Vaginal Microbicide Formulations Workshop

Editor

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Contraceptive Research and Development Program
Arlington, Virginia
Proceedings of the Fifth Contraceptive Research and Development Program Workshop
Bethesda, Maryland
November 7, 1997

Held in collaboration with the
National Institute for Allergy and Infectious Diseases
and the
National Institute of Child Health and Human Development

Major financial support was provided by
United States Agency for International Development
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Preface

At the Natcher Conference Center on the National Institutes of Health campus in Bethesda, Maryland, this workshop brought together 120 participants from six countries—academic, government, and industry researchers interested in the challenge of developing effective vaginal contraceptives. On November 7, 1997, the National Institute for Allergy and Infectious Diseases (NIAID), the National Institute of Child Health and Human Development (NICHD) and the CONRAD Program, supported by the U.S. Agency for International Development (USAID), convened the Vaginal Microbicide Formulations Workshop.

The challenge of developing effective vaginal contraceptives and microbicides has been with us for a long time. Many of the participants supported the idea of a vaginal formulation workshop in which information about this important area could be shared among knowledgeable professionals.

The goals of the workshop were as follows:

- To present the physiologic characteristics important to formulators
- Review the vaginal bacterial flora in vaginal health and the potential effects on the microflora after acute and chronic vaginal exposure to products
- To review preformulation considerations in designing a temporal and spatial drug delivery system
- To review conventional and novel formulation approaches used to promote even vaginal distribution and prolong retention
- To promote discussions to plan future work and answer the question, “What is needed to advance vaginal formulation technology?”

Science is propelled by the exchange of information. This volume includes the presentations made at the plenary session and at the following discussions. The discussions illuminate our challenge to improve current in vitro, animal model, and in vivo techniques and to remove the uncertainty of product behavior after vaginal administration. The workshop focused mainly on contraception and sexually transmitted disease prevention; however, the knowledge presented can be applied to other intravaginal therapies. This book transcribes thoughts and ideas elicited by a lively meeting. Although the printed work can never fully capture the dynamics of the event nor the formal and informal exchanges that occurred, it is being published so that a wider audience might benefit.

William F. Rencher, Ph.D.
Acknowledgment

We are greatly indebted to Ms. Marlene LaMont for her tireless efforts in registration and in tending to many, many details of the event.

Special thanks go to Ms. Mary Williams, freelance copy editor, for her help in revising several chapters and the meeting transcript.
Opening Remarks

CONRAD Program

It was with great pleasure that I welcomed the participants on behalf of the CONRAD Program. The great interest that was shown in attending this workshop attested, I believe, to the importance of the topic and, unfortunately, to the rather neglected state of formulation development that has existed with respect to spermicides and microbicides until recently. We hope that the information presented will be useful in guiding those of you who face the task of finding esthetically acceptable and efficacious ways of delivering the active compounds that you are investigating.

I thank the United States Agency for International Development, the National Institute of Allergy and Infectious Diseases, and the National Institute of Child Health and Human Development for providing the funding for this workshop. I also thank Bill Rencher, who took over at CONRAD for Paul Krause and who ably completed the organization, and most of all, I thank the speakers for their presentations and manuscripts.

Henry Gabelnick, Ph.D.
Director, CONRAD Program

National Institutes of Health

On behalf of both the National Institute of Allergy and Infectious Diseases (NIAID) and the National Institute of Child Health and Human Development (NICHD), I took great pleasure in welcoming the participants to this conference. CONRAD put a monumental effort into organizing the meeting, and we all recognized how outstanding was the list of speakers and topics. I think that the results of the meeting will be a real benefit to everyone working in this area. The background and support of NICHD is fairly obvious to those who have worked in the area of contraceptive development. The support and the interest of NIAID may be a little less obvious. About 3 years ago, the division of AIDS in NIAID decided to supplement their traditional approaches to treating HIV infection, those being vaccine and therapy development, by encouraging the development of products that could be used by women intravaginally to prevent the transmission of HIV in heterosexual situations. Shortly after that decision was made, we in the Division of AIDS began using our extramural contract resources to help academic and small pharmaceutical sponsors in developing the database that was needed to go to the FDA with an IND for these kinds of products.

In the development of an IND and in the preclinical data to support this
research, we very rapidly discovered a major void of information and knowledge in how to develop the semi-solid delivery vehicles that were needed for this kind of approach. At about the time I began developing the idea for the meeting, I discovered that Drs. Gabelnick and Krause at CONRAD were virtually in lockstep with me in developing a parallel meeting. The pooling of our resources led to the present proceedings.

CONRAD developed the list of speakers, organized the topics, and provided suggestions for the speakers, and I think that they did an outstanding job. It was a stimulating and productive meeting.

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The Vagina: Physiologic Characteristics Important to Formulators of Microbicides

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The physiology and anatomy of the vagina have been relatively poorly studied compared to those of other female pelvic organs. Traditionally, anatomic descriptions have focused on surgical correction or repair, with few well-designed investigations pertaining to physiology. However, our understanding and appreciation of human sexuality and the vagina have grown considerably in the past few decades. When not sexually aroused, the lumen of the human vagina is considered a potential space and the vagina is considered to have little function. In contrast, during sexual arousal there are dramatic physiologic changes. This chapter reviews human vaginal anatomy and physiology, with special considerations pertinent to the formulation of microbicides and the design of vaginal delivery systems.

ANATOMY

The vagina is a fibromuscular tube that exists in a relaxed state. However, this tube is not symmetrical or similar to any known geometric shape. The vaginal lumen is a potential space with walls that are easily distensible. The overall shape of the vaginal canal and its distensibility are constrained by the elasticity of the vaginal wall and its relationship to other pelvic organs.

The vagina extends from the introitus to the uterus and connects the two. The introitus of the vagina is located between the urethra and the symphysis pubis, which are superior boundaries, and between the posterior fourchette, rectal sphincter, and rectum, which are the posterior boundaries of the introitus (1). In a woman standing upright, the lower one-third of the vagina is directed obliquely upward and backward at approximately a 45° angle to the horizontal axis. The vagina has a convex curve with the upper vagina being at
almost a right angle to the lower vagina. The upper two-thirds of the vagina lies almost parallel with a horizontal axis (1). Although the cervix, uterus, and upper vagina have considerable mobility, they are fixed in position over the muscular levator plate by the cardinal ligaments. The length and flexibility of these ligaments permit the cervix and upper vagina to be moved somewhat in any direction over the pelvic structures. The vagina is also attached to the lateral pelvic wall by condensations of connective tissue and smooth muscle intimately adherent to the adventitia of the vaginal vessels. These attachments tend to stabilize it in position from side to side, and the muscle elements supply a certain amount of tone, enabling the vagina to adapt to changes in intravaginal and extravaginal pressure.

The anterior and posterior walls of the vagina are slack and remain in contact with each other. The lateral walls of the vagina are fairly rigid and are defined by the anatomic pelvic support. The lower third of the vagina is supported by connections between fibers of the pelvic and urogenital dia­phragms. The middle third receives its main support from the lateral and inferior segments of the cardinal ligament. The upper third of the vagina mainly rests on the rectum, which overlies the pubbococcygei of the levator plate. These walls remain separated. Therefore, in cross-section, the vagina has a classic H-shaped appearance (1). The dimensions of the vagina vary considerably from woman to woman, depending on both sexual arousal and reproductive stage. In a normal reproductive-aged woman (after puberty and before menopause), the anterior wall of the vagina measures 6–8 cm in length and the posterior wall is up to 14 cm in length (2–4). Because the cervix is incorporated into the anterior vaginal wall, the length of the anterior vagina plus cervix approximates the length of the posterior wall. The range of the interior diameter is 2.4–6.5 cm. The range of greatest width of the vagina is 2.1–5.0 cm, and the depth at greatest width 2–5 cm from the introitus (2).

The shape of the vagina also varies considerably among women. Vaginal casts from dental casting material have demonstrated the variety of vaginal dimensions (2). Common features of the vagina include a posterior widening around and behind the cervix, with posterior fornices being quite deep in some subjects. Anterior to the cervix, the caliber of the vagina is constricted, especially near the introitus. The pattern of rugae in the vaginal mucosa is distinct in almost all subjects. Vaginal shapes can be divided into at least four groups. Type 1 is characterized by long, almost parallel lateral walls. Type 2 is conical, with the posterior fornix noticeably wider than the anterior canal. Type 3 is heart-shaped, characterized by marked distention of the posterior fornix and a constriction at the mid-fornix to produce a scallop or heart shape. Type 4 has distention of the anterior and lateral walls into a distinct bulge or balloon shape (2). Dimensions and shape are not significantly associated with parity. These studies provide important evidence of the great variety of vaginal shapes, sizes, and introital diameters. Clearly these findings call into question the wisdom of a “one size fits all” vaginal product or design.
BLOOD SUPPLY

The uterine and pudendal arteries, both of which arise from the internal iliac artery, are the chief sources of blood supply to the vagina (1). In general, the arteries are bilateral with multiple collateral vessels. They enter the vagina laterally and unite at the midline through diffuse anastomoses. Branches of the uterine and pudendal arteries also form anastomotic branches with the inferior vesical and middle rectal arteries. They form a plexus around the vagina from which a median artery arises on the anterior and posterior walls. These arteries are sometimes called the azygos vaginal arteries. A rich venous plexus surrounds the vagina and communicates with the vesicular, pudendal, and hemorrhoidal venous plexuses, which empty into the internal iliac veins. Venous drainage of the vagina begins in small sinusoids that drain into adjacent venous plexuses. The vaginal veins communicate with plexuses in the paravaginal tissue, perineum, rectum, and bladder (1). The veins are usually thin-walled, containing few valves. They drain the pelvic plexuses along the course of their corresponding arterial supply.

VAGINAL ABSORPTION

The vagina’s proximity to the rich surrounding anastomoses and to the arterial supply make it an ideal route for drug administration. The vagina is lined by stratified squamous epithelium that is rich in glycogen during the reproductive years. The rugal folds of the vagina provide a certain degree of distensibility to minimize laceration. The vaginal epithelium is composed of five layers (5). The surface of the vaginal cells is made up of many microridges that run longitudinally (6). It is believed that the morphology and pattern of the microridges affect the firmness of the epithelium (7). Traversing the vaginal epithelium is a system of intercellular channels. This network provides a route for transport of macromolecules, fluids, and cells from the basal lamina to the vaginal lumen. Migration may also proceed in the opposite direction, a fact that may have important implications for drug absorption.

The mechanisms involved in vaginal drug absorption obey many of the basic concepts developed from studies of gastrointestinal drug absorption (8–12). Drug absorption depends on the physicochemical properties of the drug in terms of molecular weight, dissolution characteristics, and ionization properties. Absorption may proceed by simple diffusion and/or by active transport. Many drugs, however, are weakly acidic or basic. Therefore, the equilibrium dissociation constant of the drug, the microenvironment pH of the delivery vehicle, and the vaginal pH have an important influence on the extent of drug absorption. Under steady-state conditions, drug diffusion across the vaginal mucosa can be described by Fick’s Law:

\[-dM/dt = D_mSK/h (C_v - C_p)\]
where \(M\) is the amount of drug in the vagina at time \(t\), \(D_m\) is the diffusivity in the vaginal membrane, \(S\) is the surface area, \(K\) is the partition coefficient between the aqueous medium of the vagina and the vaginal epithelium or membrane, \(h\) is the thickness of the vaginal epithelium, and \(C_v\) and \(C_p\) are the drug concentrations in the vagina and plasma, respectively.

The equation can be rearranged to describe the permeability of drug across the vaginal epithelium. By converting the left side of the equation to concentration units, the permeability coefficient is described below:

\[
P_v = -\frac{V}{C_v} \left( \frac{dC_v}{dt} \right)
\]

where \(V\) is the volume of the vaginal compartment and \(P_v\) is the permeability coefficient (cm/sec) for drug passage from vagina to plasma. It should be noted that certain stimulating hormones, such as estrogen, increase the thickness of the vaginal epithelium and thus alter drug permeability (8). In short, vaginal drug absorption is influenced by the interplay of pH, drug dissociation and lipophilicity, membrane permeability via lipid and aqueous pore pathways, and permeability across the aqueous diffusion layer.

**VAGINAL DRUG DISTRIBUTION**

Drug distribution in the vagina is dependent on a number of mechanisms. A drug may distribute topically through the vaginal canal in an antegrade or retrograde manner or by absorption across the vaginal epithelium, as mentioned above. Therefore, topical distribution may account for drug loss through the introitus and for transport from the vaginal fornices to the upper reproductive structures. After vaginal absorption, simple diffusion across parenchymal and interstitial structures may also account for local distribution. In this case, drug penetration into proximal tissue structures depends on similar physiochemical factors to those previously discussed for drug absorption. The predominant route of drug distribution after vaginal absorption, however, occurs via exchange with the rich venous plexuses of the vagina, followed by systemic transport. Studies show that vaginal drug absorption leads to elevated levels of the drug in all the reproductive structures within 4–6 hr (13–15). Intuitively, venous transport is a unidirectional process favoring systemic absorption rather than redistribution to proximal pelvic structures. This raises the possibility that other mechanisms are involved in distributing the drug after vaginal absorption. As mentioned above, retrograde transport through the endocervical canal, uterine cavity, and fallopian tubes may be partially responsible. A countercurrent model involving exchange of material between arterial and venous segments of capillaries has also been proposed to explain the relatively rapid kinetics of drug uptake by the upper reproductive organs (16–19). Researchers have described countercurrent ex-
change in the uterine adnexa, kidney, and small intestine (20–22). The mechanism is dependent on the close association between venous and arterial limbs in a vascular loop. A close anatomic relationship exists between the tortuous arteries and veins of the reproductive organs. First, drug absorption is followed by distribution along various regions of the reproductive tract via venous plexuses. Next, countercurrent exchange may occur between adjacent arterial vessels, leading to drug distribution to organs perfused by their respective arterial supply (16–19).

PELVIC AND VAGINAL MUSCULATURE

The complex musculature surrounding the vagina can be categorized as voluntary or involuntary. The muscles surrounding the introitus of the vagina and the muscles that make up the pelvic diaphragm are under direct voluntary control (1). The size, power, and control of these voluntary muscles vary enormously among women. The effect of contractions of these muscles on the distribution of vaginal fluid has never been studied. The vagina passes through the muscles of the pelvic floor at a distance approximately one-third from the introitus. The posterior two-thirds of the vagina sits atop a muscular plate. Therefore, it can be hypothesized that voluntary contraction of the muscles of the pelvic wall may cause dependent vaginal secretions to remain in the posterior aspect of the vagina.

An additional confounding group of muscles is represented by the muscles of the vagina itself. The smooth muscles are arranged in bands completely surrounding the vagina, without a definitive border at the posterior end of the vagina. The temporal patterns and exact manner of contractions of these muscle groups, including those involuntarily controlled, are largely unknown. Spontaneous contractions occur in the resting, non-sexually stimulated vagina. Women are unaware that such contractions occur. Constrictions originating from the cervical end of the vagina have been reported to occur as frequently as every 8–10 min. However, there is debate about the existence of these contractions. Opponents of this theory suggest that the balloons inserted into the vagina to test these contractions may actually cause them. Other studies have only demonstrated large spontaneous contractions of the vagina infrequently over a 4–6 hr period (23). Although the pattern of contractions of the vagina is poorly understood, it is not unreasonable to assume that there is some sort of mesenteric reflex of the vaginal mucosa similar to peristalsis.

VAGINAL HISTOLOGY AND ULTRASTRUCTURE

The mucosal layer of the vagina in a woman of reproductive age is lined with stratified squamous epithelium that is rich in glycogen. The epithelium of
the vagina is comparable to that of the oral cavity in cell structure, but the vaginal mucosal layer is composed of multiple rugal folds, dramatically increasing the surface area (1,3). The epithelium thickens with the estrogenic stimulation of puberty and thereafter responds cyclically to ovarian production of estrogen and progesterone. These cyclic changes are not grossly evident, although they are reflected fairly accurately in vaginal smears and type of discharge. With estrogen alone, the mucosal cells undergo a process of maturation, becoming progressively thinner and flatter. Progesterone inhibits this maturation to some extent, causing the epithelium to mature up to the superficial layer (3). The superficial epithelial cell layers in the vagina do not appear to have a barrier function similar to that of keratinized epithelia. Although the vagina itself contains no glands, there are remnants of the mesonephric and paramesonephric ducts, which can be found at almost any location within the walls. Because the vagina is lined by squamous epithelium and not a mucous membrane, it is incapable of producing mucus or secretions. However, cervical mucus or secretions from endometrial or salpingeal mucous membranes collect in the vagina. A modified serum transudate also contributes to the fluid found in the vagina.

Electron micrographs of human vaginal epithelium show densely packed cells in the superficial layer with narrow, cycle-independent intercellular spaces (3). Deeper in the epithelium the intercellular spaces widen and become large. The epithelial surface is moistened by fluid, some of which originates from the uterus and cervix and some of which is a transudate from the epithelial vascular bed that is passively transported through the epithelial intercellular space to the surface. The intraepithelial transport system allows large molecules, including proteins, to pass.

The vaginal mucus contains macrophages, lymphocytes, plasma cells, Langerhan's cells, eosinophils, and mast cells. During infection, lymphocytes, Langerhan's cells, and macrophages can migrate into the intercellular channels. Leukocyte invasion, especially that of lymphocytes, is cycle-dependent, with a peak at menstruation. Lymphoid nodules with macrophages and T and B cells are present in the vaginal wall. Macrophages are stimulated by estrogen to phagocytic activity, which could be a positive factor in vaginal macrophage function with respect to resistance to infections (3). However, these cells, and especially Langerhan's cells, may be recipients of cell-free or cell-associated viruses, such as HIV. Cell-associated HIV virus may be primarily responsible for transmission across intact epithelial surfaces (24). It has also been hypothesized that cell-free viruses can infect mucosa when present in high concentrations or when epithelial surfaces have been disrupted. Studies of discordant couples have demonstrated that older and postmenopausal women, who have low estrogen levels and atrophic vaginal mucosa, are at increased risk for HIV seroconversion because of increased risks of epithelial disruption during sex (24). This argument can also be used to explain why HIV transmission is augmented in the presence of genital ulcerative diseases.
that cause mucosal disruption (25). In addition, any compound that causes toxicity to the vaginal mucosa may disrupt the epithelium. This has been suggested to explain why a high dose or frequent use of nonoxynol-9 can disrupt the epithelium and increase a woman’s risk of seroconversion with HIV (24). It should be pointed out that the precise cells and tissues in which the female reproductive tract is infected with HIV are not known. A study of HIV transmission in hysterectomized monkeys suggests that the presence of the cervix is not required for transmission (24). However, this finding may not be easily generalized to humans and does not exclude an additional route of infection via the cervix or the upper genital tract.

**VAGINAL FLUID**

The amount, consistency, and character of the fluid that accumulates in the vagina are difficult to describe because these parameters change with the menstrual cycle and with reproductive age. However, there is a definitive type of normal vaginal fluid. The fluid is usually a clear mucus. It is a complex mixture, made up of a combination of secretions of the female genital tract and desquamated epithelial cells (26). The vaginal fluid can contain components from organs as far removed as the fallopian tube, but its main constituents are vaginal fluid transudate mixed with uterine and cervical secretions. The bulk of the fluid lies on the dependent portion of the vagina, and the secretions are not visible at the introitus (26). The predominant organisms of the vaginal flora are the anaerobic and facultative *Lactobacillus* species, *Bacteroides* species, *Peptococcus* species. *Corynebacterium* species, *Staphylococcus epidermis*, *Peptostreptococcus* species, and *Eubacterium* species. The vaginal pH of a normal menstruating woman is 3.5–5.0 but can change under a variety of circumstances (27). Common vaginal infection, such as *Gardnerella vaginalis*, increase the pH from 5.0 to 6. A woman infected with *Trichomonas vaginalis* has a vaginal pH greater than 6.

Vaginal fluid has lower levels of glucose and Na⁺ but higher levels of K⁺ than plasma. A hypothesis for these findings is that the plasma filtrate from the blood leaks out of the vascular capillaries and passes through the vaginal epithelium via the intercellular channels between the vaginal epithelial cells (3). These cells have a limited capability of reabsorbing Na⁺ ions. In the basal, sexually quiescent state, the slow trickle of plasma through the epithelium allows sufficient contact time for the vaginal cells to reabsorb a considerable fraction of the Na⁺ from the plasma so that the concentration of Na⁺ in the vaginal fluid at the surface of the epithelium is approximately half that of plasma. In contrast, the concentration of K⁺ in the basal fluid is much greater than that of plasma; values between 5 and 15 times that of that plasma have been recorded (3). Possible mechanisms for the increased K⁺ concentration include direct secretion of K⁺ by the vaginal epithelial cells or cell desqua-
tion from the vaginal surface and liberation of the contained intracellular K+ from cell disruption (3).

The quantity and quality of vaginal discharge in a healthy, normal woman have been subjected only to limited objective study. Most comments written in gynecologic textbooks are broad, sweeping statements such as, "there is a constant small amount of vaginal discharge present under normal circumstances" (25) or, "the physiological vaginal discharge is sufficient to keep the vagina and introitus moist without becoming externally apparent" (25). Historically, two methods have been used to try to quantify the volume of vaginal fluid. The amount of vaginal discharge determined by using tampons (weighed after use) demonstrates a mean of 1.55 gm of fluid produced in an 8-hr period (28). The greatest amount of fluid was noted on day 14 of the menstrual cycle, where the mean was 1.96 gm/8 hr. The lowest discharge was on day 7 (1.3 gm/8 hr) and on day 26 (1.37 gm/8 hr). The range and variability were quite significant. For example, the range of weight per 8 hr at day 14 was 0.74-3.49 gm. Another interesting point is that there was no difference between overnight and daytime sampling. Quantification of vaginal fluid by the use of a tampon may be an overestimate of vaginal discharge, given the inherent absorbency of the tampon, which may dry the vaginal mucosa (2).

Recently, investigators have advocated the use of a small plastic graduated vaginal aspirator to quantify the amount of fluid contained in the vagina, perhaps yielding more consistent results (29). Using these volume meters, a distinct pattern of amount and characteristic fluid was confirmed throughout the menstrual cycle. Less than 0.1 ml of fluid per day was collected in the few days after menstruation had ceased. The fluid volume then increased, starting on day 9 of a normal 28-day cycle and reaching a peak fluid volume of 0.9 ml/day on day 14 (coincident with ovulation). Thereafter, the volume decreased to less than 0.2 ml by day 19 of the cycle. It remained below 0.2 for 6 additional days and below 0.1 for the 6 days preceding the onset of menses (29).

CERVICAL MUCUS

A significant contributor to the vaginal fluid is cervical mucus. Cervical mucus varies in amount and viscosity depending on the stage of the menstrual cycle. Mid-cycle human cervical mucus varies in viscosity from 2,840 centipoise (cp) to 10,000 cp, with an average of 4360 cp (30). The amount of cervical mucus in the human vagina is approximately 2–5 ml. Estrogen stimulates the production of a thin isotonic mucus containing increased amounts of high molecular weight glycoproteins called mucins. Progesterone dominance in a menstrual cycle leads to the production of relatively dry, viscous mucous. (31) Mucus can be readily seen on the external os of the cervix and in the cervical canal. The contribution of cervical mucus to the vaginal fluid has never been fully quantified. However, it is common in normal, reproductive-
aged women to note the presence of a clear mucus-like external vaginal discharge around the expected time of ovulation (days 13–15 of the cycle).

**CHANGES IN THE VAGINA WITH SEXUAL AROUSAL**

According to Masters and Johnson's work (32), changes in the vagina during sexual arousal include an increase in vaginal lubrication, “sweating.” The inner two-thirds lengthens, distends, and dilates, resulting in “tenting.” During sexual arousal there is also engorgement of blood vessels in the genital region. The mechanism is believed to involve a closing of the normal arteriovenous anastomoses, preventing shunting of blood from tissue in the non-aroused state and resulting in a temporary decrease in venous drainage (22). The net effect of these physiologic changes is a decrease in the amount of blood exchanged in the pelvic area during sexual arousal. The mechanisms underlying the changes appear to be mediated by vaginal release of vasoactive intestinal peptide (VIP) (33). It is unknown whether these changes in pelvic circulation alter the absorption of potential agents into the blood supply of the pelvis.

The amount and character of vaginal secretions before and after sexual arousal have been analyzed. The predominant changes in vaginal secretions caused by sexual arousal appear to be quantitative, increasing two- to threefold. There do not appear to be any consistent qualitative changes in the small, volatile organic compounds during stimulation. Compounds noted to be increased by sexual arousal include lactic acid, glycerol, cholesterol, and stearic acids. These compounds may be plasma-derived and may reflect, in part, the composition of vaginal transudate (34).

A small but significant increase in the pH of the vaginal fluid is associated with sexual arousal. This change is approximately 0.2, or from a pH of 4.8 to 5.0. The baseline vaginal fluid is probably altered by the modified plasma transudate associated with sexual arousal. Overall, the change in pH found in this study was not felt to be significant compared to the possible change in vaginal pH after intercourse (35).

There may also be changes, in the muscular contractions, or peristalsis, of the vagina associated with sexual arousal. Masters and Johnson reported a series of vaginal contractions every 0.8 sec during orgasm (32). Although there has been extensive subsequent research to characterize the contractions of the vagina in both the sexually aroused and the non-aroused state, no clear pattern has emerged. At this time, therefore, there is no evidence to support any specific pattern of muscular contractions that would recreate the normal physiology of a woman in either the aroused or the unaroused state. It is also unclear if this pattern would be best replicated with a random pattern of simulated contractions.

During the resolution phase, after orgasm, the vaginal congestion disperses and the vaginal tenting resolves. On cessation of sexual arousal, the vasodi-
latation of the blood vessels subsides and the production of vaginal fluid by transudation gradually decreases to the basal level. The limited Na⁺-reabsorptive capacity of the epithelium can now cope with the absorptive load, and slowly the fluid is reabsorbed into the plasma as an osmotic consequence of the sodium transfer.

The most important change in the vagina occurs with sexual activity. During vaginal intercourse the vagina is no longer a potential space. Air may be introduced into the vaginal canal, changing the oxygen tension of the tissues. The vaginal fluid is moved to all areas of the vagina and is no longer confined to the dependent portions. After ejaculation, the amount of fluid in the vagina is, on average, tripled, and the pH rises rapidly to neutrality. The normal male ejaculate is 2–6 ml, has a pH of 7.2, and contains a higher concentration of fructose, prostaglandins, volatile amines, alkaline phosphate, and bicarbonate buffers than vaginal fluid (36). The ejaculate initially has a very high viscosity due to a coagulum that increases adherence of the semen to the cervix and the cervical mucus. This coagulum breaks down after approximately 15–20 min and the ejaculate liquefies. After liquefaction, much of the ejaculate is often lost as part of a vaginal discharge. The remaining volume of fluid in the vagina, a mixture of semen and vaginal fluid, is only slightly greater than the baseline. The changes in the vaginal microenvironment after intercourse must be considered in the development of any vaginal device or microbicide.

**SUMMARY**

Although the human vagina has not been subjected to the same rigorous investigation as other pelvic structures, there are certain known aspects of vaginal anatomy and physiology that will affect the development of future vaginal devices and microbicides. The anatomy of the vagina is variable among women, and care must be taken to avoid the misconception that one type of a vaginal product will fit all women. The mucosa of the vagina is very distensible and has a very large surface area. The stratified squamous epithelium actively alters the fluid contained in the vagina and readily absorbs many compounds directly into the pelvic blood supply. There is growing evidence that the pelvic blood supply acts to locally concentrate absorbed compounds in the pelvis. The epithelium contains an active immune system, including phagocytic activity. This system may act to decrease local infection but may also be a portal of entry for cell-mediated viruses such as HIV. Vaginal fluid consists of a combination of secretions from the uterus and cervix and a modified serum transudate. The amount of fluid varies throughout the menstrual cycle, with the peak amount coincident with ovulation. Most importantly, the vagina should not be considered as a quiescent potential space, but instead as an organ with dramatic anatomic and physiologic changes in response to sexual arousal. These changes must be considered when vaginal microbicides or devices are under development.
ACKNOWLEDGMENT

I thank Dr. Jay Baker at the CONRAD Program for sharing his unpublished research on vaginal models and for his suggestions on the manuscript.

REFERENCES


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**QUESTION AND ANSWER DIALOGUE**

*David Katz:* What changes in the configuration or the compliance of the rugae are associated with sexual arousal? Looking at casts generated by Dr. Jay Baker, it didn't look like the geometry had changed a lot.

*Kurt Barnhart:* Rugae certainly change in the property of distensibility with estrogen. Clearly, there is a greater distensibility in a reproductive-aged woman. Whether distensibility changes more dramatically with sexual arousal is unknown. In theory, I would like to say that distensibility increases with sexual arousal. However, no one has objectively measured that.

*David Sokal:* You discussed the vaginal epithelium but you didn’t discuss the cervix, which may be also susceptible to irritation. Could you describe the cervix a little bit?

*Kurt Barnhart:* The external portion of the cervix has a stratified squamous epithelium. There is a transformation zone which this epithelium changes to columnar epithelium. The transformation zone is usually visible on the external portion of the cervix, but sometimes it is actually inside the canal of the cervix. The location of the transformation zone is called ectropion. The transformation zone is important because of its predilection to dysplasia and cancer. This area is more fragile and more absorp-
tive than squamous epithelium. So yes, there is also a concern that the transformation zone can be a portal of entry for infection as well as an area for mechanical disruption.

Kenneth Mayer: You mentioned that the vagina is an immunologically active organ. Please comment about the distribution of different cells in the tissues and pre- and post-coital distribution of the kind of cells in the cervical or vaginal secretions, particularly with regard to CD4 markers.

Kurt Barnhart: I do not have the objective answer you want, but anything that's going to stimulate the mucosa is probably going to stimulate these cell-mediated immunologic cells to move out of the circulation and onto the epithelium of the vagina. We just lack information of how much these cells are stimulated after sexual intercourse. I would say certainly it's increased, but the quantification is very difficult.

Nancy Alexander: What do prostaglandins do to the vaginal epithelium, either those produced during delivery or those that are found in semen?

Kurt Barnhart: Prostaglandins have many functions, and there are many types of prostaglandins. Certainly, prostaglandins mediate the process of dramatic cervical change in labor and parturition. The cervix changes from a firm 3-cm structure to a paper-thin structure that almost completely disappears. Therefore, prostaglandins are able to change the composition of connective tissue. How prostaglandins in the ejaculate change the morphology of the vagina is really unknown. Prostaglandins are postulated to cause uterine contraction. The teleologic explanation of this is that the prostaglandins cause a lowering of the cervix into a pool of semen. This is also an area that clearly has not been investigated enough.

Jay Baker: You spoke of the vagina as a fibromuscular tube, and one of the things that I'm impressed with in the differences in the vagina is that the fibromuscular tube part is flexible, has a very soft and pliant wall, and the epithelium has an ability to stretch and expand and contract, except around the cervix, where it is mated to its stroma and there are no rugae, and there is no cushioning effect. Do you know of any difference in the ability of the cervical tissue to withstand trauma, whether it is more easily injured with intercourse and whether that tight epithelium without cushioning is more susceptible to injury?

Kurt Barnhart: The only way I can answer your question is from clinical experience. In cases of vaginal trauma, for whatever reason, the area of trauma is invariably in the most dispensable portion of the vagina. Very rarely is the trauma directly on the cervix. Often the area of injury is in the vaginal fornices or in the area of vaginal epithelium that has rugae. This may be directly related to the injury to the vagina, but it also may be directly related to the very firm connective tissue underlying the mucosa of the cervix. It is plausible that the cervix is less susceptible to injury by virtue of the denseness of the tissue. Remember, I am not speaking from any objective evidence or clinical study but making a correlation from clinical medicine.

John Marvel: Could you comment on the functional volume of the vagina as it relates to the amount of the application volume?

Kurt Barnhart: That, again, is a matter of debate. There's anywhere from 0.5 to 2 ml of fluid in the vagina at a given time. I think with any more than that a woman has a normal discharge. For example, at the time of ovulation, when you have very thick copious mucus, it's often seen as a discharge. I think that's because you are exceeding the 1–2 ml that can be held in the normal vagina. Now, you can put more fluid in, and it will stay in the distensible, dependent portion of the vagina, but I think it's not
physiologic. I think if you exceed 1 ml conservatively maybe 2 ml, you’re talking about exceeding the capacity of the normal non-distended vaginal walls. In this case, a woman will experience a discharge and lose your product.

Manzer Durrani: I published a paper in 1981 in the International Journal of Pharmaceutics. I did an in vitro study of a guinea pig vaginal membrane transport of antiviral drugs through different phases of the menstrual cycle. I took several hundred smears from guinea pig vaginas, and I looked at the pathology and histologic changes, and studied the ability of these antiviral drugs to penetrate through the vaginal membranes. What I found was that, at different phases of the menstrual cycle, the thickness of the vaginal epithelium changed as well as the transport and flux of the drugs. My question is, when we introduce a product during different times in a woman’s cycle, does it become more viable or toxic?

Kurt Barnhart: That is one of the main points I wanted to emphasize in this short talk. The vagina really is very dynamic. The epithelium changes in response to hormones. Estrogen, which is unopposed in the first 2 weeks of the menstrual cycle, causes thickening and a maturation of epithelial cells. This results in a thicker epithelium. The progesterone effect, apparent in the latter 2 weeks of the menstrual cycle, affects maturation of the epithelial cells. We have every indication that it is going to change cell-to-cell interactions and anchoring. The difficulty is that no one has yet been able to quantify these changes. Certainly, these changes are going to affect the bioactivity of a vaginal compound.

Joseph Robinson: I wonder if you would make a comment or two about the changes that you see in the postmenopausal female as to volume and integrity of the tissue.

Kurt Barnhart: That is a very good point. The simplest way to describe the postmenopausal vaginal epithelium is atrophic, without estrogen. The mucosa is thinner, paler, with less blood supply, less distensible, and probably the vagina is smaller in dimension. Quantification of these changes has not been performed. The postmenopausal vagina is more susceptible to trauma and more susceptible to disruption of the vaginal mucosa. Remember, the postmenopausal vaginal epithelium does not go through changes with the menstrual cycle. So there are very dramatic changes in the vagina after menopause. In general, the mucosa is thinner and more fragile. The size of the vagina may change but, again we are talking about a potential space. More dramatic is the loss of the distensibility of the vagina. Overall, the non-sexually aroused postmenopausal vagina is probably not all that much different. However, the sexually aroused vagina may not expand to the same extent or have the same distensibility. One reason for this might be that the postmenopausal vagina has fewer rugae, resulting in less mucosal flexibility.

Arthur Mldozeniec: You talked about the probability that if you increased the fluid flow much beyond 2 ml there’s going to be a tendency for spontaneous discharge. Could you comment about the sensation of fullness that may be perceived? What is the woman’s response? How can that be moderated or controlled? And could she learn to adapt to and accept 3–5 ml volumes?

Kurt Barnhart: The simple answer is probably, but we are talking about two areas. The distensibility, especially the fornices and the posterior vaginal area, probably can accept the volume without difficulty and without too much sensation. The problem is getting it there. In the delivery system, most of the sensation of pressure and fullness is close to the introitus, where you’ve got much stronger musculature involved and
voluntary control of the musculature. So you probably can accept fluid there without too much of a problem. The issue in my mind is how do you get it there and is it going to stay there. I don't think it's her perception of fullness as much as the difficulty of her normal anatomy forcing the fluid back out again. There may be discomfort in using an applicator or dispenser that's large enough to dispense 5 ml.

Bill Rencher: How does the vaginal tract change during pregnancy and after childbirth?

Kurt Barnhart: During pregnancy there is a high-estrogen state and a greater blood supply to the uterus, cervix, and vagina. During labor and delivery there is even a more dramatic engorgement of pelvic tissues, and the cervix and uterus go through dramatic changes as I described. There are probably also dramatic changes in the vaginal epithelium, but these are not well characterized. After delivery, there is a dramatic resolution and restoration of the cervix and uterus to the normal prepregnancy state. Again, there are probably changes in the vagina and vaginal mucosa as well. Basically, we think of pregnancy as a high-estrogen state, so its most likely effect on the vaginal mucosa is to produce a thicker mucosa with a greater blood supply. This is probably exaggerated at the time of delivery with a very rapid return postpartum. The actual specifics and magnitude of these changes have not been characterized.
Vaginal Bacterial Flora in Vaginal Health

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The microbial population of the vagina dynamically changes in response to various stresses, including menses, intercourse, contraceptive products, and douching (1). One of the most important determinants of the vaginal ecosystem is a woman’s estrogen status. The squamous epithelium of the vagina undergoes morphologic and fundamental changes in response to estrogen (2). Changes in estrogen affect the levels of glycogen deposited in the epithelium, which in turn affect the production of acid in the vagina and the microbial flora of that ecosystem. Before menarche, a female child has a non-estrogenized vaginal epithelium that is deficient in glycogen. The vaginal pH of a pre-menarchal female is usually 7 and the epithelium is quite thin. Under estrogenic influence, glycogen is deposited on the vaginal epithelium, the epithelium thickens, and the pH drops to 4-5 (3). In postmenopausal women who do not receive estrogen replacement therapy, estrogen depletion results in a thinning of the vaginal epithelium and an increase in vaginal pH to 6-7 (3). What is the normal bacterial flora of the vagina? The answer to that question depends on the age and estrogen status of the female. Prepubertal girls (4) have low frequencies of lactobacilli, Gardnerella vaginalis, Prevotella bivia, genital mycoplasmas, and yeast (Table 1). In contrast vaginas of reproductive-aged women without infections are usually colonized by lactobacilli (92%), and over half are colonized by G. vaginalis (5). Likewise, anaerobes such as P. bivia are frequently recovered. In postmenopausal women who have not received estrogen replacement therapy, only about half remain colonized by lactobacilli, and the frequencies of G. vaginalis, P. bivia and the genital mycoplasmas are also decreased (6). Other vaginal microflora do not appear to be under estrogen control. As shown in Table 1, the frequency of coliforms such as Escherichia coli, viridans streptococci and staphylococci are relatively constant among women regardless of estrogen status. E. coli may, in fact, be somewhat less common in women with adequate estrogen levels, (7)
BACTERIAL FLORA IN VAGINAL HEALTH

TABLE 1. A comparison of the vaginal microflora of prepubertal girls, women of childbearing age, and postmenopausal women (6)

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Prepubertal (n = 19)</th>
<th>Pregnant (n = 132)</th>
<th>Postmenopausal (n = 73)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Facultative lactobacilli</td>
<td>11 (ND)</td>
<td>92 (10^6.4)</td>
<td>49 (10^5.7)</td>
</tr>
<tr>
<td>Gardnerella vaginalis</td>
<td>0</td>
<td>58 (10^6.4)</td>
<td>27 (10^5.3)</td>
</tr>
<tr>
<td>Coryneforms</td>
<td>42 (10^5.5)</td>
<td>78 (10^5.6)</td>
<td>58 (10^4.6)</td>
</tr>
<tr>
<td>Yeasts</td>
<td>32 (10^6.3)</td>
<td>18 (10^6.7)</td>
<td>41 (10^5.4)</td>
</tr>
<tr>
<td>Coliforms</td>
<td>32 (10^6.1)</td>
<td>62 (10^6.5)</td>
<td>78 (10^4.3)</td>
</tr>
<tr>
<td>Anaerobic gram-negative rods</td>
<td>89 (10^6.9)</td>
<td>90 (10^6.5)</td>
<td>89 (10^4.1)</td>
</tr>
<tr>
<td>Prevotella bivia</td>
<td>11 (ND)</td>
<td>61 (10^4.9)</td>
<td>33 (10^3.8)</td>
</tr>
<tr>
<td>Fusobacterium species</td>
<td>26 (10^6.7)</td>
<td>12 (10^3.1)</td>
<td>7 (ND)</td>
</tr>
<tr>
<td>Peptostreptococcus</td>
<td>68 (10^6.5)</td>
<td>86 (10^4.1)</td>
<td>49 (10^3.4)</td>
</tr>
<tr>
<td>Staphylococci</td>
<td>68 (10^6.1)</td>
<td>59 (10^4.4)</td>
<td>7 (10^3.7)</td>
</tr>
<tr>
<td>Viridans streptococci</td>
<td>32 (ND)</td>
<td>33 (10^5.2)</td>
<td>38 (10^4.4)</td>
</tr>
<tr>
<td>Group B Streptococcus</td>
<td>16 (10^4.2)</td>
<td>23 (10^3.6)</td>
<td>7 (10^2.5)</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>32 (ND)</td>
<td>33 (10^5.2)</td>
<td>38 (10^4.4)</td>
</tr>
<tr>
<td>Mycoplasma hominis</td>
<td>20 (ND)</td>
<td>82 (10^5.2)</td>
<td>13 (10^4.5)</td>
</tr>
<tr>
<td>Ureaplasma urealyticum</td>
<td>32 (10^6.9)</td>
<td>8 (10^2.5)</td>
<td>15 (10^1.5)</td>
</tr>
</tbody>
</table>

* Data in parentheses are cfu/gm; mean concentration calculated for those categories in which five or more subjects were positive.

but the role of estrogen vs. the role of sexual activity in encouraging growth of this organism has not been fully described.

Vaginal acidity is widely held to be an important deterrent to genital tract infection. Therefore, any formulation to be placed in the vagina should opti­
mally be buffered to a pH of 3.8–4.5. In our experience, the pH of the introitus and vaginal vault do not differ significantly, whereas the cervix has a pH of approximately 7. Why is the vagina acidic? Lactic acid is principally responsible for vaginal acidity. Both vaginal and cervical epithelial cells have the capacity to convert glycogen to glucose, which is further metabolized to lactic acid through cell glycolysis (8). The resident glucose can be converted to lactic acid by lactic-acid producing bacteria that may also be present in the vaginal microflora. Therefore, vaginal acidity depends on adequate levels of estrogen as well as the presence of lactic-acid producing bacteria such as lactobacilli. Concentrations of lactobacilli are probably important determinants of vaginal pH as well. In prepubertal females, lactobacilli are usually present at concentrations of less than 100,000 colony-forming units (cfu) per gram of vaginal fluid (4). In contrast, in women of reproductive age, the median concentration of lactobacilli in vaginal fluid is 10–100 million/gm vaginal fluid (5). The increased concentration of lactic acid-producing bacteria in the vaginal fluid results in a lower vaginal pH in women colonized by lactobacilli. Another important determinant of vaginal pH in reproductive-aged women is whether bacterial vaginosis is present. Among women with bacterial vaginosis, lactobacilli are
decreased relative to anaerobes and mycoplasmas in the vagina. In these women, the vaginal pH increases to 4.7–5.5 (median 5.0) (9).

Some investigators have suggested a racial variation in vaginal pH among sexually active young women. In one study of 273 sexually active adolescents in Denver, vaginal pH was measured by using pH paper (10). Women with lower genital tract infections, such as bacterial vaginosis, were excluded. These authors reported that the mean vaginal pH among African–American women was 5.3 ± 0.7, whereas women of other races had a mean pH of 4.7 ± 0.6. Some recent studies have also documented that African–American women are less likely to be vaginally colonized by lactobacilli than are Caucasian women (Nugent, personal communication). This finding may explain the increased vaginal pH in African–American women but requires confirmation in additional studies.

The vaginal microflora are also affected, in large part, by the presence of strains of lactobacilli that produce hydrogen peroxide (H₂O₂) (11). As shown in Table 2, the prevalence of H₂O₂-producing lactobacilli varies broadly but ranges from 42 to 74% of reproductive-aged women. The frequency of H₂O₂-producing lactobacilli in premenarchal girls is estimated at 11% (4), and 38% of postmenopausal women are colonized by this organism (6).

Data suggest that the prevalence of bacterial vaginosis is lower in women with H₂O₂-producing lactobacilli than in women with lactobacilli that do not produce H₂O₂. These data suggest that H₂O₂-producing strains of lactobacilli are better deterrents to bacterial vaginosis compared to lactobacilli that do not produce this compound (5,12,13). Moreover, longitudinal studies have shown that women already having H₂O₂-producing lactobacilli are more likely to be persistently colonized with these strains than are women colonized by lactobacilli that do not produce H₂O₂ (14). These studies suggest that both the presence of lactobacilli and their production of H₂O₂ play a role in maintenance of the vaginal ecosystem and prevention of vaginal infections, such as bacterial vaginosis.

The use of products in the vagina can alter its pH and inhibit the growth of lactobacilli or can promote growth of pathogens. Vaginal products may also coat the epithelial cells of the vagina, thereby altering the binding of organ-

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Pregnant</th>
<th>Age</th>
<th>No. women</th>
<th>Prevalence of LB+ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hillier (12)</td>
<td>1992</td>
<td>Yes</td>
<td>16–42</td>
<td>275</td>
<td>46</td>
</tr>
<tr>
<td>Hillier (15)</td>
<td>1993</td>
<td>Yes</td>
<td>16–40</td>
<td>170</td>
<td>42</td>
</tr>
<tr>
<td>Hawes (14)</td>
<td>1996</td>
<td>No</td>
<td>16–45</td>
<td>182</td>
<td>65</td>
</tr>
<tr>
<td>Puaprapoonsiri (13)</td>
<td>1996</td>
<td>Yes</td>
<td>21–37</td>
<td>118</td>
<td>74</td>
</tr>
<tr>
<td>Hill (4)</td>
<td>1985</td>
<td>No</td>
<td>1–6</td>
<td>19</td>
<td>11</td>
</tr>
<tr>
<td>Hillier (6)</td>
<td>1997</td>
<td>No</td>
<td>55–79</td>
<td>73</td>
<td>38</td>
</tr>
</tbody>
</table>
isms in the vaginal ecosystem. The spermicides or microbicides in vaginal products may also act against the normal flora. Every vaginal product contains preservatives that may also act against potentially beneficial members of the microbial flora. Although the effects of the different vaginal preparations on the microflora have not been completely studied, there are several examples of vaginal products for which the effects on the vaginal microflora were carefully evaluated. One such study (15), evaluated the effects of various vaginal douche preparations on the vaginal microflora. They evaluated various organisms at baseline, 10 min after application of a product, again after 4 hr and 1 day after application. Within 10 min after application of the povidone-iodine douche, lactobacilli had decreased in concentration 100-fold and did not return to pretreatment levels for 24 hr after application of the douche product (15). *G. vaginalis* was decreased 100-fold after 10 min, but this organism rebounded by 4 hr after application. On the other hand, vaginal colonization by *E. coli* increased 1,000-fold 24 hr after application of povidone-iodine. This is one example of how application of a vaginal product would decrease vaginal lactobacilli and promote the growth of potential pathogens.

A number of studies have evaluated the effects of nonoxynol-9 (N-9) on the vaginal microflora. *In vitro* studies have suggested that N-9 acts against lactobacilli (16,17). However, longitudinal studies of women who have used N-9 during intercourse have not shown an increased prevalence of bacterial vaginosis. If lactobacilli were killed through the use of N-9, one would expect to see an increase in the incidence of BV (18). Other studies have looked at short term N-9 exposure. In one such study (19), women used a diaphragm plus N-9 foam containing N-9 plus condoms, or oral contraceptives for intercourse. The vaginal lactobacilli were evaluated before intercourse, again the next morning after intercourse, and 24 hr after intercourse. In this study there was no decrease in either the concentration or frequency of vaginal lactobacilli after intercourse using N-9. These studies suggest that *in vitro* evaluation of a vaginal product may not predict the biologic effects that will be observed when women use such a product.

How can we design testing systems that are more relevant for use in evaluating the effects of potential microbicides? *In vitro* susceptibility testing of microorganisms has routinely evaluated the concentration of an antimicrobial agent required to kill organisms or inhibit growth over 18–24 hr. Whether or not an agent is suitable as an antibiotic depends on the achievable levels of the compounds in body fluids or tissues. Although all in vitro studies ignore host factors and conditions that may affect the success of an antimicrobial agent in treating infections, these studies have been useful predictors of clinical efficiency of systemic antibiotics for treating infections. For classic susceptibility testing, bacteria are usually grown in the presence of various dilutions of an antibiotic at pH 7. This is a sensible approach, given that systemic antibiotic therapies are designed to provide high sustained levels of antibiotics in body
BACTERIAL FLORA IN VAGINAL HEALTH

fluids and tissues that have a neutral pH. However, this model of *in vitro* susceptibility testing has less relevance to the vaginal use of microbicides because it does not simulate the actual conditions under which a microbicide will function.

Topical microbicides could function to decrease incident infections in women by a variety of mechanisms. Microbicides that could be used vaginally include broad-spectrum agents such as disinfectants, compounds that could block the receptor molecules for binding of pathogens to the target cell, or antimicrobial agents that block replication of the pathogen. The type of *in vitro* testing to be conducted is somewhat dependent on the mechanism of action of the microbicide. For example, both N-9 and chlorhexidine gluconate are compounds that would act as surface active disinfectants which have activity against vaginal microflora (20) and HIV (21). Because these broad-spectrum agents would have to be fast-acting to be effective, evaluation of their ability to inhibit the growth of target organisms is probably not relevant. Instead, a better method might be to evaluate the effects of short-term (30 min) exposure of a compound against the target organism. Compounds that prevent infection by blocking attachment of organisms to their target cells would not usually be expected to have broad activity against members of the normal flora *in vitro*. Therefore, these types of compounds could be screened for their activity against selected members of the normal vaginal flora, but limited activity might be expected. For compounds that inhibit viral or bacterial replication, classic growth inhibition assays may be the most relevant. For example, PMPA, an antiviral compound that has been shown to have activity against retroviruses (22), would be tested for activity against HIV using growth inhibition assays. These compounds would be expected to have little or no activity against bacterial STDs or members of the vaginal microbial ecosystem.

Vaginal microbicides will be placed directly into the vagina, usually at higher concentrations than would be possible with a systemically applied antibiotic. The concentration of microbicide in the vagina will probably decrease rapidly as a result of dilution by vaginal fluid, deposition of ejaculate and leakage of the product from the vagina over time. Therefore, the actual time that a microorganism is exposed to a vaginal microbicide depends on the volume and concentration of the microbicide and vehicle inserted into the vagina, the viscosity of the vehicle, and finally, the mucoadhesive properties of the product. *In vitro* testing of microbicides and vehicles should keep in mind the conditions of pH, the presence or absence of blood, and the probable time that organisms may be exposed to the experimental microbicide.

Any *in vitro* testing of microbicides in vehicles should be conducted over the natural pH range of the vaginal ecosystem. A pH of 4 represents the optimal vaginal pH of a woman having *Lactobacillus*-predominant vaginal flora. A vaginal pH of 5 is common among women with bacterial vaginosis, and a vaginal pH of 6 is often seen in postmenopausal women who do not
receive estrogen replacement therapy and in women completing menstrual cycles. Finally, a vaginal pH of 7–8 can occur in women immediately after deposition of ejaculate or during menses.

How should the *in vitro* assays be conducted? As noted above, many experimental microbicides have been tested using classic MIC assays. It may be more relevant to assess whether exposure of a test organism to an experimental microbicide is lethal over a short interval. In this case, the test organism would be exposed to various dilutions of the potential microbicide for intervals ranging from 30 to 120 min. After incubation of the test organism with the microbicide, an aliquot of the testing solution would be removed and assayed to determine whether or not the test organism was viable. These so-called exposure assays assess whether short-term exposure to a microbicide can be expected to sufficiently destroy potential pathogens in the vaginal-cervical ecosystem without destroying potentially beneficial organisms.

Which organisms should be tested to evaluate whether a microbicide formulation has adverse affects on the vaginal ecosystem? A suggested list is presented in Table 3. *Lactobacilli* are recognized as important determinants in protecting against genital tract infection. Because of the importance of *Lactobacillus* in acidifying the vagina and preventing overgrowth of potential pathogens, any topical microbicide, active ingredient, or vehicle should have limited or no activity against *Lactobacillus* at the concentrations that will be used in the vagina. Two urinary pathogens, *E. coli* and *Enterococcus*, frequently increase in the vagina when the ecosystem is disturbed. For example, women who use N-9 during intercourse have an increase in both the frequency and the concentration of *E. coli* and *Enterococcus*. An optimal microbicide formulation would be neutral or inhibitory to *E. coli* at concentrations that are not inhibitory to lactobacilli. Likewise, microbicidal active agents and vehicles should not promote the growth of *Candida albicans*, the primary cause of yeast vaginitis, in the vaginal ecosystem because this could result in yeast vaginitis. Therefore, potential microbicides in vehicles should

**TABLE 3.** Organisms that should be considered in performing *in vitro* testing of vaginal formulations

<table>
<thead>
<tr>
<th>Organism</th>
<th>Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactobacillus crispatus</em></td>
<td>Common species in normal vaginal ecosystem; may increase resistance to infection</td>
</tr>
<tr>
<td><em>Gardnerella vaginalis</em></td>
<td>Association with bacterial vaginosis</td>
</tr>
<tr>
<td><em>Prevotella bivia</em></td>
<td>Association with bacterial vaginosis</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Primary cause of urinary tract infections; frequently increases when vaginal ecosystem perturbed</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>Common to vagina among women of all ages; increases after ecosystem is stressed</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Etiologic agent of toxic shock syndrome</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>Most frequent cause of yeast vulvovaginitis</td>
</tr>
</tbody>
</table>
be evaluated for their affects on *Candida*. The final two target organisms that should be screened are *G. vaginalis* and *P. bivia*, both of which can cause bacterial vaginosis. Products should be tested to ascertain whether levels of compound that act against *Lactobacillus* also act against these two pathogens.

An optimal vehicle or formulation for vaginal use as a microbicide would have minimal or no activity against lactobacilli, would not selectively promote the growth of pathogens such as *Candida*, *E. coli*, or *Staphylococcus aureus*, would not disrupt the vaginal epithelium, and would not sensitize the vaginal epithelium. In terms of use for women, the product should not cause any stinging or burning or provide the sensation of excess discharge. Another issue to consider in formulating a vaginal microbicide is whether or not there are interactions with latex condoms or diaphragms. It may also be important to know whether there is synergy or antagonism with other spermicides or vaginal medications. Finally, to understand its effects on the vaginal ecosystem, we must know how long a product persists in the vagina after application.

A product with a reasonably successful profile *in vitro* could be moved into Phase I clinical studies. It is very important that any product to be used as a vaginal microbicide be evaluated for its effects on the vaginal ecosystem and the epithelium, early in Phase I clinical studies.

**REFERENCES**


**QUESTION AND ANSWER DIALOGUE**

_Nancy Alexander:_ In your pH studies, have you looked at the pH of the vagina through sexual excitement with condom use and diaphragm use to determine what part might come from the cervix and how much the exudate might change the pH?

_Sharon Hillier:_ We haven't done the sexual excitement studies. Masters and Johnson studies—which were actually very good—did show a slight increase. We did measure vaginal pH after intercourse when condoms were absolutely used. We found there was an elevation in vaginal pH of about 0.2 units. A woman might go from 4.5 to 4.7, or 4.7 to 5.0, with intercourse without ejaculate in the vagina. Where it comes from, the cervix vs. the vagina, I have no clue.

_Nancy Alexander:_ Have you conducted any studies with agents besides nonoxynol-9, such as benzalkonium chloride (BZK)?

_Sharon Hillier:_ We have not performed BZK studies in humans but we are submitting a manuscript in a monkey model. We found that BZK was much more active in vivo against normal flora organisms than N-9. We are completing a study of three groups of women, using three different formulations of N-9. The results will be available next year. I think that this will be a good way to look at the effects of formulations on the flora. My sense from the animal model data and from our in vitro data is that BZK has more activity than N-9.

_Charles Litterst:_ I was fascinated by the studies that you did on differences in microflora among Caucasians, Hispanics, and African–Americans. Do we know anything about the effect on microflora of things like economics, developing vs. developed nations, or rural vs. urban living conditions?

_Sharon Hillier:_ One interesting component of my job is that I study vaginal flora all
over the world, and what it's taught me is that the prevalence of Lactobacilli truly varies in different populations. When we look in Africa, for example, rural Ugandan women—a study we just published recently with Ron Gray—we found that about 50% of the African women had bacterial vaginosis—a very high rate—and a very low prevalence of lactobacilli. A head lab tech from my lab went to Nairobi for a couple of months to set up a Lactobacillus testing laboratory. There we found that the prevalence of Lactobacilli in commercial sex workers in Nairobi is around 45%, quite low. When we look at pregnant women in Jakarta, Indonesia, we find that it's around 60%. In Lima, BV is around 40%, and Lactobaccillus colonization is about 50%. So it's very widely variable. Whether or not it's due to diet, behavior, or something else is a topic for research. I've had this recent notion that perhaps we get normal flora from other people. Certain groups of people may be less likely to carry it and therefore less likely to pass it to the people with whom they live.

Jay Baker: I had a similar question about rates of colonization of lactobacilli in African-American and Hispanic women compared to Caucasians. Is there a difference? You answered the first part about Lactobacillus concentration, but what about the difference between those that produce hydrogen peroxide and those that do not?

Sharon Hillier: African-American women are the most deficient in peroxide producing Lactobacillus. We are currently trying to use Lactobacillus suppositories to recolonize women. We're targeting young African-American women and find that it is possible to colonize women with an exogenous strain of Lactobacilli. My sense is that the variability in lactobacilli by race isn't a genetic difference.

Jay Baker: Thank you. My other question involves women who have been exposed to N-9 and you said there was about a log drop-off of Lactobacillus concentrations early on but that 24 hr later, the lactobacilli recovered to their normal level.

Sharon Hillier: Actually, a log drop was reported in one publication, but the Lactobacillus didn't change at all in my studies.

Jay Baker: But E. coli looks like it increased by three logs. Do you know whether this is related to the availability of E. coli binding sites, epithelial cells that are newly exposed, or if it's some sort of a toxin growth factor that is now present for E. coli?

Sharon Hillier: The person who's studied that most is Anne Stapleton in collaboration with Walt Stamm at the University of Washington. They've done a lot of work looking at the effects of N-9 on binding of these cells. They have not completed this study.

Kenneth Mayer: I'm curious about the role of E. coli because they are a pretty heterogeneous population. Have you looked to see whether you overselect for uropathogenic E. coli, because certainly in other microenvironments, in the gut, for example, we wouldn't say the overgrowth of E. coli compared to anaerobes was necessarily a bad thing.

Sharon Hillier: We recently started doing a PCR for the uropathogenic strains, the ones that have the fimbriae. We are looking at some of the different strains in the vagina. It's troubling to see that the prevalence of the fimbriated strains in the vagina is pretty high. It's around 20%, so I think if they have them, you're going to select for their growth and they are going to grow to a very high level, which is why I'm concerned about the link with UTI and pyelonephritis.

Willard Cates Jr.: This is probably the only chance we'll have to talk about an ideal vehicle (placebo) for comparison in blinded studies, to minimize real differences be-
tween an active product and the undesired "effective" vehicle. As many of you in the room know, Sharon especially, this has been the Achilles heel in Phase 3 trials. Any comments on what might be the ideal vehicle from a comparison group?

Sharon Hillier: I think that the ideal is placebo-controlled trials. I nevertheless have a great concern that actually using a vehicle, be it a Carbomer gel or whatever, as a placebo is truly not a placebo, because the gel by itself can alter the attachment sites to the epithelial cells and vastly change the ecosystem. One of the things we learned in our randomized, placebo-controlled trial of BV treatment with Metrogel vaginal vs. clindamycin vaginal cream was that the placebos had some effect because they acted as acidifiers, they had preservatives or some antimicrobial properties. So you observed placebo cure rates that were higher than you would see in untreated women. As we move to Phase 3 trials and we try to decide how to structure those trials, it's incredibly important to consider whether or not you can ever construct a true placebo vehicle for use in the vagina. My argument is that you have to run a three-armed study with a no-treatment group, a placebo group, and the active in the vehicle. But the vehicle is a key part in this whole thing. It's not like a pill coating. It has volume in the vagina, it can change the consistency of the vaginal fluid, it can change the ability to bind, and I believe very strongly that we have to consider the possibility that these placebo vehicles may not be placebos.

E. Taha: There are several reports showing strong association between bacterial vaginosis and acquisition of HIV, including my own work. Could this be due to pH or lactobacilli?

Sharon Hillier: I believe that keeping the Lactobacillus intact and promoting colonization is really important. Your studies and other cross-sectional studies suggest that the incidence of HIV is decreased in women who have Lactobacillus. Personally, I don't think it's BV that's the risk factor, it's the lack of Lactobacillus. I think that it's probably multifaceted, and probably more than we can get into here, but when women have predominant vaginal Lactobacilli, the vaginal fluid is a much different environment than in a woman with BV. Women with Lactobacillus flora have peroxide, lactic acid, and so on. Women with BV have a number of virulence determinants which could alter cell function. We know we get cytokine activation with BV. So we know that it's a vastly different ecosystem.

Valerie Kitchen: I'd like to mention what we found in a placebo-controlled study in N-9 gel with 40 women, 20 in each group, all non-sexually active. My comment centers around the issue of placebo use and the choice of placebo. We used a placebo that matched the commercial formula minus N-9. We saw a reduction in lactobacilli after daily use for 7 days in both groups. The N-9 gel produced a greater reduction. Baseline levels were observed after a 7-day recovery. In tandem, we saw incidence of vaginal inflammation in both groups, 35% in the N-9 group and 10% in the placebo group. So I think the issue of the placebo usage is crucial.

Jeffery Spieler: My question relates to the diaphragm and spermicides. For the purpose of this discussion right now, let's separate the vagina from the upper reproductive tract, defined as the cervix and above. When we isolate the cervix from the vagina, we probably reduce the efficiency of HIV transmission, although we don't know the exact amount. There is some controversy about whether or not diaphragms and spermicides protect against HIV. We don't have those data, but we think we have some data that show it protects against chlamydia and gonorrhea. How do a dia-
phragm and spermicide affect the vaginal flora and infection? What percent of the contribution of HIV infection comes from the vagina vs. the cervix and above?

Sharon Hillier: With diaphragm use, we do not observe a higher incidence of BV, other than a shift to greater numbers of *E. coli*, for example. We didn't find an increased incidence of yeast vaginitis, bacterial vaginosis, trichomoniasis, gonorrhea, or chlamydia in our studies. In terms of shifting the ecosystem, no, I don't think diaphragms are a real player. But the issue is to better understand the link between the cervix and the role of the cervix in prevention of HIV.
3

Preformulation Considerations in Designing a Temporal and Spatial Drug Delivery Pattern

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Safe and effective dosage forms designed to deliver dose-sparing, bioavailable active ingredients are the prime consideration of the development scientist. A well-designed vaginal microbicide product depends on a vehicle that effectively delivers the active pharmaceutical ingredient (API) in a pattern that does no harm to the active pharmacologic agent and is simultaneously safe for the patient. Therefore, primary dosage-related preformulation goals must render the product preserved, stabilized, and bioavailable.

All components in the formulation, as well as the API, are treated as subsystems of the dosage form. The chemistry of each formulation component is usually selected for its inactivity and chemical stability. Therefore, these chemical interactions are investigated through compatibility studies, preferably performed in the desired form of the product (e.g., solid, gel, liquid, foam).

The most influential preformulation studies assess the product’s physical properties, that affect stability, drug release, distribution, and vaginal retention. These include solubility in various pH environments and electrolytes, to simulate vaginal fluids and semen, which may be influenced by buffer and surfactant components in the vehicle. The physical form of the API may change, for example, from drug dissolved in the surfactant’s micelles to finely precipitated particles on proteins when the drug interacts with vaginal or seminal fluid. Once formulated into a stable and effective matrix of acceptable components, the API is designed to release from the formulated vaginal product in a predictable pattern. Drug release may be the result of the crystallinity, hygroscopicity, and/or polymorphism of the drug. This pattern is both time-controlled (temporal release) and targeted (spatial release) to create an effective microbicide action. Distribution and vaginal retention are
characterized in vitro by rheometric evaluation; when neat, diluted with simulated fluids and applied to either epithelium (animal buccal/vaginal tissue) or simulated tissues (resins).

The most optimal product features of a vaginal gel product are selected by using the House Of Quality approach. The consumer’s desired attributes and your technical requirements are obtained by surveys, listed, and ranked before formulation development. In fact, this permits the best assessment of product esthetics and therapeutic activity during the clinical study phases. Feedback on the product’s clinical performance during Phase I studies is essential to correlate in vivo performance to the physical properties measured in the preformulation laboratory.

Finally, later preformulation studies are performed to investigate the properties, particularly the limits, of the product to help define the product specifications. This provides you with confidence in the formula before manufacturing the product for long-term toxicology and pivotal clinical investigations.

**QUESTION AND ANSWER DIALOGUE**

*Bill Rencher:* How did you select the viscosity and rheology specifications for your vaginal cream? There is such a wide variety on the market, and we have heard from Kurt Barnhart that the vagina can’t hold a great deal of volume.

*Arthur Mlodozeniec:* We developed a vehicle with a bulk viscosity consistent with commercial creams as a starting point. Our interest is in controlling the distribution of an enzyme in the product uniformly throughout the vagina. The product contains hydroxypropyl methyl cellulose and is thermotropic. It undergoes an unusual solid gel transformation as it warms to body temperature. This is a desirable feature for vaginal gel delivery systems and, in fact, for a lot of other biologic sites. The viscosity in vitro has no relationship to the biologic dispersion. In vivo, we looked for residue gel over a certain increment of time in the vagina, measured by swab sampling. We do not have a good model that correlates viscosity to the biological behavior—the retention and distribution. Now, if you ask me if we have done this in the past for rectal delivery, yes, we have, and we have a good lead. Many of the slides I showed you here were from the work we did for rectal drug delivery.

*Howard Levine:* I want to agree with you. When we developed our spermicide, Advantage 24®, we looked at viscosity as a measure. Everybody looks at viscosity as a measure. However, we found through various studies there is no relationship between viscosity and product performance in terms of drug dispersion. What you just suggested at the end is more important. Is there a measure of the drug present at a point in time after application rather than the vehicle? If you look at some of the methyl cellulose gels they have very, very high viscosities, but once you put them into the vagina they liquefy and run out very quickly. Other products, with lower viscosities, stay in the vagina for a long period of time. It’s really a measure of what’s present after vaginal application. The laboratory in vitro viscosity really means nothing.

*Henry Gabelnick:* What techniques do you recommend for in vivo determination of distribution?

*Arthur Mlodozeniec:* The in vivo monitoring technique we use is gamma scintigraphy.
David Katz: I think you hit on a critical issue here in your discussion of the thermal setting properties of your gel. Once the gel goes into this new environment, it may behave differently from the way it did in the packaged form. It is that bioresponsiveness we must exploit if we’re going to improve these products.

E. S. Nuwayser: I am faced with the same issues that you have just discussed. We estimated the desired range of viscosity by measuring the viscosity of commercial vaginal gels and creams. To monitor the distribution of a product in the vagina, we used rabbits. We mixed the formulation with a fluorescent dye, applied a set volume in the vagina of the rabbit, sectioned the vaginal tract of the anesthetized rabbits, and recorded the level of florescence to map the distribution.

Arthur Mlodozeniec: I’m most enthusiastic about the use of animal modeling. The more simulation work performed before Phase I clinicals, the better our formulations will become. However, we have to be cautious about individual species responses. We were very much deceived and confused with our data obtained from an ophthalmic rabbit model because of the problem with blinking. Thermally-induced viscosity was particularly difficult for us to assess, and we missed the problem with eyelid response. That was very manifest in human beings. People did not like feeling as if their eyelids were glued shut. Even though we had designed a formulation with the desired gelling temperature and wonderful contact on the corneal surface, the subjects didn’t understand the science and felt very spooky about the viscosity behavior. We got none of that from our rabbit studies.
4

Vaginal Formulations: The Quest for Prolonged Effectiveness

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A critical function of vaginal drug delivery systems is residence time. Simple solution or suspensions are removed in a matter of a few hours; and since the success of both local and systemic delivery depends on the intimacy and duration of drug contact with the tissue surface, prolonged contact is essential. During the past decade, a number of biocompatible, bioadhesive polymers have improved both the contact time and performance of vaginal products, allowing them to work for 3–4 days rather than a few hours. In addition to bioadhesive polymers, there are phase-change polymers that respond to environmental conditions such as temperature, pH, and specific ions. Each approach can be used in developing a vaginal drug delivery product, but the formulation has to be developed to preserve esthetic and functional attributes.

There are a number of esthetic and functional qualities that must be incorporated into a vaginal drug delivery system. Some of the esthetic qualities include ease of application, minimal to no staining of undergarments, and no objectionable odors. Functional qualities include the following:

1. Prolonged retention to minimize the need for frequent dosing
2. Ability to solubilize water soluble and semi-water-soluble drugs
3. Lack of interference with sexual intercourse
4. Usefulness in both pre- and postmenopausal women
5. Lack of toxicity and irritation

Traditional dosage forms, such as suppositories, gels, and foams, can meet several but not all of the esthetic or functional characteristics just described. The functional characteristic most difficult to achieve is prolonged retention in the vagina, especially if the remaining attributes are to be addressed. An erodible or non-erodible solid insert undoubtedly prolongs retention, but it also interferes with normal sexual activity and may be uncomfortable or
painful for some postmenopausal women with dry vaginas. Fortunately, there are a number of more user friendly, alternative approaches maintain the desired functional attributes. This review examines these alternative approaches.

BIOADHESION

Bioadhesives are polymeric systems that adhere to biologic surfaces (1). These surfaces can be mucosal or non-mucosal and the nature of the forces of attachment can vary considerably. These are the types of forces that can be involved in bioadhesion:

- Covalent (e.g., cyanoacrylate)
- Hydrogen bonding (e.g., carbopol and cellulosic polymers)
- Electrostatic (e.g., cationic polymers)
- Hydrophobic (e.g., most transdermal adhesives)

Clearly, noncovalent bioadhesive polymers are preferred over covalent polymers for safety reasons.

It is easy to screen a variety of polymers for their bioadhesive potential by simply allowing contact of the polymer solution suspensions with freshly excised vaginal tissue and determining the force of detachment of that polymer from the tissue (2-4). A number of polymers commonly used in pharmaceutical products have shown good bioadhesion to rabbit vaginal tissue. These include carbopol, carboxymethyl cellulose, and hydroxypropyl cellulose. Although a number of cationic polymers show good bioadhesion, cationic polymers are commonly more toxic than anionic or neutral polymers. Moreover, anionic polymers are typically better bioadhesives than neutral polymers.

Having established the chemical form of the bioadhesive, we must choose either a crosslinked, and hence water-insoluble, or a non-crosslinked polymer. Longer contact time is expected from the crosslinked polymer, given that the non-crosslinked polymer contact time is limited by the polymer's dissolution rate.

Natural mucin, which picks up approximately 40 times its weight in water, is a limited-swelling hydrogel. In seeking a mucomimetic bioadhesive polymer, a limited swelling capacity is desirable for a variety of reasons. Polycarbophil, whose structure is shown in Fig. 1, is a lightly crosslinked polyacrylic acid that picks up 60–100 times its weight in water and is deemed a useful candidate for vaginal drug delivery. Moreover, because of the sialic and sulfonic acids in mucin, both of which have dissociation constants in the 1–2 range, there is a net negative charge on the mucin molecule. The apparent pKa of the polyacrylic acid polymers, such as polycarbophil, is in the 4–5 range. These polymers also carry a net negative charge and, therefore, are similar to natural mucin.
The attachment of polycarbophil to a variety of tissues, including rabbit vaginal tissue, has been extensively investigated. The normally weak adhesive force of hydrogen becomes a strong primary force in polycarbophil attachment because of the number of hydrogen bonds. Indeed, a small portion of this gel adheres to the finger, even under a forceful stream of running water.

These partially hydrated polyacrylic acid polymers act by drawing water from the mucus membrane to draw themselves into the mucus. At this point the polymer chains become entangled with the mucous chains, increasing the surface area of contact and the opportunity for bonding (5–7). Additional diffusion of polymer chains into mucus increases entanglement and surface area. Contact with vaginal tissue, which is not a mucus membrane tissue, is expected to conform to the same process.

**PHASE CHANGE POLYMERS**

A number of polymers that are liquid at room temperature become solid at body temperature. Poloxamer is a typical polymer in this regard. In addition to temperature, there are other environmental triggers of gelation, such as calcium and pH. Such phase-change polymers, which solidify in the vaginal cavity, might indeed have a long contact time but will probably interfere with sexual intercourse. However, there are polymers or polymer blends that soften but do not become solid. The great advantage of these polymers is that they can be inserted as liquids, suspensions, or gels. Moreover, although the polymers do prolong contact time and have some ability to modulate drug
release from gels, water-soluble drugs, in particular, release from such systems quickly.

Having decided that a bioadhesive hydrogen is a useful platform, we must construct the rest of the vaginal drug delivery system. The following parameters must be included in a final formulation.

**VISCOSITY**

A low-viscosity product may leak out of the vaginal cavity, and too high a viscosity may interfere with sexual intercourse. A gel in the range of 50,000–80,000 cps is deemed suitable for both ease of administration and retention. At this viscosity, a dose volume of about 3.0 gm was found suitable for the premenopausal women, and a dose of about 2.2 gm was suitable for the postmenopausal subjects to avoid leakage. It is important to recognize that we are relying on bioadhesion, rather than viscosity, as the retentive mechanism. Viscosity should therefore be viewed more from the points of view of ease of application and dose volume required.

**pH**

It is desirable to maintain an acidic environment in the vaginal cavity to mimic normal physiologic conditions in the healthy premenopausal woman. Addition of a typical acidic buffer will probably have only a transitory effect on resident pH, whereas employing a bioadhesive polymer with an acid pKa builds the acidifying component into the polymer, sustaining the pH. A number of existing polymers—most notably the carboxopolys, which are polyacrylic acid-based—have useful pKas (i.e., apparent pKas in the 4–5 range). Indeed, polycarbophil has an apparent pKa of 4.56 (8). Below the pKa, at pHs of 2–5, the polymer has modest swelling capabilities, and above this pH the viscosity rises dramatically. Naturally, the swelling and viscosity depend heavily on the electrolyte concentration, such that any increase in solution ionic strength reduces solution viscosity. It would be desirable to have new bioadhesive polymers with apparent pKas somewhat lower and in the 3–4 range, which could then be formulated to maintain a vaginal tissue pH of approximately 4.5. Experiments with polycarbophil, a lightly crosslinked polyacrylic acid, show good vaginal retention (3–4 days) and an ability to convert a postmenopausal vaginal pH of approximately 6.0–6.5 to approximately 4.5 (3).

**SLIP**

Hydrogels do provide initial lubrication during sexual intercourse but often become tacky with the friction that is associated with sexual intercourse. This can be appreciated by simply rubbing a drop of the commercial lubricant KY
Jelly between the thumb and forefinger. The product quickly develops a resistance to shear and a tacky quality is produced.

Adding a small amount of lipid to the product creates more slip to facilitate normal sexual intercourse. This is important for offsetting the vaginal dryness associated with menopause and making the product more user friendly for local or systemic treatment of pathologies.

**DRUG SOLUBILITY**

A common characteristic of hydrogels is the rapid diffusion of water-soluble drugs out of these systems. Thus, whereas the apparent viscosity may be high (80,000–100,000 cps), the intrinsic viscosity is low (close to 1 cps) and the restraining forces on a diffusing molecule are minimal. The expectation that a delivery system will remain in the vagina for 3–4 days carries with it the assumption that the drug will be slowly released over this same time period. A semi-soluble drug, such as progesterone or testosterone, will be automatically sustained because of its slow dissolution rate. The remaining drugs (i.e., those with a log oil-water partition coefficient of −2 to +2) are much more difficult to sustain, especially those drugs that are very water soluble. The best solution to this problem is to build the drug-sustaining effect into the bioadhesive polymer by synthesizing new polymers. However, in the short term there are at least two other solutions to the problem.

1. Employ a reconstitutable microsphere in which the drug is coated with a rate-controlling membrane. The system must be reconstitutable to enjoy a satisfactory shelf life because, if the microsphere is simply placed in the gel at time of manufacture, the drug will leach out in a period of days.

2. Employ various lipids within the formulation both to structure the gel (reducing the total free water content) and to permit partitioning of at least those drugs with modest oil solubility into the lipid phase, thereby gaining additional sustaining effect.

For very water-soluble drugs, rapid release from hydrogels continues to be a problem, and it is likely that a time period of less than 3–4 days will be necessary to accommodate the relatively rapid release of drug.

**PRESERVATIVE**

Dispensing a vaginal product from a unit dose container would avoid the need for a preservative. Sorbic acid, which degrades reasonably rapidly, can be used during manufacture to lower the microbial bioburden in the product.

Choice of a preservative is difficult. A preservative in a vaginal product containing a bioadhesive polymer may upset the fragile ecosystem of the vagina, given the long contact time of the product with the tissue. In addition, a cationic preservative, such as benzalkonium chloride, will probably be dam-
aging to vaginal tissues (as it is to other tissues), and may be incompatible with anionic polymers. Other preservatives may be unacceptable in foreign countries (e.g., the mercurials), and still others may be sensitizers. The final decision about which preservative to use must be made on a case by case basis.

If a gel or foam is selected as the final delivery system, a number of routine components will be needed in a commercial product. For example, the addition of a humectant, such as glycerin or propylene glycol, will be needed to slow evaporation of water from the system.

In my view, the recognition that bioadhesive polymers substantially increase vaginal resident time produced a major conceptual change in the formulation of vaginal delivery systems. Indeed, twice-weekly application appears routine for a gel or cream product. The first-generation bioadhesive polymers were able to alter the vaginal environment to treat a number of local pathologies (9). Yet, however valuable the first-generation bioadhesive polymers may have been, what is needed now are new multifunctional polymers to fully address the functional demands described herein.

REFERENCES


QUESTION AND ANSWER DIALOGUE

*Jay Baker:* You mentioned the presence of pores in the vagina. Can you tell me what the distribution of these pores is?

*Joseph Robinson:* I can't, but it's published information. The purpose of the pores, I postulate, is to move IgA and IgG into the vaginal area. This area is often subjected to foreign proteins, so it's not too surprising that you have to move immune material to this area. To do that, you need a mechanism. Also, if you manually or sexually stimulate a woman, there is a transexudate that moves across very, very rapidly, so you need a pathway for that. It's not going to cross that tissue quickly. The pores provide
the pathway for movement of fluids from the vascular bed into the vaginal orifice. So my sense is that it is used for both movement of the material and vascular secretions.


George Digenis: Just a comment first, then a question. The unassociated form of nonoxynol 9 has the advantage that you have mentioned. Polymers such as polycarbophil interfere with N-9 micelle formation. In addition, we have good data to show that polymers bring spermicides closer to the cell membrane of some bacterial cells and human growth cells than N-9 micelles. Povidone–N-9 formulations made in our laboratory are less irritating than N-9 solutions and a commercial control.

The questions I have are these. Men who suffer from inflammatory diseases, including HIV, have ejaculum very rich in proteolytic activity. What happens to the chemistry of the epithelium lining when such an ejaculate is introduced into the vaginal cavity? What happens if the woman already suffers from vaginosis and her proteolytic activity is probably increased? If this is the case, then the woman is probably more susceptible to STDs. If we are delivering a vaginal peptide product, we have to be concerned with loss of potency and efficacy.

Joseph Robinson: My sense is that inflammatory tissue almost invariably has higher proteolytic activity, but I have no idea.

Lourens Zaneveld: Semen naturally contains many proteinases. Some are plasminogens and some are in the active form. Sperm are proteolytic and contain other proteinases. In regard to disease states of the vagina, I can think of two articles. One was in a case of gonorrhea. The proteolytic activity of the vagina increases greatly. Second was in a case of vaginosis, in which there are high hyaluronidase levels in the vagina.

Kenneth Mayer: What we do know in the HIV-infected male and female genital tract is that HIV in and of itself can cause genital tract leukocytosis. Somebody referred to papers from Harvard. At Brown, there is collaboration with Deborah Anderson in which we’re measuring cytotoxins. There are proteolytic enzymes in the cervix and vaginal secretions in otherwise non-acidic HIV-infected women. HIV is present in the genital tract both as cell-free and cell-associated. Will the addition of polymer to the formula modulate the antiviral activity of the N-9 product?

Howard Levine: Yes, we had some work done several years ago with the Pasteur Institute which actually looked at that and found that bioadhesives adhere to cells and thus increase the antiviral effect of nonoxynol-9. In vitro we found that free virus was killed by N-9 at much lower concentrations than N-9 in our delivery system. However, the cell-associated virus in the affected lymphocyte was in fact killed by the polymer delivery system with N-9 in an order of magnitude less than N-9 itself. Hence the toxicity issue: If the issue of irritation and toxicity is overarching for Nonoxynol 9, it would be interesting to go back and look at the unassociated form relative to irritation. I am willing to bet that there’s far less damage to cells in the unassociated N-9 form.

E.S. Nuwayser: What is the current state of delivery of peptides through the vagina. Do you know of anyone who has delivered vaccines through the vagina?

Joseph Robinson: It’s a great route of administration, but I think that the cultural barriers would prevent you from ever commercializing such a product. Insofar as
peptides and proteins are concerned, I've done a fair amount of animal exploratory work. I don't know of anybody who is looking beyond animal work, so my work is restricted. I think it’s an interesting area. Would you consider calcitonin to be delivered vaginally for osteoporosis? As for vaccines, I haven’t tried them, but it wouldn’t surprise me in the slightest if it were successful.

Richard Gandour: Any correlation with polymer molecular weight and efficacy?

Joseph Robinson: Many years ago there was a study that was done that found the higher the molecular weight of a polymer, the better the bioadhesion. That’s not quite true anymore. There are better bioadhesives within a homologous series that are not molecular weight-dependent. And it was probably true because of issues of entanglement and hydrogen bonding and the like. Nowadays, we have a much better understanding of the system, and although there is a rough correlation within a homologous series, overall, when I compare several different polymers, I’m not going to find that molecular weight dependency. I will find inversions of it.
Principles in Microbicide Formulations
with Emphasis on Hydrophobe-Modified
Cationic Polysaccharides

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The organizers of the Vaginal Microbicide Workshop are to be commended for arranging the conference and requesting that speakers address “mistakes to avoid as well as examples of success.” The work presented here had its share of both.

Work on delivery systems for topical dermatologic and topical ophthalmologic actives was under way at the Union Carbide Corporation Laboratories when I was invited to attend an NIH symposium on Vaginal Contraceptives in 1991 (1). A specific goal of that meeting was to reduce irritation from topical contraceptives. Our work in ophthalmology appeared to be directly relevant. Topical delivery systems to enhance its performance were under study involving a new active for treating Herpes simplex virus. Our work in dermatology also appeared relevant.

It is a well-accepted axiom that the efficacy of any biomaterial is determined at the interface between the active and the targeted substrate. Optimizing the interfacial chemistry was a major focus in the dermatology and ophthalmology programs and likewise became a major focus in the contraceptive program. After all, from commodities, such as reinforced thermoplastics and thermosets, to orthopedic biodegradable, through esoteric applications such as cell adhesion molecules (integrins), what happens at the interface determines the performance. Our earlier studies indicated that certain cationic polymers were especially attractive candidates for drug delivery, when substantivity (adhesion) to the dermatologic or ophthalmologic substrate was important. The fundamentals from those studies appeared directly applicable to topical microbicide delivery to mucosal substrates.
R' = Hydrophobe \[ R = -N^\oplus(CH_3)_3 \text{Cl}^\ominus \text{ or } R' \]

**FIG. 1.** Double-substituted cationic cellulose ethers (DCEs).

Many factors were considered at the outset of our program in contraceptives, including the following:

Composition of the vaginal mucosa
Molecular structure of active [originally nonoxynol-9 (N-9)]
Delivery system options: e.g., polymer type, vehicle, buffers, preservative
Compatibility of the active and delivery system
Biocompatibility/toxicology of the active and delivery system
User-friendly aspects of formulated products
Formulation efficacy and stability
Economics

One mistake that was made early on was not including physiologic saline compatibility in the list. Some time was lost in correcting that discrepancy, but fortunately it led to a new class of materials (2) that will be referred to as double-substituted cationic cellulose ethers (DCEs). These novel materials, schematically depicted in Fig. 1, contain both a cationic substituent and a hydrophobic substituent, attached to a cellulose ether backbone. Although the focus of the talk will largely center on these materials, as delivery systems for a wide variety of microbicides (3), the principles to be established should apply to other polymers as well.

**FIG. 2.** Sialic acid.
TABLE 1. Chemical composition of cervical mucus

<table>
<thead>
<tr>
<th>Segment</th>
<th>g/100 g mucin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sialic acid</td>
<td>15.0</td>
</tr>
<tr>
<td>N-Acetylgalactosamine</td>
<td>9.7</td>
</tr>
<tr>
<td>N-Acetylglucosamine</td>
<td>12.8</td>
</tr>
<tr>
<td>Galactose</td>
<td>-19.0</td>
</tr>
<tr>
<td>Fucose</td>
<td>7.5</td>
</tr>
<tr>
<td>Peptide</td>
<td>20.0</td>
</tr>
<tr>
<td>Salt, water, etc.</td>
<td>-15.0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>65%</strong></td>
</tr>
</tbody>
</table>

**SUBSTRATE CHEMISTRY**

The chemistry of the substrate, mucosa, was a primary consideration in the design of the delivery system. Mucins, whether gastrointestinal, respiratory, ophthalmologic, cervical, or vaginal, are glycoproteins whose major function is to protect the epithelial surface (5). These extremely hydrophilic substances are composed of a polypeptide backbone covalently linked to oligosaccharide and polysaccharide units carrying a negative charge. Sialic acid, Fig. 2, is present in all mucins and is mainly responsible for their anionic nature (pH 4.5). Table 1 shows the components in cervical mucus, which are saccharide-type molecules and extremely hydrophilic (6). No significant differences are found in women belonging to different blood groups (7).

On the basis of earlier delivery work and the chemistry of the vaginal mucosa, it was our hypothesis that a biocompatible cationic polymer could complex with the anionic mucosa and possibly reduce irritation of vaginal and cervical epithelia associated with certain actives, e.g., (N-9) (8). By complexing the hydrophobe portion of N-9 with DCE (without decreasing spermicidal activity), the speculation was that irritation associated with the strong detergent properties of N-9 would be reduced. Moreover, reducing epithelial damage would remove a pathway for entry of the human immunodeficiency virus (HIV) (9,10). In the latter phases of this work, formulations with DCEs containing either a spermicide, a virucide, or both were evolved. In some of the later cases, new problems in formulation surfaced, the solution to which stemmed from dermatologic complexes studied earlier (see below).

**CATIONIC POLYMER CANDIDATES**

Because sialic acid is dissociated at normal vaginal pH, it contains a large hydration sphere that strongly attracts cationic substances. Therefore, cationic polymers were designed, synthesized and screened to bioadhere to these sites.

Ultra high molecular weight cationic polyacrylamide polymers (PAMs) (Fig. 3), were engineered with hydrophobic substituents to possess high anionic substrate substantivity and varying mucin binding strengths. However, further work with this class was abandoned because of practical difficulties in
FIG. 3. PAMs. 

FIG. 4. Poly DADMAC. 

FIG. 5. Chitosan lactate. 

R = C_{12}H_{23} 
R = CH_{3} 

QUATRISOFT® 
Polymer JR® 

reducing the residual monomer to acceptable levels; acrylamide is a known neurotoxin.

Diallyldialkyl ammonium halide copolymers (DADMAC) (Fig. 4) were also briefly considered. Optimum MW was a problem with this system, as was true of poly (N-acyl) alkylenimines (11).

Chitosan salts were also evaluated. Chitosan lactate (Fig. 5) displayed very interesting results. Depending on the degree of deacetylation of the precursor, chitin, high molecular weight chitosans are water-soluble, substantive, economical, and have good rheologic properties (12). Chitosans are used in personal care formulations and convey excellent moisturizing properties (13). The mucin binding and other properties are good. Unfortunately, incorporation of a hydrophobe may not be perceived to be as economically feasible as alternative polymers.

Cationic and hydrophobe modified polysaccharides are preferred excipients for personal care products because they are substantive (adherent) to anionic or hydrophobic substrates (skin, hair, mucosa), hydrophilic, film-forming, compatible with many therapeutic agents, non-penetrating and non-irritating. Polymer JR® and Quatrisoft® (Fig. 6) are two such materials routinely used in topical formulations for cosmetic and personal care products. The objective in this case was to evaluate these polymers and to modify the cationic and hydrophobic moieties to improve mucin substantivity and spermicidal effects. Discovery of DCE polymers is discussed below.

**HYDROPHOBE MODIFIED SYSTEMS**

Topical spermicides such as N-9 (Fig. 7) and benzalkonium chloride (Fig. 8) act on sperm membranes through a detergent effect, i.e., hydrophobe-hydrophobe bonding between the active and the substrate (spermatozoa). It was our idea to optimize the cationic/hydrophobic polymer in the drug delivery system so that epithelial cells were protected without sacrificing the drug’s spermicidal activity. One of the questions that needed to be answered in designing an optimal cationic/hydrophobe-modified polymer was the effect of the hydrophobe on the activity of the drug (N-9 initially. and other actives subsequently).

A number of hydrophobic groups on various hydrophilic polymer types were studied to answer this question. Table 2 displays the functional groups most studied. Cationic/hydrophobic entities (a. and b.) differ in hydrophobe type. Entities c. and d. are non-ionic. Entity e. is a cationic control, devoid of hydrophobe. These substituents were used to modify various water-soluble polymers, which were then tested with and without N-9 for spermicidal activity (see Table 3).

Table 3 summarizes sperm penetration in cervical mucus as a function of molecular structure for various polysaccharides, with the substituents shown in Table 2. The results are extremely instructive. Hydroxyethyl cellulose (A)
TABLE 2. Functional derivatives

<table>
<thead>
<tr>
<th>Derivative</th>
<th>Composition</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>a.</td>
<td>CH₃</td>
<td>Cationic/Hydrophobic</td>
</tr>
<tr>
<td></td>
<td>CH₃</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C₁₂H₂₃</td>
<td></td>
</tr>
<tr>
<td>b.</td>
<td>CH₃</td>
<td>Cationic/Hydrophobic</td>
</tr>
<tr>
<td></td>
<td>CH₃</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C₁₈H₃₇</td>
<td></td>
</tr>
<tr>
<td>c.</td>
<td>-CH₂CH(OH)CH₂ C₆H₁₉</td>
<td>Non-ionic/Hydrophobic</td>
</tr>
<tr>
<td>d.</td>
<td>-C₁₆H₃₁</td>
<td>Non-ionic/Hydrophobic</td>
</tr>
<tr>
<td>e.</td>
<td>-N(CH₃)₃</td>
<td>Cationic (no hydrophobe)</td>
</tr>
</tbody>
</table>

TABLE 3. Sperm penetration vs. molecular structure (3)

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Derivative (from Table 2)</th>
<th>Class</th>
<th>Sperm penetration in cervical mucus (of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Hydroxyethyl cellulose</td>
<td>None</td>
<td>Non-ionic</td>
<td>100</td>
</tr>
<tr>
<td>B. Carboxymethyl cellulose/polyvinyl pyrrolidone</td>
<td>None</td>
<td>Anionic</td>
<td>97</td>
</tr>
<tr>
<td>C. Polymer JR®</td>
<td>e.</td>
<td>Cationic</td>
<td>77</td>
</tr>
<tr>
<td>D. Carboxymethyl cellulose</td>
<td>c.</td>
<td>Anionic/hydrophobe</td>
<td>22</td>
</tr>
<tr>
<td>E. QUATRISOFT®</td>
<td>a.</td>
<td>Cationic/hydrophobe</td>
<td>0</td>
</tr>
<tr>
<td>F. DEC (Fig. 1)</td>
<td>a. and e.</td>
<td>Cationic/hydrophobe</td>
<td>0</td>
</tr>
</tbody>
</table>
or a mixture of carboxymethyl cellulose/polyvinyl pyrrolidone (B) did not effect sperm penetration. These polymers do not contain a hydrophobic moiety. Cationic polymer C (hydroxyethyl cellulose with a cationic moiety without a hydrophobic group) has a minor effect on sperm penetration. Hydrophobe incorporation has an important effect, independent of the polymer charge. Hydrophobe modified carboxymethyl cellulose (D) reduces penetration. Cationic/hydrophobe-modified hydroxyethyl celluloses (E and F) eliminate sperm penetration, even though they are not spermicidal. These DCEs physically impede sperm penetration, without effecting motility. The effect in (E and F) is primarily hydrophobe-related rather than cationic concentration-related. (C) has a much higher cationic content than (E), but sperm penetration in the presence of (C) is significantly higher than (E), 77 vs. 0, respectively. Composition (E), Fig. 6 \( R = C_{12}H_{23} \) had many of the sought-after attributes. However, its borderline saline compatibility in topical formulations was viewed as a weak point. By contrast, composition (F) (Fig. 1) had the same desirable features, with the added advantage of saline compatibility in formulated products. Therefore, composition (F), a double-substituted cationic cellulose ether (Fig. 1) became a leading candidate for topical contraceptive development.

**DCE MICROBICIDE FORMULATIONS**

**Hydrophobe-Modified Polymer Selection**

In the course of screening N-9/cationic polymer formulations at Eastern Virginia Medical School, an important observation was made. Hydrophobe-modified cationic polysaccharides (14), in which \( R \) in Fig. 6 is C8 or greater, displayed unique sperm impedance properties. By contrast, the related non-hydrophobe modified material, \( R = CH_3 \) in Fig. 6, was devoid of that effect.

It is important to state that hydrophobe incorporation into water-soluble polymers, at the desired level for optimal efficacy, can be complex. Chemical efficiency can be low in the derivatized polymer and solubility characteristics change dramatically. Of the various hydrophobes evaluated in these studies, the \(-C_{12}H_{23}\) hydrophobe was preferred. Composition (E) [Fig. 6 \( R = C_{12}H_{23} \)] had many of the sought-after attributes. However, borderline saline compatibility in topical formulations was viewed as a weak point. By contrast, composition (F), which contained \( R = CH_3 \) and \( R = C_{12}H_{23} \), had the same desirable features, with the added advantage of saline compatibility in formulated products. Therefore, composition (F), a double-substituted cationic cellulose ether (DCE) (Fig. 1), became a leading candidate for topical contraceptive development (U.S. Patents 5,407,919 and 5,595,980) (15–17).
DCE formulations were evaluated in combination with spermicide, virucide or both. As formulations evolved, new surprises surfaced.

Modified versions of the Sander-Cramer assay and the Cervical Mucus Penetration Double End Test (DET) were used to assess in vitro spermicidal activity and cervical mucous biodiffusion, respectively (18). Table 4 compares the results of 4.0% N-9/DCE gels to a leading commercial product, used consistently as a control. Excellent spermicidal activity was observed in all cases (Sander-Cramer). Unexpectedly, DCE facilitated the diffusion of N-9 into cervical mucus (DET). This result is especially important in contraception because the greater the diffusion the higher the spermicide concentration in the cervical mucus through which the sperm must pass to reach the ovum.

It is well accepted that a correlation exists between the potential for irritation of vaginal contraceptives in rabbits and humans (19). Rabbit vaginal irritation studies are used extensively in the pharmaceutical industry, because the rabbit vagina is more sensitive to irritation.

A 10-day double-blind study was conducted at an FDA regulated facility to measure irritation as well as microscopic histopathology, after necropsy on day 11. A total of 45 animals were utilized, receiving 1 gm/day of cationic polysaccharide/N-9-formulated gels or a leading commercial product (as a control). Forty animals had no visual signs of irritation. Five had barely perceived responses. In the postmortem histopathology assay, interior middle and posterior sections of excised tissue were examined microscopically, from which irritation scores were assigned for the epithelium, leukocytes, congestion, and edema. The scoring system is as follows: 1–4 = minimal irritation; 5–8 = mild irritation; 9–11 = moderate irritation; and 12–16 = marked irritation.

**TABLE 4. Spermicidal/biodiffusion properties**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Wt. %</th>
<th>(mg/ml)</th>
<th>(% CTL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCE-1</td>
<td>4.0</td>
<td>0.201</td>
<td>71</td>
</tr>
<tr>
<td>DCE-2</td>
<td>4.0</td>
<td>0.113</td>
<td>71</td>
</tr>
<tr>
<td>DCE-9</td>
<td>4.0</td>
<td>0.163</td>
<td>74</td>
</tr>
<tr>
<td>Control</td>
<td>4.0</td>
<td>0.114</td>
<td>89</td>
</tr>
</tbody>
</table>

**N-9/DCE Compositions**

**TABLE 5. Vaginal tolerance in rabbits**

<table>
<thead>
<tr>
<th>Sample</th>
<th>N-9 (wt %)</th>
<th>Histopathology score</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCE (Fig. 1)</td>
<td>4.0</td>
<td>1</td>
</tr>
<tr>
<td>DCE (Fig. 6) + chitosan lactate</td>
<td>4.0</td>
<td>1</td>
</tr>
<tr>
<td>Commercial control</td>
<td>4.0</td>
<td>4</td>
</tr>
</tbody>
</table>
Correlating to human irritation potential, 0–8 is acceptable, 9–11 is borderline acceptable, and >12 is potentially irritating.

Table 5 displays the histopathology scores from this study. All of the compositions were acceptable. Not surprisingly, the irritation potential of the compositions containing hydrophobically modified cationic polysaccharides was lower than that of the commercial control.

In concluding this section, the preliminary results of a rabbit contraceptive efficacy trial (20) are shown in Fig. 9. Despite the fact that DCE (Fig. 1) is not spermicidal, a DCE gel composition without N-9 showed a pregnancy rate almost equivalent to the commercial control (with 4% N-9). Furthermore, the DCE gel with only 2% N-9 had the lowest pregnancy rate (mean number of implantation sites).

**DCE Complexes with Other Actives**

One path for HIV infection may involve damaged (or healthy) vaginal epithelia (21,22) through cell surface adhesion (23). Recently, it has been

**TABLE 6. Efficacy of DCE gels in vitro**

<table>
<thead>
<tr>
<th>Assays*</th>
<th>Cell free</th>
<th>Cell assoc.</th>
<th>Viral binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composition</td>
<td>0.1%</td>
<td>0.01%</td>
<td>0.1%</td>
</tr>
<tr>
<td>I. DCE gel 2% N-9; 2% D.S.</td>
<td>&gt;2.8</td>
<td>2.7</td>
<td>&gt;3.5</td>
</tr>
<tr>
<td>II. Comm. gel 4% N-9</td>
<td>&gt;3.8</td>
<td>2.5</td>
<td>&gt;3.5</td>
</tr>
<tr>
<td>III. Comm. gel 2% N-9</td>
<td>&gt;3.8</td>
<td>0.8</td>
<td>2.3</td>
</tr>
<tr>
<td>IV. DCE gel 2% D.S.; 0% N-9</td>
<td>100</td>
<td>95</td>
<td>75</td>
</tr>
<tr>
<td>V. Comm. gel 4% N-9</td>
<td>5.7</td>
<td>4.4</td>
<td>0.0</td>
</tr>
</tbody>
</table>

* Results are expressed in logs of infectivity reduction for cell-free and cell-associated; in % inhibition for viral binding.
reported that the major HIV envelope protein is held intact through hydrophobic residues (24). For these and other reasons, we sought to combine hydrophobe substituted cationic polymers, particularly DCEs (Fig. 1), with active anionic polymers, with or without N-9 present.

Anionic pharmaceutical delivery can be more complex, especially if the active is a polyanion (25,26). Carrageenan, dextran sulfate, and others are anionic polysaccharide actives, capable of blocking various viral infections in vitro (27). The anionic co-polymer, acrylic acid–vinyl alcohol sulfate, also displayed anti-HIV activity in mice (28).

We were successful earlier in achieving anionic/cationic complexes with glycosaminoglycans, e.g., hyaluronic acid/cationic polysaccharide compositions (15), now used in personal care applications. Those compositions are substantive, stable, and user-friendly. Unfortunately, despite the highly anionic nature of hyaluronic acid (and other glycosaminoglycans), these materials do not block viral infections (27). Therefore, emphasis was placed on sulfated polysaccharides that are anti-viral. Table 6 summarizes the in vitro virucidal and spermicidal properties of a DCE/dextran sulfate/N-9 gel formulation. Not only were the desired rheologic properties and long-term stability achieved, but the activity of the dextran sulfate or N-9 was not compromised. Furthermore, as shown by the in vivo rabbit irritation studies (Table 7), such compositions displayed minimal irritation.

The versatility of DCE as a broad-spectrum delivery system was further indicated with C31G/DCE gel formulations. C31G is a broad-spectrum microbicide (29,30) based on a combination of amphoteric actives, i.e., hydrophobe-modified betaine and amine oxide, respectively. In vitro studies with C31G/DCE gels at two separate laboratories showed excellent microbicide performance. In addition, the gel compositions showed good stability in preliminary studies. Work with other actives is also being considered, such as peptide/DCE compositions, also predicted to have the required efficacy and user-friendly properties.

**CONCLUSIONS**

1. Hydrophobe, cationic-modified cellulose ethers (DCEs; Fig. 1) are drug delivery vehicles specifically designed to interact with mucin. DCEs are
closely related to cationic polysaccharides, used safely and effectively for many years in topical personal care products.

2. DCEs are not spermicidal. However, DCEs effectively retard sperm travel (impedance). Incorporation of N-9 facilitates impedance and cidal properties.

3. DCE gel formulations displayed minimal irritation in two separate in vivo rabbit studies.

4. In a preliminary in vivo animal study, DCE gel, without pharmaceutical active, reduced sperm implantation to the same degree as a leading commercial product containing 4% N-9. The addition of 2% N-9 to DCE gel had a significantly lower implantation rate than the commercial product containing 4% N-9.

5. We have demonstrated that DCE formulations with non-ionic, cationic, and anionic actives may be formulated successfully.

6. The addition of sulfated polysaccharides to DCE gels are effective microbicides in vitro, including HIV.

FUTURE WORK

DCE-containing delivery systems targeted to mucin or epithelial surfaces will continue to be investigated, optimized, and the results shared with others in the scientific community.

Exploration of the hydrophobic effects on cellulosics should be applied to “polymer-like drugs” to enhance microbicide specificity and activity, e.g., hydrophobe modification of sulfated polysaccharides.

Topical formulations based on “off-the-shelf” excipients are being used successfully. However, in some instances there is a need and opportunity to design enhanced delivery systems for specific target substrates, such as the vaginal mucosa. More attention should be focused there.

The “hydrophobe effect” is already being used in several commercial applications (e.g., latex paints, personal care products) to enhance rheological and surfactant properties, in both synthetic and natural water-soluble polymers. This effect should be explored further in the cases of actives and excipients in the drug area. For example, hydrophobe modification of sulfated polysaccharides, or hydrophobe modification of amphoteric polymer systems, may provide a number of advantageous properties (31).

ACKNOWLEDGEMENTS

A number of collaborators have made especially significant technical contributions to the work covered in this article, including: Gustavo Doncel, MD, PhD, Eastern Virginia Medical School; George Salensky, (formerly) Union Carbide Corporation; Russell L. Kreeger, PhD Union Carbide Corporation;
James Kawakami, PhD (formerly) Union Carbide Corporation; and John Kemnitzer, PhD Integra LifeSciences Corporation.

Technical collaborators in the earlier dermatology and ophthalmology programs, which provided the basis for this work, are also acknowledged, including: E. Desmond Goddard, PhD (formerly) Union Carbide Corporation; Frederick M. Merritt, PhD (formerly) Union Carbide Corporation; Emmett M. Partain, PhD Union Carbide Corporation; and Phillip A. Band, PhD Biomatrix Corporation.

The financial support of CONRAD and Integra LifeSciences Corporation, as well as the early support from Union Carbide Corporation, is also gratefully acknowledged.

REFERENCES

6

Physical and Chemical Principles in the Design and Evaluation of Vaginal Formulations

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(In Collaboration with Eastern Virginia Medical School)

The problem of sexually transmitted disease (STD) is steadily growing, with increasing numbers of people contracting more severe and drug-resistant infections. Worldwide, 333 million new STD cases were estimated for 1995, including trichomoniasis (170 million), chlamydia (89 million), gonorrhea (62 million), and syphilis (12 million) (1). Persistent viral infections, including HIV, HSV, HBV, and HPV, have afflicted millions, with no definitive cures at hand. The World Health Organization estimated that by the end of 1995, 20 million people worldwide would be infected with HIV (2). In the United States, 14 million new STD cases have been reported, with 86% occurring among the 15- to 29-year-old age group (13). In the United States, AIDS accounts for the largest proportion of STD-related deaths, with 319,849 deaths among 513,486 reported cases in 1995 (4). A 1992 CDC study found that the most common causes of STD-related deaths among United States women were cervical cancer (57%), AIDS (29%), hepatitis B and C (10.5%), and other STDs (3.5%) (4).

The burden of STDs and associated sequelae on the United States 1994 economy has been estimated at approximately $10 billion for the most common STDs, a figure that increases to $17 billion when sexually transmitted HIV infections are included (4). More than 10% of new AIDS cases reported to the CDC in 1990 were from heterosexual transmission (5), and it was estimated that the number of cases of heterosexual transmission doubled between 1990 and 1995 (6). Clearly, effective STD prevention can dramatically reduce death, morbidity, and health care costs.

In spite of these sobering statistics, STD prevention is not currently addressed by today's vaginal contraceptive formulations. Infection prevention,
the surest way to reduce the adverse consequences of STDs, is currently performed by barrier methods, such as condoms. Formulations that address this major health need are becoming increasingly critical. Unfortunately, in many cultural and social settings, women not only bear the primary responsibility for contraception but also frequently contract STDs, including HIV. The risk for HIV transmission appears to be higher in people who already have an STD and in women with unbalanced vaginal flora (7–9).

Appropriate barrier methods and vaginal formulations are the first line of defense against pregnancy and STDs. Recognition of this fact in working to develop truly novel systems is crucial. Indeed, major efforts are under way to develop new and more effective microbicides, excipient delivery vehicles, and integrating formulations.

The use of a double-substituted hydrophobe-modified cationic polysaccharide (DCE) is fundamentally different from current commercial vaginal formulations, which rely exclusively on non-ionic or anionic vehicles.

The key objective of our efforts has been to develop a vaginal formulation that optimizes spermicidal and antiviral activity while enhancing spreading and true bioadhesiveness. The DCE (10–12) formulation is the result of research to find an excipient delivery vehicle that included substantivity to vaginal mucosa, saline compatibility, compatibility with a wide range of spermicidal and anti-viral compounds, low irritation potential, sperm impedance, and system stability and efficacy after stressed storage conditions. On the basis of results from in vitro studies, the DCE vehicle was selected for clinical development.

This report describes the physical and chemical considerations in the design of such an excipient vehicle, characterization of the DCE formulation and, finally, comparison of DCEs with commercial non-ionic/anionic excipient-based contraceptive vaginal products. The design principles in the development of the DCE formulation may serve as a useful guideline for research into new water-soluble polymers for vaginal drug delivery applications.

**GENERAL REGULATORY AND MARKET CONSIDERATIONS**

Careful review of FDA regulatory requirements is necessary before researching and developing contraceptive and anti-STD systems (13). Depending on the system and its medical indications, the FDA generally requires a full package of toxicology, pharmacokinetic, irritation, hypersensitivity/photosensitivity, genotoxicity, reproductive toxicology, and carcinogenicity studies to support the submission of Investigational New Drug (IND) applications. Discussion with the review division appropriate to each IND application is highly recommended.

To provide contraceptive and/or anti-STD products to the general population, business objectives, in addition to FDA regulations, must be met. Con-
siderations include raw materials, manufacturing, and packaging costs, liability, and demand for such products. In the United States, the use of spermicides accounts for a steady 3.5% (over a 10-year period) of total contraceptive sales, with projections of $52 million in total sales. Condoms—which, combined with spermicide, offer the most effective contraceptive and anti-STD method in the marketplace—had projected sales in 1997 of $183 million (14).

A female-controlled, combined anti-STD and contraceptive product is an unmet need with critical health and economic ramifications. Product claims for combined contraceptive and STD prevention with clearly defined risk levels are expected to meet with wide acceptance. This need provides social and economic justification for government and industry to fund research to bring these technologies to the public.

TECHNICAL VEHICLE DESIGN AND FORMULATION CONSIDERATIONS

Vaginal products need to be designed for women's convenience. In addition to effective contraceptive and antiviral protection, a product's esthetic qualities are important to ensure proper compliance and continued use.

The design of vaginal formulations for women's control should address the technical and aesthetic requirements outlined in Table 1. Traditionally, design of such vaginal formulations for contraceptive indications have relied on the items listed in Table 2.

<table>
<thead>
<tr>
<th>Technical requirements</th>
<th>User requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug (spermicide, antiviral) activity is not significantly reduced by the vehicle</td>
<td>Ease of application before intercourse</td>
</tr>
<tr>
<td>Drug is active immediately</td>
<td></td>
</tr>
<tr>
<td>Is non-irritating and has no adverse effects to either partner</td>
<td>Is not messy, cold or wet, and does not give a &quot;fullness&quot; feeling</td>
</tr>
<tr>
<td>Has metered dosage</td>
<td>Does not stain</td>
</tr>
<tr>
<td>Has no systemic absorption or local toxicity</td>
<td>Does not negatively affect coitus for either partner</td>
</tr>
<tr>
<td>Has product use and activity retention over a wide pH range</td>
<td>Is odorless and tasteless</td>
</tr>
<tr>
<td>Vaginal microflora are not adversely affected</td>
<td>Has substantiated spermicidal and antimicrobial claims</td>
</tr>
<tr>
<td>Has long shelf life under storage stress</td>
<td>Has discreet packaging</td>
</tr>
<tr>
<td>Gives coating retention for a specific length of time (bioadhesion)</td>
<td></td>
</tr>
<tr>
<td>Gives complete coverage of the vagina and cervix</td>
<td></td>
</tr>
<tr>
<td>Has microbial selectivity, with activity against HIV and STDs</td>
<td></td>
</tr>
<tr>
<td>Is compatible with barrier method materials</td>
<td></td>
</tr>
</tbody>
</table>
TABLE 2. Traditional design of vaginal formulations

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spermicide</td>
<td>N-9 (most common active in U.S.)</td>
</tr>
<tr>
<td>Water-soluble polymer vehicle</td>
<td>Thickener (viscosity control); reduces seepage; bioadhesiveness</td>
</tr>
<tr>
<td>Preservative</td>
<td>Meets USP microbiologic challenge (multiuse), or prevents growth during manufacture (unit dose)</td>
</tr>
<tr>
<td>Additives</td>
<td>Vehicle property enhancement (pH, fragrance, system compatibility)</td>
</tr>
<tr>
<td>Water</td>
<td>Formulation solvent (cheap and nonirritating)</td>
</tr>
</tbody>
</table>

The majority of OTC formulations combine these basic components with variable success in terms of contraceptive efficacy, convenience, and aesthetics. The United States-marketed contraceptive products all contain N-9 as the spermicide in various concentrations. Because the carrier system has little effect on sperm motility, these products must rely on the spermicide's non-surfactant effects to destroy sperm. No claim of activity against STDs and HIV are made by current contraceptive products, with the exception of full barrier methods such as condoms.

An interesting feature of current commercial products is that the polymer vehicles available for formulation have been limited to non-ionic and anionic materials. The vehicles include carboxymethylcellulose, soluble starch, hydroxyethylcellulose, polyvinyl alcohol, or poly(acrylic acid). Commercial products have a viscosity range between 32,000 and 120,000 cp and a pH range between 4.2 and 5.6.

The formulator must choose the additives carefully for optimal component compatibility, aesthetics, and efficacy. However, because of reliance on available systems and limitations on bioadhesion, drug bioavailability, contraceptive efficacy, and end-use characteristics have been limited. Some formulations use conventional additives that may have unwanted effects for the intended application. Each additive introduces an additional variable that must be evaluated from product performance and regulatory viewpoints.

Researchers sometimes overlook the potential of a delivery vehicle to improve application performance. Vehicle design requires knowledge of general polymeric structure-property relationships, and especially prediction of solution properties. By using "off the shelf" excipients that do not take full advantage of the knowledge base that exists for the vaginal environment [structure (15,16), pH (17), and rheology(18) of the cervical mucus, for example], although solution structure-property evaluation is used, current formulation technology will continually encounter the same limitations.

Water solubility is determined by polymer structure (e.g., linear, branched); concentration and placement of charged species [ionomeric (cationic or anionic) or amphoteric (cationic and anionic)], hydrophilic/hydrophobic substituents, and hydrogen bonding, to name the more common factors. In general, polymeric water solubility requires polar functional groups and sites for
specific interactions to occur. The solution properties, such as viscosity, result from the interactions of all these factors. Viscosity control in vaginal formulations is addressed by adding a low concentration of a water-soluble, high molecular weight polymer (natural, synthetically modified natural, or synthetic-based) that falls within the families of non-ionic and anionic polymers. It is important to note, however, that there is a weak correlation between vaginal retention time and bulk viscosity measurements.

At present, the only purpose of these polymers is to tailor the viscosity. The polymer is rarely an active ingredient, with a few notable exceptions. Those polymers that confer strong anti-HIV activity in vitro include low molecular weight povidone-iodine (19), the sulfated polysaccharides/glycosaminoglycans (e.g., dextran sulfate, carageenan, heparin) (20), PAVAS (a co-polymer of acrylic acid with vinyl alcohol sulfate) (21) and a sulfated polystyrene

<table>
<thead>
<tr>
<th>Table 3. Examples of commonly encountered water-soluble polymers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water-soluble polymer</td>
</tr>
<tr>
<td>Hydroxyethylcellulose</td>
</tr>
<tr>
<td>Hydroxypropylcellulose</td>
</tr>
<tr>
<td>Hydroxypropylmethylcellulose</td>
</tr>
<tr>
<td>Amylopectin</td>
</tr>
<tr>
<td>Carboxymethylcellulose                      +</td>
</tr>
<tr>
<td>Xanthan                                     -</td>
</tr>
<tr>
<td>Hyaluronic Acid                             -</td>
</tr>
<tr>
<td>Poly(acrylic acid)                          -</td>
</tr>
<tr>
<td>Polyacrylamide                              N</td>
</tr>
<tr>
<td>Poly(ethylene oxide)                        N</td>
</tr>
<tr>
<td>Poly(vinyl alcohol)                         N</td>
</tr>
<tr>
<td>Poly(N-vinyl pyrrolidinone)                 N</td>
</tr>
<tr>
<td>Poly(diallyldimethyl-ammonium chloride)     +</td>
</tr>
<tr>
<td>&quot;Cationic&quot; polyacrylamide                   +</td>
</tr>
<tr>
<td>Chitosan                                    +</td>
</tr>
<tr>
<td>Polymer JR®                                 +</td>
</tr>
<tr>
<td>Quatrisoft®                                 +</td>
</tr>
<tr>
<td>DCE                                         +</td>
</tr>
<tr>
<td>Product (Company)</td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>Advantage 24 (Lake Pharmaceutical)</td>
</tr>
<tr>
<td>Conceptrol (Ortho Pharmaceutical)</td>
</tr>
<tr>
<td>Gynol II Jelly, original formula (Ortho Pharmaceutical)</td>
</tr>
<tr>
<td>Gynol II Jelly, extra strength (Ortho Pharmaceutical)</td>
</tr>
<tr>
<td>Koromex Clear Gel (Quality Health Products)</td>
</tr>
<tr>
<td>Koromex Jelly (Quality Health Products)</td>
</tr>
<tr>
<td>Delfen Foam (Ortho Pharmaceutical)</td>
</tr>
<tr>
<td>KY-Plus (Johnson &amp; Johnson)</td>
</tr>
<tr>
<td>Vaginal Contraceptive Film (Apothecus Pharmaceutical)</td>
</tr>
<tr>
<td>Semicid Vaginal Inserts (Whitehall-Robins)</td>
</tr>
<tr>
<td>Today Sponge* (Whitehall-Robins)</td>
</tr>
</tbody>
</table>

* No longer commercially available in the U.S.
derivative (22,23) [which is also strongly contraceptive but non-spermicidal (24)]. Surprisingly, cationic polymers are not encountered in vaginal preparations but are found in cosmetic products (25,26) (e.g., skin creams, conditioners, mousse) because of their excellent substantivity to anionic surfaces (such as skin and hair). Typically encountered polymer systems in water-soluble applications are listed in Table 3. Of course, many other water-soluble polymers exist that can be incorporated to meet the demands of a particular application (27). Some examples of viscosity ranges encountered in commercial vaginal products are shown in Table 4.

Salt, pH, temperature, and lipids must be taken into consideration for their possible effects on viscosity and solubility in the vagina. A pH range of 4–7 and a relatively constant temperature of 37°C can generally be expected. Observed solution properties as a function of salt and polymer concentration can be referred to as saline compatibility. Polyelectrolyte solution behavior (28) is generally dominated by ionic interactions, as are other materials of like charge (repulsive), opposite charge (attractive), solvent ionic character (dielectric), and dissolved ions (i.e., salt). In general, at a constant polymer concentration, an increased salt concentration decreases the viscosity because the hydrodynamic volume of the polymer is decreased. At a critical salt concentration, precipitation may occur.

Rheologic consideration (measurement of viscosity at different shear rates) is an important aspect of the application and user-friendliness of the product. Viscosity reduction during shear is seen as desirable and, in theory, will lead to increased vaginal coating and enhanced miscibility of introduced seminal fluids or microorganisms. The DCE formulations exhibit such behavior (Fig. 1) and are fully reversible.
Study of bioadhesion to the vaginal substrate requires knowledge and demonstration of the mechanism of interaction. In the present case, the terms mucoadhesion, bioadhesion, and substantivity can be used interchangeably when the mucosa is evaluated as a substrate. Several forces may be active, e.g., electrostatic, hydrophobic, and hydrogen bonding, giving rise to surface interactions between the polymer and the mucosal substrate or causing diffusion into the mucous layer (29,30).

It is fairly well established that the vaginal mucous glycoproteins have a net negative charge (31). Mucosal tissue has also been shown to possess appreciable hydrophobicity, as determined by contact angle measurements (32). To demonstrate its adhesion to a biologic surface, a material must be unequivocally shown to interact with a substrate. ATR-IR (33–36) (attenuated total reflectance-infrared), ESCA (37,38) (electron spectroscopy for chemical analysis), and SIMS (39) (secondary ion mass spectrometry) are techniques that allow chemical depth profiling distinctions in the surface analysis of *in vitro* models.

Interaction with the cervical mucus is expected to be highest with cationic species (40), such as benzalkonium chloride, chlorhexidine, and vantocil (polyhexamethylene biguanide). A clear exception is the water-soluble sulfated polystyrene derivative ORF 13904 (24). In general, sperm penetration is lower for water-soluble cationic polymers than for anionic or nonionic polymers (41).

Use of ESCA showed the substantivity (25) of a series of hydrophobe-modified cationic polysaccharides to skin, hair, and an anionic vinyl substrate ("skin substitute"), even after repeated washings with distilled water. A "skin substitute." after exposure to a fluorescent dye such as fluorescein, can qual-

### Table 5. Formulation substantivity to a "simulated" mucosal substrate

<table>
<thead>
<tr>
<th>Test system</th>
<th>Remarks</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCARMAG 527 Control</td>
<td>Fluorescein before saline wash</td>
<td>Not substantive (very light violet)</td>
</tr>
<tr>
<td>DCE Sol'n</td>
<td>Fluorescein before saline wash</td>
<td>Substantive (strong green color)</td>
</tr>
<tr>
<td>2% DS/4% N9/1.25% DCE</td>
<td>Fluorescein before saline wash</td>
<td>Substantive (strong green color)</td>
</tr>
<tr>
<td>2% DS/4% N9/1.25% DCE</td>
<td>Fluorescein after saline wash; repeat wash</td>
<td>Substantive (strong green color)</td>
</tr>
<tr>
<td>2% DS/0% N9/1.25% DCE</td>
<td>Fluorescein before saline wash</td>
<td>Substantive (strong green color)</td>
</tr>
<tr>
<td>2% DS/0% N9/1.25% DCE</td>
<td>Fluorescein after saline wash; repeat wash</td>
<td>Substantive (strong green color)</td>
</tr>
<tr>
<td>Advantage 24®</td>
<td>Fluorescein before saline wash</td>
<td>Partially substantive (faint violet with streaks of green)</td>
</tr>
<tr>
<td>Advantage 24®</td>
<td>Fluorescein after saline wash; repeat wash</td>
<td>Partially substantive (faint violet with streaks of green)</td>
</tr>
</tbody>
</table>
tatively corroborate ESCA, which is an expensive technique not suitable for screening purposes.

Using this technique, substantivity (Table 5) was assessed using a negatively charged vinyl substrate that is often used to simulate skin (the anionic UCARMAG Binder 527 resin). Fluorescein, sodium salt, at 0.5% in water was added before and/or after a 0.9% saline wash (2 × 25 ml) step. If added later, then samples were rewarshed to determine whether any substantive coating remained.

Greater substantivity of DCE formulations was observed against a bioadhesive-claiming commercial product. This substantivity was maintained after rinsing with saline. Substantivity to the vaginal mucosa and epithelia over a prolonged period is anticipated and will be clinically evaluated.

ANTIMICROBIAL EVALUATION OF FORMULATION VEHICLES

The mucosal surface has a plethora of defenses against infection (42), but it can be damaged by physical or chemical irritation. Some microbicides and bactericides may actually subvert their own purposes by irritating the mucosal surface and compromising its natural defenses. For example, complexation of cationic structures with *Escherichia coli* has been observed (43). Inactivation of this organism with a lipophilic cation, specifically 2-(4-dimethylaminostyryl)-1-ethylpyridinium (DMP+) (44), is a strong indication to include such parameters in an overall vehicle design strategy.

The DCE systems were not designed for bactericidal properties. Fortuitously, by combining cationic and hydrophobic substituents in DCE, bactericidal activity has been observed. The cation/hydrophobe ratio is an important design parameter responsible for the observed properties of sperm impedance and mucoadhesion. (It is important to note that the DCE is a non-irritating vehicle, as demonstrated in rabbit vaginal irritation studies.)

Bactericidal activity was studied using a 2.5% DCE concentration. Subsequent dilutions were made with soybean casein digest (SCDB) or lactose broth. Samples were inoculated with prepared test organisms, which included *Staphylococcus aureus* ATCC 6538, *Salmonella typhimurium* ATCC 6994, *E. coli* ATCC 8739, and *Pseudomonas aeruginosa* ATCC 9027. Test cultures did not demonstrate growth at DCE concentrations of 0.23% or greater. At a DCE concentration of 0.06%, it was not possible to culture *E. coli* and *S. typhimurium*.

USP guidance attributes failure to isolate inoculated organisms to bactericidal activity of the test compound and indicates that the material is not likely to be contaminated with these organisms. DCE is categorized as bactericidal, with future screening to be done only for *S. aureus* and *P. aeruginosa*, which was isolated only at the lower concentrations.

A bactericidal vehicle has tremendous potential in HIV prevention by pre-
venting bacterial STDs, considering that the risk for HIV infection appears to be higher when one already has an STD (45,46).

**ANTI-VIRAL DESIGN PARAMETERS**

Prevention of HIV transmission is a major worldwide concern. A recent recommendation by the International Working Group on Vaginal Microbicides states (47) that "it is desirable that an agent be evaluated for activity against HIV and other STD regardless of its intended HIV indication since a clinical outcome of HIV prevention may be achieved by the prevention of other STD." Clearly, from a health standpoint—formulations that have only contraceptive indications are doing only half the job. Formulations need to be intentionally designed to exhibit anti-viral properties (48).

Specific polyanions such as dextran sulfate (DS) appear to exhibit strong anti-HIV activity *in vitro* (49,50). Human orally administered DS is poorly absorbed, but i.v. administration does result in increased plasma lipolytic activity (51).

Polyanions that have been considered for intravaginal anti-HIV activity include DS, carrageenan, heparin, heparan sulfate, dermatan sulfate, pentosan polysulfate, fucoidan chondroitin sulfate, keratan sulfate, and PAVAS (20,21,52,53).

DCE formulations containing DS display strong anti-HIV activity *in vitro* in comparison with negative (not shown) and positive controls (Fig. 2). This is an important first step in the screening process toward clinical effectiveness.

![Figure 2](imageurl)  
*FIG. 2. Anti-HIV results of vaginal formulations via viral binding assay.*
### TABLE 6. Spermicidal/cervical mucus blocking/cervical mucus biodiffusion activity of various formulations

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Sperm dilution (1/x)</th>
<th>MEC (mg/ml)</th>
<th>DET</th>
<th>MOET Conc (mg/ml)</th>
<th>MOET % CTL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% N9/0% DS/</td>
<td>Failed</td>
<td>NA</td>
<td>89.5 ± 4.9</td>
<td>1:10</td>
<td>2.3 ± 1.2</td>
</tr>
<tr>
<td>1.25% DCE</td>
<td></td>
<td></td>
<td>1:160</td>
<td>13.7 ± 3.1</td>
<td></td>
</tr>
<tr>
<td>0% N9/0% DS/</td>
<td>Failed</td>
<td>NA</td>
<td>98.9 ± 0.9</td>
<td>1:10</td>
<td>2.6 ± 1.3</td>
</tr>
<tr>
<td>1.25% DCE</td>
<td></td>
<td></td>
<td>1:160</td>
<td>13.5 ± 2.9</td>
<td></td>
</tr>
<tr>
<td>0% N9/2% DS/</td>
<td>Failed</td>
<td>NA</td>
<td>98.9 ± 0.9</td>
<td>1:10</td>
<td>5.2 ± 1.7</td>
</tr>
<tr>
<td>1.25% DCE</td>
<td></td>
<td></td>
<td>1:160</td>
<td>25.7 ± 4.5</td>
<td></td>
</tr>
<tr>
<td>0% N9/2% DS/</td>
<td>Failed</td>
<td>NA</td>
<td>100.0 ± 0.0</td>
<td>1:10</td>
<td>9.4 ± 2.5</td>
</tr>
<tr>
<td>1.25% DCE</td>
<td></td>
<td></td>
<td>1:160</td>
<td>19.7 ± 3.2</td>
<td></td>
</tr>
<tr>
<td>0% N9/2% DS/</td>
<td>—</td>
<td>—</td>
<td>100.0 ± 0.0</td>
<td>1:10</td>
<td>8.3 ± 4.0</td>
</tr>
<tr>
<td>1.25% DCE</td>
<td></td>
<td></td>
<td>1:160</td>
<td>35.2 ± 7.7</td>
<td></td>
</tr>
<tr>
<td>1% N9/2% DS/</td>
<td>13.3 ± 1.5</td>
<td>0.084 ± 0.012</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1.25% DCE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2% N9/2.5% DS/</td>
<td>21.3 ± 3.1</td>
<td>0.104 ± 0.012</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1.25% DCE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4% N9/0.85% DS/</td>
<td>85.3 ± 31.7</td>
<td>0.076 ± 0.016</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1.25% DCE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4% N9/2% DS/</td>
<td>64 ± 3.3</td>
<td>0.087 ± 0.017</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1.25% DCE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4% N9/2.5% DS/</td>
<td>90.7 ± 30.6</td>
<td>0.060 ± 0.012</td>
<td>90.0 ± 2.6</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1.25% DCE</td>
<td></td>
<td></td>
<td>1:160</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2% N9/2% DS/</td>
<td>54.4 ± 9.1</td>
<td>0.091 ± 0.011</td>
<td>90.5 ± 2.7</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1.25% DCE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2% N9/2% DS/</td>
<td>28.8 ± 2.0</td>
<td>0.150 ± 0.016</td>
<td>95.8 ± 1.8</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1.25% DCE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2% N9/2% DS/</td>
<td>40.0 ± 9.4</td>
<td>0.128 ± 0.016</td>
<td>95.8 ± 0.9</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1.25% DCE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2% N9/2% DS/</td>
<td>76.8 ± 20.8</td>
<td>0.106 ± 0.009</td>
<td>90.5 ± 0.9</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1.25% DCE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2% N9/2% DS/</td>
<td>40.0 ± 5.2</td>
<td>0.119 ± 0.016</td>
<td>93.7 ± 3.8</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1.25% DCE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conceptrol®</td>
<td>80.0 ± 10.4</td>
<td>0.119 ± 0.016</td>
<td>88.4 ± 4.1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Conceptrol®</td>
<td>35.2 ± 3.0</td>
<td>0.119 ± 0.006</td>
<td>82.1 ± 4.4</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Conceptrol®</td>
<td>28.8 ± 2.0</td>
<td>0.150 ± 0.016</td>
<td>NA</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>KY® Plus</td>
<td>28.8 ± 2.0</td>
<td>0.151 ± 0.016</td>
<td>89.5 ± 3.9</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>KY® Plus</td>
<td>24.0 ± 2.5</td>
<td>0.207 ± 0.022</td>
<td>85.3 ± 3.1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Igepal CO-630 Special</td>
<td>30.4 ± 1.5</td>
<td>0.128 ± 0.011</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Igepal CO-630 Special</td>
<td>28.0 ± 3.8</td>
<td>0.107 ± 0.012</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>KY Jelly®</td>
<td>Failed</td>
<td>NA</td>
<td>NA</td>
<td>1:10</td>
<td>53.2 ± 9.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1:160</td>
<td>97.7 ± 0.3</td>
</tr>
<tr>
<td>KY Jelly®</td>
<td>Failed</td>
<td>NA</td>
<td>NA</td>
<td>1:160</td>
<td>82.8 ± 6.1</td>
</tr>
<tr>
<td>KY Jelly®</td>
<td>Failed</td>
<td>NA</td>
<td>NA</td>
<td>1:10</td>
<td>26.8 ± 5.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1:160</td>
<td>71.1 ± 5.3</td>
</tr>
<tr>
<td>Replens®</td>
<td>NA</td>
<td>NA</td>
<td>—</td>
<td>1:160</td>
<td>82.8 ± 6.1</td>
</tr>
<tr>
<td>0.9% NaCl</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
<td>100</td>
</tr>
</tbody>
</table>

*a Measurements made at Eastern Virginia Medical School.
CONTRACEPTIVE DESIGN PARAMETERS

The most important aspect of a contraceptive vaginal formulation is obviously its effectiveness in the prevention of pregnancy. Initial evaluations before any clinical study should include the following:

1. Spermicidal effectiveness [Sander-Cramer Assay, sperm dilution and minimum effective concentration (MEC)] (54).
2. Ability to inhibit sperm penetration in cervical mucus [the Modified One End Test (MOET)]
3. Biodiffusion in cervical mucus [Double End Test (DET)]

A series of DCE-based formulations containing Igepal CO-630 Special (N-9) and dextran sulfate (DS) were submitted to Eastern Virginia Medical School (EVMS) for in vitro contraceptive testing. Also included in the screening tests were placebos, N-9, two commercial spermicidal products (Conceptrol® and KY® Plus), vaginal moisturizer products (KY® Jelly and Replens®), and saline. Test results are summarized in Table 6.

The N-9-containing products all exhibited similar spermicidal activity, as illustrated by MEC. Samples without N-9 did not have spermicidal activity.

The DCE placebo vehicle inhibits sperm penetration into the cervical mucus, as illustrated by the very low MOET values after 1:10 and 1:100 dilutions. This activity has not been reported for anionic or nonionic polymer vehicles. There are no striking differences observed in the cervical mucus biodiffusion (DET) among the contraceptive products and the DCE systems. Hence, a contraceptive effect presumably exists for the DCE vehicle—by a sperm impedance mechanism, not spermicidal—that does not exist with currently used anionic and nonionic vehicles. This activity is seen in an in vivo model and is described in more detail elsewhere (12).

N-9 is currently the only approved spermicide in the United States, and formulators must take this fact into strategic account. However, in the design of new polymer vehicles, use of an approved spermicide is a logical choice for product introduction. At the same time, compatibility of such excipients is an important consideration in the formulation of new chemical spermicides.

A correlation exists for a surfactant's spermicidal activity and partition coefficient, such that the MEC is on the order of nonionic > cationic > anionic for given structural variables (55). Researchers need to consider such parameters in designing polymer vehicles and formulations with ionic and hydrophobic features.

STORAGE AND STABILITY

Stability of the final formulation is extremely important and can be influenced by such parameters as formulation components, mixing, temperature of preparation and storage conditions. Formulation stability can generally be
addressed by observing of phase separation or changes in viscosity (usually a decrease).

These characteristics, however minor, may greatly influence the contraceptive profile of a formulation. For example, the spermicide N-9 may aggregate (micelle formation), thus altering its bioavailability. Furthermore, contact of these micelles with vaginal epithelia may result in an increased rate of observed irritation.

Formulation instability due to a prolonged storage period or temperature compromise is very likely the cause of variable contraceptive efficacy and irritation with existing products containing similar N-9 concentrations.

For comparison purposes, we evaluated product formulations using viscosity as an indication of stability after prolonged 40°C (104°F) storage (Fig. 3). The DCE gel formulations exhibited no significant change in appearance or viscosity over 6 months at 40°C. In contrast, the commercial products K-Y®

### TABLE 7. Spermicidal activity\(^a\) of aged samples

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Sander-Cramer Assay MEC (mg/ml)(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t = 0</td>
</tr>
<tr>
<td></td>
<td>t = 6 months</td>
</tr>
<tr>
<td>2% N9/2% DS/1.25% DCE</td>
<td>0.136 ± 0.022</td>
</tr>
<tr>
<td></td>
<td>0.208 ± 0.031</td>
</tr>
<tr>
<td></td>
<td>0.115 ± 0.016</td>
</tr>
<tr>
<td></td>
<td>0.193 ± 0.032</td>
</tr>
<tr>
<td></td>
<td>0.136 ± 0.016</td>
</tr>
<tr>
<td>KY®-Plus</td>
<td>0.229 ± 0.019</td>
</tr>
<tr>
<td>Conceptrol®</td>
<td>0.177 ± 0.018</td>
</tr>
<tr>
<td>Nonoxynol-9</td>
<td>0.151 ± 0.017</td>
</tr>
<tr>
<td></td>
<td>0.138 ± 0.018</td>
</tr>
<tr>
<td></td>
<td>0.504 ± 0.030</td>
</tr>
<tr>
<td></td>
<td>0.193 ± 0.020</td>
</tr>
<tr>
<td></td>
<td>(t = 2.5 months)</td>
</tr>
<tr>
<td></td>
<td>NA</td>
</tr>
</tbody>
</table>

\(^a\) Measurements made at Eastern Virginia Medical School.
\(^b\) \(n = 12\).
TABLE 8. Effect of viscosity on spermicidal activity$^a$

<table>
<thead>
<tr>
<th>Formulation</th>
<th>$\eta^{25}$ (cp)</th>
<th>$\eta^{37}$ (cp)</th>
<th>MEC (mg/ml) $n = 10$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2% N9/2% DS/1.25% DCE</td>
<td>13,959</td>
<td>NA</td>
<td>0.085 ± 0.011</td>
</tr>
<tr>
<td>2% N9/2% DS/1.25% DCE</td>
<td>63,698</td>
<td>61,339</td>
<td>0.088 ± 0.012</td>
</tr>
<tr>
<td>Nonoxynol-9</td>
<td>NA</td>
<td>NA</td>
<td>0.188 ± 0.020</td>
</tr>
</tbody>
</table>

$^a$ MEC measurements made at Eastern Virginia Medical School.

Plus, Conceptrol®, and Advantage 24® could not tolerate storage under similar conditions.

Further evaluation of the impact of aging on the spermicidal effectiveness was also assessed (Table 7). The spermicidal properties of the aged Conceptrol® (2.5 months) and DCE (6 months) formulations were unaffected at 40°C. KY® Plus lost half of its in vitro spermicidal activity after 6 months under this storage condition.

The DCE formulations were initially designed for sponge incorporation, with evolution of the product to include gel applications. Gels with two viscosities were evaluated by the Sander-Cramer technique (Table 8). The spermicidal effectiveness was not affected by viscosity. The viscosity of the clinical formula was set at 50,000–65,000 cp to be similar to commercial products. The viscosity of these formulations did not alter appreciably when measured at 25°C or 37°C.

Maintenance of formula viscosity and effectiveness over prolonged storage at higher temperatures is imperative for product distribution to regions of the world where hot and humid conditions routinely exist. Ideally, the properties of a given formulation should be unaffected by storage, application temperature, or changes in vaginal pH. These properties include substantivity as well as spermicidal, antiviral, and antimicrobial effectiveness.

**SUMMARY AND FUTURE CONSIDERATIONS**

Contraceptive formulations based on available water-soluble polymers are used quite extensively and successfully. However, to meet the challenge of today's emerging health crisis, rationally designed water-soluble polymer vehicles are poised to play an important role in the arsenal against pregnancy and STDs. Double-substituted, hydrophobe-modified cationic polysaccharides, designed specifically for mucosal applications, begin to fulfill this role by displaying sperm impedance, bactericidal activity, and excellent formulation characteristics with a broad range of active ingredients and additional excipients, as well as being nonirritating.

Incorporation of an antiviral component brings an additional element of protection to the user. Dextran sulfate in current DCE formulations has excellent antiviral activity in vitro and demonstrates the potential usefulness of a highly discussed strategy.
Exploitation of the cationic/hydrophobic effect for mucosal substrate use is a design principle that lends itself to the investigation of a variety of structural analogues. However, the measure of success for any vehicle, combination of active ingredients, and final formulated product will be the demonstration of unequivocal efficacy \textit{in vivo}.

\section*{ACKNOWLEDGMENTS}

This broad range and highly interdisciplinary work could not have taken place without the excellent technical contributions and insights of Dr. James Kawakami (formerly Union Carbide), Dr. George Brode (Integra LifeSciences), Dr. Gustavo Doncel (Eastern Virginia Medical School), and Dr. Henry Gabelnick (CONRAD). Thanks to Dr. Bill Rencher for editorial assistance. The financial support of CONRAD and Integra LifeSciences Corporation is also gratefully acknowledged.

\section*{REFERENCES}


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**QUESTION AND ANSWER DIALOGUE**

**Joseph Robinson:** I'd like to correct something relative to this issue of bioadhesion. You can have two negatively charged surfaces that attract each other very well; bacteria do it all the time and so can negatively charged polymers. In fact, I can give you a polymer that you can place in your mouth, rinse with water to try to remove but it won't until you manually pull it off. After peeling it off, you will strip the mucus off with it.

**Unidentified:** Forgive my ignorance, but I'm trying to understand this barrier issue. During mid-cycle there is a spike in calcium which binds to the sialic acid groups and reduces charge repulsion, which automatically drops the viscosity of mucus. Now your polymeric (DCE) material is expected to penetrate into the cervical os and bind to this very dilute mucus to present a barrier. This is quite hard for me to picture.

**Joseph Robinson:** This is a flow of cervical mucus through the cervical canal, and the issue of trying to get something back up in sufficient concentration—to at least present a barrier for diffusion—is a very difficult challenge. This is a relatively inherent weakness of all of these spermicidal systems.

**Gustavo Doncel:** It is true that the current formulations of DCE do not "biodiffuse" into cervical mucus very well, but they probably do it a little better than the commercially available controls. However, that is not the primary objective. DCE prevents
sperm from penetrating into cervical mucus by acting on both sperm and mucus. This is what our *in vitro* and *in vivo* models have demonstrated for this class of compound.

*Henry Gabelnick:* I think the issue is not to look on the mucus as the sole target for the carrier but the DCE’s (Q-2) ability to adhere to skin, epithelium, and other cells. It is the CDE’s general property of coating a variety of cellular surfaces that makes it a useful drug delivery system.

[Editor’s insert: Later in the meeting, Dr. Gabelnick stated: “Q2 (DCE) is available in many different viscosities ranging from low enough to be incorporated in a sponge to high enough for use in films. We have chosen to use the gel in the first clinical tests because it’s the most straightforward formulation to work with and it is the easiest thing to compare with other commercial products. The other thing that I want to make clear in the context of Q2 is that CONRAD paid for the research and is co-owner of the patent, and Q2 will be made available to everyone in this room and throughout the world, to use as an adjunct with their own active(s). This is not something that we have developed with public money to create a royalty flow for CONRAD. I encourage people to contact CONRAD or Integra.”]

*Adriane Fugh-Berman:* To add to the cervical mucus discussion, cervical mucus protects and helps sperm. If a substance doesn’t get into the cervical mucus, there are pockets of protected sperm. The spermicide’s goal is to kill sperm, and other target microorganisms in the vagina. We shouldn’t be fixated on cervical mucus within the cervical canal. Cervical mucus also decreases the chance of live organisms penetrating.

*Bill Rencher:* John, you presented polymer substantivity experiments using a fluorescence method. My understanding is that fluorescein can differentiate between hydrophilic and hydrophobic material. How do you correlate this with substantivity of polymers to mucus?

*John Kemnitzer:* We use this technique as a qualitative screening tool. I’m not saying that this dye is the most appropriate one. I could name others, but it’s a good indication for the type of adhesion or substantivity that we are looking for. This is a very inexpensive and quick screening tool, used to give us essentially a yes or no answer.

*Arthur Mlodozeniec:* Have you tested your compounds’ bioadhesion by using the polymer-tissue detachment force method presented by Dr. Robinson?

*John Kemnitzer:* Not yet. However, some skin stripping tests were performed back in 1987 with the singly substituted cationic and cationic-hydrophobe systems. The results show very high adherence to skin and artificial skin substrates.
Considerations for the Formulation of Protein- and Peptide-Based Therapeutic Molecules

Michael D. Pierschbacher

Telios Pharmaceuticals, Inc., San Diego, California 92121

A large proportion of the physiologic processes that occur in the body are regulated or carried out by proteins. These molecules have been engineered by time to efficiently interact in complicated arrays or cascades that are normally controlled in a robust balance. Therefore, it is intuitive to try to correct a situation in which this balance has been upset by replacing the protein that is missing or deficient, with the expectation being that this approach should carry an increased chance of success coupled with a decreased risk for toxicity compared with more classic drug design. Proteins have also been put to use in situations in which they would not normally be involved, e.g., in enzymatic debridement of wounds. However, proteins pose special challenges when they are considered for therapeutic application. Proteins are generally expensive to produce and difficult to purify, and they are frequently unstable in solution and susceptible to enzymatic degradation, making repeated parenteral administration necessary. In addition, proteins often have multiple functions, not all of which may be needed or desirable for a particular application. A solution to some of these concerns can be derived by identifying a fragment of the protein that retains the desired function and/or stability while eliminating the problems caused by the rest of the protein. An example of this would be Fab antibody fragments that retain the antigen-binding properties of the whole protein but not the ability to activate complement. Fragments such as these are relatively large and usually require cell culture or transgenic animal facilities for production. Taking this approach further, small peptide subsequences of a protein may retain a particular function, e.g., the Arg-Gly-Asp sequence that was identified in fibronectin to be responsible for one of the cell attachment properties of that protein. In the latter case, such peptides can be approached synthetically. Although the use of totally amino acid-based com-
pounds, especially for topical applications, has much to recommend it (safety being high on the list), formulation issues must be carefully considered.

This chapter presents some of the situations that we have encountered in the formulation of proteins and peptides for pharmaceutical applications. It is not meant to be comprehensive, because treatises on this subject already exist. Rather, I will try to highlight challenges that we have encountered and illustrate how these challenges were met. The proteins and peptides that we have worked with may have direct application to reproductive health. Although we have not focused our work in this area, the lessons that we have learned can, I am confident, be applied broadly.

The concept of replacement therapy, in other words, the adding back of a missing or deficient protein (e.g., insulin, tissue plasminogen activator, erythropoietin) or peptide (e.g., oxytocin, LHRH, somatostatin) has been used for many years with reliable success. Yet, synthetic organic chemistry has dominated the pharmaceutical industry. The justification for this has been that small organic molecules are easier to make, less expensive, orally available, and stable. Even though these considerations will remain strong incentives to consider small molecule alternatives to replacement therapy, events of the past decade and ongoing work will continue to make it increasingly attractive to take the replacement therapy approach. These events include the increasingly rapid identification and association of specific peptide mutations with specific disease states and the continuing elucidation for individual proteins of their functions and involvement in biologic pathways. The possibilities for cloning and production of large quantities of proteins in transgenic plants and animals has vastly decreased the cost associated with protein and peptide production as well as the degree of difficulty associated with obtaining pure material. The regulatory bodies are becoming increasingly comfortable with handling recombinant proteins, and methods of continuous delivery of proteins over time have been developed. The last great challenge remains the formulation and stabilization of the proteins themselves.

STABILIZATION AGAINST PROTEOLYSIS

Proteolytic activity in mucous membranes can be a major obstacle to the effective application of peptide-based drugs, severely limiting the extent and duration of their activity. Although less proteolytic activity was detected in a 10% homogenate of the rat vaginal membrane than in the small intestine by the agar plate method using casein as a substrate (1), a significant amount of activity does exist there. Moreover, a comparative study of aminopeptidase activities against enkephalins in the absorptive mucous membranes in the rabbit demonstrated that the supernatants of homogenates of the vaginal, nasal, buccal, rectal, and ileal mucous membranes exhibited similar activity (2). Although many protease inhibitors do exist, inhibition of protease activity


may in fact be detrimental to the desired physiologic outcome for any drug being applied. Fortunately, alternative strategies can be employed.

Our work with the extracellular matrix (ECM) protein fibronectin can illustrate some of the possible strategies we have used to overcome the protease sensitivity problem. Fibronectin is extremely sensitive to proteases in its environment as are many of the other ECM proteins, a property that probably facilitates the efficient remodeling of tissue. Attempts have been made to take advantage of the cell adhesive qualities of fibronectin to condition the surface of prosthetic implantable devices for improved tissue integration. However, proteolytic sensitivity results in the rapid removal of this protein from the surface of the device and the resulting proteolytic fragments may be inflammatory, thus causing more problems than are solved. To identify the structure within the fibronectin polypeptide chains that was responsible for the cell adhesive properties of the protein, we took advantage of the protease sensitivity to cleave the molecule into small fragments that could then be tested for their interaction with the cell surface. We identified a small (108 amino acids of the 2,500 amino acid polypeptide chain of intact fibronectin) peptic fragment that retained the cell adhesive properties of the parent molecule (3). This fragment was extremely resistant to a wide variety of proteases (4) but suffered from two striking drawbacks. The first was readily apparent, the second more subtle. Whereas intact fibronectin is sticky and readily coats surfaces in such a way as to expose its cell adhesive properties, the fragment was highly soluble in aqueous environments and required chemical methods to immobilize it on surfaces. However, what eventually became apparent was that the fragment was not bound by the same cell surface receptor as was the intact protein (5). The importance of this distinction has only recently been appreciated.

The ultimate identification of the amino acid sequence arginine-glycine-aspartic acid (abbreviated RGD) as the minimum epitope within fibronectin that could be recognized by cells (6) led to the identification of the first of what has become a large family of cell surface adhesion receptors that we now call collectively integrins (7). As it turns out, most proteins that make up the ECM contain one or more RGD sequences, and these sequences are recognized by one or more members of the integrin super family. To date more than 20 integrins have been identified and about half of them have been reported to bind to the RGD sequence (8). The striking thing is that, whereas short synthetic peptides that contain the RGD sequence will bind (albeit weakly) to all of the RGD binding integrins, when this sequence is presented in the context of an individual protein it may be recognized by only one or a limited number of the integrins. This led us and others to question the structure–function relationship underlying this remarkable specificity. Ultimately, we came to the conclusion that it was the three-dimensional environment immediately surrounding the RGD sequence itself that determined which of the receptor binding pockets the RGD peptide could enter (9).
opened up the possibility for us to synthesize small RGD-containing molecules that could mimic the receptor specificity of individual intact ECM proteins or to combine the receptor specificity of more than one protein together in the same molecule (10).

The discovery that the cell attachment activity of the ECM could be mimicked by short, immobilized synthetic peptides containing the RGD sequence was significant, as was the related observation that in solution these same peptides were capable of inhibiting cell attachment to RGD-containing ligands. This meant that the potential existed to develop RGD-based therapeutics that function either as agonists to promote the interaction of cells and tissues with artificial matrices or as antagonists to control the nature of cell–cell and cell–ECM interactions. In addition, the pharmacophore was small enough to encourage broad analogue development and has led to the development of a number of molecules that have therapeutic applicability.

As mentioned above, there is a need to condition the surface of implantable prosthetic devices so as to minimize the foreign body response and the encapsulation that is frequently seen with such devices and to maximize their incorporation into the body in a functional manner. One way to accomplish this may be to coat the surface with a molecule that will promote the physiologic interaction of cells with that surface, hence the previous attempts with fibronectin coatings. We therefore sought to coat devices with short synthetic RGD-containing peptides. This was not straightforward, however, because short peptides, particularly those that contain polar residues such as arginine and aspartic acid, are highly soluble and do not stick to surfaces. Moreover, most materials that are used to fashion prosthetic devices are by design chemically inert, thus limiting the options for chemically coupling peptides to their surface.

To overcome these hurdles, we designed a peptide that is hydrophobic at one end and carries an RGD sequence at the other end (Sequence 1) so that it would be energetically favorable for the hydrophobic end to come out of aqueous solution and self-assemble onto surfaces while the hydrophilic end would remain in solution and present the RGD to the appropriate integrin on the cell surface.

Sequence 1. Sequence of PepTite™ Biocompatible Coating peptide:

\[ \text{Ac-G(dR)GDIPASSKGGGG(dR)LLLLL(dR)} - \text{NH}_2 \]

This peptide also has a number of other important features built into it. We observed that cells could not bind to the RGD sequence bound to surfaces in this way unless it was separated from the hydrophobic end by at least a few residues, so we optimized the length and structure to elicit the best cell adhesion response on coated surfaces. The addition of arginines flanking the hydrophobic leucine stretch improved the solubility of the peptide in water and, together with the lysine in the center of the spacer region, served to
further condition the surface of the coated material to be compatible with the cell surface. The R-G bond in the RGD sequence is highly susceptible to cleavage by serine proteases such as trypsin and plasmin. We observed, however, that although integrins could still bind to the RGD sequence when the R position was replaced with the D-conformer of arginine, these proteases could no longer cleave this bond. To further protect the peptide from proteases, the NH$_2$- and COO$^-$-termini were acetylated and amidated, respectively. These modifications have resulted in a peptide that is stable over long periods of time either on the dry coated device or in contact with plasma or serum (unpublished observations). A wide variety of materials have been coated with this peptide and implanted in preclinical animal models with surprisingly favorable results (11.12; and unpublished observations). Whereas this peptide interacts with a relatively wide spectrum of cell surface integrin receptors, another strategy can be applied not only to achieve a high degree of metabolic stability but also to impart a high degree of receptor selectivity as well.

RECEPTOR AFFINITY AND SELECTIVITY

The approach of coating surfaces with a peptide that mimics the function of natural ECM proteins can be correlated with the possibility of inhibiting the interaction of cells with the natural ECM by providing soluble ligands for the integrins to antagonize that interaction. This requires that the antagonists have a high affinity for the integrin to avoid the need for high concentrations of the antagonist to achieve the desired effect. However, because a number of integrins exist that can bind the RGD sequence, each performing a unique function, it is also necessary to design molecules that are specific for only one or a small subset of the integrin family of receptors. One way to achieve this is by cyclizing small peptides that contain this sequence (9).

As an example, we have designed a nine amino-acid peptide that is selective for the fibrinogen binding integrin on platelets and at the same time shows an affinity for this integrin that is three orders of magnitude greater than that of the linear counterpart (Sequence 2). This peptide, like the one described above, is acetylated and amidated, but in this case the R-G bond is protected from relevant proteases by a relative lack of conformational freedom that does not allow the protease to bind. This peptide is cyclized by virtue of a disulfide bond between the terminal cystine residues, but other options are available to accomplish the cyclization. The peptide can be bound by an NH$_2$- to COO$^-$-terminal lactam (peptide) bond or by forming a peptide bond from the side chain of one amino acid to the terminal functional group at the other end of the peptide. These approaches accomplish the protection of the termini by incorporating them into the backbone of the peptide itself. The metabolic stability of such peptides is evidenced by their pharmacologic profile (13). It was observed that the above-mentioned peptide (i.e., Sequence 2) circulates
TABLE 1. Concentrations of representative peptide pharmacophores required to inhibit the binding of four different RGD binding integrins to their respective ligands by 50% (IC₅₀, nM)

<table>
<thead>
<tr>
<th>Integrin—</th>
<th>Peptide—</th>
<th>α₁β₃</th>
<th>α₄β₃</th>
<th>α₅β₁</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. 8X</td>
<td>12</td>
<td>330</td>
<td>6,100</td>
<td>8,700</td>
</tr>
<tr>
<td>B. 11D</td>
<td>6</td>
<td>200</td>
<td>10,000</td>
<td>1,500</td>
</tr>
<tr>
<td>C. 29M</td>
<td>120</td>
<td>6</td>
<td>550</td>
<td>390</td>
</tr>
<tr>
<td>D. 32M</td>
<td>84</td>
<td>1</td>
<td>14</td>
<td>42</td>
</tr>
<tr>
<td>E. 5U</td>
<td>3,400</td>
<td>2,700</td>
<td>46</td>
<td>1,800</td>
</tr>
<tr>
<td>F. 23T</td>
<td>n.d.</td>
<td>63</td>
<td>2</td>
<td>350</td>
</tr>
<tr>
<td>G. 6Z</td>
<td>30</td>
<td>52</td>
<td>330</td>
<td>2</td>
</tr>
<tr>
<td>H. 13H</td>
<td>57</td>
<td>n.d.</td>
<td>110</td>
<td>9</td>
</tr>
<tr>
<td>I. 27O</td>
<td>190</td>
<td>2</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>J. 27S</td>
<td>15</td>
<td>4</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

in the bloodstream of rabbits into which it has been injected for many hours, and almost 100% can be recovered from the urine intact. This is not necessarily true for all cyclic peptides, and some trial and error may be required to optimize this property. However, as this particular peptide illustrates, complete protection can be achieved by using this approach. In general, the smaller and more tightly constrained the ring, the more resistant the peptide will be to proteases.

Sequence 2. Sequence of the antithrombotic peptide TP-9201:

\[ \text{Ac-CNPRGDY(O-Me)RC-NH₂} \]

With regard to receptor specificity and the degree of affinity for a given receptor, we have found that a great deal can be accomplished through cyclization (Table 1). By systematically varying the orientation and structure of the pendant side-chains around the ring, keeping the RGD intact, we have been able to identify multiple different pharmacophores (pharmacologically distinct structures) that display high affinity and distinctly different preferences for various integrins.

STABILITY AFTER FORMULATION

Many proteins and most peptides, when stored as lyophilized powders in a cool, dry environment, remain stable for extended periods of time. However, refrigeration, or freezing and desiccation is often not practical, and where these can be accommodated it adds cost. Furthermore, reconstitution at the time of use adds an unwelcome burden to the healthcare giver and the uncertainty of complete reconstitution. Such considerations also severely hinder the ease of shipping and burden the distribution process with considerable cost, although they do remain options where the efficacy of the product warrants.
Whereas immunoglobulin molecules are exceptionally stable in solution, at neutral pH and room temperature, many other large proteins are not. Even minor contamination with proteases or catalytic metals can result in substantial degradation over time, and serious consideration must be given to removing or neutralizing these contaminants before formulation. As stated above, one way to overcome the shortcomings in stability of a particularly sensitive protein is to identify a more stable fragment that retains the desired activity from the whole protein and, ideally, one small enough to lend itself to economical synthesis. This is often not possible, as in the case of another ECM protein called decorin, the body's natural regulator of the growth factor TGF-β. The results of extensive fragmentation studies with this protein showed that the binding site in decorin for TGF-β is noncontiguous (unpublished observations). Furthermore, we found that this protein acts via a concerted mechanism combining several different functions to regulate the scarring process. However, when fragmentation is possible, other concerns arise.

Proteins are relatively stable to processes such as oxidation and deamidation because of their tightly folded and compact tertiary structure. Individual amino acids in short peptides, on the other hand, have very little protection by surrounding residues from the environment. For example, asparagine and to a lesser extent, glutamine are susceptible to deamidation at pH higher than 7.5, and this degradation process accelerates as the pH rises. This can often change the activity of the peptide markedly. Consequently, formulations should always be held below pH 7.5 but not below about 5.5, because considerable discomfort could ensue on application of acidic formulations to the patient. It is important that the appropriate buffer be chosen to adequately buffer the solution at the pH chosen because it should be kept in mind that degradation products from the peptide could have an influence on the pH.

The peptide in Sequence 2 contains a disulfide bond and, whereas disulfide bonds are quite stable in the environment of the body, our experience has taught us that extremely low levels of reducing agent, be that in the form of an unrelated contaminant or a small amount of uncyclized peptide, can catalyze significant instability. With careful removal of such contaminants, however, this peptide has a projected shelf life of 10 years at room temperature when formulated in a succinate buffer at pH 5.8 (unpublished observations). Incidentally, the choice of counter-ion can also contribute to the overall stability of peptides, and several should be considered before one is chosen.

The peptide in Sequence 1 was originally designed to contain an --RGDS----- sequence. However, as has been well studied, in unconstrained peptide (and to some extent in flexible regions of proteins) the D-S bond can undergo a β-shift rearrangement and in this case destroy the activity that is dependent on the aspartic acid. The residues surrounding this bond can apparently influence the rate and degree to which this rearrangement proceeds. Although the rearrangement is an equilibrium process, this particular peptide proceeds rapidly to a greater than 60% population of the rearranged form.
Different residues following the aspartate residue rearrange at widely differing rates. Thus, replacing the serine with an isoleucine slows this process to barely detectable levels, resulting in a very practical shelflife for this peptide.

**IMMUNOGENICITY**

Although most applications of peptides and proteins in the vagina might be envisioned as topical, it must be kept in mind that absorption through the vaginal mucosa may be substantial. Aref et al. (14) have reviewed extensive work that has been done to investigate the delivery of peptides into the blood through the vaginal mucosa and, in fact, proteins as large as insulin have been shown to be absorbed through this route at pharmacologic concentrations (15). Therefore, immunogenicity must be considered. Fortunately, peptides of less than 20–30 amino acids are usually poorly immunogenic. However, our experience has been that peptides made up primarily of positively charged and neutral amino acids are consistently less immunogenic than peptides that carry an overall negative charge, and that a certain amount of trial and error with respect to conservative replacement of amino acids can quickly lead to peptides that completely evade the immune system.

The flip side of this issue is that frequently it is desirable to have an antibody reagent with which to detect the presence of the peptide. We were successful in raising antibodies that crossreacted with the peptide in Sequence 2 by immunizing with an analogue of that peptide, even though the peptide itself is totally non-immunogenic. The affinity was rather low but it was good enough to enable us to develop an antibody-based quantitation assay for detection of the peptide in the blood. We then used this assay to develop an HPLC method for monitoring this peptide in the blood, which we now use routinely.

**POTENTIAL USE OF RGD-BASED COMPOUNDS IN CONTROLLING FERTILITY**

Recently issued patent U.S. #05578306 cites “Methods of diagnosing infertility in a mammal and methods of detecting the window of embryo implantation in endometrium. Methods of in vitro fertilization, methods of preventing embryo implantation and a method of monitoring endometrial maturation are also within the scope of the present invention. The present invention is also directed to contraceptives. Diagnostic kits useful in the practice of the methods of the invention are also provided.” This work establishes \( \beta_3 \)-integrin binding to an RGD sequence as a necessary event for successful embryo implantation, thus offering a clear opportunity for either enhancement or intervention.
REFERENCES


QUESTION AND ANSWER DIALOGUE

Bill Rencher: Have you thought of applying PepTite to contraceptive implants? With Norplant® there appears to be a very slow cellular capsule formation around each implant that reduces levonorgestrol release. I was thinking that a PepTite-coated implant may stimulate production of a thin biologic capsule rather than a thicker capsule, and thus prolong zero-order release at higher drug rates.

Michael Pierschbacher: I've made that suggestion. We've not pursued it at this time. In fact, a problem with the current biologic encapsulation is that removal of the implant is painful and major tissue reconstruction occurs.

Kurt Barnhart: I'm intrigued about the integrins. Most of my naive knowledge of integrins is in the endometrium, the window of implantation, and the work that's been done with $\alpha_3\beta_3$. Clearly, that has a very cyclical change, in accord with hormones in the menstrual cycle. If we're talking about applications of the same integrin elsewhere, does that integrin have the same selectivity in the vagina as other areas you've studied?

Michael Pierschbacher: The generalization that I think I'm comfortable making is that, whenever you have cells moving around in the body, there are two integrins that seem to be playing critical roles, pretty much all the time, everywhere we look. They are $\alpha_3\beta_3$ and $\alpha_4\beta_1$, the first fibronectin receptor. I think that makes sense when we've looked at the pharmacology of those two receptors. $\alpha_3\beta_3$ is tied in with a number of different second messenger systems that control the degree of response to growth factors. So it makes sense that they would be functioning when cells are responding to those growth factors. The $\alpha_3\beta_1$ is tied very closely to the BCL2 pathway and elevates BCL2, protecting cells from apoptosis. So while they are moving and not, perhaps, coupled to their normal matrix, but interacting with fibronectin matrix, they are pro-
tected from apoptosis through that integrin. Then, in some cases, other integrins come into play for particular cell types; but, pretty much all the time that you see a cell moving or tissue morphogenesis happening, you will find elevated $\alpha_\beta_3$ and $\alpha_\beta_1$. Interestingly, TGF-β elevates $\alpha_\beta_1$ as well, and TGF-β is known to protect cells from apoptosis and may actually use that pathway to do it.

*Jeffery Spieler:* Any known integrin interaction with sperm?

*Michael Pierschbacher:* There has been a report of an RGD interaction between sperm and egg during fertilization, but I have not seen any follow-up.
8

Formulation of C31G as a Vaginal Microbicide

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*Biosyn, Inc., Philadelphia, Pennsylvania 19104; and †Philadelphia College of Pharmacy and Science, Philadelphia, Pennsylvania 19104

Our studies have focused on a new microbicide, C31G, composed of two amphoteric surface active molecules, an alkyl amine oxide and an alkyl betaine. In vitro, C31G is an effective microbicide when tested against a large number of Gram-positive and Gram-negative bacterial species and strains, and also demonstrates potent activity against yeast, HIV, and HSV. In addition, C31G has spermicidal activity. In comparison with nonoxynol-9 (N-9), C31G demonstrates a wider range of bioactivity. Formulation development presented several unique challenges based on the chemistry of the active components in C31G. For example, the amphoteric nature of both the amine oxide and the betaine eliminated the possibility of using most common anionic excipients. We were able to successfully formulate both a suppository and a gel dosage form, develop a validated HPLC analytic method, and demonstrate in vitro availability of the actives.

Because heterosexual intercourse by an infected man with an uninfected woman is the most significant route for HIV transmission, the need for new methods to prevent sexual transmission of HIV is critical. Although latex condoms remain an effective barrier to the virus, in many cultures women have too little power to demand the use of male condoms (1). It is therefore crucial to develop new, female-controlled methods designed to prevent HIV transmission. These methods could involve physical barriers (e.g., female condom), chemical microbicides, or combinations of the two.

The ideal microbicide would be a broad-spectrum antimicrobial and antiviral agent with a low toxicity for the vaginal epithelium. It might also be important for the microbicide to display spermicidal activity or in some instances, it might be preferable to have a microbicide that does not interfere with contraception. At present, most of the over-the-counter spermicidal products contain N-9. Although N-9 has been demonstrated to be a good
lubricant with spermicidal activity, a number of problems are associated with its use. N-9 has been shown to be active in vitro against a number of pathogens (2,3), yet there is little evidence to support effective microbicidal activity in vivo. Several studies have reported that repeated use of N-9 is associated with vaginal irritation and disruption of the mucosal surface (4,5). In addition, in vitro studies indicated that the compound can increase Candida adherence (6), which could explain the reports of increased incidence of vaginal candidiasis in N-9 users (7). In a recent study evaluating an N-9-containing vaginal film as a potential inhibitor of HIV transmission, there was no added value of the N-9 film over the use of condoms alone and there was a modest increase in vaginal irritation (8). There are conflicting reports with respect to the efficacy of N-9 against Chlamydia (9,10). In general, there is a strong feeling in the community, that the development of new microbicides could have a significant effect on reducing the transmission of a variety of viruses and bacteria associated with STDs, including HIV, HSV, Treponema, Neisseria, Hemophilus, and Chlamydia. Furthermore, because STDs are clearly a cofactor for HIV acquisition (11,12), any new microbicide must have a broad range of activity against the pathogens associated with these STDs.

Although N-9 is the only commercially available spermicide with documented antimicrobial activity against some STD pathogens in vitro, a number of chemical types of microbicides are under development. Some of these involve new delivery systems for N-9, some are combination products incorporating known antimicrobial agents, and others represent new chemical entities. Table 1 summarizes some of the microbicides under development. This list is intended to be representative rather than exhaustive.

Once an active ingredient is selected, the issue of formulation of this material becomes paramount. As discussed elsewhere in this volume, the nature of the active microbicide usually plays a major role in the approach taken to

<table>
<thead>
<tr>
<th>Type of compound</th>
<th>Example</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface-active agents</td>
<td>Advantage-24</td>
<td>N-9 in a slow-release gel</td>
</tr>
<tr>
<td></td>
<td>Protectate sponge</td>
<td>Sponge containing N-9 and benzalkonium chloride</td>
</tr>
<tr>
<td></td>
<td>Chlorhexidine</td>
<td>Quaternary ammonium with high substantivity</td>
</tr>
<tr>
<td></td>
<td>C31G</td>
<td>Broad-spectrum antibacterial and antiviral</td>
</tr>
<tr>
<td>Sulfated polysaccharides</td>
<td>Dextran sulfate carrageenan</td>
<td>Blocks HIV, HSV, and Chlamydia infections</td>
</tr>
<tr>
<td>Proteins/peptides</td>
<td>Magainins, protegrins</td>
<td>Hydrophobic peptides that disrupt membranes</td>
</tr>
<tr>
<td></td>
<td>Monoclonal antibodies</td>
<td>Pathogen-specific</td>
</tr>
<tr>
<td></td>
<td>Pro 2000</td>
<td>Blocks gp120-CD4 binding</td>
</tr>
<tr>
<td></td>
<td>Buffergel</td>
<td>Maintains low pH to kill microbes</td>
</tr>
<tr>
<td></td>
<td>Lactobacillus</td>
<td>Maintains low pH</td>
</tr>
</tbody>
</table>
formulate the vaginal microbicide. In terms of the final product, the ideal microbicide formulation would be odorless and tasteless, should dissolve quickly and spread evenly throughout the vagina, should be able to be used discreetly, and would be effective for several hours after application. Focus group studies have shown that different populations prefer different types of delivery systems (e.g., gels, foams, tablets, film, suppository), and it appears likely that a variety of types of formulations may be desirable, considering the divergent attitudes of individuals in different cultural situations.

C31G: CHEMISTRY AND BIOACTIVITY

Our own studies have focused on a new surface-active microbicide that is based on a patented technology referred to as C31G (13–15). C31G consists of a mixture of synthetic amphoteric molecules, typically an alkyl amine oxide and an alkyl dimethyl glycine (betaine). The two surface-active molecules are mixed in equimolar concentrations and buffered with citric acid to a pH of 4.8. The molecules form a mixed micelle with the amphoteric polar head groups on the surface of the micelle available to interact with bacteria or enveloped viruses. C31G also demonstrates potent spermicidal activity (16). The mechanism of action of C31G appears to involve binding of the micelle to the phospholipid membrane of the microbe, followed by a disruption of this membrane. A typical structure for C31G, shown below (Fig. 1) is a C14 alkyl amine oxide and a C16 alkyl betaine. Other betaines that can be used include alkyl sulfobetaines, acyl betaines, and alkylimidazolinium betaines; the amine oxides include alkyl-N, N-dimethylamine oxides, N-dihydroethylamine oxides, or acylamide t-amide oxides. Typically, the alkyl chain lengths may vary from C12 to C18.

Extensive studies have been carried out to evaluate the antimicrobial efficacy of C31G (14,15,17). Typically, this involves the determination of the minimal inhibitory concentration (MIC) and/or the minimum cytocidal concentration (MCC). Shown in Table 2 are representative MIC data for C31G against a variety of common microorganisms. For comparison we have included the MIC values for N-9. It should be noted that C31G is a broad-spectrum antibacterial, with similar MIC values for a wide variety of Gram-

\[
\begin{align*}
\text{Alkyl Dimethyl Amine Oxide} & \quad \text{Alkyl Dimethyl Glycine (betaine)} \\
\text{CH}_3 & \quad \text{CH}_3 \\
\text{C}_{14} \text{H}_{29} \text{N}^-\text{O}^- & \quad \text{C}_{16} \text{H}_{15} \text{N}^-\text{CH}_2 \text{C}^\text{O}^- \\
\text{CH}_3 & \quad \text{CH}_3 \\
\end{align*}
\]

FIG. 1. Composition of C31G.
negative. Gram-positive bacteria and yeast. In addition, C31G gives an MCC value equal to the MIC value in all cases tested, demonstrating that the mechanism of action involves killing of microbes rather than merely inhibiting their growth. In contrast, N-9 is almost as effective as C31G against certain organisms (e.g., *N. gonorrheae* and *T. pallidum*), moderately effective against other organisms (e.g., *S. sanguis*), and without effect against other known pathogens (e.g., *E. coli*, *S. aureus*, *C. albicans*).

In addition, C31G is effective against antibiotic-resistant bacteria and azole-resistant *C. albicans* (Table 3). With the rapid emergence of antibiotic-resistant organisms, microbicides demonstrating activity against these resistant strains will become increasingly important. Furthermore, because the mechanism of action of C31G involves membrane disruption rather than inhibition of a specific enzymatic pathway, the emergence of C31G-resistant organisms is unlikely.

We have also demonstrated that C31G is effective against HIV-1, HSV-1, and HSV-2, all enveloped viruses. Representative data are shown below in Figs. 2 and 3. In contrast, C31G and other surface-active agents, including N-9, are inactive when tested against nonenveloped viruses (SV40, Polio, HPV). It may therefore be desirable, particularly in the case of HPV, to develop formulations with additional ingredients designed to inactivate these pathogens.

<table>
<thead>
<tr>
<th>Organism/strain</th>
<th>Phenotype</th>
<th>MIC (%) C31G</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em></td>
<td>Wild-type</td>
<td>0.004</td>
</tr>
<tr>
<td><em>MR S. aureus</em></td>
<td>Methicillin-resistant</td>
<td>0.004</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>Wild-type</td>
<td>0.004</td>
</tr>
<tr>
<td><em>FR C. albicans</em></td>
<td>Fluconazole-resistant</td>
<td>0.010</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Wild-type</td>
<td>0.012</td>
</tr>
<tr>
<td><em>PsA1</em></td>
<td>Resistant</td>
<td>0.012</td>
</tr>
<tr>
<td><em>PsA5</em></td>
<td>Resistant</td>
<td>0.100</td>
</tr>
<tr>
<td><em>Pseudomonas cepacia PsA6</em></td>
<td>Resistant</td>
<td>0.003</td>
</tr>
<tr>
<td><em>Pseudomonas sp. PsA2</em></td>
<td>Resistant</td>
<td>0.050</td>
</tr>
</tbody>
</table>
The antimicrobial action of C31G is extremely rapid. When tested in a
time-kill assay against *E. coli*, there was essentially 100% kill within seconds
(Fig. 4). We believe that this is a most important consideration in the devel­
opment of new microbicides, because it is likely that incoming pathogens will
need to be rapidly inactivated to prevent infectious agents in the male genital
secretions from infecting cells of the female genital tract.

C31G: FORMULATION

The overall goal in formulating C31G into a vaginal dosage form was to
retain maximal flexibility to respond to clinical testing and market demands.
A suppository was considered as a convenient delivery system and provided
for finite dosing, i.e., predetermined fixed dose. A gel dosage form was also
considered to provide an alternative to suppositories, with a range of proper-
ties and the potential for a variable dose. Final selection would depend on clinical testing, climate conditions in the market area, patient acceptability, and the target patient base.

**Suppository Dosage Form**

The suppository dosage form was addressed first, with the initial decision being the choice of base. This selection process centered on two factors: the intended use of the product and the physical form of the active ingredient, C31G.

The two components of C31G are both water-soluble and available commercially as concentrated aqueous solutions. Subsequently, the alkyl amine oxide has become available in solid form. Incorporation of such an active ingredient into a lipid base, such as cocoa butter or glycerol esters, is difficult because of solubility problems or limited water uptake, although lipid bases are known to accommodate an appreciable amount of aqueous solutions by emulsification or physical entrapment. Release of a drug from such bases would require a dissolution step (in the case of a solid water-soluble active) and at least one partitioning step in the diffusion of the active from the suppository base into vaginal fluid. Both of these steps are potentially slow and could compromise the overall bioavailability. The use of a water-soluble active in a water-soluble base, such as polyoxyethylene glycol, would eliminate the potentially slow dissolution or partitioning step. Bioavailability would depend simply on the disintegration or dissolution of the suppository itself. General properties of potential suppository bases are outlined in Table 4.

The selection process identified polyoxyethylene glycol (PEG) as a reasonable choice for the suppository base. The qualitative formula for the C31G/PEG suppository dosage form is summarized in Table 5. A series of polyoxyethylene glycols, from liquid to waxy solid, was used to provide for flexibility in physical properties.
TABLE 4. General considerations in the selection of a suppository base

<table>
<thead>
<tr>
<th>Suppository base</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid: cocoa butter or fatty acid esters</td>
<td>Many successful therapeutic products but not many contraceptive products</td>
</tr>
<tr>
<td></td>
<td>Would require a dissolution and partitioning process with a water-soluble drug, which could result in slow or incomplete bioavailability</td>
</tr>
<tr>
<td>Glycerinated gelatin</td>
<td>Historically used for vaginal suppositories</td>
</tr>
<tr>
<td></td>
<td>Perceived potential for local irritation</td>
</tr>
<tr>
<td>Polyethylene glycols (PEGs)</td>
<td>Widely used as a suppository base</td>
</tr>
<tr>
<td></td>
<td>Water-soluble so that dissolution and partitioning of the drug would not be a problem</td>
</tr>
</tbody>
</table>

The finished suppositories complied with USP Uniformity of Dosage Units requirements (USP 23 <905>) with a content uniformity of 95–103% of label (1.7–3.0% RSD) and were easy to manufacture. Content determination was carried out with an HPLC assay, which presented a challenge because neither component absorbs in the UV. Consequently, refractive index was used as the detection method. Disintegration testing was done using the USP procedure (USP 23 <701>) and indicated disintegration times of 9–15 min for the entire range of PEG compositions. Typical data are summarized in Table 6.

Dissolution profiles were obtained using a dissolution procedure with parameters intended to mimic in vivo conditions as follows:

Suppository
37°C
15 ml water in a 50-ml Erlenmeyer flask
Platform shaker at 180 rpm
0.5 ml sample, replacement with 0.5 ml water
HPLC assay

Profiles are summarized in Fig. 5, comparing the dissolution of C31G from a PEG base with the dissolution of N-9 from two commercial products, Encare and Conceptrol. The profiles demonstrate that the in vitro performances of the suppository products are similar.

Biologic testing of C31G dissolved from suppositories showed equivalent bioactivity to the unformulated C31G for both bacteria (MIC) and sperm (16), indicating that the formulation did not compromise activity. In addition,
a spermicidal test on sections of individual suppositories confirmed a uniform distribution of C31G throughout the dosage form. Concerns regarding the clinical acceptability of a suppository dosage form remain. However, the above *in vitro* data demonstrate that C31G is effective in a PEG suppository dosage form.

**Gel Dosage Form**

The formulation of C31G into a gel presented special problems with compatibility. Through an initial screening program it was determined that C31G was incompatible with many typical gelling agents. Because both components of C31G are amphoteric, they either carry a permanent positive charge or are positively charged under certain pH conditions. Consequently, C31G has solubility problems with anionic gelling agents; these observations are summarized in Table 7.

Early trials were made with the compatible excipients as shown in Table 8, utilizing several gelling agents to provide flexibility in manipulating physical properties of the gel.

![Graph showing dissolution profiles](image)

**FIG. 5.** Comparison of dissolution profiles of C31G/PEG and commercially available N-9 suppositories.
TABLE 7. Compatibility of C31G with gelling agents

<table>
<thead>
<tr>
<th>Material</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incompatible</td>
<td></td>
</tr>
<tr>
<td>Carrageenan</td>
<td>Copious, stringy precipitate</td>
</tr>
<tr>
<td>Carbopol</td>
<td>Stringy precipitate</td>
</tr>
<tr>
<td>Polycarbophil</td>
<td>Stringy precipitate</td>
</tr>
<tr>
<td>Na carboxymethyl cellulose</td>
<td>Stringy precipitate</td>
</tr>
<tr>
<td>PVP</td>
<td>Clear to cloudy</td>
</tr>
<tr>
<td>Pluronic acid</td>
<td>Phase separation</td>
</tr>
<tr>
<td>KY lubricant</td>
<td>Phase separation</td>
</tr>
<tr>
<td>Sodium lauryl sulfate</td>
<td>Precipitate</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>Hard white precipitate</td>
</tr>
<tr>
<td>TEA stearate</td>
<td>Thick white precipitate</td>
</tr>
<tr>
<td>Compatible</td>
<td></td>
</tr>
<tr>
<td>Gelatin</td>
<td></td>
</tr>
<tr>
<td>Starch</td>
<td></td>
</tr>
<tr>
<td>Non-ionic cellulose derivatives</td>
<td>A rather wide range of physical</td>
</tr>
<tr>
<td>Double-substituted cellulose ether (DCE, Integra)</td>
<td>properties could be obtained with</td>
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<tr>
<td></td>
<td>the above general formula, ranging</td>
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<td></td>
<td>from clear, brittle to soft, cloudy</td>
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<tr>
<td></td>
<td>gels. Of primary interest in ph</td>
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<td>ysical properties were the appear</td>
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<tr>
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<td>ance, thickness or viscosity, and</td>
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<td>tackiness. It soon became apparent</td>
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<tr>
<td></td>
<td>that few quantitative guide</td>
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<tr>
<td></td>
<td>lines have been published at least</td>
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<td></td>
<td>on the viscosity of vaginal products.</td>
</tr>
<tr>
<td></td>
<td>Our approach was to evaluate initia</td>
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<td></td>
<td>lly on a subjective basis using KY</td>
</tr>
<tr>
<td></td>
<td>lubricant as a reference point.</td>
</tr>
<tr>
<td></td>
<td>Although many samples were prepared</td>
</tr>
<tr>
<td></td>
<td>with the above formula with suitab</td>
</tr>
<tr>
<td></td>
<td>le viscosity and texture at room tem</td>
</tr>
<tr>
<td></td>
<td>perature, most of them became fluid</td>
</tr>
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<td></td>
<td>at 37°C. Attempts to increase viscos</td>
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<td>ity at 37°C resulted in products th</td>
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<td></td>
<td>at either had excessive brittleness</td>
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<td></td>
<td>or exhibited phase separation at ro</td>
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<td>om temperature. The observation th</td>
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<tr>
<td></td>
<td>at KY lubricant maintained its visc</td>
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<td>osity at 37°C led to a reevaluation</td>
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<td>of hydroxyethyl cellulose, even th</td>
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<td>ough initial testing had shown an i</td>
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<tr>
<td></td>
<td>mmiscibility between C31G and KY lu</td>
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<tr>
<td></td>
<td>bricant. Further experimentation es</td>
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<td></td>
<td>tablished a phase diagram of C31G w</td>
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<tr>
<td></td>
<td>ith hydroxyethyl cellulose, identif</td>
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<td></td>
<td>ying areas of phase separation but</td>
</tr>
<tr>
<td></td>
<td>also areas of compatibility. These</td>
</tr>
<tr>
<td></td>
<td>compatible areas were explored and</td>
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<td></td>
<td>resulted in a composition of C31G a</td>
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<tr>
<td></td>
<td>nd hydroxyethyl cellulose with clea</td>
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<tr>
<td></td>
<td>r gel properties at room temperature</td>
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<tr>
<td></td>
<td>capable of maintaining viscosity a</td>
</tr>
</tbody>
</table>

TABLE 8. Viscosity/temperature profile of C31G in hydroxyethyl cellulose gels

<table>
<thead>
<tr>
<th>Flow parameter (sec)</th>
<th>Gel lot no.</th>
<th>20°C</th>
<th>37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G</td>
<td>2.6</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>6.8</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>7.6</td>
<td>6.9</td>
</tr>
</tbody>
</table>
TABLE 9. Qualitative formula for C31G vaginal gel

<table>
<thead>
<tr>
<th>Ingredient</th>
</tr>
</thead>
<tbody>
<tr>
<td>C31G (as a 10% concentrate)</td>
</tr>
<tr>
<td>Type A gelatin</td>
</tr>
<tr>
<td>Purified corn starch</td>
</tr>
<tr>
<td>Hydroxypropylmethyl cellulose</td>
</tr>
<tr>
<td>Glycerin</td>
</tr>
<tr>
<td>Water</td>
</tr>
</tbody>
</table>

The change in viscosity with temperature can be seen in Table 8, using an empirical drainage test (time for a standard sample to flow through a standard tube). These differences are relatively small in comparison with the fluidization with temperature exhibited by the formula in Table 9.

An HPLC-based analytic method has been developed and validated for analysis of C31G in the gel formulation. Clinical batches have been prepared under GMP conditions and are currently on stability.

Biologic testing on the gel confirms that C31G in the gel formulation has activity equivalent to that of the unformulated C31G and the suppository dosage form.

ACKNOWLEDGMENTS

This research was supported in part by grants from the NIAID (#PO1AI37829), NICHD (#NO1-HD33193), and CONRAD.

REFERENCES

QUESTION AND ANSWER DIALOGUE

Bill Rencher: What’s the minimum micellar concentration of C31G? How does this compare to the Sander-Cramer (spermicide) MIC value?

Roger Schnaare: The CMC is between \(10^{-5}\) and \(10^{-4}\) M. The antimicrobial MICs are about 10 times higher than the CMC, we believe, when C31G is acting in its micellar form.

George Brode: Just a comment. Among the water-soluble polymers used in personal care products, the amphoterics have had some of the least irritation. This may explain why C31G is so innocuous to epithelium whereas N-9 and benzalkonium chloride are very strong detergents and more irritating.

Arthur Mlodozeniec: Have you looked at the surfaces and self-association of the molecules (C31G) at increasing concentrations? In systems like this, it wouldn’t be surprising that they would form lyotropic mesophases (crystal faces with ordered structure).

Daniel Malamud: We have some ongoing studies as part of a graduate student thesis. What he is doing is using a Langmuir trough with DPPC monolayers and looking at the way a variety of surface active molecules, including N9, amine oxide, and betaine, interact with that monolayer. You can watch the dissolution of the phospholipid membrane, which is fluorescent-labeled. There are clear differences between each of the compounds, and he is building a model to demonstrate the mechanism of this interaction. Looking back to my MIC slide, what’s interesting to me is not that C31G has similar MICs for all those bacteria but that N-9 does not. We are studying this with model phospholipids such as DPPC. One approach is to extract phospholipid from bacteria that are sensitive and insensitive, say, to N-9 and to look at the different ways in which they interact.

Roger Schnaare: Also, another graduate student is looking at how the molecules interact with each other in solution, to answer the question of whether stoichiometry is critical for the paired compounds.

Arthur Mlodozeniec: This is the first time I’ve been exposed to your molecule, but I’ve worked with similar systems in the past. If you find the unique stoichiometry that gives you symmetrical order, then you begin to develop new properties of the system, including electrical properties in potential differences. And this would give you a stunning move toward biologic surface properties. The basis for membrane mimetic properties is to develop potential differences, and a lot of that depends on the symmetry of molecules, because then you begin to layer bifolds and so forth and you can get much more structure. This also begins to affect the selected diffusion and pathways that might exert some effect in terms of spermicidal activity and so forth. These systems can be studied quite simply. There are smectic and pneumatic liquid crystal phases—not the kind that we’re used to in liquid crystal displays but the kind that you would have in the biologic systems. And they are found that way in nature, including in ejaculum.

Roger Schnaare: We know that we have monomers, micelles, and rods. We do not think that we are high enough in concentration to get other structures. This should certainly be explored.
What is Needed to Advance Vaginal Formulation Technology?

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A vaginal formulation is affected by chemical, physical and biological interactions with its environment. Accordingly, advances in vaginal formulation technology will derive from a synergy of expertise in chemistry, pharmacology, engineering, physiology, microbiology, and medicine. Improved products (vehicles and active ingredients) should be developed, with their molecular properties designed to fulfill certain functions. These functions relate to product dispersion throughout the vagina, retention for intended intervals, physicochemical interaction with the vaginal milieu, including release of bioactive constituents, and consequent effects on target organisms. Some details of these functions differ, depending on whether the primary goal is contraception or prophylaxis against STD pathogens. The latter is a more demanding goal in that it requires coating the vaginal epithelium with a protective/bioactive layer rather than simply preventing spermatozoa from entering the cervical os. To some extent, we can study different functional requirements of vaginal formulations empirically using animal models. Functions can also be defined and studied in terms of basic scientific principles that are amenable to both in vitro and in vivo experimental evaluation in animals and in humans. Experimental assessment of physical and chemical properties of formulations, as well as specific interpretation of resulting data, should be guided by characterization of product functions at a mechanistic level. The following sections summarize a strategy for experimental testing and analysis of vaginal formulations that is being undertaken in our laboratory. Both in vitro and in vivo studies of formulation functioning are being conducted. The perspective and methodology presented do not include all formulation functions and testing.
A woman typically introduces a topical intravaginal contraception/prophylaxis with expression into the vagina of a bolus of a product (vehicle). Thereafter, the time-dependent distribution of this vehicle [and its active ingredient(s)] throughout the vagina is a complex phenomenon deriving from multiple physical and chemical processes. Therefore, it is a deterministic process, albeit a complex one, i.e., properties of the vehicle, and its intravaginal environment, determine its spreading over the epithelium (coating) and subsequent retention. Thus, we can identify the primary interacting elements of the overall process whereby a vehicle coats the vaginal epithelium, fills open spaces, and resists deformation or is “eroded” over time:

Squeezing of a vehicle by movements of apposing epithelial surfaces returning to their undistended configuration (on applicator removal), by visceral contractions, or by movements of the penis

Shearing of a vehicle, e.g., due to motions of the penis. apposing epithelial surfaces

Sliding of a vehicle over those surfaces caused by gravity

Seeping of a vehicle into vaginal rugae

Sticking of a vehicle to vaginal surfaces and/or their coatings

On the basis of fundamental principles of physics and chemistry, we can measure a set of vehicle properties that affect these processes of vehicle deployment:

Rheologic properties—e.g., viscosity, elasticity, yield stress, plasticity

Surface tension, which depends on the surface contacted by vehicle

Density

Boundary conditions between the vehicle surfaces and the vaginal surfaces.

These are related to adhesion of the vehicle to contact surfaces, i.e., to epithelium that may be naked, or coated with mucus or other vaginal “fluid”

Miscibility refers to vehicle erosion over time caused by interactions of a layer of vehicle on an epithelial surface with semen. Miscibility is a thermodynamic effect of the vehicle and semen in contact

These properties conform to basic biophysical principles that apply to spreading and retention of a vehicle over time. The spreading of a deformable material results from a trade-off between applied forces (to the surface and mass of the material) and the inherent resistance of the material to movement and deformation. As the spermicidal/microbicidal vehicle is placed in the vagina, the product applicator is removed, and the walls of the vagina are returned to their undistended configuration, several types of applied forces immediately act to displace and deform the vehicle.
Adhesion and the rheologic properties of the vehicle are the two principal phenomena that resist movement and deformation of the vehicle. For many polymeric materials, including those of typical intravaginal vehicles, the ability of the rheologic properties to resist deformation depends on other factors that influence the rate of deformation. For example, viscosity may decrease or increase with increasing shear rate. Therefore, rheologic properties of vehicles should be characterized with respect to the biologically relevant scales of stress and strain that are estimated to occur within the vagina.

The real in vivo spreading and retention of a vehicle are quite complex, and a mathematical model of the entire process cannot be immediately posed and solved. However, this does not invalidate the study of how vehicle properties influence deployment. Our concept is characteristic of engineering research and development and involves a "divide-and-conquer" strategy. That is, we conceive of the total process (spreading and retention) as comprising a set of simpler, core subprocesses that occur simultaneously (i.e., squeezing, shearing, sliding, seeping, adherence/erosion). These subprocesses can each be modeled theoretically and measured experimentally. In each, salient vehicle properties (independent variables, e.g., viscosity) predict relevant features of vehicle performance (the end points or dependent variables, e.g., spreading rate). After applying data to predictive mechanistic, mathematical models, integrating results from all these simplified subproblems provides clues about which properties are significant or predictive.

Just as we study product dispersion and retention within the vagina so, too, can we study and describe release and transport of active ingredients into the vagina. The process of drug release from a vehicle and subsequent interaction with local fluids (semen, vaginal fluid, or mucus), epithelium, and individual cells is influenced in part by the mechanisms of vehicle deployment. However, additional physicochemical factors are involved, e.g., pH and other ion transport processes, solubility, and drug partitioning phenomena at the vehicle margins. These processes have been addressed elsewhere in this volume and only a few comments will be offered here. Target organisms for active ingredients may reside in semen, cervical mucus, or other vaginal fluid. Therefore, formulations should be tested with respect to all three fluids. There is significant species variability in the physiology, histology, and chemistry of the vagina and cervix, and these differences must be taken into account in choosing animal models. For example, only primates and ruminants have a mucus-laden cervix, whose contents may appreciably spill out into the vagina during the periovulatory period. This is an important fact to consider in designing animal studies that focus on mucus-related phenomena. The pH of the vagina and the natural sperm survival therein vary among species: for example, the human vagina is less hospitable to sperm than is the rabbit vagina.

The physicochemical interaction of the vehicle with the target fluid will influence the release of active ingredients and their transport through the fluid. For example, in recent work we have begun to investigate the effects of
VAGINAL FORMULATION TECHNOLOGY

Vehicle pH and ionic strength on nonoxynol 9 (N-9) permeation of cervical mucus and its ability to immobilize sperm in the mucus (1,2). This work involves direct measurement of the transfer of N-9 molecules from a vehicle to the mucus, as well as the use of the double ended test (DET; 3). We have found that low pH enhances the effects of N-9 and increases the depth of the lethal zone in mucus into which sperm cannot penetrate (DET results). However, the effects of vehicle pH and osmolarity on N-9 diffusion *per se* are more complicated and depend on N-9 concentration (i.e., the micelle to monomer ratio). There is clearly a trade-off here. Vehicles of high osmolarity and pH >6 will prove more effective in delivering increased net amounts of N-9 into mucus adjacent to the vehicles, enhancing the barrier effects of N-9. Increasing osmolarity, however, will also reduce the lethal penetration distance of N-9 into the mucus interior. Such results illustrate the potential for developing improved vehicles that are more effective in delivering their bioactive payloads and that, themselves, may have significant bioactivity. The DET is a *passive* test, as is our measurement of N-9 diffusion, in that there is no mixing of formulation and mucus, as may occur within the vagina. In contrast, the Sander-Cramer test of direct spermicidal activity against semen involves mixing semen and spermicide (either in a solution or in vehicle itself) via vortexing. This is a simple and standard technique that is useful as an initial screen of the potency of spermicidal molecules. However, in evaluation of bioactive molecules in gel formulations, the test is less useful and is more difficult to standardize. Indeed, there is a need to assess the range of conditions in the mixing of semen, vehicle, and mucus, that may occur within the vagina. This information could be used to set standards for mixing vehicles, semen, and mucus in *in vitro* tests, which could help in the development of improved formulations.

**HYPOTHESES THAT CAN BE TESTED WITH IN VITRO EXPERIMENTS AND THAT COULD LEAD TO IMPROVED VEHICLE DESIGN**

The following are questions regarding vehicle functioning that might be studied through *in vitro* experimentation. This list is intended to be illustrative and does not include questions involving drug delivery functions *per se*, i.e., transport mechanisms of release and molecular migration.

1. Vehicle rheologic properties resist all causes of spreading, but in different ways. Non-Newtonian behavior, including yield stresses, may be significant.

2. Vehicle density drives *sliding* due to gravity but viscosity and local layer thickness may significantly inhibit sliding. Thus, sliding may be important to different extents in different regions of the vagina, and it may also be important in vaginas with various undistended configurations (e.g., in nulliparous vs. multiparous women).
3. Surface tension between a vehicle and epithelial surface drives *seeping into rugae*. Such surface tension depends on the nature of the epithelial surface, i.e., mucus-covered, liquid-coated, or moist. Seeping *per se* also depends on the size and shape of individual rugae.

4. *Slippage* at the boundary between a vehicle and epithelial surface (i.e., due to inadequate adhesion) can increase the rates of squeezing and sliding, and can make adhesion yet more uneven. The tendency for such slippage depends on the vehicle and epithelial interaction.

5. Vehicle properties may vary with time *in situ*, resulting in redistribution of the coating of the epithelium and erosion.

6. Specific contact of semen with a vehicle might erode or alter a vehicle’s distribution on the epithelium over time (*adhesion/erosion*).

These hypotheses can be developed and tested through mathematical models of the mechanics of vehicle deployment, primarily squeezing, shearing, sliding and seeping. One illustrative example is presented here, for the squeezing process.

### EXAMPLE MODEL: SQUEEZING OF VEHICLE AS APPOSING EPITHELIAL SURFACES RESUME UNDISTENDED CONFIGURATION

We consider a simple geometric model of initial spreading after release of a bolus of vehicle into the vagina (Fig. 1). Apposing vaginal surfaces return to their undistended positions (as the applicator is removed). The vehicle, a viscous fluid, is squeezed between the surfaces. These surfaces are assumed to behave as elastic solids. This problem involves the mechanics of fluid motions

\[
R = \left[ \sqrt{\frac{V}{\pi}} \right] \left[ \frac{24 \pi \sqrt{E \mu (1 - \nu^2)}}{V^{3/2}} \right] + h \left[ \frac{h}{(2 - \nu)} \right]^{1/9}
\]

\[
S = \left[ \frac{4E}{3V} \mu (1 - \nu^2) \right] \left[ \frac{24 \pi \sqrt{E \mu (1 - \nu^2)}}{V^{3/2}} \right] + h \left[ \frac{h}{(2 - \nu)} \right]^{1/9}
\]

**FIG. 1.** Geometric model of initial spreading after release of a bolus of vehicle into the vagina.
Algebraic expressions can be derived for the vehicle radius $R$, thickness of vehicle layer $2h$, and rate of spreading $S$ (the time derivative of $R$) as functions of vehicle viscosity, tissue elasticity, and time (Fig. 1).

In Fig. 1, $V$ is the volume of the vehicle applied, $E$ is the Young’s (elastic) modulus of the vaginal tissue, $t$ is time, $\mu$ is the viscosity of the vehicle, $\nu$ is the Poisson’s ratio of the tissue, and $2h_0$ is the initial separation of the apposing epithelial surfaces after insertion of the bolus of the vehicle.

It is instructive to consider the predictions of this model for fluids with viscosities that approximate those that may be expected to occur within the vagina. Consider three viscosities $\mu$: one similar to water ($\mu = 0.01$ poise); one typical of a relatively low viscosity cream ($\mu = 5 \times 10^5$ poise) and one equal to that measured at room temperature for both Conceptrol and Advantage 24 at low shear rate ($\mu = 3.3 \times 10^7$ poise). The last is clearly an upper bound for the in vivo viscosity of a vehicle (that can be diluted and be lower than its room temperature value). We consider a vehicle volume of $4 \text{ cm}^3$, a density of $1 \text{ gm/cm}^3$ (similar to water), and an initial tissue spacing (i.e., thickness of bolus of vehicle) of $0.5 \text{ cm}$. Thus, the initial radius of the vehicle bolus (assumed cylindrical) is $R = 1.6 \text{ cm}$. For the vaginal tissue, we take a Young’s (elastic) modulus $E = 1 \text{ MPa}$ and a Poisson’s ratio of 0.5. These values are estimates, based on properties of other body tissues. As a frame of reference, $E = 0.6 \text{ MPa}$ for an elastin molecule, and $E = 1.4 \text{ MPa}$ for lightly vulcanized rubber. We calculate the following results:

<table>
<thead>
<tr>
<th>$\mu$ (poise)</th>
<th>$t = 1 \text{ sec}$</th>
<th>$t = 1 \text{ min}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$0.01$</td>
<td>0.62</td>
<td>0.02</td>
</tr>
<tr>
<td>$R$ (cm)</td>
<td>11.15</td>
<td>17.58</td>
</tr>
<tr>
<td>$2h$ (cm)</td>
<td>0.01</td>
<td>0.004</td>
</tr>
<tr>
<td>$5 \times 10^5$</td>
<td>0.041</td>
<td>0.002</td>
</tr>
<tr>
<td>$R$ (cm)</td>
<td>1.71</td>
<td>2.46</td>
</tr>
<tr>
<td>$2h$ (cm)</td>
<td>0.44</td>
<td>0.21</td>
</tr>
<tr>
<td>$3.3 \times 10^7$</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>$R$ (cm)</td>
<td>1.602</td>
<td>1.691</td>
</tr>
<tr>
<td>$2h$ (cm)</td>
<td>0.499</td>
<td>0.447</td>
</tr>
</tbody>
</table>

From these illustrative and preliminary results we can infer the following:

For a low-viscosity vehicle, squeezing of vaginal epithelium back toward its undistended state occurs almost instantly and the vehicle spreads rapidly.

For intermediate viscosities, squeezing is appreciable but occurs within minutes (not seconds).

For the highest viscosity, squeezing of vaginal epithelium back toward its
undistended state is slow and the vehicle spreads very slowly. The highest viscosity may be ineffective for the vehicle spreading process.

Notably, this model can be elaborated further to incorporate non-Newtonian vehicle properties and effects of slip on surfaces (due to a mucous or fluid coating).

**THE RANGE OF PUTATIVE THICKNESSES OF FORMULATION COATINGS**

It is possible to estimate the thickness of a layer of formulation on the vaginal epithelium on the basis of the volume of formulation applied and the surface area that is coated (effective surface area). Here, a simple analysis is performed based on the assumption of complete and uniform dispersion of the formulation over the surface area. The thickness, \( h \), of the layer is then simply

\[
h = \frac{V}{A}
\]

where \( V \) is the applied volume of formulation and \( A \) is the effective surface area. The suggested volumes to be applied of different commercial products range from approximately 1 to 5 ml. Estimating the effective surface area \( A \) of the average human vagina is complicated because vaginal epithelial surfaces vary greatly in the number and size of their rugae, and because the dimensions and shapes of vaginas vary greatly. The effective surface area of the coating also depends on whether or not the vagina is distended and whether or not the formulation penetrates the rugal folds. Here, we estimate lower and upper bounds for the effective surface area \( A \). The former is based on a minimal vaginal size in the absence of rugae and distension. The latter is based upon a maximally distended vagina. Our calculations derive from results of several studies of the size and shape of the human vagina.

Masters and Johnson (4) studied the dimensions of the vaginal "barrel," which was assumed to be cylindrical. They presented data, based on 100 nulliparous women, for "sexually unstimulated" and "stimulated" barrels, both with and without dilation by a speculum. The smallest vaginal size reported by Masters and Johnson is the smallest among those surveyed by us. We obtained 50.3 cm\(^2\) as the surface area of this minimal size. Of course, the real human vagina is not shaped like a simple circular cylinder. For example, Pendergrass et al. (5) presented results based on vinyl polysiloxane casts of the human vagina in 39 women. Four archetypal shapes were described: conical, parallel sides, heart, and slug. Typical dimensions were provided for each shape. Presumably, the vagina was partially distended during the casting process. However, the presence of folds in the surface of the molds and the unstimulated state of the subjects suggest the potential for further distension. Therefore, only the general shapes were used in our estimate of maximal surface areas. We used larger values for the greater dimensions measured or
TABLE 1. *Thickness of coating of vehicle on epithelium*

<table>
<thead>
<tr>
<th>Formulation volume <em>V</em></th>
<th>Effective vaginal surface area <em>A</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50 cm²</td>
</tr>
<tr>
<td>2.0 ml</td>
<td>400 μm</td>
</tr>
<tr>
<td>5.0 ml</td>
<td>1,000 μm</td>
</tr>
</tbody>
</table>

referenced by Pendergrass et al. (5). The resulting calculations ranged from 368 cm² for the conical shape to 599 cm² for the parallel-sided model, and we use the latter as our upper bound for effective surface area. We obtain (using rounded values) the results listed in Table 1.

**IN VIVO ASSESSMENT OF THE DISTRIBUTION OF FORMULATIONS IN THE VAGINA**

Objective assessment of the spatial distribution within the vagina of a formulation—vehicle and/or active ingredient(s)—is a critical need in evaluation of current products and in development of improved ones. Sustained coating of the vaginal epithelium and external cervical os is mandatory for prophylactic efficacy. Prior work has not attempted to measure vehicle distributions, but rather total loading of active ingredients into the vagina by monitoring amounts of systemically absorbed materials (e.g., peptides and proteins; cf. ref. 6), or by lavaging the vagina and analyzing the derived fluid [e.g., recent studies of N-9 by Barditch-Crovo, et al., (7) and Mauck, et al., (8)]. There is anecdotal information on the use of colposcopy in sheep for visual assessment of formulations within the vagina (Hahn, D.W., personal communication). There does not appear to have been a systematic follow-up to these colposcopic studies, or at least not reported in the public domain.

Our laboratory is now engaged in development and application of technology for direct *in vivo* visualization and analysis of the distribution of formulations in the vagina (9). We are using fiberoptics to provide both visual images and spectrometric information for local fields distributed throughout the vaginal surfaces. The spectrometric information is interpreted with respect to the fluorescent labeling of formulations. The degree of fluorescence correlates with the local thickness of the layer of formulation, provided that the label has not appreciably separated from the formulation. In our initial studies, sodium fluorescein (which is approved for human use) is mixed directly into the formulation. This approach is suitable for inaugural work and method troubleshooting, especially when the formulation is in the vagina for only a short time. In follow-up, we plan to take measures to restrict the fluorescein to the formulation, either by direct binding to an immobile formulation constituent or by encapsulation in particles mixed into the formulation.
Our current intravaginal measurement device is a 1-inch diameter, hollow, transparent acrylic tube with a hemispherical distal end. An internally contained 70° 4-mm rigid medical-grade endoscope (Storz) transmits light and images in and out of regions of focus outside the tube. A compact gear mechanism positions the endoscope throughout the interior surface of the tube for systematic measurement. A 500-W mercury lamp with cold mirror (Oriel) and dual magenta filters (OptoSigma) provides broad visual spectrum illumination (low UV) while excluding the fluorescent wavelengths 500-560 nm. A beam splitter (Storz) divides the output light from the endoscope for simultaneous imaging and fluorescence measurement. An integrating camera (Optronics) generates the image for real time viewing and recording. A photomultiplier (Oriel) with a 520-nm interference filter (Oriel) measures fluorescence. Test formulations are rendered fluorescent by addition of 0.1% USP injectable grade sodium fluorescein. This fluorescein emits yellowish-green light near 519 nm when excited by blue light near 460 nm. Thus, the filtering in our system optimizes the trade-off between taking a pure measurement of fluorescence intensity and providing an adequate light spectrum for the video imaging.

In experiments in which fluorescein did not appreciably separate laterally from the gel, calibration in vitro has demonstrated a linear relationship between thickness of a test gel layer (range 40-500 μm) and photomultiplier response (fluorescence intensity). This relationship exists because, for such relatively thin layers (as exist in coating), the intensity is an integral over all fluorescein molecules throughout the depth of the layer. In our device, the internal positioning mechanism for the endoscope enables sighting at 45° polar angle increments and 10-mm longitudinal increments over a 150-mm length along the tube, thereby mapping its external surface and adjacent vaginal epithelium. Such mapping is systematically undertaken when the device is in place after insertion into the vagina, and requires approximately 15 min (although this time will be reduced with more automated data acquisition). The 70° angle of the endoscope lens also enables direct sighting over the hemispherical distal end of the tube. This sighting can be performed when the device is in place or during the insertion. During the insertion, observations are thus made of the areas of the vaginal epithelium that have not been contacted by the surface of the device. Optical power levels are safe for 8-hr epithelial exposure. The variable integration time capability of the camera accommodates restrictions of light imposed by volunteer comfort, safety, and photobleaching of the fluorescent label. Color perception and visual quality of the video image are good and suitable for digital analysis.

During clinical application, an examination table is fitted with a positioning structure that holds the intravaginal sensing device and that has a 6° leeway for ease of insertion and positioning. This enables comfortable and proper positioning of the device. Initial in vivo studies in women using current intravaginal products are now under way. Initial results have already demon-
strated the ability to detect formulation coatings on local vaginal surfaces, to assess the continuity of coating, and to define margins between coated and non-coated regions of the epithelium.

ACKNOWLEDGMENT

Support for the project work presented here was provided by the Contraceptive Research and Development Program (CONRAD), Eastern Virginia Medical School, under a Cooperative Agreement (CCP-A-00-92-00015-15) with the United States Agency for International Development (USAID). The views expressed by the authors do not necessarily reflect the views of USAID and CONRAD.

REFERENCES

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Panel Discussion

David Katz: I would like to open the workshop for general discussion.

Lourens Zaneveld: I have a handful of people working in this area [of vaginal formulation], and I would like to suggest that the highest priority in this field be to take whatever compound we have as quickly as possible to the clinic. There are no in vitro or animal models that can mimic the human situation. Maybe over time we can develop those models, but we are still many years away. I suggest we proceed with urgency to Phase 1 clinical trials—with or without coital testing—to assess safety, formulation properties, and preliminary efficacy. Then, go back and try to improve your formulation(s).

Henry Gabelnick: I think that we would be missing the point of the synergy between the active ingredient and the formulation that's been stated today on several occasions. We cannot simply plunge forward with new active ingredients without understanding how the formulation affects the utility.

To do a real assessment of the utility of any active ingredient, particularly against HIV, you are talking about enormous resources. For Phase 2-3 clinical studies, there are very limited resources in people and centers, never mind the money. To have confidence in our decision to invest that resource, we need a way to discriminate among potential candidates. We have to study the formulation effects before Phase 1, so we give the drug candidate its best shot. Models and rheologic studies need to be refined to address the human system.

One of the things that I find discriminatory is the way we take for granted that there should be no obvious irritation. How do we clinically determine that irritation? For quite some time, we've been relying on colposcopy. I was recently at a meeting of the Population Council [October 1997] where their ethical review board says you can't do colposcopy in a Phase 1 study because colposcopy is so uninterpretable that it's unethical to ask a woman to be involved in a procedure that doesn't give definitive results. I don't know if that's completely true. I still think we can learn more from colposcopy if we refine the technique. But, nevertheless, that's an opinion expressed by people who have been in the field for some time.

When we come to drug distribution and we're talking about HIV, I know Jeff Spieler mentioned [earlier today] that a lot of transmission takes place in the cervix and not in the vagina. We also know that women who have had hysterectomies can get AIDS. And to me, just how the vehicle and the active ingredient distribute in the vagina is something we really need to know. And I do believe that there is money available to do it.

Lourens Zaneveld: I want to respond because I basically agree with everything you said. I didn't want to trivialize the formulation. You must be absolutely sure that the active ingredient is compatible with the formulation. I took for granted that that
would be examined. I also took for granted that, if you got things into the Phase 1 clinical trial, you would have done the adequate toxicity studies such as rabbit vaginal irritation.

What I want to probe is whether or not we should spend a lot of time on animal models. Would you, for example, recommend that, before we go even to Phase 1 clinical trials, we do primate HIV testing or that we use the mouse model for herpes, the chlamydial model, or any of those other things before we get to the clinical trial? Do you feel that we should do a lot of engineering studies in vitro before we go to Phase 1 clinical trial?

I couldn't agree more with David Katz. I think distribution studies using his new technique or the new gamma scintigraphy are extremely important. A year ago we didn't know how to address problems like these. You have come up with a relatively simple way of doing it. However, I still feel that, if we had sufficient funds, I would go to the clinic quickly to see how a formulation behaves before spending a large effort in formula optimization.

Bill Rencher: I would focus on the in vitro parameters and epithelial tissue models that I feel will predict the product's behavior in the vagina, then proceed to the clinic to establish an in vivo—in vitro correlation. Several pharmaceutical researchers have dedicated part of their career to this approach, including Dr. Robinson. Unfortunately, correlating the two was rarely part of the clinical objective.

In product design, our goal is to optimize distribution, retention, and drug effectiveness at the target. In spermicide:STD preventative products discussed today, retention of both properties at the cervical os is critical. Also critical for STD prevention is vaginal wall coating. To achieve our goal, we need to learn more about vaginal morphology in healthy and ill states and how it affects the vehicle's properties. We need to study distribution and retention by noninvasive and invasive techniques in the clinic, to begin correlating in vivo behavior with in vitro quantitation.

Performing basic formulation—development research to optimize vaginal drug delivery needs to be taken to the next level, like we have with other delivery sites. GI, ophthalmic, nasal, and transdermal drug delivery have been studied extensively for years. Liposome technology, for example, may be tailored to enhance drug uptake. Novasomes® is an example of liposome technology modified for transdermal drug delivery. I feel that optimal vaginal delivery can only be achieved if your goals always include correlating in vivo results to in vitro quantitation.

Arthur Mlodozeniec: I want to endorse what Bill said and give a couple of additional arguments. I think it is always exciting to want to get into Phase I as soon as possible to see if you've got some kind of therapeutic advantage over what is already in the competitive marketplace. My discomfort with that, however, is that when we rush, we get exciting Phase I results, then we repeat Phase I with an improved formula and we have a whole new set of exciting Phase I results from the second run. Unfortunately, we don't know why we didn't reproduce what we had the first time. Bill Rencher talked about how we have done wonderful things in certain drug delivery areas in the last decade, for example, ophthalmic drug delivery, which 20 years ago was a mystery. And until we began to understand the physiologic barriers to drug delivery, the metabolism, the role the vehicle plays, and the other players, we could not optimize drug delivery. You notice when I put my fishbone on the overhead, the vehicle was only one component.
I don’t count the buffering systems and all these other components as vehicle, and let me digress to say that I think it’s a mistake to study active ingredients separately and vehicle separately. That’s an all-or-none kind of comparison. If you really want to do that kind of a study, which I am not warmed up to but can appreciate why you want it, I would start with the full potency of your active ingredient in the vehicle, go to 50%, 25%, and lower, because when you get to zero it’s a very unusual system. That’s not even a true vehicle anymore. The vehicle behaves differently when it’s by itself than when it’s delivering something.

I believe that looking for a vehicle that’s going to serve all drugs is going to be a difficult thing to do. I think we just have to understand all the theoretical portions of drug delivery as we did with other routes of administration. I am amazed, having been in this field just a few months now, how understudied vaginal drug delivery is. It’s an absolute disgrace compared to transdermal with 25 years of highly publicized research. The skin is a barrier to drug delivery, just like a wall. The vagina is a wonderful opportunity, and when Joe Robinson said there are cultural barriers against permitting the vagina as a systemic route for systemic drug delivery, that could be overcome. I mean, if you can cure using the vaginal drug delivery as a route, I think that’s exciting.

I would like to see basic funding that addresses the issue of the physiologic barriers to drug delivery, so we understand them. I think that’s where I see the greatest advances being made, where all of us would benefit. Then the polymer systems will come. Right now, if we just keep studying new vehicles I fear that we are going to have results that are going to be very difficult to interpret.

Zeda Rosenberg: I would like to give part of one perspective of a funding agency. The perspective of our institute is to develop a product that will protect against HIV transmission and other STDs. For me, contraceptive or noncontraceptive is incidental. The main goal for me, personally as well as professionally, is to get a product out there that will prevent transmission of HIV. So I would like to see some consideration of development of products without considering their spermicidal activity. What I’ve heard today is some discussion of whether the product should be more or less viscous depending on whether it needs to impede sperm vs. to protect the mucosal surface from infection. I think that these may be two distinct phenomena and that you may end up not being able to develop a product that can do both well. I would look for something that could prevent transmission through mucosal surfaces.

Second, I agree very strongly that we need to get as many things into the clinic and into Phase 1 as possible. Where I see some of the good formulation learning is in the Phase 1 studies. You’re not just doing safety testing in Phase 1, you are asking critical questions to help move forward the formulation development issue. Also, here I think you can also start looking, depending on your active ingredient, at some preliminary biologic plausibility of your product. If you are looking at the effects of the product in HIV-infected women, in vaginal load, there are some things that you can do in a Phase 1 study, domestically and internationally. If highly active retrograde therapy is reducing the viral load secretion, some of these studies can be done internationally. So I would like to look at Phase 1 as much broader than a traditional safety study. This way you can keep a lot of this information altogether.

I agree with Henry regarding the difficulty in doing these studies and the amount of resources required — we are going to have to find some way to screen products before we go into Phase 3. The HIV Network for Prevention Trials (HIVNET) has been
discussing this at length, and we feel that the Phase 2-3 trials can be lumped together. There is a break between Phase 1 and moving on into larger studies, and that's where I think we need some evidence of biologic plausibility.

I agree that there is no consensus on what the animal models mean. We haven't validated the animal models because we don't have any human efficacy data. But I think that if we were able to compare some of these models in primate models to human, it would give us more information than we have now. It may or may not help us make some very difficult decisions. I'd rather try them and see what information the animal models will yield.

*John Kemnitzer:* I'd like to pose a question. How can I, as a company representative, go to my manager and say *I just want a product that's going to prevent HIV transmission,* and I go into the clinic and one person out of 5,000 is infected? Now we're talking about a liability issue, so how do we balance this issue with the prevention or the slow-down of the rate of transmission? Because I think that will be a limiting factor. I think many companies will say, well, I don't even want to touch this. And if you make that stipulation I think you put yourself at risk.

*Zeda Rosenberg:* That's a very serious concern, and liability has been raised by most companies. That's the same issue that we're going to have with vaccines. These are not unique to microbicides. An HIV vaccine is not going to be 100% effective, either. There will be people who get infected using the vaccine, so these are issues, in terms of HIV prevention, across the board. There is no simple answer to that. There are some companies that are choosing to go forward with an HIV indication and that have nonspermicidal products knowing the liability issues. They are writing letters to the AIDS task force of President Clinton to try to get this on the table. These companies will need support if they're going to move forward with these products, so I think political discussion needs to occur. And yes, companies are taking a risk. I completely understand that. Actually, some of the big companies are considering moving back in.

*Daniel Malamud:* Two comments. One, in addition to this testing liability there is also a viability issue, which is one of the problems for the small company. Although I'm very much for rushing ahead to the clinical trials, a small company will find it very, very difficult to survive a failed test if it's not the right formulation. So that is an issue that worries me.

Second, because of something that Zeda Rosenberg said with which I agree very strongly, I think we need help with, I'll call them Phase I testing algorithms. Because I think in terms of speeding up the process, I agree that once we go to all the trouble to make a GMP formulation and recruit subjects, just to have them use it for a few days and say *everything's fine!*—I do not think we are getting our money's worth. A number of studies could be done in microbiology, recovering cervical mucus and looking for an active drug. We need guidance from the science community on how to effectively utilize this small population.

*Kenneth Mayer:* With regard to the Phase 1, as a clinical investigator I think there are also great opportunities to answer more basic scientific questions, such as product effects on microflora or on cytokines. These kinds of basic studies should not interfere with the interpretation of Phase 1 safety data. Also, we have to go back to the ultimate use of these products, as part of a Phase 1 study, and conduct coital studies to look at the effects on the male urethra.

Going back to a point made earlier today, that some products may work differently with cell-associated virus and cell-free virus, attention needs to be paid to that as well.
[Editor’s comment: Perhaps collecting post-coital mucus (containing drug and semen) for anti-STD bioactivity in vitro to help correlate in vitro methods.]

Kurt Barnhart: I want to bring back a clinical perspective for a second. I remember not all that long ago hearing Willard Cate’s talk about contraception. He said that even though we were looking for ways for family planning, we couldn’t separate family planning from the prevention of STDs. And now I’m hearing that it’s almost the opposite. Now we are trying to separate STDs from contraception. There are probably reasons to do that, political and funding, but we have a tremendous opportunity for growth in terms of vaginal products, formulas, and delivery systems, and I don’t think that we should focus only in one direction.

Henry Gabelnick: The point that you make is well taken. You have to recognize also that there are women who want to protect themselves against STDs but want to maintain their fertility. Particularly in the community that we serve with CONRAD and USAID, that is a significant problem. Some cultures demand that a woman maintain fertility, but she needs to protect herself as well. So there are needs for microbicidal products. The extent to which that’s needed is debatable, but there is no question that there is at least a segment of the population that needs such a product. I personally believe that it is going to be a lot easier to get a combination product through the FDA. On the other hand, I’m sure David Phillips would like to comment on some compounds in their portfolio that appear to have a clear differentiation of activities.

David Phillips: Well, I agree with you. You need both.

Unidentified: I just want to point out that, at any given time in the U.S., only about 4% of women are trying to get pregnant. In any given year, most women are concerned about preventing both pregnancy and STDs. Not only is it a lot easier and cheaper to do a 6-month contraceptive efficacy trial, it’s a lot faster way to get a product on the market than waiting for the results of an STD—especially HIV—transmission trial. We at the Contraception and Reproductive Health branch have a clinical trials network that will be testing contraceptives in Phase 1 through 3 trials.

I also want to point out that it’s not just in other countries that women’s fertility is valued but also in the U.S. With vaginal contraceptives or vaginal products, there is a certain amount of negotiation, partner cooperation, or partner knowledge. It is a lot easier for a woman to make a case to a guy that it’s not a good time for her to have a kid than that she’s using the product because she does not trust his disease status.

Lori Heise: We need both contraceptive and noncontraceptive microbicidal products. I think we forget that the global burden of disease from traditional STDs is much greater than HIV. And we had women who came to our symposium, who said quite frankly. We don’t care if it turns us green, we will use it. I believe users’ perspectives are very important! I think we become very complacent, because we don’t understand what it means to have every second friend you have dying, which is what women in Harare and Uganda are facing. Literally, if it works, they will use it, I will promise you. . . . I encourage us not to forget the urgency to get something out. The eyes on the prize is if it works.

Zeda Rosenberg: No, I would be delighted if we had a spermicidal microbicide that was the first thing out, and it worked beautifully. All I’m saying is that we need to also consider the nonspermicidal. If the combination is the quickest way to get a product out there, I think that’s the way we ought to go. But in terms of getting a product on the market with spermicidal efficacy claims, you still need clinical studies. So it doesn’t
speed up the process in terms of STD prevention. It does clearly help companies, and that's an important perspective. In conclusion, I want both paths to be pushed simultaneously.

Lourens Zaneveld: My question is directed to the formulators in the audience. Is there a general formulation approach that we should use to study our prototype products before we go to the clinic? I have heard a variety of different possibilities.

Bill Rencher: I'm sure every formulator in here would like to come up. I'll give my opinion. Using the surface-active agents we discussed today, N-9 and C331G, as examples, I would study how the sperm and STD activity is affected by drug concentration below and above the critical micellular concentration and by oils, co-solvents and polymers in the vehicle (excipients that will alter the partitioning of the drug out of the vehicle). Also, I would study the effect of bioadhesive polymers (mixed with the drug in vehicle) on drug distribution and retention on vaginal epithelium in vivo by using gamma scintigraphy in rabbits and women, and in vitro by using adhesion techniques.

I would not be too concerned with the product rheology until I had feedback on the product's esthetics from women in the clinic. If the cream/gel is truly bioadhesive, a bulk viscosity above 10,000 cps should provide the necessary body to apply it vaginally without it immediately running out.

John Marvel: I'd like to give another formulator's opinion. I think what we're looking for is an adequate formula vs. a perfect formula. I think that we've got to address that. If we have a new active compound, we have to look for that adequate formulation. If we're looking for the perfect one, that's second generation, and we will have to wait for clinical remarks to really tell what to do to further improve it.

Gustavo Doncel: David just asked me if I would comment on one of the topics from his presentation. I've been involved with screening, testing, and evaluating of contraceptive active ingredients and formulated materials for a long time. I think we are doing the best we can at the moment. But I feel that the current tests used to evaluate these active ingredients and formulated materials, particularly in terms of bioactivity, are a little primitive. There is a lot of room for innovation. For example, I have not seen a clear, direct correlation between the in vitro data and the in vivo behavior of the formulated products.

Carefully designed studies to correlate in vivo outcomes and activities with in vitro data would give me greater confidence to design or improve a product on the basis of in vitro results. With an established correlation, prototype formulas could be altered much earlier in development (pre-IND). Today we assume that our in vitro data are predictive of the activity in the future clinical trials. However, reality usually proves us only partially correct.

Henry Gabelnick: I just want to elaborate a little on Bill's comment about the viscosity. And because there's nobody left here from Integra, I will speak for them. We believe the Q-2 — hydrophobic, cationic cellulose—polymers in development by Integra to have beneficial drug delivery properties. They are bioadhesive, muco-protective and safe. Q-2 is available in many viscosities, ranging from low enough to incorporate into a sponge to high enough to form into a film. We have chosen to use the gel [middle chain length range] in the first tests because it's a straightforward formulation and the easiest to compare with other commercial formulas.

The other thing I want to make clear in the context of Q2—and not because CONRAD paid for the research—is that Q2 is available, to everyone in this room
and throughout the world, to use as an adjunct with their own active ingredients. This is not something we have developed with public money to create a royalty flow for CONRAD. I encourage people to contact CONRAD or Integra.

Billing Rencher: To wrap up the discussions, I would like to say a few words summarizing what has been said. This workshop has highlighted the need for more thorough understanding of vaginal drug delivery and has called for improvement in the following areas:

- Sander-Cramer assay, and modified one-end and double-end biodiffusion in cervical mucus tests, to simulate in vivo spermicide activity (using PCT results)
- Clinical studies to correlate in vivo to in vitro results, to make in vivo simulations possible
- Clinical assessment of vaginal irritation, contraceptive efficacy, and product behavior (distribution, retention, and effectiveness).

Also mentioned was the need for improvement in the following techniques:

- Colposcopy to assess irritation
- Measurement of spermicide efficacy in vivo immediately after coitus
- Gamma scintigraphy, fluorescence, and other techniques to quantitate drug/product distribution and retention.

As Dr. Doncel mentioned earlier, as scientists we love solving problems. I have no doubt that, with the attention that these issues have received today, we will dramatically improve our laboratory and clinical techniques over the next 2 to 3 years and, with a little luck, develop highly effective vaginal microbicidal products.

In closing, on behalf of the sponsors of this workshop, I thank the speakers for their preparation, presentations, and written chapters for publication. And thanks to all for your attendance and participation.
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