cleavage of rabbit and pig ova can occur in the absence of the isthmus, although the incidence of polyspermic fertilization is increased following isthmic resection. The establishment of pregnancy following proximal isthmic reversal in the present report furnishes additional data regarding the ability of the oviduct to function despite radical alteration.

The availability of a functional, microsurgically modified animal oviduct model may furnish valuable insights into tubal function which could clearly have clinical relevance. The present availability of the operating microscope, microsurgical instruments and extremely fine suture plus the growing willingness of surgeons to embrace microsurgery as an accepted technique will enhance utilization of microsurgery as an import research tool and clinical technique.

Tumor Promoting Constituent of *Euphorbia serrata* L. Latex

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Summary. Euphorbia serrata latex has initially ingenol-3-palmitate, which by action of silica gel is converted to ingenol-20-palmitate. The former is responsible for the irritant and cocarcinogenic activity of the latex on mouse ear and on mice back skin.

The plants of Euphorbiaceae family are known to be toxic and poisonous. They excude skin irritant and inflammatory, white milky latex, when the stems or leaves are cut or broken[1]. Human and animal sufferings due to the accidental use of these plants are well documented[1]. The irritant latices of these plants have been shown to contain esters of polycyclic, polyfunctional diterpenes, such as phorbol[2], ingenol[3] and their various derivatives[4,5], which exhibit cocarcinogenic activity on mice back skin[3,6]. In the present communication we wish to report on the irritant and cocarcinogenic constituent of the latex of *Euphorbia serrata*.

Materials and methods. The latex of *E. serrata* was collected near Tabriz in summer 1975 and was stored in methanol. This methanolic latex preparation was filtered and the residue remaining was washed with acetone repeatedly, till no irritation was noted on mice ear. Combined acetone extract on evaporation under reduced pressure gave a pale yellowish amorphous mass, which was used for the separation of irritant factors by column and TLC and for biological assays. The irritation dose 50 (ID_{50}) was determined following the standard method on the ears of NMRI-mice[7]. Cocarcinogenic activity was determined using 0.1 μM, 7,12-dimethyl benz (a)-anthracene (DMBA) as initiator for 2 groups of 20 NMRI-mice, each (male, female, 1:1), in standard assay on mice back skin[7]. One group of 20 mice received on back skin an acetone extract of *E. serrata* latex, for 16 weeks, twice weekly (32 dose p); another group of 20 mice was painted only with 0.1 μM, 7, 12-dimethyl-benz(a)-anthracene, on back skin, and used as controls. Cocarcinogenic activity was expressed as tumor rate and average tumor yield, at 12 and 16 weeks, after initiation in case of *E. serrata* latex. The mice were kept on standard laboratory diet and were given water ad libitum. All tumors 1 mm in diameter or more were recorded and diagnosed histologically (Table).

Results. Fraction ES-1 (1.4 g, 63%, ID_{50}: > 100 μg/ear) on further purification by preparative TLC gave a semisolid mass, responding positively for diterpenes. From its physical data and irritation value, it was found to be ingenol-20-palmitate, which was isolated from the seed oil of *E. lathyris* and from the latex of *E. ingens*[3]. Parent diterpene ingenol of this ester was isolated by alkaline hydrolysis followed by acetylation, as ingenol,

Irritant and cocarcinogenic activities of the acetone extract of the latex of *Euphorbia serrata* as compared to that of an acetone extract of the latex of *Euphorbia ingens*, assays on the mouse ear and on the back skin of mice respectively

Acetone extract from the latex of	Irritant[a] activity ID_{50} (μg/ear)	Cocarcinogenic activity[b,c]						
		Single dose *p* (μg/ear)	Tumor rate (tumor bear/surv) after weeks		Average tumor yield (Tumor/surv) after weeks		Histologic diagnoses tumors in treated area	
			12	> 12	12	> 12	Total/mice investigated histol.	Type of tumors in total
E. ingens	0.74[d]	5000	0/26	1/22[f]	0/26	1/22[f]	6/3	1 Fibrosarcoma
E. serrata	1.5[e]	5000	0/17	4/10[g]	0/17	5/10[g]	10/4	Multiple squamous cell papilloma

[a] Standard assay: SD δ = 1.3[d] and 1.3[e]; [b] Standard assay: start, 28 male, female (1:1) NMRI mice, initiator: 0.1 μM, 7,12-dimethyl-benz-(a)-anthracene, promotor: *p*, twice weekly, after 12 and 24 weeks, 24 and 48 applications respectively for *E. ingens*, *E. serrata*, start: 20 male, female (1:1) NMRI mice, initiator: 0.1 μM, 7,12-dimethyl-benz-(a)-anthracene, promotor dose *p*, twice weekly, after 12 and 16 weeks, and 24 and 32 applications respectively; [c] *E. ingens* experiment was stopped at 48 weeks and *E. serrata* ended at 16 weeks due to the scarcity of the material; [f] 24 weeks; [g] 16 weeks.

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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