Use of trimethoprim to prevent overgrowth by *Proteus* in the cultivation of *N. gonorrhoeae*

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The gonococcus often has to be isolated from sites heavily contaminated by other bacteria, especially in cultures from the female genital tract and the rectum. The introduction by Thayer and Martin (1964, 1966) of ristocetin or vancomycin, colistimethate sodium, and nystatin (VCN) as selective agents made it possible to suppress overgrowth by most contaminating organisms and this greatly facilitated the isolation of the gonococcus. These agents fail to affect most strains of *Proteus* and swarming by this organism often prevents the isolation of the gonococcus in culture.

Trimethoprim acts synergically with sulphonamides and the combination is effective against the gonococcus (Csonka and Knight, 1967). However, it seemed from the results of Darrell, Garrod, and Waterworth (1968) that strains of *Proteus* were more sensitive to trimethoprim alone than were gonococci; the effect of trimethoprim on the suppression of *Proteus* in the presence of gonococci has therefore been studied.

Material and methods

The standard medium used in these experiments was Oxoid brain heart infusion (code number CM 225), prepared according to the manufacturers instructions, which was agarized with 1.35 per cent. New Zealand agar. The molten base was cooled to 50°C., and 10 per cent. defibrinated horse blood (Wellcome Reagents Limited) was added and the plates were poured. For isolation of gonococci, the Thayer—Martin combination of antibiotics (Vancomycin 3 µg./ml., Colistin sulphomethate sodium 7.5 µg./ml. and Nystatin 12.5 units/ml.) were added before pouring the plates. Trimethoprim lactate was dissolved in sterile distilled water at a concentration of 3200 µg./ml. and stored at -20°C.

Specimens were obtained from male and female patients attending the Whitechapel Clinic on charcoal-treated swabs in Stuart's transport medium. After plating, cultures were incubated for 48 hours in candle jars at 36°C. and gonococci identified by their colonial and microscopical appearance, oxidase reaction, and fermentation tests.

Results

Comparison of the minimum inhibitory concentration of trimethoprim lactate for strains of Proteus and gonococci
Trimethoprim lactate was incorporated into plates or slopes of brain heart infusion blood agar medium at concentrations from 4 to 64 µg./ml. The *Proteus* strains were isolated from the genital tract and identified as *P. mirabilis*. They were cultivated overnight in nutrient broth. This culture was inoculated into fresh broth which was incubated for 5 hours. A loopful of this was inoculated into 2 ml. fresh broth and a loopful of this broth suspension was used as the inoculum for a set of slopes. The gonococcal inoculum was a loopful of suspension of gonococci from a plate culture diluted in broth to a standardized turbidity approximately equal to a 1 in 5 dilution of the stock suspension prepared according to the U.S. Public Health Service Manual (1961). This was inoculated on to a set of plates containing the same range of trimethoprim concentrations, eight strains being tested on the set of plates. Plates and slopes were read after 48 hrs' incubation at 36°C. the results are shown in Table I.

TABLE I *Comparison of inhibition of strains of Proteus and gonococci by trimethoprim lactate*

Species	Number of strains	Minimum inhibitory concentration of trimethoprim lactate (µg./ml.)					
		4	8	16	32	64	> 64
<i>P. mirabilis</i>	30	15	11	4	—	—	—
<i>N. gonorrhoeae</i>	44	—	—	3	9	24	8

From this experiment a concentration of 8 µg./ml. trimethoprim lactate seemed suitable for the suppression of most strains of *Proteus* while allowing the growth of gonococci.

Recovery of gonococci from suspensions artificially contaminated with Proteus

Strains of *Proteus* were grown overnight in broth and diluted in 10-fold steps up to 10⁻⁴. These were mixed with an equal volume of a suspension of

gonococci prepared as described above and two loopfuls of the mixed suspension were plated on (a) basal medium + VCN antibiotics and (b) basal medium + VCN antibiotics + 8 µg./ml. trimethoprim lactate.

These experiments with seven strains of *Proteus* are summarized in Table II.

TABLE II *Growth of Proteus and gonococci in mixed culture in the presence of trimethoprim lactate*

Separation of gonococcus from <i>Proteus</i> strain	Basal medium + VCN					Basal medium + VCN + Trimethoprim lactate 8 µg./ml.				
	Dilution of overnight broth culture of <i>Proteus</i>					Dilution of overnight broth culture of <i>Proteus</i>				
	0	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	0	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴
P1	P	P	P	P	P	P	P	G	G	G
Pi	P	P	P	P	P	P	P	P+G	G	G
Pj	P	P	P	P	P	P	P	P	G	G
Ph	P	P	P	P	P	P	P	G	G	G
Pm	P	P	P	P	P	P	P	G	G	G
Pv	P	P	P	P	P	P	P	G	G	G
Pr	P	P	P	P	P	P	P	G	G	G

P = Confluent growth of *Proteus* Pl, Pi, Pj, and Ph = Laboratory strains of *Proteus mirabilis*
 G = Growth of gonococci alone Pm = *Proteus morganii* 10041
 P = Colonies of *Proteus* NCTC
 Pr = *Proteus rettgeri* 7475 NCTC Pv = *Proteus vulgaris* 4175 NCTC

These results showed complete inhibition of a 10⁻³ dilution of the broth culture of *Proteus*. A viable count showed that this contained approximately 10⁶ organisms.

Effect of addition of trimethoprim on isolation of gonococci from routine clinical specimens

Specimens of exudates from 152 selected patients (82 males, 70 females) were plated on basal medium + VCN and on basal medium + VCN + trimethoprim lactate 8 µg./ml. The order of inoculation was rotated on alternate days. The results are shown in Table III.

TABLE III *Comparison of parallel cultures for gonococci with and without trimethoprim lactate in the medium from 152 selected cases*

Result	Basal medium + VCN	Basal medium + VCN + Trimethoprim lactate 8 µg./ml.
<i>Proteus</i> grown	58	1
Gonococci grown	35	42
No <i>Proteus</i> or gonococci grown	59	59

There was some selection in the choice of specimens for study in that when previous routine cultures were found to grow *Proteus*, the original swabs were re-plated on the medium containing trimethoprim. This explains the high incidence of cultures for which *Proteus* was grown on the routine medium. The results showed that, whenever gonococci had been grown on the basal medium + VCN, they were

grown on the same medium with the addition of trimethoprim lactate. Also, the addition of trimethoprim suppressed overgrowth by *Proteus* in all except one instance. Seven positive cultures for gonococci were lost through swarming by *Proteus* on the routine medium, but they were all isolated from the new medium with trimethoprim lactate. These results indicate the potential diagnostic value of the proposed medium.

It should be stressed that, while trimethoprim is bacteriostatic and prevents swarming by *Proteus*, viable organisms can remain and may grow out on trimethoprim-free medium. This may hinder isolation of gonococci in pure culture for fermentation studies but despite this a presumptive diagnosis can be made from the primary plates. It was also noticed that the size of gonococcal colonies was slightly smaller on the medium containing trimethoprim than on the same medium without it; this did not prevent easy recognition of colonies.

Summary

The addition of trimethoprim lactate at a concentration of 8 µg./ml. to brain heart infusion blood agar plus the Thayer-Martin inhibitors was found to suppress the growth of *Proteus* species without interfering with the growth of *N. gonorrhoeae*. Its addition to routine media for isolation of gonococci seems worthy of trial.

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Emploi de la triméthoprime pour prévenir l'envahissement par *Proteus* lors de la culture de *N. gonorrhoeae*

SOMMAIRE

L'addition de lactate de triméthoprime à la concentration de 8 µg./ml. au milieu cerveau-cœur, gélosé au sang et supplémenté des inhibiteurs de Thayer-Martin, fut trouvée capable de supprimer la pousse des espèces *Proteus* sans interférer avec celles de *N. gonorrhoeae*. Cette addition aux milieux de routine pour l'isolement des gonocoques semble mériter l'essai.