Pathogenesis of pelvic inflammatory disease

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Our current concept of the pathogenesis of pelvic inflammatory disease (PID) is a synthesis of information from many sources. The main part is indirectly derived from epidemiological, clinical, and laboratory observations, as well as from deductions by analogy from findings in experimental systems. Documented facts are scarce, fragmentary, and in part contradictory. PID is defined as “the acute clinical syndrome associated with ascending spread of microorganisms (unrelated to pregnancy or surgery) from the vagina or cervix to the endometrium, fallopian tubes, and/or contiguous structures”. The definition is wide and includes a multitude of inflammatory conditions of the female pelvic organs. This review will focus upon infection of the prognostically most important anatomical structure—the Fallopian tube; that is, on acute salpingitis.

Aetiological microorganisms in acute salpingitis Bacterial species isolated from the Fallopian tubes in cases of salpingitis fall into two main categories; sexually transmitted (disease; STD) organisms, and species indigenous to the lower genital tract (endogenous organisms).2

STD-organisms Neisseria gonorrhoeae and Chlamydia trachomatis are documented as causative of acute salpingitis.2 In young women, up to 60% of cases are associated with a genital infection with one or both of these two organisms.3

Aetiological roles of sexually transmittable mycoplasmas—viz. M hominis and M genitalium—have also been discussed. In studies from the 1970s, M hominis was isolated “in pure culture” from the Fallopian tubes of women with salpingitis.4 Serological studies and animal experiments have supported a hypothetical role of both M hominis5 and M genitalium.6 However, later studies have not confirmed that M hominis can cause acute salpingitis by itself.7 Studies on M genitalium have met with difficulties because of the long term required for the isolation of the organism. Recent studies8 do not support an important role for this mycoplasmal species in the aetiology of urogenital infections. The use of genome amplification procedures may clarify the role—if any—of M genitalium in acute salpingitis.

In adult women, N gonorrhoeae and C trachomatis cause cervicitis and/or urethritis.9 10 Cervicitis is usually self-limiting, although chlamydial cervicitis may last for months11 12. In STD-associated salpingitis, cervicitis caused by the corresponding STD-organism is always present.

Co-infections with N gonorrhoeae and C trachomatis are common. Experimentally, simultaneous infection with N gonorrhoeae enhances the replication of C trachomatis in cervical epithelia by 80- to 1.15-fold.14 This suggests that endocytosis initiated by one organism (N gonorrhoeae) facilitates engulfment of a second organism (C trachomatis). It remains to be determined if microorganisms other than N gonorrhoeae (e.g. those of bacterial vaginosis15) can elicit a similar response.

“Endogenous” organisms In women with acute PID, many facultative and anaerobic bacterial species have been isolated—alone, or together with STD-organisms—from abscess material and or cul-de-sac fluid.12 16 This gave rise to the concept of “polymicrobial PID”.16 The concept was based on analyses of material most often obtained by culdocentesis or from transcervical endometrial cultures. From intra-pelvic specimens obtained transabdominally during laparoscopy, usually no more than one bacterial species can be recovered (Wolner-Hanssen et al, unpublished). The distribution of species in “polymicrobial” cul-de-sac isolates16 show a striking resemblance to that of vaginal organisms usually found in bacterial vaginosis (BV).17 The possibility of contamination with BV-organisms in transvaginally obtained culdocentesis specimens makes it difficult to distinguish between true growth and contamination. Recent findings indicate that bacterial vaginosis per se might play a role in the pathogenesis of PID (vide infra).

In anecdotal cases, viruses (viz. Cocksackie B5, ECHO 6, and HSV-2) have been isolated from the upper genital tract in women with salpingitis.2 However, no systematic studies have been presented on the role of viruses in PID.

In spite of the recovery of many microbial species from the upper genital tract in cases of PID, a survey of published studies reveals that no micro-organisms at all were recovered from the Fallopian tubes in 20–30% of the cases studied.2

Spread of organisms to the upper genital tract Canicular spread It is generally agreed, that
from cervical infections caused by micro-organisms in the ejaculate (for example gonococci and chlamydiae). If spread of pathogens by spermatozoa were of significance in the pathogenesis of salpingitis, use of condoms should protect also infected women. On the contrary, in women already infected with C trachomatis or N gonorrhoeae, condom use does not protect from PID. Evidently, the role—if any—of spermatozoa in the pathogenesis of acute salpingitis must be further clarified.

Influence of sexual steroid hormones STD-associated acute salpingitis more often starts during or shortly after a menstrual bleeding than in the luteal phase. In ovulating women, gonorrhoea is diagnosed more often in the follicular than in the luteal phase of the menstrual cycle.

A number of case-control studies have shown that users of combined oral contraceptives (OCs) more often than non-users are culture-positive for C trachomatis from the cervix. In a case-control study of randomly selected women attending an STD clinic, a corresponding association was found, and the strength of this association was highest among women using OCs with low oestrogen content or with levonorgestrel. A logical conclusion from this would be that the use of hormonal contraception should increase also the risk of chlamydia-associated acute salpingitis. On the contrary, chlamydia-infected women using combined OCs less often than non-users develop acute salpingitis; and if they do, the inflammatory reactions of the Fallopian tubes tend to be less intense than in non-users. Consequently, the fertility prognosis after PID in pill-users is more favourable than in non-users.

The above observations have raised questions of whether the acquisition and ascent of an STD-associated cervicitis might be modified by hormonal influences.

The cervix is considered a barrier against ascent of micro-organisms. The functions of the endocervical glands are under hormonal influence. During the follicular (oestrogen dominated) phase, the cervical mucus is abundant and watery. Its glycoprotein molecules are arranged in parallel rows which make possible the penetration of spermatozoa. During the luteal (progesterone dominated) phase, the water content of the cervical secretion is low and the glycoprotein molecules are arranged in an interlacing network, rendering it impenetrable to spermatozoa. By analogy, the likelihood of spread of (cervical) micro-organisms into the endometrial cavity might be higher through follicular than through luteal phase mucus.

In vitro, 17-beta-oestradiol enhanced the adherence and growth of C trachomatis in a dose dependent manner in human endometrial cells, HeLa cells, and McCoy cells. By contrast, adding of ethinylestradiol and contraceptive progestins to cultures of human endometrial cells did not have any effect on the replication of...
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C trachomatis. In vitro, progesterone depressed the growth of N. gonorrhoeae.

In animal experiments, administration of oestriadiol to ovarioctomised guinea-pigs resulted in prolongation of a genital chlamydial infection and promoted its spread to the upper genital tract. Administration of mestranol/norethynodrel to guinea pigs enhanced the course of experimental genital infection with the agent of guinea pig inclusion conjunctivitis (GPIC), and ascending infection was seen in hormone-treated but not in untreated animals. By contrast, administration of progesterone alone to guinea pigs experimentally infected with GPIC prevented acute endometritis.

In a series of ten promiscuous women at high risk of PID and using contraception with long acting injections of medroxy-progesterone acetate, acute salpingitis was not seen during a 2-3 year follow-up (Westrom, unpubl.). Women using levonorgestrel-medicated intrauterine contraceptive devices (ICUDs) had a cumulative 36-month PID-related discontinuation rate of 0.5 as compared with 2.0 in women using copper-mediated ICUDs (p < 0.013).

The apparent protective effect of progestins against ascending infection has been explained inter alia by the luteal-type cervical mucus as well as by the low inactive endometrium induced by the hormones. However, observations that use of OCs to some extent protects against PID in women infected with C trachomatis but not in those with gonorrhoea argue against such a postulated "either/or" cervical barrier effect.

Menstruation Symptoms and signs of STD-associated acute salpingitis often start during or shortly after a menstrual bleeding. Hypothetically, the cervical mucus barrier might not be effective during menstruation, leaving an open route into the endometrial cavity.

During menstruation, retrograde bleeding into the Fallopian tubes and pelvic cavity is common. Retrograde menstrual blood flow would facilitate the spread of endometrial organisms into the Fallopian tubes. Furthermore, the menstrual blood itself contains compounds that support micro-organisms (such as iron).

As indicated above, cycle-related hormonal influences might also explain the frequent development of salpingitis just after menstruation. The post-menstrual period of the cycle is estrogen dominated, and oestrogen stimulates chlamydial infection in human endometrial cells in vitro as well as experimental genital GPIC-infection in guinea-pigs.

Bacterial vaginosis and iatrogenic factors. In non-gonococcal/non-chlamydial PID, a large number of different anaerobic and facultative species have been recovered from pelvic specimens. Isolates include inter alia streptococcus spp., Escherichia coli, bacteroides spp., peptococci, Haemophilus influenzae, and mycoplasmas. Although these bacterial species may occasionally be isolated from the vaginal secretion of healthy women, the ecological disturbances in the vagina seen in bacterial vaginosis (BV) cause significant numerical increases of potentially pathogenic species. Women with BV might therefore be at increased risk of PID. This has been documented for women who douche, and in women subjected to legal abortions.

Women who have frequent sexual intercourse and women who use IUCDs more often have bacterial vaginosis than comparable controls. Both frequent intercourse and IUCD-use have been implicated as risk-factors for PID. In fact, the association between frequent intercourse and PID was evident only in women with bacterial vaginosis (Wolner-Hanssen et al, unpublished). Thus, it seems that micro-organisms involved in bacterial vaginosis might ascend to the upper genital tract and cause PID if "assisted" by the "co-factors" mentioned above.

Use of intrauterine contraceptive devices (ICUDs) The role of ICUDs in the spread of infection in the female genital tract is one of the most controversial topics in contemporary medicine. By the mid 1980s at least 25 studies had reported an increased risk of PID among IUCD-users as compared with nonusers; ranging from twofold to sevenfold (see ref 59). Recent re-analyses, however, suggest that this risk was overestimated. When the control groups exclude women using methods protecting women from PID, the relative risk in IUCD-using women drops below 2:0. The risk is lower for progesterone medicated IUCDs than for copper- or non-medicated devices. The risk of PID is clearly increased during the first month after insertion; thereafter it drops to insignificant levels. More important than the influence of IUCD-use per se is probably the fact that IUCDs were marketed for use in young women presenting (other) risk factors for PID in the midst of world-wide epidemics of gonorrhoea and chlamydia.

Epidemiological risk indicators of salpingitis As is evident from the above, PID does not strike blindly. A number of factors determine a woman's risk of developing the disease. Risk factors and risk markers in PID have recently been analysed by Washington et al.

The risk indicators can be divided into (a) risk of acquiring an STD, and (b) risk of developing salpingitis. Risk factors for developing PID include acquisition of an STD (which has risk factors of its own), age, contraceptive practice, and health care behaviour (douching). Corresponding markers for PID include age, socioeconomic status, contraceptive practice, smoking. Knowledge of risk indicators "will help doctors identify women who need more intensive education and counselling to modify risk behaviour or practices" and also it may increase or decrease suspicion of PID in a given case.
The presence of micro-organisms and their antigens on mucosal surfaces including those of the Fallopian tubes may start cascades of events involving humoral and cellular defence mechanisms. Published studies mainly have been concerned with tubal infections caused by gonococci and chlamydiae.

**N. gonorrhoeae** Gonococcal strains may differ in colony types (T1-T4), piliation, and nutritional requirements. Strains that produce acute salpingitis differ from strains causing inter alia disseminated gonococcal infection. Because gonococcal salpingitis in animal models bears little resemblance to that of human gonococcal PID, studies on tubal infections have been limited to experimental systems.

Gonococcal strains capable of producing salpingitis invade cells lining the tubal mucosa. In studies on tissue culture of human Fallopian tube, gonococci selectively adhere to and enter into nucleated cells, leaving the ciliated cells uninfected. However, soon the ciliated cells show sloughing and ciliostasis. In similar studies, the ciliary activity in tubes infected with T1-T2 strains of gonococci ceased within 18 hours, as did the activity in tissue cultures grown in media containing supernatant from T1-T2 strains grown in liquid media. In control cultures the ciliary activity continued for more than 54 hours. This indicates that sloughing of the ciliated cells and ciliostasis is mediated by some cell-free toxic product from the organisms.

Once inside the non-ciliated cells, the gonococci are protected from immune defence factors, traverse the cells, and are eventually released from their basal surfaces by exocytosis. During growth, gonococci shed outer membrane blebs which contain lipo-oligosaccharide and peptidoglycan. The binding of antibodies to the LOS antigens activates the complement cascade with generation of, inter alia, C5a. Complement is present in Fallopian tube secretion. C5a exerts a strong stimulus to the influx of polymorphonuclear leucocytes (PMNs). The presence of PMNs starts a new series of events including "common" pathways of inflammation such as release of oxygen metabolites and proteinases, which can cause cell death and tissue destruction. Release of phospholipase A2 may start the prostanoid cascade with ensuing production of PGE2 and PGE3, leucotrienes, PGFalpha, thromboxane A2 and prostacycline. Some of these compounds are involved in the inflammatory reaction and cause oedema, vasodilatation, and tissue destruction.

In gonorrhoea-associated salpingitis, the rates of isolation of the organism from Fallopian tube specimens have been inversely related to the duration of symptoms. It has therefore been speculated that other (endogenous) microorganisms might "take over" and continue the infection in tubes compromised by the gonococcal infection. When reviewing the background for this speculation, it becomes clear that the original studies leading to this hypothesis were all performed before chlamydia was recognised as a cause of acute PID. Women with chlamydia-associated salpingitis generally have a clinically milder disease than those with gonorrhoea-associated disease, and also tend to delay seeking medical care. It is therefore possible that the more long-standing cervix-positive/tubal-negative gonorrhoeal cases of the older studies would have been chlamydia positive from both sites if examined with modern methods.

Gonococci have not been isolated from normal Fallopian tubes.

**Chlamydia trachomatis** This bacterium is now established as an important aetiological agent in salpingitis—indeed, chlamydia-associated salpingitis is currently more common than gonococcal disease. Like *N. gonorrhoea, C. trachomatis* has been demonstrated in the epithelial linings of the cervix, endometrial cavity and Fallopian tubes. Unlike gonococci, *C. trachomatis* has been demonstrated also in cases of chronic salpingitis, as well as in some instances from apparently healthy, uninflamed Fallopian tubes.

In experimental studies using organ culture of human Fallopian tube infected with *C. trachomatis*, the organism could be recovered over a 5-7 day period, and chlamydial inclusions containing all forms of the organism were observed in mucosal cells 72 hours after inoculation in both ciliated and non-ciliated cells. Disruption of cell junctions and cell rupture with release of elementary bodies were observed during the maintenance of the cultures. Similar findings were made in organ cultures of experimentally infected human McCoy-cells as well as in the oviducts of mice experimentally infected with the mouse pneumonitis biovar of *C. trachomatis*. In the natural disease, use of scanning and transmission electron microscopy have revealed that *C. trachomatis* attaches to the epithelial surface without apparent ligand binding and are taken into the cytoplasm of the infected cells by endocytosis. Chlamydial elementary bodies are released from cytoplasmic inclusions into the lumen of the Fallopian tube. In experimental chlamydial salpingitis in pig-tailed macaques, damage of ciliated cells in the Fallopian tube was demonstrated. Ciliated cells are lost and on remaining ciliated cells, cilia are lost or "clubbed". Ciliostatic effects have been observed also in studies on chlamydia-infected cells in cultures of human nasal polyps. Like the gonococci, *C. trachomatis* will meet mucosal defence mechanisms before adhering to and entering into the target cells. Chlamydiae can elicit a polyclonal response of human lymphocytes as well as complement activation and stimulation of chemotaxis upon normal antibody-negative
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In vitro, incubation of *C. trachomatis* with complement and specific antibody caused chemotaxis of human polymorphonuclear leucocytes (PMNs).90 The majority of chlamydial elementary bodies were internalised in the PMNs (also in the absence of opsonisation) and rendered noninfectious. However, a small percentage of chlamydiae were able to survive internalisation and surviving organisms may establish infection.91 An initial tissue infiltration with PMNs occurs in a primary chlamydial infection of genital mucosal surfaces in humans92 as well as experimentally infected mice.93 Mononuclear cells soon take over and form germinal centres.92 Eventually, plasma cells dominate and are regarded as hallmarks of chlamydial infection.91 In areas with heavy mononuclear cell infiltration, epithelial cells deteriorate.97

Chlamydial infections induce production of cytokines including tumour necrosis factor (TNF), interferons (IFN), and interleukins (IL).94 In vitro systems, stimulation with *C. trachomatis* elementary bodies elicited cytokine production by lymphocytes including IL-6 from B-cells and IFN-gamma from T and/or non T/non B cells.97 TNF inhibits growth of *C. trachomatis* in HEP-2 cells.94,98

In women with chlamydial cervicitis, cervical secretions contain increased levels of IFN-gamma,99 and women with acute PID significantly more often have IFN-gamma in their sera than women without PID.100 IFN-gamma has a cytotoxic effect on chlamydial-infected McCoy-cells101,102 and has been shown to inhibit experimental infection in mice with serovar L1.103

IFN-gamma can induce expression of Major Histocompatibility Complex (MHC) class II (Ia antigens) on epi- and endothelial cells as well as on macrophages. Two hypothetical effects of Ia antigen expression have been presented:100 (a) Ia antigen expression might down-regulate cellular immune responses via the autologous mixed lymphocyte reaction enabling opportunistic organisms to invade the Fallopian tubes, and (b) Ia antigen expression might result in the activation of both humoral and cell-mediated immune responses directed against the expressing cells, resulting in destruction of infected tubal epithelial cells. In ocular trachoma, class II antigen expression is associated with active inflammation.104

In women with PID, monocytes may produce increased amounts of interleukines (IL), viz. IL-1 and/or IL-6.105 Further increases in monokine-production may be elicited by IFN-gamma. Accumulation of monokines in the Fallopian tubes might induce scarring and tissue damage.105

A mucosal chlamydial infection elicits a humoral immune response with production of local and serum immunoglobulins of the IgA, IgG, and IgM classes.116-108 In prospectively followed women, specific serum antibodies of the IgG class were detectable up to 6 years after an episode of acute salpingitis.109 In one experimental study on guinea pigs, serum derived antibody was found to provide a partial protection against reinfection.110 In experimental genital chlamydial infections in pig-tailed macaques,111 guinea-pigs,112 and cynomolgus monkeys,113 a protective immunity against repeated challenges could be demonstrated. However, the protective immunity against *C. trachomatis* infections seems to be both shortlived and serotype specific.114

In a study on CB/nu mice, transfer of congenic cells or specific antibody did not eliminate the chlamydial infection,115 and B-cell deficient mice have been shown to resolve genital chlamydial infections as well as reinfections.116 These findings indicate that cell-mediated immune mechanisms play an important role in the resolution of chlamydial infection.

There is cumulative evidence that the immunity acquired during chlamydial infections in some individuals might cause delayed hypersensitivity reactions during reinfections. This may be the cause of progressive scarring in ocular trachoma. In experimental studies, progressive conjunctival and tubal scarring has been observed after repeated inoculations with *C. trachomatis*.117,118 In cynomolgus monkeys a soluble extract of *C. trachomatis* induced a marked inflammatory reaction when inoculated into the eyes of immune monkeys.118

In one study on mice, repeated challenges with *C. trachomatis* after experimentally induced salpingitis in the majority of animals caused a more severe inflammatory reaction than in control animals. In other studies using pig-tailed macaques, a single tubal inoculation after repeated cervical inoculations caused tubal oedema and peritubal adhesions.3,120 By contrast, a single direct tubal inoculation with *C. trachomatis* in animals not previously infected with the organism failed to cause any tubal scarring.120

Recently, a chlamydial 57-kD protein belonging to the so called “heat-shock proteins” was identified and characterised.121 This protein is loosely attached to chlamydial elementary bodies. In experimental studies on ocular trachoma, a delayed hypersensitivity reaction was reproduced by adding the 57-kD protein to the conjunctivae of previously infected animals.122 It is tempting to hypothesise that an endocervical chlamydial infection (challenge) in an immunologically “primed” woman might induce delayed hypersensitivity reactions and inflammation in her Fallopian tubes—allogenic with what has been demonstrated in animals.3,120 However, in natural genital chlamydial infections in women, a delayed hypersensitivity reaction to repeated challenges with *C. trachomatis* has not yet been confirmed.

Tubo-ovarian abscess (TOA) Abscess formation is a late manifestation of PID in a proportion of cases. In experimental studies in rats, it was shown that neither *N. gonorrhoeae* nor *C. trachomatis* alone produced abscesses.123 However, when mixed with fac-
ultative and anaerobic bacteria—abscess formation followed.\textsuperscript{123} Clinical findings support the results of the experimental studies and have revealed the presence of a mixed bacterial flora in TOAs in a proportion of cases.\textsuperscript{5, 13, 15} Tissue destruction and bacterial metabolism will produce an environment with low or no oxygen tension.\textsuperscript{124} This favours anaerobic bacteria which will eventually be the dominating isolate in abscesses.\textsuperscript{5, 15} It remains to be determined whether women with the mixed anaerobic flora of bacterial vaginosis\textsuperscript{15} are at higher risk of TOAs if they acquire PID.

Silent PID In studies on infertile women, at least four observations indirectly indicate that asymptomatic genital chlamydial infection may produce tubal factor infertility (TFI): (a) about half of women with TFI claim that they have never had PID\textsuperscript{125, 126}; (b) in women with no history of PID, specific serum IgG antibody to \textit{C trachomatis} has been demonstrated significantly more often in those with TFI than in those with other causes of infertility\textsuperscript{125-127}; (c) in infertile women with antibody to \textit{C trachomatis}, those with TFI had significantly higher geometrical mean values of antibody than women with other causes of infertility\textsuperscript{125-127}; and (d) histopathological studies, and functional studies (as measured by ciliary beat frequency) on Fallopian tube biopsies from infertile women with scarring or adhesions revealed no differences between women reporting and not reporting a history of previous PID\textsuperscript{128}.

These findings indicate that in many cases a tubal chlamydial infection may occur with few or no symptoms.\textsuperscript{13} Apart from the absence of symptoms, silent salpingitis does not seem to differ from symptomatic PID.\textsuperscript{125, 128} If the existence of "silent chlamydial salpingitis" can be further confirmed, its clinical manifestations must urgently be identified to permit treatment before there is tubal damage.

Tissue repair
During the process of repair, dead cells are substituted by ingrowing fibroblasts which cause scarring and eventually functional impairment of the Fallopian tubes. Morphologically, tubal occlusion,\textsuperscript{19} intraluminal adhesions,\textsuperscript{26} and deciliation\textsuperscript{128} may be found after natural disease in women as well as after experimental infections in animals.\textsuperscript{130-132} These sequelae seem to be irreversible.

The serosal inflammation may lead to intrapelvic adhesions—initially fibrous and easily breakable, but latter collagenised and permanent. Second look laparoscopies after PID have revealed that peritubal adhesions usually persist, but may spontaneously disappear in some patients.\textsuperscript{13} Fibrinolysis might be instrumental in the dissolution of adhesions. Experimental studies in animals have shown that the peritoneal fibrinolytic system is inhibited during infection. In peritonitis, peritoneal fluid plasminogen activator inhibitor II (PAI II) is increased.\textsuperscript{134} In Beagle dogs and in rats, postoperative adhesions could be prevented with plasminogen activator.\textsuperscript{135} In naturally occurring peritonitis, inhibition of fibrinolysis might be of importance for preventing the spread of a localised infection. It is tempting to speculate whether stimulation of fibrinolysis in addition to antibiotic treatment might prevent (peri)tubal adhesions in acute salpingitis.

The functional impairment of the tubes caused by salpingitis may lead to infertility or increased risk of a tubal pregnancy.\textsuperscript{43} The initiation and continuation of the tubal infection is a procedure that may take place in hours. The beginning of tubal scarring may be a matter of days. In follow-up studies of women who had one laparoscopically verified episode of acute salpingitis, those who delayed seeking care for more than two days after the onset of pain were three times more likely to experience post-salpingitis infertility or ectopic pregnancy than those who sought care promptly.\textsuperscript{136} In experimental chlamydial salpingitis in mice, the ensuing infertility rate was inversely proportional to the time between inoculation and start of treatment with tetracyclines.\textsuperscript{131}

Notwithstanding the amount of information that we have on the pathogenesis of acute PID, our review shows that we are only at the beginning of a full understanding of its complex dynamics. Knowledge of the pathogenesis of acute salpingitis will help to prevent sequelae such as infertility and ectopic pregnancy. More studies are needed in order to reach that goal.

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McGue ZA, Johnson AP, Taylor-Robinson D. Pathogenic mechanisms of Neisseria gonorrhoeae: observations on damage to human fallopian tubes by gono-


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