3, 5, 20, triacetate: $C_{26}H_{34}O_8$ (MS), parent ion; *m/e* 474, UV (MeOH) λ_{max}: 212, 290, ε = 16300, 220, IR(CH_2Cl_2): 1740, 1705, 1640 cm^{-1}. These values are in accordance with the values of ingenol triacetate reported elsewhere[8]. The fatty acid attached to ingenol was found to be palmitic acid by GLC[9].

Fraction ES-2 (0.42, 19.09%, ID_{50}: > 200 μg/ear) from its UV data; [UV (MeOH) λ_{max}: 212 and 290, ε = 12480, 280], appears to be another C-20 ingenol mono-ester. The presence of the ingenol was established as usual[8] and fatty acid was identified as capric acid by GLC[9].

As compared with the acetone extract of *E. ingens* latex, *E. serrata* latex exhibits nearly half the irritant and slightly less cocarcinogenic activity, considering the fact that at similar single dose *p* and relatively short period of treatment with acetone extract it produces multiple squamous cell papilloma (Table). However, no tumors developed in the control group of 20 mice painted only with 0.1 μM, 7, 12-dimethyl-benz (a)-anthracene.

Discussion. It is well established that in contact of silica gel tumor promoting ingenol-3-palmitate (ID_{50}: 0.08 μg/ear)[3] translocates the acid residue attached at C-3 to C-20 position of ingenol, producing thus, like other C-20 non-irritant, non-cocarcinogenic ingenol esters, ingenol-20-palmitate (ID_{50}: > 100 μg/ear)[3]. *E. serrata* latex, which is irritant on mice ear (ID_{50}: 1.5 μg/ear) before being chromatographed on silica gel column, gives, after column chromatography, only a non-irritant fraction similar to ES-2 (Ingenol-20-palmitate, ID_{50}: > 100 μg/ear) as seen by the similar effect. It can thus be concluded that one of the irritant substances present initially in *E. serrata* latex was ingenol-3-palmitate, due to which it shows skin irritant and tumor promoting activities, on mice ear and on back skin (Table) and ingenol-20-palmitate is an artefact[3] formed during the isolation procedure.

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Pregnancy Specific β_1-Glycoprotein – a Product of the Syncytiotrophoblast

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Summary. Pregnancy specific β_1-glycoprotein (PSβG) has been identified in vitro in trophoblast cultures and in vivo, using transmission electron microscopy, in the syncytiotrophoblast, PSβG may, like other pregnancy proteins, have immunosuppressive properties.

Pregnancy specific β_1-glycoprotein (SP_1 or PSβG) is a glycoprotein which was first identified in human placenta by Bohn[2]. Using an immunofluorescence technique he later showed that it could be detected in the cytoplasm of the syncytiotrophoblast[3].

We present here evidence that PSβG is synthesized by human syncytiotrophoblast. Preliminary in vitro studies were also undertaken to determine whether or not PSβG, like some other pregnancy proteins[4–7], had immunosuppressive properties.

Materials and methods. A monospecific rabbit antiserum to PSβG, provided by Bohn, was used in all studies. Explants of placentae (menstrual age 10–12 weeks) were cultured according to Beck and Ewen[8]. At day 7, cultures were fixed in 10% neutral buffered formalin and, using an enzyme-bridge immunoperoxidase technique[9] were examined for the presence of PBβG in the trophoblast cytoplasm. Evidence of active protein synthesis in the cultured placental explants was obtained by addition of [U-^{14}C] L-isoleucine and L-lysine (1 μCi of each; specific radioactivity approximately 300 μCi/μmol) to the medium on each of the first 5 days. After culture for 7 days, the pooled supernatant culture media and tissue were homogenized in the presence of 0.1% Triton X-100. Cell debris was sedimented by centrifugation and purified human IgG (Sigma, London) was added to the supernatant to a concentration of approximately 15 mg/100 ml. Protein was precipitated at 4 °C with a 50% saturation of ammonium sulphate, the protein precipitate sedimented, redissolved in phosphate buffered saline pH 7.4 and dialyzed against several changes of this buffer. The protein solution was concentrated by membrane ultrafiltration, mixed with an equal volume of term pregnancy serum and used for immunoelectrophoresis followed by autoradiography with Kodak DF-46 occlusal film. In addition, autoradiographs of the cultured chorionic villi were prepared using a liquid emulsion (Ilford G5).

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Ultrastructural localization of PSβG within formalin prefixed chorionic villi dissected from 12 week placentae was carried out by an indirect immunoperoxidase procedure with appropriate controls[10] using rabbit anti-PSβG and horseradish peroxidase conjugated goat anti-rabbit IgG[11]. The bound peroxidase conjugated antibody was revealed by the cytochemical procedure of GRAHAM and KARNOVSKY[12] and, following fixation in 0.2 *M* S-Collidine buffered osmium tetroxide, the specimens were block stained in uranyl acetate[13] and subsequently embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate[14] and lead citrate[15] prior to examination within an AEI EM6B transmission electron microscope.

To examine the effects of fractions containing PSβG, and pregnancy-associated α_2-glycoprotein (α_2-PAG) on cultured lymphocytes, protein fractions were prepared from term pregnancy plasma as described by BOHN[2]. Chorionic gonadotrophin (hCG) was obtained from Organon as 'Pregnyl'. All proteins were dialyzed against phosphate buffered saline pH 7.2 and sterilized by membrane filtration.

Human lymphocytes were isolated from heparinized venous blood by density gradient centrifugation[16]. Proteins were added to microcultures (2×10^5 cells in Eagle's medium supplemented with 10% heat-inactivated human AB serum) at the commencement of the culture period immediately before addition of either 0.5 μg phytohaemagglutinin (PHA-P, Wellcome) or 5.0 μg Concanavalin A (Con A, Sigma, London). At 48 h, [methyl-^{3}H]-thymidine (0.2 μCi; specific radioactivity 5 Ci/mmol) was added to each culture and the cells harvested, at 72 h, on to glass fibre discs using a multiple cell culture harvester (Skatron AS, Norway).

Results and discussion. Using the indirect immunoperoxidase technique, it has been shown that following culture

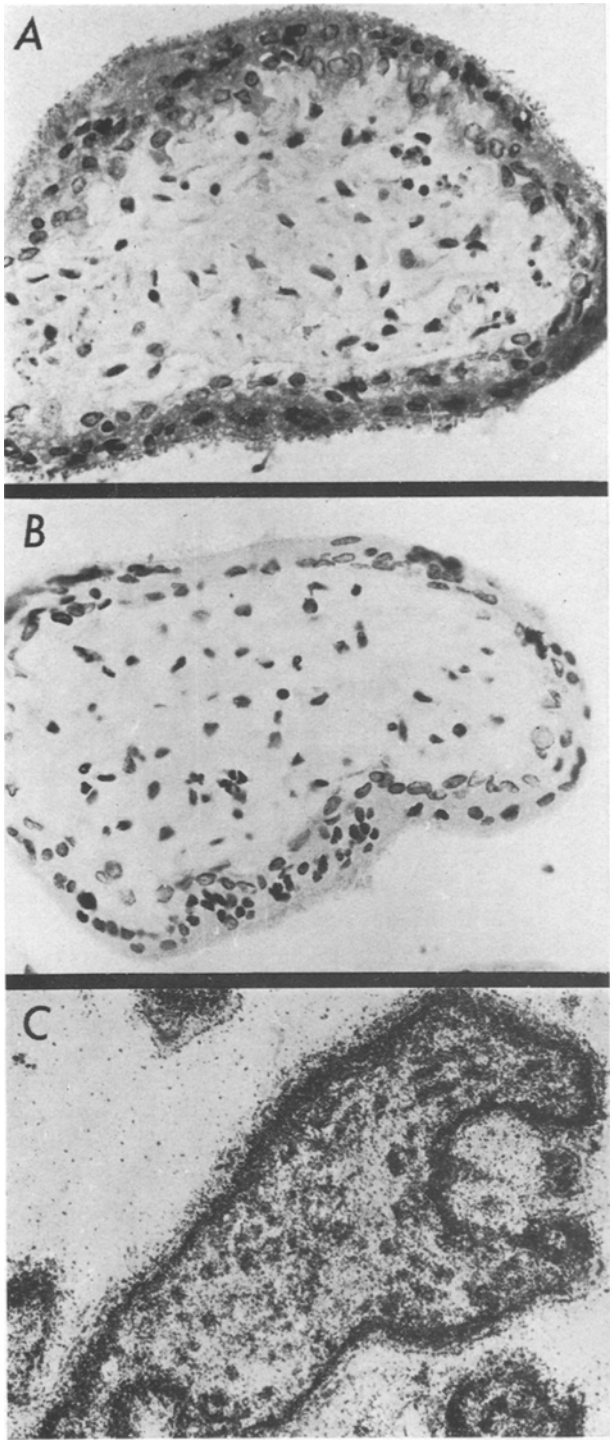

Fig. 1. First trimester placenta after 7 day culture, 5 days in the presence of [U-^{14}C]-L-lysine and [U-^{14}C]-L-isoleucine counterstained with haematoxylin a) indirect immunoperoxidase technique to demonstrate presence of PSβG. $\times$430. b) Immunoperoxidase negative control. $\times$430. c) Autoradiograph of cultured tissue. $\times$170.

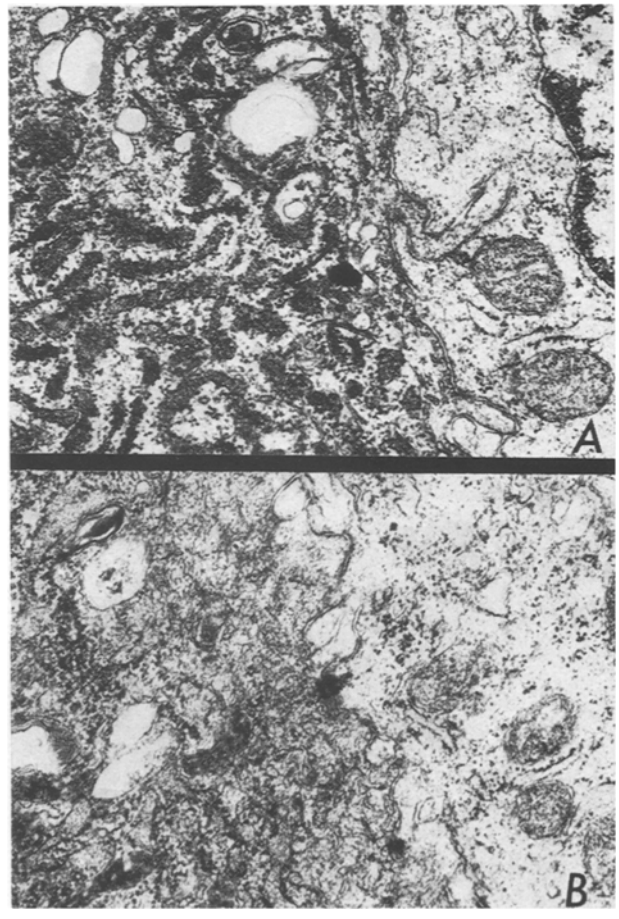

Fig. 2. a) Indirect immunoperoxidase technique to demonstrate the presence of PSβG in the rough endoplasmic reticulum of a syncytiotrophoblast. $\times$33,000. b) Negative control $\times$33,000.

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.

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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

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