Identification of a murine pregnancy-associated serum protein (α_1 -PAP) as a female-specific acute-phase reactant*

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Summary. A murine pregnancy-associated protein (α_1 -PAP) with α_1 -electrophoretic mobility and an estimated molecular weight of 150 000 was present in serum from pregnant C57BL/10 mice but could not be detected in serum of mature non-pregnant females and males. During pregnancy α_1 -PAP was first detected on Day 7, rose to maximum levels between Days 12 and 14, and thereafter declined during the remainder of pregnancy and was undetectable by Day 8 *post partum*. The protein was also detected in the serum of females, but not males, subjected to an inflammatory stimulus. Examination of the α_1 -PAP levels during an acute-phase response in females demonstrated that the protein behaved as a typical classical acute-phase reactant, although the levels were only 10% of those observed during Days 12 to 14 of pregnancy. α_1 -PAP therefore appears to represent a hitherto undescribed female-specific acute phase reactant in the mouse.

Introduction

Many attempts have been made to establish laboratory animal models to aid the study of human pregnancy-associated serum proteins since their physiological importance is largely unknown (see supplement by Klopper, 1982). Four pregnancy-specific serum proteins have been detected in mice and rats after immunization of rabbits with late pregnancy serum and absorption of the antiserum with plasma powder from non-pregnant animals (Lin, Halbert & Kiefer, 1974). However, none of these proteins exhibited cross-reactivity with human pregnancy-associated proteins, by immunoelectrophoresis or by immunoelectrodiffusion, although one of the proteins showed cross-reactivity between rats and mice. Using a similar approach, 4 pregnancy-associated proteins have also been detected in murine pregnancy serum (Hau, Svendsen, Teisner & Svehag, 1978; Hau, Svendsen, Teisner & Brandt, 1981a; Hau, Svendsen, Teisner, Thomsen Pederson & Kristiansen, 1981b), but only one was suggested to be pregnancy-specific: a protein of molecular weight 140 000 and α_1 -electrophoretic mobility, 'pregnancy-associated murine protein-1' (PAMP-1), present in the serum of non-pregnant females, increased 5-fold in concentration during pregnancy to reach maximum levels at mid-pregnancy, after which it decreased to non-pregnant levels at parturition. Immunological cross-reactivity between PAMP-1 and human pregnancy-associated globulin (α_2 -PAG) has been demonstrated by using an antiserum raised in chickens (Hau, Westergaard, Svendsen, Bach & Teisner, 1981c). In contrast, PAMP-2 only occurred in the serum of pregnant animals from mid- to late gestation (Hau *et al.*, 1980), decreased in concentration before parturition, possessed α_2 -electrophoretic mobility and a molecular weight of 70 000 and exhibited

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cross-reactivity with human SP-1 (Hau *et al.*, 1980). The relationship between these proteins and those described by Lin *et al.* (1974) is unknown.

There appear to be features common between pregnancy and the response of animals to tissue injury, the acute-phase response, because certain pregnancy-associated serum proteins have been identified as acute-phase reactants. In the rat 'reproduction-associated protein' (Heim, 1962) is identical to acute-phase α_2 -macroglobulin (Gordon, 1976), although the site of synthesis is different: in pregnancy synthesis occurs in the fetal liver (Sarcione & Bohne, 1969), decidual tissue and the metrial gland (Bell, 1979a, b), whereas during an acute-phase response the liver is the sole site of production (Benjamin & Weimer, 1966; Koj, 1974). The levels of the acute-phase reactant of the mouse, serum amyloid P-component (SAP), are increased 5-fold during allogeneic but not syngeneic pregnancy (Waites, Bell & Bell, 1983). SAP is the major acute-phase reactant of the mouse, increasing 4–50-fold depending on the strain examined (Pepys, 1979; Pepys, Baltz, Gomer, Davies & Doenhoff, 1979) and in this respect resembles the major acute-phase reactant in man, C-reactive protein (CRP). Both are members of a family of pentameric proteins termed pentraxins (Osmand *et al.*, 1977).

As part of an investigation concerned with characterization of, and the elucidation of the function of, pregnancy-associated serum proteins in the mouse, we have identified an apparently pregnancy-specific serum protein as a female-specific acute-phase reactant.

Materials and Methods

Animals

The inbred strains of mice used were C57BL (H-2^b), C57BL/10ScSn (H-2^b), CBA/Ca (H-2^k) and B10 BR (H-2^k). An outbred colony of BM mice was also used. Pregnant animals were obtained by overnight pairing of females of an appropriate strain with males of proven fertility and the morning on which a vaginal plug was detected was designated Day 1 *post coitum* (*p.c.*) of pregnancy. Pregnant mice were bled by cardiac puncture (Days 11–19 *p.c.*) or by retro-orbital bleeding under light diethyl ether anaesthesia (Days 3–9 *p.c.*). Serum samples collected from individual fetal and neonatal animals within a litter were pooled. Virgin females and males were bled by cardiac puncture. Blood was allowed to clot for 1 h at room temperature, the clot ringed and left overnight at 4°C. After centrifugation at 800 *g* for 10 min the serum was removed and stored at –20°C. Serum was also obtained from male and female mice subjected to injury by subcutaneous (*s.c.*) injection of 0.1 ml turpentine/arachis oil (1:1 *v/v*) at 72 and 24 h before bleeding. For determination of the profile of α_1 -PAP and serum amyloid P-component during an acute-phase response, a single *s.c.* injection of 0.2 ml turpentine/arachis oil (1:1, *v/v*) was given and animals were bled by retro-orbital puncture at 12, 24, 48 and 72 h. Serum was similarly obtained from blood removed by cardiac puncture of hooded female rats 48 h after *s.c.* injection of 2 ml turpentine/arachis oil. Blood for serum was also obtained from normal female and male rats and golden hamsters.

Preparation of antisera

Pooled serum (1 ml) from C57BL/10 ♀ × CBA/Ca ♂ mice 7–17 days pregnant was fractionated by gel filtration chromatography on a Sephadex G200 (Pharmacia) column (dimensions 31.5 × 2.5 cm) equilibrated with Dulbecco A phosphate-buffered saline (PBS) (Oxoid Ltd) pH 7.3 at 4°C with a flow rate of 15 ml/h. Fractions from each of the three major peaks eluted were pooled and concentrated. Aliquants of each pool were emulsified with Freund's complete adjuvant (Difco Laboratories, Detroit, Michigan, U.S.A.) to a final protein concentration of 0.25 mg/ml. Young adult New Zealand White rabbits were injected twice with 1 ml of the appropriate emulsion in both hind legs with an interval of 2 weeks between injections. At 2 weeks after the

second injection, 2 ml emulsion were injected i.p. and 10 days later animals were bled by puncture of the ear artery. A similar schedule was adopted using pooled whole serum from pregnant animals (adjusted to a final protein concentration of 0.25 mg/ml), and all animals were maintained on a 17-day rotation schedule of booster injection (2 ml emulsion i.p.) and bleeding with an interval of 10 days between, with 7 days between bleeding and the next injection. The rabbit antiserum was absorbed with serum from C57BL/10 males for 1 h at 37°C in ratios predetermined by a series of precipitin tests to optimize absorption. Samples were centrifuged at 800 *g* for 10 min and the supernatants removed and tested for antibody activity by crossed-immunoelectrophoresis against serum from male, virgin female and pregnant mice. In some instances absorption was with serum from virgin C57BL/10 females. Murine serum amyloid P-component (SAP) and anti-SAP antiserum were prepared as previously described (Waites *et al.*, 1983).

Immuno-electrophoretic techniques

Crossed-immunoelectrophoresis was performed in a 1% (w/v) agarose gel with a Tris-barbitone buffer pH 8.6 (Crowle, 1977). Electrophoresis in the first dimension was performed with voltage of 25 V/cm for 1.5 h and the second dimension with a voltage of 10 V/cm overnight. The relative electrophoretic mobility of the proteins was estimated by this method. Rocket immunoelectrophoresis (Laurell, 1972) was performed using the same gel-buffer system and the wells received 5 μ l serum. Levels of α_1 -PAP were expressed as arbitrary units per ml (AU/ml), with 1 AU being defined as the peak height obtained with a 1:30 dilution of a standard of pooled pregnancy serum. The lower limit of sensitivity of the assay was 0.03 AU/ml. Levels of SAP were determined by comparison with purified protein standard (Waites *et al.*, 1983).

Molecular weight estimation

Rocket immunoelectrophoresis was used to determine the elution volume of α_1 -PAP from pooled pregnancy or acute-phase serum fractionated on a Sephadex G200 column. The chromatographic constant (K_{av}) value was calculated for α_1 -PAP and its molecular weight estimated by interpolation from a standard curve of K_{av} values calculated for 4 different proteins of known molecular weight: chymotrypsinogen A (M_r 25 000), bovine serum albumin (M_r 67 000), aldolase (M_r 158 000) and catalase (M_r 232 000).

Anion exchange column chromatography

After Sephadex G200 fractionation of pooled pregnancy or acute-phase sera, α_1 -PAP-containing fractions were pooled, dialysed against the equilibration buffer and applied to a DEAE Sephacel (Pharmacia) column pre-equilibrated at 4°C in 0.05 M-Tris-HCl pH 8.6. The bound proteins, including α_1 -PAP, were eluted with a NaCl gradient from 0 to 0.3 M in the starting buffer.

Results

Production of antisera against a murine pregnancy-associated serum protein

After immunization of rabbits with pooled whole pregnancy serum from C57BL/10 females mated allogeneically with CBA/Ca males, an antiserum was obtained which, after absorption with male murine serum, produced two immunoprecipitates in crossed-immunoelectrophoresis against pregnancy serum (Pl. 1, Fig. 1). Due to their observed electrophoretic mobilities these proteins were termed α_1 and α_2 -pregnancy-associated proteins (α_1 -PAP and α_2 -PAP). Only α_2 -PAP was detected in serum from non-pregnant females; neither protein was present in male serum. Absorption of the antiserum with serum from non-pregnant mice yielded an antiserum which reacted only with α_1 -

PAP. Immunization of rabbits with the intermediate or low molecular-weight pools obtained from Sephadex G200 gel filtration of pregnancy serum yielded antisera which, after absorption with male serum, produced single precipitates in crossed-immunoelectrophoresis against pregnancy serum and none against serum from non-pregnant or male mice. The protein detected by these antisera was α_1 -PAP (Pl. 1, Fig. 2). Analytical rocket immunoelectrophoresis confirmed that, whereas α_1 -PAP appeared to be pregnancy-specific, α_2 -PAP was also detectable in the serum of non-pregnant females (Pl. 1, Figs 3 & 4).

Serum samples from rats and hamsters were tested for proteins cross-reacting with α_1 -PAP serum. Cross-reactivity was noted only with serum from normal female rats and those in the acute phase of an inflammatory response.

Serum concentrations of α_1 -PAP

A rocket immunoelectrophoretic assay with a lower limit of sensitivity of 0.03 AU/ml was used to determine serum concentrations of α_1 -PAP. The protein was undetectable in sera of C57BL/10 males ($N = 53$) and in a variety of normal males of other strains including C57BL, C3H, CBA/Ca, B10BR and BM ($6 < N < 11$ for each strain). The concentrations of α_1 -PAP were undetectable in sera of virgin females of the C57BL/10 ($N = 30$), C57BL, CBA/Ca, B10BR and BM strains ($6 < N < 9$).

As shown in Text-fig. 1, α_1 -PAP was first detectable in C57BL/10 females mated allogeneically with CBA/Ca males on Day 7 of pregnancy (0.1 ± 0.07 AU/ml) and thereafter increased rapidly to Day 12. Maximum levels, representing at least a 2800-fold increase, were observed between Days 12 and 14 (84 ± 7.9 AU/ml); values then fell dramatically during the remainder of pregnancy and further after parturition, being undetectable by 7 days after parturition. Fetal serum obtained on Day 19 of pregnancy and the serum of neonates, did not contain α_1 -PAP. Substantial levels of α_1 -PAP were detected in sera obtained on Days 12–14 of pregnancy in other strain combinations, although strain-dependent differences in absolute levels were observed: CBA/Ca ♀ $\times$ C57BL/10

PLATE 1

Fig. 1. Crossed-immunoelectrophoresis using mouse pregnancy serum in the first dimension against anti-whole pregnancy antiserum absorbed with male serum in the second dimension. This shows the detection of two immunoprecipitates, α_1 -PAP and α_2 -PAP, the latter also being detectable in sera from non-pregnant females (see text). Under some conditions, as shown here, α_1 -PAP appears to be heterogeneous.

Fig. 2. Crossed-immunoelectrophoresis using mouse pregnancy serum in the first dimension against antiserum raised by the immunization of rabbits with the intermediate or low molecular weight pools obtained from Sephadex G200 gel filtration of pregnancy serum following absorption with male serum. A single immunoprecipitate was detected which was termed α_1 -PAP.

Fig. 3. Rocket immunoelectrophoresis using the dispecific antiserum shown above and pregnant (P), non-pregnant (NP) and male (M) serum as antigens at a 1:4 dilution (Wells 1–3, left to right) and pregnant serum at 1:30 (Well 4). α_1 -PAP (the upper rocket) is seen only in pregnant serum whereas α_2 -PAP is seen in non-pregnant serum.

Fig. 4. Rocket immunoelectrophoresis using the monospecific antiserum against α_1 -PAP (as above) showing that the protein is undetectable in non-pregnant females (NP) but is seen in the serum of females in the acute phase of an inflammatory response (AP) but not in males. Serum samples were used at 1:30 dilution (use of neat NP, M and AP ♂ serum also failed to reveal α_1 -PAP).

PLATE I

(Facing p. 584)

Text-fig. 1. Serum levels of α_1 -PAP in C57BL/10 female mice during pregnancy and the post-partum period. Values are mean $\pm$ s.e.m. when $N = 5$ –14 and otherwise as mean. Levels were undetectable in the sera of virgin females ($N = 30$) and males ($N = 53$).

σ , 125.5 ± 10.2 AU/ml ($N = 4$); B10BR $\sigma \times$ C57BL/10 σ , 94.2 ± 9.9 AU/ml ($N = 6$); C57BL/10 $\sigma \times$ B10BR σ 100 ± 24.7 AU/ml ($N = 2$); B10BR $\sigma \times$ C57BL σ , 70.5 ± 8.4 AU/ml ($N = 4$); and BM $\sigma \times$ BM σ , 69 ± 4.2 AU/ml ($N = 24$).

Detection of α_1 -PAP in sera of females in the acute phase of an inflammatory response

During a preliminary study rabbit anti- α_1 -PAP was demonstrated to produce a precipitate in Ouchterlony and rocket immunoelectrophoretic assays with sera from C57BL/10 females subjected to an inflammatory stimulus (Pl. 1, Fig. 4) although no such reactivity was observed in sera from control virgin females. Several observations suggested that this reactivity in acute-phase sera was due to α_1 -PAP, rather than reflecting cross-reactivity of another antigen with the rabbit antiserum. Firstly, in crossed-immunoelectrophoresis with acute-phase serum or a mixture of acute-phase and pregnancy sera, a single precipitate of α_1 mobility was obtained. Also, tandem immunoelectrophoresis of pregnancy and acute-phase sera resulted in two precipitin peaks which were confluent. Secondly, after fractionation of acute-phase sera on Sephadex G200 gel filtration and anion exchange column chromatography, the component reactive with rabbit anti- α_1 -PAP serum behaved identically to α_1 -PAP, i.e. had a molecular weight of 150 000 and eluted at 0.16 M-NaCl.

During determination of the molecular weight of α_1 -PAP in acute-phase serum by fractionation on Sephadex G200 gel filtration, it was found that serum amyloid P-component (SAP), assayed with rabbit anti-SAP in rocket immunoelectrophoresis, co-eluted with α_1 -PAP. Since levels of murine SAP are also increased during allogeneic pregnancy, the possibility was considered that α_1 -PAP was related to SAP. However, it appeared from a number of observations that α_1 -PAP and SAP did represent distinct entities. (1) The rabbit anti- α_1 -PAP serum exhibited no cross-reactivity with pure SAP. (2) In crossed-immunoelectrophoresis, the electrophoretic mobility of SAP was only 35–40% that of α_1 -PAP. (3) Although further studies showed that α_1 -PAP was detectable in sera of females of the C57BL/10 and BM strains after induction of an acute-phase response (1–10% of the peak levels observed during pregnancy), there were no detectable levels of α_1 -PAP in males

Text-fig. 2. Serum levels of α_1 -PAP and SAP during an acute-phase response in BM male and female mice. Values are mean $\pm$ s.e.m. for 7–4 mice. In sera of male animals α_1 -PAP was undetectable in controls ($N = 53$) and throughout the period examined. In females, serum α_1 -PAP was undetectable before treatment ($N = 30$).

of the C57BL/10 ($N = 13$) or BM ($N = 6$) strains after induction of an acute-phase response (Text-fig. 2).

Concentration of α_1 -PAP and SAP during an acute-phase response

To determine whether α_1 -PAP behaved as a classical acute-phase reactant the concentration of α_1 -PAP was compared with that of SAP after the induction of an acute-phase response. As shown in Text-fig. 2, in male and female BM mice SAP levels increased 3-fold to reach maximal levels between 24 and 48 h and thereafter declined. In the male, levels at 24 and 48 h were not significantly different, although in the female the peak SAP response occurred at 48 h. Although classically the peak SAP response is expected at 24 h, this does seem to be dependent on the method of induction of the acute-phase response and the strain of mouse used (Pepys *et al.*, 1979). In sera of male animals α_1 -PAP was undetectable in controls and throughout the period examined. In sera of females, although detectable at 12 h after induction, peak levels were similarly observed between 24 and 48 h (6.9 ± 1.7 AU/ml; $N = 7$), representing more than a 200-fold increase, and thereafter levels declined. Maximum levels of α_1 -PAP achieved after an acute-phase response were 10% of those achieved during allogeneic pregnancy

Discussion

Two murine pregnancy-associated serum proteins have been detected using antisera raised in rabbits against whole and fractionated pregnancy sera. The first, α_1 -PAP, initially appeared to be pregnancy-specific due to its absence in sera from non-pregnant females, whereas the second, α_2 -PAP, was also detected in sera of non-pregnant females and thus was pregnancy-associated. α_1 -PAP could be similar to one of the 4 murine pregnancy-specific proteins demonstrated by Lin *et al.* (1974). In many respects the protein also resembles the pregnancy-associated protein (PAMP-1) described by Hau *et al.* (1978) in its similar electrophoretic mobility and molecular weight, i.e. 150 000 compared to 140 000 for PAMP-1. A protein in sera from normal $\varnothing$ rats and those in the

acute phase, probably rat α_2 -PAG (Porstmann & Schmechta, 1980), exhibits cross-reactivity with antisera to α_1 -PAP and PAMP-1. The profiles of α_1 -PAP and PAMP-1 during pregnancy were also comparable in that both reached maximum levels at mid-pregnancy. However, PAMP-1 was detected in the serum of mature non-pregnant females, albeit at 13–18% of the mid-pregnancy levels (Hau *et al.*, 1978), whereas in the present study no α_1 -PAP was ever found in the serum of non-pregnant females. The lower limit of sensitivity of the assay used in the present study would indicate that, even if present in the sera of non-pregnant C57BL/10 females, levels could be only 0.03% of mid-pregnancy levels. The identification of α_1 -PAP as a female-specific acute-phase reactant at levels up to 10% of the mid-pregnancy levels could possibly account for these discrepancies if the mouse strain studied by Hau *et al.* (1978) was already subject to an acute-phase response by a subclinical infection, or, more likely, exhibited high endogenous levels of acute-phase reactant. The ability to detect acute-phase α_2 -macroglobulin in normal rats depends upon the strain studied (Bosanquet, Chandler & Gordon, 1976; Bell, 1980) and the endogenous levels of the major murine acute-phase reactant, SAP, in normal animals is genetically controlled, as is the relative increase in SAP levels after an acute-phase response (Pepys *et al.*, 1979; Mortensen, Beisel, Zeleznik & Le, 1983).

The identification of α_1 -PAP as a female-specific acute-phase reactant represents the first report of a sex-restricted acute-phase reactant whose synthesis is also pregnancy-associated. Murine SAP as an acute-phase reactant is not sex-restricted and, although allogeneic pregnancy can stimulate serum levels, the profile during pregnancy differs from that for α_1 -PAP (Waites *et al.*, 1983). The inability to detect α_1 -PAP in sera of males subjected to inflammatory stimuli implicates gonadal steroids in the regulation of its synthesis during an acute-phase response. In hamster females a protein which is a sex-restricted protein and structural homologue of murine SAP and human CRP, i.e. a pentraxin, does not occur in normal male serum due to its suppression by testosterone (Coe, Margossian, Slayter & Sogn, 1981; Coe, 1982). Levels of acute-phase α_2 -macroglobulin also exhibit sex-dependent differences in rats and this appears to be due to suppression by oestradiol in the female (Bell, 1980). Human pregnancy-associated α_2 -glycoprotein (α_2 -PAG), considered to be the human analogue of PAMP-1 (Hau *et al.*, 1981c), is also found at higher levels in sera of non-pregnant females than in males, is strongly influenced by female hormones and is elevated in a variety of clinical conditions (see Horne, Thomson & Armstrong, 1982). Although described as an acute-phase reactant (Bohn, 1972), this has been disputed since the kinetics of the α_2 -PAG response after injury are dissimilar to the response of classical acute-phase reactants (Horne, Bohn, McLay, Wood & Thompson, 1975). α_1 -PAP synthesis during an acute-phase response may in fact be under complex hormonal control, exhibiting many features of the aforementioned proteins and influenced by testosterone, oestradiol and progesterone (G. T. Waites & S. C. Bell, unpublished observations).

The appearance of α_1 -PAP in serum of pregnant females and non-pregnant females in the acute phase of an inflammatory response leads to the question of whether the site of synthesis and mechanism of control is the same in these physiological conditions. During an acute-phase response tissue damage is thought to stimulate mononuclear phagocytes to release interleukin-1 which in turn induces synthesis of SAP and other acute-phase reactants by hepatocytes (Le, Muller & Mortensen, 1982; Sipe, Vogel, Szein, Skinner & Cohen, 1982). Allogeneic pregnancy in C57BL/10 females also induces SAP synthesis and serum levels are equivalent to those of injured animals; paternally inherited alloantigens on the feto-placental unit may be processed by phagocytes and stimulate hepatocytes by the same route (Waites *et al.*, 1983). However, although this route may also apply to synthesis of α_1 -PAP, the mechanism of induction must be different because α_1 -PAP occurs in allogeneic and syngeneic pregnancy, although an alternative tissue source may be involved. Although α_2 -macroglobulin is synthesized by the maternal liver during an acute-phase response in rats and is subject to hormonal regulation (Bell, 1980), during pregnancy it is synthesized by hormone-insensitive routes in the fetal liver and maternal decidual tissues (Bell, 1983). Multiple sites also appear to be involved in the synthesis of human α_2 -PAG, including the

liver (Stimson, Farquarson, Shepherd & Anderson, 1979), peripheral blood monocytes (Stimson, 1977; Horne, Thomson, Towler, MacMillan & Gibb, 1978) and IgA-producing plasma cells (Horne, Armstrong, Thomson & Thompson, 1983).

The detection of an apparently pregnancy-specific protein only in the serum of females subjected to injury raises many questions concerning its relationship to the well characterized murine acute-phase reactant SAP and similar proteins in other species. Studies on this relationship and on the site of synthesis, regulation and function during pregnancy and inflammation are now in progress.

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References

- Bell, S.C. (1979a) Synthesis of 'decidualization-associated protein' in tissues of rat uterus and placenta during pregnancy. *J. Reprod. Fert.* **56**, 255–262.
- Bell, S.C. (1979b) Immunohistochemical identity of 'decidualization-associated protein' and α_2 acute-phase macroglobulin in the pregnant rat. *J. Reprod. Immunol.* **1**, 193–206.
- Bell, S.C. (1980) Effects of oestradiol and progesterone on the concentration of α_2 -macroglobulin in the sera of injured male and female rats. *J. Endocr.* **86**, 189–191.
- Bell, S.C. (1983) Decidualization: regional differentiation and associated function. *Oxford Rev. Reprod. Biol.* **5**, 220–271.
- Benjamin, D.C. & Weimer, H.E. (1966) Synthesis of α_2 -AP (acute-phase) globulin of rat serum by the liver. *Nature, Lond.* **209**, 1032–1033.
- Bohn, H. (1972) Characterization of the pregnancy-associated glycoprotein as acute phase reactants. *Arch. Gynäk.* **213**, 54–72.
- Bosanquet, A.G., Chandler, A.M. & Gordon, A.H. (1976) Effects of injury of the concentration of α_1 -macroglobulin and α_2 -macroglobulin in the plasmas of male and female rats. *Experientia* **32**, 1348–1349.
- Coe, J.E. (1982) Female protein of the Syrian hamster: a homolog of C-reactive protein. *Ann. N. Y. Acad. Sci.* **389**, 299–305.
- Coe, J.E., Margossian, S.S., Slayter, H.S. & Sogn, J.A. (1981) Hamster female protein: a new pentraxin structurally and functionally similar to C-reactive protein and amyloid P-component. *J. exp. Med.* **153**, 977–991.
- Crowle, A.J. (1977) Templates for antiserum application in immunoelectrophoresis and two-dimensional electroimmunodiffusion. *J. Immunol. Methods* **14**, 197–200.
- Gordon, A.H. (1976) The α -macroglobulins of rat serum. *Biochem. J.* **159**, 643–650.
- Hau, J., Svendsen, P.E., Teisner, B. & Svehag, S.E. (1978) Studies of pregnancy-associated murine serum proteins. *J. Reprod. Fert.* **54**, 239–243.
- Hau, J., Westergaard, J.G., Svendsen, P., Bach, A. & Teisner, B. (1980) Comparison of the pregnancy-associated murine protein-2 and human pregnancy-specific β_1 -glycoprotein. *J. Reprod. Fert.* **60**, 115–119.
- Hau, J., Svendsen, P., Teisner, B. & Brandt, J. (1981a) Pregnancy-associated murine protein-3 (PAMP-3), characterization and serum levels. *Biol. Reprod.* **24**, 163–169.
- Hau, J., Svendsen, P., Teisner, B., Thomsen Pederson, G. & Kristiansen, B. (1981b) Correlation between fetal weight and maternal serum levels of murine α -fetoprotein and quantitation of four molecular forms. *Biol. Reprod.* **24**, 683–689.
- Hau, J., Westergaard, J.G., Svendsen, P., Bach, A. & Teisner, B. (1981c) Comparison of pregnancy-associated murine protein-1 and human pregnancy zone protein. *J. Reprod. Immunol.* **3**, 341–349.
- Heim, W.G. (1962) A rat serum protein related to reproduction, tissue synthesis and lactation. *Nature, Lond.* **193**, 491.
- Horne, C.H.W., Bohn, H., McLay, A.L.C., Wood, E.H. & Thompson, W.D. (1975) Serum pregnancy-associated α_2 -glycoprotein (α_2 -PAG)—an acute phase reactant? *Behring Instit. Mitt.* **58**, 50–53.
- Horne, C.H.W., Thomson, A.W., Towler, C.M., MacMillan, F.K. & Gibb, L.M. (1978) Relationship of pregnancy-associated alpha-2-glycoprotein (α_2 -PAG) to peripheral blood leukocytes. *Scand. J. Immunol.* **8**, 75–80.
- Horne, C.H.W., Thomson, A.W. & Armstrong, S.S. (1982) Clinical and immunological significance of pregnancy-associated α_2 -glycoprotein (α_2 -PAG). *Placenta, Suppl.* **4**, 33–50.
- Horne, C.H.W., Armstrong, S.S., Thomson, A.W. & Thompson, W.D. (1983) Detection of pregnancy-associated α_2 -glycoprotein (α_2 -PAG), an immunosuppressive agent, in IgA producing plasma cells and in body secretions. *Clin. exp. Immunol.* **51**, 631–638.
- Klopper, A. (1982) Immunology of human placental proteins. *Placenta, Suppl.* **4**.
- Koj, A. (1974) Acute phase reactants: their synthesis, turnover and biological significance. In *Structure and Function of Plasma Proteins*, Vol. 1, pp. 73–132. Ed. A. C. Allison. Plenum Press, London.

- Laurell, C.B. (1972) Electroimmunoassay. *Scand. J. clin. Lab. Invest.* **29**, Suppl. 125, 21–37.
- Le, P.T., Muller, M.T. & Mortensen, R.F. (1982) Acute phase reactants of mice. I. Isolation of serum amyloid P-component (SAP) and its induction by a monokine. *J. Immunol.* **129**, 665–672.
- Lin, T.M., Halbert, S.P. & Kiefer, D. (1974) Pregnancy-associated serum antigens in the rat and mouse. *Proc. Soc. exp. Biol. Med.* **145**, 62–66.
- Mortensen, R.F., Beisel, K., Zeleznik, N.J. & Le, P.T. (1983) Acute phase reactants of mice. II. Strain dependence of serum amyloid-P-component (SAP) levels and response to inflammation. *J. Immunol.* **130**, 885–889.
- Osmand, A.P., Friedenson, B., Gewurz, H., Painter, R.H., Hoffman, T. & Shelton, E. (1977) Characterization of C-reactive protein and the complement subcomponent Clt as homologous proteins displaying cyclic pentameric symmetry (pentraxins). *Proc. natn. Acad. Sci. U.S.A.* **74**, 739–743.
- Pepys, M.B. (1979) Isolation of serum amyloid P-component (protein SAP) in the mouse. *Immunology* **37**, 637–641.
- Pepys, M.B., Baltz, M., Gomer, K., Davies, A.J. & Doenhoff, M. (1979) Serum amyloid P-component: an acute phase reactant in the mouse. *Nature, Lond.* **278**, 259–261.
- Porstmann, T. & Schmechta, H. (1980) Isolation of a pregnancy-associated globulin (PAG) from the rat and preparation of a specific antiserum (anti-PAG). *Acta biol. med. germ.* **39**, 449–487.
- Sarcone, E.J. & Bohne, M. (1969) Synthesis of alpha₂ (acute phase) globulin by fetal and neonatal rat liver *in vitro*. *Proc. Soc. exp. Biol. Med.* **131**, 1454–1456.
- Sipe, J.D., Vogel, S.N., Sztein, M.B., Skinner, M. & Cohen, A.S. (1982) The role of interleukin-1 in acute phase serum amyloid A (SAA) and serum amyloid P (SAP) biosynthesis. *Ann. N.Y. Acad. Sci.* **389**, 137–149.
- Stimson, W.H. (1977) Identification of pregnancy-associated α_2 -macroglobulin on the surface of peripheral blood leucocyte populations. *Clin. exp. Immunol.* **28**, 445–452.
- Stimson, W.H., Farquarson, D.M., Shepherd, A. & Anderson, J.M. (1979) Studies on the synthesis of pregnancy-associated globulin by the liver, placenta and peripheral leucocyte populations. *J. clin. Lab. Immunol.* **2**, 235–238.
- Waites, G.T., Bell, A.M. & Bell, S.C. (1983) Acute phase serum proteins in syngeneic and allogeneic mouse pregnancy. *Clin. exp. Immunol.* **53**, 227–232.

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