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OLD HERBORN UNIVERSITY SEMINAR ON THE VAGINAL FLORA IN HEALTH AND DISEASE: MINUTES AND REVIEW OF THE DISCUSSION

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DISCUSSION PARTICIPANTS (in alphabetical order):

Philip B. Carter, Sabina Cauci, Edward V. De Buysscher, Catherine C. Davis, Anders Hallén, Peter J. Heidt, Donna R. Hill, Thomas M. Hooton, Marijane A. Krohn, Helen McDonald, Dominiek Maes, Inger Mattsby-Baltzer, Franco Quadrifoglio, Gregor Reid, Volker D. Rusch, David Taylor-Robinson, and Dirk van der Waaij

The day and one-half period of discussion among the invited speakers touched on a variety of subjects which did not always proceed in an organised fashion. At times a subject was revisited from a slightly different perspective. Thus, for ease of reading, the discussion points are not reported in a chronological fashion but are organised according to topic as far as possible.

The discussion opened with a focus on urinary tract infection (UTI). Hooton questioned whether giving lactobacilli would be helpful in addressing recurrent UTI which has been observed in women aged 14 to 68 in Seattle studies. It was agreed that we are lacking knowledge about the value of such therapy in the young, prepubertal, and the postmenopausal. Hooton reported that knowledge of the incidence of UTI in postmenopausal women, with or without hormone replacement therapy (HRT) is not known or even under study. Some data are coming from Seattle, in studies that are currently underway, regarding lactobacilli and UTI in women aged 50-70.

Data do exist regarding bacterial vaginosis (BV) in postmenopausal women. There is a significant incidence of BV in postmenopausal women and it is known that lactobacilli populations are highly variable in postmenopausal women and is not related to age of the individual. The relationship of numbers of lactobacilli to glycogen content of the vaginal epithelium is not firm (Cauci).

British workers are studying postmenopausal women in an STD clinic as well as younger women in a clinic for lesbians. In the latter, approximately 50% are presenting with BV while in the former, low numbers of lactobacilli are observed and a higher incidence of BV than would be expected (Taylor-Robinson).

Discussion ensued regarding the clinical and laboratory basis for a BV diagnosis. There was agreement that elevated numbers of *Gardnerella vaginalis*, a diminished number of lactobacilli, an increase in anaerobes, a pH of greater than 4.5, the presence of clue

cells, a positive "whiff" test, and a Nugent score of 5-6 was consistent with BV. These assessments are based on the Gram-stain of a vaginal swab and are quite subjective; it was felt that a more quantitative approach is desired in order to better standardise BV diagnosis among different laboratories and clinics.

Taylor-Robinson described a longitudinal study of BV in women, based upon vaginal swab slides, in which the participants were going along fine and then observed a sudden fluctuation in bacterial numbers. This change tended to correlate with the early part of the cycle when the menstrual flow was heaviest. Carter asked if there might be growth factors for *G. vaginalis* in the sloughed endometrium similar to erythritol in the mammalian placenta which is a growth factor for brucellae. This remains to be determined.

The discussion moved to biofilms, described by Reid and others referring to William Costerton's work, in which a community of different microbes exist in a matrix of protein and polysaccharide on mucosal and other surfaces, such as tampons, with the anaerobic flora predominating close to the bottom. De Buysscher led the discussants through a review of Freter's scheme for microbial homeostasis on mucosal surfaces in the intestine. This scheme is based on antibody selection of populations of bacteria expressing continually changing surface antigens: The bacteria initially present (Colony A) express antigens (Ag) 1,2 which grow and expand their populations. The antigens drain, in the case of the vagina, to the iliac lymph node where an immune response is initiated. The antibody produced against Ag 1,2 selects against the population of bacteria present in the vagina and induces that population to change by expressing slightly different Ags (1',2',3) creating Colony A'. These bacteria would grow and expand the concentration of Ags 1',2',3 in the vagina and lymph and a subsequent immune response would select for a new colony, B. Since it has been suggested that the amount of antibody present in the vaginal mucosa and the thickness of the mucosa varies with the time in the menstrual cycle, it may be that the shower of antigens from the vagina into the lymphatic drainage also varies with the cycle. In this case the stimulation of the draining nodes would rise and fall on a 28 day cycle in women between menarche and menopause. What happens in postmenopausal women would, presumably, be different. Cauci questioned whether the thickness of the vaginal mucosa, known to be thinner in postmenopausal women not on HRT, did in fact change with the time of the month. Hooton reported that vaginal biopsies of university-aged women, done as part of a study conducted in Seattle, did not show any variation in thickness with the time taken during the menstrual cycle. This contradicts the published information cited by De Buysscher that indicates high progesterone present after menses correlates with a thinner vaginal epithelium which is rich in IgG-containing cells. Taylor-Robinson showed data from experiments in London in which mice were given exogenous oestrogen. Such treatment increased the number of indigenous bacteria in the vagina of the mice and increased the susceptibility of the mice to vaginal infection with other microbes, such as mycoplasmas. It was noted that the vaginal specimens showed an increase in the number of sloughed epithelial cells, without evidence of neutrophils, and these cells were covered with bacteria, suggestive of clue cells in the human. Mattsby-Baltzer commented that oestrogen is known to increase the viscosity of vaginal mucus.

In discussing such exogenous affects on the flora, Taylor-Robinson commented that semen appears to affect vaginal flora composition, that semen

from different men can cause a change in a woman's flora, and asked if amoxicillin therapy would do the same and predispose to BV. Krohn said that Hillier's reports do not support such an affect.

Hooton mentioned that UTI in older women has been suggested to be related to inadequate bladder emptying (and the same for men). This is in the literature but there is little or no evidence supporting it; the data on UTI in postmenopausal women is for the most part lacking. Reid asked what protects young or elderly women from UTI? Hooton reported that higher concentrations of *E. coli* have been observed in women with recurrent UTI but the enigma is that you can find such high counts in women without UTI. This may relate to differences in virulence factors. Reid mentioned that mannose-resistant, low fimbriae strains are also found in UTI.

Cauci stated that *E. coli* produce haemolysins and toxins in addition to having attachment factors – is antibody to these present in vaginal fluids and, if so, does this antibody diminish with age as is typical of other immune responses in the elderly? De Buysscher drew on what is already established in animals where antibody to *E. coli* attachment factors is highly protective. He also commented that, although new immune responses are not so active in older animals, they have a larger "library" to draw upon than the younger animal or person. Mattsby-Baltzer stated that no correlation has been observed between UTI and *E. coli* haemolysin production, attachment factors, or serum resistance. Hooton looked at fimbriae and haemolysin production in UTI strains from patients with symptomatic UTI or asymptomatic bacturia and found no correlation; there was a correlation with strains from cases of pyelonephritis and the presence of P fimbriae. By convention, asymptomatic bacturia is $>10^5$/ml of urine from several samples over a period of days. Mattsby-Baltzer reported than in a Swedish study, symptomatic versus asymptomatic bacturia was correlated with the presence of K1 capsules in the *E. coli* strains isolated; so-called "high responders" presented with symptoms. De Buysscher asked if the *E. coli* from asymptomatic bacturia patients are coated with antibody since in animal models they are. This was not known. Hooton reported that ~60% of *E. coli* from pyelonephritis cases lack P fimbriae.

Cauci reported on her study of 800 premenopausal and 2,000 postmenopausal women not on HRT. In the latter group, no lactobacilli and no bacteria, generally, were observed on Gram-stain (estimate: >1,000/ml in order to observe) and that the incidence of BV was approximately half that of premenopausal women or postmenopausal women on HRT (related to change in sexual activity?). It was suggested that Hillier or Hill might have additional data on postmenopausal women.

Hill asked what factors affect vaginal health. What is the normal bacterial population and how is it partitioned in the sense of biofilms and vaginal site (introitus, vaginal vault, cervix)? How do spermicides, intercourse, tampons, and diet affect the population? It was agreed that current knowledge is soft and that better defined studies on larger numbers of women are necessary to fully answer these questions. Krohn commented that physician or self-collected vaginal specimens show that bacterial populations are fairly consistent for an individual over time but that there is variation among individuals. Reid asked if there is a change in flora during times of tampon use; over one hour or eight hours. It is known that tampons, as one biomaterial which has been studied, do not induce an anaerobic environment, such as has been associated with BV, if anything, just the opposite (Davis). But it is not known

whether tampons alter the vaginal flora. Reid commented that we know that in a study of stents, over 90% had biofilms on their surface. This opened a discussion of whether we need a new paradigm for quantifying bacteria. We already recognise that plate counts do not accurately assess the number of bacteria in a sample which is why we speak of colony-forming units (CFU) but do we need to find a method which takes into account the mixture of bacterial types found closely associated in biofilms? In referring to the Seattle studies of vaginal wall biopsies, Hooton questioned whether there is a total turnover of vaginal epithelium and its attendant biofilm. The consensus was that the epithelium turns over continually and thus the biofilm would not be disrupted. Reid mentioned that *Candida* is the major cause of diaper rashes and that the *Candida*-biofilm interface is an important area for research into understanding yeast invasion of the mucosa.

Carter raised the issue of how food may influence the vaginal flora, not just the intestinal flora, and may sensitise individuals to yeast antigens as Dr. Orian Truss and others have suggested. Reid asked if yeast antigens are found in the blood stream which might cause systemic effects and whether they might be detected. (Note: German workers have found whole yeast cells in the blood stream of people following ingestion.) McDonald suggested that an appropriate experiment would be to compare vegetarians and nonvegetarians in regard to their complaints of suggested *Candida*-related symptoms. Van der Waaij and Krohn both commented on the difficulty of doing dietary studies correctly and there was general concern about the controls used by Truss and others. Van der Waaij indicated that people in dietary studies could not be trusted to stick with the protocol without being virtually held in confinement. Krohn mentioned that groups working for years on the influence of diet on heart disease or cancer have established consensus protocols which are well-accepted for conducting dietary studies. Hard data is urgently needed in this regard since people are reacting to anecdotal reports and trying a variety of remedies, reported in the popular press, in attempts to find some resolution of their chronic/recurrent UTI or vaginal discharge.

Hooton asked whether probiotics in food or vaginal suppositories work better if the strains are peroxide producers. Reid suggested using a mixture of peroxide positive and negative strains and collect data on colonisation from human volunteers after per oral administration. Taylor-Robinson reported that in their hands, a peroxide-negative, bacteriocin-positive lactobacillus strain inhibited *Gardnerella*. Maes stated that probiotics have had broad use in food animals but not for vaginal problems. Reproductive tract problems in food animals are generally localised in the uterus without involvement of the vagina (although studies of vaginal microecology have not been published and vaginal discharge, without metritis is not pursued). The point was made that food animals vary from primates in that they mate only during oestrus and so may not be as susceptible to vaginal infections outside of pregnancy. Taylor-Robinson cited Hillier's report of the use of peroxide-positive *Lactobacillus acidophilus crispatus* in women: Administration of either 10^5 or 10^8 CFU results in vaginal colonisation for months at the level of 10^6 CFU. If the woman was already colonised with peroxide-producing lactobacilli, colonisation with *L. a. crispatus* was better than when the woman was not. Reid commented that relying on one strain was probably not the correct approach and that a cocktail would be better. Carter asked Heidt and van der Waaij to comment in this regard based upon their work in animals and immunodeficient patients. Van der Waaij

suggested looking at the immune response to the lactobacilli to determine which may be more persistent. When autologous probiotics were suggested, he cautioned against the "French kitchen" approach of a little bit of this and a little bit of that. We need hard data to make these decisions. McDonald mentioned that she is aware that. *L. a. crispatus* can colonise individuals for five years and this is not autologous. McDonald added the cautionary note of being careful not to introduce lysogenic phages, the plague of food manufacturers, which might destroy a person's natural flora of lactobacilli. Reid mentioned that exogenous lactobacilli will not displace *Candida, Proteus*, etc. which are already established in the vagina; antibiotic therapy must be given to deplete these organisms before *Lactobacillus* therapy can be successful.

Mattsby-Baltzer noted that BV is sometimes difficult to diagnose if the patient lies on the fringes of what is accepted, i.e. lacking one or more of the diagnostic features or having a vaginal pH not much above normal ranges. She asked if there might not be subgroups of BV which could be recognised if the diagnostic parameters were better characterised or standardised; should we culture all cases and emphasise a decrease in lactobacilli and an increase in anaerobes in the diagnosis? Currently, some studies report only the Nugent or Amsell score. McDonald mentioned that BV patients with *P. mobiluncus* present with an intense inflammatory response which may suggest we are dealing with a different disease. Krohn felt that it was time to go past the Nugent criteria, which represented an initial attempt at standardisation of clinical evaluation and help predict an uncommon outcome in large study populations, and determine which anaerobes are associated with which clinical presentations. The Amsell score is based upon odour, which is very subjective and pH (it is easy to determine pH 4.0 or 5.0 with litmus paper but pH 4.4 – 4.7 is a problem; thus if pH 4.5 is diagnostic, you have variability in the determination of this diagnostic criterion). The Nugent scoring presents a problem in the evaluation of intermediate flora. In clinical BV, Cauci felt the Nugent score was not all that dependable because bacteria other than *Gardnerella vaginalis* matter. She demonstrated this with data presented in the following table:

Lactobacilli	*G. vaginalis*	*P. mobilis*	Nugent Score
2* (2)	>30 (4)	-	6
5 (1)	>30 (4)	-	5
25	5	1	5
25	2	1	4

* bacteria seen / microscopic field

Taylor-Robinson stated that BV is certainly not homogeneous and that the lack of uniformity among laboratories, reported at the recent Aspen meeting, was astounding. The strongest association with preterm labour is in women with BV, especially those with BV plus *M. hominis*. He asked whether antibiotic therapy was known to affect preterm delivery. Mattsby-Baltzer asked whether BV during pregnancy might indicate upper tract infection. Only a subgroup, responded Krohn, who cited Hillier's finding of *Bacteroides* in the chorionic membranes of some BV women but not all. Cauci and Mattsby-Baltzer mentioned that BV was observed in about 20-30% of pregnant women (and even nonpregnant) in Italy and Sweden. Krohn mentioned that bacteria are found in the amniotic fluid of less than 1% of women not in labour.

Mattsby-Baltzer asked about the basis for recurrences in BV. Taylor-Robinson said there are two groups: Those clinically cured who then develop a new infection and those not really clinically cured who have a recurrence. Krohn estimates that there are 10-20% of women of child-bearing age in the USA who have BV at any one time (12% in the UK [Taylor-Robinson] and the same in Australia [McDonald]); approximately 80% of treated women are cured. Blacks have consistently higher prevalence of BV that is an apparent enigma since, in general, they douche quite a bit more than white women. Taylor-Robinson mentioned "difficult" cases, women who keep coming back, and back, and back. The approach has been to treat with metronidazole or clindamycin at intervals so that the *G. vaginalis* populations get lower and lower.

Taylor-Robinson asked if vaginal bacteria were able to infect cells like mycoplasmas do. Mattsby-Baltzer reported that *Prevotella* strains adhere closely to the cell membrane of HeLa cells, as is typical of mycoplasmas, but only a few appear to enter. McDonald mentioned in pregnancy, Gram-stains show 1+ to 2+ neutrophils. Taylor-Robinson maintained that a PMN response in BV is rare. Several discussants mentioned that after 41 weeks gestation, there are a lot of inflammatory cells invading the term placenta.

Hooton reported that a recent Medline literature review he did, reflected the pitifully low number of researchers world-wide addressing the problem of UTI (20-30). He made a free-hand drawing of the incidence of UTI in females throughout life which reflected the blip among the very young, low levels through childhood with increasing incidence in the adolescent and fertile woman to menopause, with the highest incidence being in the elderly woman. This latter may be related to elderly care facilities in which the cleanliness of the elderly person may not be as good, due to inability of the person to care properly for herself and inadequate staffing. Hooton felt that the data on UTI on the postmenopausal woman, below 70, was the softest and felt that more epidemiological assessments needed to be made in this age group. BV, on the other hand, peaks in the 20's and 30's and tapers off soon after menopause. The statistics are somewhat difficult to assess since a lot of UTI are just asymptomatic bacturias. 50, 60, 70% in institutionalised men and women and this is not really proven to be due to anatomical changes with age which lead to inadequate bladder emptying. Citing Stamey's work, Hooton mentioned that women who develop UTI are observed to have the causative organism in their vagina months prior to presenting with UTI. No genetic typing of the microbes has been done to confirm that it is the same organism; most studies in this area only use culture. The question was asked whether quantitation of the microorganisms should also be done. It was mentioned that heavy growth of *E.*

coli is associated with adverse pregnancy outcomes. A further question was asked if recurrent UTI are caused by the same risk factors as sporadic cases. It was admitted that there is very little data on premenarchal girls since pelvic exams and thorough work-ups are usually only done on such girls if raped or abused.

Hill returned to the need for data on normal flora saying that you cannot know the importance of the vaginal flora in disease if more isn't known about it in health. Very little work has been done on normal flora and its role in candidosis. With the availability of over-the-counter vaginal creams for the treatment of yeast infections, young women are using these at the first sign of itching. Hooton said these creams, themselves, are known to alter the lactobacilli in the flora and so their broadened and increased use may have a negative effect and predispose the user to subsequent vaginal infections. Krohn reported that, in a recent study, 20-25% of university-aged women had used Monistat® in the past five months and that a higher incidence of Group B streptococci were noted in these women; there has been no longitudinal study of vaginal floral changes after anti-fungal use.

Reid returned to BV maintaining that we need much better data on its incidence and prevalence. To do this, BV needs to be better defined. This was echoed by McDonald who felt that more research into the parameters defining BV was desperately needed. Cauci felt that the clinical diagnoses, when made, are correct but as many as 30% of cases were being missed. Hooton felt that the same problem with definition exists with UTI and the vaginal flora. UTI is diagnosed if the bacturia is $>10^5$/ml but in Seattle, experienced clinicians will go as low as 10^2/ml.

Carter raised discussion of the work of Truss and others suggesting a role for subclinical candidosis and diet causing local and systemic health problems in many women. Heidt mentioned that dietary influence is not known but in studies of bone marrow transplant patients, antibiotics and chemotherapeutics did cause the intestinal flora to change and were associated with an increase in *Candida* (this increase was not profound).

In response to comments by Carter and McDonald on the ability of ureaplasmas to produce disease, Taylor-Robinson said that ueaplasmas were strongly associated with chorioamnionitis but their role in spontaneous abortion is undetermined. He cited a 1M pound MRC study, based upon the assumption that *U.urealyticum* is a sole cause of disease. Maurice Shepard's work on non-gonnococcal urethritis in male Marines could be faulted by his lack of data on *Chlamydia* which might have been present. Also, as demonstrated by Japanese and British workers, the presence of *M. genitalium* can only be confidently assessed using PCR since this organism is so difficult to culture. This having been stated, it was admitted that *U. urealyticum*, in pure culture (10^5 intraurethrally), will cause NGU.

Cauci noted that cultivation of anaerobes is difficult and expensive and some clinical microbiology laboratories are reluctant to undertake it. She maintained that a woman can have increased numbers of bacteria but if the host is able to counteract them immunologically or with other defence mechanisms, there is no clinical problem. It was suggested that a survey of IgA-deficient women should be performed. Others felt that IgA-deficient individuals generally do well because they have compensatory local responses. A thorough assessment of local immune responses in the vagina needs to be pursued.

De Buysscher returned to a discussion of local immune responses. Cauci reported that vaginal histamine and IgE increased in candidosis so perhaps in-

gestion of yeasts or yeast products induces an enhanced reaction in persons who are presensitised.

The session ended with a comment about HIV infection. It is apparent that vaginal inflammation increases the risk of HIV infection in unprotected sex with infected partners. If BV, even without inflammation, also enhances risk of HIV infection, this would represent a significant at risk population.

Carter said that he is left with the conclusion that *Chlamydia* are the only recognised frank pathogens of the urogenital tract. Others felt that this was true to a certain extent and that appropriate animal models would be very helpful in advancing the field. Maes discussed the problems of using animals which only mated during oestrus. Swine are reasonable models for uterine infections but little else. However, he did admit that this view might be based on the fact that vaginal discharge is not associated with a drop in fecundity and therefore has not been important in the pork industry and thus not studied by swine veterinarians. A closer look at the vaginal flora and vaginal infections in swine may reveal useful information relevant to the human. It was agreed that rhesus monkeys may provide the best model for studies of human relevance because of menstruation and their sexual habits. Such animals could also be used in controlled dietary studies since they, like swine, have a digestive tract similar in many respects to the human.