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The Ljubljana IUDS: further observations on surface morphology

L. G. KEITH*, R. BAILEY† and M. METHOD*

**Department of Obstetrics and Gynecology, Northwestern University Medical School and Prentice Women's Hospital and Maternity Center, Chicago, Illinois, USA*
†Materials Science Laboratory, Department of Geology, Northern Illinois University, DeKalb, Illinois, USA

Abstract

Twelve IUDs that had been worn from 8 years 10 months to 24 years were examined by SEM. Photomicrographs of selected samples are shown, and a discussion of the nature of the surface encrustations is provided. The authors are of the opinion that surface encrustations are generic to different types of IUDs and that their clinical significance is presently unknown.

Introduction

Numerous studies have documented the presence of adherent materials on the surface of previously worn IUDs [1–13]. As early as 1967, Potts and Pearson selected a combination of light and electron microscopy to describe surface adherences [1]. More recently, transmission electron microscopy [11], along with energy-dispersive spectrometry (EDS) and X-ray diffraction (XRD) [14], has been used to define more precisely the exact nature of these deposits. The evidence to date suggests that calcium is probably the major cationic component of IUD surface encrustations, although the exact number and nature of the anionic binding compounds remain to be clarified. Other major questions of a general clinical nature also await clarification. These include: (1) What is the clinical relevance of IUD encrustations? (2) Are encrustations substantially different in symptomatic women compared to their asymptomatic counterparts? (3) What role, if any, does the presence of bacteria have in the process of calcium deposition? (4) Why is it that calcium encrustations do not cover all IUDs to the same extent? (5) To what degree does the woman's biochemical makeup (as determined by her place of residence and natural diet) influence the rate and likelihood of calcium deposition?

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The micro-technical nature of the methods of analysis of IUD surface encrustations poses additional problems to research investigators. Such problems include: (1) selection of a technical vocabulary to describe the changes noted; (2) determination (if possible) of a standard magnification at which relevant details of surface structures are easily and accurately identified; (3) delineation of those structural details which represent artifacts from processing; and (4) understanding of the appearance of calcified biologic materials such as micro-organisms and red and white blood cells.

The use of precise terminology to describe photographic observations is of particular importance. For example, if the surface of an IUD is described as being degraded [15] when it is actually heavily encrusted, this imprecision in terminology fails to recognize the stable nature of the plastic IUD body or tail and suggests a process which has not been shown to occur.

Review of the literature [1–11] and the results of our own experiments [12–14] support the following statements. Firstly, the topographic morphology of surface encrustations varies considerably between different sites on a given IUD, between various IUDs of the same type and between IUDs of different types. Secondly, it is possible that these variations are more dependent on the specific biochemical and physical milieu surrounding a given site on an IUD than they are on specific IUD types. Thirdly, there is no indication that the surfaces of IUDs or their strings undergo a physical degradation with time.

Our prior investigations [12,13] described the surface changes on 6 IUDs removed from asymptomatic women living in America; 3 new IUDs were also studied as controls. Only one of these IUDs had been used for more than 10 years. Because it was recognized that the number of long-term wearers of IUDs (more than 10 years: author's definition) throughout the world was considerable [13], a decision was made to seek out a group of IUDs for study which might assist in clarifying the nature and extent of surface deposits which had had at least 10 years to accumulate.

On 1 May 1984 the presentation of data from Ljubljana, Yugoslavia on the long-term use of non-medicated IUDs in women over 40 years of age [16] at an international workshop on intrauterine contraception provided the opportunity to initiate this work. Professor Lidija Andolšek-Jeras of the Department of Obstetrics and Gynecology at the University Medical Centre of Ljubljana, Yugoslavia, graciously agreed to send for scanning electron microscope (SEM) analysis a series of IUDs which had been worn for 10 or more years. These devices were removed from women who came for routine health care and requested device removal, or agreed to device removal upon physician request. The Ljubljana IUDs form the basis of this paper.

Materials and methods

In April 1985, a package of previously used IUDs was sent from Yugoslavia to the office of the senior author. Each of the 12 IUDs was received in a separate plastic envelope-packet with a label which contained the following information: patient age at removal; date of insertion; date of removal; reason for removal; clinic number of patient.

Table 1 Characteristics of Ljubljana IUDs and planned analyses

IUD No.	*Master code*	*Weight (g)*	*Length of use*	*Age of patient*	*Reason for removal*	*Planned analyses*
1	STC-1	0.5641	15 y/7 mon	39	Infection	SEM, EDS, XRD
2	Cu-7-4	0.1749	12 y/8 mon	35	Wanted pregnancy	SEM
3	LL-5	0.7164	15 y	48	Amenorrhea	Acid etch
4	LL-6	0.6359	24 y	49	Personal	SEM, EDS, XRD
5	LL-7	0.7562	13 y/8 mon	40	Bleeding fibroids	Acid etch
6	Cu-7-5	0.1992	9 y/4 mon	34	Personal	SEM, EDS, TEM
7	Cu-T-2	0.1921	10 y/2 mon	49	Personal	SEM
8	LL-8	0.7865	17 y/3 mon	52	Menopause	SEM, EDS, XRD
9	LL-9	0.7343	12 y/3 mon	51	Premenopause	Acid etch
10	LL-10	0.7998	13 y/10 mon	36	Partial expulsion	SEM, EDS, XRD
11	Cu-T-3	0.2027	8 y/10 mon	33	Planned pregnancy	SEM
12	Cu-7-6	0.2022	11 y	53	Menopause	SEM

In June 1985, each IUD was catalogued into the laboratory book, photographed in black and white and weighed. Table 1 lists the IUDs by name and code number and the initially projected methods of study. The numbers assigned to the IUDs for the master code were in sequence with the numbers of IUDs previously described [12,13] so that each IUD had a unique master number, regardless of device type. Initial SEM studies were begun in July and continued through August 1985. Stereolight photos of selected IUD segments were performed in July. Because of the large number of IUDs available for study and the almost limitless numbers of micrographs that theoretically could be obtained from them, it was decided to perform SEM examinations only on representative portions of these IUDs or their strings, especially in areas with visible surface encrustations.

Results

The experimental results for each IUD examined by SEM are noted below.

Cu-7-4

A solid film overlies numerous rounded protuberances scattered over a cracked encrustation surface covering the entire cap-like structure which connects the arm with the body of the IUD. In the foreground of Figure 1, 4 smaller (and possibly younger) structures lie in front of a solitary protuberance. The distal end of one of the tail strings is shown in Figure 2. Several encrustation morphologies are seen. In the foreground, a flat encrustation surface is present with fractures throughout resembling dried river beds. Behind and toward the distal end, several rounded protuberances are seen. The lowermost end of the string is totally obscured by a combination of these two forms of encrustation.

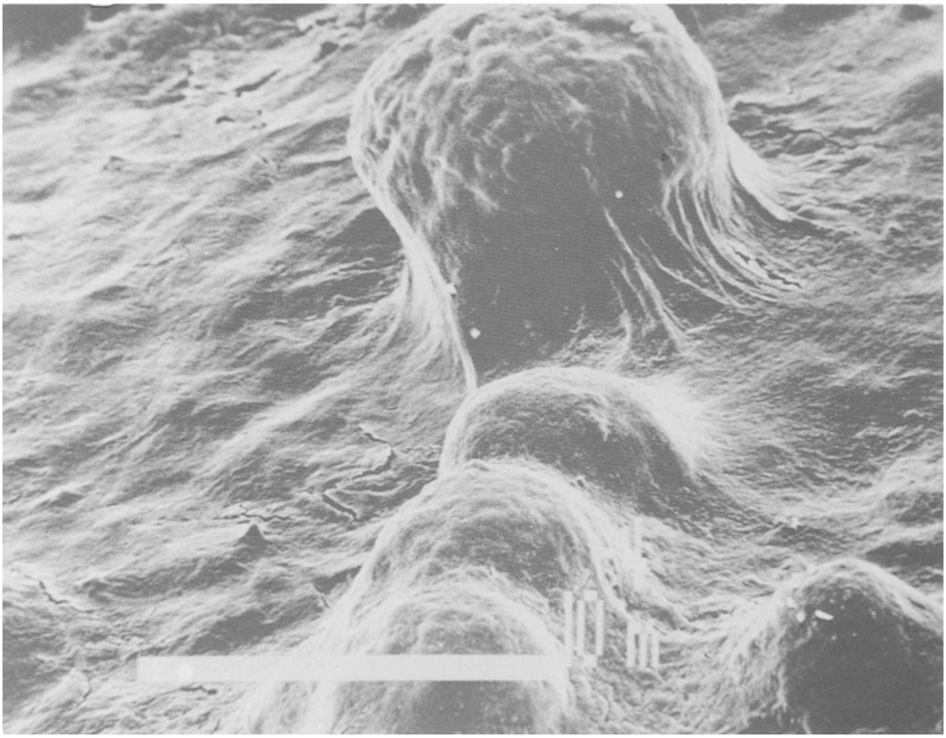

Figure 1 Cu-7-4-IUD$_4$ Numerous rounded protuberances are noted over a cracked encrustation surface and covered by what appears to be a solid film OM×500

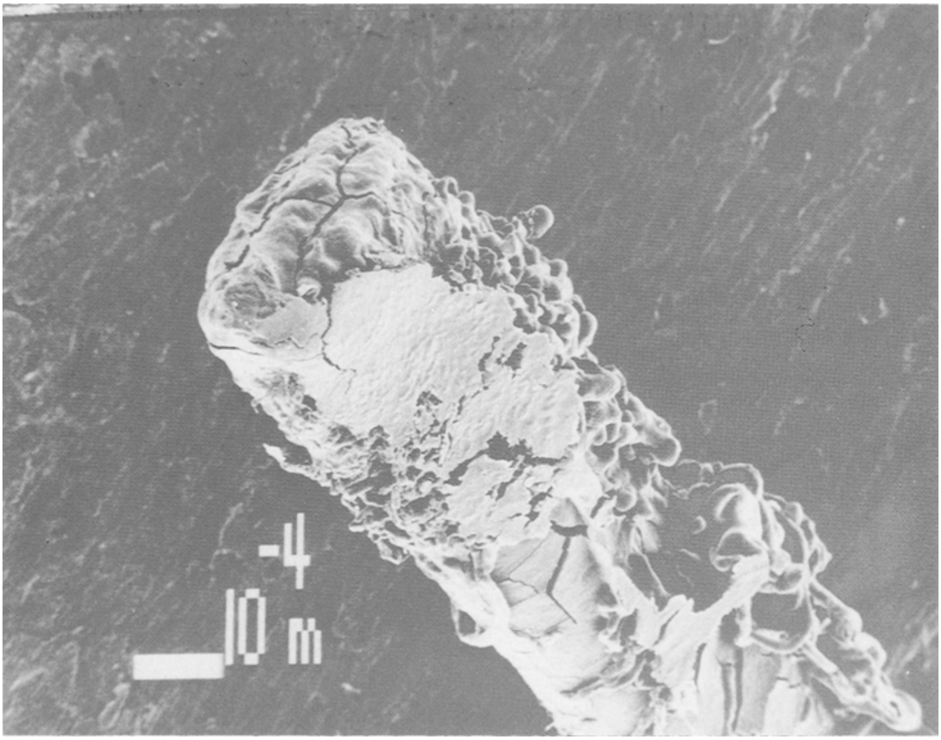

Figure 2 Cu-7-4-LS end$_1$ The distal end of one of the tail strings reveals several encrustation morphologies. In the foreground, a flat surface is present with fractures throughout, resembling a dried riverbed. Behind, and toward the distal end, several rounded proturbances are seen. The lowermost end of the string is totally covered by a

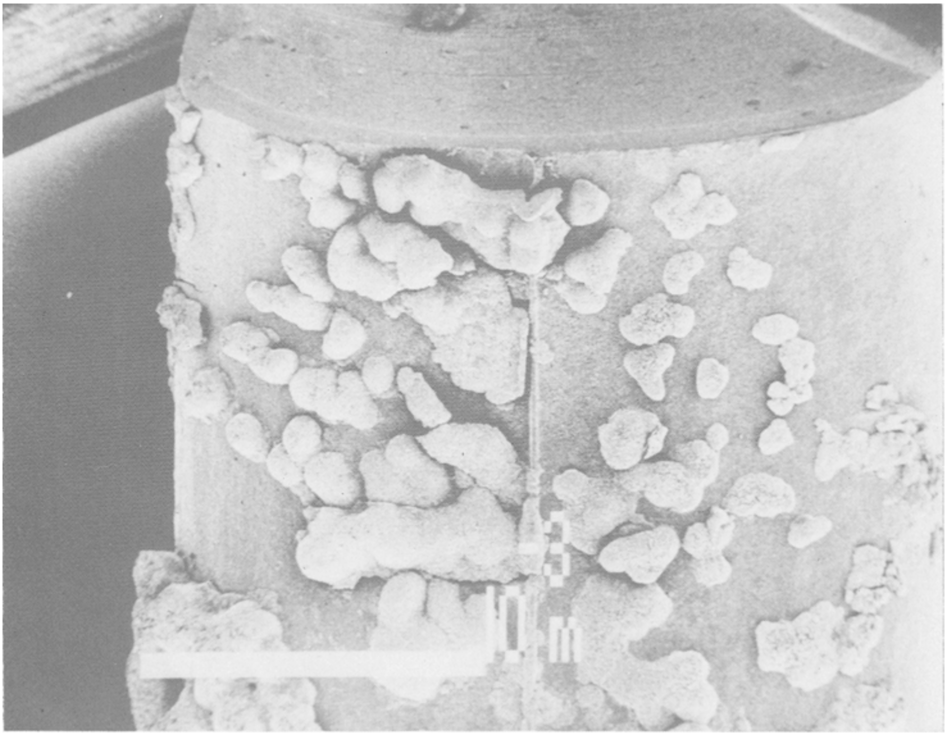

Figure 3 LL-6-IUD$_1$ A relatively clean undersurface is visible on the IUD body itself. The molding mark is easily discernible. Islands of irregular shaped masses with smooth and rounded contours overlie the IUD surface OM×40

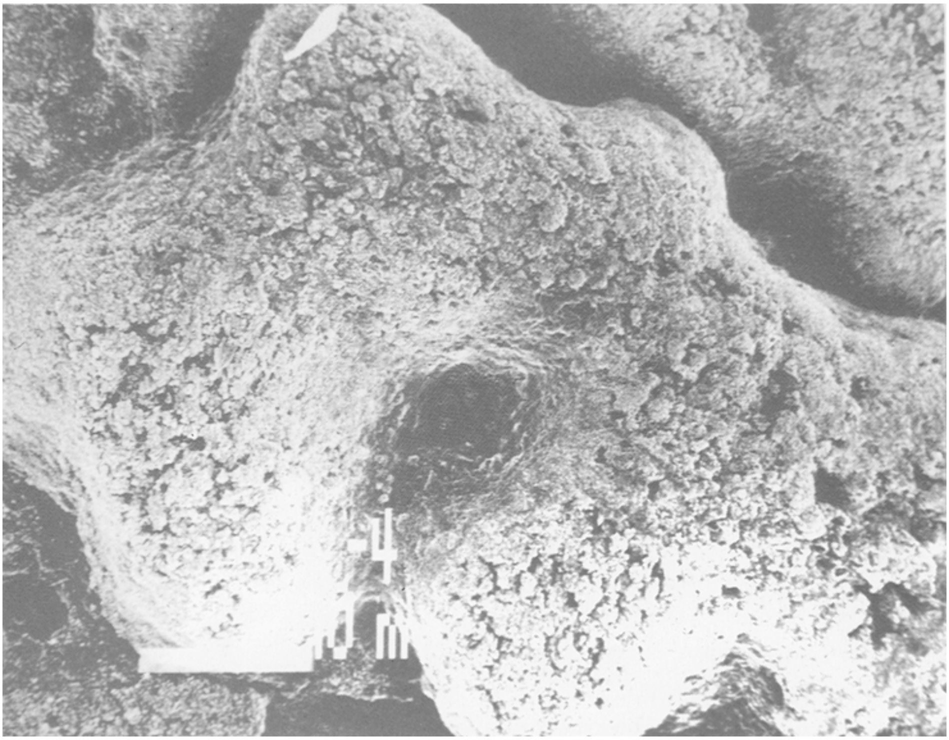

Figure 4 LL-6-IUD$_4$ High magnification of one of the rounded protuberances illustrated in Figure 3 reveals the surface to be irregular and pock-marked OM×200

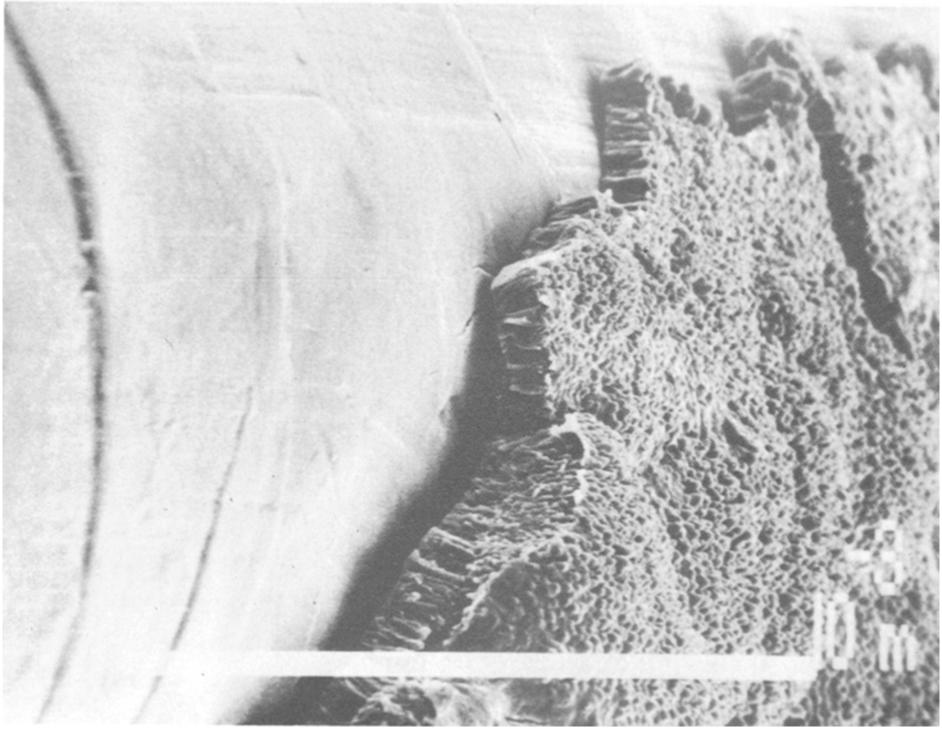

Figure 5 LL-6-LS end$_5$ A thick encrustation overlies the IUD surface and is separated from it by a distinct space OM×80

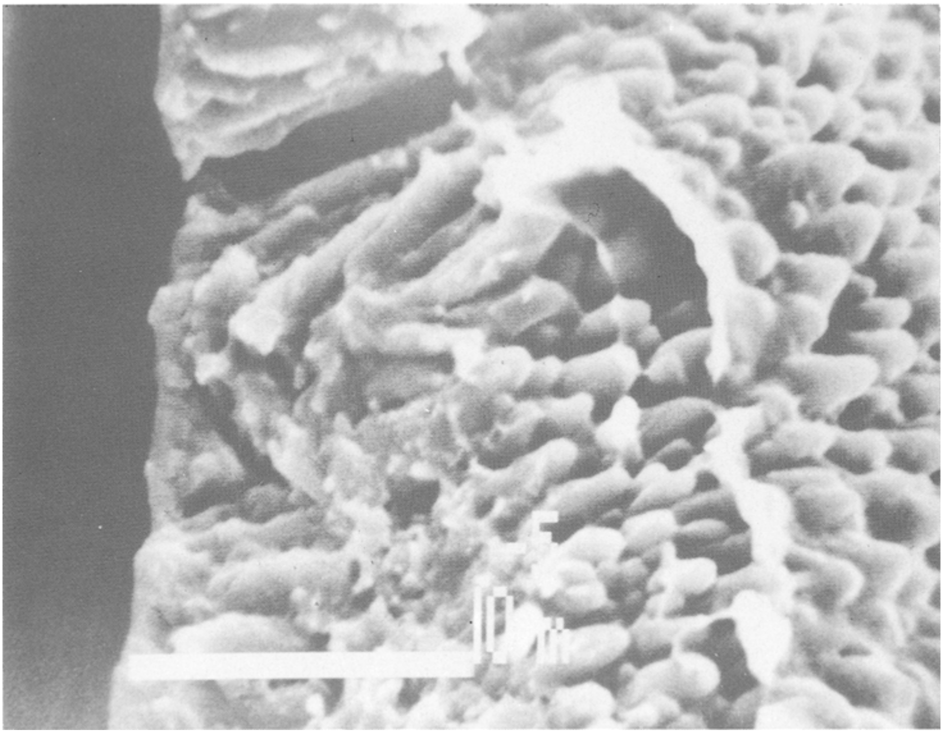

Figure 6 LL-6-LS end$_7$ A higher magnification of Figure 5 illustrating numerous, erect, finger-like projections coalescing into a common structure OM×4000

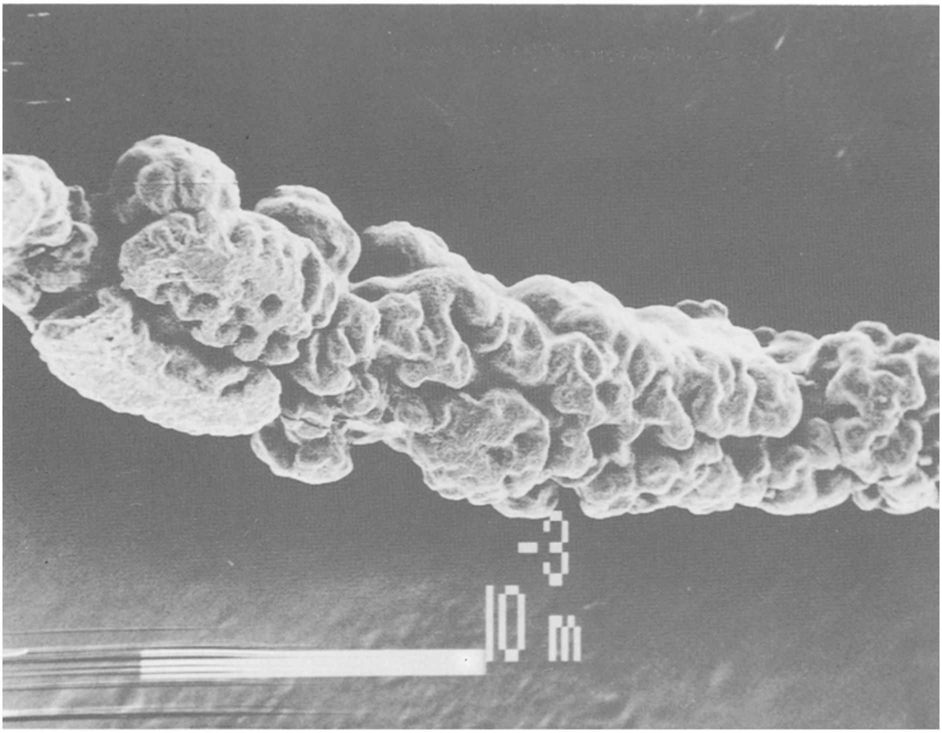

Figure 7 Cu-7-5-MS$_5$ This string is totally surrounded by a highly convoluted encrustation with rounded, smooth edges separating individual surfaces OM×40

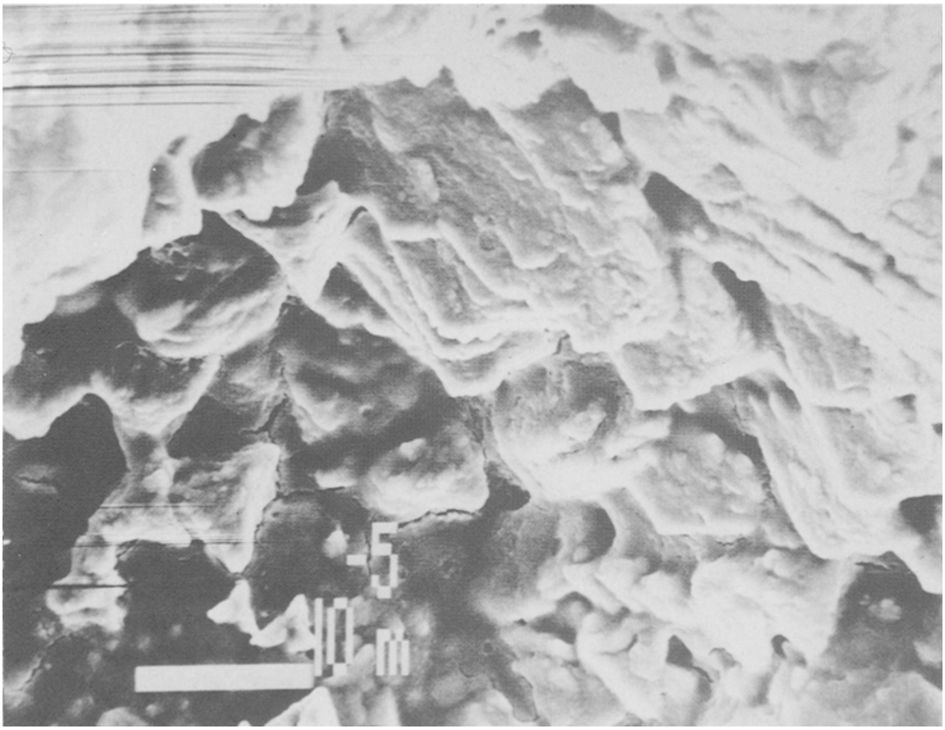

Figure 8 Cu-7-5-MS$_6$ A higher magnification of Figure 7 in which the undersurface of one of the convolutions is examined. The angular and plate-like nature of the surface and its crystal faces is easily visible OM×2000

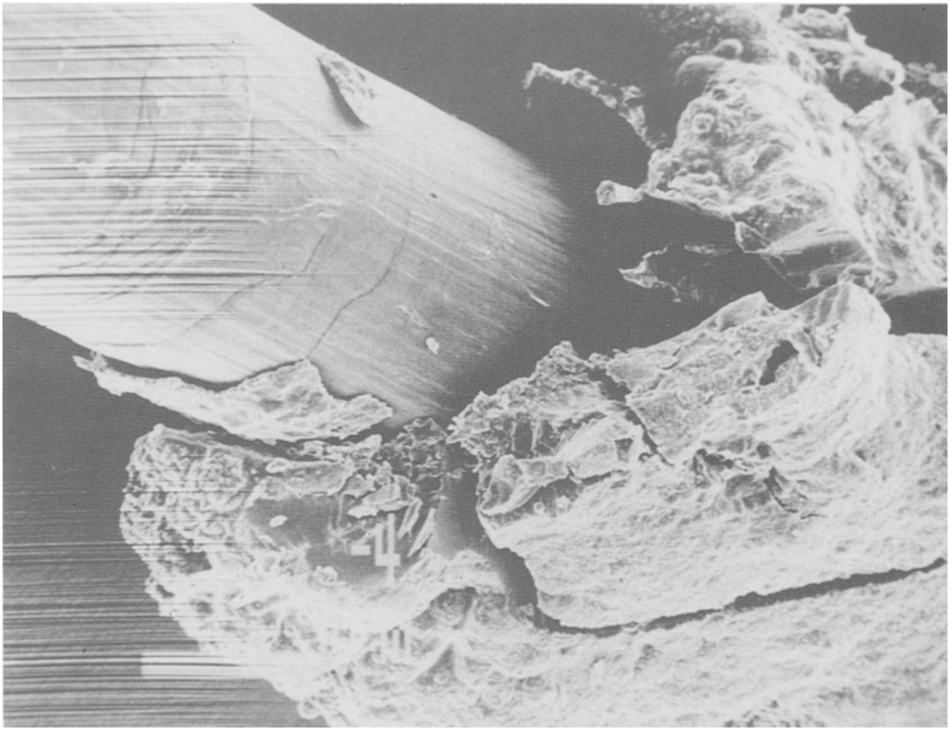

Figure 9 Cu-T-2-US$_2$ A portion of one of the strings slightly below its attachment to the IUD shows a thick encrustation with a fractured surface demonstrating crystal faces OM×200

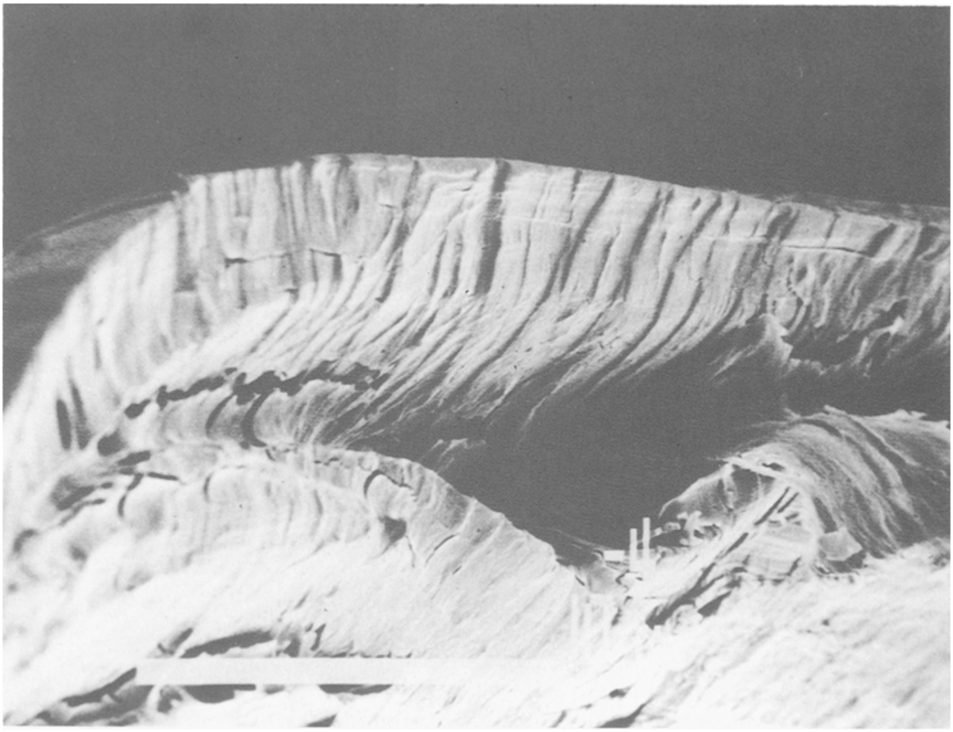

Figure 10 LL-8-LS end$_3$ A highly convoluted encrustation totally covering the distal end of one of the strings is marred by fractures running at right angles to each other OM×500

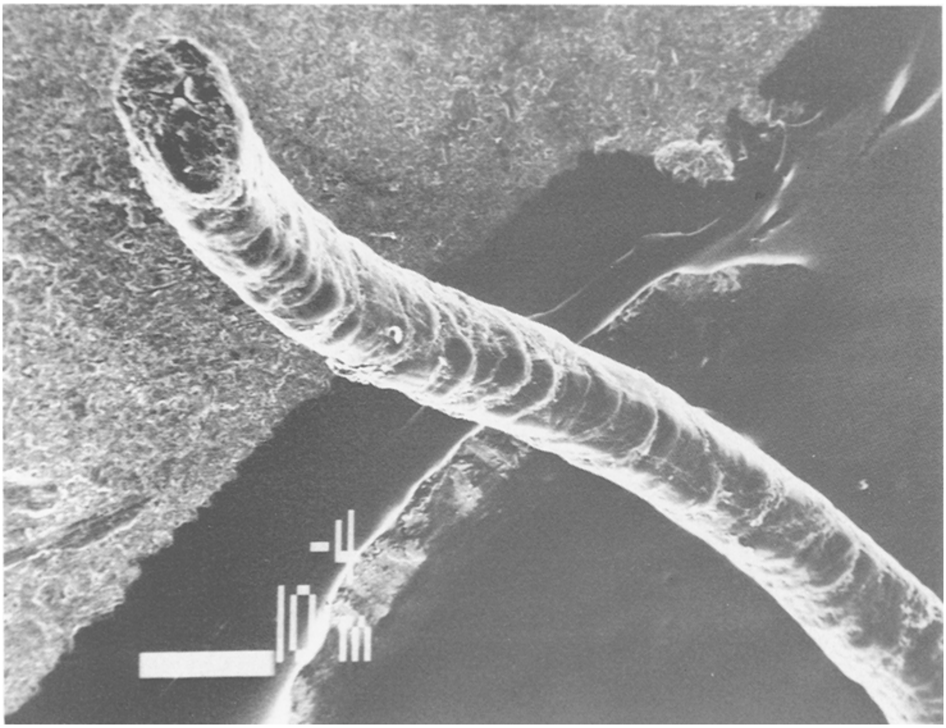

Figure 11 LL-10-LS end$_3$ The distal end of the tail is completely encrusted OM×150

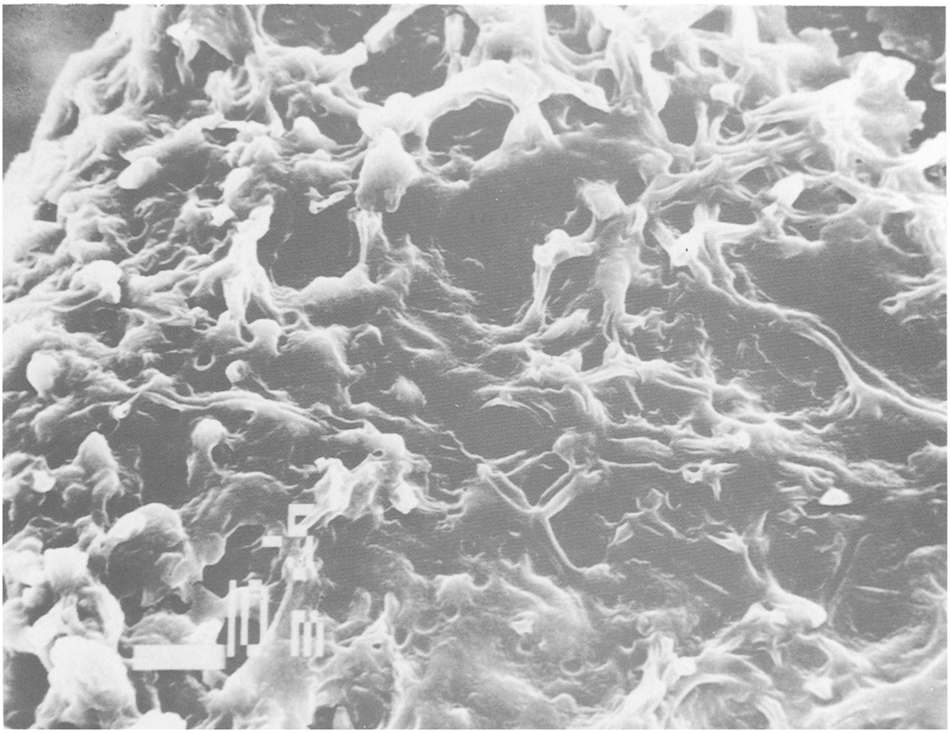

Figure 12 LL-10-LS end$_4$ A high magnification of Figure 11 reveals numerous spaghetti-like structures crisscrossing the distal-most surface of the IUD tail OM×100

Figure 13 LL-10-IUD$_2$ Numerous rounded protuberances are seen on the body of the IUD OM×100

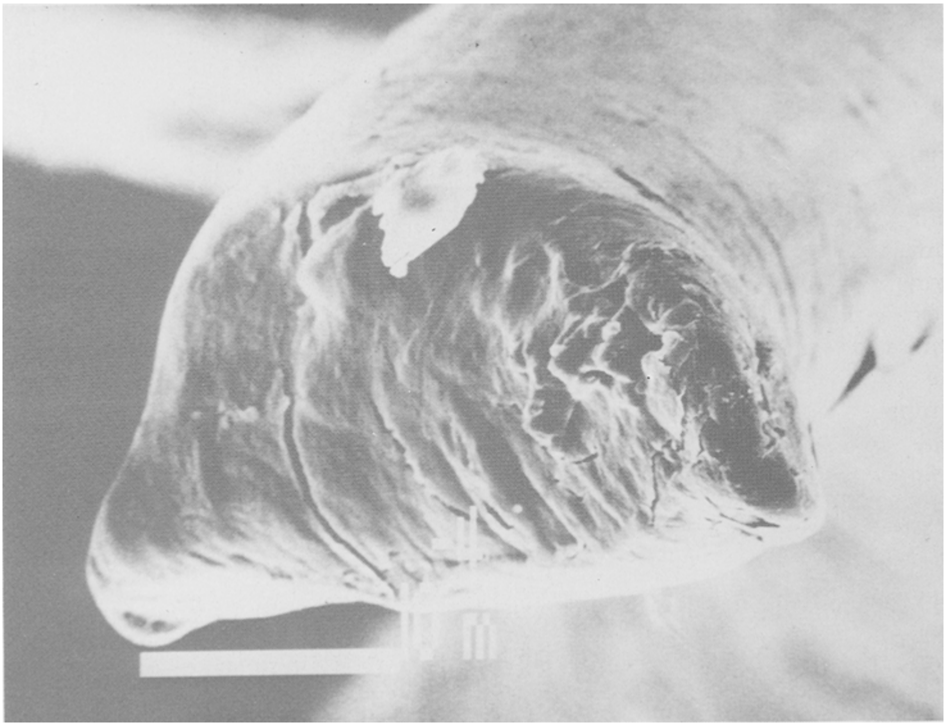

Figure 14 Cu-T-3-LS end$_5$ The distal end of the tail is completely covered by encrustation material OM×300

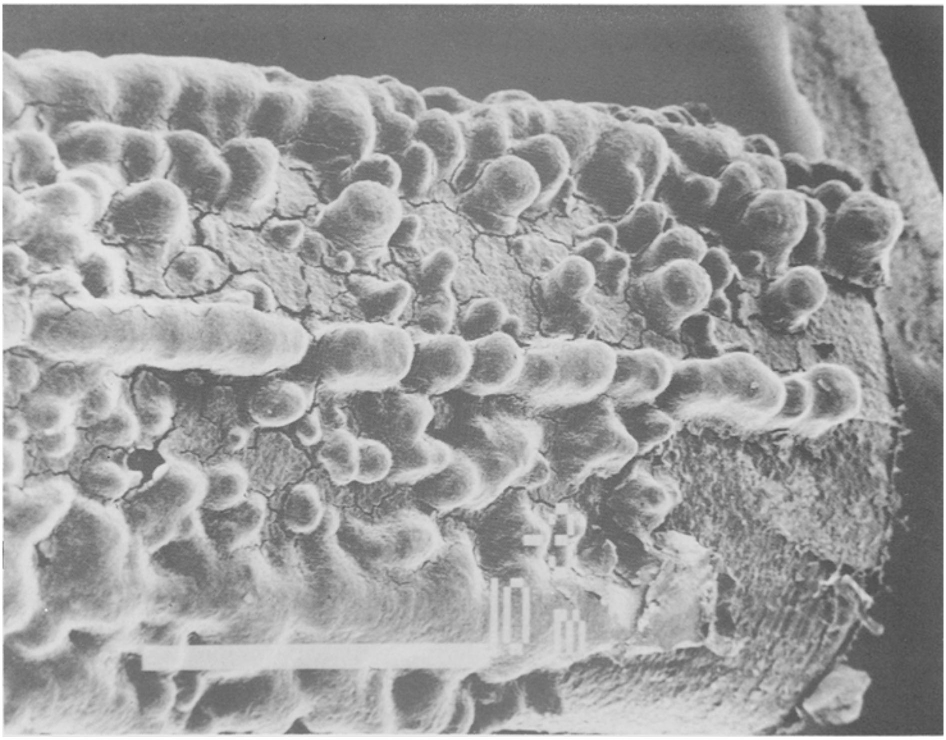

Figure 15 Cu-7-6-UI end$_3$ Rounded protuberances are seen throughout; their location appears regular and in some places the spaces between individual protuberances appear to be in the process of being filled in OM×40

LL-6

Figure 3 shows a relatively clean undersurface on the IUD body (the molding mark is still visible) marred by islands of irregular-shaped masses with smooth and rounded contours. A higher power examination of the above (Figure 4) shows the surface to be irregular and pock-marked. Examination of the end of one of the strings reveals a new and distinct morphology (for this series) (Figure 5). A thick encrustation overlies the IUD surface and is separated from it by a distinct space. This platform-like structure appears to have two parts: an exterior film overlying the entire surface and numerous, erect, finger-like projections coalesced into a common structure. A higher magnification (Figure 6) of this view shows the separate and distinct nature of each of the upright projections and the film which overlies them.

Cu-7-5

Views of the midstring show that it is totally surrounded by a highly convoluted encrustation with rounded, smooth edges separating the individual surfaces (Figure 7). At a higher magnification (Figure 8) which examines the undersurface of one of the convolutions, the angular and plate-like nature of crystal faces is easily visible.

Cu-T-2

A view of a portion of one of the strings slightly below its attachment to the IUD shows a thick encrustation (Figure 9). Of interest is the fractured surface demonstrating crystal faces. This morphology contrasts with that described on LL-6 (see above). A lower power view (not shown) of this area corresponds to the external morphology shown intact in Figure 7.

LL-8

A view of one of the lower strings (distal end) (Figure 10) shows a highly convoluted pattern of encrustation with fractures in the surface often running at right angles to each other. The underlying string is no longer visible.

LL-10

The distal end of one tail string is shown to be completely encrusted (Figure 11). Once again, fracturing is present. A higher magnification of this end (Figure 12) reveals numerous spaghetti-like structures criss-crossing the surface. When the IUD body was examined (Figure 13) numerous rounded protuberances were observed.

Cu-T-3

The distal tail is totally covered by a fractured encrustation (Figure 14).

Cu-7-6

The upper IUD surface has a mud-cracked basement layer of encrustation with rounded protuberances arising from its surface (Figure 15). In the background and foreground the individual masses appear to be overlaid by a relatively thick film. On the molding mark distinct individual protuberances are seen between others in which the intervening space has been filled in or overlaid by encrustation.

Discussion

The major findings of one of our prior studies (pure crystalline forms) [12,14] and the present report (solitary rounded protuberances) have recently been independently confirmed in two publications from Hungary [17,18]. Both reports were unknown to us before initiation of this study. With regard to the crystalline forms, we described euhedral crystals forming a rosette of calcium apatite [$(Ca_5(PO_3)_3(F,Cl,OH)$] [13]. The crystals in the Hungarian report were also euhedral crystals and in some instances formed rosettes. They were similar in morphology to our findings but appeared somewhat flattened in their *a* and *b* axes (commonly described as width and breadth) [17,18]. It is not clear whether these two examples represent the totality of possible intrauterine crystalline forms of calcium salts.

To date the *in vitro* studies of the kinetics of crystal growth of hydroxy apatite have described processes which take place at constant pH [19]. Such is not the case in biological systems, and the composition of the intially precipitating solid phase often does not correspond with that of the final crystalline phase [20]. Thus, the final crystalline structure may depend on the presence or absence of specific precipitating or reacting ions [20]. At least *in vitro*, the process of deposition of hydroxy apatite proceeds through a gradual transition in which amorphous material is deposited on a 'seeding substance' and forms needle-like material which ultimately may become highly ordered crystalline forms [20].

Variations in composition of calcium precipitates are common in dental calculus, especially as they relate to the ratios of calcium and phosphorus [21]. Such variations may be due to the presence of a factor impeding the precipitation of apatite. According to Jensen and Dano [21], this factor is present in the saliva. When calcification of dental plaque (the underlining basis of calculus) was studied in one *in vitro* experiment, it was observed that calcification was inhibited by testicular hyaluronidase [22]. More recent investigation has shown the presence of a male inhibitory material (HuSePl-Frl) which prevents spermatozoal rejection during fertilization by acting on natural killing activities [23]. It can only be speculated upon at this time whether such activity may affect the longevity of bacteria which enter the uterus at the time of coitus.

Since bacterial plaque represents a major component of dental calculus [24–26], it has been suggested that micro-organisms may also be integral parts of IUD encrustations [8,10,11]. To what extent this is true is unknown, because calcium deposition *in vivo* also takes place in closed systems where bacteria are not normally present, i.e. brain, coronary arteries, breast. It is also unclear whether similar biochemical processes precede calcification in various locations on the same IUD, since the biochemical milieu surrounding these various locations differs markedly from the leading end of the IUD to its distal tail. *In vitro* studies of dental calculus document two morphologically distinct patterns of calcification: in the first, mineralization progresses from the inside outward within a bacterial cell; in the second, calcification appears to proceed in the opposite direction [27]. In contrast to the mouth in which the processes of mineralization [28] and calcification [27] have been studied in detail, little is known about these same processes as they relate to IUDs.

Any consideration of the possibility that bacteria play a role, albeit ill-defined, in the process of deposition of calcium encrustations on IUDs may reasonably be followed by re-examination of the question of whether the uterine cavity is sterile. Traditional gynecologic teaching holds that it is, unless some condition or circumstance renders it otherwise. Childbirth and abortion, gynecologic surgery such as dilatation and curettage, and insertion of foreign objects such as hysteroscopes, chorionic villus biopsy needles and IUDs are all thought to represent instances in which the transmission of microorganisms from the lower (cervix and vagina) to the upper (uterus, tubes and ovaries) generative tract is facilitated [29]. Recently, it has been suggested that other factors, i.e. bacteriospermia, ascent of trichomonads and passive ascent of bacteria, may also act, either alone or together to facilitate ascent of microorganisms into the uterine cavity [30]. Since these processes can take place repeatedly over time, the concept of uterine cavity sterility, especially among sexually active women, needs re-examination. This is particularly true for women who wear IUDs, as the literature after 1974 commonly states that the risk of infection is higher in this subset of the general population [31]. However, as DeFoort [32] has recently stated, 'All reasoning about pelvic inflammatory disease (PID) and IUDs starts from what logicians call a "default assumption". This is an assumption made automatically and not as a result of consideration and elimination – by its nature, an implicit assumption [33]. In this case, the assumption is that some women will never get PID if they refrain from wearing IUDs.' The fallacy of the latter assumption is well known to clinicians. The vast majority of women who develop PID have never worn IUDs and the vast majority of women who wear IUDs do not develop PID. Prior to 1974, PID generally was thought to derive from sexually transmitted infection. After this date, many studies associated IUD use with PID and failed to consider the role of sexually transmitted agents [33a]. More recently, the role of these agents has been reconsidered vis-à-vis any potential contribution of the IUD [29].

At present, it is not possible to state with certainty what happens to bacteria once they enter the uterine cavity. The experiments of Mishell and colleagues performed two decades ago suggested that the uterus cleared itself of bacteria which entered at the time of IUD insertion [34]. These experiments were not designed to consider the possibility of recurrent contamination and were performed prior to recognition of the mechanisms by which bacteria attach to solid surfaces. It is now generally accepted that the smooth capsules of bacteria are not really smooth but are covered with a variety of different types of projections, all of which are designed to assist the bacteria to attach to other structures, be they animate or inanimate. In the case of gonococcal infection, for example, the presence of pili mediates bacterial attachment to epithelial cells [35], sperm [36] and erythrocytes [37]. Other examples of attachment by diverse micro-organisms abound [38,39].

The microenvironment of the uterus at the IUD–mucosal interface is aqueous. As such, the formation of microbial surface films in nature is complex and often involves the successful attachment of diverse organisms which may interact with one another [40]. Environmental factors such as pH, temperature and nutrient concentration are obviously important [40], but make the *in vivo* study of such phenomena difficult. In general, the adhesion of bacteria to surfaces in aquatic environments is a function of the nature of the surface and the molecular architecture of the bacterial glycocalyx [41]. This action is so strong that more than 99.9% of bacteria in flowing aquatic systems grow in glycocalyx-enclosed microcolonies adherent to submerged surfaces [42]. A more complete discussion of this phenomenon is provided by Costerton and colleagues [41].

The tendency for bacteria to form adherent microcolonies when they come in contact with a solid surface is not compatible with the hypothesis advanced by Tatum and colleagues in 1975 that bacteria 'wick' up multifilament IUD tails [43], but is compatible with the observations of Purrier and colleagues [44] that bacteria can be found on the exterior surfaces of multifilament as well as monofilament tails. It is also compatible with the SEM observations of Morgos and colleagues [45] who demonstrated the presence of microcolonies of various organisms on the tails of IUDs removed from a group of Arab women and examined directly. The presence of bacteria on either the outside or the inside of an IUD tail has never been shown to be associated with the presence of infection. Both Tatum and colleagues [43] and Bank and Williamson [46], who later examined an additional series of Dalkon Shield tail strings noted that all of the IUDs in their studies had been removed from asymptomatic women. Of the IUDs examined by us, only one (STC-1) (IUD no. 1, Table 1) was removed as part of the treatment of infection. There were no visible differences in its surface characteristics.

Since it is possible that bacteria enter the uterine cavity repeatedly along with sperm [30], it is reasonable to question whether presently accepted methods of disinfection generally used prior to IUD insertion are sufficient [47]. Cleansing of the vagina with Betadine is normally advocated. Some clinicians also advocate a Betadine soak of the IUD prior to its insertion [48]. However, it is often unappreciated that Betadine and similar solutions are formulated as antiseptics and it is not the intent of manufacturers that they be used as disinfectants [49]. Indeed, microbial contamination of iodine sources is possible and may, in some cases, be the source of 'pseudobacteremia' *in vivo* [49]. The same may be said for other antiseptics such as Benzalkonium chloride [50].

The possibility of using the IUD body itself as a carrier of an antimicrobial substance has been raised in the past, but to our knowledge has not yet been brought to practical application.

Acknowledgement

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Resumé

Douze stérilets qui ont été utilisés pendant une période allant de 8 ans et 10 mois à 24 ans ont fait l'object d'un examen sous microscope électronique. Des microphotographies de spécimens sélectionnés illustrent ce document. Après avoir examiné la nature des incrustations superficielles, les auteurs en ont tiré la conclusion que les différents types de stérilets présentent tous les mêmes incrustations dont on ne connaît pas encore la signification clinique.

Resumen

Doce DIU que fueron usados de 8 años 10 meses a 24 años, se examinaron por microscopio de barrido. Se muestran microfotografías de especímenes seleccionados y se especula sobre la naturaleza de las incrustaciones en la superficie. Los autores son de opinión que las incrustaciones en la superficie son comunes para los distintos tipos de DIU y que actualmente se desconoce su significado clínico.