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A Parotid Salivary Protein Present during Late Pregnancy and Postpartum[1] (39519)

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Saliva provides a potential means for detection and investigation of physiologic changes which occur during and after pregnancy. Differences from nonpregnant individuals in the concentration of salivary calcium, sodium, and phosphorus were observed in normal pregnancy (1, 2). A decrease in flow rate and an increase in protein concentration of parotid saliva have also been detected in pregnant subjects (2). Until now apparently there are no published reports of changes in specific salivary proteins during pregnancy and postpartum. This paper describes a change in a human parotid salivary protein or proteins observed during late pregnancy, immediately postpartum, and 4–6 weeks postpartum detected by polyacrylamide-gel electrophoresis.

Methods. Approximately 10 ml of stimulated parotid saliva (sour lemon drops) were collected using a Teflon suction cup device (3) from eight pregnant subjects at each clinic visit (Obstetrics and Gynecology Clinic, University of Minnesota), when permitted by the clinic schedule, from about 2–3 months gestation to 2 weeks prepartum and at 4–6 weeks postpartum. Samples were procured from three of the above eight donors 4 months postpartum. Parotid saliva was collected from an additional individual on each of the 9 days prior to delivery and 1, 2, and 3 days postpartum. Single samples were obtained 1–4 days postpartum from 55 other subjects. In addition, parotid saliva was collected by the same technique as used with the above subjects from 12 male donors and 12 female subjects not pregnant for the previous 12 months. Flow rates and protein concentration determined by the procedure of Lowry *et al.* (4) were recorded for parotid saliva samples collected from pregnant subjects at various times during pregnancy and postpartum. The salivary specimens were dialyzed immediately after collection against several changes of distilled water at 4° for 48 hr and lyophilized. Multiple samples from the same subject were stored after lyophilization until all except the 4 months postpartum samples had been collected.

Each lyophilized residue was reconstituted to 10 mg/ml in 0.9% NaCl. The proteins in duplicate 30-μl aliquots of these solutions were separated by electrophoresis at pH 9.0 on 15-cm-wide, 2-mm-thick polyacrylamide gel slabs, 3.0 cm of 6% gel over 7.5 cm of 10% gel according to a previously described technique (5). Electrophoresis was conducted at 4° and at 40 mA constant current (150–200 V) until bromphenol blue marker dye reached the bottom of the gel (approximately 3.5 hr). All samples from a donor, except the 4 month postpartum specimens, were placed on the same gel. Proteins in the gels were fixed by immersion of the gels in 50% trichloroacetic acid for 30 min and were stained for 30 min with 0.1% Coomassie brilliant blue in 50% trichloroacetic acid. Excess stain was removed with several changes of 10% glacial acetic acid. The preparations were subjected to continual mechanical shaking during fixing, staining, and destaining. The stained salivary protein patterns were recorded by photographing the gels under standardized conditions. In addition, a portion of the samples was scanned with an Ortec Model 4310 densitometer. Reproducibility of the above technique has been verified and discussed in previous publications (6, 7).

Results. The flow rate (single gland) and protein concentration of parotid saliva from pregnant subjects were (mean ± SD) 1.02 ± 0.42 ml/min and 154 ± 69 mg/100 ml, respectively. No consistent and significant change in flow rate and protein concentration was noted for individual subjects during pregnancy and postpartum.

Figure 1 gives the parotid salivary protein patterns obtained from all samples collected

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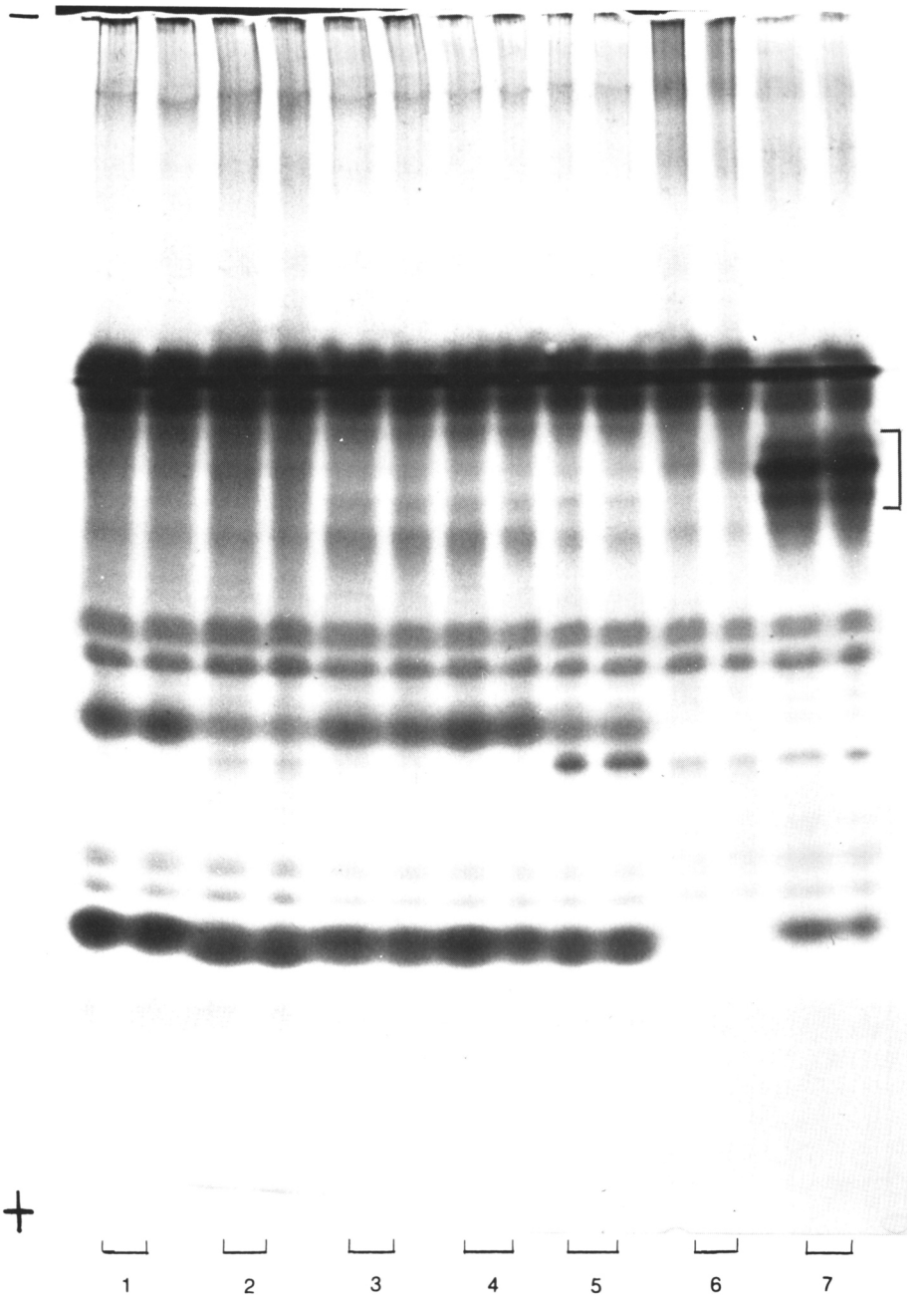

FIG. 1. Polyacrylamide gel slab patterns of parotid salivary proteins collected from one subject during pregnancy and postpartum. Each sample was run in duplicate on adjacent channels. Sample 7, located at the right margin of the gel, was collected 4 weeks postpartum. Samples obtained from 2 months gestation (sample 1) to 6 weeks prepartum (sample 6) are placed in sequence of collection from the left margin of the gel to the sample adjacent to the postpartum specimen. The electrophoretic zone containing pregnancy-associated protein is indicated by the bracket on the right margin of the figure. Anode (+) and cathode (−) are indicated on the left margin of the gel.

from one subject. A large increase in Coomassie blue-staining material is evident in the 10% gel about one-third the distance from the top to the bottom of the gel from the sample collected 4 weeks postpartum in comparison to the samples collected at 2 months gestation to 6 weeks prepartum. For simplicity of discussion, the increased Coomassie blue-staining substance or substances present in the electrophoretic zone indicated in Fig. 1 will be subsequently designated as pregnancy-associated protein. Figure 2 further demonstrates the 4 week postpartum increase in pregnancy-associated protein by comparison of the postpartum protein pattern of a second individual with four control subjects. No difference was observed between male donors and female subjects not pregnant within the previous 12 months in Coomassie blue staining within the electrophoretic zone which contains the pregnancy-associated protein. Pregnancy-associated protein also was detected 4 to 6 weeks postpartum from the other six subjects who were studied throughout pregnancy and postpartum.

Densitometry also demonstrated increased staining in the electrophoretic zone which contains pregnancy-associated protein. Quantitation of the amount of protein in this zone, however, was found impractical because of incomplete resolution of the multiple bands present on the gels, the large range in concentration of the various proteins present in the gels, and the unavailability of pure pregnancy-associated protein for quantitative calibration.

Only minimal, if any, pregnancy-associated protein was present in any subject prior to 1 month before term. A high concentration of protein in the electrophoretic zone which contains pregnancy-associated protein was evident in all samples collected from the one subject on each of the 9 days prior to delivery and at 1, 2, and 3 days postpartum (Fig. 3). Staining within the zone which contains pregnancy-associated protein as intense as observed with this pregnant subject has been seen in only three of over 400 parotid saliva samples from nonpregnant subjects analyzed by the present technique. The amount of pregnancy-associated protein present at these times, however, was less than observed 4–6 weeks postpartum with the other subjects. The parotid salivary samples collected from the 55 donors 1–4 days postpartum also contained less pregnancy-associated protein than at 4–6 weeks postpartum. No pregnancy-associated protein was evident 4 months postpartum in the three subjects investigated.

Differences in the parotid protein patterns of samples collected at different times during pregnancy and postpartum, besides that noted above, are evident in Figs. 1 and 3. None of these other differences, however, appeared to be consistent among subjects. Samples must be collected at more frequent intervals and at carefully controlled time periods to define the potential relationship of these additional differences to pregnancy. The variation among gel patterns of parotid salivary samples collected from a subject throughout pregnancy was much greater than that among multiple samples collected from individual nonpregnant subjects at different times (5–7).

Discussion. The data in this paper show a marked increase in or appearance of an additional protein or proteins in a particular electrophoretic zone from human parotid saliva collected during late pregnancy, shortly after term, and 4–6 weeks postpartum. Careful examination of Figs. 1 and 2 suggests an apparent increase in staining intensity in more than a single band within the pregnancy-associated protein zone from samples collected 4–6 weeks postpartum. The specific event during pregnancy or postpartum with which the protein change is associated is not clear. However, since the amount of pregnancy-associated protein present in parotid saliva is much greater 4–6 weeks postpartum than either before term or immediately postpartum, it appears that the salivary change we have noted is more closely associated with postpartum processes, perhaps activation of lactation, than with pregnancy per se. Several hundred parotid salivary protein samples have been analyzed by the procedure used in the present study. The only other subject in whom a protein change comparable to that observed in 4–6 weeks postpartum subjects was a 16-yr-old male with severe acne. Parotid salivary differences other than the pregnancy-

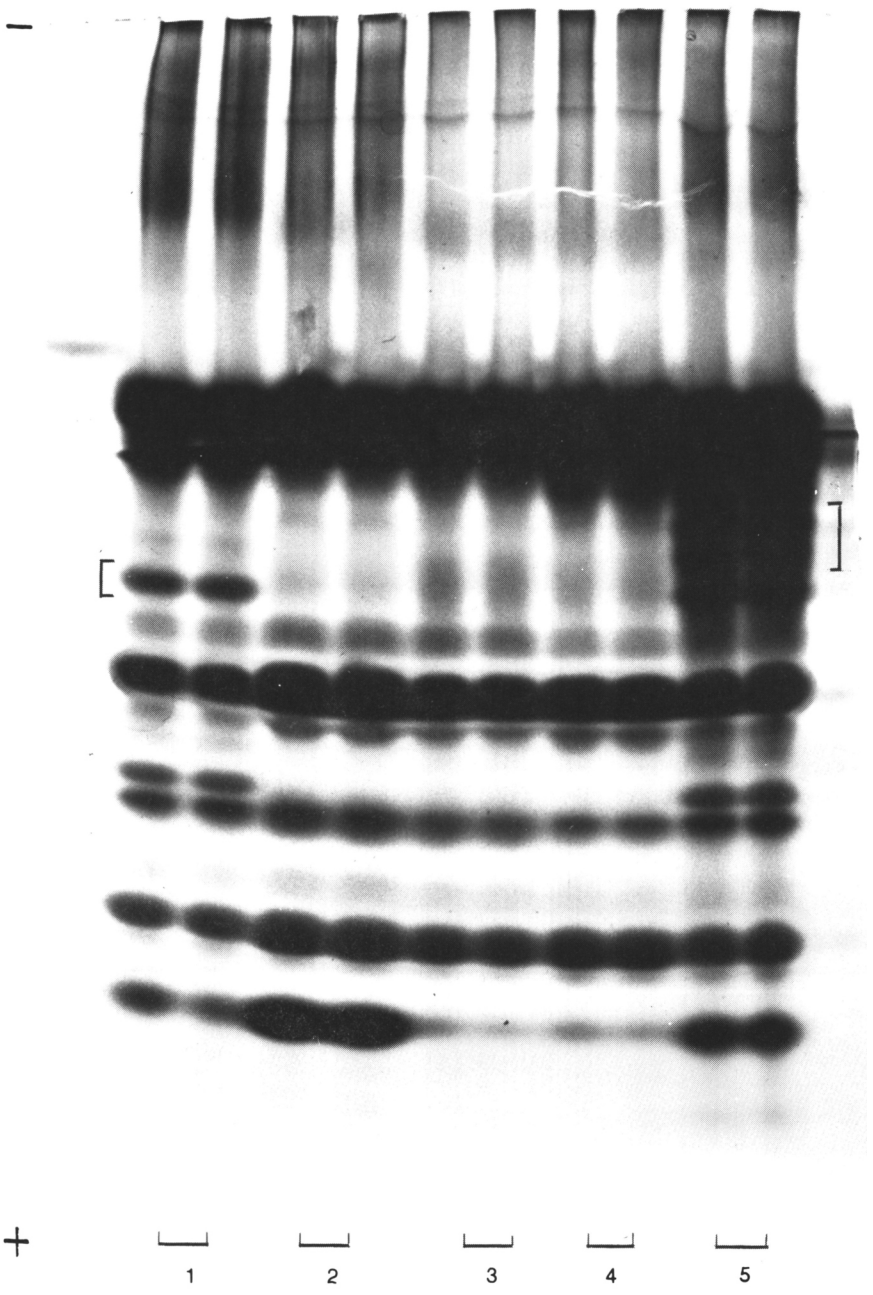

FIG. 2. Polyacrylamide gel slab patterns of the parotid salivary proteins of a 4 week postpartum sample (sample 5) compared with four control donors (samples 1–4). The bracket on the right margin of the figure indicates the electrophoretic zone which contains pregnancy-associated protein. The prominent staining area in sample 1 indicated by the bracket is not a pregnancy-associated protein and has an electrophoretic mobility slightly greater than the pregnancy-associated protein. This latter protein, also seen in sample 5 just below the pregnancy-associated protein, frequently occurs in high concentration in parotid saliva. Anode (+) and cathode (−) are indicated on the left margin of the gel.

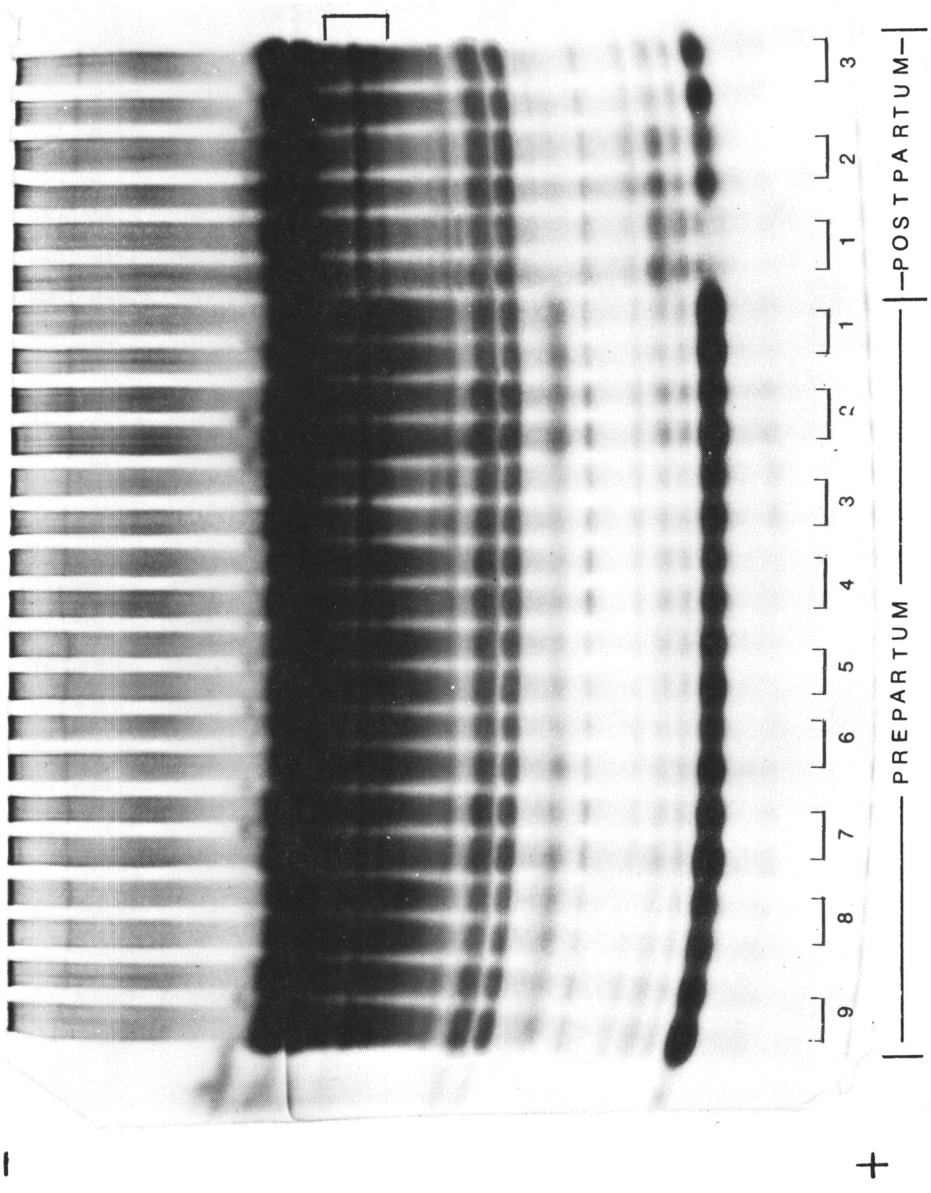

FIG. 3. Polyacrylamide gel slab patterns of parotid salivary proteins collected from one subject on each of the 9 days prior to delivery and 1, 2, and 3 days postpartum. Time of sample collection (days pre- or postpartum) is indicated by the numbers on the bottom of the figure. The electrophoretic zone containing pregnancy-associated protein is shown by the bracket on the right margin of the gel.

associated protein were evident in some but not all subjects during pregnancy and postpartum. These additional changes, although not equivalent in all subjects, indicate that multiple changes are likely to occur in salivary proteins during pregnancy and postpartum.

The biochemical nature of the pregnancy-associated protein has not been identified yet. It occurs in an area of the gel in which small amounts of protein are obtained from parotid saliva of most control subjects. Thus, the pregnancy-associated protein may be a large increase in secretion of a normal salivary protein or proteins. Characterization studies performed in our laboratory and electrophoretic mobility indicate that the pregnancy-associated protein is probably not one of the several given in Table I. Amylase, the prominent staining area at the interface between the 6 and 10% gels, has a lower electrophoretic mobility than the pregnancy-associated protein. Immunoglobulins IgA and IgG are restricted to the 6% portion of the gel. The protein is not strongly bound by hydroxyapatite, nor does it contain unusually large quantities of proline. The pH of the gel system is such that proteins with very basic isoelectric points, such as lysozyme and small molecular weight basic proteins, are excluded from the gel. Albumin and transferrin have been demonstrated by crossed immunoelectrophoresis to have greater electrophoretic mobilities than the pregnancy-associated protein. Recent studies in our laboratory with purified proteins show that lactoferrin and lactoperoxidase also have electrophoretic mobilities less than the pregnancy-associated protein. The pregnancy-associated protein has an electrophoretic mobility similar to acid and alkaline phosphatases, substances with molecular weights of 80,000–100,000. However, no other data are presently available to indicate that the pregnancy-associated protein is either of these enzymes. Periodic acid–Schiff stains of the gels suggest that the pregnancy-associated protein may contain a significant amount of carbohydrate.

TABLE I. SALIVARY PROTEINS TENTATIVELY EXCLUDED AS THE PREGNANCY-ASSOCIATED PROTEIN.

1.	Amylase
2.	IgA, IgG
3.	Proline rich proteins
4.	Proteins strongly bound by hydroxyapatite
5.	Small molecular weight highly basic proteins
6.	Lysozyme
7.	Serum albumin
8.	Transferrin
9.	Lactoferrin
10.	Lactoperoxidase

Summary. Stimulated parotid saliva was collected from: (i) eight pregnant subjects at intervals from about 2–3 months gestation to 2 weeks prepartum and at 4–6 weeks postpartum; (ii) three of the above eight donors 4 months postpartum; (iii) one individual each of the 9 days prior to delivery and at 1, 2, and 3 days postpartum; (iv) 55 individuals 1–4 days postpartum; (v) 12 male and 12 female control subjects. A marked increase in or appearance of an additional protein or proteins was detected in a particular electrophoretic zone from the saliva collected during late pregnancy, shortly after term, and 4–6 weeks postpartum. The amount of this yet unidentified protein or proteins is greater in parotid saliva collected 4–6 weeks postpartum than obtained either immediately pre- or postpartum.

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