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Morphology of human and experimental hydrosalpinges: a comparative study

J. A. M. OTUBU, R. M. L. WINSTON, M. WINEMAN AND T. RYDER
Institute of Obstetrics and Gynaecology, Hammersmith Hospital and Queen Charlotte Hospital, London, U.K.

Summary

Infection, and possibly raised intra-luminal pressure, may be responsible for the morphological changes and associated infertility that occur with hydrosalpinx formation. Biopsies from human hydrosalpinges were studied by light microscopy and scanning and transmission electron microscopy. Loss of mucosal folds, atrophied epithelium and varying degrees of deciliation were the main features found. Mechanical hydrosalpinges were produced in rabbits by double clip application (duration of clip application varied from 8 to 52 weeks). Morphological study of animal hydrosalpinges showed partial or complete loss of mucosal folds, atrophied epithelium and varying degrees of deciliation. The severity of morphological changes was related to duration of clip application. It is concluded that raised intra-luminal pressure plays a role in the morphological changes seen in human hydrosalpinges and infertility in women after anatomically successful salpingostomy. The rabbit would appear to provide a suitable model for the study of human hydrosalpinges. The significance of these findings is discussed in relation to the clinical management of hydrosalpinges.

Résumé

Infection, et peut-être bien une tension intraluminale élevée, sont les causes des modifications morphologiques et l'infertilité parallèle qui se produit avec la formation hydrosalpinx. Les biopsies venant des hydrosalpinges humaines furent étudiés par la microscopie éclairée, par un appareil sondeur, et par une microscopie de la transmission de'électron. La perte des plis muqueux, l'épithelium atrophié, et la déciliation à degré varié, voila les traits principaux relevés. En utilisant les lapins on a produit les hydrosalpinges mécaniques en faisant l'application de double agrafe (durée de l'application d'agrafe était entre 8 et 52 semaines). L'étude morphologique des hydrosalpinges animales révelèrent une perte partielle ou complète des plis muqueux, l'épithelium atrophié et des degrés variés de la déciliation. La sévérité des transformations morphologiques était en rapport avec la duration de l'application d'agrafe. On est arrivé à la conclusion qu'une tension intraluminale élevée joue un rôle dans les transformations morphologiques aperçues dans les hydrosalpinges humaines et dans l'infertilité chez les femmes après une salpingostomie réussie anatomiquement. Le lapin fournit un modèle approprié pour l'étude des hydrosalpinges humaines. L'importance de ces découvertes est traitée à propos de la maîtrise médicale des hydrosalpinges.

Correspondence: Dr J. A. M. Otubu, Department of Obstetrics and Gynaecology, Faculty of Medical Sciences, University of Jos, Jos, Nigeria.

Introduction

Marked variability in morphological findings is a feature recognized in the early studies of human hydrosalpinges (Frankl, 1930). Woodruff and Pauerstein (1969) described two types of hydrosalpinges; hydrosalpinx follicularis and hydrosalpinx simplex. They reported that the epithelium in hydrosalpinx simplex is compressed, varying from low cuboidal to extremely flattened mesothelial type. Normal epithelium with cilia preserved is present in areas where mucosal folds are also preserved. David, Garcia and Czernobilsky (1969), in a

light microscopic study, also noted that the epithelial lining is somewhat impaired, although in scattered areas it seemed to them to appear normal. They reported that the levels of protein and bicarbonate in the tubal fluid of hydrosalpinges is very low. Patek and Nilsson (1977) reported their findings from light microscopic study of five human hydrosalpinges. The epithelium was compressed to one-sixth of its usual height; however, according to them, scanning electron microscopy (SEM) did not show any deciliation despite a survey of a large area. Vasquez *et al.* (1979) obtained biopsies at the time of salpingostomy from thirty patients with hydrosalpinges. They found that flattening and loss of mucosal folds, or an altered fold relationship, is always present. They reported that varying degrees of marked deciliation is usual. Bernhardt-Huth, Schlosser and Frantzen (1980), using an animal model to study the most appropriate site for tubal sterilization, applied clips at the ampullary–isthmic junction (AIJ) and the fimbrial end. This resulted in hydrosalpinx in the ampulla but not in the isthmus. The duration of clip application varied from 1 to 12 weeks. They showed marked flattening of mucosal folds with cuboidal type epithelium. They also found progressive deciliation, which was maximal at 12 weeks after clip application. They concluded that the isthmus was a most appropriate site for sterilization if reversibility was a consideration, since the degree of morphological changes that occurred at this site were slight compared with those seen in the ampulla. Vasquez *et al.* (1981) studied morphological changes in mechanical hydrosalpinges, produced in a similar study. They reported progressive atrophy of mucosal folds, flattened epithelium and progressive deciliation maximal at 16 weeks after clip application. Halbert and Patton (1981) induced hydrosalpinges in rabbits and monkeys by ligating the ovarian end of the oviduct. Although they reported a general flattening of mucosal folds, the flattened epithelial surface showed normal ciliation after 2–5 months of ligation. Patton and Halbert (1981), in a comparable study, obtained similar morphological results when the oviduct had been ligated for 28–52 weeks. In both these studies, the physiology of ovum transport was shown to be unimpaired. The discrepancies between the results of human and animal studies summarized above remain unexplained. Irrespective of these differences, general flattening of the mucosal folds has been widely reported in both animal and human hydrosalpinges; this suggests that raised intraluminal pressure due to accumulated fluid may be a factor in the changes described in human hydrosalpinges. The aim of this study is to compare the morphology of human and experimentally produced hydrosalpinges (clip application varying from 8 to 52 weeks) in rabbits.

Materials and methods

Ten ampullary biopsies were obtained from human hydrosalpinges for SEM. Experimental hydrosalpinges were induced in New Zealand white rabbits by double clip application. One clip was applied at the fimbrial end and the other at the AIJ. The duration of clip application varied from 8 to 52 weeks. The hydrosalpinges were induced on the right oviducts while the left oviduct served as control.

Twenty ampullary biopsies were obtained from mechanical hydrosalpinges in rabbits, for SEM. In two animals, intra-arterial perfusions with 10% buffered formalin were used to fix the whole oviduct and serial sections were studied by light microscopy.

Ten ampullary biopsies from rabbits and five from humans were obtained for transmission electron microscopy (TEM). Biospies for SEM were immediately fixed in 10% buffered formalin. They were later processed through graded alcohols and then critical-point dried in carbon dioxide. An Emscope sputter coater was used to coat specimens. The thickness of gold was kept to the minimum required to prevent charging. Scanning was done at magnifications between ×50 and ×2k in a Cambridge Instrument stereoscan 5600 and pictures were taken.

Biopsies for TEM were immediately fixed in 3% glutaraldehyde in phosphate buffer for 1 to 4 h at 4°C. They were washed and then stored in phosphate buffer. They were then postfixed in 1% Millonigs buffered osmium tetroxide and processed through graded alcohols. They were then embedded and polymerized. Semi-thin sections were stained with 1% toluidine blue in 1% borax and viewed with a phase contrast microscope. Ultra-thin sections cut with a diamond knife, stained with 1% aqueous uranyl

acetate and then Reynolds lead nitrate, were examined in an AEI SOI. Electron microscope and representative pictures were taken.

Results

Light microscopy

Human. The serosa showed evidence of perisalpingitis in some areas. The inflammatory cells consisted mainly of lymphocytes and plasma cells. Polymorphonuclear leucocytes were rarely present. Most of the blood vessels in the subserosa were thick; others were thin-walled and dilated. The smooth muscle showed remarkable thickening. This thick layer showed variable pathologic changes. Some areas showed normal smooth muscle cells while others showed atrophy, fibrosis and/or oedema. No hypertrophy was demonstrable. The walls of blood vessels in this area showed marked hypertrophy. In two specimens, abscess cavities were seen within the smooth muscle layer with massive infiltration of polymorphs and lymphocytes. Apart from these two specimens, others showed few inflammatory infiltrates. Most of these were plasma cells (more than 20%) and a few lymphocytes and histiocytes. The submucosa was considerably reduced in thickness and the inflammatory infiltrate was predominantly of plasma cells. The mucosa showed atrophy and marked flattening of folds. A few plical remnants could be identified.

The epithelium was flattened and varied from cuboidal to mesothelial in some areas. There was evidence of pseudo-stratification in some areas. Semi-thin sections examined under phase contrast microscope also showed atrophied epithelium with cuboidal cells and varying degrees of deciliation.

Animal model. Figure 1 shows a mechanical hydrosalpinx produced in a rabbit. The serosa of mechanical hydrosalpinx was normal but lined by mesothelial cells. There was no evidence of perisalpingitis. Subserosal blood vessels were thin-walled. Circular and longitudinal muscle layers could not be identified in the muscularis. Although individual muscle cells were flattened and elongated, there was hypertrophy of the muscle layers. These changes were marked in hydrosalpinges produced after clip application for 8 weeks. Increased collagen deposit was noted. In hydrosalpinges, after clip application of 18–52 weeks, evidence of atrophy in the muscle cells was noticed at irregular intervals. The submucosa was reduced in thickness. Progressive atrophy of mucosal folds and flattening of the epithelium were noticed. (Figs 2, 3 and 4). Cells became cuboidal and pale while early pseudo-stratification was noticed. Cilia were preserved in some areas.

Scanning electron microscopy

Human. The mucosal folds were flattened and in some areas were completely lost. Where



Fig. 1. Mechanically produced hydrosalpinx in the rabbit. Duration of clip application is 24 weeks.



Fig. 2. Transverse section through normal (control) rabbit ampulla, demonstrates complex mucosal folds and thin musculature. H&E stain (×26).

remnants of folds existed, they were attenuated and unduly separated (Fig. 5). In other areas, folds were agglutinated and bridges between folds appeared to be formed. Desquamation of epithelium was common and its loss in places left gaps in the epithelium (Fig. 5). Ciliated cells were preserved in some areas but ciliation was patchy. Deciliation, usually more severe in between folds, was the main feature. The percentage of the mucosal surface that was ciliated was less than 30%. Non-ciliated cells showed varied shapes, being flattened, stretched, hyperplastic and swollen, or rounded and polygonal. When these combinations occurred in the same field, there was a 'cobble-stone' appearance. Microvilli were retained to a varying degree. Both deciliated cells and secretory cells showed numerous holes and depressions on their surfaces (Fig. 6).

Animal model. Complete loss of mucosal folds was common. Where a few folds were left, they were flattened and thinned. Polyp-like folds formed in some areas (Fig. 8). Deciliation was a marked feature. When remnants of fold were left, deciliation was more marked in areas between folds. Surface that was deciliated was 30.3 ± 1.4% for hydrosalpinges whose duration of clip application was 8 weeks whereas it was 9.9 ± 9.60% for hydrosalpinges whose duration of clip application was 24 weeks. When these measurements were individually compared with the ampulla from normal rabbit control oviducts (71.1 ± 10.6%), the difference was

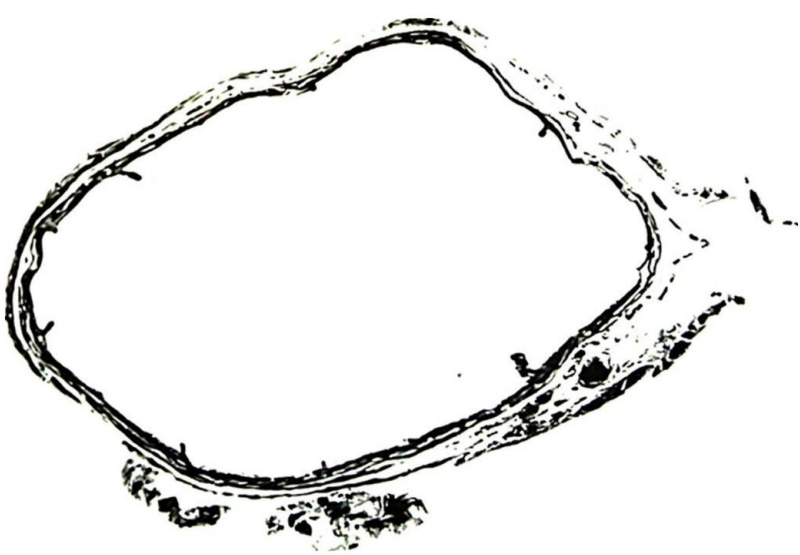


Fig. 3. Transverse section through mechanical hydrosalpinx at an equivalent site in the oviduct to Fig. 2. Fixation by intra-arterial perfusion of formalin. The mucosal folds are extremely atrophied and the epithelium is flattened. H&E stain (×12).

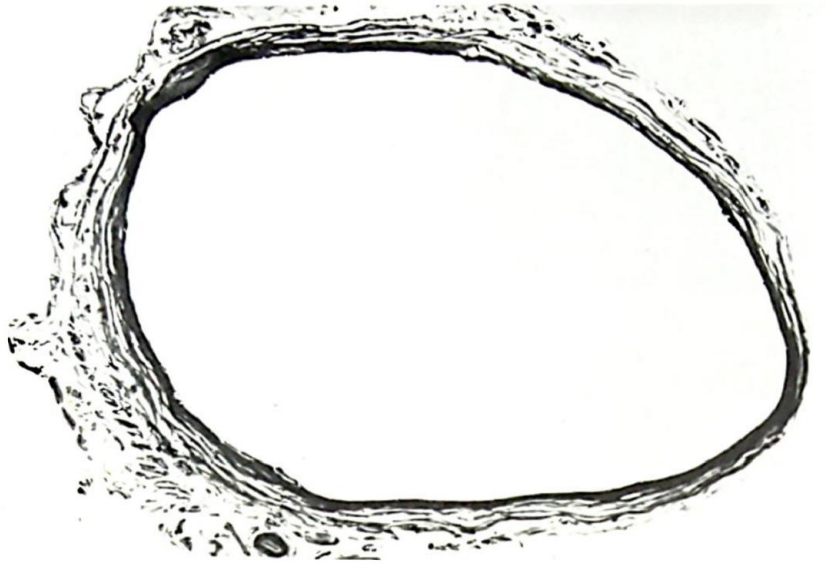


Fig. 4. Transverse section through mechanical hydrosalpinx. Fixation as in Fig. 2. This section shows total loss of mucosal folds and atrophy of the epithelium. H&E stain (×26).

statistically significant ($P < 0.001$). The non-ciliated, or secretory cells, and deciliated cells demonstrated varied shapes, flattened, stretched, polygonal, round, or swollen and hyperplastic. The cells also showed varying loss of microvilli, with an extreme loss of microvilli giving the cells a partially bald appearance. Depressions or holes on the surface were rarely seen. All these morphological changes were remarkably similar to those seen in specimens from human.

Transmission electron microscopy

Human. The epithelium was considerably flattened. The cells were cuboidal in shape and

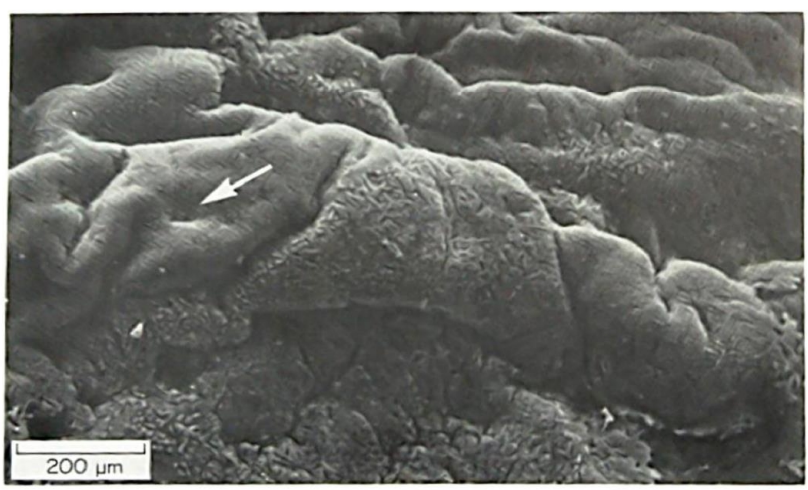


Fig. 5. Scanning electron microscopy: endosalpinx. Human hydrosalpinx, demonstrates attenuated folds. There are hardly any cilia in certain areas (black arrow). There is marked desquamation in some areas with total loss of epithelium (◀). Secretory phase (×75).

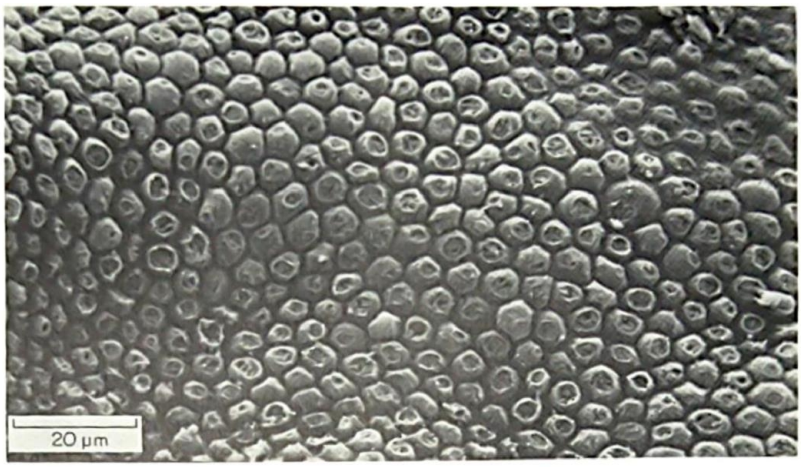


Fig. 6. Scanning electron microscopy: higher power view of area arrowed in Fig. 5. Demonstrates complete deciliation, cells are round and fairly uniform in shape. Numerous cells show holes or depressions in their surface. Secretory phase (×1500).

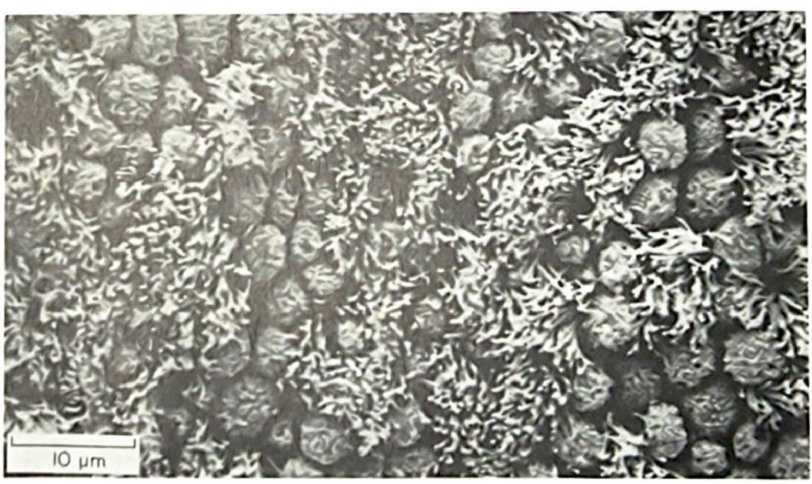


Fig. 7. Scanning electron microscopy: normal rabbit endosalpinx (×1500).

showed signs of degeneration. There was an increased nuclear–cytoplasmic ratio. The nuclear membrane was irregular in some parts and completely rounded in others. Occasionally prominent nucleoli could be seen. The cytoplasm was completely devoid of cell organelles and contained intracytoplasmic vacuoles (Fig. 9). These vacuoles looked like empty secretory granules in places. There was total deciliation in some places. The submucosa had completely disappeared in some areas. The smooth muscle layer showed areas of atrophy and fibrosis. Excessive collagen fibrils were noted.

Animal model. In early hydrosalpinges (duration of clip application of 8 weeks), the epithelium showed typical secretory protrusions and contained two types of secretory granules; (i) numerous electron dense circular granules and (ii) a few electron-luscent granules (Fig. 10). Cilia were preserved in some areas. In ciliated cells, the supra-nuclear area showed

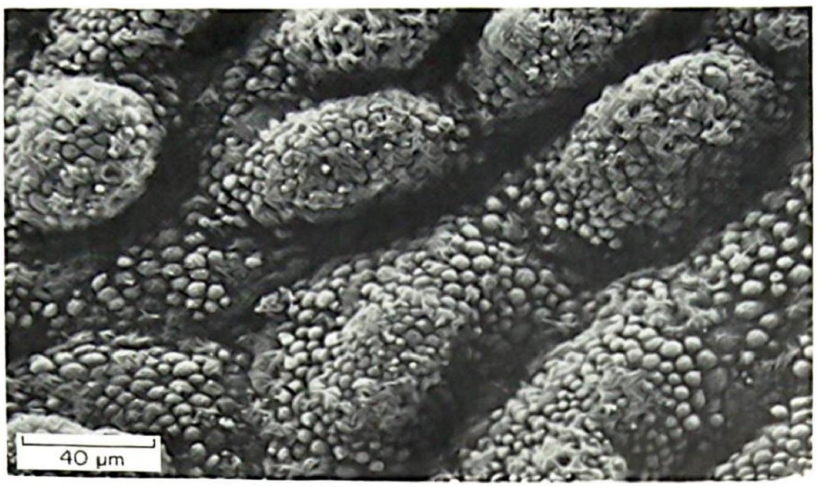


Fig. 8. Scanning electron microscopy: endosalpinx. Mechanical hydrosalpinx duration of clip application is 8 weeks. Some folds have become rather polypoid (×375).

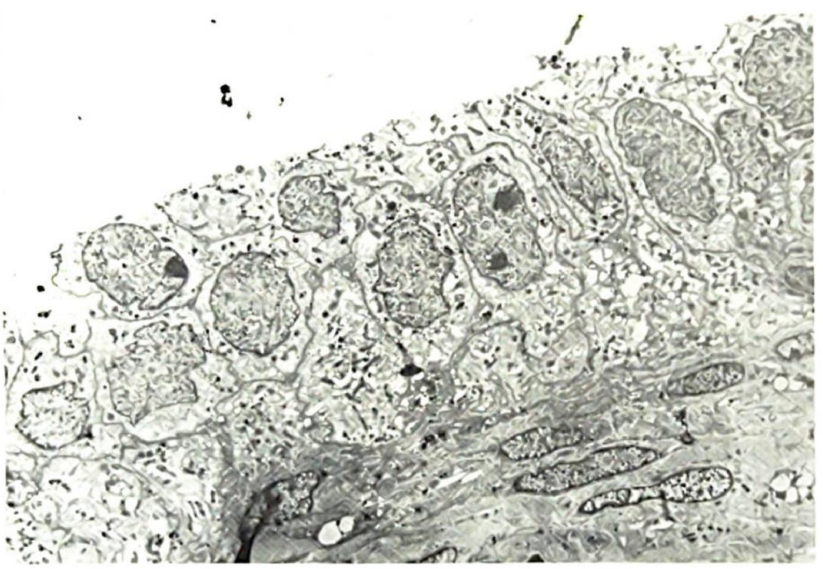


Fig. 9. Transmission electron microscopy: ampullary endosalpinx of a human hydrosalpinx. There is increased nuclear–cytoplasmic ratio. Epithelial cells show large irregular nuclei with sparse chromatin. Mitochondria are decreased, the cytoplasm is vacuolated and there are a few scattered secretory granules only. There are no cilia or cell organelles. Secretory phase (×2100).

numerous mitochondria. The nucleus itself tended to have a serrated edge. In the endosalpinx of later hydrosalpinges (duration of clip application of 24 weeks and longer), the picture was similar to that seen in the human (Fig. 9). The cells showed degenerative changes with an increased nuclear–cytoplasmic ratio, loss of cell organelles and the presence of numerous cytoplasmic vacuoles. Cilia were mostly absent. The submucosa was compressed and in some areas it was completely absent.

Discussion

This study shows a remarkable morphological

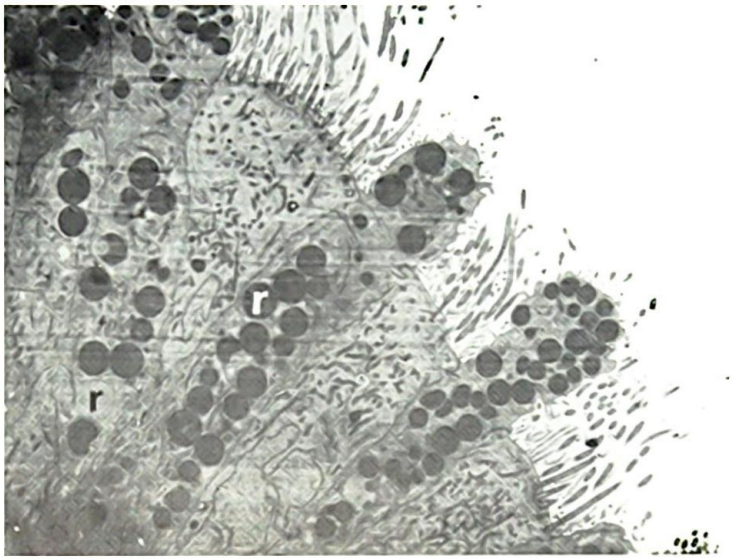


Fig. 10. Transmission electron microscopy: rabbit ampullary endosalpinx after 8 weeks' clip application. Increased secretory activity. Three secretory cells show typical secretory protrusions (◀). Two types of secretory granules are present. Numerous electron dense circular granules (white r) and a few electron lucent granules (black r). Loss of microvilli on the secretory cells. Ciliated cells show cilia cut in transverse and longitudinal sections. The supranuclear area shows numerous mitochondria (×1625).

similarity between mechanically induced and clinical hydrosalpinges. The induction of mechanical hydrosalpinges in this study and the morphological features we describe are in agreement with those of Bernhardt-Huth *et al.* (1980), Vasquez *et al.* (1981) and Kleinstein, Neubuser and Mussman (1982), but contradicts those of Halbert and Patton (1981) and Patton and Halbert (1981). Most studies published on human and experimental hydrosalpinges are in agreement with regard to light microscopic findings, namely flattening of folds and flattening of the epithelium. The main point of disagreement is the extent of deciliation. This is important in view of the presumed role of cilia in ovum transport. It is now generally agreed that deciliation and surface morphology of the hydrosalpinx is best studied by SEM. It has the advantage that it allows the observer to examine large areas of the epithelium and, in assessing the degree of deciliation, to take into account the remarkable variation that occurs over the surface. This raises the question of bias in taking biopsies. Taking biopsies under the microscope could introduce a bias, as there might be a tendency to take biopsy from 'bad-looking' areas in preference to normal or 'good-looking' areas. Taking multiple biopsies can, however, reduce this bias. In the present study, biopsies were taken from some experimental and human hydrosalpinges without the microscope. The method used in processing specimens may be another source of error. Although it is more usual to use buffered glutaraldehyde as fixative for SEM, our method using 10% buffered formalin has been found to be very effective, in our study, and causes no artefacts. Many details of structure may be obscured depending on the method of drying. Critical point drying is the preferred method for reproductive tract tissues (Gould, 1973). Earlier studies (Patek, Nilsson & Johannisson, 1972) used freeze drying methods. The natural state of biological specimens is not frozen and, therefore, it is desirable to examine specimens in their natural state and/or in contact with water.

The phase of the menstrual cycle may be an important source of difference despite a body of evidence against a cyclic ciliogenesis in humans (Ferenzcy & Richart, 1974; Brosens & Vasquez, 1976; Cristoph & Dennis, 1977). In

monkeys, cyclic ciliogenesis has been shown to occur (Brenner, 1969; Rumery *et al.*, 1978). Rumery *et al.* (1978) noted that these cyclic changes in ciliation of the mucosa were pronounced in the fimbriae, less distinct in the ampulla and very slight in the isthmus.

The phase of the cycle was recorded in every patient and taken into consideration in this present study. The method of measurement of the percentage of surface area that is ciliated may be a source of difference. Counting ciliated cells from SEM photo micrographs is inaccurate and subject to bias as well. Figure 7 is a photomicrograph of normal endosalpinx of rabbit ampulla. It is clear from this photograph, that it is impossible to tell one ciliated cell from another and a count is bound to be inaccurate. Measurement of the surface area by planimetric methods (Brosens & Vasquez, 1976) is possible in this photograph and was done accurately. The planimetric method correlates well with the very cumbersome method of counting individual cells seen in cross-section in semi-thin sections examined under light microscope. (Vasquez *et al.*, 1979). In this study, planimetry method was used to show a statistically significant difference between the percentage of ciliated cells in normal rabbit ampulla compared to mechanical hydrosalpinges induced for different durations. How cilia or whole ciliated cells are actually lost from the epithelium is a matter for speculation. Brenner (1969) suggested that in oviduct of the Rhesus monkey, deciliation in the luteal phase of the cycle was accomplished by a pinching off of the apical portion of the ciliated cells. This cellular fragment with the cilia still attached may then be discarded from the surface. In this study, we demonstrated deciliated cells that showed numerous holes or depressions in their surfaces. Although these may represent sites of desquamated cells or parts of cells, we feel that they are more likely to be due to artefacts.

Agglutination of mucosal folds and varying pathologic damage to smooth muscle are some of the features directly attributable to infection and were not noticed in mechanical hydrosalpinx. Although no attempt was made in our study to divide human hydrosalpinges into thin-walled or thick-walled, it was noticed that most of the tubes that had extensive fibrosis in the smooth muscle wall were the thick-walled hydrosalpinges. The thin-walled hydrosalpinges (Vasquez *et al.*, 1979) and the hydrosalpinx simplex (Woodruff & Pauerstein, 1969) show morphological features very similar to those in experimental hydrosalpinges and one is tempted to suggest that raised intra-luminal pressure is the added factor producing the changes. Less smooth muscle damage was found in this group in this study.

In thick-walled hydrosalpinx, the initial infection may have been extensive and more penetrating, producing damage to the tubal wall. Subsequent closure of the fimbrial end does not, therefore, produce marked dilatation of the fibrotic inelastic tube wall. Folds are remarkably preserved in these although agglutination of folds forming bridges, and pseudo-glandular spaces are prominent. It is not surprising that the percentage of ciliated cells and the height of the epithelium were higher in these hydrosalpinges compared to the thin-walled ones (Vasquez *et al.*, 1983), although these differences were not statistically significant. The changes seen in thick-walled hydrosalpinges are, therefore, histologically similar to those seen in hydrosalpinges with follicular salpingitis and chronic interstitial salpingitis. In our study, the histological findings in the tubal wall are similar in some respects.

It would also be argued that, since the histological changes in the tube wall are those of a non-specific chronic inflammatory condition, continuous raised intra-luminal pressure could be a factor in producing the smooth muscle changes (atrophy and wall fibrosis). In experimental hydrosalpinges, although the early response of smooth muscle to distension was hypertrophy, atrophic changes became more obvious, when hydrosalpinges had been *in situ* for longer periods. It is not possible at the moment to speculate on the early response of human fallopian tube to distension. It may indeed prove to be similar to that in animal model.

Although no direct extrapolation from animal data to human is intended, these interesting animal data allow a few conclusions to be drawn. Human hydrosalpinges were characterized in this study by flattening and sometimes complete loss of mucosal folds, atrophy of epithelium and varying degrees of deciliation. The smooth muscle layer showed varying degrees of damage; atrophy, oedema, fibrosis and possibly hypertrophy, all these changes have

been shown also to occur in the experimental hydrosalpinges. The severity of the damage in animal model is related to time and is increasingly severe after 24 weeks of double clip application. On the basis of this finding we feel that raised intra-luminal pressure may play a major contributory role in the damage to the human fallopian tube with hydrosalpinx. Our findings suggest that 8-week hydrosalpinges produced mechanically in a rabbit is a fair model and that they are sufficiently chronic to give meaningful data.

Although the duration of tubal blockage cannot usually be ascertained in women, most human hydrosalpinges probably develop over several months or even years. It is probable that most of the more severely damaged human material we examined came from long standing hydrosalpinges. Unfortunately, evidence for this is hard to produce but the absence of any infection in most of these biopsies tends to suggest this claim. The absence of infection also suggests that pressure may be an important factor in the amount of damage. The clinical relevance of this study is that there is a need to re-examine the role of intra-luminal pressure in continuing damage of the human fallopian tube. On the basis of animal findings, we feel that once a diagnosis of tubal obstruction is made the need to release the pressure in the tube may be urgent.

Acknowledgments

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