PRIMARY ISOLATION OF *NEISSERIA GONORRHOEAE* ON HEMOGLOBIN-FREE THAYER-MARTIN MEDIUM

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IN THE PAST, *Neisseria gonorrhoeae* has been isolated on chocolate agar, and it has been taken for granted that the inclusion of hemoglobin in the medium was necessary.

The purpose of this study was to determine whether the omission of hemoglobin from the medium would affect the growth of *N. gonorrhoeae* in primary isolates from discharges of patients. Growth of *N. gonorrhoeae* on the hemoglobin-free medium was compared with growth on the standard Thayer-Martin (T-M) medium containing hemoglobin.

Materials and Methods

Patients for this study were men and women seen in the Norfolk, Va., venereal disease and family planning clinics between July 15 and August 19, 1969. The majority of the patients were women since gonorrhea in men is diagnosed by smear rather than by culture.

Urethral specimens for culture were taken from men before treatment when the smear was negative and after treatment for followup evaluation. Specimens were also taken from the rectal area from male homosexuals who admitted rectal exposure.

Although urethral specimens from males are usually obtained by loop, it was felt that a swab would collect more of the discharge, thereby assuring an adequate inoculum for each of the two plates. Specimens from women were routinely taken from both cervix and rectum by swab except for those patients examined in the family planning clinic where only cervical cultures were taken.

The swabs, immediately after being taken, were placed in 1.0 ml. of trypticase soy broth as a temporary holding medium before being inoculated onto plates. Most specimens remained in the broth for less than 2 hours because an effort was made to plate them as quickly as possible; however, on a few occasions swabs remained in the broth as long as 5 hours. We realize that trypticase soy broth may not be a good holding and transport medium since certain strains of *N. gonorrhoeae* might be lost which might otherwise have grown with direct application of patient discharges to culture mediums. This procedure was employed so that a uniform inoculum for both plates could be obtained.

The swabs were pressed against the inside of the tube to remove as much of the specimen as possible, and the solution in the tube was mixed for 5 to 10 seconds on a Vortex-Genie to insure that there was uniform suspension of the material in the broth. An inoculum of 0.1 ml. was then placed on each of the two plates (one with hemoglobin, the other without) which were incubated under CO_2 tension (candle extinction) for 18 hours.

The medium that was used was originally described by Thayer and Martin in 1964 (*1*), modified by them in 1966 (*2*), and further modified by Martin and co-workers in 1967 (*3*). The medium was prepared using gonococcal (GC) medium base, IsoVitaleX enrichment (vitamin B–12, l-glutamine, adenine, guanine, p-aminobenzoic acid, l-cysteine, glucose, diphosphopyridine nucleotide, cocarboxylase, ferric nitrate, thiamine hydrochloride, and cysteine), and VCN (vancomycin-colistimethate-nystatin) with and without hemoglobin. This medium is similar to that described by White and Kellogg

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in 1965 (*4*) containing GC base, 1 percent defined supplement (glucose, glutamine, cocarboxylase, and ferric nitrate), and the antibiotics ristocetin and polymyxin B.

Each patient's specimen was inoculated onto both mediums for comparison of the efficacy of each in supporting the growth of *N. gonorrhoeae* in primary isolation.

Cultures were examined for the oxidase enzyme by use of the standard indicator (dimethylparaphenylenediamine monohydrochloride), and all oxidase-positive colonies containing gram-negative diplococci were confirmed to be *N. gonorrhoeae* by the rapid immunofluorescent procedure described by Kellogg and Deacon (*5*). When colonies of oxidase-positive gram-negative diplococci were observed on both plates inoculated with the same specimen, it was assumed that they both represented the same organism and fluorescent antibody (FA) confirmation was done only from the Thayer-Martin plate containing hemoglobin; however, when colonies of oxidase-positive gram-negative diplococci were observed on the hemoglobin-free plate and not on the other, the FA procedure was carried out on organisms from the hemoglobin-free plate only.

Since cultures on the T-M and hemoglobin-free plates were examined by different observers, those specimens which showed growth on one plate and not on the other were carefully compared by the authors to eliminate error in examining the plates.

Results

The results of this study are summarized in the accompanying table. A total of 724 specimens were cultured. *N. gonorrhoeae* was isolated from 112 cultures on T-M medium, while 124 were isolated on the same medium without hemoglobin. Even though more specimens were isolated on the hemoglobin-free medium, it should be pointed out that nine strains did not grow on the hemoglobin-free medium but did grow on the standard T-M medium after 18 hours of incubation. Thayer-Martin medium containing hemoglobin grew *N. gonorrhoeae* on one more rectal culture from a male than did the hemoglobin-free medium. It may be that *N. gonorrhoeae* grows more slowly on the hemoglobin-free medium since in three of the nine instances in which the organism was not demonstrable after 18 hours of incubation, another 24 hours of incubation showed confirmed *N. gonorrhoeae* growth on the hemoglobin-free plate.

Discussion

Before the use of IsoVitaleX enrichment in gonococcal culture mediums, the hemoglobin may have served as a nutritional source of iron; however, since the IsoVitaleX contains ferric nitrate, hemoglobin as an iron source may no longer be needed. The buffering effect of the protein fraction of hemoglobin also seems to have been provided by the IsoVitaleX enrichment. It would be interesting to see if growth on hemoglobin-free medium would be as satisfactory as it was in this study if some other enrichment not containing iron were used in place of the IsoVitaleX.

Summary and Conclusions

From 724 specimens, *N. gonorrhoeae* was isolated after 18 hours of incubation in 112 cultures, using standard Thayer-Martin medium, and in 124 cultures using the same medium with-

Comparison of Thayer-Martin and hemoglobin-free mediums for isolation of *Neisseria gonorrhoeae*

Medium	Women		Men		Total
	Cervix	Rectum	Urethra	Rectum	
Total specimens cultured	416	241	60	7	724
Total confirmed isolates	90	32	7	4	133
On Thayer-Martin and hemoglobin-free mediums	67	26	7	3	103
On Thayer-Martin medium only	5	3	0	1	9
On hemoglobin-free medium only	18	3	0	0	21

out hemoglobin. The hemoglobin-free medium seems to support isolation of *N. gonorrhoeae* as well as, or better than, the medium with hemoglobin.

Because of the cost of the hemoglobin and the additional care needed in preparing a medium containing it, it seems that eliminating hemoglobin from routine gonococcal culture media should be given further rigorous evaluation and consideration.

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Tearsheet Requests

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Admissions to Nursing Schools Increasing

Schools of nursing across the country reported substantial gains in the numbers of men and women preparing for careers as registered nurses during the academic year 1968–69, according to an annual survey made by the National League for Nursing, New York.

Admissions to the three types of programs which prepare registered nurses (associate degree, baccalaureate degree, and diploma) increased to 64,157 during the last academic year, or 2,768 more than for the preceding 12 months. Graduations rose by 641 to a total of 42,196, while enrollments reached 150,795, compared to 145,588 the previous year.

There were 1,339 nursing education programs on October 15, 1969, a rise of 46 over 1968. The highest gain was in the rapidly growing junior and community college programs, which increased by 60 during that period. There were 19 more baccalaureate programs in senior colleges and universities, and 33 fewer hospital-based diploma programs. These figures reflect the continuing gradual movement of nursing education into institutions of higher education. The following table summarizes the changes in registered nurse education from 1967–68 to 1968–69:

Item	*Associate degree*	*Baccalaureate*	*Diploma*
Number of programs	+60	+19	−33
Admissions	+4,037	+1,092	−2,361
Graduations	+2,488	+1,236	−3,083
Enrollments	+7,066	+3,119	−4,978

Margaret E. Walsh, general director of the league, attributes the increase in the number of students preparing for nursing largely to the availability of Federal funds for nursing education. The Health Manpower Act of 1968 provides scholarship and loan funds for nursing students, traineeships for registered nurses going on for further education, and construction grants to schools of nursing.

Despite the growth of the nursing student body, the demand for nurses continues to outstrip the supply of nursing personnel. In 1969 there were 680,000 registered nurses. According to the Public Health Service, an estimated 1 million will be needed by 1975.