

STUDIES ON DEVELOPMENT OF A VAGINAL PREPARATION PROVIDING BOTH PROPHYLAXIS
AGAINST VENEREAL DISEASE, OTHER GENITAL INFECTIONS AND CONTRACEPTION

III. IN VITRO EFFECT OF VAGINAL CONTRACEPTIVE AND SELECTED VAGINAL
PREPARATIONS OF CANDIDA ALBICANS AND TRICHOMONAS VAGINALIS.

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ABSTRACT

A study is presented in which the antimicrobial effects of a number of commonly used intravaginal contraceptives and other preparations were tested on C. albicans and T. vaginalis. This was ancillary to a study of the potential use of such compounds as a topical means of prophylaxis against venereal diseases. Two methods of testing were used to determine the bactericidal and bacteriostatic properties of these products. The preparations which were effective by the time exposure (1,5,10 minutes) technique were also effective by the plate dilution method. The results indicated that six out of 21 contraceptives and ten out of 20 non-contraceptive preparations tested were effective in inhibiting the growth of C. albicans while most of the preparations effectively inhibited the growth of T. vaginalis. These results are encouraging, but further studies in this area are suggested. It is postulated that any agent that would simultaneously provide prevention from pregnancy and other genital infections would be of great value to existing family planning and V.D. control programs.

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CONTRACEPTION

INTRODUCTION

Vaginal discharge is a most frequent symptom in women attending gynecological and family planning clinics. In majority of cases, the cause of abnormal vaginal discharge is either Trichomonas vaginalis, Candida albicans or Neisseria gonorrhoeae. The incidence of vaginal candidosis is increasing as reported by several workers (1,2,3). Candida albicans has now replaced Trichomonas vaginalis as the commonest cause of genital symptoms in women as reported by these investigators. Oral contraceptives are blamed for a high incidence of genital Candidiasis and Trichomoniasis (4,5). Trichomoniasis is usually transmitted sexually and genital Candidiasis can also be sexually transmitted once it has been established in the female sex partner. Thus, it would appear that in addition to the use of the condom to prevent transmission, it might be possible to utilize certain chemical agents as a prophylaxis just as in the control of gonorrhoea and syphilis.

With this in mind, we have tested certain commonly used intravaginal chemical contraceptives to explore their potential for topical use in prevention of transmission of venereal disease (6,7) as well as in prevention of transmission of T. vaginalis and C. albicans. Many topical intravaginal contraceptives were found to have bactericidal and bacteriostatic effects against Neisseria gonorrhoeae and Treponema pallidum (7). Therefore, this observation was extended to study the antimicrobial effect of the same chemical contraceptives on minor venereal disease microorganisms, such as C. albicans and T. vaginalis.

METHODS AND MATERIALS

Two methods were used to determine the effect of contraceptive and certain non-contraceptive preparations on the growth of C. albicans and T. vaginalis. These methods were basically the same as those described previously (7). Respective dilutions of various preparations (w/v) were prepared in physiological saline solution to get 50,20,10, or 1% concentrations and tested for their antimicrobial effect.

Candida albicans culture:

C. albicans culture was obtained through the courtesy of Dr. D.S. Kellog from the Center for Disease Control, Atlanta, Georgia. It was subcultured on Sabouraud Maltose agar slant and, for the experiments, a 48-hour-old culture grown on Sabouraud agar plate at 37°C was used. The culture suspension for the inoculum was prepared by washing the colonies in physiological saline solution and standardized by reading percent transmission (42 + 3%) as determined by Colorimeter, B&L spectronic "20" at 620 m μ . The total number of microorganisms was also determined by the standard plate dilution method.

(a) Time exposure method

To 1 ml of various dilutions of test samples of the preparation under study, 0.1 ml of yeast culture suspension containing 10^7 to 10^6 organisms was added and mixed. After intervals of 1, 5, and 10 minutes, the mixture

was inoculated on Sabouraud Maltose agar plates using a 3mm loop. The plates were incubated at 37°C and read after 48 hours. The concentration of test material which completely inhibited the visible growth of C. albicans following a period of exposure, is taken as the effective concentration for this exposure interval.

(b) Plate dilution method

In the second method, 1 ml of each dilution of the test sample was added to 9 ml of melted Sabouraud Maltose agar at 50°C, mixed and poured into 60 x 15 mm diameter petri dishes. After the plates were dry, they were inoculated by streaking with a 3 mm wire loop from culture suspension containing 10^5 to 10^6 colony forming units (CFU) per 0.1 ml. The plates were incubated at 37°C and read after 48 hours. The lowest concentration of test material completely inhibiting the visible C. albicans growth was considered as the end point.

Trichomonas vaginalis culture:

T. vaginalis (Atcc #30001) culture was grown at 37°C in Simplified Trypticase Serum (STS) base medium (B.B.L.) with 5% horse serum. The 2-day-old culture was used for these experiments. The culture was considered satisfactory if more than 5 Trichomonads were found per field, on an average, when a wet smear from the culture was examined under a microscope using 10x objective (low dry) and five different fields.

(a) Time exposure method

To 1.5 ml of the appropriately diluted sample of the agent to be tested, an equal volume of T. vaginalis culture was added and mixed. After 5- and 10-minute intervals, 0.5 ml aliquots were taken from the mixture and inoculated in two separate tubes containing 10 ml of STS base medium with 5% Horse Serum. The tubes were incubated at 37°C and read after 2 days.

(b) Tube dilution method

The various dilutions of contraceptive and non-contraceptive preparations were made in physiological saline solution. 1.0 ml of a desired dilution of the test material was added to 9.0 ml of STS base medium with 5% Horse Serum. After proper mixing, each tube was inoculated with 0.5 ml of a 2-day-old T. vaginalis culture. The inoculated tubes were incubated at 37°C and read after 2 days.

RESULTS

Effect on growth of C. albicans:

Forty-one preparations consisting of twenty-one contraceptives and twenty non-contraceptives were tested for their effect on inhibition of growth of C. albicans and T. vaginalis. The results in Table I show the effect of contraceptives on the growth of C. albicans. By the time exposure method, two preparations (Nos. 1, 16) at 10%, two (Nos. 4, 15) at 20%

Table I

Highest Dilution of Contraceptives Required to Inhibit
the Growth of *C. albicans* by Two Methods^a

No.	Contraceptives	Time Exposure Method					Plate Dilution Method			
		Conc %	pH on Dilution	Growth after exposure			Conc %	pH in medium ^b	Growth on plates	
				1 min	5 min	10 min			I	II
1	Certane Vaginal Jelly	10	4.6	-	-	-	1	6.4	-	-
2	Conceptrol	50	4.5	+	+	+	50	5.4	+	+
3	Contra Creme	50	6.9	+	+	+	50	5.4	+	+
4	Contra Foam	20	7.4	+	-	-	10	6.1	-	-
5	Cooper Creme	50	6.5	+	+	+	50	6.1	+	+
6	Delfen Cream	50	5.1	+	+	+	50	4.9	+	+
7	Delfen Foam	50	4.9	+	+	+	50	4.7	+	+
8	Emko Concentrate	50	7.4	+	+	+	50	6.0	+	+
9	Emko Concentrate + Spermicide	50	8	+	+	+	50	6.2	+	+
10	Emko Foam	50	7.1	+	+	+	50	6.1	+	+
11	Finesse	50	5.8	+	+	+	50	6.0	+	+
12	Immolin Vaginal Cream-Jel	50	4.9	+	+	+	50	5.8	+	+
13	Koromex A Vaginal Jelly	50	5.3	+	-	-	10	5.7	-	-
14	Lanesta Gel	50	5.5	+	+	-	50	5.5	-	-
15	Lorophyn Suppositories	20	5.7	+	-	-	1	6.1	-	-
16	Lorophyn Jelly	10	8.5	+	-	-	1	5.6	-	-
17	Milex Crescent Jelly	50	5.5	+	+	+	50	5.9	+	+
18	Ortho Creme	50	7.0	+	+	+	50	6.4	+	+
19	Ortho-Gynol Jelly	50	4.4	+	+	+	50	5.3	+	+
20	Preceptin Gel	50	4.6	+	+	+	50	5.3	+	+
21	Ramses Vaginal Jelly	50	6.7	+	+	+	50	5.7	+	+

+ = growth

- = no growth

a) Culture suspension for these experiments contains 10^5 - 10^6 CFU/0.1 ml. The inoculated Sabouraud Agar plates were incubated at 37°C and read after 48 hours.

b) pH of the medium after adding above contraceptive at different concentrations.

Table II

Highest Dilution of Non-contraceptives and Other Antiseptic Preparations
Required to Inhibit the Growth of *C. albicans* by Two Methods^a

No.	NonContraceptives	Time Exposure Method					Plate Dilution Method			
		Conc %	pH on Dilution	Growth after exposure 1 min 5 min 10 min			Conc %	pH in medium ^b	Growth on plates I II	
1	Betadine Vaginal Gel	0.1	4.5	-	-	-	50	5.6	-	-
2	Candectin Vaginal Tablets	10	7.5	-	-	-	1	6.7	-	-
3	Demure ^c	10	3.8	-	-	-	1	5.7	-	-
4	Emko Concentrate (no active ingredient)	50	7.3	+	+	+	50	6.3	+	+
5	Feminique ^c	50	2.6	+	-	-	10	5.2	-	-
6	Iso-Sol Argyrol ^c	20	8.4	-	-	-	10	6.1	-	-
7	Koromex A Base only	50	5.5	+	+	+	50	5.3	+	+
8	Neo-Silvol	20	6.9	-	-	-	50	6.0	+	-
9	Norform	10	4.4	+	+	-	10	5.7	-	-
10	Penigin	50	5.1	+	+	+	50	4.8	+	+
11	Penigin C	50	5.0	+	+	+	50	4.8	-	-
12	Progonasyl	50	7.7	+	+	+	50	7.6	+	+
13	Propion Gel	50	6.6	+	+	+	50	6.0	-	-
14	Silver Protein	10	9.6	+	-	-	1	6.3	-	-
15	Sporostacin Vaginal Cream	50	3.0	+	+	+	10	5.9	-	-
16	Trib Vaginal Cream	50	5.3	+	+	+	50	5.7	+	+
17	Trimo San Vaginal Jelly	20	4.6	+	-	-	10	6.0	-	-
18	Vabal D Base	50	4.8	+	+	+	50	5.7	+	+
19	Vabal D Cream	10	5.8	-	-	-	1	6.5	-	-
20	Vagisec Liquid Douche ^c	50	9.2	+	+	+	20	5.9	-	-

+ = growth

- = no growth

a) Culture suspension for these experiments contains $10^5 - 10^6$ CFU/0.1 ml. The inoculated Sabouraud Agar plates were incubated at 37°C and read after 48 hours.

b) pH of the medium after adding the non-contraceptive at different concentrations.

c) Dilutions of liquid test samples prepared (V/V) in physiological saline solution.

Table III

Effect of pH on Viability of *C. albicans* in Physiological Saline Solution

pH	Experiment I			pH	Experiment II	
	Growth After Exposure (Minutes) ^a				Growth After Exposure (Minutes) ^a	
	1	5	10		5	10
2.15	+	+	+	2.20	+	+
2.65	+	+	+	3.40	+	+
3.35	+	+	+	3.70	+	+
6.30	+	+	+	5.60	+	+
6.50	+	+	+	7.00	+	+
7.40	+	+	+	9.70	+	+
8.60	+	+	+			
10.00	+	+	+			

+ = growth

a) Culture suspension for these experiments contains $10^5 - 10^6$ CFU/0.1 ml. The inoculated Sabouraud Agar plates were incubated at 37°C and read after 48 hours.

and another two (Nos. 13, 14) at 50% concentration were found effective in inhibiting the growth of C. albicans. All exposure times, including one minute, were detrimental for the growth of C. albicans with one preparation (No. 1). At 20% concentration, Nos. 4 and 15 were not effective at 1 minute but inhibited the growth when organisms were exposed for 5 and 10 minutes. The same 6 contraceptives out of 21 were effective when tested by the plate dilution technique; however, their effective concentrations were lower. Preparation Nos. 1, 15, and 16 were effective at 1% concentrations, Nos. 4 and 13 at 10%, and No. 14 at 50%.

The results with non-contraceptive preparations are shown in Table II. Out of 20 preparations tested, 10 were effective in inhibiting the growth of C. albicans by the time exposure method. Preparation No. 1 was effective at 0.1%. Preparation Nos. 2, 3, 9, 14, and 19 were effective at 10% concentration and preparation Nos. 6, 8, and 17 at 20% concentration.

By plate dilution, 14 preparations out of 20 inhibited the growth of C. albicans. All preparations which were effective by the time exposure method were also effective by the plate dilution method. Effective concentrations were always low in the plate dilution method with the exception of two preparations (Nos. 1 and 8) in which effective concentration by the time exposure method was low as compared to the plate dilution method. The effect of exposure of C. albicans to different pH ranges as encountered with contraceptive and non-contraceptive preparations was also studied. The results of two different experiments are shown in Table III. The growth of C. albicans was not inhibited by exposure up to 10 minutes in either acid or alkaline pH from 2.2 to 10.

Effect on growth of T. vaginalis:

All contraceptive preparations when tested by the time exposure or tube dilution technique were effective in inhibiting the growth of T. vaginalis as shown in Table IV. In the time exposure method, most of the preparations were found effective at 1% concentration. By the tube dilution method 19 preparations were effective at 10% concentration and the minimum inhibiting concentration (MIC) was higher for most of the preparations as compared to their MIC by the time exposure method.

The results with non-contraceptive preparations are shown in Table V. Again, all test preparations, except for two (Nos. 7 and 13), effectively inhibited the growth of T. vaginalis by both methods.

DISCUSSION

Vaginitis is a common gynecological problem in which pathological conditions due to T. vaginalis or C. albicans occur with much greater frequency than that caused by other etiologic agents. The socio-economic and medical importance of this disease is sometimes underestimated. In recent years, several investigators have reported on the prevalence of Candida among antenatal patients (3) and what the risks are of infecting the fetus from the maternal genital tract (8, 9). A change in recent years in the ratio of patients with trichomonal vaginitis and vaginal candidosis and analysis of

Table IV

Highest Dilution of Contraceptives Required to Inhibit
the Growth of *T. vaginalis* by Two Methods^a

No.	Contraceptive	Time Exposure Method			Tube Dilution Method	
		Conc %	No. Live/Total		Conc %	No. Live/Total
			5 min	10 min		
1	Certane Vaginal Jelly	10	NG ^b	NG	50	2/4
2	Conceptrol	1	0/1	0/2	10	NG
3	Contra Creme	1	NG	NG	10	NG
4	Contra Foam	1	NG	NG	20	NG
5	Cooper Creme	1	NG	NG	10	NG
6	Delfen Cream	1	NG	NG	100	NG
7	Delfen Foam	1	NG	NG	10	NG
8	Emko Concentrate				10	NG
9	Emko Concentrate + Spermicide	10	0/3	NG	100	NG
10	Emko Foam	1	NG	NG	10	NG
11	Finesse	1	0/2	NG	10	NG
12	Immolin Vaginal Cream-Jel	1	NG	NG	10	NG
13	Koromex A Vaginal Jelly	10	NG	NG	10	NG
14	Lanesta Gel	1	NG	NG	10	NG
15	Lorophyn Suppositories	20	NG	NG	10	NG
16	Lorophyn Jelly	1	1/1	NG	10	3/5
17	Milex Crescent Jelly	1	NG	NG	10	NG
18	Ortho Creme	1	NG	NG	10	NG
19	Ortho-Gynol Jelly	1	NG	NG	10	NG
20	Preceptin Gel	1	NG	NG	10	NG
21	Ramses Vaginal Jelly	1	NG	NG	10	NG
	Control (mean average)		13/16	12/15		7/10

a) Growth in STS medium (B.B.L.) with 5% Horse Serum. Mean count from 10 fields of duplicate tubes.

b) NG indicates no growth when examined under microscope using low dry (10X) objective.

Table V

Highest Dilution of Non-contraceptives and Other Antiseptic Preparations
Required to Inhibit the Growth of *T. vaginalis* by Two Methods^a

No.	Non-contraceptives	Time Exposure Method			Tube Dilution Method	
		Conc %	No. Live/Total 5 min 10 min		Conc %	No. Live/Total
1	Betadine Vaginal Gel	1	NG ^b	NG	20	0/1
2	Candectin Vaginal Tablets	1	NG	NG	1	0/1
3	Demure ^c	1	NG	NG	1	NG
4	Emko Concentrate (no active ingredient)	50	NG	NG	10	NG
5	Feminique	10	NG	NG	10	NG
6	Iso-Sol Argyrol ^c	20	0/3	NG	20	NG
7	Koromex A Base only	50	2/5	1/4	50	5/10
8	Neo-Silvol	1	NG	NG	1	NG
9	Norform	10	NG	NG	10	NG
10	Penigin	10	0/2	NG	10	NG
11	Penigin C	10	NG	NG	10	NG
12	Progonasyl	50	NG	NG	10	NG
13	Propion Gel	50	1/4	1/3	50	2/3
14	Silver Protein	1	NG	NG	10	NG
15	Sporostacin Vaginal Cream	1	NG	NG	10	0/1
16	Trib Vaginal Cream	1	NG	NG	20	NG
17	Trimo San Vaginal Jelly	50	NG	NG	10	NG
18	Vabal D Base	10	NG	NG	20	NG
19	Vabal D Creme	10	NG	NG	10	NG
20	Vagisec Liquid Douche ^c	1	NG	NG	1	NG
	Control (mean average)		13/16	12/15		7/10

- a) Growth in STS medium (B.B.L.) with 5% Horse Serum. Mean count from 10 fields of duplicate tubes.
 b) NG indicates no growth when examined under microscope using low dry (10X) objective.
 c) Dilutions of the liquid test samples prepared (V/V) in physiological saline solution.

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causative factors suggests that the use of oral contraceptive preparations is related to the epidemiology of the condition (4,10). In fact some, gynecologists have recommended cessation of oral contraception during therapy for both trichomonal and monilial vaginitis. Such claims have been accepted widely; however, more systematic studies are needed in this area. A well-designed cross-sectional and prospective study based upon contraceptive practices in different population groups may provide further information on the role of contraceptive practices in vaginal infections.

Chemical contraceptive preparations for vaginal application are used and well-accepted by many groups as effective birth control agents. Some of the topical contraceptives are known to have antimicrobial properties in vitro as shown in our recent studies (6,7). Therefore, it was decided to test chemical contraceptives and other preparations for their effect on the growth of C. albicans. The MIC of various preparations required to inhibit the growth of C. albicans as reported here are relative values and should not be considered as the absolute effective value of these contraceptives. However, under these experimental conditions, the results are repeatable and the MIC of various products can be compared. The inhibiting effect on the growth of C. albicans was due to active ingredients of the test sample since pH alone in a range of 2.2 to 10 did not inhibit the growth of C. albicans.

Two types of tests were used which reflect bactericidal and bacteriostatic properties of these products. The preparations which were effective by the time exposure technique were also effective by the plate dilution method. The difference in MIC values by these two types of tests are probably due to various factors, such as solubility, compatibility, stability and other relationships between the test samples and the medium.

The protozoan parasite, T. vaginalis, is a very delicate microorganism and requires very special conditions for growth. Nutritional requirements and diagnostic procedures for Trichomonas vaginalis, as reported and recommended by several investigators (11,12), were used in this study. As seen from these results, most of the contraceptive and non-contraceptive preparations have a detrimental effect on T. vaginalis in vitro. However, it is implausible to extrapolate from in vitro to in vivo effects, especially in an uncontrolled clinical situation, without supporting experiments with these compounds in a simulated vaginal milieu. The pH, electrolytes, mucous and other flora of the vaginal secretions may all be expected to have effects upon the interaction of these compounds and the microorganisms of interest. Therefore, further studies in this area should be done to elucidate these points.

Before the advent of systemic chemotherapy against T. vaginalis and C. albicans, it is a matter of clinical record that vaginal infections by these two organisms were very difficult to eradicate in many females - by the use of topical trichomonocides and fungicides alone. It is therefore extremely doubtful whether any topical chemical compound used primarily for its contraceptive effect, and employed sporadically by the female according solely to her contraceptive requirements, would be curative of established vaginal infections by T. vaginalis or C. albicans. However, if the initial source of these infections to the female is sometimes the male sexual partner, carrying the organism usually asymptotically in the genito-urinary tract, an intra-

vaginal chemical compound inimical to these two microorganisms may well significantly reduce the female's risk of contracting either infection by sexual intercourse.

The findings reported here and a previously reported study on the effect of chemical vaginal contraceptives on T. pallidum and N. gonorrhoeae (7) suggest that the use of certain of these preparations might have value in prevention of sexual transmission of C. albicans and T. vaginalis. Any preparation that would simultaneously provide prevention of conception and protection against a broad spectrum of genital infections would be a most useful adjunct to public health programs to control venereal diseases and other genital infections transmitted by sexual intercourse.

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