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Changes of serum anti-thyroid antibodies during and after pregnancy in autoimmune thyroid diseases

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SUMMARY

Changes of serum anti-thyroglobulin haemagglutination antibodies (TGHA) and anti-thyroid microsomal haemagglutination antibodies (MCHA) were observed during pregnancy and after delivery in Graves' disease and autoimmune thyroiditis. Both TGHA and MCHA decreased as pregnancy progressed, and sometimes they became negative in late pregnancy. Transient increases of TGHA and MCHA were observed after delivery and the antibody titres reached peaks about 3–4 months post-partum in more than half the patients. In some patients, antibodies developed after delivery. Similar transient increases of antibodies were observed after spontaneous and therapeutic abortion. These changes seem to be induced by physiological and immunological changes occurring during pregnancy and after delivery.

INTRODUCTION

Pregnancy has been described as a successful allograft of foreign tissue, and maternal immunological inertness has been postulated as one mechanism for protecting the foetal allograft (Beer & Billingham, 1971). Recently, studies have indicated a depression of cell-mediated immunity during pregnancy (Finn *et al.*, 1972; Purtilo, Hallgren & Yunis, 1972; Thong *et al.*, 1973), but little is known about humoral immunity in pregnancy. We observed gradual decreases in the serum concentrations of IgG, IgA and IgM during normal pregnancy, and suggested that this immunoglobulin depletion might result from both immune suppression and haemodilution (Amino *et al.*, 1977c). Very recently we found that pregnancy and delivery strikingly influenced the clinical course of autoimmune thyroid diseases, and discovered transient post-partum hypothyroidism in autoimmune thyroiditis (Amino *et al.*, 1976b) and transient post-partum hyperthyroidism in Graves' disease (Amino *et al.*, 1977b) and in autoimmune thyroiditis (Amino *et al.*, 1977a). We suggested that these changes might be induced by physiological and immunological changes associated with gestation and parturition (Amino *et al.*, 1977a).

Accordingly, we studied changes of serum anti-thyroid antibodies during and after pregnancy in patients with autoimmune thyroid diseases. In this paper describing these studies, the biological significance of the changes is also discussed.

PATIENTS AND METHODS

Eleven patients with Graves' disease and six patients with autoimmune thyroiditis were observed during pregnancy, and for 6–8 months post-partum, at the Endocrine Clinic and the Obstetrics Clinic of Osaka University Hospital. Pertinent clinical data on these patients are summarized in Table 1. The diagnoses of Graves' disease and autoimmune thyroiditis were established on the basis of clinical and laboratory findings. Two of the patients with Graves' disease (cases 5 and 10) and two other patients with autoimmune thyroiditis were observed after spontaneous or therapeutic abortion. The gestational age

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TABLE 1. Pertinent clinical data on seventeen patients with autoimmune thyroid diseases

Patient No.	Age at last delivery (years)	Age at the time when diagnosis was made	Diagnosis	Treatment			Thyroid function	
				Before pregnancy	During pregnancy	After delivery	During pregnancy	After delivery
1	30	28	Graves' disease	Subtotal thyroidectomy	None*	None	Euthyroid	Transient thyrotoxicosis
2	26	25	Graves' disease	Subtotal thyroidectomy	None*	None	Euthyroid	Euthyroid
3	31	29	Graves' disease	Subtotal thyroidectomy	Desiccated thyroid*	None	Euthyroid	Latent hypothyroidism
4	26	24	Graves' disease	Anti-thyroid drug	None†	None	Euthyroid	Euthyroid
5	33	19	Graves' disease	Anti-thyroid drug	None	None	Euthyroid	Transient thyrotoxicosis
6	29	26	Graves' disease	Anti-thyroid drug	None	None	Euthyroid	Transient thyrotoxicosis followed by transient hypothyroidism
7	26	23	Graves' disease	Anti-thyroid drug	None*	None	Euthyroid	Euthyroid
8	27	27	Graves' disease	None‡	Anti-thyroid drug	Anti-thyroid drug	Mild thyrotoxicosis to euthyroid	Thyrotoxicosis aggravated
9	28	24	Graves' disease	Anti-thyroid drug	None	None	Euthyroid	Euthyroid
10	23	21	Graves' disease	Anti-thyroid drug	None	Anti-thyroid drug	Mild thyrotoxicosis	Thyrotoxicosis aggravated
11	30	24	Graves' disease	Anti-thyroid drug	None	None	Euthyroid	Transient thyrotoxicosis
12	28	26	Autoimmune thyroiditis	Desiccated thyroid	Desiccated thyroid	Desiccated thyroid	Euthyroid	Euthyroid
13	27	25	Autoimmune thyroiditis	None	None	None	Euthyroid	Euthyroid
14	21	21	Autoimmune thyroiditis	None‡	None	None	Euthyroid	Euthyroid
15	21	19	Autoimmune thyroiditis	None	None*	None	Euthyroid	Transient thyrotoxicosis
16	23	23	Autoimmune thyroiditis	None	None	None	Euthyroid	Transient thyrotoxicosis followed by transient hypothyroidism
17	23	18	Autoimmune thyroiditis	None	None	None	Euthyroid	Transient thyrotoxicosis followed by transient hypothyroidism

* Treated with iron for anaemia in late pregnancy.
† Treated with diuretic drug for toxaemia.
‡ Thyroid abnormality was first found in early pregnancy.

was determined on the basis of the date of the last menstrual period. Fourteen subjects delivered healthy babies at full term and two patients (cases 8 and 11) had healthy babies after 36 and 37 weeks of gestation. In one patient (case 14), the baby was born prematurely at 30 weeks and it did not survive. Blood was withdrawn from subjects, in the morning, at roughly monthly intervals from early pregnancy until 6–8 months post-partum, and the serum was separated and stored at −20°C for examination.

Anti-thyroglobulin antibodies (TGHA) and anti-thyroid microsomal antibodies (MCHA) (Amino *et al.*, 1976a) were measured by the tanned red cell haemagglutination technique (Boyden, 1951), using commercially available test kits (Thyroid Test and Microsome Test, respectively, Fujizoki Pharmaceutical Co. Ltd, Tokyo). The thyroglobulin haemagglutination antibody test, known as the tanned red cell (TRC) haemagglutination test, is here abbreviated to TGHA, because the MCHA test also utilizes tanned red cells. The assay was performed by a slight modification of the method described previously (Amino *et al.*, 1976a), using an automatic dilutor and a 96 channel automatic pipetter (Cooke Laboratory Products, Division of Dynatech Laboratories Inc., Alexandria, Virginia, U.S.A.). Serial two-fold dilutions of initial 1:20 dilutions of sera were made to estimate the titre of antibodies, and titres of 1:20 or more were considered to be positive. The serum immunoglobulins, IgG, IgA and IgM, were measured by the single radial immunodiffusion technique using a commercial test kit (Partigen plates, Behring Institute, Marburg). Peripheral leucocyte counts were made with a Coulter Counter Model S (Coulter Electronics Inc., Hialeah, Florida, U.S.A.), and lymphocyte counts were calculated from the product of leucocyte counts and the percentage of lymphocytes, estimated by differential analysis on smears. The free thyroxine index (FTI) was calculated from the product of serum total thyroxine and the ratio of the patient's resin T_3 uptake to the control resin T_3 uptake. The serial serum samples obtained during and after pregnancy from one subject were all stored at −20°C, and were later assayed together in a single run for anti-thyroid antibodies and immunoglobulins to avoid inter-assay variation.

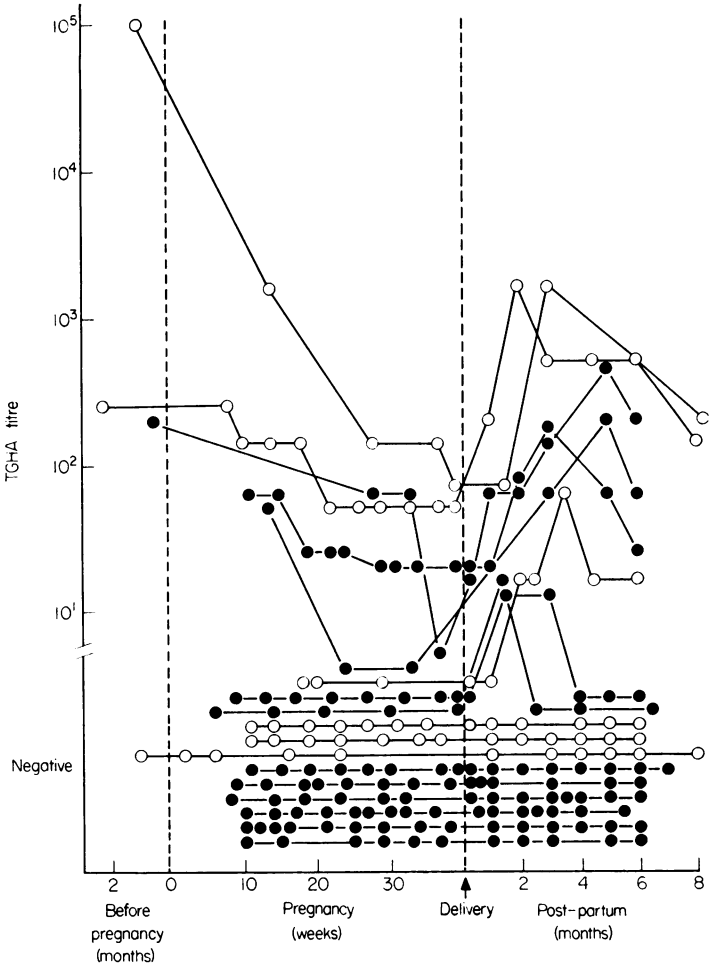

FIG. 1. Changes of serum anti-thyroglobulin haemagglutination antibodies (TGHA) during pregnancy and after delivery in patients with Graves' disease (●) and autoimmune thyroiditis (○).

RESULTS

The changes of serum TGHA and MCHA are shown in Figs 1 and 2. As shown in Fig. 1, nine patients gave negative reactions for TGHA throughout the observation period. Three patients gave negative reactions for TGHA during pregnancy, but developed a low positive titre at 1·5–3 months post-partum,

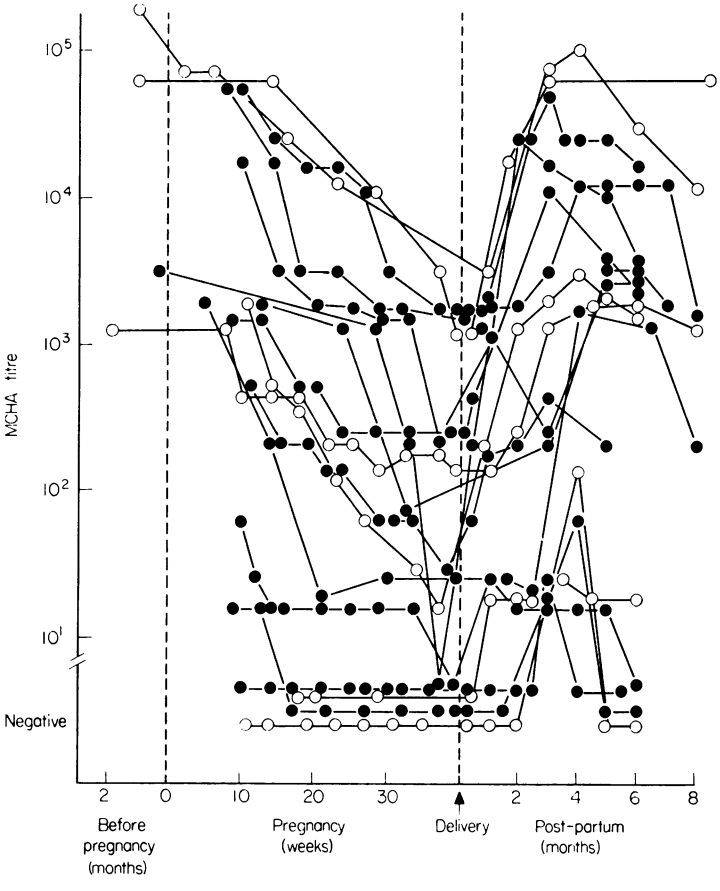

FIG. 2. Sequential change of serum anti-thyroid microsomal haemagglutination antibodies (MCHA) during pregnancy and after delivery in patients with Graves' disease (●) and autoimmune thyroiditis (○).

which was transient in two cases. In all the five patients with positive TGHA in early pregnancy, the titres gradually decreased during pregnancy and in two patients they became negative at the end of pregnancy. After delivery, transient increases in the TGHA titre were observed in eight cases. As shown in Fig. 2, the titres of MCHA similarly decreased gradually and were lowest at the end of pregnancy in fourteen patients; in three of these, MCHA became negative. After delivery, the MCHA titres increased; the increase was transient in fifteen cases, but the higher titres persisted in two cases. As observed for TGHA, three patients gave a negative reaction for MCHA throughout pregnancy but a positive reaction after delivery, which was transient in two and sustained in one. The patterns of change of antibodies in Graves' disease and autoimmune thyroiditis were similar.

The extent of the decrease in antibodies during pregnancy varied in different patients; when the decrease in antibodies is expressed as the ratio of the titre in late pregnancy to that in early pregnancy, it was found to be $1/2^2$–$1/2^{11}$ for TGHA and $1/2^2$–$1/2^8$ for MCHA. The ratio of the decrease in MCHA was $1/2^5$–$1/2^6$ in eight out of eleven patients. The extent of increase after delivery also varied, and the ratio of the maximum titre to that in late pregnancy varied from 2^1 to 2^7. Seven out of eight patients

whose ratio of increase in MCHA was more than 2^5 showed transient thyrotoxicosis or aggravation of thyrotoxicosis after delivery. The time of the peak of antibody titres varied from 1 to 6 months post-partum, but the peaks of TGHA and MCHA were observed at 3–4 months post-partum in four out of eight and ten out of seventeen patients, respectively. The post-partum peak of TGHA coincided with that of MCHA in four out of eight cases.

Similar transient increase of antibodies was observed after spontaneous and therapeutic abortions. The change of MCHA is shown in Fig. 3. The peak was seen 2–3 months after abortion.

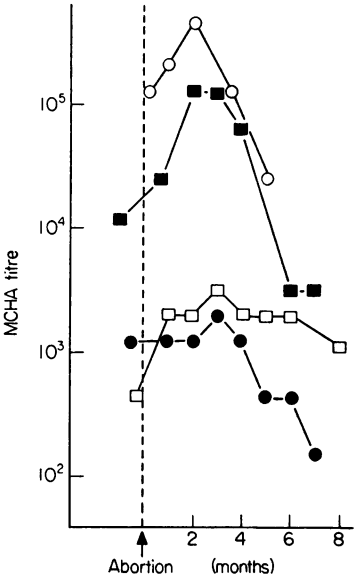

FIG. 3. Change of serum anti-thyroid microsomal haemagglutination antibodies (MCHA) after therapeutic or spontaneous abortion in patients with: Graves' disease (●, therapeutic; ■, spontaneous) and autoimmune thyroiditis (○, therapeutic; □, spontaneous).

Fig. 4 shows the sequential changes of serum antibodies, immunoglobulins, the size of goitre, free thyroxine index and peripheral leucocyte and lymphocyte counts during and after pregnancy in a patient with autoimmune thyroiditis (case 15). The titre of MCHA decreased during pregnancy and increased transitorily after delivery, but TGHA was negative throughout the observation period. All serum immunoglobulins except IgA also decreased during pregnancy and increased after delivery. The goitre decreased in size during pregnancy and increased transitorily after delivery concomitantly with the increases in antibodies and immunoglobulins. Transient increase of the free thyroxine index was also observed at this time. Peripheral lymphocyte counts decreased during pregnancy and increased after delivery in this case. Similar decreases during pregnancy and transient increases after delivery were seen in serum anti-thyroid antibodies, immunoglobulins and peripheral lymphocyte counts in the other two patients (cases 1 and 12). Ten healthy subjects did not show antibodies at any time during, or after, pregnancy.

DISCUSSION

There are few reports on changes of serum antithyroid antibodies during pregnancy. Parker & Beierwaltes (1961) observed pregnant women with a demonstrable TGHA and found that the titre decreased during pregnancy. They also observed a transient increase in the titre after delivery in three patients. Hill (1961) found that TGHA was positive in 1·5% out of 140 pregnant women and in 5% out of 174 non-pregnant women in the 20–39 year age-group without overt thyroid disease, but the difference was only just significant at $P = 0{\cdot}10$. Hjort & Pedersen (1962) observed the decrease in the titre of thyroid-

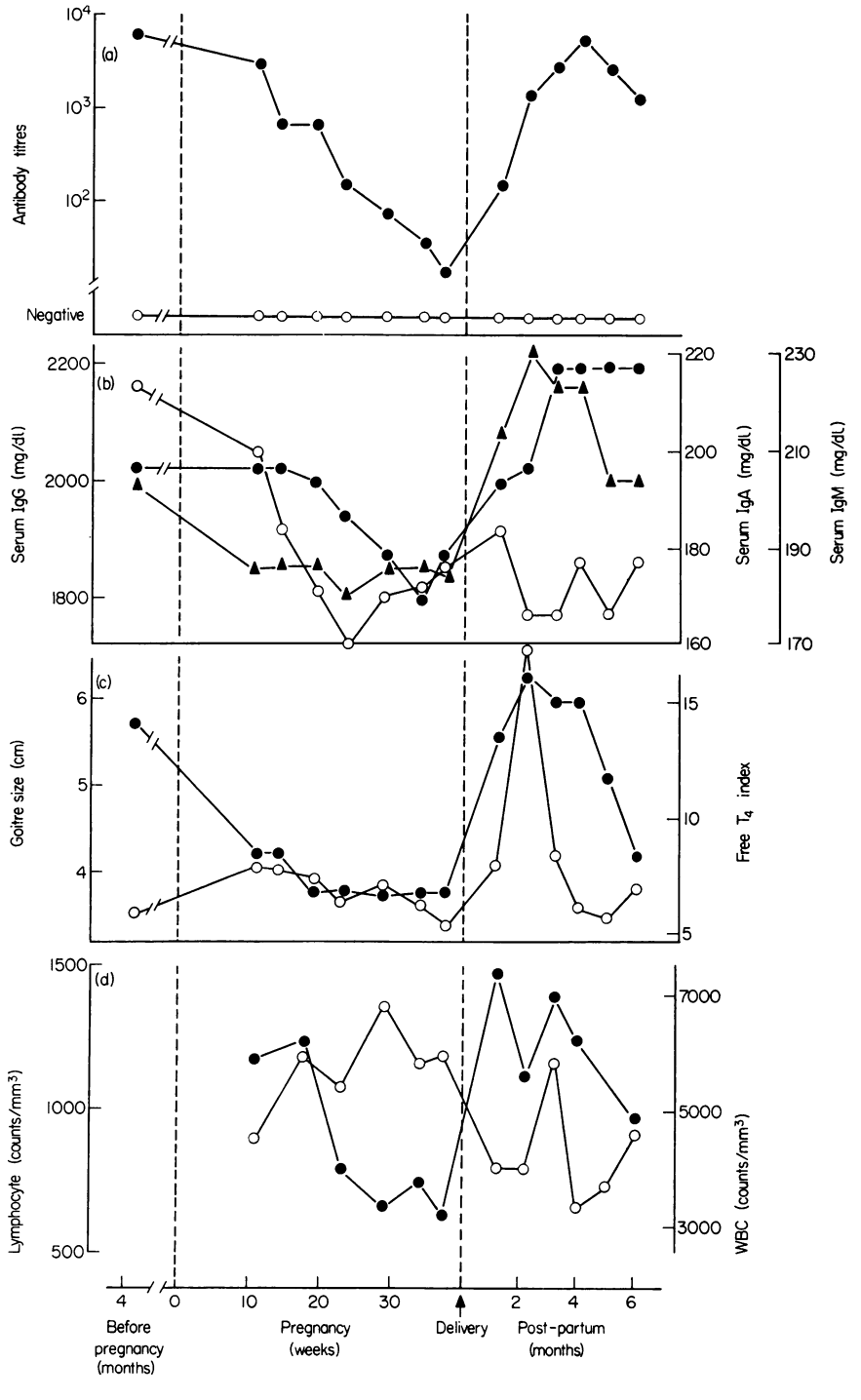

FIG. 4. Sequential changes of serum anti-thyroid antibodies, immunoglobulins, the free thyroxine index, the size of goitre and peripheral leucocyte and lymphocyte counts before, during and after pregnancy in a patient with autoimmune thyroiditis (case 15). The size of goitre is expressed as transverse width. (a) Antibody titres, (●) MCHA, (○) TGHA. (b) Serum levels, (●) IgG, (○) IgA, (▲) IgM. (c) Goitre size and free thyroxine index, (●) goitre, (○) thyroxine. (d) Counts, (●) lymphocyte, (○) WBC.

specific complement-fixing antibody during pregnancy in one case, and the development of antibody after delivery in another case. There are no previous reports of systematic studies on changes of anti-thyroid microsomal antibodies during and after pregnancy in patients with Graves' disease and autoimmune thyroiditis.

In this work, we observed a reduction in the titres of TGHA and MCHA during pregnancy, and transient increases of both TGHA and MCHA after delivery. There are several possible reasons why the antibody titres decreased during pregnancy. The decrease was probably not due to haemodilution, because the reduction was greater than could be expected from haemodilution. Moreover, the decrease was probably not due to administration of desiccated thyroid or anti-thyroid drugs, because patients who were not given drugs showed a similar reduction, and the titres rose again after delivery, while on drug therapy. The changes may be explained by supposing that antigens are released from the maternal thyroid gland during pregnancy, and that these antigens may neutralize the antibodies present in circulation. The concentration of serum thyroglobulin has been reported to increase in late pregnancy (Torrigiani, Doniach & Roitt, 1969; Van Herle *et al.*, 1973). However, this increase is so small that serum from normal pregnant women had no effect on the titres of TGHA and MCHA in our preliminary experiment (unpublished data). The possibility that circulating thyroid microsomal antigen may increase in pregnancy has not yet been proven. Moreover, the possibility that serum from pregnant women may contain some factor which inhibits haemagglutination *in vitro* was excluded by our finding that the sera from normal women in late pregnancy did not affect the titres of TGHA and MCHA, as described above. Most anti-thyroid antibodies are IgG (Torrigiani & Roitt, 1963), and the mother may lose circulating antibodies to the foetus through transplacental transfer, but this is not supported by the apparent decrease in the titres in early pregnancy and the absence of apparent thyroid damage in the newborn, although passive transfer of serum antibodies has only been shown to induce thyroid abnormality in humans in neonatal thyrotoxicosis (McKenzie, 1964).

An alternative explanation is that antibody production is suppressed in pregnancy. Strelkauskas *et al.* (1975) reported that T cells decreased in early pregnancy, and if this is true, the formation of T cell-dependent antibody, such as TGHA (Bankhurst, Torrigiani & Allison, 1973), might be reduced. Recently, we observed gradual decreases of the serum immunoglobulins IgG and IgA in normal pregnancy, and suggested that the decreases were mainly due to immunosuppression (Amino *et al.*, 1977c). The similar patterns of decrease in MCHA, immunoglobulins, goitre size and peripheral lymphocyte counts during pregnancy in a patient with autoimmune thyroiditis (Fig. 4) strongly suggest that immunosuppression causes the change of MCHA. Hormonal changes associated with gestation, such as increased free serum cortisol (Burke & Roulet, 1970) and chorionic gonadotropin, conceivably have an effect on homeostatic immune regulation, preventing rejection of the foetal allograft (Adcock *et al.*, 1973), although the exact mechanism of this effect requires further study.

The transient increase of antibodies after delivery is very striking. A similar pattern of decrease in pregnancy and increase after delivery was observed in the insulin antibody in the sera of insulin-treated diabetic patients (Exon, Dixon & Malins, 1974). As shown in Fig. 4, post-partum changes in serum immunoglobulins IgG and IgM, goitre size and the peripheral lymphocyte counts are roughly correlated with that of MCHA. Thus the transient post-partum increase of antibodies may also result from immunological changes in the mother after delivery.

The symptoms of Graves' disease often improved during pregnancy and become aggravated again after delivery (Gardiner-Hill, 1929; Astwood, 1951). Nelson & Palmer (1975) reported a remission of goitrous hypothyroidism during pregnancy in a patient with autoimmune thyroiditis. Recently, we observed transient post-partum hypothyroidism and thyrotoxicosis (Amino *et al.*, 1976b, 1977a,b) in autoimmune thyroid diseases. Amelioration during late pregnancy and relapse or aggravation of the disease 2–6 months post-partum have also been reported in other autoimmune diseases, such as rheumatoid arthritis (Hench, 1949; Oka, 1953), systemic lupus erythematosus (Friedman & Rutherford, 1956; Garsenstein, Pollak & Kark, 1962) and idiopathic thrombocytopenic purpura (Lorz & Frumin, 1961).

On the basis of all these data, it seems possible that immunosuppression in pregnancy may disappear at delivery, and that 'transient enhancement' of the immune reactions may occur after delivery by a

somewhat similar mechanism to the 'rebound phenomenon' observed after withdrawal of immunosuppressive glucocorticoid therapy. Transient increases in antibody levels after abortion may be induced by a similar mechanism. Thus patients suspected of having autoimmune diseases in pregnancy should be re-evaluated 1–6 months post-partum, even though they give a negative test for antibodies during pregnancy.

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