Nuclear bodies were occasionally found within the nuclei of some tumor cells. Similar structures have been found in normal, diseased tissues and in tumors[10–13]. Morphologically, the nuclear bodies observed in this study correspond to Type I and Type II nuclear bodies classified by BOUTEILLE et al.[10]. WEBER et al.[14] believe that the nuclear body is a frequent internuclear inclusion which seems to be related to cellular hyperactivity. This hyperactivity may be physiological, hormonal, drug-induced, viral or tumoral. The appearance of nuclear bodies in Walker tumor cells is likely to be related to hyperactivity.

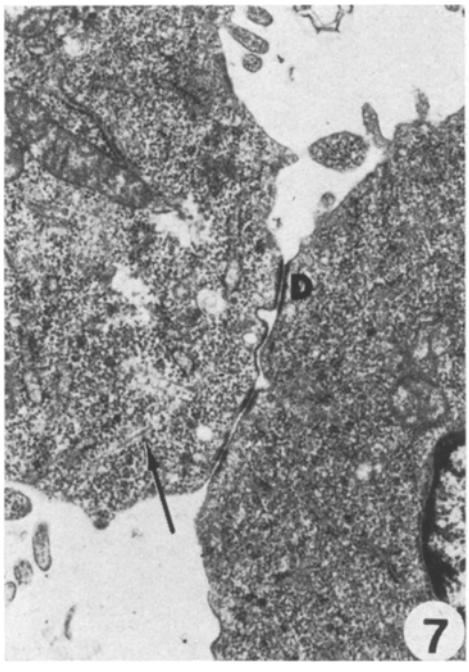

Fig. 7. Walker 256 tumor cells showing desmosomes (D) between the adjacent cell membranes. Arrow indicates tubular structure within the cytoplasm. × 18,900.

Cytofilaments have been observed in many fibrogenic and non-fibrogenic cells as well as epidermal cancer cells[15,16]. In these observations, bundles of cytofilaments were always seen close to the nucleus. However, in the present investigation, cytofilaments were found to be either perinuclearly located or at the periphery. They were also present in cytoplasmic projections. Some authors believe that they play a role in cytoplasmic viscosity or act as a cytoskeleton[15,17]. MALECH and LENTZ[16] suggested that the presence of cytofilaments in malignant cells may be correlated with the motile, invasive properties of these cells. The Walker 256 tumor cells do exhibit ameoboid movement and invasiveness (unpublished results). Thus the author is tempted to agree with MALECH and LENTZ.

The presence of desmosomes is usually regarded as a feature of differentiated cells. They have been observed in several types of tumor cells such as Wilm's tumor[18] and mouse sarcoma cells[19]. The presence of desmosomes in Walker 256 cells has not been reported before. We previously discounted as a Walker 256 cell, any showing desmosomes, but there is clear proof from examining solid Walker 256 tumors that they are formed.

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Pregnancy Following Segmental Isthmic Reversal of the Rabbit Oviduct[1]

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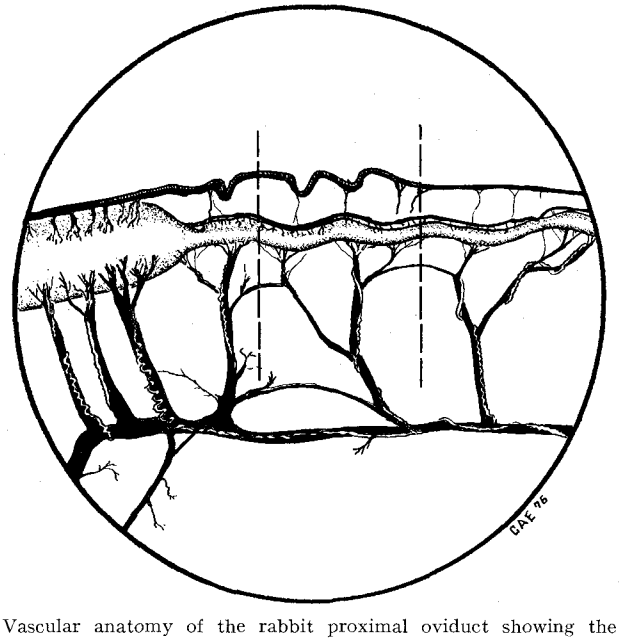
Summary. Microsurgical reversal of a segment of rabbit proximal tubal isthmus has been followed by normal pregnancy in the first two animals to undergo the procedure. Establishment of pregnancy despite radical modification of the oviduct furnishes the opportunity to gain new insights into the mechanisms controlling tubal ovum transport and emphasizes the evolving feasibility and importance of tuboplastic microsurgery both as a research tool and clinical procedure.

Transport of ova through the oviduct into the uterus is a complex, discontinuous process characterized by a pause of varying, species-specific duration at the ampullary-isthmic junction (AIJ) with or without an additional pause at the tubo-uterine junction (TUJ) before final entrance of ova into the uterus[4]. In order to investigate the hypothesis that the tubal isthmus and its junctions constitute the primary physiologic mechanism controlling tubal transport and entrance of ova into the uterus, microsurgical modification of the rabbit oviduct isthmus has been employed. In the course of preliminary investigations, reversal of a segment of proximal tubal isthmus with end-to-end reanastomosis has been followed by pregnancy despite an earlier report that segmental reversal results in infertility[5]. A future report will examine the physiologic consequences of tubal isthmic reversal with particular emphasis upon electrophysiologic, contractile and ciliary activity following completion of this procedure in additional subjects. We describe here in detail the microsurgical technique being used and the resulting pregnancies obtained following our initial isthmic reversal procedures.

Materials and methods. The initial subjects were virgin female New Zealand White rabbits weighing 3300 g each. Following i.v. sodium pentabarbitol anesthesia, a mid-ventral laparotomy was performed and the reproductive tract gently exteriorized and examined. A 1 cm segment of proximal isthmus with a well defined 'vascular tree' composed of caudal branches of the utero-ovarian artery and vein was selected for reversal (see Figure). Peritubular ligatures were placed above and below the area of isthmus to be reversed by piercing the mesosalpinx and mesotubarium superius, drawing through a length of elastic cord (0.5 mm diameter) and pinching the ends together sufficiently tight against the tube with bulldog clamps to occlude the tubal vascular arcade but not the tubal lumen.

Using Vannas scissors, the oviduct was completely severed perpendicular to its long axis several millimeters inside the tubal ligatures. The dissection was extended 1.5 cm into the mesosalpinx. A segment of tubal isthmus approximately 1 cm long with an intact pedicle of mesosalpinx was thus created. A small stab wound was made in the uterine horn and a length of polyethylene tubing (external diameter 0.60 mm) was introduced into the uterine lumen. The segment of isthmus was rotated 180° about its pedicle and the cannula introduced as far as the AIJ. A single 4–0 silk suture anchored the stent at its uterine insertion. Throughout the procedure the tube and adjacent tissue was kept moist with normal saline. Hemostasis of unligated blood vessels was achieved by constant irrigation with a 1% solution of 1:1000 adrenaline in normal saline.

Using the indwelling polyethylene stent for appositional control and a Zeiss OPMI-6 binocular operating microscope for visualization, end-to-end anastomosis was accomplished using 10–0 monofilament nylon suture mounted on a 130 μm needle. 5 sutures were placed around the perimeter of the tube through the serosa and myosalpinx excluding the mucosa[6]. Both anastomosis sites were reperitonealized and the stent removed. The defects in the mesosalpinx were repaired with interrupted 10–0 nylon sutures. The contralateral oviduct served as a control. The abdomen was closed in 2 layers and an i.m. injection of penicillin. 1,000,000 units and streptomycin, 100 mg, was administered. Following a 2 week recovery period the animals were mated and given an i.v. injection of 100 IU HCG to insure ovulation.



Vascular anatomy of the rabbit proximal oviduct showing the location of the transection incisions (broken lines) used to create a segment of isthmus supported by a pedicle of mesosalpinx. Note that although the tubal vascular arcade is severed, branches of the utero-ovarian vessels within the pedicle remain intact and continue to perfuse the segment.

Results. The postoperative course was unremarkable. Both animals returned to estrus and mated without difficulty. 15 days after mating laparotomy was performed. In the first rabbit there was total absence of adhesions and lack of palpaple thickening at either anastomosis site. The normal uterine-tubal-ovarian anatomic relationship was undisturbed. Apart from a slight obliteration of the mesotubarium superius along the length of the reversed segment and the presence of sutures, the operated oviduct was not grossly distinguishable from its contralateral unoperated counterpart. Results obtained in the second rabbit were similar with the exception of the presence of a single thin adhesion between a loop of small intestine and the pedicle of the reversed segment.

The ovary on the unoperated side of the first rabbit contained 2 corpora lutea. A single normal size for date embryo was located in the uterine horn midway between the TUJ and uterine cervix. The ovary on the operated side contained 7 corpora lutea. 6 normal size, evenly spaced embryos were present in the uterine horn. In the second rabbit the control ovary contained 6 corpora lutea. A single, normal size embryo was located in the distal portion of the uterine horn. The ovary on the operated side contained 8 corpora lutea. 3 normal size, evenly spaced embryos were present in the uterine horn.

Discussion. We believe these to be the first reported pregnancies following reversal of a segment of the oviduct. Earlier attempts by Kuo and Lim[5] to reverse segments of rabbit and pig oviducts and obtain pregnancy were unsuccessful. This led to the conclusion that such procedures were incompatible with pregnancy due primarily to reversal of ciliary action in the reversed segment which prevented downward passage of ova as well as upward passage of spermatozoa although in fact the direction of the ciliary beat was not determined. In light of our results it seems more probable that failure to obtain pregnancy stemmed from the small calibre of the oviducts and the extreme difficulty of achieving successful end-to-end anastomosis without adequate equipment – limitations acknowledged by Kuo and Lim.

More recent attempts to investigate the role of the isthmus and its junctions using microsurgical techniques have furnished important insights into tubal functions. David et al[7], have observed that migration of spermatozoa, fertilization, and ovum transport can occur in the rabbit following excision of the TUJ. Hunter and Leglise[8,9] concluded that normal fertilization and

[1] This study was partly supported by an N.I.H. Institutional Research Grant.
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[3] Supported by the Rockefeller Foundation.
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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.