Clin. exp. Immunol. (1977) 28, 306–314.

Differential effect of pregnancy or gestagens on humoral and cell-mediated immunity

N. FABRIS, L. PIANTANELLI & M. MUZZIOLI *Experimental Gerontology Center, I.N.R.C.A. Research Department, Ancona, Italy*

(*Received* 19 *May* 1976)

SUMMARY

The reactivity of spleen lymphocytes in a mixed lymphocyte culture and the *in vivo* PFC response to sheep erythrocytes have been evaluated in pregnant female mice and data compared with those observed in virgin sexually mature female mice daily treated either with progesterone or human chorionic gonadotropin (HCG) or human prolactin.

The mixed lymphocyte reactivity is depressed at mid-pregnancy, whereas PFC response is increased. Comparable immunological modifications have been found in mice treated with HCG, but not in animals treated with progesterone or prolactin. The similarity between HCG treatment and pregnancy suggests that the rate of gonadotropin release may be one of the earliest events responsible for the immunological disturbances present during pregnancy, although its action on the lymphoid system seems to require the presence of the ovary.

From these data and from the observation that HCG increases the PFC response also in thymusless nude female mice, it can be deduced that it acts on both T and B cells.

INTRODUCTION

The immunological implications of pregnancy and, more generally, of femininity still pose a number of unanswered questions. It has been reported that during pregnancy cell-mediated immunity is depressed (Purtilo *et al.*, 1972; Thong *et al.*, 1973; Morton, Hegh & Clunie, 1974; Olding & Oldstone, 1974), although according to other authors is unmodified (Carr, Stites & Fundenberg, 1973), and that such a depression may be either generalized or specific, the later being a kind of immunological tolerance (Schwarz, 1968; Silvers *et al.*, 1970). In order to explain the generalized depression, serum-blocking antibody (Hellstrom, Hellstrom & Brawn, 1969) as well as known hormones, such as corticosteroids (Kasakura, 1973), progesterone (Munroe, 1971), oestrogens (Waltmann, 1971), chorionic gonadotropin (Contractor & Davies, 1973; Adcock *et al.*, 1973), or still undefined serum factors (Hill, Finn & Denye, 1973) have been taken into consideration. That some hormonal factors may depress cell-mediated immunity is supported also by the observation that women taking oral contraceptives show a reduced *in vitro* blastic transformation to PHA (Barnes *et al.*, 1974) and that the menstrual cycle itself may modulate PHA responses (Fabris, Ghisilieri & Bevilacqua, 1976 to be submitted for publication).

On the other hand, immunoglobulin levels do not seem to change during pregnancy or after oral contraceptive treatment (Horne *et al.*, 1970). Also the incidence of autoantibodies is not modified by these conditions (Barnes *et al.*, 1974). We have shown that the number of PFCs against sheep erythrocytes increases during pregnancy in the mouse (Fabris, 1973a), and increased numbers of PFCs have been recorded in peritoneal exudate cells from retired female breeder mice when compared to age-matched virgin females (Nossal *et al.*, 1970).

The observation, however, that females outperform males in terms of antibody response and that

Correspondence: Dr N. Fabris, Experimental Gerontology Center, Research Department, Via Birarelli, 8–60100 Ancona, Italy.

males may adopt female patterns after castration (Eidinger & Garrett, 1972), strongly supports the idea that female hormones do affect humoral immune responses.

This paper presents evidence that human chorionic gonadotropin (HCG) but not progesterone or human prolactin (LTH) modifies both cell- mediated immunity and humoral immune responses, and that these alterations are similar to those observed during syngeneic or allogeneic pregnancy. Cell-mediated immunity has been measured by unidirectional mixed leucocyte (UML) culture; humoral antibody response has been evaluated by plaque-forming capacity against sheep erythrocytes.

MATERIALS AND METHODS

Animals. Charles River (Charles River, Italy), C3H (Kindly supplied by Hoffmann-La Roche) and BALB/c carrying the nude thymusless mutation were used. Animals were kept at 22–24°C (nude mice at 26°C) and fed with the usual pellets and water *ad libitum.* Tetracyclin (Terramycin–Pfitzer) was monthly given in the drinking water in the amount of 100 μg/ml for 4 successive days. In order to establish the time of pregnancy, Charles River mice were mated for a 48-hr period with allogeneic (C3H) or syngeneic males.

Animals under hormonal treatment were weekly weighed and at killing the weights of thymus and spleen have been recorded.

When not otherwise specified each experimental group was constituted of five to seven animals.

Operative procedure. Ovariectomized female mice were prepared according to the method of Ingle and Griffith (Ingle & Griffith, 1962).

Hormonal preparations. Progesterone was kindly supplied by Farmitalia (Italy). For injection progesterone was dissolved in sesame oil and administered s.c. Human chorionic gonadotropin (batch no. 5658, 6 i.u./mg) and prolactin (batch R 1339) were kindly supplied by Richter through the courtesy of Professor Matscher. They were dissolved in sterile physiological saline and injected s.c. Saline and sesame oil injected mice served as controls respectively.

Unidirectional mixed leucocytes (UML) cultures. Spleen cells were obtained by teasing the spleen in cold RPMI 1640 (Eurobio–France) through a 60-mesh net with two successive passages through a 25-gauge needle. Cells were washed and resuspended in RPMI 1640 supplemented with 5% fresh human serum.

The number of viable cells was determined by trypan blue exclusion and the cell suspension diluted to 4×10^6 viable cells/ml.

Aliquots of 0·25 ml of this suspension were distributed in 12 × 75 mm plastic tube (Falcon) together with 0·25 ml of stimulator cell suspension diluted to a concentration of 8×10^6 viable cells/ml. Double aliquots of either responder or stimulator cells were used as controls. Culture were then incubated at 37°C for 5 days. ^{3}H-labelled thymidine ([^{3}H]Td; Amersham, sp. act. 5000 mCi/mM) was added in the amount of 0·5 μCi/tube 22 hr before the end of the culture. The amount of radioactivity present in the trichloracetic acid precipitable material was measured in a liquid scintillation counter (Packard). Results are expressed in ct/min/culture. The standard error among triplicate cultures was lower than 7·5%.

Humoral immune responses. Primary immune responses against sheep red blood cells (SRBC) were measured after one i.p. injection with 0·1 ml of 20% erythrocyte suspension in saline.

In one experiment immunization with 0·1 ml of 2% SRBC suspension in saline was used. Plaque-forming-cells (PFC) were determined by plating $0\cdot5\times 10^6$ spleen cells prepared as described above with 0·1 ml of 10% SRBC suspension in agar Petri dishes, and adding, after incubation, lyophilized guinea-pig serum diluted 1:20.

RESULTS

(a) *UML reaction by spleen cells from pregnant mice*

The UML reaction mounted by spleen cells from pregnant Charles River mice against (Charles River × C3H) F_1 spleen cells is reduced when compared to that mounted by cells from unmated controls (Fig. 1). In particular, spleen cells removed at 9 or 12 days of pregnancy show a 50% reduction, while cells from 15- or 18-day pregnant mice respond as well as cells from virgin females. The reduction of UML reactivity can be observed during either syngeneic or allogeneic pregnancy (Fig. 1).

Moreover, the spontaneous blastic transformation significantly increases in cultures from pregnant mice, the higher values being observed at 9 and 12 days of pregnancy. In the following stages of pregnancy the high spontaneous transformation progressively disappears and by 18 days of pregnancy there are no differences compared to unmated controls (Fig. 1). It is of interest to note that, parallel with the decrease of UML reactivity and the increase of spontaneous blastic transformation, a nearly proportional increase of the total number of nucleated cells harvested per spleen is recorded during pregnancy (Table 1). In fact the spleen at 8 and 12 days of pregnancy contains more cells than the spleen

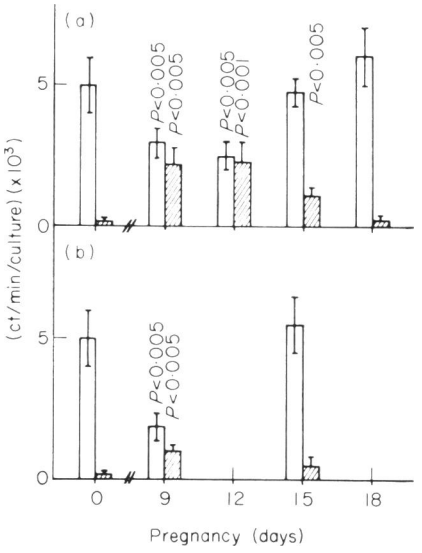

FIG. 1. UML reactivity of spleen cells removed from pregnant or virgin Charles River female mice, when stimulated with (Charles River × C3H) F_1 spleen cells. Animals at different stages of either syngeneic (Charles River × Charles River) (a) or allogeneic (Charles River × C3H) (b) pregnancy have been used. Open columns (□) indicate the amount of radioactivity incorporated by stimulated cells during the last 22 hr of culture, the background of either responder or stimulator cells being subtracted. Hatched columns (▨) indicate the spontaneous transformation of responder cells. I = ± s.e.

of virgin females or of 15- or 18-day pregnant mice, the last group showing even lower numbers of spleen cells than unmated controls.

(b) *Effect of progesterone on PFC response and UML reaction*

As reported in Fig. 2, virgin female Charles River mice, treated with progesterone at two different daily doses (100 and 500 μg/mouse/day) for 15 days show a slightly increased number of PFC/1 × 10^6 spleen cells, when compared with oil-treated controls.

On the other hand, the reactivity of spleen cells in UML cultures does not seem to be greatly modified by progesterone treatment, although the spontaneous blastic transformation is higher than that recorded in spleen cultures from oil treated mice (Fig. 2).

(c) *Effect of prolactin on PFC response and UML reaction*

As shown in Fig. 3, treatment with prolactin, given at three different daily doses, does not modify either the number of PFC or the reactivity of spleen cells in UML cultures, or finally, the total number of nucleated spleen cells.

TABLE 1. Number of nucleated spleen cells in syngeneic or allogeneic pregnant mice (× 10^6)

Mating	Days of pregnancy				
	Unmated	9	12	15	18
Syngeneic	168 ± 13	253 ± 16	245 ± 28	146 ± 23	89 ± 12
Allogeneic		221 ± 18	—	143 ± 19	—

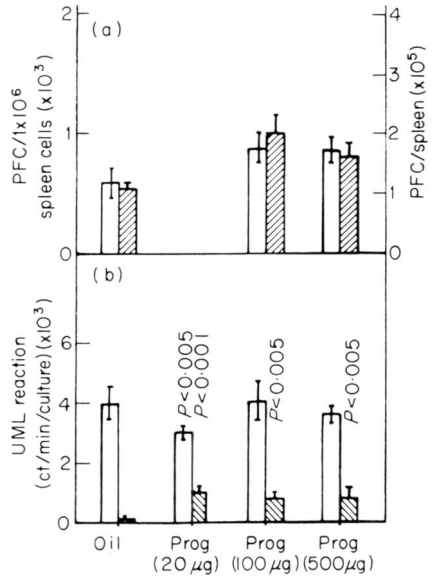

FIG. 2. PFC response and UML reactivity of spleen cells from virgin female Charles River mice, treated for 15 days with progesterone at different daily doses. PFC response was determined 4 days after immunization with 0·1 ml of a 20% erythrocyte suspension in saline. UML reactivity of spleen cells was determined as reported in Fig. 1. (a) Open columns (□) = PFC/1 × 10^6 spleen cells; hatched columns (▨) = PFC per spleen. (b) Open columns (□) = amount of radioactivity incorporated by stimulated cells (background of either responder or stimulator cells being subtracted); stippled columns (▧) = spontaneous transformation of responder cells. I = ± s.e.

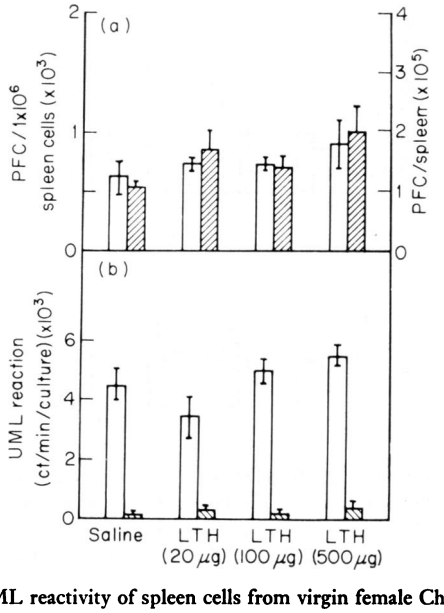

FIG. 3. PFC response and UML reactivity of spleen cells from virgin female Charles River mice, treated for 15 days with prolactin (LTH) at different daily doses. PFC response was determined 4 days after immunization with 0·1 ml of a 20% erythrocyte suspension in saline. UML reactivity of spleen cells was determined as reported in Fig. 1. (a) Open columns (□) = PFC/1 × 10^6 spleen cells; hatched columns (▨) = PFC per spleen. (b) Open columns (□) = amount of radioactivity incorporated by stimulated cells (background of either responder or stimulator cells being subtracted); hatched columns (▧) = spontaneous transformation of responder cells. I = ± s.e.

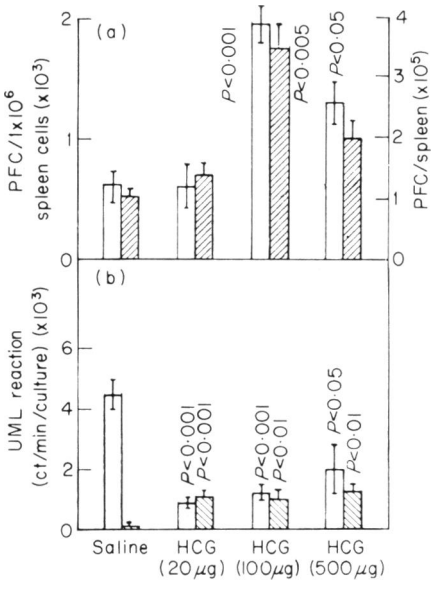

FIG. 4. PFC response and UML reactivity of spleen cells from virgin female Charles River mice, treated for 15 days with human chorionic gonadotropin (HCG) at different daily doses. PFC response was determined 4 days after immunization with 0·1 ml of a 20% erythrocyte suspension in saline. UML reactivity of spleen cells was determined as reported in Fig. 1. (a) Open columns (□) = PCF/1 × 10^6 spleen cells; stippled columns (▨) = PFC per spleen. (b) Open columns (□) = amount of radioactivity incorporated by stimulated cells (background of either responder or stimulator cells being subtracted); stippled columns (▧) = spontaneous transformation of responder cells. I = ± s.e.

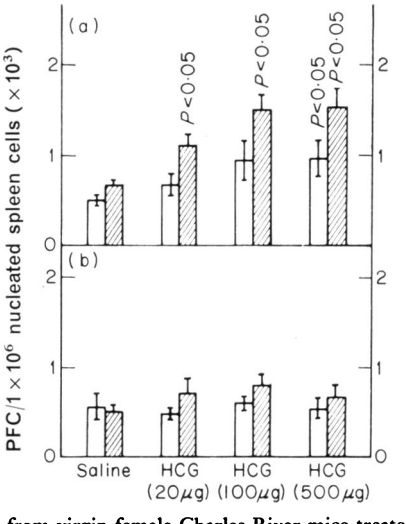

FIG. 5. PFC response of spleen from virgin female Charles River mice treated with different doses of HCG from the day of antigenic challenge to the day of sacrifice. PFC response was determined 4 days after immunization with 0·1 ml of a 20% erythrocyte suspension in saline (a) or with 0·1 ml of a 2% erythrocyte suspension (b). Open columns (□) indicate PFC/1 × 10^6 spleen cells; stippled columns (▨) indicate PFC per spleen. I = ± s.e.

(d) *Effect of chorionic gonadotropin on PFC response and UML reaction*

The number of PFC/1 × 10^6 spleen cells is significantly increased in mice daily treated with 100 or 500 μg HCG for 15 days when compared to the values observed in saline-treated controls (Fig. 4). The total number of nucleated spleen cells is not greatly modified although a statistically significant decrease is observed with the highest dose of HCG.

TABLE 2. Decreased thymus weights in unimmunized Charles River female mice daily treated with HCG

Experimental groups	Body weight (g)	Thymus weight (mg)	Thymus weight (%)	Spleen weight (mg)	Spleen weight (%)
Saline	22·6±0·8	51±4	0·23±0·03	118±7	0·52±0·04
HCG	21·1±0·9	26±3	0·12±0·02	102±7	0·48±0·05
Significance test	$P>0·05$	$P<0·001$	$P<0·001$	$P>0·05$	$P>0·05$

By contrast the reactivity of spleen cells in UML culture is depressed and concomittantly the spontaneous blastic transformation is significantly increased (Fig. 4). Such a deep alteration is observed in animals treated with 20, 100 or 500 μg of HCG per day.

The high sensitivity of PFC response to HCG treatment prompted us to investigate whether shorter hormonal treatments were as active as those used in the previous experiments.

Animals treated with HCG from the day of antigenic challenge (for a total of four hormone injections) show an increased number of PFC, only the two highest HCG doses being, however, effective (Fig. 5).

If animals are immunized with ten-fold less antigen, the hormonal treatment does not significantly increase the number of PFCs reached four days after challenge.

Finally, unimmunized animals treated with the hormone alone do not form consistent numbers of PFCs. It is of interest to note that unimmunized mice treated with HCG for 15 days show a reduction of both absolute and relative weights of the thymus, while the weights of the spleen are unmodified (Table 2).

(e) *Effectiveness of HCG treatment in ovariectomized mice*

In order to investigate whether HCG acts on PFC response directly or through the release of other hormonal factors by the ovary, virgin sexually mature BALB/c mice were ovariectomized and the effect of HCG treatment on their PFC capacity examined.

From the data reported in Table 3 it can be deduced that ovariectomized mice do not respond as well as sham-operated mice to HCG daily given in that amount (100 μg/day), which, from previous experi-

TABLE 3. PFC response by spleen cells from ovariectomized normal or thymusless nude BALB/c female mice with or without HCG treatment

Experimental groups	no. of spleen cells ($\times 10^6$)	PFC/1 $\times 10^6$ spleen cells	PFC/spleen ($\times 10^3$)
Sham-operated controls	196±35	804±112	148·4±15·7
Sham-operated+HCG	236±9	1591±202 ($P<0·01$)*	372·2±32·8 ($P<0·05$)*
Ovariectomized	228±21	659±78	156·4±25·0
Ovariectomized+HCG	171±18	986±115	171·9±32·3
Normal controls	114±13	583±65	68·5±16·5
Normal+HCG	124±9	864±63	104·3±12·2 ($P<0·05$)*
Nude controls	39±8	40±13	1·8±0·9
Nude+HCG (follicular hyperplasia)	75±5 ($P<0·05$)†	140±22 ($P<0·05$)†	10·4±1·2 ($P<0·01$)†
Nude+HCG (without follicular hyperplasia)	18±2 ($P<0·05$)†	27±6	0·5±0·1

* When compared with sham-operated or normal controls.

† When compared with nude controls.

ments, seemed to be the optimal dose acting on PFC response. These findings would suggest that the effect of HCG may be mediated, at least partially, through the release of other factors synthesized by the ovary.

This interpretation is supported also by observations made in thymusless nude female mice. In order to test the relevance of the thymus for the effectiveness of HCG treatment on PFC response, 20-day-old female nude mice were treated with the same injection schedule used in the previous experiments.

At the time of killing, it has been observed that only half of thymusless nude treated mice showed the usual hyperplasia of ovarian follicoli found in normal female mice after HCG therapy. This fact may well be connected with the retardation of sexual development in nude mice recently documented (Besedowski & Sorkin, 1974). By selecting data on PFC response of HCG treated nude mice showing follicular hyperplasia from unresponsive ones, it was found that HCG increases PFC responses only in the first group. (Table 3). Also the total number of nucleated spleen cells is significantly increased only in nude mice responding with ovarian hyperplasia to the HCG treatment. Such an increment is far higher than that observed in normal mice after HCG treatment. In nude mice not responding with follicular hyperplasia to HCG treatment, the total number of spleen cells is significantlv diminished (Table 3).

DISCUSSION

The data reported above demonstrate that, in our exprimental conditions UML reactions of spleen cells are depressed by the pregnancy status. These observations together with our previous findings on contact allergic reactions (Fabris, 1973a) support the data of other authors, who demonstrated a depressed cell-mediated immunity in human pregnancy (Purtilo *et al.*, 1972; Thong *et al.*, 1974; Olding & Oldstone, 1974). On the other hand primary PFC response to SRBC is increased in pregnant mice and particularly during the first two weeks of pregnancy (Fabris, 1973a). No clear data on antibody responses in human pregnancy are available at present; it has been recently shown, however, that the level of human B cells in peripheral blood increases during early pregnancy (Strelkausas *et al.*, 1975).

Since one of the most typical features of pregnancy is the progressive modification of hormonal balance, the observed alterations of the immune system during pregnancy may well depend on the day by day readjustment of hormonal equilibrium. According to the present knowledge, the hormonal changes in short-gestation animal species involve primarily gonadotropins, oestrogen and progesterone and, as consequences of these hormonal modifications, corticosteroids and thyroxine with minor homeostatic readjustment of other hormones (Davies & Ryan, 1972). Some of these hormones, however although secreted in different amounts during pregnancy, may be of little relevance for the interpretation of our findings.

Plasma levels of corticosteroids, for instance, increase progressively during pregnancy, probably under oestrogen stimulation, and in the mouse reach the maximal peak the 16th day of gestation (Barlow *et al.*, 1973). Although it has been suggested that raised plasma levels of corticosteroids may be responsible for the depressed MLC responses in humans (Kasakura, 1973), they can not explain the findings in the mouse, because at 15 days of pregnancy, i.e. when the highest plasma levels of corticosteroids are found, UML reactivity is normal and PFC response still increased (Fabris, 1973a).

Also an increment of thyroxine synthesis, according to its effect on the immune system (Fabris, 1973b) does not explain all the immunological disturbances observed during pregnancy in the mouse.

With regard to the hormones primarily involved in pregnancy, all of them have the capacity to depress cell-mediated immunity, in some particular experimental models (see the Introduction). Since, however, oestrogens increase progressively in rodent pregnancy until delivery, they do not offer a reasonable interpretation of all our findings. By contrast, plasma levels of gonadotropins and of progesterone which gradually rise to a peak around mid-pregnancy and progressively decline to low levels at term, (Davies & Ryan, 1972), may fit with the kinetics of both UML reactions and PFC responses during pregnancy.

The evaluation of our data on immunological responses in mice treated either with progesterone, or with prolactin or with HCG, show that chorionic gonadotropin, and only this hormone, induces a statistically significant increase of PFC capacity, while strongly depressing UML reactions. It is of interest to note that the administration of HCG and, to a lesser extent, of progesterone as well as the pregnant status significantly increase the spontaneous blastic transformation of spleen cells when transferred in culture.

The similarity between the immunological behaviour of HCG treated mice and that observed during pregnancy is quite striking and favours the assumption that HCG may play a primary role in the immunological disturbances during gestation. The demonstration of an inversion of levels of human T and B cells, which seems to parallel the rise and fall of plasma HCG, during early pregnancy (Strelkausas *et al.*, 1975) is in agreement with our findings.

With regard to the mode of action of HCG, it has been proposed that HCG may directly interfere with T-cell function, since it depresses *in vitro* the blastic transformation of lymphocytes (Contractor & Davies, 1973; Adcock *et al.*, 1973). This interpretation would agree with our observation on the increased PFC response under HCG treatment provided it can be proved that HCG acts also on suppressor T cells.

Two experimental observations are however against this interpretation. Firstly, the action of HCG, at least on PFC capacity, does not seem to be directly exerted on the cells involved in the PFC response. In fact, the removal of ovaries or the failure of nude mice to react with follicular hyperplasia to HCG treatment prevents HCG exerting its action on PFC response.

Moreover, since HCG treatment increases by a factor of ten the PFC response in nude mice, its action or, more precisely, the action of hormonal factors released from the ovary under HCG treatment, is directed also on B cells. This assumption is further supported by the observation that pregnant nude female mice form many more PFCs than virgin mice (Rowley, personal comunication).

With regard to the high spontaneous blastic transformation shown by spleen cells from pregnant mice we may agree with other authors (Carr *et al.*, 1973) that a low level antigenic stimulation of the mother may occur during pregnancy by foetal antigens. The high spontaneous transformation observed in spleen cells from HCG or progesterone-treated mice, may suggest, however, that, in our case, the common daily level of antigenic stimulation is involved as well.

Taken together, our findings suggest that HCG may modulate both T- and B-cell response, but we can not say, at present, whether different hormones, released under HCG treatment, may act independently on T- or B-cell response.

From a speculative point of view the possibility that some hormones such as gonadotropins may, directly or indirectly shift the immunological response towards antibody production and, eventually, towards enhancing antibody synthesis, is very significant in relation to the problem of the safety of foetuses as allografts. Other biological problems, however, may be involved as well. Thus the high incidence of autoantibodies in humans, particulary in women, in the 6th and 7th decades of life (for review see: Walford, 1969), and the progressively decreased incidence in the following decades correlate with the gonadotropin patterns (Albert *et al.*, 1956) to such an extent that an investigation in this direction is certainly justified.

An other interesting speculation is the high incidence of plasmocytomas, as well as of other tumours in that period of life which follows the onset of sexual decline, i.e. when pituitary gonadotropins are synthetized at their highest rate. Whether this concurrence is casual or related to a direct effect of hormones on tumor growth, as suggested by some observations on oil-induced mouse plasmocytomas (Hollander *et al.*, 1968) or virus- (Bentley *et al.*, 1974) or X-ray-induced lymphosarcomas (Pierpaoli & Haran-Ghera, 1975), or finally due to a hormone dependent alteration of the immunological response, remains to be investigated.

We extend our gratitude to R. Stecconi, R. Ricciotti and Q. Bernacchia for their excellent technical assistance and Dr T. Makinodan for revising the manuscript.

REFERENCES

ADCOCK, III., E.W., TEASDALE, F., AGUST, C.S., COX, S., MESCHIA, G., BATTAGLIA, F.C. & NAUGHTON, M.A. (1973) Human chorionic gonadotrophin: its possible role in maternal lymphocyte suppression. *Science*, **181**, 835.

ALBERT, A., RANDALL, R.W., SMITH, R.A. & JOHNSON, C.E. (1956) Urinary excretion of gonadotrophin as a function of age. *Hormones and the Aging Process.* (ed. by E.T. Engle and G. Pincus), p. 49. Academic Press New York.

BARLOW, S.M., MORRISON, P.J. & SULLIVAN, F.M. (1973) Plasma corticosterone levels during pregnancy in the mouse. *J. Endocrinol.* **59**, 31.

BARNES, E.W., MACCUISH, A.C., LOUDON, N.B. JORDAN, J. & IRVINE, W.J. (1974) Phytohaemagglutinin-induced lymphocyte transformation and circulating autoantibodies in women taking oral contraceptives. *Lancet*, **i**, 898.

BENTLEY, H.P., HUGHES, E.R. & PETERSON, R.D.A. (1974) Effect of hypophysectomy on a virus-induced T-cell leukaemia. *Nature (Lond.)*, **252**, 747.

BESEDOWSKI, U. & SORKIN, E. (1974) Thymus involvement in female sexual maturation. *Nature (Lond.)*, **249**, 356.

CARR, M., STITES, D.P. & FUDENBERG, H.H. (1973) Cellular immune aspects of human fetal maternal relationship. II. *In vitro* response of gravida lymphocytes to phytohaemagglutinin. *Cell Immunol.* **8**, 448.

CONTRACTOR, S.F. & DAVIES, H. (1973) Effect of human chorionic gonadotrophin on phytohaemagglutinin-induced lymphocyte transformation. *Nature: New Biology*, **243**, 284.

DAVIES, J. & RYAN, K.J. (1972) Comparative endocrinology of gestation. *Vitam. u. Horm. (New York)* **30**, 223.

EIDINGER, D. & GARRETT, T.S. (1972) Studies of the regulatory effects of sex hormones on antibody formation and stem cell differentiation. *J. exp. Med.* **136**, 1098.

FABRIS, N. (1973a) Immunological reactivity during pregnancy in the mouse. *Experientia*, **29**, 610.

FABRIS, N. (1973b) Immunodepression in thyroid-deprived animals. *Clin. exp. Immunol.* **15**, 601.

HELLSTROM, K.E., HELLSTROM, I. & BRAWN, J. (1969) Abrogation of cellular immunity to antigenically foreing mouse embryonic cells by a serum factors. *Nature (Lond.)*, **224**, 914.

HILL, C.A.ST., FINN, R. & DENYE, V., (1973) Depression of cellular immunity in pregnancy due to a serum factor. *Brit. med. J.* iii, 513.

HOLLANDER, V.P., TAKAKURA, K. & YAMADA, H. (1968) Endocrine factors in the pathogenesis of plasma cell tumors. *Recent Progress in hormone research* (ed. by E.B. Astwood), p. 81. Academic press, New York.

HORNE, C.H., HOWIE, P.W., WEIR, R.J. & GOIDIE, R.B. (1970). Effect of combined oestrogen-progestogen oral contraceptives on serum-levels of 2-macroglobulin transferrin, albumin and IgG. *Lancet*, **i**, 49.

INGLE, D.J. & GRIFFITH, J.Q. (1962) Surgery of the rat. *The rat in laboratory investigation*, (ed. by E. Farris and J.Q. Griffith), p. 434 Hafner Publishing Co. New York.

KASAKURA, S. (1973) Is cortisol responsible for inhibition of MLC reactions by pregnancy plasma? *Nature (Lond.)*, **246**, 496.

MORTON, H. HEGH, V. & CLUNIE, G.J.A. (1974) Immunosuppression detected in pregnant mice by rosette inhibition test. *Nature (Lond.)*, **249**, 459.

MUNROE, J.S. (1971) Progesteroids as immunosuppressive agents. *J. Reticuloend. Soc.* **9**, 361.

NOSSAL, J.V., BUSSARD, A.E., LEWIS, H. & MAZIE, J.C. (1970) Formation of haemolitic plaques by peritoneal cells in vitro. I: A new technique enabling micromanipulation and yielding higher plaque numbers. *Developmental Aspects of Antibody Formation and Structure*, (ed. by U. Sterzl and J. Riha), p. 655. Czecoslovak Acad. Press, Prague.

OLDING, L.B. & OLDSTONE, M.B.A. (1974) Lymphocytes from human newborns abrogate mitosis of their mothers lymphocytes. *Nature (Lond.)*, **249**, 161.

PIERPAOLI, W. & HARAN-GHERA, N. (1975) Prevention of induced leukaemia in mice by immunological inhibition of adenohypophysis. *Nature (Lond.)*, **254**, 334.

POWELL, A.E. (1974) Maternal lymphocytes: suppression by human chorionic gonadotrophin. *Science*, **184**, 913.

PURTILO, D.T., HALLGREN, H.M. & YUNIS, E.J. (1972) Depressed maternal lymphocyte response to phytohaemagglutinin in human. *Lancet*, **i**, 769.

SCHWARZ, M.R. (1968) The mixed lymphocyte reaction: an *in vitro* test for tolerance. *J. exp. Med.* **127**, 879.

SILVERS, W.K., LUBAROFF, D.M., WILSON, D.B. & FOX, D. (1970) Mixed lymphocyte reactions and tissue transplantation tolerance. *Science*, **167**, 1624.

STRELKAUSAS, A.J., WILSON, B.S., DRAY, S. & DODSON, M. (1975) Inversion of levels of human T and B cells in early pregnancy. *Nature (Lond.)*, **258**, 331.

THONG, Y.H., STEELE, R.W., VINCENT, M.M., HENSEN, S.A. & BELLANTI, J.A. Impaired *in vitro* cell-mediated immunity to rubella virus during pregnancy. *New Engl. J. Med.* **289**, 604.

WALFORD, R.L. (1969) *The Immunological Theory of Ageing.* Munkssgaard, Copenhagen.

WALTMAN, S.R., BURDE, R.M. & BERRIOS, J. (1971) Prevention of corneal homograft rejection by estrogens. *Transplantation*, **11**, 194.