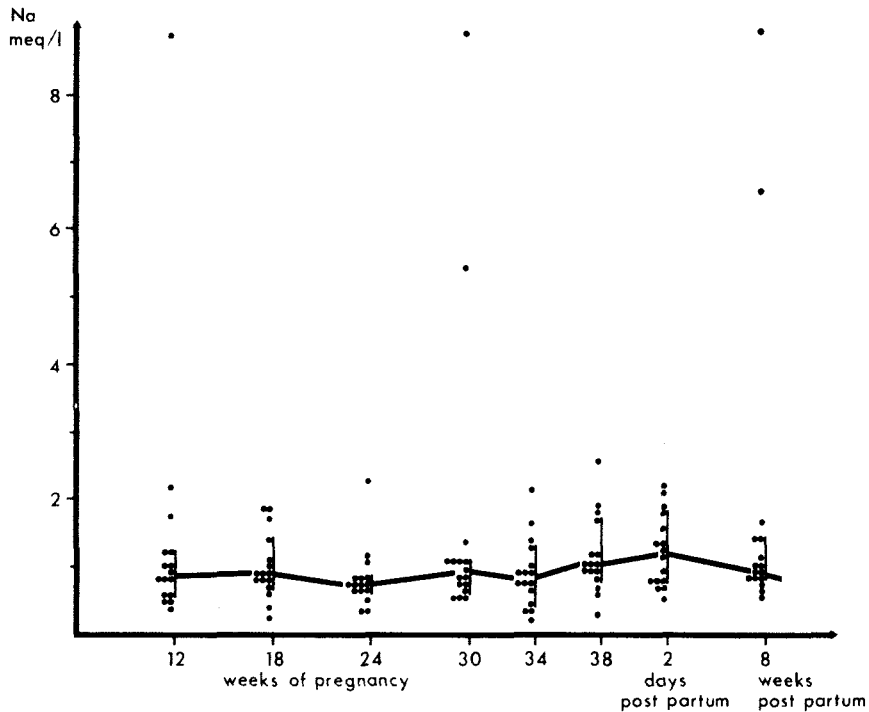


Fig. 5. Original and medium values surrounded by 95 % confidence intervals for parotid saliva sodium concentration at the various weeks of observation. Resting saliva.

concentration was 2.4 and 1.8, respectively. The negative regression of calcium concentration in resting saliva on saliva flow rate was significant.

The corrected mean rank for calcium concentration in both resting and stimulated saliva varied with time ( $0.001 > p$  resp.  $0.001 > p$ ).

#### DISCUSSION

To diminish the effect of individual and general factors the investigation was performed as an intraindividual study where the method of collection, posture of the person studied, time of last meal etc had been standardized as far as possible.

Yet large differences were found between different persons studied. The statistical methods used were justified because values found for different persons were so different that direct comparison between the values found in different persons was usually not possible. The assumption of a normal distribution was therefore unreasonable and only comparisons of determina-

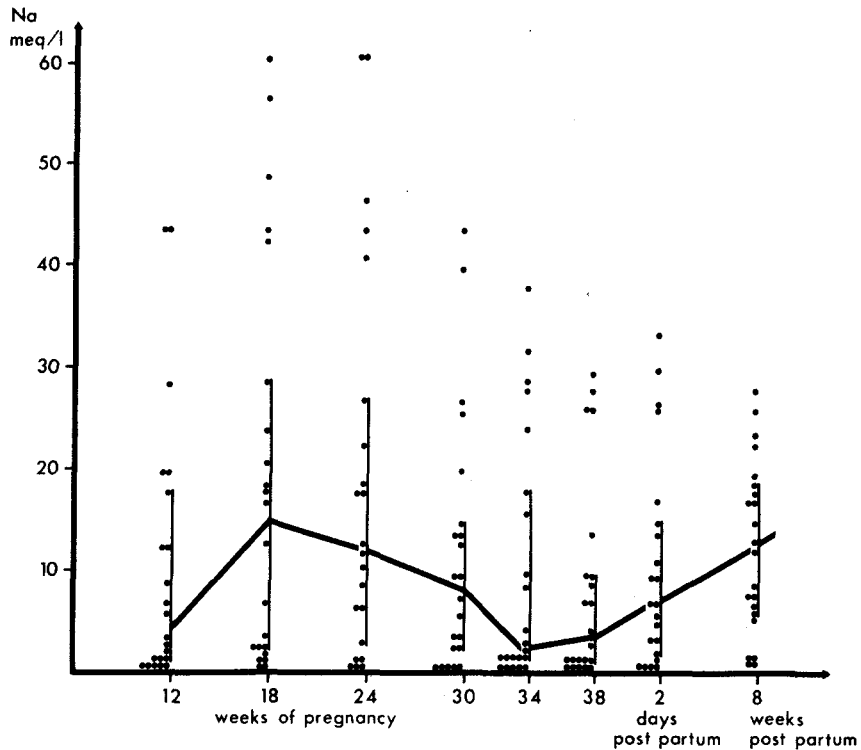


Fig. 6. Original and medium values surrounded by 95 % confidence intervals for parotid saliva sodium concentration at the various weeks of observation. Citric acid stimulated saliva.

tions made in one and the same person are of any value. Significant rank variations, however, imply that the corresponding relationship also exists in the original values found. Since some of the persons studied produced very little saliva, it was not always possible to determine the concentration of total protein, sodium, potassium or calcium.

Since the saliva constitutes the external environment of the teeth and the mucosa its significance in the maintenance of health and disease of these tissues has been the subject of several investigations. According to such investigations, the saliva protects these tissues, the protection being of both physical and chemical nature (Rölla, 1965a, b). The physical function is related to mechanical cleansing, while the chemical defence is believed to consist of the salivary content of inorganic and organic substances and the effect of these components on the oral bacterial flora.

During pregnancy there was a decrease in the rate of secretion of both resting and stimulated parotid saliva. This is in agreement with what *Kullan-*

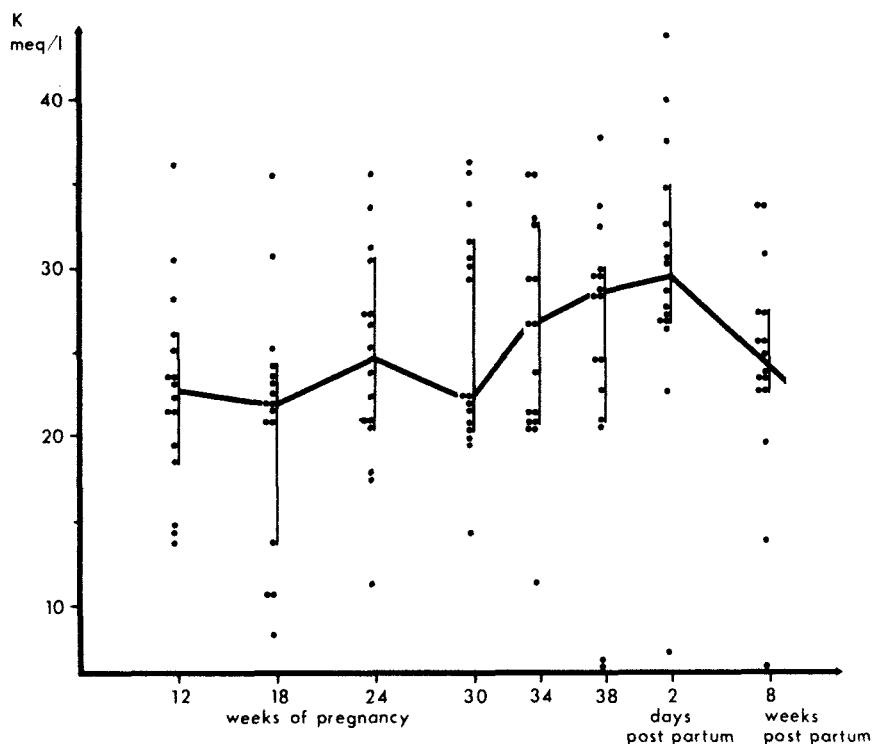


Fig. 7. Original and medium values surrounded by 95 % confidence intervals for parotid saliva potassium concentration at the various weeks of observation. Resting saliva.

*der* and *Sonesson* (1965) found in a cross-sectional study of the rate of flow of saliva from the submandibular glands during pregnancy.

A reduction of the rate of salivary secretion may be of significance in disease of the periodontium and oral mucosa, and the frequency of caries has been found to be increased in persons with xerostomia (*Bertram*, 1957). Periodontal changes have been found to be more common and severe in desalivated experimental animals than in controls (*Gupta et al.* 1960; *Ruben & Goldman*, 1963; *Hugoson & Persson*, 1966) with a pronounced increase in the amount of soft deposits (*Hugoson & Persson* 1966). The patients in the present investigation were also examined regarding the condition of the gingiva and the state of the oral hygiene (*Hugoson*, 1971), but no increase in the amount of bacterial plaque could be demonstrated. Judging from this examination and other clinical investigations (*Bertram*, 1957) and animal experiments (*Hugoson & Persson*, 1966), only extreme changes in the rate

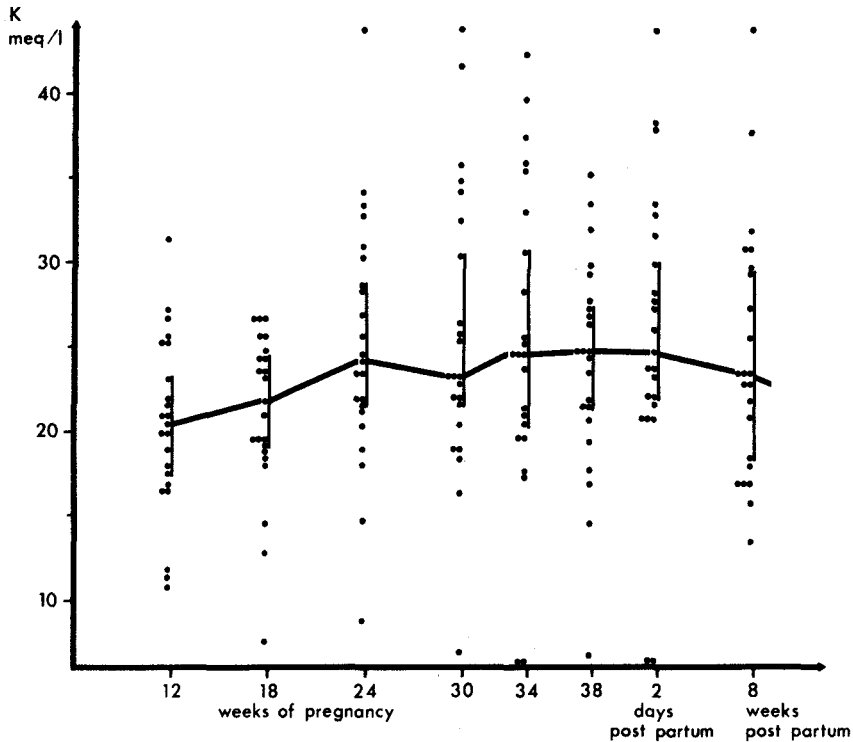


Fig. 8. Original and mean values surrounded by 95 % confidence intervals for parotid saliva potassium concentration at the various weeks of observation. Citric acid stimulated saliva.

of flow of saliva have any significant effect on the state of hygiene of the mouth as reflected in the amount of plaque.

No really satisfactory method is available for determining the total protein content of saliva. In the present investigation *Daughaday's* (1951) modification of Lowry's method was used, in which the intensity with which the protein stain varies mainly with the concentration of the protein tyrosin. Bovine serum albumin was used as a standard. Since saliva contains glycoproteins, the use of Lowry's method and bovine serum albumin as a standard result in the development of a colour whose intensity does not truly reflect the protein content (*Wolf & Taylor, 1964; Dawes, 1965*). The purpose of the present investigation was, however, not to study the true protein content of the saliva but changes in the content, as reflected in a number of determinations made during pregnancy and post partum. The method was therefore considered satisfactory.

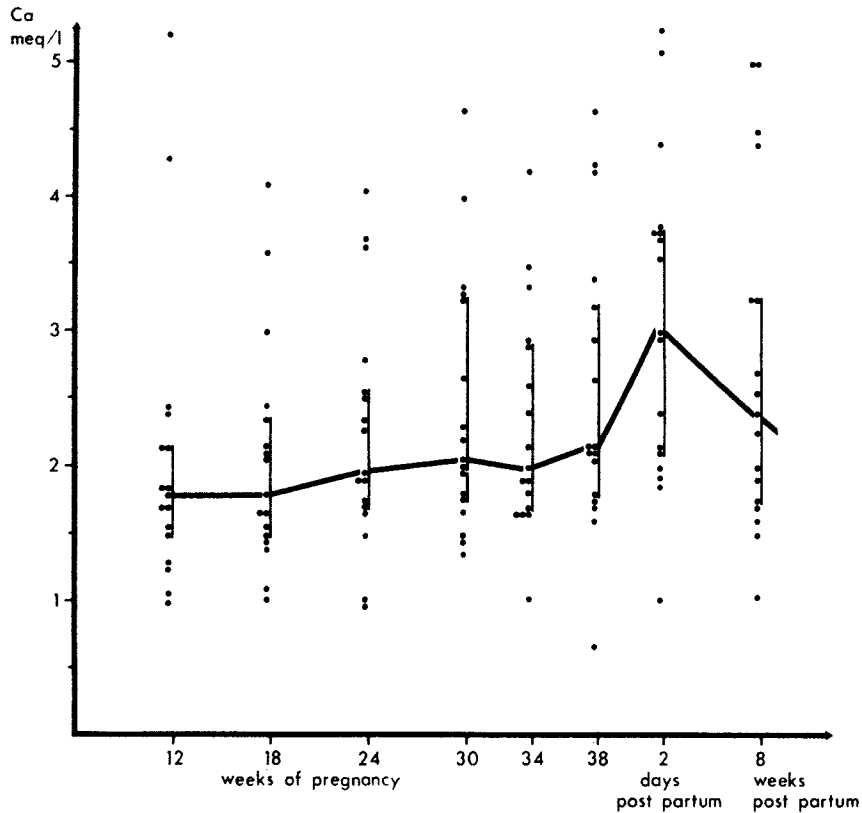


Fig. 9. Original and mean values surrounded by 95 % confidence intervals for parotid saliva calcium concentration at the various weeks of observation. Resting saliva.

In investigations by *Brankamp* (1936), *Araki* (1951) and *Caldwell & Pigman* (1966), *Windeler & Shannon* (1967) it has been shown that the protein content increases with the rate of secretion of the saliva. In the present investigation the concentration of protein in the saliva was found to increase during pregnancy until partus, at the same time as the flow of saliva decreased. It is therefore conceivable that the synthesis of saliva proteins varies with the hormone pattern prevailing at the end of pregnancy. This possibility is supported by the structural and histological changes observed in the salivary glands under the influence of steroid hormones (*Bixler et al.*, 1957; *Shackelford & Klapper*, 1961; *Baker et al.*, 1964, *Travill*, 1966) as well as by the knowledge of the effect of these hormones on intracellular and extracellular membranes (*Rothstein*, 1968).

The change observed in the concentration of sodium between resting saliva

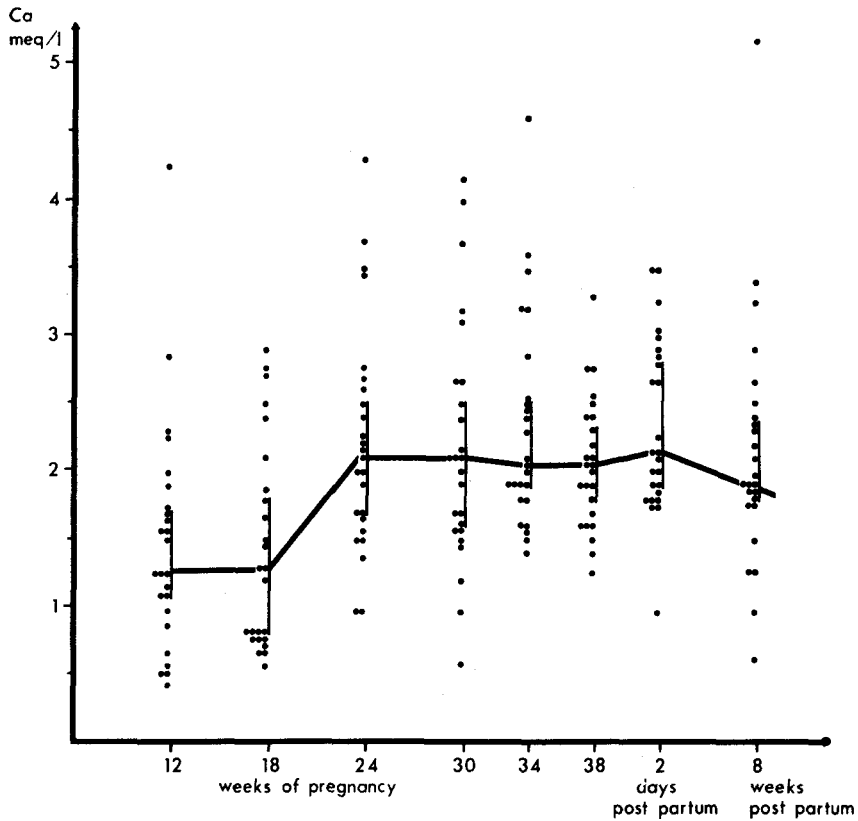


Fig. 10. Original and medium values surrounded by 95 % confidence intervals for parotid saliva calcium concentration at the various weeks of observation. Citric acid stimulated saliva.

and stimulated saliva as well as the variation with time found for stimulated saliva agree with earlier published observations of an increase in the concentration of sodium with increasing secretory rate (*Thaysen et al.*, 1954; *Mangos & Brown*, 1966; *Petersen & Poulsen*, 1967). Samples obtained by micropuncture of rat salivary glands (*Martinez et al.*, 1966; *Young & Schögel*, 1966) have been found to contain sodium, chloride and potassium in almost the same concentrations as in plasma, and the composition of the primary secretion has proved to be independent of secretory rate. The amount and direction of the net electrolyte transport in the duct system can therefore be calculated from the concentration of electrolytes in the final saliva (*Petersen & Poulsen*, 1967).

Progesterone and estrogen are believed to have sodium retaining properties and premenstrual edema has been thought to be of endocrine origin. Edema,

primarily due to renal retention of sodium by aldosterone may depend on other adrenocortical hormones, such as testosterone, progesterone and estrogen. On the other hand, potassium helps to maintain the osmotic equilibrium between intracellular and the extracellular fluid.

No relationship has been found between the potassium concentration in the saliva and the secretory flow, except at very low rates (*Thaysen et al.*, 1954; *Erikson*, 1959, *Petersen & Poulsen*, 1967). The decrease in flow rate of resting and stimulated parotid saliva in the present study may therefore be explained by hormone-induced changes in the water retaining capacity of the parotid salivary gland. This situation is reflected as an increase of the concentration of potassium in the saliva.

The relationship found between salivary flow rate and calcium concentration are not in agreement with earlier investigations, according to which the calcium concentration varies positively with (*Windeler & Shannon*, 1966 and *Dawes*, 1967) or independently of (*Dawes & Jenkins*, 1964), flow rate. In the present study the calcium concentration increased with decreasing salivary flow rate. However, *Sullivan & McCoomde* (1960) have shown that 37 % of the calcium is protein-bound. The increase in the total protein content of parotid saliva during pregnancy may therefore also explain the simultaneous increase in calcium concentration.

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