A NOTE ON THE USE OF THAYER AND MARTIN'S SELECTIVE MEDIUM FOR *N. GONORRHOEAE**

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The isolation of gonococci from infected secretions is often made difficult because of overgrowth by other organisms. In latent and chronic infections in women the numbers of gonococci present are often scanty and the sites from which cultures are taken are usually heavily colonized by other organisms such as staphylococci, diphtheroids, and coliform bacilli; these grow profusely on the enriched media which are necessary to support growth of the gonococcus.

Many agents have been added to media to improve the chances of isolating gonococci by selectively inhibiting the growth of other organisms. Such substances as crystal violet, Nile blue, aerosporin, tyrothricin, thallium acetate, and boric acid have been tried; none has proved entirely satisfactory in practice because the concentrations needed to inhibit the growth of contaminants are usually too close to those inhibiting gonococci. Thayer and Martin (1964) have advocated the use of a combination of polymyxin and ristocetin added in conventional media at concentrations of 25 units/ml. and 10 µg/ml. respectively. Polymyxin is active against many Gram-negative organisms and ristocetin against Gram-positive flora; the combination allows the growth of gonococci but gives a marked suppression of the growth of many other organisms present in the genital tract. Wende, Forshner, and Knox (1964) cultured material from 350 patients and isolated gonococci from 209 on the Thayer-Martin medium compared with 162 isolations on a modified Lankford medium.

Materials and Methods

Cultures were examined from 443 patients attending the Whitechapel Clinic at their first visit or when coming for tests of cure. They included both cases of gonorrhoea and non-specific genital infection and were unselected, except that an attempt was made to include as many rectal cultures as possible.

Specimens of secretions were collected on charcoal-impregnated swabs which were left in Stuart's transport

medium (Moffett, Young, and Stuart, 1948) at refrigerator temperature until they could be plated out, usually after an interval of 18 to 24 hours. The swabs were plated first on Macleod's chocolate agar with 10 per cent. heated horse blood (Macleod, Coates, Happold, Priestley, and Wheatley, 1934) and then on the same medium to which 25 units/ml. of polymyxin and 10 µg/ml. ristocetin were added just before the plates were poured. The inoculated plates were incubated at 36°C. in candle jars for 48 hours. Organisms were reported as "presumptive gonococci" on the basis of their colonial and microscopical appearance and a positive oxydase test and subcultured to glucose and maltose hydrocele agar slopes to complete their identification.

The sequence of inoculation may have favoured the routine at the expense of the selective medium, as the latter probably received smaller inocula; this was a deliberate choice as it was desired to compare the performance of the selective medium with the exact technique in current use in the laboratory.

TABLE I
RESULTS OF PARALLEL CULTURES FOR GONOCOCCI ON
ROUTINE AND SELECTIVE MEDIA FROM 443 PATIENTS

Patients	Medium	Result				Total Positive
Males (212)	Routine	—	+	+	—	152
	Selective	34	147	5	26	173
Females (231)	Routine	—	+	+	—	51
	Selective	175	49	2	5	54

Results

The results in terms of the patients tested are shown in Table I. Gonococci were grown on one or both media from 234 patients. The routine medium gave 203 (86.8 per cent.) isolations compared with 227 (97 per cent.) on the selective medium, so that the use of the latter increased the numbers of patients in whom a full bacteriological diagnosis could be made by 10 per cent. Cultures from seven patients grew only on the routine medium without antibiotics; six of these seven strains grew when subcultured on the selective medium so that the original

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TABLE II
ANALYSIS OF RESULTS ACCORDING TO SITES CULTURED

Patients	Site	No. of Cultures	Positive Results With:	
			Routine Medium	Selective Medium
Males	Urethra ..	193	150	168
	Rectum ..	19	2	5
Females	Urethra ..	229	41	43
	Cervix ..	223	45	45
	Vagina ..	231	33	38
	Rectum ..	94	9	15
	Bartholin ..	1	1	1

failure of isolation may have been due to a scanty inoculum rather than to inhibition by the antibiotics. The remaining strain failed to grow, suggesting that it was sensitive to the polymyxin-ristocetin combination. These results suggest that only a small minority of strains of gonococci would be lost through their sensitivity to the action of polymyxin and ristocetin in the concentrations used in the selective medium. Thayer and Martin in tests on 59 strains found one which showed complete and two which showed partial inhibition of growth in the presence of the combined antibiotics.

The results of cultures from individual sites are shown in Table II. The selective medium gave slightly better results with cultures from male patients; in females there was little difference except in cultures from the rectum. Suppression of the growth of contaminating organisms on the selective medium was often marked and sometimes complete compared with the mixed growth usually seen on the same medium without antibiotics. This greatly facilitated subculture of gonococci from the primary plates for fermentation and sensitivity studies. It was usually found possible to obtain a pure subculture from the primary plates, even from rectal cultures. When examined after 24 hours' incubation colonies of gonococci on the selective medium were usually smaller than when grown in the absence of antibiotics; after the customary 48 hours' incubation

there was little, if any, difference in the colonial size of most strains on the two media. The antibiotic combination did not inhibit the swarming of *Proteus* and this remains a frequent bar to the isolation of gonococci, particularly in cultures from the female genital tract.

Summary

Cultures for gonococci were made from 443 patients on chocolate agar containing 25 units of polymyxin and 10 µg ristocetin per ml. and on the same medium without antibiotics. The combined antibiotics markedly suppressed the growth of contaminating organisms and facilitated the isolation of gonococci in pure culture. This was found of most value when dealing with rectal cultures.

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Un mot sur l'emploi du milieu sélectif de Thayer et Martin dans le cas de la gonococcie N.

RÉSUMÉ

Ou cultiva des gonocoques provenant de 443 malades, d'une part sur un milieu d'agar chocolaté contenant 25 unités de Polymixine et 10 µg de ristocétine par ml. et d'autre part sur le même milieu sans antibiotiques. Cette combinaison d'antibiotiques facilita remarquablement l'isolement des gonocoques en culture pure d'éléments parasites. Ce fut surtout dans le cas de cultures d'origine rectale que cette découverte fut jugée importante.