of placental explants for 7 days it is still possible to demonstrate PSβG in the cytoplasm of the trophoblast (Figure 1a). That the trophoblast is actively synthesizing protein is clearly shown by the autoradiograph of the cultured placental explant (Figure 1c). Radioimmunoelectrophoresis of the supernatant and homogenized tissue from such explant cultures further showed labelling of the PSβG precipitin arc.

Ultrastructural studies of a series of first trimester placentae using the immunoperoxidase procedure outlined above, confirmed that PSβG is produced by the trophoblast and, furthermore, revealed that synthesis of this protein in vivo is localized within the syncytiotrophoblast. The presence of PSβG in the cisternae of the rough endoplasmic reticulum within the syncytiotrophoblast was revealed by the positive peroxidase staining observed in the test preparations (Figure 2a) which was not observed in the control preparations (Figure 2b). However, positive staining for PSβG was not only confined to the cisternae of the endoplasmic reticulum, but was observed also within vesicles beneath the apical plasma membrane, and also on the extracellular surface of the plasma membrane investing the microvilli which project from the apical surface of the syncytiotrophoblast.

Effect of 50 µg PSβG and α_2-PAG-containing fractions and hCG on tritiated thymidine incorporation by unstimulated and phytomitogen treated human lymphocytes

Treatment	Mean counts/min ± 1 SD	Stimulation index
untreated	39 ± 3	1
PSβG	42 ± 15	1.1
α_2-PAG	18 ± 4	0.5
hCG	56 ± 25	1.4
PHA	8,719 ± 262	224
PSβG + PHA	2,172 ± 253	57
α_2-PAG + PHA	1,283 ± 199	33
hCG + PHA	4,121 ± 665	106
Con A	2,105 ± 220	54
PSβG + Con A	1,919 ± 498	49
α_2-PAG + Con A	223 ± 33	8
hCG + Con A	1,526 ± 219	39

Results are expressed as the mean cpm of 4 cultures, then divided by the mean cpm of the untreated, unstimulated cells to give the 'stimulation index'.

The Table shows that a protein fraction containing PSβG but not containing the known immunosuppressive proteins human placental lactogen[5], chorionic gonadotrophin (hCG)[4] or pregnancy associated α_2-glycoprotein (α_2-PAG)[6,7] exerts a marked inhibitory effect on PHA stimulation of human lymphocytes but not on Con A stimulation. Only purified hCG and a semi-purified preparation of α_2-PAG inhibited both PHA and Con A stimulation of lymphocytes. Proof that PSβG is an immunosuppressive protein must await is purification.

Our studies clearly indicate that, in vivo, PSβG is a product of the syncytiotrophoblast but that, in vitro, the cytotrophoblast may be involved in its production. This latter observation is in keeping with the previous observation of Beck and Ewen[8] that in organ culture of placental tissue some proteins normally synthesized by the syncytiotrophoblast appear in the cytotrophoblast.

Although the functional role of pregnancy specific β_1-glycoprotein is as yet unknown, our own studies and those of Bohn[17] indicate it may have immunosuppressive properties. This finding may be of importance since PSβG can be detected on the syncytiotrophoblast cell membrane.

[10] G. A. Andres, K. G. Hsu and B. C. Seegal, in *Handbook of Experimental Immunology*, 2nd edn. (Ed. D. M. Weir; Blackwell, Oxford 1973), p. 341.
[11] P. K. Nakane and G. B. Pierce, jr., J. Cell Biol. *33*, 307 (1967).
[12] R. C. Graham and M. J. Karnovsky, J. Histochem. Cytochem. *14*, 291 (1966).
[13] J. G. Hirsch and M. E. Fedorko, J. Cell Biol. *38*, 615 (1968).
[14] M. L. Watson, J. Biophys. Biochem. Cytol. *4*, 475 (1958).
[15] E. S. Reynolds, J. Cell Biol. *17*, 208 (1963).
[16] A. Boyum, Scand. J. clin. Lab. Invest. *21*, Suppl. 97, 1 (1968).
[17] H. Bohn, R. Schmidtberger and H. Zilg, Blut, in press (1976).

C-Reactive Protein-like Precipitins in Lumpsucker (*Cyclopterus lumpus* L.) Gametes

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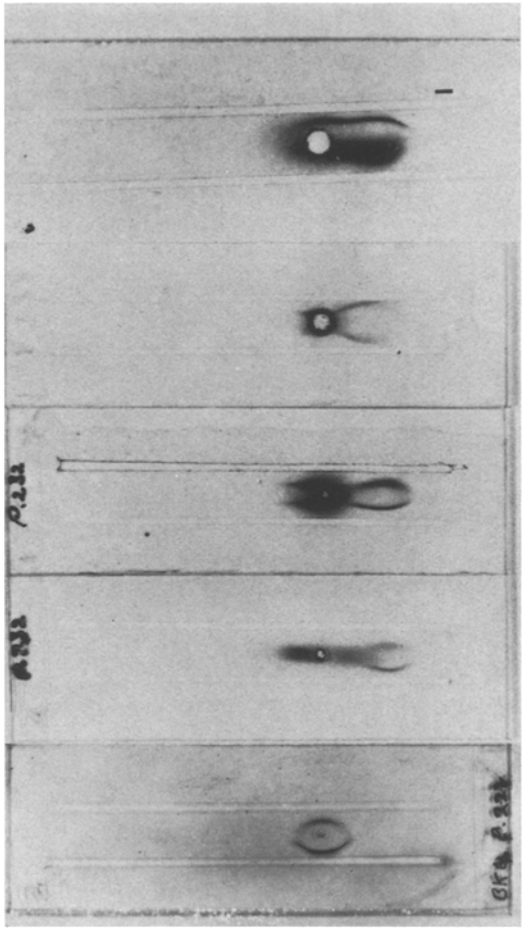
Summary. A precipitin to pneumococcal and fungal C-substance, found in both ova and spermatozoa from a marine teleost fish, has similarities to mammalian C-reactive protein (CRP). This is the first demonstration of a CRP-like protein in the gametes of any species and adds further interest to the as yet unknown physiological role of CRP in vertebrates.

C-reactive protein (CRP) is an acute phase or pathological protein found in the sera of many vertebrates following infection, burns, injury or carcinoma[1,2] although we recently found a CRP-like protein in the sera of apparently healthy plaice, *Pleuronectes platessa* L., and some other marine teleosts[3]. The plaice CRP-like protein, like that of higher vertebrates, is inhibited by very small amounts of phosphorylcholine, and calcium ions are necessary for precipitation to occur with pneumococcal and fungal C-substances[3]. We now report the discovery of a CRP-like precipitin in extracts from the ova and spermatozoa of the lumpsucker, *Cyclopterus lumpus* L., a teleost found in the North Sea and in the Arctic and American Atlantic. Precipitins with the same specificity have also been found in the serum, liver and spleen of this species but not in the urine or bile. Lumpsuckers were caught in salmon nets off the Aberdeen coast during their shoreward breeding migration between March and July, 1975. They were maintained in the aquarium in aerated seawater at 12–15 °C for periods of up to 1 month. Fish were

[1] W. S. Tillett and T. Francis, J. exp. Med. *52*, 561 (1930).
[2] A. Koj, in *Structure and Function of Plasma Proteins* (Ed. A. C. Allison; Plenum Press, London 1974), vol. 1, p. 73.
[3] B. A. Baldo and T. C. Fletcher, Nature, Lond. *246*, 145 (1973).

killed by a blow on the head and blood collected either directly from the heart or the caudal vein. Urine could usually be collected by syringe from the bladder when it was visibly distended. Liver, spleen and gonads were dissected from the body cavity but in some cases unfertilized egg masses were collected from the aquarium tanks where they had been laid overnight. All the organs were homogenized with the minimum amount of water, centrifuged at 50,000 *g* for 15 min and the supernatant fractions freeze-dried.

Extracts from lumpsucker ova and spermatoza were examined in Ouchterlony gel diffusion experiments with water soluble extracts from the nematode *Ascaris lumbricoides*, a number of fungi and purified pneumococcal C-substance. Clear precipitin lines were formed with the pneumococcal and *Ascaris* preparations and with extracts from the fungi which contain C-substance-like components[3,4]. The precipitin lines formed with each preparation were continuous and no spurring was visible. Precipitin lines formed between C-substance and gamete extracts, serum and liver extracts were also found to be confluent. These precipitin lines were soluble, within 60 to 90 min, in saline containing sodium citrate (5%), EDTA (0.1 *M*) or phosphorylcholine (0.01 *M*) and Ca^{2+} (0.01 *M*). In these properties at least, the lumpsucker precipitins are similar to mammalian CRP and the CRP-like proteins that we have detected in other teleosts[3,5,6]. The lumpsucker egg and serum precipitins eluted in the same position from Sephadex G-200 columns and were slightly retarded with respect to a mammalian 7S 1gG marker. The similarities between the egg and serum precipitins were not due to contamination of the eggs with body fluids since precipitins were present in eggs that had been laid and left in seawater for several hours.



Immunoelectrophoresis of lumpsucker tissues and serum. Cathode to the right. a) well: lumpsucker serum; upper trough; *E. floccosum* (2.5 mg/ml); lower trough; pneumococcal C-substance (1.8 mg/ml) b) well: lumpsucker sperm extract; upper and lower troughs: *E. floccosum* (10 mg/ml) c) well: lumpsucker egg extract; upper trough: *T. schoenleinii* (10 mg/ml); lower trough: *E. floccosum* (10 mg/ml) d) well: lumpsucker liver extract; upper and lower troughs: *E. floccosum* (5 mg/ml and 2.5 mg/ml) e) well: purified lumpsucker egg CRP-like protein (5 mg/ml); upper trough; *E. floccosum* (2.5 mg/ml); lower trough: rabbit antiserum to lumpsucker serum.

Lumpsucker preparations were also examined by immunoelectrophoresis in agar (1%) at pH 8.6 with fungal extracts and pneumococcal C-substance. Precipitin arcs with mobilities equivalent to that of a slow γ-globulin were seen with serum and with both male and female gamete extracts (Figure a–c). The egg CRP-like protein, purified by a method using L-α-lecithin[7], had β-mobility on immunoelectrophoresis (Figure e). This variation in electrophoretic mobility found for lumpsucker CRP-like precipitins is consistant with that described for mammalian CRP[8]. With some liver and egg preparations, precipitation in the α_2-region was also seen (Figures c and d). Unlike the γ-migrating arcs, these precipitated proteins did not dissolve in citrate, EDTA and phosphorylcholine solutions. The nature of these components is under investigation.

Stress and trauma are known to promote the production of mammalian CRP but no information is available on the factors which lead to the appearance of CRP-like proteins in fish. Except for the stress induced by the brief handling immediately prior to bleeding, many of the fish examined in this and our previous studies had not been subjected to conditions likely to induce the production or release of acute phase proteins. It remains important however, to clearly establish whether the fish CRP-like proteins are normal constituents of sera, liver and gametes of healthy fish or if they appear only after subacute infection, trauma and/or when the animals are removed from their natural environment. However, CRP-like proteins have been described in the sera of other normal fish and sensitive assay methods have shown it to be a normal constituent of plasma from healthy human adults[9,10]. We have found precipitins in every sample of lumpsucker eggs examined and in over 100 serum samples. Only one spent female appeared to lack serum precipitins and this was also the only fish found with a fungal infection.

Human CRP interacts with the complement system and has been implicated in a variety of biological processes including opsonization and the promotion of phagocytosis, lysis and agglutination of bacteria, mutagenesis and binding to cell surfaces[11,12]. The plaice CRP-like protein

[4] J. L. LONGBOTTOM and J. PEPYS, J. Path. Bact. *88*, 141 (1964).
[5] T. J. ABERNETHY and O. T. AVERY, J. exp. Med. *73*, 173 (1941).
[6] J. E. VOLANAKIS and M. H. KAPLAN, Proc. Soc. exp. Biol. Med. *136*, 612 (1971).
[7] Y. HOKAMA, R. TAM, W. HIRANO and L. KIMURA, Clin. chim. Acta *50*, 53 (1974).
[8] H. D. CROXATTO, E. T. NISHIMURA, K. YAMADA and Y. HOKAMA, J. Immun., *100*, 563 (1968).
[9] L. J. WATSON, Ph. D., University Arkansas, 1969, p. 214.
[10] C.-O. KINDMARK and J. I. THORELL, Scand. J. clin. Lab. Invest. Suppl. *124*, 49 (1972).
[11] L. T. PATTERSON, J. M. HARPER and R. D. HIGGINBOTHAM, J. Bact. *95*, 1375 (1968).
[12] R. F. MORTENSEN, A. P. OSMAND and H. GEWURZ, J. exp. Med. *141*, 821 (1975).

may be involved in immediate hypersensitivity responses[13]. Until now, CRP-like proteins have not been found in the ova or spermatozoa of any species. The presence of such a protein in lumpsucker gametes once again raises the question of the function, if any, of CRP in vertebrates. Agglutinins for some cells and in particular for erythrocytes, have been found in extracts of the ova of a number of lower vertebrates and invertebrates[14,15]. These agglutinins have been called 'protectins'[16], since they may act to protect the eggs from invasion by pathogens. Whether the lumpsucker CRP-like protein we have described is protective in any way for the fish gametes is at present unknown. The occurrence of the protein however, in both eggs and sperm adds further interest to the speculation and predictions of the biological role of CRP in vertebrates.

[13] T. C. Fletcher and B. A. Baldo, Science *185*, 360 (1974).
[14] B. A. Baldo, and G. Uhlenbruck, Vox Sang. *27*, 67 (1974).
[15] H. Kothbauer, Naturwiss. Rundsch. *28*, 73 (1975).
[16] O. Prokop, G. Uhlenbruck and W. Köhler, Dt. Gesundheitswes. *23*, 318 (1968).

Immunosuppressive Effect of a Mouse Placenta Fraction on H-2 Incompatible Split Heart Allografts[1]

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Summary. A soluble placenta fraction from mice (A.CA) mated with H-2 histoincompatible males (A/Sn) significantly prolonged the survival of heterotopic A/Sn heart transplants in A.CA recipients. No prolongation of A/Sn heart graft survival was obtained with the corresponding A.CA placenta fraction after A.CA × A.CA mating.

Pregnancy represents an exception to the rule of allograft rejection[3]. An intriguing biological question is how the fetus, possessing paternal histocompatibility antigens, is protected from the expected immunological destruction. Ample evidence of immune stimulation is found in the mother in the form of hypertrophy of regional lymph nodes and the production of sensitized lymphocytes and antibodies to fetal histocompatibility antigens[4]. There is, however, also substantial data in support of a partial impairment of the mother's immune response, particularly the cell-mediated, during pregnancy[5–8]. The mechanisms responsible for this impairment are poorly understood[4,9]. Most likely several mechanisms are responsible for the fetal exemption from the consequences of the immune response. The theory that uterus represents an immunologically privileged site has been rejected[4] and there are no special features of the maternal immune system that makes it tolerant to fetal tissue. There is, however, growing evidence that mucoproteins in the glycocalyx on trophoblasts can mask transplantation antigens and possibly interfere with the effect of sensitized lymphocytes[10,11]. Furthermore, trophoblasts have been shown to inhibit the spreading of macrophages in vitro[12]. Finally, maternal antibodies or antigen-antibody complexes may exert a blocking effect on the cell-mediated immune response[4] and various gestational hormones[11,13–16], and certain α-globulins[17] in maternal plasma have been reported to have immunosuppressive effect. We report here a significant immunosuppressive effect of a soluble mouse placenta fraction on the survival of split mouse heart grafts transplanted over a strong histoincompatibility (H-2^a→ H-2^t) barrier.

Materials and methods. A technique for heterotopic grafting of split allogeneic mouse hearts earlier described[18] was used. A.CA mice served as recipients and A/Sn baby mice (24–48 h age) as donors in the present study. The electrical activity of the transplants was monitored with a Tektronic 410 cardioscope. Placenta was excised from A.CA mice on days 16–18 after mating, homogenized in cold, sterile 0.25 *M* sucrose solution and ultrasonicated with a Branson B-12 sonifier equipped with a microtip. 5 ml samples were treated in glass tubes submerged in ice under constant stirring. Six 30 sec pulses of sonic energy (20 kHz 50 watt) were applied at 30 sec intervals. A.CA recipients were given 0.3 ml of this placenta homogenate i.p. on every 2nd day after transplantation, starting on day 0.

The placenta homogenate was centrifuged further at 20,000 *g* for 20 min at +4°C. The pellet was resuspended in 5 ml 0.25 *M* sucrose solution, the sonication procedure was repeated and after centrifugation as above the 2 supernatants were pooled and concentrated 6× by ultrafiltration. The final pellet was resuspended in 2.5 ml 0.25 *M* sucrose solution with the aid of gentle sonication.

[1] The work was supported by the Danish Medical Research Council (Project No. 512-3088).
[2] Present address: Department of Immunology, National Bacteriological Laboratory, S–105 21 Stockholm, Sweden.
[3] J. B. Solomon, *Foetal and Neonatal Immunology, Frontiers of Biology* (Eds. A. Neuberger and E. L. Tatum; North-Holland Publ. Co., Amsterdam 1971), vol. 20.
[4] A. E. Beer and R. E. Billingham, Scient. Am. *230*, 36 (1974).
[5] R. H. Andresen and C. W. Monroe, Am. J. Obstet. Gynec. *84*, 1096 (1962).
[6] R. H. Buckley, R. I. Schiff and D. B. Amos, J. Immun. *108*, 34 (1972).
[7] R. W. Heslop, P. L. Krohn and E. M. Sparrow, J. Endocr. *10*, 325 (1954).
[8] S. Kasakura, J. Immun. *107*, 1296 (1971).
[9] R. E. Billingham, Am. J. Obstet. Gynec. *111*, 469 (1971).
[10] G. A. Currie, W. Van Doorninck and K. D. Bagshawe, Nature, Lond. *219*, 192 (1968).
[11] B. J. Martin, S. S. Spicer and N. M. Smythe, Anat. Rec. *178*, 769 (1974).
[12] R. M. Fauve, B. Hevin, H. Jacob, J. A. Gaillard and F. Jacob, Proc. natn. Acad. Sci., USA *71*, 4052 (1974).
[13] S. F. Contractor and H. Davies, Nature New Biol. *243*, 284 (1973).
[14] J. S. Munroe, J. reticuloend. Soc. *9*, 361 (1971).
[15] R. Rembiesa, W. Ptak and M. Bubak, Experientia *30*, 82 (1974).
[16] J. S. Thompson, M. K. Crawford, R. W. Reilly and C. D. Severson, J. Immun. *98*, 331 (1967).
[17] A. H. Glasgow, S. R. Cooperband, K. Schmid, J. T. Parker, J. C. Occhino and J. A. Mannick, Transpl. Proc. *3*, 835 (1971).
[18] S.-E. Svehag and W. Schilling, Transplantation *15*, 345 (1973).