VARIATIONS IN THE SERUM CONCENTRATION OF A HUMAN PREGNANCY-ASSOCIATED α -MACROGLOBULIN DURING PREGNANCY AND AFTER DELIVERY

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Human pregnancy is associated with the appearance in the blood of additional proteins not commonly identifiable in the plasma of non-pregnant subjects (MacLaren, Thornes, Roby & Reid, 1959; Bohn, 1971; Lin, Halbert, Kiefer, Spellacy & Gall, 1974). One of these is readily detectable by electrophoretic and immunochemical methods (Smithies, 1955; Afonso & de Alvarez, 1964; Studd, Blainey & Bailey, 1970), and has been isolated, characterized and found to be a high molecular weight α -globulin containing approximately 10% carbohydrate (Stimson & Eubank-Scott, 1972). This pregnancy-associated α -macroglobulin (PAM) is not unique to pregnancy and its concentration has been determined in the serum of subjects with a variety of conditions (Stimson, 1973a, 1974). Treatment of both males and females with oestrogens results in elevations in the PAM serum level (Horne & co-authors, 1973; Berne, 1973; Stimson, 1974), but it is not involved in steroid transport (Stimson, 1973b; Bohn & Kranz, 1973; Von Schoultz, Stigbrand, Carstenson, Sodergard & Shanbhag, 1973). Recent investigations have shown that peripheral blood leucocytes are capable of synthesizing PAM but that lymphocytes are not the cell type responsible (Stimson & Blackstock, 1975).

Previous studies of changes in the concentration of this serum protein throughout pregnancy have been carried out with quantitative immunoelectrophoresis (Stimson, 1974) and single radial immunodiffusion (Von Schoultz, 1974). In the present study, a greater degree of sensitivity was achieved by employing an immunoassay technique, detailed elsewhere (Stimson & Sinclair, 1974). Briefly, serum samples were incubated in antibody-coated polystyrene tubes for 45 min at 37°C. After washing with 0.1% Triton X-100 solution, an antibody-horse radish peroxidase conjugate was added and the tubes were reincubated for 2 hr at room temperature. The test-tubes were then washed, and the peroxidase activity remaining bound was determined by measuring the absorbance at 450 nm that develops over 1 hr in a substrate solution containing 5-aminosalicylic acid and hydrogen peroxide. This 'sandwich-type' enzyme immunoassay has a limit of sensitivity of approximately 200 ng/ml and the results have been found to be highly reproducible. Fourteen assays of the same sample gave a mean $\pm$ S.D. value of 113 ± 3.8 μ g/ml. In this

study, all estimations were carried out in duplicate on serum obtained from healthy primigravidae of 17 to 30 years of age.

Text-figure 1 depicts typical changes in PAM concentration that were found throughout pregnancy and up to 11 weeks *post partum*. There were considerable individual variations but PAM levels normally rose sharply in the first trimester and fell rapidly after delivery. The mean concentration of PAM in fifty-eight women, who were sampled serially during and after their pregnancies, is shown in Table 1. Considerable individual variations were apparent from the large standard deviations obtained. The level reached a plateau during the third trimester and within 6 weeks after delivery declined to a comparatively minimal concentration. Most of the women showed the highest concentration of PAM

TEXT-FIG. 1. Changes in the concentration of a pregnancy-associated α -macroglobulin (PAM) throughout pregnancy in six primigravidae.

at birth, but eleven gave a peak value at approximately 28 to 30 weeks and then showed a small, steady decline until delivery. As oestrogen levels still increased in these women after this time, oestrogen concentrations do not appear to be wholly responsible for PAM blood level and it is possible that oestrogen may only function as a method of stimulating PAM synthesis *in vivo*, as it has been found capable of achieving in leucocyte cultures (Stimson & Blackstock, 1974).

Kasakura (1971) has shown that human pregnancy plasma contains a non-specific immunosuppressive factor which may aid in preventing rejection of the fetus by the mother. The α -macroglobulin has the capacity to inhibit leucocyte transformation induced in mixed leucocyte cultures (Stimson, 1972a) and by phytohaemagglutinin stimulation (Von Schoultz, Stigbrand & Tarnvik, 1973) and could therefore be involved in this phenomenon. All the women from whom samples were obtained for this study, however, had uncomplicated

pregnancies and gave birth to healthy children in spite of the great individual variations in PAM levels encountered. It may therefore be inferred that if the macromolecule does contribute to maternal immunological unresponsiveness, it must act with other factors also capable of inducing suppression.

Table 1. Concentration of pregnancy-associated α -macroglobulin in fifty-eight primigravidae before and after birth

	<i>No. of samples</i>	<i>PAM ($\mu\text{g/ml}$)</i>
Gestation (weeks)		
5 to 6	22	28 ± 19
7 to 8	23	53 ± 41
9 to 10	32	72 ± 56
11 to 12	21	105 ± 82
13 to 14	34	322 ± 242
15 to 16	29	481 ± 321
17 to 18	30	580 ± 359
19 to 20	31	664 ± 423
21 to 22	33	682 ± 456
23 to 24	38	720 ± 507
25 to 26	37	760 ± 521
27 to 28	41	800 ± 583
29 to 30	44	858 ± 641
31 to 32	38	847 ± 627
33 to 34	46	851 ± 638
35 to 36	51	863 ± 642
37 to 38	54	832 ± 659
39 to 40	43	851 ± 647
41 to 42	26	873 ± 621
After parturition		
1 day	58	808 ± 581
1 week	49	571 ± 312
2 to 3 weeks	18	329 ± 182
4 to 5 weeks	23	150 ± 112
6 to 7 weeks	38	59 ± 43
8 to 9 weeks	26	35 ± 22
10 to 11 weeks	24	29 ± 24

Of the women examined, nineteen at 5 to 6 weeks, twenty at 7 to 8 weeks, and thirty-one at 9 to 10 weeks had detectable levels of PAM. Values are Means $\pm$ S.D.

The PAM concentration was also determined in two groups of 110 primigravidae aged 18 to 32 years and 38 to 41 weeks pregnant from Dundee and Glasgow. Mean $\pm$ S.D. values of 872 ± 584 and 979 ± 682 $\mu\text{g/ml}$, respectively, were obtained but no explanation for this difference has yet been found. In all late pregnancy serum samples so far examined, however, it has proved possible to detect the presence of PAM in measurable quantities by the immunoassay procedure. The distribution curves of PAM can be very asymmetrical (B. H. Berne, personal communication). In this study, the values exhibited both normal and log-normal distributions and the results were treated accordingly.

Changes in individual serum protein concentrations in pregnancy are common (Studd *et al.*, 1970; Stimson, 1972b) but the increases associated with this glycoprotein are outstanding (60-fold increases have been noted). Although

the biological rôle of this α -globulin is as yet uncertain, such increases during pregnancy must be associated with a definite function.

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