DIAGNOSTIC

ASPECTS

OF

GONORRHOEA

E. Stolz

DIAGNOSTIC ASPECTS OF GONORRHOEA

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE GENEESKUNDE

AAN DE ERASMUS UNIVERSITEIT TE ROTTERDAM

OP GEZAG VAN DE RECTOR MAGNIFICUS

PROF. DR. P.W. KLEIN

EN VOLGENS BESLUIT VAN HET COLLEGE VAN DEKANEN.

DE OPENBARE VERDEDIGING ZAL PLAATSVINDEN OP

WOENSDAG 11 DECEMBER 1974,

DES NAMIDDAGS TE 15.00 UUR

DOOR

ERNST STOLZ

GEBOREN TE SOERABAJA

Promotores   : Prof. Dr. M. F. Michel

                     Prof. Dr. C. H. Beek

Coreferenten   : Prof. Dr. L. Burema

                     Prof. Dr. H. A. Valkenburg

Aan mijn vrouw en kinderen.

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INTRODUCTION

In 1971 an investigation into the epidemiological, clinical, bacteriological and therapeutic aspects of gonorrhoea was started in collaboration with the Bacteriological Laboratory of the University Hospital/Medical Faculty Rotterdam. In the framework of this investigation, the data were recorded on optically readable forms. These forms could be processed to punched cards and fed to a computer for storage and processing, and could also be used as such for filing purposes. In order to make efficient use of these forms, it now became necessary to standardize the questioning technique used for collection of the epidemiological data, and the methods of clinical examination, bacteriological investigation and treatment.

This thesis deals with a part of the data covered by the above-mentioned investigation - mainly those parts relating to the diagnostic aspects of gonorrhoea. However, in order to place these aspects in the total framework of the investigation and in order to allow better comprehension of further publications resulting from this thesis, a full description is given of the methods of examination, treatment and follow-up (Chapter I), the methods of bacteriological investigation (Chapter II) and the registration and processing of the data (Chapter III). The epidemiological data on the gonorrhoea patients covered by this investigation are also summarized (Chapter IV).

The diagnostic aspects of gonorrhoea dealt with in this thesis relate to the optimum detection of gonorrheal infection (Chapters V - VIII), the sensitivity of gonococci to various antibiotics (Chapters IX - XI), anti-gonorrheal therapy in relation to the sensitivity of the gonococci to the antibiotic used (Chapter XII) and finally means of detecting various extragenital forms of gonorrhoea (Chapters XIII - XV).

We shall now discuss each of these points in somewhat greater detail.

Various culture media for gonococci were compared, to determine which one gave the best conditions for growth (Chapter V). The optimum selective gonococcal medium found in this investigation was used for the following studies.

The relative value of the various possible sampling sites for gonococci for the detection of gonorrhoea in females was assessed (Chapter VI).

Selective gonococcal culture was compared with the use of smear preparations (stained with methylene-blue or Gram-stained) for the detection of urethral gonorrhoea in males and urogenital/rectal gonorrhoea in females; the methylene-blue-stained and Gram-stained smears were also compared with one another. (Chapter VII).

The value of selective gonococcal culture was also compared with that of delayed direct immunofluorescence (performed with the aid of a fluorescent antigonococcal conjugate prepared in our own laboratories) for the detection of urethral gonorrhoea in males and urogenital/rectal gonorrhoea in females (Chapter VIII).

The sensitivity of all gonococcal strains isolated to ampicillin, penicillin and tetracycline was determined by the agar dilution method (Chapter IX). In addition, the sensitivity of a smaller number of strains to rifampicin, spectinomycin, sulphamethoxazole, trimethoprim and a combination of sulphamethoxazole and trimethoprim in a ratio of 5:1 was determined (Chapter X). These strains were stored by the low-temperature method described in Chapter II until required for this purpose. The patterns of resistance of the various strains to the different antibiotics were compared, and we also investigated whether significant differences in sensitivity to the various antibiotics existed between strains from different epidemiological groups of

patients (Chapters IX and X).

The sensitivity of gonococcal strains isolated by Heimans (1967) from women in Amsterdam and stored by freeze-drying to all the above-mentioned antibiotics was also determined, and the data obtained in this way were compared with those for gonococcal strains isolated from women in Rotterdam during the present investigation to see whether significant differences in resistance to the various antibiotics had arisen in the period from 1967 to 1972 (Chapter X).

On the basis of the sensitivities to sulphamethoxazole, trimethoprim and a combination of sulphamethoxazole and trimethoprim in the ratio 5:1 determined for all gonococcal strains, rules are given for the manner and extent of the potentiation of sulphamethoxazole by trimethoprim (Chapter XI).

The results of our standard treatment of uncomplicated urogenital/rectal gonorrhoea in males and females (1 gram of ampicillin intramuscularly (i.m.), followed after 4 hours by 2 gram of ampicillin orally) were correlated with the ampicillin-sensitivity of the gonococcal strains isolated before and in certain cases after the treatment. The serum ampicillin level was also determined in 6 male volunteers with urogenital gonorrhoea who has been given this standard treatment (Chapter XII).

Finally, two extragenital forms of gonorrhoea, oropharyngeal gonorrhoea (Chapters XIII and XIV) and the septic gonococcal dermatitis syndrome (Chapter XV) are discussed.

A number of the chapters of this study have already been published in article form. In order to give a certain uniformity of presentation, the other chapters in which results are discussed are also written in

article form. This inevitably leads to a certain repetition in the descriptions

of methods of clinical and bacteriological investigation.

References

Heimans, A. L. (1967)

"Diagnosis and Treatment of Gonorrhoea in Women" (Thesis)

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Chapter I

METHODS OF EXAMINATION, TREATMENT AND FOLLOW-UP.

THE EXAMINATION OF PATIENTS IN THE VENEREAL DISEASES CLINIC.

Routine examination

A brief medical history was taken from each patient visiting the Clinic. In the ensuing examination, the abdomen and anogenital region were inspected first, particular attention being paid to symptoms of diseases which can be conveyed by sexual intercourse.

Routine examination of male patients:

. A Gram-stained smear from the urethra. This preparation was examined under the microscope for Gram-negative diplococci and for other bacteria, yeasts, Trichomonas vaginalis, leukocytes and other cells.

. A culture for gonococci from the urethra.

. A wet, unstained preparation from the urethra, which was examined under the microscope for motile, live Trichomonas vaginalis.

. The two-glass test. For this test, the bladder is emptied in two phases. The first glass should only be filled one-third full of urine, the remaining urine being discharged into the second glass. In the event of an infection of the anterior urethra only, leukocytes will only be found in the first glass, while infections higher up will also give leukocytes in the second glass.
This two-glass test is superficial, but certainly very useful for the diagnosis of urethritis anterior, urethritis posterior, etc.

. A culture for gonococci from the oropharynx in all patients (from August 1, 1972) (see Chapters VI and XIV).

. A gonococcal culture of the prostatic discharge in patients with chro-

nic urethritis and patients suspected of being asymptomatic carriers of go-

nococci.

. A gonococcal culture from the rectum in patients suspected to be suf-

fering from rectal gonorrhoea.

All male patients were examined on an examination table.

Material for investigation was withdrawn as follows. Before withdrawal

of the discharge from the urethra, the urethra was massaged from proximal

to distal. The material was withdrawn with the aid of a flame-sterilized pla-

tinum loop, and then smeared out on to one microscopic slide for Gram-

staining and on to another slide previously prepared with a drop of physio-

logical saline for a wet unstained preparation. A charcoal-impregnated swab

(C. I. S.) was then held against the pus (if any) coming from the urethra and

transferred to Stuart transport medium, after which this medium was sealed

at once.

If the urethra produced no discharge whatsoever after massage, the loop

was inserted into the urethra. It was often possible to obtain some materi-

al in this manner. The C. I. S. was also inserted as far as possible into the

urethral orifice. When the material was so sparse as not to be clearly vi-

sible on the slides, a sterilized inoculating loop was inserted into the ure-

thra instead of the C. I. S. for the Stuart medium. With the aid of this loop,

the material was inoculated directly on the selective medium for gonococcal

culture. The two-glass test was carried out with the aid of two polythene

vessels as described previously. The prostate massage was carried out in

a knee-elbow position for a maximum of five minutes or until a drop of prosta-

tic fluid became visible. The material was withdrawn as described above

for the urethra. Material from the rectum was withdrawn by inserting a

C. I. S. per anum. The C. I. S. was inserted at least three centimetres be-
yond the sphincter and pressed against the front wall of the rectum.

Routine examination of <u>female patients</u>:

.   A culture for gonococci from the rectum.

.   A Gram-stained smear from the urethra.

.   A culture for gonococci from the urethra.

.   A Gram-stained smear from the ostium of the cervix uteri.

.   A culture for gonococci from the ostium of the cervix uteri.

.   A wet unstained preparation from the fornix posterior of the vagina,
which was examined for the presence of <u>Trichomonas vaginalis</u>.

.   A Gram-stained smear from the walls of the vagina, which was exa-
mined for the presence of yeasts.

.   A culture for gonococci from the oropharynx (from August 1, 1972).
(see Chapters VI and XIV).

The examination of female patients was always performed
in a gynaecological chair. The examination was carried out at all times of
menstrual cycle,even during menstruation. The material for investigation
was withdrawn as follows. After one finger had been inserted intravaginal-
ly, a C. I. S. was inserted into the rectum with the other hand. The intrava-
ginal finger was then pressed against the C. I. S. through the posterior wall.
In this way, any infected glands of Lieberkühn in the front wall of the rec-
tum get squeezed out.
The urethra was then massaged with the intravaginal finger passing over
the symphysis pubis in the direction of the urethral orifice. In a number of
patients a drop of pus could be discerned emerging from the paraurethral

passages or from the urethra after the massage. The material for a Gram-stained smear was withdrawn with the aid of a metal scoop, while the material for gonococcal culture was withdrawn with the aid of a C.I.S. Finally the portio was adjusted with the aid of a speculum. The material from the ostium of the cervix uteri for the Gram-stained smear was withdrawn with a metal scoop, while the material for gonococcal culture was withdrawn with a C.I.S. Material was also taken from the fornix posterior of the vagina with a scoop for an examination for Trichomonas vaginalis (wet unstained preparation) and from the vaginal walls for an examination for Candida albicans (Gram-stained smear). The above-mentioned sequence of withdrawal from the rectum, urethra and cervix has been chosen because when we started with the speculum examination, material spilled over the rectum in cases where vaginal discharge was abundant; in some of these cases the vaginal discharge also reached the urethra. It is surprising that other authors do not generally mention the sampling sequence they used. It would seem quite possible that the high percentages of positive rectal cultures mentioned by certain other authors might be caused by leakages during the examination. This matter is also discussed in Chapter VI.

Blood samples were taken from each patient for determination of serological reactions for syphilis (the Kolmer reaction and the Venereal Disease Research Laboratory (VDRL) test) in the District Laboratory, Rotterdam.

Additional serological tests for civilian patients with gonorrhoea.

When the routine examination described above showed that a civilian patient had gonorrhoea, a blood sample was taken and sent

to the National Institute for Public Health (Rijks Instituut voor Volksgezond-
heid - R.I.V.), Bilthoven, the Netherlands, for performance of the following
serological tests: The Kolmer reaction, the VDRL test, the Reiter Protein
Complement Fixation (RPCF) test, the Treponema Pallidum Immobilisation
(TPI) test, the Fluorescent Treponema Pallidum Antibody-Absorbtion (FTA/
ABS) test and the Gonococcal Complement Fixation Test (GCFT).

The above mentioned additional tests were not carried out on
sailors, since they are without value in connection with the clinical follow-up
of the patients; and while this follow-up is generally possible with civilians, it
is rarely so in the case of sailors who are always on the move.
The results of the serological tests will not be presented in this study.

THE TREATMENT AND FOLLOW-UP OF GONORRHOEA PATIENTS.

For all patients with acute, uncomplicated urogenital/rectal
gonorrhoea, the treatment consisted of 1 gram of ampicillin intramuscularly
(i.m.), followed by 2 gram of ampicillin orally after 4 hours. In the event
of penicillin allergy the following treatment was administered orally:
tetracycline HCl 2 gram daily for 5 days. The treatment of patients with
chronic/complicated urogenital/rectal gonorrhoea consisted of oral adminis-
tration of tetracycline HCl 2 gram daily for 10 days or 4.8 mega units (m.u.)
of Bicillin (a combination of procaine penicillin G and sodium penicillin G in
a ratio of 3:1) i.m. per day for 10 days.
The treatment of patients with oropharyngeal gonorrhoea will be discussed in
Chapter XIV.

As a rule blood was taken for serological examinations two,
six and twelve weeks after the treatment had started. The other tests on male
patients were repeated one week and two weeks after the start of treatment.
For the female patients the other tests were repeated one, two, six and
twelve weeks after the start of treatment.

Chapter II

BACTERIOLOGICAL METHODS

SMEAR PREPARATIONS

Smears were stained according to Gram and methylene-

blue.

TRANSPORT MEDIUM

Stuart transport medium (Oxoid) was used. Screwcapped

tubes with 5 ml of this medium were stored before utilisation at 4°C for one

week at most. The tubes were controlled at regular intervals to see whether

replacement was necessary. Material which had been withdrawn with the aid

of a charcoal-impregnated swab (C.I.S.) was stored for at most 4 hours in

the transport medium before plating on the gonococcal medium.

CULTURE AND FERMENTATION REACTIONS OF GONOCOCCI FOR THE

IDENTIFICATION.

Culture medium

The gonococcal medium had the following composition:

Bacto GC (Gonococcal Culture) medium base (Difco), Bacto haemoglobin (Dif-

co), Isovitalex TM (Thayer Martin) enrichment (BBL) and VCN (vancomycin,

colistimethate, nystatin) inhibitor (BBL). It was made up as follows.

First a solution of Bacto GC medium base of twice the final concentration re-

quired was prepared according to the manufacturer's instructions and then

autoclaved. Bacte haemoglobin was then suspended in distilled water by

means of a mixer and autoclaved. After cooling to a temperature of 50-60°C

this suspension was mixed with the Bacto GC medium base, and Isovitalex

TM enrichment and VCN inhibitor were added. Twenty-five ml of this me-

dium were used per Petri-dish.

Culture

The medium was inoculated as follows. Material from the swab in the Stuart medium was spread out evenly over one half of the plate (Figure 1A). This material was then streaked over the other half of the plate with the aid of a flame-sterilized platinum loop (Figure 1B). Finally a flame-sterilized loop was used to streak the material perpendicular to the previous streaks. (Figure 1C). After inoculation the plates were placed in a copper kettle with a capacity of 20 litres and incubated for 48 hours at a temperature of $37^{O}C$. in a CO_2-rich environment. The cultures were then examined visually and growth of gonococcal colonies was scored as follows:

Table I

Scoring of growth of gonococcal colonies on gonococcal medium.

Score 0:   No gonococcal colonies visible.

Score 1:   1-5 gonococcal colonies on the first half of the plate (Figure 1D).

Score 2:   5-20 gonococcal colonies on the first half of the plate (Figure 1E).

Score 3:   More than 20 gonococcal colonies on the first half of the plate (Figure 1F).

Score 4:   The first half of the plate shows dense growth and a striped pattern is visible on the second half of the plate (Figure 1G).

Score 5:   The first half of the plate shows dense growth and a checkered pattern is visible on the second half of the plate (Figure 1H).

Figure 1

Inoculation of the medium (Figures A, B, C) as described
in section concerning "Culture" and scoring of growth of gonococcal colo-
nies on gonococcal medium (Figures D, E, F, G, H) as described in Table I.
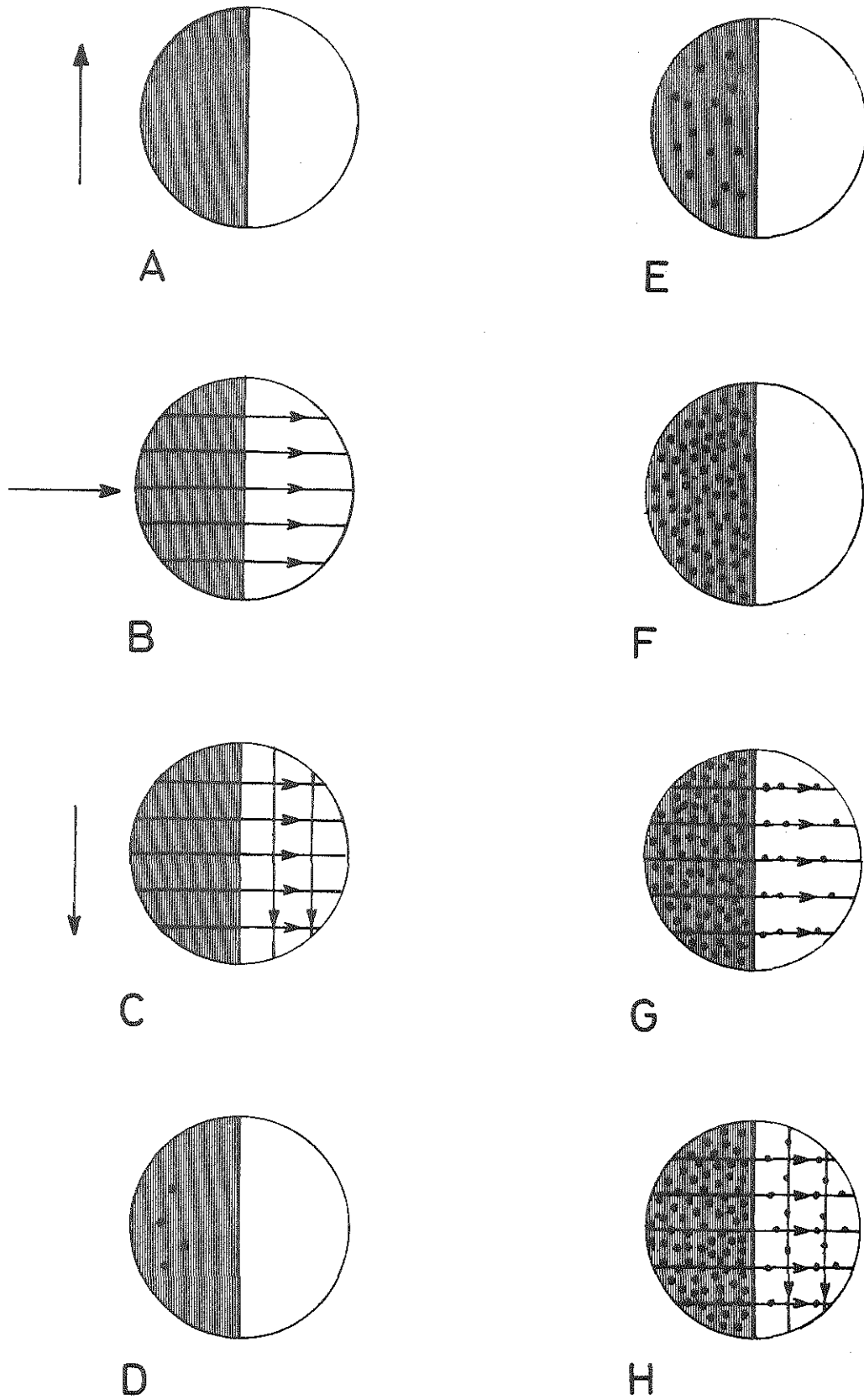
If colonies thought to be due to gonococci were observed, the colonies were investigated further to see whether they were formed by Gram-negative diplococci, whether the Gram-negative diplococci fermented glucose but not lactose, maltose and saccharose and whether the colonies in question were oxidase-positive. All colonies which passed these three tests for gonococci were reinoculated on a medium containing Bacto GC medium base, Bacto haemoglobin and Isovitalex TM enrichment for storage.

Sugar fermentation

A medium without ascites was developed for sugar fermentation. With this medium a satisfactory growth was obtained. The medium used to test for fermentation of glucose, maltose, lactose and saccharose was prepared as follows. Three grams of Columbia agar base (BBL) + 17 mg of phenol red were dissolved in 1000 ml of distilled water. This mixture was sterilized for 15 minutes at $121^{o}C$ in the autoclave. After cooling, 50 ml of a 20% sugar solution was added. The culture medium was poured into narrow tubes (about 4 ml per tube) and allowed to set. The tubes were inoculated rather generously with a 20-hour pure sub-culture and incubated 24 hours in an environment enriched with CO_2. When the sugar fermented, the color turned from red to yellow.

Oxidase reaction

The oxidase reaction was carried out with the aid of p-aminodimethylanilineoxalate (Difco). A few drops of the reagent were poured over a number of the colonies under examination. The reaction was considered to be positive when the colonies initially turned pink and then black.

IMMUNOFLUORESCENCE

Preparation of a fluorescent antigonococcal conjugate.

The preparation of antigonococal serum and of globulins from

the antigonococcal serum as well as the conjugation of these globulins with flu-

orescent isothiocyanate (FITC) were carried out as described by Mouton (1966).

The fluorescent antigonococcal conjugate was diluted before

use to the titre giving +++ fluorescence of the gonococcus (see Table II).

Dilution was performed at 4°C in phosphate buffered saline (P.B.S.) pH 7.2,

just before the conjugate was to be used.

Preparation of direct immunofluorescence slides.

The slides used were 1.2 mm thick. Before use they were

cleaned with 96% alcohol or chromic acid and wiped dry with clean paper.

One drop of physiological saline was deposited on a slide and 1-2 gonococcal

colonies not older than 24 hours were suspended into the saline. The prepa-

ration was dried for half an hour at room temperature and then fixed in a flame.

One or two drops of conjugate were deposited on the fixed smear, which was

then incubated for 30 minutes in a humid Petri dish at room temperature.

The slide was rinsed with physiological saline and then put in a measuring

cylinder with physiological saline or tap water for ten minutes. The slide

was wiped dry round the smear. Finally, the smear was covered with gly-

cerol buffer (9 parts of glycerol and 1 part PBS), over which a cover plate

was placed. The edges were sealed with paraffin.

Immunofluorescence microscopy

Microscopy was carried out with transmitted light. A Leitz

Ortholux microscope was used with a Periplan G 12.5 x ocular and an ultra

dark area condensor (u.v. 1.2 - 1.4 Super Wide Tioyda) and Fluorid 95 - 1.32

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oil-immersed objectives. The light source was a high-pressure mercury
lamp (C.S.150 W). BG 38 and BG 12 filters (3 mm) and a yellow filter K 530
were used.

Scoring of fluorescence of micro-organisms

Fluorescence was scored as follows, depending on the de-
gree of fluorescence of the edge of the micro-organisms and the sharpness
of the edge:

Table II

Scoring of fluorescence of micro-organisms

++++   = higly fluorescent edge with a clear outline

+++   = rather less highly fluorescent edge with the contours still visible

++   = dimly fluorescent edge; diffuse outline.

+   = almost no fluorescence at the edge and a diffuse outline.

Cross-reaction

In order to determine whether our unabsorbed antigonococ-
cal conjugate was specific for the detection of gonococci, a number of gono-
coccal and other bacterial strains were tested with the following result:

Table III

Fluorescence of gonococcal and other bacterial strains in direct immuno-
fluorescence slides, using our unabsorbed antigonococcal conjugate.

Strains	Fluorescence score
Neisseria gonorrhoeae	++++ – +++
Neisseria meningitidis	+++
Neisseria catarrhalis	+++
Staphylococcus aureus	++
Staphylococcus albus	+
Streptococcus haemolyticus	–

In our experience meningococci were larger and staphylo-
cocci smaller than gonococci.

Clinical use of direct immunofluorescence.

Direct immunofluorescence can be used in two ways for
clinical applications.

A.   The direct direct method

Pus from the patient, smeared out on a microscopic slide,
is to be tested for the presence of gonococci. The smear is processed as
described above under "Preparation of direct immunofluorescence slides".
The direct direct method was not used in the present study.

B.   The delayed direct method

Material from an 18 hour culture, which has been inocula-
ted with pus from the patient, is to be tested for the presence of gonococci.
The material is smeared out on a microscopic slide, and processed as
described above under "Preparation of direct immunofluorescence slides".

Application of immunofluorescence in this investigation.

Only delayed direct immunofluorescence was used for this
investigation. The gonococcal medium used for this purpose was a selec-
tive medium as described by Thayer and Martin (1966). This medium is
selective for both Neisseria gonorrhoeae and Neisseria meningitidis.
Other Neisseriae , staphylococci and streptococci generally do not grow on
this medium. However, according to Lind (1967) meningococci are very sel-
dom found in urogenital and rectal sites. Providing the delayed direct immuno-
fluorescence is only used for examination of these sites, the risk of error will
be cut down by making the diagnosis of gonorrhoea dependent on a ++++ or +++

fluorescence of diplococci. In the present investigation, delayed direct immuno-
fluorescence was only used for the detection of gonorrhoea in the above-mentio-
ned sites and the diagnosis of gonorrhoea was made dependent on ++++ or +++
fluorescence of diplococci.

GONOCOCCAL COMPLEMENT FIXATION TEST. (GCFT)

A monovalent antigen (i.e. one prepared from one single
strain of gonococci) was used for this reaction. The tests were performed by
the National Institute for Public Health (RIV) at Bilthoven. The results will
not be presented in this study.

ANTIBIOTIC SENSITIVITY DETERMINATION

Sensitivity to ampicillin (Am), penicillin (P), tetracycline (T),
rifampicin (Ri), spectinomycin (Sp), sulphamethoxazole (Su), trimethoprim
(Tr) and a combination of sulphamethoxazole and trimethoprim in a ratio of
5:1 (Su/Tr 5:1) was determined by the agar dilution method (Ericsson and Sher-
ris, 1971). The sensitivity was expressed as the minimum inhibitory concen-
tration (MIC in μg/ml). The medium used for sensitivity determinations for
Am, P, T, Ri and Sp consisted of Bacto GC medium base, Bacto haemoglobin
and Isovitalex TM enrichment. The medium used for sensitivity determinati-
ons for Su, Tr and Su/Tr 5:1 consisted of DST agar (Oxoid) enriched with
7.5 % haemolysed horse blood.

A series of plates with increasing concentrations of the antibiotic
under investigation and a control plate without antibiotic were prepared. The con-
centrations of Am were in the range between 0.005 and 0.64 μg/ml, those of P
between 0.0025 and 1.28 μg/ml, those of T between 0.04 and 2.56 μg/ml, those
of Ri between 0.0025 and 0.64 μg/ml, those of Sp between 7.5 and 25.0 μg/ml,
those of Su between 0.16 and 20.48 μg/ml, those of Tr between 2.56 and 40.96 μg/ml

and those of Su/Tr 5:1 between 0.10 and 12.29 µg/ml.

For sensitivity determinations organisms were suspended in trypticase soya broth (TSB) to a density of $10^6 - 10^8$ viable units (v.u.)/ml. P.B.S. pH 7.2 was used instead of TSB, when the sensitivity to Su, Tr of Su/Tr 5:1 was determined. Using a multipoint replicator the suspensions were inoculated on to the plate series, resulting in spot inocula covering a circle of 4-6 mm diameter and containing $10^3 - 10^4$ v.u.

In each run three gonococcal strains and one Staphylococcus (Oxford strain) with known sensitivity to the antibiotics under investigation were tested simultaneously.

After incubation (18-20 hours) the MIC was determined by observing the lowest concentration of antibiotics in which bacterial growth was completely or almost completely inhibited, as judged by the naked eye. A haze of growth or a single colony was disregarded.

The following products were used for the preparation of the stock solutions of the above-mentioned antibiotics: ampicillin (Beecham), penicillin G (Specia), tetracycline HCl (Nogepha), rifampicin (Lepetit), spectinomycin (Gist- en Spiritusfabriek), sulphamethoxazole and trimethoprim (Hoffmann-La Roche).

STORAGE OF GONOCOCCAL COLONIES

A.   Freezing on agar at -20^oC to -35^oC.

An agar block measuring about 4 x 10 mm was cut from a full-grown 24-hour culture on a medium containing Bacto GC medium base, Bacto haemoglobin and Isovitalex TM enrichment, and transferred to a sterilized tube with screw cap and a rubber ring. This tube was well sealed and stored at a temperature of -20°C to -35°C. Cultures could be preserved for about

two months in this way. This method was only used incidentally.

B.   Freezing in a suspension at a temperature of -195°C

A sample from an overnight culture on the medium described under A was transferred with the aid of a swab to 8% glycerine tryptone water (tryptone water = 1% tryptone and 0.5% Na Cl in distilled water) to give a dense suspension. Polythene straws (also used for the storage of sperm for artificial insemination in animal breeding) were half filled with the aid of a Pasteur pipette and sealed at both ends with polythene glue. The straws containing gonococcal strains were stored in liquid N_2 (-195°C).Strains thawed out after two years were still alive. This method was used for the investigation described in Chapters X and XI.

References

Ericsson, H.M. and Sherris, J.C. (1971)

Acta path.microbiol.scand. , Section B 1971, Suppl. 217.

Lind, I. (1967)

Acta path.microbiol.scandinav. , 70, 613.

Mouton, R.P. (1966)

Dermatologica, 132, 343.

Thayer,J.D. and Martin, J.E. (1966)

Publ.Hlth.Rep. (Wash.), 81, 559.

Chapter III

REGISTRATION AND PROCESSING OF THE DATA.

DEVELOPMENT OF OPTICALLY READABLE FORMS FOR REGISTRATION AND PROCESSING OF THE DATA.

The system for the computer processing of data on patients suffering from gonorrhoea and syphilis was worked out by the author together with H. E. Menke, Department of Dermatology, Faculty of Medicine, Erasmus University.

The large numbers of gonorrhoea patients attending the Venereal Disease Clinic of the Academic Hospital, Rotterdam, and the large amount of data to be handled make registration in the traditional way well-nigh impossible. For this reason data collection forms were drafted in collaboration with Mr. J. M. A. Jeurissen of the Municipal Computer Centre, Rotterdam, which after processing to a punched card could be fed into the computer and at the same time could be used for filing purpose.

When an IBM computer terminal became available to the Medical Faculty Rotterdam (M. F. R.) in 1971, the forms were modified in collaboration with the staff of the System Development Department of the Faculty of Medicine of Erasmus University to make them directly readable (so-called optically readable forms). The System Development Department was also responsible for the final lay-out.

REGISTRATION AND PROCESSING OF THE DATA.

The forms were converted into punched cards with the aid of an IBM 1232 optical mark page reader and a coupled IBM 534 card punch. In view of the standard method used for the punching of data, rearrangement and recording of the punched-card data was required for the project in question.

This was done by the computer, which performed plausibility checks at the same time. A separate data set was drafted for each type of form. The total collection of data consisted of the following data sets:

A1. Epidemiology

A2. Addendum females. (Extra form for gynaecological and epidemiological data.)

A3. Contact/Source (1)

A4. Contact/Source (2)

A5. Serology

G1. Clinical data

G2. Bacteriology

G3. Therapy

FILE - NUMBER

```
_0_ _1_ _2_ _3_ _4_          _5_ _6_ _7_ _8_ _9_
_0_ _1_ _2_ _3_ _4_          _5_ _6_ _7_ _8_ _9_
_0_ _1_ _2_ _3_ _4_          _5_ _6_ _7_ _8_ _9_
_0_ _1_ _2_ _3_ _4_          _5_ _6_ _7_ _8_ _9_
_0_ _1_ _2_ _3_ _4_          _5_ _6_ _7_ _8_ _9_
_0_ _1_ _2_ _3_ _4_          _5_ _6_ _7_ _8_ _9_
```

STARTING DATE EXAMINATION = DATE 0

```
_00_ _10_ _20_ _30_
_0_ _1_ _2_ _3_ _4_      day      _5_ _6_ _7_ _8_ _9_

_10/_ _1_ _2_ _3_ _4_    month    _5_ _6_ _7_ _8_ _9_

_70_ _71_ _72_ _73_ _74_  year    _75_ _76_ _77/_ _68_ _69_
```

Name physician:
Code: _1_ _2_ _3_ _4_   _5_ _6_ _7_ _8_ _9_

Name social worker:
Code: _1_ _2_ _3_ _4_   _5_ _6_ _7_ _8_ _9_

Patient: male   or   female

                      civilian or   sailor

Case history possible   _yes_   _no_

Occupation:
```
0000 1000 2000 3000 4000 5000 6000 7000 8000 9000
_000 _100 _200 _300 _400 _500 _600 _700 _800 _900
_00_ _10_ _20_ _30_ _40_ _50_ _60_ _70_ _80_ _90_
_0_ _1_ _2_ _3_ _4_ _5_ _6_ _7_ _8_ _9_
```

Nationality:
```
                        100 200 300 400 500
_00_ _10_ _20_ _30_ _40_  _50_ _60_ _70_ _80_ _90_
_0_ _1_ _2_ _3_ _4_       _5_ _6_ _7_ _8_ _9_
```

Civil status: A. unmarried married divorced widow(er)

                B. engaged conc. hom.con. other

Age (in years):
```
_00_ _10_ _20_ _30_ _40_   _50_ _60_ _70_ _80_ _90_
_0_ _1_ _2_ _3_ _4_       _5_ _6_ _7_ _8_ _9_
```

Domicile: abroad   Netherlands   no fixed adress

Home adress:

* town

* street and house number

* Postal district code (if domiciled in the Netherl.)
```
0000 1000 2000 3000 4000 5000 6000 7000 8000 9000
_000 _100 _200 _300 _400 _500 _600 _700 _800 _900
__00 __10 __20 __30 __40 __50 __60 __70 __80 __90
___0 ___1 ___2 ___3 ___4 ___5 ___6 ___7 ___8 ___9
```

Ever visited our clinic before ?	yes	no
Reason for examination:		
* asked to attend	go.s.c. lues.s.c.	not app.
* came voluntarily	go.s.c. lues.s.c.	not app.
* afraid	_yes_	_no_
* warned	_yes_	_no_
* complaints	_yes_	_no_
* other	other reason	no reason	reinfected
* previous examination pos. serology	_yes_	_no_

Penicillin-allergy	_yes_	_no_	unknown
Allergy to other medicines	_yes_	_no_	unknown
If so, to which ?			

Addictions: alcohol   drugs   other   none

Former venereal infections:

* Syph.I:
```
yes  10 20 30 40    year    _50 _60 _70 _80 _90
no  _1_ _2_ _3_ _4_         _5_ _6_ _7_ _8_ _9_
```
* Syph II:
```
yes _10 _20 _30 _40         _50 _60 _70 _80 _90
no  _1_ _2_ _3_ _4_         _5_ _6_ _7_ _8_ _9_
```
* Other early Syph.
```
yes _10 _20 _30 _40         _50 _60 _70 _80 _90
no  _1_ _2_ _3_ _4_         _5_ _6_ _7_ _8_ _9_
```
* Other kinds of Syph. ?
```
yes _10 _20 _30 _40         _50 _60 _70 _80 _90
no  _1_ _2_ _3_ _4_         _5_ _6_ _7_ _8_ _9_
```
* Go.I (previous Go)
```
yes _10 _20 _30 _40         _50 _60 _70 _80 _90
no  _1_ _2_ _3_ _4_         _5_ _6_ _7_ _8_ _9_
```
* Go II (penultimate Go)
```
yes _10 _20 _30 _40         _50 _60 _70 _80 _90
no  _1_ _2_ _3_ _4_         _5_ _6_ _7_ _8_ _9_
```
* Go III
```
yes _10 _20 _30 _40         _50 _60 _70 _80 _90
no  _1_ _2_ _3_ _4_         _5_ _6_ _7_ _8_ _9_
```
* Go IV
```
yes _10 _20 _30 _40         _50 _60 _70 _80 _90
no  _1_ _2_ _3_ _4_         _5_ _6_ _7_ _8_ _9_
```
* Suffered more than 4 times from GO   yes no
* Suffered from other venereal diseases   yes no

If so, which ?

Was an antibiotic/chemotherapeutic therapy prescribed for :	present complaint	other illness	both	_no_
- if so, which ?				

Last therapy:

Where: by whom:

Medicament:

Total dosage:

Method of administration:

Dates: from   to

How many days before the date of 0 :
```
000 100 200 300 400         500 600 700 800 900
_00 _10 _20 _30 _40         _50 _60 _70 _80 _90
_0_ _1_ _2_ _3_ _4_         _5_ _6_ _7_ _8_ _9_
```

Penultimate therapy	yes	no
Where:	by whom:	
Medicament:		
Total dosage:		
Method of administration:		
Dates: from	to	

How many days befor the date 0 ?
```
000 100 200 300 400         500 600 700 800 900
_00 _10 _20 _30 _40         _50 _60 _70 _80 _90
_0_ _1_ _2_ _3_ _4_         _5_ _6_ _7_ _8_ _9_
```

More than 2 therapies ?	_yes_	_no_

MEDICAL FACULTY ROTTERDAM
ACADEMIC HOSPITAL ROTTERDAM - Dijkzigt

Department of Dermatology
File number :

FILE - NUMBER

_0_ _1_ _2_ _3_ _4_ _5_ _6_ _7_ _8_ _9_
_0_ _1_ _2_ _3_ _4_ _5_ _6_ _7_ _8_ _9_
_0_ _1_ _2_ _3_ _4_ _5_ _6_ _7_ _8_ _9_
_0_ _1_ _2_ _3_ _4_ _5_ _6_ _7_ _8_ _9_
_0_ _1_ _2_ _3_ _4_ _5_ _6_ _7_ _8_ _9_
_0_ _1_ _2_ _3_ _4_ _5_ _6_ _7_ _8_ _9_

STARTING DATE EXAMINATION = DATE 0

_00_ _10_ _20_ _30_
_0_ _1_ _2_ _3_ _4_   day   _5_ _6_ _7_ _8_ _9_
_10_/_1_ _2_ _3_ _4_ month   _5_ _6_ _7_ _8_ _9_
_===_ _71_ _72_ _73_ _74_ year _75_ _76_ _77_/_=_ _69_

MENSTRUATION DATA (also if using contraceptive pil)

Date last menstruation (first day):
_00_ _10_ _20_ _30_
_0_ _1_ _2_ _3_ _4_   day   _5_ _6_ _7_ _8_ _9_
_10_/_1_ _2_ _3_ _4_ month   _5_ _6_ _7_ _8_ _9_
_===_ _71_ _72_ _73_ _74_ year _75_ _76_ _77_/_=_ _69_

Cycle: regular   irregular
                     =====   ====

If regular,
average duration of cycle (in days) :

_00_ _10_ _20_ _30_ _40_   _50_ _=_ _=_ _=_ _=_

_0_ _1_ _2_ _3_ _4_   _5_ _6_ _7_ _8_ _9_

How long does menstruation last (in days):

_=_ _1_ _2_ _3_ _4_   _5_ _6_ _7_ _8_ _9_

Menstruation pattern: changed   not
                                      changed

Remarks:

Pregnancy: not   diagnosed   examined
                examined   but not
                 ===   ===   diagnosed
                                       ===

Times marries:

_0_ _1_ _2_ _3_ _4_   _5_ _6_ _7_ _8_ _9_

Year of first marriage:

_00_ _10_ _20_ _30_ _40_   _50_ _60_ _70_ _80_ _90_

_0_ _1_ _2_ _3_ _4_   _5_ _6_ _7_ _8_ _9_

Children: yes   no
                           ===   ===

Abortion: yes   no
                           ===   ===

How many children alive at birth:

_00_ _10_ _20_ _=_ _=_

_0_ _1_ _2_ _3_ _4_   _5_ _6_ _7_ _8_ _9_

Abortion(s) performed bij a physician:

_0_ _1_ _2_ _3_ _4_   _5_ _6_ _7_ _8_ _9_

Abortus provocatus in any other way:

_0_ _1_ _2_ _3_ _4_   _5_ _6_ _7_ _8_ _9_

Spontaneous abortions/children still-born:

_0_ _1_ _2_ _3_ _4_   _5_ _6_ _7_ _8_ _9_

Year first abortion:

_00_ _10_ _20_ _30_ _40_   _50_ _60_ _70_ _80_ _90_

_0_ _1_ _2_ _3_ _4_   _5_ _6_ _7_ _8_ _9_

Year first child:

_00_ _10_ _20_ _30_ _40_   _50_ _60_ _70_ _80_ _90_

_0_ _1_ _2_ _3_ _4_   _5_ _6_ _7_ _8_ _9_

FILE - NUMBER

_0_ _1_ _2_ _3_ _4_   _5_ _6_ _7_ _8_ _9_
_0_ _1_ _2_ _3_ _4_   _5_ _6_ _7_ _8_ _9_
_0_ _1_ _2_ _3_ _4_   _5_ _6_ _7_ _8_ _9_
_0_ _1_ _2_ _3_ _4_   _5_ _6_ _7_ _8_ _9_
_0_ _1_ _2_ _3_ _4_   _5_ _6_ _7_ _8_ _9_
_0_ _1_ _2_ _3_ _4_   _5_ _6_ _7_ _8_ _9_

STARTING DATE EXAMINATION = DATE O

_00_ _10_ _20_ _30_
_0_ _1_ _2_ _3_ _4_   day   _5_ _6_ _7_ _8_ _9_
_10/_ _1_ _2_ _3_ _4_   month   _5_ _6_ _7_ _8_ _9_
_70_ _71_ _72_ _73_ _74_   year   _75_ _76_ _77_ _78_ _79_

Serial number of contact/source:
_1_ _2_ _3_ _4_   _5_ _6_ _7_ _8_ _9_

(left margin, vertical) serial number of contact/source

IF PATIENT HAS NO COMPLAINTS, PLEASE RECORD:

Date of last sexual contact: _00_ _10_ _20_ _30_
_0_ _1_ _2_ _3_ _4_   day   _5_ _6_ _7_ _8_ _9_
_10/_ _1_ _2_ _3_ _4_   month   _5_ _6_ _7_ _8_ _9_
_20_ _71_ _72_ _73_ _74_   year   _75_ _76_ _77//68_ _69_

Date of penultimate sexual contact: _00_ _10_ _20_ _30_
_0_ _1_ _2_ _3_ _4_   day   _5_ _6_ _7_ _8_ _9_
_10/_ _1_ _2_ _3_ _4_   month   _5_ _6_ _7_ _8_ _9_
_70_ _71_ _72_ _73_ _74_   year   _75_ _76_ _77//68_ _69_

Regular sexual intercourse: yes ===   no ===

IF THE PATIENT HAS COMPLAINTS, PLEASE RECORD:

Date of first complaints: _00_ _10_ _20_ _30_
_0_ _1_ _2_ _3_ _4_   day   _5_ _6_ _7_ _8_ _9_
_10_ _1_ _2_ _3_ _4_   month   _5_ _6_ _7_ _8_ _9_
_70_ _71_ _72_ _73_ _74_   year   _5_ _6_ _7_ _8_ _9_

Sexual intercourse before complaints   yes === no ==

Date of last sexual intercourse before complaints arose: _00_ _10_ _20_ _30_
_0_ _1_ _2_ _3_ _4_   day   _5_ _6_ _7_ _8_ _9_
_10_ _1_ _2_ _3_ _4_   month   _5_ _6_ _7_ _8_ _9_
_70_ _71_ _72_ _73_ _74_   year   _75_ _76_ _77//68_ _69_

Date of penultimate sexual contact before complaints arose: _00_ _10_ _20_ _30_
_0_ _1_ _2_ _3_ _4_   day   _5_ _6_ _7_ _8_ _9_
_10_ _1_ _2_ _3_ _4_   month   _5_ _6_ _7_ _8_ _9_
_70_ _71_ _72_ _73_ _74_   year   _75_ _76_ _77//68_ _69_

Regular sexual intercourse before complaints arose:
    yes ===   no ===
After the complaints arose:
    yes ===   no ===

Date of last sexual intercourse after the complaints arose: _00_ _10_ _20_ _30_
_0_ _1_ _2_ _3_ _4_   day   _5_ _6_ _7_ _8_ _9_
_10/_ _1_ _2_ _3_ _4_   month   _5_ _6_ _7_ _8_ _9_
_70_ _71_ _72_ _73_ _74_   year   _75_ _76_ _77//68_ _69_

Name of contact/source in question:

Nickname:

Brief description, if possible, of contact/source

Domicile   abroad   The Netherlands   no fixed
      ======   ======   adress.
Adress:
   - town:
   - street and house number

   - code place + postal district
_00_ _10_ _20_ _30_ _40_   town   _50_ _60_ _70_ _80_ _90_
_0_ _1_ _2_ _3_ _4_   _5_ _6_ _7_ _8_ _9_
_00_ _10_ _20_ _30_ _40_   district   _50_ _60_ _70_ _80_ _90_
_0_ _1_ _2_ _3_ _4_   _5_ _6_ _7_ _8_ _9_

More precise definition of contact/source:

spouse	fiancé(e)	concubine	friend	prostitute	amateur	pick up	homosexual partner	partner exchange	other
===	===	===	===	===	===	===	===	===	===

If the reply is other, please specify which:

Occupation (code) of contact/source:

0000 1000 2000 3000 4000 5000 6000 7000 8000 9000
_000_ _100_ _200_ _300_ _400_ _500_ _600_ _700_ _800_ _900_
_00_ _10_ _20_ _30_ _40_ _50_ _60_ _70_ _80_ _90_
_0_ _1_ _2_ _3_ _4_ _5_ _6_ _7_ _8_ _9_

Age of contact/source (in years)
_00_ _10_ _20_ _30_ _40_   _50_ _60_ _70_ _80_ _90_
_0_ _1_ _2_ _3_ _4_   _5_ _6_ _7_ _8_ _9_

Nationality of contact/source:
000 100 200 300 400   500 600 700 800 900
_00_ _10_ _20_ _30_ _40_   _50_ _60_ _70_ _80_ _90_
_0_ _1_ _2_ _3_ _4_   _5_ _6_ _7_ _8_ _9_

Department of
Dermatologie
File number:

CONTACT/SOURCE | no. A 4

FILE - NUMBER

_0_ _1_ _2_ _3_ _4_	_5_ _6_ _7_ _8_ _9_
_0_ _1_ _2_ _3_ _4_	_5_ _6_ _7_ _8_ _9_
_0_ _1_ _2_ _3_ _4_	_5_ _6_ _7_ _8_ _9_
_0_ _1_ _2_ _3_ _4_	_5_ _6_ _7_ _8_ _9_
_0_ _1_ _2_ _3_ _4_	_5_ _6_ _7_ _8_ _9_
_0_ _1_ _2_ _3_ _4_	_5_ _6_ _7_ _8_ _9_

STARTING DATE EXAMINATION = DATE 0

_00_ _10_ _20_ _30_		
_0_ _1_ _2_ _3_ _4_	day	_5_ _6_ _7_ _8_ _9_
_10_ _1_ _2_ _3_ _4_	month	_5_ _6_ _7_ _8_ _9_
70 71 72 73 74	year	75 76 77//68 69

Serial number of contact/source
_1_ _2_ _3_ _4_   _5_ _6_ _7_ _8_ _9_

First place of encounter with contact/source

not asked ===   no data supplied ===

at the patient's home	at home of contact/source	in the street	in a bar/café	brothel/massage esta-blishment	hotel	sauna	on board ship.	other place of encounter	at a dance hall
===	===	===	===	===	===	===	===	===	===

abroad ===   in the Netherlands ===   elsewhere ===

Adress:
   -town:
   -street ans house number:

   -code place and postal district (Netherlands)

_00_ _10_ _20_ _30_ _40_	town	_50_ _60_ _70_ _80_ _90_
_0_ _1_ _2_ _3_ _4_		_5_ _6_ _7_ _8_ _9_
_00_ _10_ _20_ _30_ _40_	district	_50_ _60_ _70_ _80_ _90_
_0_ _1_ _2_ _3_ _4_		_5_ _6_ _7_ _8_ _9_

Method of contraception employed:

=== not asked

=== no data supplied

=== none

=== unknown

If answer is "other",

how ?

coïtus interruptus	_yes_ _no_ sometimes
sheath	yes no sometimes
periodic abstinence	_yes_ _no_ sometimes
contraceptive pill	_yes_ _no_ sometimes
spiral	_yes_ _no_ sometimes
other	yes no sometimes

Manner of sexual intercourse:

=== not asked

=== no data supplied

=== none

If answer is
other, how ?

genitogenital	_yes_ _no_
genito-oral	_yes_ _no_
genito-anal	_yes_ _no_
anogenital	_yes_ _no_
orogenital	_yes_ _no_
other	_yes_ _no_

Place of coitus (p.c.)

not asked ===   not supplied ===   abroad ===
Netherlands ===   Abroad and Netherlands ===
elsewhere ===

code p.c.	1.Patient's home	_1x_	more often	_no_
	2.Home of contact	_1x_	often	_no_
	3.In the street	_1x_	often	_no_
	4.In a automobile	_1x_	often	_no_
	5.At a brothel/massage establ.	_1x_	often	_no_
	6.In a hotel	_1x_	often	_no_
	7.On board ship	_1x_	often	_no_
	8.Sauna	_1x_	often	_no_
	9.elsewhere	_1x_	often	_no_

Is the behaviour of the patient generally:

heterosexual	bisexual	homosexual	no data sup-plied	other
===	===	===	===	===

Code locations (place and postal district, if in the
Netherlands) of place of coitus, stating code p.c.
as given above.

_00_ _10_ _20_ _30_ _40_	I town	_50_ _60_ _70_ _80_ _90_
_0_ _1_ _2_ _3_ _4_		_5_ _6_ _7_ _8_ _9_
_00_ _10_ _20_ _30_ _40_	district	_50_ _60_ _70_ _80_ _90_
_0_ _1_ _2_ _3_ _4_		_5_ _6_ _7_ _8_ _9_
_1_ _2_ _3_ _4_	p.c.	_5_ _6_ _7_ _8_ _9_
_00_ _10_ _20_ _30_ _40_	II town	_50_ _60_ _70_ _80_ _90_
_0_ _1_ _2_ _3_ _4_		_5_ _6_ _7_ _8_ _9_
_00_ _10_ _20_ _30_ _40_	district	_50_ _60_ _70_ _80_ _90_
_0_ _1_ _2_ _3_ _4_		_5_ _6_ _7_ _8_ _9_
_1_ _2_ _3_ _4_	p.c.	_5_ _6_ _7_ _8_ _9_
_00_ _10_ _20_ _30_ _40_	III town	_50_ _60_ _70_ _80_ _90_
_0_ _1_ _2_ _3_ _4_		_5_ _6_ _7_ _8_ _9_
_00_ _10_ _20_ _30_ _40_	district	_50_ _60_ _70_ _80_ _90_
_0_ _1_ _2_ _3_ _4_		_5_ _6_ _7_ _8_ _9_
_1_ _2_ _3_ _4_	p.c.	_5_ _6_ _7_ _8_ _9_

TO BE COMPLETED WITHIN 3 MONTHS

Contact/source is:

| | | examined | | examined and treated |
| not traced === | by us | elsewhere | by us | elsewh. |

File number:

_0_ _1_ _2_ _3_ _4_	_5_ _6_ _7_ _8_ _9_
_0_ _1_ _2_ _3_ _4_	_5_ _6_ _7_ _8_ _9_
0 1 2 3 4	5 6 7 8 9
_0_ _1_ _2_ _3_ _4_	_5_ _6_ _7_ _8_ _9_
_0_ _1_ _2_ _3_ _4_	_5_ _6_ _7_ _8_ _9_
_0_ _1_ _2_ _3_ _4_	_5_ _6_ _7_ _8_ _9_

Has contact/source suffered from GO/Lues ?
   yes   possible   no   unknown
   ===   ===   ===   ===

Are more than three items to be coded ?

   yes   no
   ===   ===

If so, please list additional codes.

Department of Dermatology   SEROLOGY
File number :   No. A 7

FILE - NUMBER									
0	1	2	3	4	5	6	7	8	9
0	1	2	3	4	5	6	7	8	9
0	1	2	3	4	5	6	7	8	9
0	1	2	3	4	5	6	7	8	9
0	1	2	3	4	5	6	7	8	9
0	1	2	3	4	5	6	7	8	9

STARTING DATE EXAMINATION = DATE 0

00	10	20	30							
0	1	2	3	4	day	5	6	7	8	9
10/1	2	3	4		month	5	6	7	8	9
70	71	72	73	74	year	75	76	77/68	69	

DATE OF INVESTIGATION (with respect to date 0)

before date0:
- 1 week
- 1 day
- 2 weeks - 1 week
- 6 weeks - 2 weeks
- 3 months
- 6 weeks - 3 months
- more than 6 months

====== = Date 0

after date 0:
- 1 - 4 days
- 5 - 7 days
- 8 -14 days
- 15 -21 days
- 22 -28 days
- 29 -42 days
- 43 -56 days
- 57 -90 days
- 90 days ∧

SEROLOGY										
	UTRECHT	(R.I.V.)				DIJKZIGT				
neg.	1/1	1/2	1/4	1/8		neg.	1/1	1/2	1/4	1/8
1/16	1/32	1/64	1/128	1/256	VDRL	1/16	1/32	1/64	1/128	1/256
1/512		n.p.	a.c.	doubtf.		1/512		n.p.	a.c.	doubtf.
neg.	pos.	n.p.		doubtf.	TPCF	neg.	pos.	n.p.		doubtf.
neg.	1/1	1/2	1/4	1/8	FTA	neg.	1/1	1/2	1/4	1/8
1/16	1/32	1/64	1/128	1/256		1/16	1/32	1/64	1/128	1/256
1/512		n.p.	a.c.	doubtf.		1/512		n.p.	a.c.	doubtf.
neg.	±	+	++	+++	FTA abs	neg.	±	+	++	+++
++++		n.p.		failure		++++		n.p.		failure
neg.	±	+	++	+++	TPI	neg.	±	+	++	+++
++++		n.p.		failure		++++		n.p.		failure
neg.	pos.	in %	trepon.			neg.	pos.	in %	trepon.	
		n.p.	a.c.					n.p.	a.c.	

DEPARTMENT OF DERMATOLOGY :

RPR: neg   +   ++

```
┌─────────────────────────────────────────┐
│              FILE - NUMBER                │
│  0  1  2  3  4        5  6  7  8  9       │
│  0  1  2  3  4        5  6  7  8  9       │
│  0  1  2  3  4        5  6  7  8  9       │
│  0  1  2  3  4        5  6  7  8  9       │
│  0  1  2  3  4        5  6  7  8  9       │
│  0  1  2  3  4        5  6  7  8  9       │
└─────────────────────────────────────────┘
```

STARTING DATE EXAMINATION = DATE 0

00	10	20	30		
0 1 2 3 4	day	5 6 7 8 9
0 1 2 3 4	month	5 6 7 8 9
70 71 72 73 74	year	75 76 77/68 69

SUBJECTIVE SYMPTOMS

	yes	no
. symptoms	yes	no
. discharge	yes	no
. dysuria	yes	no
. stranguria	yes	no
. pollakisuria	yes	no
. haematuria	yes	no
. tenesmi	yes	no
. painful inquinal glands	yes	no
. swollen inquinal glands	yes	no
. anal itching/pain	yes	no

Specific to males:

	yes	no
. oedema of glans/prepuce/penis	yes	no
. desquamation/irritation of glans/prepuce/penis	yes	no
. painful swollen scrotum	yes	no

Specific to females:

	yes	no
. swelling/pain of labiae	yes	no
. pruritus vulvae	yes	no
. lower abdominal pain	yes	no
. other symptoms	yes	no

if so, which ?

Were other venereal complaints found during the examination or within 3 months following the examination ?

. genital/anal/oral ulcers	invest.	3 m.	none
. early syfylis (SI,SII,S.R.)	invest.	3 m.	none
. non specific urethritis	invest.	3 m.	none
. trichomoniasis	invest.	3 m.	none
. candidiasis	invest.	3 m.	none
. scabies	invest.	3 m.	none
. pediculosis pubis	invest.	3 m.	none
. condylomata accuminata	invest.	3 m.	none
. genital herpes	invest.	3 m.	none
. other venereal diseases which ?	invest.	3 m.	none
. other diseases, which ?	invest.	3 m.	none

Name physician:

no. code:
1 2 3 4   5 6 7 8 9

Case History (own words):

	yes	no
Special notes	yes	no

Clinical

	yes	no
. swollen inquinal glands	yes	no
. proctitis	yes	no
. anal eczema	yes	no

Specific to males:

	yes	no
. discharge	yes	no
. balanitis/posthitis	yes	no
. oedema glans/prepuce/penis	yes	no
. prostatitis	yes	no
. funiculitis	yes	no
. epididymitis	yes	no

Specific to females:

	yes	no
. urethral fluor	yes	no
. vaginal fluor	yes	no
. oedema of labiae	yes	no
. vulvitis	yes	no
. bartholinitis	yes	no
. vaginitis	yes	no
. cervicitis	yes	no
. fluor found with aid of speculum	yes	no
. salpingitis	yes	no

	yes	no
. Other symptoms of gonorrhoea	yes	no

If so, which ?

. Colour discharge   yellow
                          green   yellow green white
. Consistency of discharge   watery slimy creamy

Temperature: 30 40   (c°)

0 1 2 3 4		5 6 7 8 9

0,0 0,1 0,2 0,3 0,4   0,5 0,6 0,7 0,8 0,9

MEDICAL FACULTY ROTTERDAM
ACADEMIC HOSPITAL ROTTERDAM - Dijkzigt

Department of Dermatology   BACTERIOLOGY
File number :   no. G 2

FILE - NUMBER

0	1	2	3	4	5	6	7	8	9
0	1	2	3	4	5	6	7	8	9
0	1	2	3	4	5	6	7	8	9
0	1	2	3	4	5	6	7	8	9
0	1	2	3	4	5	6	7	8	9
0	1	2	3	4	5	6	7	8	9

STARTING DATE EXAMINATION = DATE O

00	10	20	30							
0	1	2	3	4	day	5	6	7	8	9
10/	1	2	3	4	month	5	6	7	8	9
70	71	72	73	74	year	75	76	77//68	69	

DATE OF INVESTIGATION (with respect to date O)

before date	1 week - 1 day	2 weeks - 1 week	1 month - 2 weeks	3 month - 6 weeks	more than 6 month
==== = date O					
after date	1 - 4 d.	5 - 7 d.	8 -14 d.	15 -21 d.	22 -28 d.	29 -42 d.	43 -56 d.	57 -90 d.	90 d. A

Concerning:	rectum	urethra	cervix	prostatic fluid	other site
. MB	pos.	neg.	doubtf.	n.p.	extr.cell diplococc.
. Gram	pos.	neg.	doubtf.	n.p.	extr.cell diplococc.
. Culture	pos.	neg.	overgr.	n.p.	
. Delayed I.F.	pos.	neg.	crossr.	n.p.	

Sensivities:

Am.	0,005	0,01	0,02	0,04	0,08	0,16	0,32	0,64		
P	0,0025	0,005	0,01	0,02	0,04	0,08	0,16	0,32	0,64	0,128
T	0,04	0,08	0,16	0,32	0,64	1,28	2,56			

Other bacteriological investigations:		perf.	n.p.
Trich.vag.smear	neg.	pos.	n.p.
Trich vag.culture	neg.	pos.	n.p.
Cand. smear	neg.	pos.	n.p.
Cand. culture	neg.	pos.	n.p.
Bact. culture	pathogen non-pathogen sterile		n.p.

Concerning:	rectum	urethra	cervix	prostatic fluid	other site
. MB	pos.	neg.	doubtf.	n.p.	extr.cell diplococc.
. Gram	pos.	neg.	doubtf.	n.p.	extr.cell diplococc.
. Culture	pos.	neg.	overgr.	n.p.	
. Delayed I.F.	pos.	neg.	crossr.	n.p.	

Sensivities:

Am.	0,005	0,01	0,02	0,04	0,08	0,16	0,32	0,64		
P	0,0025	0,005	0,01	0,02	0,04	0,08	0,16	0,32	0,64	0,1
T	0,04	0,08	0,16	0,32	0,64	1,28	2,56			

Other bacteriological investigations:		perf.	n.p.
Trich.vag.smear	neg.	pos.	n.p.
Trich.vag.culture	neg.	pos.	n.p.
Cand. smear	neg.	pos.	n.p.
Cand. culture	neg.	pos.	n.p.
Bact. culture	pathogen non-pathogen sterile		n.p.

Concerning:	rectum	urethra	cervix	prostatic fluid	other site
. MB	pos.	neg.	doubtf.	n.p.	extr.cell diplococc.
. Gram	pos.	neg.	doubtf.	n.p.	extr.cell diplococc.
. Culture	pos.	neg.	overgr.	n.p.	
. Delayed I.F.	pos.	neg.	crossr.	n.p.	

Sensivities:

Am	0,005	0,01	0,02	0,04	0,08	0,16	0,32	0,64	
P	0,0025	0,005	0,01	0,02	0,04	0,08	0,16	0,32	0,64	1,28
T	0,04	0,08	0,16	0,32	0,64	1,28	2,56		

Other Bacteriological investigations:		perf.	n.p.
Trich.vag.smear	pos.	neg.	n.p.
Trich.vag.culture	pos.	neg.	n.p.
Cand. smear	pos.	neg.	n.p.
Cand. culture	pos.	neg.	n.p.
Bact. culture	pathogen non-pathogen sterile		n.p.

G.C.F.T.	n.p.	neg.	a.c.	1 1	1 2	1/4	1 8	1 16	1 32	1 64	1/128	1 256	1 512

Department of Dermatology　　　　　　　THERAPY
File number:　　　　　　　　　　　　　　| no. G 3 |

FILE - NUMBER
_0_ _1_ _2_ _3_ _4_　　　　　_5_ _6_ _7_ _8_ _9_
_0_ _1_ _2_ _3_ _4_　　　　　_5_ _6_ _7_ _8_ _9_
_0_ _1_ _2_ _3_ _4_　　　　　_5_ _6_ _7_ _8_ _9_
_0_ _1_ _2_ _3_ _4_　　　　　_5_ _6_ _7_ _8_ _9_
_0_ _1_ _2_ _3_ _4_　　　　　_5_ _6_ _7_ _8_ _9_
_0_ _1_ _2_ _3_ _4_　　　　　_5_ _6_ _7_ _8_ _9_

STARTING DATE EXAMINATION = DATE 0

_00_ _10_ _20_ _30_		
_0_ _1_ _2_ _3_ _4_	day	_5_ _6_ _7_ _8_ _9_
_10/_1_ _2_ _3_ _4_	month	_5_ _6_ _7_ _8_ _9_
_70_ _71_ _72_ _73_ _74_	year	_75_ _76_ _77/_68_ _69_

. 1 g. ampicillin i.m. followed by

　2 g. ampicillin orally　　　yes　　　no
　after 4 hours　　　　　　=====　　　====

. other manner of administration:
　　　　　　　　　　　　　　　yes　　　no
　　　　　　　　　　　　　　=====　　　====
　if so, which :

. penicillin

　(Bicillin, PPC) - 4,8 m.u.　yes　　　no
　　　　　　　　　　　　　　=====　　　====

-other manner of administration:
　　　　　　　　　　　　　　　yes　　　no
　　　　　　　　　　　　　　=====　　　====
　if so, which :

: tetracycline 250 mg 4 dd 2

+ vit. B-complex 4 dd 1-5 days
　　　　　　　　　　　　　　　yes　　　no
　　　　　　　　　　　　　　=====　　　====

. other manner of administration:
　　　　　　　　　　　　　　　yes　　　no
　　　　　　　　　　　　　　=====　　　====
　if so, which :

. Other treatment
　　　　　　　　　　　　　　　yes　　　no
　　　　　　　　　　　　　　=====　　　====

　if so, which :

. no treatment
　　　　　　　　　　　　　　　yes　　　no
　　　　　　　　　　　　　　=====　　　====

　reason :

In which of the following periods did the
patient undergo further examinations ?

1-4

　　5-7

　　　　8-14

　　　　　　15-21

　　　　　　　　22-28

Periods:	29-41
no. of days after date 0	42-56

　　　　　　　　　　　　　　　57-90

　　　　　　　　　　　　　　　　　≥ 90

In which of the following periods was the
first positive Gram-smear, culture and/or
I.F. smear found ?

1-4
　5-7
　　8-14
　　　15-21
　　　　22-28

Periods:	29-41
no. of days after date 0	42-56

　　　　　　　　　　　　　　57-90
　　　　　　　　　　　　　　　　>90

Patients transferred to new list
　　　　　　　　　relapse/reinfection　other rea-
　　　　　　　　　=====　　　　　　====　　son

NEW FILE NUMBER
_0_ _1_ _2_ _3_ _4_　　　_5_ _6_ _7_ _8_ _9_
_0_ _1_ _2_ _3_ _4_　　　_5_ _6_ _7_ _8_ _9_
_0_ _1_ _2_ _3_ _4_　　　_5_ _6_ _7_ _8_ _9_
_0_ _1_ _2_ _3_ _4_　　　_5_ _6_ _7_ _8_ _9_
_0_ _1_ _2_ _3_ _4_　　　_5_ _6_ _7_ _8_ _9_

NEW STARTING DATE

_00_ _10_ _20_ _30_		
_0_ _1_ _2_ _3_ _4_	day	_5_ _6_ _7_ _8_ _9_
_10/_1_ _2_ _3_ _4_	month	_5_ _6_ _7_ _8_ _9_
_71_ _72_ _73_ _74_	year	_75_ _76_ _77_ _78_ _79_

Toxicoderma and other side effects after or during
treatment.　　pen.　　penbr.　　tetrac. other none

Has the patient received other antibiotic/chemothe-
rapeutic treatment during the 3-month follow-up pe-
riod in connection with other complaints ?

　　　　　　　　　　　　　yes　　　no
　　　　　　　　　　　　　=====　　====

If so, which ?

The required outputs (selective or non-selective lists, counts and other statistical results) could be obtained from the data sets stored in the disc memory and on the punched cards. The choice of the computer system of the System Development Department implied the use of an IBM 2780 terminal, linked by a permanent telephone line with an IBM 360 model 65 computer system of the IBM computer Centre at Rijswijk, presently at Zoetermeer, the Netherlands. The programs and data fed in by means of the terminal were processed in the central computer and the results returned to the terminal where they could be either printed out or punched on cards.

The IBM program packet offers scope for statistical processes such as frequency tables, cross tabulations and correlation calculations. In addition to the normal program packet, the System Development Department drafted extra programs for selection and sorting of the selected cases for this investigation.

Figure 1 shows the flowsheet for processing of the data registered on the optically readable forms.

F I G U R E   I

DATA - PROCESSING FLOWSHEET

Department of
Dermatology
Gonorrhoea pat.

contact/source A4
contact/source A3
add.females   A2
epidemiology   A1

epidemiological
            data

serology   A5
therapy   G3
bacteriology   G2
clinical data   G1

gonorrhoea data

IBM   1232/534

read in/convert
for each
kind of form.

punched - card
            set.
A1-A2-A3-A4-A5.

punched - card
            set.
G1 - G2 - G3 .

Gonorrhoea
data sets

IBM2780/IBM360-65

compile data-sets
(including check
and rearrange-
            ments)
output-process.

statistical proc.
counts
lists

Gonorrhoea
       surveys.

DISCUSSION OF THE OPTICALLY READABLE FORMS.

The data for each patient with a fresh gonorrheal infection, a
relapse or a reinfection are stored in a separate file. The file number is to
be found in the left-hand upper corner of each form, together with the date
of first examination = date 0. The file number is composed of six digits. The
first two digits indicate the year of the first visit to the clinic and the next
four digits stand for the patient's serial number in that year (0001 - 9999).
The starting date of the examination = date 0 is coded by a figure of six
digits. The first two stand for the day of the months, the next two for the
month and the last two digits indicate the year. In principle, date 0 is the date
when treatment was first given on the instructions of a physician from our cli-
nic. If no treatment was provided, date 0 is the date of the first positive bac-
teriological test (Gram-stained smear, gonococcal culture and/or delayed im-
munofluorescence).

All forms are filled in with a pencil. If any personal details
appeared on the forms, these were erased before reading in; consequently, the
System Development Department had no knowledge of the details.

The A1 form contains a number of epidemiological data which
are registered for each new case of disease of the patient. Some of the data
recorded in this form are summarized in Chapter IV.

The A2 form contains gynaecological and epidemiological data,
to be registered for each new case of disease of a female patient.

The A3 and A4 forms together represent one unit. One A3 and
one A4 form are completed for each possibly infected partner. The A3 and
A4 forms contain particulars of the sexual behaviour of the patient towards a
specific partner, as well as information obtained from the patient about the
said partner. Finally, the A4 form records whether the partner was traced,

was examined by us, was examined elsewhere, was examined and treated by us, or was examined and treated elsewhere. If the partner has already been given a file number by us, this is recorded on the form. Under the same heading ment ion can also be made of whether the partner was also suffering from gonorrhoea.

The A5 form is for listing the syphilis serology data obtained on different dates. One single form is used for each date.

The G1 form gives the clinical data of the patient, with details of specific venereal diseases and other diseases.

The A2, A3, A4, A5 and G1 forms are not discussed further in this thesis.

As the main attention of this thesis is focussed on the bacteriological and therapeutic part of the examination, the G2 and G3 forms will be explained in greater detail.

The G2 form gives the bacteriological data of the patient. As in the case of the A5 form, in principle one form will be employed per sampling date. This date is indicated in the top right-hand corner. The G2 form is subdivided into sections with identical content; space is left at the foot of the page for notes on the results of the Gonococcal Complement Fixation Test (GCFT). It is possible to add a second form for the same sampling date, if more than three sampling sites are investigated at the same moment. In the section on sensitivities, the minimum inhibitory concentration (MIC), determined by the plate dilution method, can be quoted for ampicillin (Am), penicillin (P) and tetracycline (T). Sensitivities to the other antibiotics tested were not registered on the G2 form, but on a blank computer form. The section on "Other bacteriological ecaminations" offers space for the listing of the observations on the <u>Trichomonas vaginalis</u> smear and culture, the

<u>Candida</u> smear and culture and the bacteriological culture.

The G3 form gives particulars of treatment and follow-up. The possibility that the patient received no treatment at all is also covered. The right-hand column indicates during which periods with respect to date 0 the patient received a check - up. Furthermore particulars about reinfection/relapse can be listed here, viz: the date of the first positive bacteriological findings, the new file number of the patient and new date of first examination. The last few sections provide space for details of toxicoderma and other side-effects after or during treatment, as well as whether the patient received any further antibiotic treatment in connection with other diseases.

Chapter IV

SOME EPIDEMIOLOGICAL DATA ON GONORRHOEA PATIENTS EXAMINED
BETWEEN 18 OCTOBER 1971 TO 1 JULY 1972.

A total of 636 gonorrhoea patients were examined in the Venereal Diseases Clinic of the University Hospital Rotterdam, Dijkzigt, in the period from 18 October 1971 to 1 July 1972. Of these 636 patients, 292 were male civilians (MC), 199 were female civilians (FC) and 145 were male sailors (MS).

In this chapter, a number of data on these patients taken from the A1 forms mentioned in Chapter III are summarized in tabular form and discussed briefly. In patients attending the Clinic with more than one gonococcal infection in the period under investigation, only data of the first gonococcal infection were used.

Since there is no registration of patients with venereal diseases in the Netherlands, and hence als not in Rotterdam, it is not possible to state what percentage of the total number of gonorrhoea patients in Rotterdam the gonorrhoea patients registered at the Venereal Diseases Clinic form.

The composition of gonorrhoea patients in Rotterdam is also not known. Therefore,it is also impossible to determine whether the composition of our group of patients is representative of the total group of gonorrhoea patients in Rotterdam. For this reason, we have not correlated the data on the gonorrhoea patients at the Clinic and demographic data for the population of Rotterdam; such an attempt would only lead to minimum estimates of the frequency of gonorrhoea. The percentages given in Table I should therefore only be regarded as characterizing gonorrhoea patients from whom material has been sampled for bacteriological investigation. The data are only discussed in general terms, but not subjected to statistical analysis.

Inspection of the distribution by occupation shows that the largest groups of MC are those of the administrative personell and of the factory workers; the other occupational groups are all more or less of the same size as one another. There are a number of quite large groups among the FC: in order of decreasing size, administrative personell, housewives, prostitutes, nurses, and barmaids and waitresses.

The percentages for the groups without occupation and student/pupil are practically equal in MC and FC. Among the MS, three large groups may be distinguished. As could be expected by far the largest is that of the seamen (not further classified), i. e. sailors of the lowest rank. The two other groups, which are of roughly the same size as one another, are those of the captains and other officers, and the engineers.

As regards the distribution of nationalities, it will be seen

that slightly more than half of the MC are Dutch. About a quarter appear to be immigrant labourers, while there is also quite a large group from Surinam. Willcox (1965) pointed out that "immigrant groups are particularly prone to venereal disease. Usually they consist mainly of males in search for employment and their ethnic and cultural background renders integration with the indigenous population difficult or slow. Their sexual potential is exploited by the prostitute and near-prostitute and venereal disease rates are consequently high".

Among the FC, practically 90% are Dutch, the only other group of any size being that from Surinam. About a quarter of the MS are Dutch (a surprising high proportion). The largest group has nationalities of the countries from which immigrant labour is drawn in the Netherlands, while MS from Western Germany also form a fair-sized group.

As regards marital status, it will be seen that about 70% of the MC and FC are single, while as many as 80% of the MS are single. The percentage of married patients is about 30% for MC, and less than this for FC and MS. Only the FC showed an appreciable group of divorced patients (> 10%).

As regards the age distribution it will be seen that FC are younger than MC and MS. About 40% of FC, 10% of MC and 15% of MS are younger than 21 (teenagers). In the same Clinic between 1954-1956 11% of 100 FC and 2.5% of 163 M (not further specified) and between 1964-1966 29.8% of 181 FC and 9.2% of 294 M were teenagers (Beek, 1968). The rising percentages of teenagers, especially among FC, observed in our Clinic are in accordance with figures from abroad. In Table I about 15% of FC, 30% of MC and 25% of MS are older than 30. Comparable data from our Clinic between 1954-1956 and between 1964-1966 were respectively: 32% of FC and 61.3% of M, and 10.5% of FC and 33.3% of M (Beek, 1968).

As regards domicile, practically all the MC and FC are domiciled in the Netherlands. About 90% of the MC and about 80% of the FC live in Rotterdam. About half of the MS are domiciled in the Netherlands, and about a quarter in Rotterdam itself. The other half has no fixed address, as is to be expected for sailors.

Many of the patients were not new to the Clinic. More than 30% of the MC, nearly 50% of the FC and nearly 20% of the MS had visited the Clinic before.

Most of the patients only mentioned one reason for consulting the Clinic; only a few gave two reasons. Of the MC, about 6% came as gonorrhoea contacts - about half at the request of the Clinic, and the other half voluntarily. About 60% of the FC came as gonorrhoea contact. It is striking that of these patients, about three out of every four came voluntarily, and only one out of four at the request of the Clinic. The percentage of gonorrhoea contacts among the MS was negligible. All but 2-3% of the MC and MS consulted the Clinic because they had complaints, while only a quarter of the FC had complaints. Quite a large group of the FC ($>$10%) had been advised to consult the Clinic. The fact that about 60% of the FC came to the Clinic as gonorrhoea contacts and 10% on the advice of other people is directly connected with the fact that only a quarter of the FC had complaints resulting from their gonorrheal infection.

The percentages of penicillin allergy found on history taking were low (between 1.5 and 3.4%) for MC, FC and MS.

Slightly more than 5% of the MC had previously had a syphilitic infection. The percentages for the FC and MS were lower. About 25% of the MC, FC and MS had previously had one gonorrheal infection, while about 20% of the MC, 10% of the FC and 25% of the MS had previously had more than

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one gonorrheal infection.

Only a small percentage of the MC and FC had previously been treated with antibiotics for their gonorrhoea, while about 30% of the MS had previously received inadequate antibiotic treatment.

Table 1

Some data on gonorrhoea patients examined in the period from 18 October 1971 to 1 July 1972. The figures in the table are percentages of the total number of patients in each group.

		MC[6]	FC[6]	MS[6]
Occupation[1]	Administrative personell	26.7	23.6	
	Commercial personell	3.8		
	Captains and other ship's officers			10.3
	Nurses		9.5	
	Shop assistants		4.0	
	Ship's engineers			11.7
	Factory workers (not further specified)	25.7		
	Welders	9.6		
	Mechanics (not further specified)	3.8		
	Barmen, barmaids, waiters and waitresses	5.1	8.5	
	Seamen (not further specified)			70.3
	Female workers, not further classified (cleaners, etc.)		4.5	
	No occupation	5.1	5.0	
	Student/pupil	5.1	5.0	
	Housewife		19.6	
	Prostitute		12.6	
	Others	15.1	7.7	7.6
Country of origin[2]	Netherlands	54.5	87.9	23.4
	Dutch Antilles			5.5
	Surinam	15.8	7.5	2.8
	"Immigrant-labour" countries[3]	24.3		39.3
	Scandinavia[4]			6.3
	Federal Republic of Germany			11.0
Marital status	Single	66.8	68.8	83.4
	Married	28.1	18.6	15.2
	Divorced	4.8	11.1	1.4
	Widow/er	0.3	1.5	

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Age-groups	11-15	0.3	3.0	
	16-20	9.6	36.7	16.6
	21-25	31.2	29.6	31.7
	26-30	27.1	14.1	26.2
	31-35	17.1	6.5	13.8
	36-40	7.9	5.0	4.1
	41-45	4.1	2.0	4.8
	46-50	0.7	2.0	2.1
	51-55	1.0		
	56-60	1.0	0.5	0.7
	> 60		0.5	
Domicile	Abroad	1.0		5.5
	Netherlands	98.6	100.0	44.8
	(Rotterdam)	(88.0)	(79.0)	(24.8)
	No fixed address	0.3		49.7
	Had visited Venereal Diseases Clinic before.	33.9	47.2	18.6
Some reasons[5] for consulting Clinic.	Requested to attend as gonorrhoea contact	3.4	15.6	0.7
	Voluntary attendance as gonorrhoea contact	2.4	45.2	0.7
	Anxious		4.5	
	Advised to attend	1.4	13.6	
	Complaints	96.6	25.0	97.9
	Penicillin allergy	3.4	1.5	2.8
Previous venereal infections.	Lues I	3.2		0.7
	Lues II	0.3	0.5	0.7
	Recent lues	0.3	0.5	
	Other forms of lues	1.4	1.0	
	1 previous case of gonorrhoea	25.3	22.6	25.5
	2 previous cases of gonorrhoea	8.9	5.0	11.0
	3 previous cases of gonorrhoea	4.5	2.0	6.9
	4 previous cases of gonorrhoea	1.0	1.0	4.8
	> 4 previous cases of gonorrhoea	5.1	1.5	2.8
	Previous antibiotic treatment for gonorrhoea	6.5	3.5	30.3

Notes

1.   Based on a list of occupations issued by the (Dutch) Central Bureau of Statistics. Administrative personell has to be regarded in a wider sense. All specified factory workers are classed among not further specified factory workers.

2.   Only percentages > 2.5% are given.

3.   Cape Verde Islands (Portugal), Spain, Italy, Yugoslavia, Greece, Turkey and Marocco.

4.   Denmark, Norway and Sweden.

5.   A few patients mentioned more than one reason for attendance.

6.   The percentages are calculated for totals of 292 MC, 199 FC and 145 MS.

In the following chapters, the period of investigation involved is given (in the section on material and methods). The investigation of Chapters VI, VII, VIII, IX, XII and XIII fell completely within the period mentioned in this chapter (IV), the investigation of Chapters X, XI and XIV only partly, while the investigations of Chapters V and XV fell entirely outside this period.

References

Beek, C.H. (1968)

Ned. T. Geneesk., 112, 989.

Willcox, R.R. (1965)

Curr. Med. Drugs, 5, 3.

Chapter V

COMPARISON OF MEDIA OF VARIOUS COMPOSITIONS FOR THE CULTURE
OF GONOCOCCI.

Introduction

According to Willcox (1964), gonococci do not grow in calf,ox-
heart or placenta broth - agar unless charcoal, starch, serum or ascitic fluid
has been added to it. Most of the fortified media in current use have a broth
agar base to which heated blood or haemoglobin and/or yeast extract, serum,
ascitic or hydrocele fluid has been added.

The isolation of gonococci from sites carrying a mixed flora
can give rise to difficulties when the nutrient medium is overgrown with other
bacteria. Especially in latent or chronic infections in women, the number of
gonococci at the sampling site may be low, and the fortified media may become
overgrown with staphylococci, diphtheroids and coliform bacilli.In the past,
many agents have been added to the fortified media in order to facilitate growth
of gonococci by selective retardation of the growth of other organisms. These
agents include triphenylmethane dyes such as gentian violet (Lagergreen and
Ouchterlony, 1948) and derivatives of phenoxazine such as nile blue A (Gard-
ner, 1940). Various authors have also experimented with thallium acetate
(Bang, 1952), boric acid and chloral hydrate (Lagergreen and Ouchterlony,
1948). In 1943 an antibiotic (tyrothricin) was used for the first time to res-
trict overgrowth of Gram-positive organisms (Stokinger, Ackerman and Car-
penter, 1953). In 1959, Crookes and Stuart recommended polymyxin B sul-
phate for suppression of the growth of Proteus. However, none of the above-
mentioned substances was entirely successful in practice, since a number of
sensitive gonococcal strains could not stand the concentrations required for

suppression of the growth of the other micro-organisms present.

The first selective medium proposed by Thayer and Martin (1964) contained ristocetin against Gram-positive and polymyxin B against Gram-negative mixed flora (RiPo medium). The basic medium used by Thayer and Martin was a chocolate agar to which haemoglobin and yeast extract had been added. Ristocetin was withdrawn from the market in 1964, and in 1966 Thayer and Martin described a new selective medium, consisting of the above-mentioned base with the addition of vancomycin (3 µg/ml) for the suppression of Gram-positive organisms, colistimethate (7.5 µg/ml) for the suppression of Gram-negative organisms and nystatin (12.5 U/ml) for the suppression of yeasts. This medium (the VCN medium) was found to be more specific and selective than the RiPo medium.

Nevertheless, overgrowth by Proteus was still sometimes a problem on the VCN medium. In 1970, Seth recommended trimethoprim (at a concentration of 8 µg/ml) for suppression of this organism.

Although the selective media offer important advantages compared with the non-selective media, a number of vancomycin-sensitive gonococcal strains were found not to grow on the selective VCN media while they did grow on the non-selective media. In a series of strains investigated by Reyn (1972), about 4% of the strains did not grow on a nutrient medium containing 2 µg/ml of vancomycin.

In order to arrive at an optimum medium for the isolation of gonococci, a comparative investigation on a number of media on different compositions was carried out in the period before 18 October 1971. The object of this investigation was to choose the best two out of four basic media and then to test various combinations of the best two basic media with two supplements. On the basis of the best combination found, the non-selective and the selective VCN

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medium were compared. Moreover the selective VCN medium was compared with the selective VCNT medium.

Material and methods

The following products were used for the preparation of the various media:

Basic media

Bacto GC Medium Base	- Difco 0289 = Medium A (GC = gonococcal culture)
GC Agar Base	- BBL 11275 = Medium B
Columbia Agar Base	- BBL 11124 = Medium C
Mueller-Hinton Agar	- Oxoid CM 337 = Medium D

Haemoglobin

Bacto haemoglobin	- Difco 0136 = Hb

Supplements

Bacto Supplement A	- Difco 0246 = S-X
Isovitalex TM Enrichment	- BBL 11976 = S-Y (TM = Thayer Martin)

Antibiotics

V-C-N Inhibitor	- BBL 12228 = V-C-N = vancomycin - colisti-methate-nystatin

Trimethoprim - Burroughs Wellcome & Co = T

The gonococcal media were prepared as follows:

1. The agar base was made up at twice the final concentration required, in accordance with the manufacturer's instructions, and autoclaved.

2. The haemoglobin was suspended with the aid of a mixer, and autoclaved.

3. After cooling to 50-60°C, the agar base and haemoglobin were mixed, and the supplement and any antibiotic used were added. Twenty-five ml of the

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medium were poured into each Petri dish, and allowed to set.

In each experiment, two media only (referred to as medium I and medium II) were compared with one another. The various pairs of media investigated are listed in Table I.

Each experiment was carried out within a certain period of time. In this period, material was sampled from the urethra of each male patient examined, and from the rectum, urethra and cervix of each female examined. The material was sampled as described in Chapter I, with the aid of charcoal-impregnated swabs (CIS's) which were placed in Stuart transport medium immediately after sampling and plated out on the media under investigation within 4 hours. The method of plating out is described in Chapter II. The inoculation sequence (medium I before medium II, or vice versa) was reversed in successive patients of each sex, as shown in the following table. Table II

The growth of the gonococcal colonies on plates I and II was scored as described in chapter II: no growth corresponds to a score of 0, while scores of 1, 2, 3, 4 or 5 were assigned to cultures showing increasing amounts of growth. All inoculated plates were scored; however, the score 0 was only included in the data for processing when the comparison plate did show some growth (i.e. was assigned a score from 1 to 5). Within a given investigation period, two series of comparisons could thus be set up: between medium I as the first medium inoculated and medium II as the second, or between medium II as the first inoculated and medium I as the second. These four different possibilities will in general give different score distributions, as illustrated (with fictive figures) in Tables III a and b.

Since the medium inoculated first may be expected to give better growth that the medium inoculated second, and since this advantage cannot be exactly expressed in numerical terms, few useful conclusions can be drawn from comparisons of the form illustrated in Tables IIIa and IIIb. However, more useful comparisons can be made on the basis of these data, by comparing medium I as the first inoculated with medium II as the first inoculated, and medium I as the second inoculated with medium II as the second inoculated. Rearrangement of the data of Tables IIIa and IIIb gives the picture shown in Tables IIIc and IIId.

Comparisons of the form illustrated in Tables IIIc and IIId were performed for all the pairs of media listed in Table I, the data being assessed with the aid of the X^2 test. Where necessary, score classes were combined to give a minimum required cell frequency for this test. Differences were considered to be significant at $p < 0.05$.

It was found that conclusions arrived at in this way on the basis of data of the form given in Table IIIc (comparison of the media inoculated first) were almost always identical with the conclusions arrived at from data of the form given in Table IIId (comparison of the media inoculated second). In view of this fact, we decided to give only the conclusions based on data of the form of Table IIIc (comparison of media I and II as first inoculated).

Results

The results of this investigation are summarized in Table IV.

Comparison of the basic media showed that medium A gave significantly higher scores than media B, C and D (comparisons 1, 2 and 3 of Table IV). Of media B, C and D, medium B gave the highest scores (comparison 1), and medium D the lowest (comparison 3). Medium A was therefore chosen as the optimum basic medium.

Comparison of supplements S-X and S-Y (comparison 4) gave no significant difference in score distributions. Comparison of medium A with supplement S-X (both Difco products) and the next best medium (B) with supplement S-Y (both BBL products) also gave no significant difference in score distributions (comparison 5). However, medium A with supplement S-Y gave a significantly better score distribution than medium B with supplement S-X (comparison 6). On the basis of these results, the combination of medium A with supplement S-Y was chosen as the best.

Comparison of this combination with and without the addition of VCN showed that the medium with VCN gave a significantly better score distribution (comparison 7). On the basis of this comparison, the medium with VCN was chosen as standard.

Comparison of this standard medium with and without addition of T gave no significant difference in scores (comparison 8). However, the plates containing T gave less frequent overgrowth with Proteus.

Discussion

The experimental set-up described in this chapter makes it possible to compare the quality of basic media, supplements and inhibitors in various combinations for the selective or non-selective culture of gonococci. One of the most surprising results of this investigation is that the best combination of basic medium and supplement consisted of a mixture of products from

different manufacturers, viz basic medium A (from Difco) and supplement S-Y (from BBL). It follows that it is advisable not to follow the maker's instructions blindly, but to experiment with the various products on the market to find the best combination.

The conclusion that the VCN medium gives better results than the non-selective medium was to be expected; it is in agreement with the publications of Thayer and Martin (1964, 1966).

Comparison of the VCN and VCNT media showed no significant difference in our investigation. The general policy followed in the Venereal Diseases Clinic of the University Hospital Rotterdam is that gonorrhoea must be diagnosed before treatment is given. The only exception to this rule is formed by women named as gonorrhoea contact by male patients in whom gonorrhoea has been diagnosed.

Patients who request an examination for venereal disease are generally examined three times for gonorrhoea in our clinic. This makes it possible to choose the VCN medium as standard medium for the first examination. If the first VCN culture gives overgrowth of <u>Proteus</u> on the plate and no visible gonococcal colonies, VCNT is used for the second and third cultures.

Summary

In this investigation, the four basic media (Bacto GC (gonococcal culture) medium base, GC agar base (BBL), Columbia agar base (BBL) and Mueller-Hinton agar (Oxoid)) were compared with the aid of gonococcal growth scores as regards their suitability for the culture of gonococci. Bacto GC medium base was found to be the best, and GC agar base the next best. Combinations of these two basic media with two supplements (Bacto supple-

ment A and Isovitalex TM (Thayer Martin) enrichment (BBL)) were then compared. The best results were obtained with the combination of Bacto GC medium base and Isovitalex TM enrichment. The best non-selective medium found in this investigation thus consisted of Bacto GC medium base, Bacto haemoglobin and Isovitalex TM enrichment. Comparison of this non-selective medium and the same medium to which VCN (vancomycin, colistimethate and nystatin) had been added gave significantly better scores for the selective medium. Comparison of this selective medium and the same medium with the addition of trimethoprim (T) gave no significant difference, although less growth of Proteus was found on the VCNT plates. The VCN medium was therefore chosen for the routine examination of patients in our clinic. When it was found that Proteus growth occurred, the VCNT medium was used for a second examination.

References

Bang, J. (1952)

Acta Dermatovener. , 32 (suppl. 29) , 34.

Cox, O.F. , Mc.Dermott, M. and Mueller, J.H. (1942)

Vener. Dis. Inform. , 23, 226.

Crookes, E.M.L. and Stuart, R.D. (1959)

J. Path.Bact. , 78, 283.

Gardner, L.W. (1940)

Amer. J. Syph. , 24, 737.

Lagergreen, B. and Ouchterlony, Ø (1948)

Nord. Med. , 40, 2410.

Reyn, A. and Bentzon, M.W. (1972)

Brit. J. vener. Dis. , 48, 363.

Seth, A. (1970)

Brit. J. vener. Dis. , 46, 201.

Stokinger, H. E. , Ackerman, H. and Carpenter, C.H. (1943)

J. Bact. , 45, 31.

Thayer, J.D. and Martin, J.E. (1964)

Publ. Hlth. Rep. (Wash.) , 79, 49.

Thayer, J.D. and Martin, J.E. (1966)

Publ. Hlth. Rep. (Wash.) , 81, 559.

Willcox, R.R. (1964)

Textbook of venereal diseases and treponematoses.

Table I

Composition of the various gonococcal culture media compared in this inves-

tigation[1].

Medium I   Medium II

1 Medium A[2] + HB + S-Y + V-C-N   Medium B + Hb + S-Y + V-C-N

2 Medium A + HB + S-Y + V-C-N   Medium C + Hb + S-Y + V-C-N

3 Medium A + Hb + S-Y + V-C-N   Medium D + Hb + S-Y + V-C-N

4 Medium A + Hb + S-X + V-C-N   Medium A + Hb + S-Y + V-C-N

5 Medium A + Hb + S-X + V-C-N   Medium B + Hb + S-Y + V-C-N

6 Medium A + Hb + S-Y + V-C-N   Medium B + Hb + S-X + V-C-N

7 Medium A + Hb + S-Y + V-C-N   Medium A + Hb + S-Y

8 Medium A + Hb + S-Y + V-C-N   Medium A + Hb + S-Y + V-C-N+T

1. For the significance of Medium I, Medium II and the other abbreviations

    used in this table, see the accompanying text.

2. The products underlined are those compared in a given set of experiments.

Table II

Inoculation sequence in the comparison of two media of different compositions (Medium I and Medium II).

	serial number of patient	sampling site	first inoculation on medium	second inoculation on medium
male patients	first	urethra	I	II
	second	urethra	II	I
	third	urethra	I	II etc.
female patients	first	rectum	I	II
		urethra	I	II
		cervix	I	II
	second	rectum	II	I
		urethra	II	I
		cervix	II	I
	third	rectum	I	II
		urethra	I	II
		cervix	I	II etc.

Table III

a) Comparison of gonococcal growth scores on Medium I as the first inoculated
    and Medium II as the second.

Scores	0	1	2	3	4	5	Total
Medium I inoculated first	5	10	32	28	18	27	120
Medium II inoculated second	8	28	32	32	15	5	120

b) Comparison of gonococcal growth scores on Medium II as the first inoculated
    and Medium I as the second.

Scores	0	1	2	3	4	5	Total
Medium II inoculated first	4	4	22	10	32	28	100
Medium I inoculated second	9	15	26	10	22	18	100

c) Comparison of gonococcal growth scores on Medium I and Medium II, both
    as first inoculated.

Scores	0	1	2	3	4	5	Total
Medium I inoculated first	5	10	32	28	18	27	120
Medium II inoculated first	4	4	22	10	32	28	100

d) Comparison of gonococcal growth scores on Media I and II, both as second
    inoculated.

Scores	0	1	2	3	4	5	Total
Medium I inoculated second	9	15	26	10	22	18	100
Medium II inoculated second	8	28	32	32	15	5	120

Table IV

Results of the comparison of the various media listed in Table I for the culture of gonococci[1]. In all cases, the gonococcal growth score for Medium I as first inoculated is compared with that for Medium II as first inoculated. The significance of the difference in score distribution found is assessed with the aid of the x^2 test.

Comparison		Score	0	1	2	3	4	5	Total	x^2 test	Medium found to be significantly better (if any)
1	I	Medium A + Hb + S-Y + V-C-N	0	1	7	6	19	27	60	$0.025 > \propto > 0.01$	I
	II	Medium B + Hb + S-Y + V-C-N	0	1	17	14	32	16	80		
2	I	Medium A + Hb + S-Y + V-C-N	1	3	0	5	22	39	70		
	II	Medium C + Hb + S-Y + V-C-N	4	4	4	14	26	23	75	$0.01 > \propto > 0.005$	I
3	I	Medium A + Hb + S-Y + V-C-N	0	1	1	5	30	22	63	$\propto < 0.005$	I
	II	Medium D + Hb + S-Y + V-C-N	15	3	2	15	19	10	64		
4	I	Medium A + Hb +S-X + V-C-N	2	0	1	8	39	44	94	$0.25 > \propto > 0.10$	
	II	Medium A + Hb + S-Y + V-C-N	0	3	6	5	28	46	88		
5	I	Medium A + Hb + S-X ∔ V-C-N	6	11	3	12	38	72	142	$\propto > 0.05$	
	II	Medium B + Hb + S-Y + V-C-N	1	5	3	20	30	47	106		
6	I	Medium A + Hb + S-Y + V-C-N	0	2	2	4	17	29	54	$\propto < 0.001$	I
	II	Medium B + Hb + S-X + V-C-N	3	6	4	14	23	10	60		
7	I	Medium A + Hb + S-Y + V-C-N	0	2	2	14	22	34	74	$\propto < 0.01$	I
	II	Medium A + Hb + S-Y	10	4	8	15	30	4	71		
8	I	Medium A + Hb + S-Y + V-C-N	2	4	8	13	34	38	99	$0.30 > \propto > 0.25$	
	II	Medium A + Hb + S-Y + V-C-N +T	2	1	13	18	52	36	122		

1. The abbreviations used are explained in the text. The products underlined are those compared in any given pair of media.

2. Differences are considered to be significant for $\propto < 0.05$.

Chapter VI[1]

COMPARISON OF SAMPLING SITES FOR GONOCOCCAL CULTURES IN THE DETECTION OF GONORRHOEA IN FEMALES.

Uncomplicated gonorrhoea in females can present itself as an infection of the endocervix, the urethra, the rectum and the oropharynx. Primary gonorrheal infection of the rectum may arise after rectopenal coitus (Jensen, 1953). Gonorrheal infection of the rectum may be secondary in females. In such cases, it is assumed that the rectum is infected by fluor containing gonococci. Gonorrheal infection of the oropharynx is generally the result of orogenital sexual contact (Bro Jørgensen and Jensen, 1973).

An investigation for gonorrhoea in females should in principle involve the culture of samples from the cervix, the urethra, the rectum and the oropharynx. Since a number of general practitioners usually take samples from the vagina instead of the cervix, the sampling sites considered in this investigation include the vagina in addition to the endocervix, the urethra, the rectum and the oropharynx. The results of this investigation are compared with those of other authors.

Material and methods

In the outpatient department for Venereal Diseases of females of the department of Dermatology of the University Hospital, Rotterdam, an average of 50 new patients and 200 old patients per month came for consultation in 1971 and 1972. All women were examined for gonorrhoea during each consultation. The data on which the comparison of the various sampling sites for the detection of gonorrhoea is based are taken from three different investigation periods. Only the result of the first examination which led to a positive diagnosis of gonorrhoea requiring treatment were

[1]Stolz, E. (to be published, accepted by Ned. T. Geneesk.)

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included in the data for comparison – with the exception of the data on 5 patients (from the third investigation period), where oropharyngeal gonorrhoea was detected after treatment for urogenital or urogenital/rectal gonorrhoea.

During the first period of investigation, from 18 October 1971 to 1 July 1972, samples from the cervix, the urethra and the rectum were taken from all women during each examination and cultured for gonococci.

The second investigation period, from 1 February 1972 to 1 July 1972, fell within the first period. In this second period, a sample from high up in the vagina was also taken from all women and cultured for gonococci.

The examination of all patients took place on a gynaecological chair. Examinations were carried out at all stages of the menstrual cycle, including during menstruation. The sampling was performed with the aid of charcoal-impregnated swabs, which were placed in a Stuart transport medium after sampling. Material was sampled from the possible sites of infection as follows:
after intravaginal insertion of one finger, a swab was inserted at least 5 cm into the rectum. The intravaginal finger was then pushed against the swab via the rear wall of the vagina. The intravaginal finger was then rotated and used to massage the urethra over the symphysis pubis in the direction of the urethral orifice. In a number of patients, this produced a drop of pus from the paraurethral glands or the urethra. Material was then sampled. Finally the portio was adjusted with the aid of a speculum and material was sampled from the cervix and from the vagina during the second investigation period.

During the third investigation period, from 1 August 1972 to 1 January 1973, a sample from the oropharynx as well as from the cervix, urethra and rectum was cultured for all women in every examination. This sam-

ple from the oropharynx was taken as follows:

after the mouth had been opened wide and the tongue depressed with a spatula,
a swab was passed over the tonsils or the tonsillar residues, or inserted in
the tonsillar cavities; the swab was then also passed over the back of the oro-
pharynx.

The sample was inoculated on a selective medium (Thayer
and Martin, 1966) from the Stuart medium within 4 hours. The selective me-
dium had the following composition:

Bacto GC medium base (Difco), Bacto haemoglobin (Difco), Isovitalex TM
enrichment (BBL) and VCN inhibitor (BBL). The VCN inhibitor contains van-
comycin, colistimethate and nystatin. After inoculation, the plates were in-
cubated at $37^{\circ}C$ for 48 hours in CO_2 rich environment. If colonies thought to be
due to gonococci were observed after this time, the colonies were investi-
gated further to see whether they consisted of gram-negative diplococci, whether
the gram-negative diplococci fermented glucose but not lactose, maltose and
saccharose, and whether the colonies in question were oxidase-positive.

Results

Table I gives the percentages of the total number of cases
of gonorrhoea in the women examined that were detected by means of cultu-
res from the cervix (C), the urethra (U), the rectum (R) and the vagina (V) or
from a combination of the cultures from these sites, for the first and second
investigation periods. From now on, these percentages will be referred to as
gonorrhoea detection percentages (GDP's). Table I also gives between brackets
the percentages of patients with negative gonococcal cultures from the cervix
and positive gonococcal cultures from the urethra, the rectum or the vagina,
singly or in combination.

We investigated whether statistically significant differences existed between the GDP's for the various sampling sites taken singly or in combination. The statistical significance was tested with the aid of the sign test on the observed frequencies. Differences were considered to be significant at a confidence level of $p < 0.05$.

In Table I, first investigation period, C was found to be significantly higher than U and R, while U was significantly higher than R. The combinations CU and CR were equal and significantly higher than UR.

In Table I, second investigation period, V was significantly lower than C, significantly higher than R and practically equal to U;CU, CR and CV were all significantly higher than RV; and CUR was significantly higher than URV - in fact, CUR gave a GDP of 100%. When cultures from the cervix, the urethra, the rectum or the vagina are used, omission of the culture from the urethra would lead to failure to detect 1.9% of the cases of gonorrhoea, omission of the culture from the rectum would also lead to failure to detect 1.9% of the cases, while omission of the culture from the vagina would not lead to failure to detect any of the cases. When cultures from the cervix, urethra and rectum are used, omission of the urethra would lead to a drop of 2.4% in the GDP, and omission of the rectum would also lead to a drop of 2.4%.

During the third investigation period, in which samples were taken from the cervix, the urethra, the rectum and the oropharynx (O),130 cases of gonorrhoea were diagnosed.

Eleven patients (8.4%) gave a positive O gonococcal culture. In 6 of the 11 patients (4.6%), the positive O gonococcal culture was found in combination with urogenital (4 patients, 3.0%) or urogenital/

rectal (2 patients, 1.5%) gonorrhoea, while in the other 5 patients (3.8%) it was found after the (successul) treatment of urogenital (3 patients, 2.3%) or urogenital/rectal (2 patients, 1.5%) gonorrhoea. It was assumed that the gonococcal culture from O before the treatment of the urogenital or urogenital/rectal gonorrhoea was missed in the case of the last-mentioned 5 patients. The data for all 11 patients with a positive O gonococcal culture were therefore included for frequency calculations. The CUR data for the patients with a negative O gonococcal culture were not subjected to further analysis.

Discussion

Phillips, Humphrey, Middleton and Nicol (1972), Olsen (1971) and Schmale, Martin and Domescik (1969) have also given percentages of the total number of cases of gonorrhoea in females which they detected with the aid of cultures from the cervix, the urethra, the rectum and the vagina or with combinations of these. These results are summarized in Table II, together with (between brackets) the percentages of patients with negative gonococcal cultures from the cervix and positive cultures from the urethra, the rectum or the vagina, taken singly or in combination, insofar as these percentages are known.

The data presented in Table II show the same trends as in our Table I. When cultures from one site only are considered, the GDP for the cervix is the highest ($>$ 90%), those for the urethra and vagina are lower and practically equal to one another, while the rectum gives the lowest score. When the data for cultures from two sites are combined, CU and CR again give the highest scores. However, in Table II the GDP for CR is always slightly higher than that for CU. The differences between CR and CU was

greatest in the investigation of Schmale, Martin and Domescik (1969). With

combinations of three sites, Table II again shows that CUR gives a GDP of

100% in all investigations. Another striking feature of Table II is the high

GDP found by Olsen (1971) for rectal cultures.

Frequencies for C, U, R and V were calculated from the per-

centages given in Tables I and II and the total numbers of patients investiga-

ted. For the two investigations carried out by Phillips, Humphrey, Middle-

ton and Nicol (1972) this leads to a slight inaccuracy, since these authors

rounded their percentages off to the nearest 0.5%.

We made use of the X^2 test on the observed frequencies

to compare the GDP's for C, U and R we found in our first investigation pe-

riod with those found by Phillips, Humphrey, Middleton and Nicol (1972) in

their second investigation period. No significant differences were found. Fur-

ther, our GDP's for C, U, R and V in the second investigation period were

compared by the same statistical method with those of Phillips, Humphrey,

Middleton and Nicol (1972) in their first investigation period, and those found

by Olsen (1971) and Schmale, Martin and Domescik (1969). No significant

differences were found between the GDP's determined by us, Phillips, Hum-

phrey, Middleton and Nicol (1972) and Schmale, Martin and Domescik (1969).

The GDP's found by Olsen (1971) for the various sampling sites, however,

did differ significantly from the following scores for the corresponding sites

found by the other authors: GDP's for the cervix, urethra, rectum and vagina

as determined by Phillips, Humphrey, Middleton and Nicol (1972); and R as

determined by Schmale, Martin and Domescik (1969) and in the present in-

vestigation.

The difference between Olsen's results and those of the other

authors is particularly striking as regards the GDP for the rectum. Unlike

the other authors, Olsen (1971) only investigated women who were stated to be contacts of male gonorrhoea patients (gonorrhoea contacts). Further, he used a selective medium containing polymyxin B sulphate and nystatin, while Schmale, Martin and Domescik (1969) and we used one containing vancomycin, colistimethate and nystatin (VCN) and Phillips, Humphrey, Middleton and Nicol (1972) used one containing trimethoprim in addition to VCN (VCNT).

It is not known whether the women investigated by Olsen (1971) had a higher percentage or rectogenital sexual intercourse in addition to genitogenital intercourse, nor whether they had more abundant vaginal discharge. We have observed that when samples are taken first from the cervix and then from the rectum in women with abundant vaginal discharge, fluor often runs over the perineum and the anus. This could lead to erroneously high scores for the GDP from the rectum. In Olsen's investigation, the women stated to be gonorrhoea contacts gave a high GDP for the culture from the vagina and a high GDP for the culture from the rectum, with a relatively low percentage of isolated positive gonococcal cultures from the rectum.

In a recent investigation, Bhattacharyya and Jephcott (1974) found that only 45% of 71 women with urogenital gonorrhoea gave a positive gonococcal culture from the rectum. Heimans (1967) also gives data on the occurrence of gonococci in the rectum from his own investigations and from the literature up to 1967.

It appears further from Tables I and II that when gonococcal cultures are taken from the cervix, the urethra and the rectum, a vaginal culture is unnecessary. Bhattacharyya, Jephcott and Morton (1973) found in an investigation of 75 women with urogenital gonorrhoea where the diagnosis was made on the basis of gonococcal cultures from the cervix, the urethra and the vagina that one-third of the cases would not have been detected

if cultures had been made from the vagina alone. Nevertheless, in two cases (2.7%), positive gonococcal cultures were obtained from the vagina even though the cultures from the cervix and urethra were negative. Since these authors did not take cultures from the rectum, it is impossible to tell whether rectal cultures would have led to detection of these two cases.

When gonococcal cultures from the cervix, the urethra and the rectum are used, we may expect omission of the urethral cultures to lead to failure to detect between 0% and 2.4% of the cases of gonorrhoea, while o-mission of the rectal cultures would lead to failure to detect between 1.9 and 6.2% of the cases (Tables I and II).

Phillips, Humphrey, Middleton and Nicol (1972), Olsen (1971) and we did not use a proctoscope in the sampling, and found low percentages of isolated positive gonococcal cultures from the rectum. Schmale, Martin and Domescik (1969), who did use a proctoscope, found the highest percentage of isolated positive gonococcal cultures from the rectum. Bhattacharyya and Jephcott (1974) found 5.6% of isolated positive gonococcal cultures from the rectum when using a proctoscope, and 2.8% with the same series of patients when a proctoscope was not used. These data suggest that when a proctoscope is used for the sampling of rectal material, higher GDP's are obtained from rectal cultures.

Bhattacharyya and Jephcott (1974) state that 44% of the patients with a positive gonococcal culture from the rectum admitted to anopenal and/or rectopenal intercourse during recent sexual contacts. This percentage was much lower in our patients, viz 10.9%.

Table III gives data from the literature and from our own investigation on the percentages of positive gonococcal cultures from the oropharynx and of isolated positive gonococcal cultures from the oropharynx

in women in whom gonorrhoea was diagnosed with the aid of cultures from the cervix, urethra, rectum and oropharynx.

It will be seen from this table that women with gonorrhoea diagnosed with the aid of cultures from the cervix, urethra, rectum and oropharynx give between 8.4 and 10.3% of positive gonococcal cultures from the oropharynx. It appears from the studies with larger numbers of patients in Table III that gonococcal cultures from the oropharynx alone give a GDP of 1.1 - 2.1%. When gonococcal cultures are made from the oropharynx, it is even more important than with sampling from other sites that the sampling should be repeated several times in order to ensure the maximum number of positive cultures (Ødegaard and Gundersen, 1973, Bro Jørgensen and Jensen, 1973, and Stolz and Schuller, 1974). Gonococcal cultures from the oropharynx are further important because the disseminated form of gonorrhoea, expressing itself as the septic gonococcal dermatitis syndrome, can arise from an undetected case of oropharyngeal gonorrhoea (Wiesner, Tronca, Bonin, Pedersen and Holmes, 1973). Moreover, oropharyngeal gonorrhoea often fails to respond to a treatment course which is sufficient to cure urogenital and rectal gonorrhoea (Bro Jørgensen and Jensen, 1971, 1972; Ødegaard and Gundersen, 1973; Stolz and Schuller, 1974; Wiesner, Tronca, Bonin, Pedersen and Holmes, 1973).

The undiagnosed oropharyngeal gonorrhoea can persist months after the treatment of the urogenital and rectal gonorrhoea, and can give rise to the disseminated form of gonorrhoea. Adequate therapies for oropharyngeal gonorrhoea are described by Wiesner, Tronca, Bonin, Pedersen and Holmes (1973) and by Stolz and Schuller (1974).

Summary

On the basis of data from our· own investigation and from the literature, we have investigated the significance of gonococcal cultures from the cervix, the urethra, the rectum and the vagina for the detection of urogenital/rectal gonorrhoea in females. The significance of gonococcal cultures from the oropharynx for detection of gonococcal infection in females has also been assessed.

More than 90% of al cases of urogenital/rectal gonorrhoea in females could be detected with the aid of a gonococcal culture from the cervix alone. However, for optimum detection of urogenital/rectal gonorrhoea cultures are needed from the cervix, the urethra and the rectum. When the three above-mentioned cultures are made, there is no need for a culture from the vagina too.

Gonococcal cultures from the oropharynx contribute little to the detection of gonorrhoea in females when made together with cultures from the cervix, the urethra and the rectum.

The importance of making gonococcal cultures from the oropharynx lies in the fact that sometimes the septic gonococcal dermatitis syndrome can arise from an undetected case of oropharyngeal gonorrhoea.

It is advisable to make gonococcal cultures from the cervix, the urethra, the rectum and the oropharynx when examining women for gonorrhoea.

References

Bhattacharyya, M. N. , Jephcott, A. E. and Morton, A. E. (1973)

Brit. Med. J., 2 , 748.

Bhattacharyya, M. N. and Jephcott, A. E. (1974)

Brit. J. vener. Dis. , 50, 109.

Bro Jørgensen, A. and Jensen, T. (1971)

Brit. Med. J. , 4 , 660.

Bro Jørgensen, A and Jensen, T. (1973)

Brit. J. vener. Dis. , 49, 491.

Heimans, A. L. (1967)

"Diagnosis and treatment of gonorrhoea in women."

(Thesis)

Jensen, T. (1953)

Brit. J. vener. Dis. , 29, 222.

Olsen, G. A. (1971)

Brit. J. vener. Dis. , 47, 102.

Ødegaard, K. and Gundersen, T. (1973)

Brit. J. vener. Dis. , 49, 350.

Phillips, I. , Humphrey, D. , Middleton, A. and Nicol, C. S. (1972)

Brit. J. vener. Dis. , 48, 287.

Schmale, J. D. , Martin, J. E. and Domescik, G. (1969)

J. Amer. med. Ass., 210, 312.

Stolz, E. and Schuller, J. L. (1974)

Brit. J. vener. Dis. , 50 , 104.

Thayer, J. D. and Martin, J. E. (1966)

Publ. Hlth. Rep. (Wash.), 81, 559.

Wiesner, P. J. , Tronca, E. , Bonin, P. , Pedersen, A. H. B. and Holmes, K. K.

(1973)

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<u>Table I</u>

Percentages of the total number of cases of gonorrhoea in women detected with
the aid of cultures from the cervix (C), urethra (U), rectum (R) or vagina (V),
or combinations of these. The percentages of patients with negative gonococ-
cal cultures from the cervix and positive cultures from the urethra, rectum
and vagina, singly or in combination, are given between brackets.

Sampling site	First investigation period 205 patients	Second investigation period 107 patients
C	94.6	94.4
U	80.0 (2.4)	84.1 (1.9)
R	45.9 (2.4)	44.9 (1.9)
V	--	79.4 (0.0)
CU	97.6	98.1
CR	97.6	97.2
CV	--	95.3
UR	86.3 (0.6)	88.8 (0.9)
UV	--	91.6 (0.9)
RV	--	84.1
CUR	100.0^2	100.0
CUV	--	98.1
CRV	--	98.1
URV	--	94.4
CURV	--	100.0^1

1= percentage of cases detected with CURV is taken as 100%.

2= percentage of cases detected with CUR is taken as 100%.

Table II

Percentages of the total number of cases of gonorrhoea in women detected with the aid of cultures from the cervix (C), urethra (U), rectum (R) and vagina (V), singly or in combination, according to the published data of Phillips, Humphrey, Middleton and Nicol (1972), Olsen (1971) and Schmale, Martin and Domescik (1969). The percentages of patients with negative gonococcal cultures from the cervix and positive cultures from the urethra, rectum and the vagina, singly or in combination, are given between brackets where known.

Sampling site	Phillips, Humphrey, Middleton and Nicol (1972)		Olsen (1971)	Schmale, Martin and Domescik (1969)
	Investigation I	Investigation II		
	262 patients	198 patients	265 patients	112 patients
C	96	90	91.3	93.8
U	78	80 (1.5)	86.4 (2.3)	77.7 (0.0)
R	42	44 (4.)	63.7 (3.0)	49.1 (6.2)
V	78	--	85.3 (0.0)	77.7 (0.0)
CU	not given	96	96.2	93.8
CR	not given	98.5	97.7	100.0
CV	not given	--	93.2	93.8
UR	not given	87	92.5 (1.5)	85.7
UV	not given	--	94.0	84.8
RV	not given	--	92.9 (0.8)	88.4
CUR	100	100^2	100.0	100.0
CUV	not given	--	97.0	not given
CRV	not given	--	97.7	100.0
URV	not given	--	98.1 (1.1)	not given
CURV	100	--	100.0^1	100.0^1

1= Percentage of cases detected with CURV is taken as 100%.

2= Percentage of cases detected with CUR is taken as 100%.

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<u>Table III</u>

Percentages of positive gonococcal cultures from the oropharynx and percentages of isolated positive gonococcal cultures from the oropharynx in women in whom gonorrhoea was diagnosed with the aid of cultures from the cervix, urethra, rectum and oropharynx.

Authors		Number of women with gonorrhoea	Percentages of positive gonococcal cultures from oropharynx	Percentage of isolated positive gonococcal cultures from oropharynx
Bro Jørgensen and Jensen, 1971		66	9.0	0.0
Wiesner, Tronca, Bonin, Pedersen and Holmes	1973	310	10.3	2.1
Ølegaard and Gundersen	1973	450	10.2	1.1
Bro Jørgensen and Jensen	1973	542	10.1	1.5
Stolz and Schuller	1974	130	8.4	0.0

Chapter VII

COMPARISON OF THE METHYLENE-BLUE-STAINED SMEAR, THE GRAM-
STAINED SMEAR AND SELECTIVE GONOCOCCAL CULTURE FOR THE DE-
TECTION OF URETHRAL GONORRHOEA IN MALES AND UROGENITAL/
RECTAL GONORRHOEA IN FEMALES.

Gonococci are Gram-negative diplococci which are generally
found in pairs. They are flattened at the contact site. Their form is reminis-
cent of that of coffee beans. The longitudinal axis of the bacterium is paral-
lel to the cleavage plane (Wilkinson, 1962). The cross-sectional diameter of
the gonococcus varies from 0.6 to 1.0 μm.

Gonococci are generally found intracellularly in the leucocy-
tes of the gonorrheal pus. However, they can also be found extracellularly
at the start of gonorrhoea (Prakken, 1956) or in chronic infections (Thayer
and Garson, 1965).

One method of detecting gonococci is based on the use of
smears of the pus on a microscope slide. The smear is fixed and stained,
and examined under a normal optical microscope with an oil-immersion ob-
jective. The form, size and position of bacteria can be assessed by examina-
tion of the smear preparation, while the use of Gram staining further makes it
possible to distinguish between Gram-positive and Gram-negative micro-orga-
nisms. When staining methods which do not distinguish between Gram-posi-
tive and Gram-negative micro-organisms are used for the detection of gonorrhoea
there is a risk that Gram-positive diplococci will be mistaken for Gram-nega-
tive diplococci. This would result in higher positive scores in examinations for
gonorrhoea than when a Gram stain is used.

The methylene-blue staining method is such a method which

can give false positive scores; its use for gonorrhoea examinations is therefore to be deprecated. Unfortunately, this method is still widely used for this purpose because it is rapidly performed. Ruys (1956), Wilkinson (1962) and Reyn (1965) advise against the use of methylene-blue staining in examinations for gonorrhoea. Ruys (1956) refers in this connection to an investigation she performed in 1932, in which gonorrheal vulvovaginitis was diagnosed in 126 children on the basis of an improperly stained preparation, while the use of Gram staining (GS) and gonococcal culture gave a positive diagnosis in only 22 of these cases.

However, the use of GS can also lead to errors. False positive diagnoses can be caused by the presence of other Neisseriae, Mima, Moraxella, Herellea and of Gram-negative staining staphylococci or streptococci occurring in pairs (Heimans, 1967). The diagnosis can also be missed if there are few Gram-negative diplococci, or none at all, in the preparation, or if there is a lot of other flora present. According to Fiumara (1967), GS is reliable in 99% of the cases of florid acute urethral gonorrhoea. In his opinion GS is no longer reliable in males with subacute or chronic urethral gonorrhoea. In females with urogenital gonorrhoea, GS performed on material from the cervix and urethra is stated to be useful only in the acute stage. Since the course of gonorrheal infection is asymptomatic in most women, and since by no means all women with symptomatic gonorrhoea are at the acute stage when examined, the value of this method for the examination of women is practically nil. GS must also be regarded as unreliable for the detection of rectal gonorrhoea in women (Heimans, 1967). GS smears of material from the oropharynx cannot be assessed because of the frequent presence of other Neisseriae.

On the other hand, GS can be a useful aid in the diagnosis of gonorrhoea in cases where the gonococci cannot grow on the culture medium used (Thayer, Schubert and Bucca, 1947). Reyn (1969), Cross, Hoger, Neibaur, Pasternack and Brady (1971), and Brorson, Holmberg, Nygren and Seeberg (1973) have published details of vancomycin-sensitive strains which do not grow on the VCN (vancomycin-colistimethate-nystatin) selective medium of Thayer and Martin (1966).

Since many physicians still use methylene-blue staining (MBS) of smears for the detection of gonorrhoea, and since the selective media introduced since 1964 give higher gonorrhoea detection percentages than the non-selective media used before that date, we thought that it would be instructive to compare the value of MBS, GS and selective gonococcal culture (SGC) for the detection of urethral gonorrhoea in males and urogenital/rectal gonorrhoea in females.

Material and methods

During the period from 18 October 1971 to 1 July 1972, material for SGC according to Thayer and Martin (1966) was taken from the urethra of every male patient examined in the Venereal Diseases Clinic of the University Hospital Rotterdam; material for this culture was taken from the cervix, urethra and rectum of each female patient in the same period. During the whole of this investigation period, the sampling of material for culture from the urethra in males and from the cervix and urethra in females was preceded by the sampling of material for GS, and in the months November and December 1971 also by the sampling of material for MBS. During a limited period within the above-mentioned total investigation period, the sampling of material for SGC from the rectum in females was also preceded by the sampling of material for GS.

Female patients were always examined in a gynaecological chair. Examinations were carried out in all parts of the menstrual cycle, even during menstruation. The material for smears was sampled with the aid of a metal scoop. The material for SGC was sampled with the aid of charcoal-impregnated swabs, which were placed in Stuart transport medium before inoculation. The material was sampled from possible sites of infection as follows. After one finger had been inserted intravaginally, a metal scoop or swab was inserted at least 5 cm into the rectum with the other hand. The intravaginal finger was then pressed against the rear wall of the vagina at the site of the scoop or swab. The intravaginal finger was then rotated and used to massage the urethra over the symphysis pubis in the direction of the urethral orifice. This massage sometimes led to production of a drop of pus from the paraurethral channels or the urethra. The material was then sampled, first with the scoop and then with the swab. Finally, the portio was adjusted with the aid of a speculum, and material was sampled from the cervix with the scoop and the swab.

Male patients were always examined on an examination table. For the sampling of material from the urethra, the urethra was first massaged from proximal to distal. A flame-sterilized platinum loop was then inserted one centimetre into the urethra for sampling of material for a smear, after which a charcoal-impregnated swab was pressed against the urethral orifice and then placed in Stuart transport medium until inoculated.

The material on the swab in the Stuart transport medium was inoculated within at most 4 hours on a selective medium composed of Bacto GC (gonococcal culture) medium base (Difco), Bacto haemoglobin (Difco), Isovitalex TM (Thayer-Martin) enrichment (BBL) and VCN inhibitor (BBL). The VCN inhibitor contains vancomycin, colistimethate and nystatin. After

inoculation, the plates were incubated at $37^{o}C$ for 48 hours in CO_2-rich environment. If colonies thought to contain gonococci were observed after this incubation period, these colonies were examined further to see whether they were formed by Gram-negative diplococci, whether the Gram-negative diplococci fermented glucose but not lactose, maltose and saccharose, and whether the colonies in question were oxidase-positive.

The MBS preparation was made by pouring Loeffler's methylene-blue over a flame-fixed smear for a few seconds. The slide was then rinsed with water and dried between blotting paper.

The GS preparation was made by staining a flame-fixed smear according to the instructions published by the Dutch National Institute for Public Health (RIV). This involves staining the smear with a solution of crystal violet and aniline water and then with an aqueous solution of iodine. The preparation is then decolorized with alcohol and finally stained with aqueous fuchsine.

In the period during which both MBS and GS smears were examined, the laboratory staff responsible for this were given two slides per sampling site, but were not told which had been sampled first. The MBS smears were scored by the same team of three analysts as the GS smears. When scoring the MBS smears, the analyst in question did not know the results for the corresponding GS smears.

A smear was scored as positive on the basis of the size and form of the micro-organisms observed; for the GS smears, the diplococci also had be be Gram-negative before a positive score could be registered.

Results

The results of the comparison of MBS, GS and SGC on ma-

terial sampled from the urethra (U) in males (M) and from U and the cervix

(C) in females (F) in the investigation period covering the months of November

and December 1971 are summarized in Table I

It will be seen that two columns of percentage frequencies are

given for each sampling site. The reason for this is as follows.

If the chances of a positive score in the MBS and GS smears

were equal, one would expect the percentage of samples giving MBS +, GS −

and SGC − to be approximately equal to the percentage giving MBS −, GS +

and SGC −. However, this is found not to be the case. The combination MBS +,

GS−, SGC− was found for 40% of the M-U samples, 46.3% of the F-U samples

and 39.5% of the F-C samples, while the combination MBS−, GS+, SGC− was

not found at all. This is strong evidence that the positive MBS scores in the com-

bination MBS+, GS−, SGC− are false positives.

The first column of percentages for each sampling site was cal-

culated with respect to the total number of samples examined in each case. If

we now exclude all samples giving MBS+, GS−, SGC− as being false positives,

and recalculate the percentages for the remaining samples we get the figures

in the second column for each sampling site. These data may be assumed to be

more reliable, and give the following picture.

For the M-U samples (99 in all), both SGC and smears (MBS

and GS) scored 98% of the total (i.e. 2% of cases finally diagnosed as gonorrhoea

were missed). In 1% of the total MBS is negative while GS and SGC are both

positive. The combination MBS+, GS−, SGC+ was not found at all.

The absolute number of F-U and F-C samples was appreciably

smaller than that of the M-U samples (22 and 23 respectively). SGC scored

100% (22 out of 22) for the F-U samples and 95.7% (22 out of 23) for the F-C

samples. The combinations MBS+, GS−, SGC+ and MBS−, GS+, SGC+ both

scored 4.5% (1 out of 22) for the F-U samples, and 13.0% (3 out of 23) and 26.1% (6 out of 23) respectively for the F-C samples. Statistical testing of the results showed that the differences between the MBS+, GS-, SGC+ and MBS-, GS+ SGC+ were not significant for any of the sampling sites (sign test, P> 0.05).

Table II gives the results of the comparison of GS and SGC for material samples from M-U, F-U, F-C and F-R in the period from 18 October 1971 to 1 July 1972.

The results for the M-U samples in Table II show the same trend as for the M-U samples in Table I: both SGC and GS scored 98.3% of the total possible. In 7 patients (1.7%) the combination SGC + GS- was found. All these patients suffered from asymptomatic urethral gonorrhoea.

The results for the F-U and F-C samples in Table II show that SGC alone would score 98.1% and 96.8% respectively, while GS alone would score 66% and 56.1% respectively. Statistical processing of these results showed that the score for SGC on the F-U and F-C samples was significantly higher than the score for GS (sign test, P< 0.05).

Although the absolute number of F-R samples was smaller than for the F-U and F-C samples, the results for the F-R samples show that GS gave a much lower score (13.8%) than for the F-U (66%) and F-C (56.1%) samples. SGC on F-R samples gave a score of 100%.

Discussion

Since MBS gives such a high percentage of false positive results when used for the detection of gonorrhoea, it should not be used as a basis for diagnosis of this disease. It could however be used as a screening method for urethral gonorrhoea in males, positive MBS smears being followed up by GS and/or SGC.

GS is found to be just as good or better as SGC for the detection of acute florid urethral gonorrhoea in males. For the detection of males with asymptomatic urethral gonorrhoea the SGC is essential. Use of both methods leads to a gain of a few percent in the gonorrhoea detection percentage.

GS is less useful for the detection of urogenital gonorrhoea in females, and is practically useless for the detection of rectal gonorrhoea. Our study shows that the use of SGC is essential for the adequate investigation of urogenital/rectal gonorrhoea in females.

The percentage of positive results found with GS which were not found by SGC was within 4% for all sampling sites. Reyn (1969) found about 4% of vancomycin-sensitive strains, which did not grow on the VCN medium, among the gonococcal strains she investigated. Vancomycin-sensitive strains are generally penicillin-sensitive too. Our clinical material contains three female contacts of males with urethral gonorrhoea, who were diagnosed as having gonorrhoea on the basis of a positive GS and a negative SGC. When material from these women was cultured on VCN medium no gonococci were found, but culture on a medium not containing VCN did permit isolation of gonococci, all of which were penicillin-sensitive. The small contribution of GS to the detection of urethral gonorrhoea in males and urogenital gonorrhoea in females probably consists of patients harbouring vancomycin-sensitive strains.

In our opinion it is advisable to use both SGC and GS for the detection of urethral gonorrhoea in males and urogenital gonorrhoea in females. For the detection of rectal gonorrhoea in females only SGC shoud be used.

Summary

The methylene-blue-stained smear (MBS), the Gram-stained

smear (GS) and selective gonococcal culture (SGC) have been compared as regards their value for the detection of urethral gonorrhoea in males and urogenital/rectal gonorrhoea in females. MBS should not be used for this purpose, because of the large number of false positive results it gives. In our material GS and SGC are of equal value for the detection of urethral gonorrhoea in males; use of both methods together leads to a gain in gonorrhoea detection percentage of a few percent. For optimal detection of asymptomatic urethral gonorrhoea in males SGC is necessary. SGC is essential for the detection of urogenital/rectal gonorrhoea in females. GS is less useful here, especially for material from the rectum. Using the SGC, GS contributes for a few percent to the detection of urethral gonorrhoea in males and urogenital gonorrhoea in females. This contribution probably consists of patients harbouring vancomycin-sensitive strains, while these strains do not grow on the vancomycin containing SGC.

In our opinion, it is advisable to use both SGC and GS for the detection of urethral gonorrhoea in males and urogenital gonorrhoea in females. For the detection of rectal gonorrhoea in females SGC should be used.

References

Brorson, J.E., Holmberg, I., Nygren, B. and Seeberg, S. (1973)

Brit. J. vener. Dis., 49, 452.

Cross, R.C., Hoger, M.B., Neibaur, R., Pasternack, B. and Brady, F.J.
(1971)

HSMHA Hlth. Rep., 86, 990.

Fiumara, N.J. (1967)

Fortschr. Med., 85, 580.

Heimans, A.L. (1967)

"Diagnosis and treatment of gonorrhoea in women".

(Thesis)

Prakken, J.R. (1956)

Leerboek der Geslachtsziekten, 2nd. ed., Scheltema en Holkema, Amsterdam.

Reyn, A. (1965)

Bull. Wld. Hlth. Org., 32, 449.

Reyn, A. (1969)

Bull. Wld. Hlth. Org., 40, 245.

Ruys, A.Ch. (1932)

Ned. T. Geneesk., 76, 2197.

Ruys, A.Ch. (1956)

Ned. T. Geneesk., 100, 3690.

Thayer, J.D., Schubert, J.H. and Bucca, M.A. (1947)

J. Vener. Dis. Inform., 28, 37.

Thayer, J.D. and Garson, W. (1965)

Ed. R.J. Dubos and J.G. Hirsch, 4th ed. Pitman, London.

Thayer, J.D. and Martin, J.E. (1966)

Publ. Hlth. Rep. (Wash.), 81, 559.

Wilkinson, A.E. (1962)

Brit. J. vener. Dis., 38, 145.

Table I

Results of the comparison of the methylene-blue-stained smear (MBS), the Gram-stained smear (GS) and selective gonococcal culture (SGC) on material sampled from the urethra (U) in males (M) and from U and the cervix (C) in females (F) for the detection of gonorrhoea.

(Investigation period November-December 1971).

MBS	GS	SGC	M-U No.	M-U %[1]		F-U No.	F-U %[1]		F-C No.	F-C %[1]	
+	–	–	66	40.0		19	46.3		15	39.5	
+	+	+	94	57.0	94.9	14	34.1	63.6	13	34.2	56.5
+	–	+				1	2.4	4.5	3	7.9	13.0
–	+	+	1	0.6	1.0	1	2.4	4.5	6	15.8	26.1
–	–	+	2	1.2	2.0	6	14.6	27.3			
+	+	–	2	1.2	2.0				1	2.6	4.3
Total			165	100.0	99.9	41	99.8	99.9	38	100.0	99.9

[1] The percentages in the first column for each sampling site are calculated with respect to the total number of samples involved. The percentages in the second column are calculated after exclusion of all samples giving the combination of results MBS+, GS-, SGC-, which most probably represent a false positive MBS score (see also accompanying text).

Table II

Results of the comparison of Gram staining (GS) and selective gonococcal

culture (SGC) on material sampled from the urethra (U) in males (M) and

from the urethra (U), the cervix (C) and rectum (R) in females (F) for

the detection of gonorrhoea.

(Investigation period 18 October 1971 - 1 July 1972)[1]

GS	SGC	M-U		F-U		F-C		F-R	
		No.	%	No.	%	No.	%	No.	%
+	+	407	96.7	100	64.1	100	52.9	4	13.8
-	+	7	1.7	53	34.0	83	43.9	25	86.2
+	-	7	1.7	3	1.9	6	3.2	0	0
Total		421	100.1	156	100.0	189	100.0	29	100.0

[1]With the exception of the F-R samples, which were taken during a limited

period.

Chapter VIII

COMPARISON OF DELAYED DIRECT IMMUNOFLUORESCENCE AND SELEC-
TIVE GONOCOCCAL CULTURE FOR THE DETECTION OF URETHRAL GO-
NORRHOEA IN MALES AND UROGENITAL/RECTAL GONORRHOEA IN FE-
MALES.

Deacon, Peacock, Freeman and Harris (1959) were the first
to describe a method for the detection of gonococci with the aid of a fluores-
cent antigonococcal conjugate. Deacon, Peacock, Freeman, Harris and Bunch
(1960) distinguished between direct direct immunofluorescence and delayed di-
rect immunofluorescence (DDIF) in this connection. In direct direct immunofluo-
rescence the pus or slime smears are treated with a conjugate of rabbit anti-
gonococcal globulins and fluorescein isothiocyanate (FITC). In DDIF the mate-
rial to be examined for gonococci is first inoculated on a gonococcal culture (GC)
medium and incubated for 16-20 hours. A smear is then made from material
taken from this culture, and treated as described above for direct direct im-
munofluorescence.

When we assume that all cases of gonorrhoea can be detected
by the combined use of GC and DDIF, we can calculate gonorrhoea detection
percentages (GDP's) for GC and DDIF separately.

Deacon, Peacock, Freeman and Harris (1960) found a slightly
higher GDP with DDIF than with GC for the various sampling sites in the inves-
tigation of women for urogenital gonorrhoea; however, there was no difference
when the results for the various sites were pooled. When non-selective gono-
coccal culture (N-SGC) media were used for the investigation of women for
urogenital/rectal gonorrhoea in women, DDIF always gave higher GDP's
than N-SGC (Harris, Deacon, Tiedemann and Peacock, 1961; Danielsson, 1963,

1965a, 1965b; Fry and Wilkinson, 1964; Holman, Koornhof and Hayden-Smith, 1964 and Simon, Gutschera, Bontemps, Schirren and Wolko, 1967).

When selective gonococcal culture (SGC) media were used for the detection of urogenital/rectal gonorrhoea in females, the results show more variation. The results of various authors are summarized in Table 1.

Thin, Williams and Nicol (1970) found a much higher GDP with DDIF than with culture. Lind (1967) found a slightly higher GDP for DDIF than for SGC she used. In a later investigation, however, Lind (1969) found no significant difference in GDP between DDIF and SGC. Lucas, Price, Thayer and Schroeter (1967), using the SGC of Thayer and Martin (1964), found a higher GDP for the various individual sampling sites investigated (cervix, vagina and urethra) than with DDIF. When the results for the various sampling sites were combined, however, DDIF and SGC were equivalent for the detection of urogenital gonorrhoea in females. Martin, Peacock and Thayer (1965) found that the SGC of Thayer and Martin (1964) gave a higher GDP for the individual sampling sites and for the detection of urogenital gonorrhoea in females than DDIF.

DDIF has not been used much for the investigation of urethral gonorrhoea in males. Moore, Vanderstoep, Wende and Knox (1963) and Danielsson (1963, 1965b) found that DDIF gave a slightly higher GDP than N-SGC. In another investigation, however, Danielsson (1965a) found no difference in GDP between DDIF and N-SGC. Lind (1967, 1969) found no difference in GDP between DDIF and SGC for the detection of urethral gonorrhoea in males.

In our clinic, material is sampled from the urethra in all male patients examined, and from the cervix, urethra and rectum in all fe-

male patients, for culture on the SGC medium of Thayer and Martin (1966).
In order to investigate the desirability of using DDIF in addition to or in-
stead of SGC, we have compared these two methods for the detection of
urethral gonorrhoea in males and urogenital/rectal gonorrhoea in females.

Material and methods

The investigation was carried out in the period from 1 January
to 1 July 1972. Material for GC was sampled from all patients consulting
the Venereal Diseases Clinic of the University Hospital Rotterdam, Dijkzigt,
during every examination. Material was taken from the cervix, the urethra
and the rectum (and from 1 February to 1 June 1972 also from the vagi-
na) in females, and from the urethra only in males. Sampling was perfor -
med with the aid of charcoal-impregnated swabs, which were placed in Stuart
transport medium after sampling and inoculated on the SGC medium of
Thayer and Martin (1966) within 4 hours after sampling. The plates
were incubated at $37^{\circ}C$ in a CO_2-rich atmosphere. When colonies thought to
be due to gonococci were found after 48 hours, a Gram-stained smear was made,
the colonies were tested for oxidase activity and sugar fermentation tests were
performed.

The fluorescent antigonococcal conjugate used was prepared
as described by Mouton (1966). Material for a DDIF preparation was sampled
from each GC started from Monday to Thursday of each week, with the
aid of a tampon soaked in physiological saline. This material was taken
from one quadrant of the half of the plate that was inoculated first (see
section Culture Chapter II, Bacteriological methods). A suspension was

made of the material sampled with the tampon, on a microscope slide on which a drop of physiological saline had already been deposited, and this suspension was smeared out over the slide in question. The smear was treated with fluorescent antigonococcal conjugate as described by Mouton (1966).

Microscopy was performed with transmitted light, using a Leitz Ortholux microscope with a Periplan G 12.5 X ocular and an ultra-dark-field condensor (UV 1.2 - 1.4 super-wide Tioyda) and a Fluorid 95 1.32 oil-immersion objective. The light source was a high-pressure mercury lamp (CS 150W). BG 38 and BG 12 filters (3 mm) and a K 530 yellow filter were used.

The fluorescence was scored as follows, depending on the degree of fluorescence of the edge of the micro-organism and the sharpness of demarcation of the edge:

++++   = strong fluorescence of edge with clear demarcation

+++   = less strong fluorescence of edge, but still clear demarcation

++   = weakly fluorescent, diffuse edge

+   = hardly any fluorescence, diffuse edge

In order to investigate whether our non-absorbed antigonococcal conjugate also gave fluorescence in other micro-organisms than gonococci, the following bacterial strains were tested with the following results:

Neisseria gonorrhoeae	++++ - +++
Neisseria meningitidis	+++
Neisseria catarrhalis	+++
Staphylococcus aureus	++
Staphylococcus albus	+
Streptococcus haemolyticus	-

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When immunofluorescence smears were evaluated for the presence of gonococci, only ++++ or +++ fluorescence was taken as positive.

Results

A total of 154 male patients were diagnosed as having urethral gonorrhoea on the basis of SGC and DDIF. The detection frequencies for the two methods combined and singly are given in Table II.

It may be seen from Table II that use of SGC alone would have led to the detection of urethral gonorrhoea in 99.4% of the male patients investigated, while use of DDIF alone would have led to detection in 94.1% of the cases. The patient with a positive DDIF smear and a negative SGC was a gonorrhoea contact, while six of the nine patients with a positive SGC and a negative DDIF smear had asymptomatic gonorrhoea. All the other patients had symptomatic urethral gonorrhoea. The plates of all nine patients with a positive SGC and a negative DDIF smear showed only a few gonococcal colonies. Statistical processing of the results showed that gonococcal culture was significantly better than DDIF (sign test, P $<$ 0.05).

Table III gives the frequencies of detection of urogenital/rectal gonorrhoea in 73 female patients by SGC and/or DDIF smear, with material samples from the cervix, the urethra and the rectum. Fifty-two of these 73 patients had also been examined for the presence of gonococci in the vagina; the results of these tests are also presented in the table.

It may be seen from Table III that negative results with both methods were obtained with 13.7% of the samples from the cervix, 20.5% of the samples from the urethra, 54.8% of the samples from the rectum and 25% of the samples from the vagina. The percentages calculated with respect to the total number of patients examined are given as "percentage 1" in Table III.

The percentages calculated with omission of the samples from a given site

which were found to be negative by both methods are given as "percentage 2".

In the following discussion, we shall only deal with percentage 2.

We see from Table III (percentage 2) that the two diagnostic

methods both give positive results more often in samples from cervix and rec-

tum (about 87%), and least often in samples from the vagina (about 72%). Use

of SGC alone would reduce the GDP by 3.2% for the cervix, 3.4% for the ure-

thra, 3.0% for the rectum and 5.1% for the vagina. However, use of DDIF

alone would reduce the GDP by 9.5% for the cervix, 13.8% for the urethra,

9.1% for the rectum and 23.1% for the vagina.

Pooling of the results for the cervix, urethra and rectum would

lead to correct diagnosis in 71 of the 73 patients (97.5%) with SGC, and in 67

of the 73 patients (91.8%) with DDIF. The two methods were both positive

in 89.0% of the patients. In nearly all cases where the DDIF smear was ne-

gative and the SGC positive, only a few gonococcal colonies were observed

on the plate.

It follows from the results of Table III that SGC gave a

higher score than DDIF in our investigation. Using SGC DDIF makes only

a slight contribution to the detection of gonorrhoea, especially where the

most important sampling sites (cervix, urethra and rectum (Chapter VI))

are concerned.

Discussion

The results of gonococcal culture depend on the technical

skills of the laboratory personnel, the culture conditions and the culture media

used. The results obtained by gonococcal culture have improved greatly

since the introduction of SGC media - especially for sampling sites where

there is much mixed flora in additon to gonococci, e.g. the vagina and the rectum. Although a SGC medium is better than a N-SGC medium there are some gonococcal strains which grow on N-SGC media but not on SGC media. For example, Reyn (1969) observed that 3% of the gonococcal strains she isolated were so sensitive to vancomycin that they did not grow on the SGC medium of Thayer and Martin (1966), which contains vancomycin, colistimethate and nystatin (VCN), but did grow on a N-SGC medium.

Cherry and Moody (1965) stated that bacterial immunofluorescence is a method with only two "dimensions", viz. morphology and serological specificity. The specificity of IF for material containing mixed flora must therefore be determined experimentally for each bacterial species. Lack of specificity can be tolerated if the morphology of the bacteria giving crossreactions is sufficiently different from that of the bacteria to be detected.

According to Reyn (1969), cross-reactions to gonococcal IF are found mainly in Neisseria meningitidis and certain strains of Staphylococcus aureus, and to a lesser extent in N. catarrhalis, N. flava and N. subflava.

The Mima, Herellea and Moraxella strains which can give rise to false positive diagnoses in Gram-stained smears give little or no crossreactions here. The cross-reactions with Staphylococcus aureus can be avoided by absorbing the fluorescent antigonococcal conjugate on Staphylococcus aureus strains giving a strong cross-reaction or by using a mixture of the fluorescent antigonococcal conjugate and non-labelled normal rabbit serum or rabbit antistaphylococcus serum. These methods do not eliminate the crossreactions with meningococci; however, this is not important in practice since meningococci are seldom found in urogenital/rectal sampling sites.

Martin, Peacock and Thayer (1965) stated that when the mor-

phology of the fluorescent bacteria is taken into consideration when evaluating slides, the occurrence of cross-reactions need not lead to difficulties; we followed this line in our investigation. In our experience, meningococci are generally larger than gonococci, and staphylococci generally smaller.

Although the above-mentioned investigations are not comparable because of the wide variety of material and methods used, the results of our investigation as regards the comparison of DDIF and SGC for the detection of gonorrhoea agree best with the results of Martin, Peacock and Thayer (1965) and Lucas, Price, Thayer and Schroeter (1967).

Our investigation on urethral gonorrhoea in males showed a higher GDP for SGC. This is not in agreement with the results of earlier investigations.

One explanation for the higher GDP's for SGC compared with DDIF in our investigation could be the fact that all men and women (including completely asymptomatic ones) who visited our VD clinic for any reason whatsoever were examined for gonorrhoea. In all the cases where the DDIF smear was negative and the SGC positive in males, and in nearly all the cases in females, only a few gonococcal colonies were found on the plate. It should be remembered in this connection that the DDIF smear is made from the culture, and that when there are few colonies on the plate the diagnosis can easily be missed in the DDIF preparation. Six of the nine men for whom the DDIF smear was negative and the SGC was positive had asymptomatic urethral gonorrhoea.

Only one of the male patients gave a positive DDIF smear and a negative SGC. This patient was a gonorrhoea contact. Of the two female patients for whom the DDIF was positive while the SGC was negative, one was also a gonorrhoea contact. This and the above-mentioned facts argue

that the fluorescent antigonococcal conjugate we used is highly specific.

The results of our investigation indicate that SGC is better than DDIF. Use of both methods together gives roughly the same increase in GDP as simultaneous use of SGC and N-SGC. We venture to doubt whether the slight increase in GDP due to the use of DDIF weighs up against the increased expenditure of time and money involved.

Summary

In an investigation of urethral gonorrhoea in males and urogenital/rectal gonorrhoea in females, more cases of gonorrhoea were detected with the aid of the selective gonococcal culture (SGC) medium of Thayer and Martin (1966) than with the aid of delayed direct immunofluorescence (DDIF). In females gonorrhoea was also diagnosed more often with SGC than with DDIF when performed on material from the various individual sampling sites involved. Of the males with urethral gonorrhoea who gave a positive SGC and a negative DDIF smear, a high proportion (6 out of 9 = 66.7%) had asymptomatic urethral gonorrhoea. In practically all cases where a positive SGC was found together with a negative DDIF smear, only a few gonococcal colonies were found on the plate.

References

Cherry, W.B. and Moody, M.D. (1965)

Bact. Rev., 29, 222.

Danielsson, D. (1963)

Acta derm. venereol. (Stockh.), 43, 451.

Danielsson, D. (1965a)

Acta derm. venereol. (Stockh.), 45, 61.

Danielsson, D. (1965 b)

Acta derm. venereol. (Stockh.), 45, 74.

Deacon, W.E., Peacock Jr., W.L., Freeman, E.M. and Harris, A. (1959)

Proc. Soc. exp. Biol. (N.Y.), 101, 322.

Deacon, W.E., Peacock, Jr., W.L., Freeman, E.M., Harris, A. and

Bunch Jr., W.L. (1960)

Publ. Hlth. Rep. (Wash.), 75, 125.

Fry, C.S. and Wilkinson, A.E. (1964)

Brit. J. vener. Dis., 40, 125.

Harris, A., Deacon, W.E., Tiedemann, J. and Peacock, W.L. (1961)

Publ. Hlth. Rep. U.S., 76, 93.

Holman, M.S., Koornhof, H.J. and Hayden-Smith, S. (1964)

S. Afr. J. Lab. clin. Med., 10, 95.

Lind, I. (1967)

Acta path. microbiol. scandinav., 70, 613.

Lind, I. (1969)

Acta path. micribiol. scandinav., 76, 279.

100

Lucas, J.B. , Price, E.V. , Thayer, J.D. and Schroeter, A. (1967)

New Engl. J. Med. , 276, 1454.

Martin, J.E. , Peacock, W.L. and Thayer, J.D. (1965)

Brit. J. vener. Dis. , 41, 199.

Moore, Jr. , M.B. , Vanderstoep, E.M. , Wende, R.D. and Knox, J.M. (1963)

Publ. Hlth. Rep. U.S. , 78, 90.

Mouton, R.P. (1966)

Dermatologica (Basel) , 132, 343.

Reyn, A. (1969)

Bull. Wld. Hlth. Org. , 40, 245.

Simon, C. , Guntschera, H. , Bontemps, M. , Schirren, M. and Welke, S. (1967)

M.M.W. , 15, 856.

Thayer, J.D. and Martin, J.E. (1964)

Publ. Hlth. Rep. (Wash.), 79, 49.

Thayer, J.D. and Martin, J.E. (1966)

Publ. Hlth. Rep. (Wash.), 81, 559.

Thin, R.N.T. , Williams, I.A. and Nicol, C.S. (1970)

Brit. J. vener. Dis. , 47, 27.

Table I

Comparison of the gonorrhoea detecting percentages (GDP's) of selective

gonococcal culture (SGC) and delayed direct immunofluorescence (DDIF) for the

detection of urogenital/rectal gonorrhoea in females, as reported by va-

rious authors.

Author(s)	DDIF ↔ SGC
Thin, Williams and Nicol (1970)[1]	>
Lind (1967)	>
Lind (1969)	=
Lucas, Price, Thayer and Schroeter (1967)[2]	=
Martin, Peacock and Thayer (1965)	<

[1] These authors used a SGC medium for the rectum and a non-selective

one for the urethra and cervix.

[2] For each individual sampling site, SGC gave higher GDP's than DDIF

> is greater than

= is equal to

< is less than

Table II

Results of selective gonococcal culture (SGC) and delayed direct immunofluo-
rescence (DDIF) for 155 male patients with urethral gonorrhoea, the diagnosis
being made on the basis of one or both of these tests.

DDIF	+	+	−	Total
SGC	+	−	+	
Numbers	144	1	9	154
Percentages	93.5	0.6	5.8	99.9

<u>Table III</u>

Results of selective gonococcal culture (SGC) and delayed direct immunofluorescence (DDIF) on samples of material from the cervix, urethra and rectum of 73 women in whom gonorrhoea was diagnosed with the aid of one or both of these tests. The results for 52 of the 73 women, in whom material was sampled from the vagina, are also given.

The percentages given as "percentage 1" are calculated with respect to the total number of patients examined. "Percentage 2" is calculated after omission of allcases in which both tests gave negative results for a given sampling site (see also accompanying text).

DDIF SGC		+ +	+ -	- +	- -	Total
Cervix	Numbers	55	2	6	10	73
	Percentages 1	75.3	2.7	8.2	13.7	99.9
	Percentages 2	87.3	3.2	9.5	-	100.0
Urethra	Numbers	48	2	8	15	73
	Percentages 1	65.8	2.7	11.0	20.5	100.0
	Percentages 2	82.8	3.4	13.8	-	100.0
Rectum	Numbers	29	1	3	40	73
	Percentages 1	39.7	1.4	4.1	54.8	100.0
	Percentages 2	87.9	3.0	9.1	-	100.0
Vagina	Numbers	28	2	9	13	52
	Percentages 1	53.8	3.8	17.3	25.0	99.9
	Percentages 2	71.8	5.1	23.1	-	100.0

SENSITIVITY TO AMPICILLIN, PENICILLIN, AND TETRACYCLINE OF GONOCOCCI IN ROTTERDAM.

Published data on quantitative determinations of the sensitivity of gonococci to penicillin and tetracycline in the Netherlands are scarce. As far as we know quantitative determinations of the sensitivity to ampicillin of gonococci isolated there have never been published.

Data on the sensitivity of gonococci to penicillin have been published by Bakker, Esseveld, and Leiker (1960), Vermeer and Schaap, (1962), Heimans (1967), and Wols-van der Wielen (1971).

Heimans (1967) investigated the quantitative sensitivity to tetracycline HCl of 192 strains isolated in Amsterdam.

The present paper reports quantitative sensitivity determinations for ampicillin, penicillin, and tetracycline, carried out on gonococcal strains isolated from male and female civilians and from male and female seafaring personnel.

The results are compared with former data from the Netherlands and some other countries and correlations are given between the observed sensitivities for ampicillin and penicillin, tetracycline and ampicillin, and tetracycline and penicillin.

MATERIAL AND METHODS.

The investigation was carried out between October 18, 1971, and July 1, 1972.

The patients consisted of male and female civilians (MC and FC) and male and female seafaring personnel (MS and FS). When patients attended with a subsequent infection in the period under investigation, only the strain

1. Stolz, E., Zwart, H. G. F. and Michel, M. F. (1974)

   Brit. J. vener. Dis. 50, 202.

from the first infection was used for this investigation.

Material was collected with carbon-impregnated cotton-wool swabs. In women, material was taken from the cervix (C), the urethra (U), and the rectum (R), and in the period from February 1, 1972, to July 1, 1972, also from the vagina (V). In men, material was taken from the urethra (U), and if indicated prostatic fluid (P) and material from the rectum (R) were also investigated. Sometimes swabs from the tonsil/oropharynx (T) were taken from both men and women. The swabs were placed in Stuart's transport medium, and inoculated on to a selective medium (Thayer and Martin, 1966) within 4 hrs.

The cultures were incubated at 37° C. in a CO_2 - rich environment. If suspect colonies were found within 48 hrs, a Gram-preparation was made, the colonies were tested for oxidase activity, and sugar fermentations were carried out.

Sensitivities to ampicillin, penicillin, and tetracycline were determined by the agar dilution method (Ericsson and Sherris, 1971). The concentrations of ampicillin were in the range 0.005 - 0.64 µg/ml., those of penicillin in the range 0.0025 - 1.28 µg/ml., and those of tetracycline in the range 0.04 to 2.56 µg/ml.

Two-fold dilutions of antibiotic in water were incorporated in a medium consisting of GC medium base (Difco), haemoglobin (Difco), and Isovitalex (BBL), and distributed on to plates. A control without antibiotic was included in each series.

The organisms to be tested were freshly suspended in trypticase soy broth (BBL) to a density of $10^6 - 10^7$ V.U./ml. With a multipoint replicator, these suspensions were inoculated on to the plate series, resulting in spot inocula covering a circle of 4-6 mm. diameter and containing $10^3 - 10^4$ V.U.

In each run, three gonococcal strains and one Staphylococcus (Oxford strain) with known sensitivity for ampicillin, penicillin, and tetra-

cycline were tested simultaneously.

After incubation (18 - 20 hrs) the minimum inhibitory concentration was determined by observing the lowest concentration of antibiotics in which bacterial growth was completely or almost completely inhibited, as judged by the naked eye. A haze of growth or a single colony was disregarded.

The results were recorded on optically readable forms. With the use of an IBM 1232 optical mark page-reader, the forms were converted into punched cards by means of a coupled IBM 534 card punch. With the assistance of the System Development Department of the Medical Faculty, use was made of an IBM 2780 terminal, connected via a permanent telephone line to an IBM 360 (model 65) computer system.

Results.

Altogether 1016 cultures were positive; from these 959 strains were available for sensitivity determination.

The distribution of the positive cultures and the cultures on which sensitivity determinations could be performed (shown between the brackets) among the groups of male and female civilians (MC and FC) and male and female seafaring personnel (MS and FS) and among the various sampling sites is shown in Table I.

The distribution of the MIC's for the strains from the groups MC-Urethra (MCU), FC-Cervix(FCC), MS-Urethra (MSU),MC-Prostatic fluid (MCP), FC-Rectum (FCR), and FC-Vagina (FCV) are given in Table II.

No significant difference was found between the distribution of sensitivities for MCU compared with MCP, and for FCC compared with FCR, FCU, and FCV at a probability level of 0.05 (X^2 test).

In our series, gonococci taken from different sites showed a different sensitivity pattern (more than one antibiotic concentration difference) in less than one patient in 200. Moreover, it was found that, if the urethra is taken as the basic site of diagnosis for men and the cervix for women, the detection of gonococci in other sites contributed only slightly to the detection

of patients with gonorrhoea (Table III). Also the numbers involved were so small that they had little influence on the distribution of the MIC's.

For these reasons, it was decided to determine the correlation between the MIC's for penicillin and ampicillin, tetracycline and ampicillin, and tetracycline and penicillin for the MCU, FCC, and MSU strains only.

Our impression that there was a relationship between the sensitivities of the gonococci for penicillin and ampicillin, tetracycline and ampicillin, and tetracycline and penicillin was 'confirmed' (i. e. not contradicted) by Spearman's rank correlation test. This test was carried out on all three groups of strains (MCU, FCC, and MSU), and the probability value was less than 0. 01 in all cases.

In Tables IV, V, and VI, the MIC's for penicillin and ampicillin, tetracycline and ampicillin, and tetracycline and penicillin in the MCU strains are set out in correlation Tables.

The rank correlation coefficient is given in each case. Similar correlation Tables were made for the FCC and MSU strains, and the rank correlation coefficients were calculated. The nine rank correlation coefficients are given together in Table VII.

Discussion.

It has been found that a representative picture of the MIC distribution for ampicillin, penicillin and tetracycline in our patient groups MC, FC, and MS can be obtained by considering only the positive cultures from the urethra in men and the positive cultures from the cervix in women.

If we define relative resistance to penicillin for the strains investigated by us as MIC $\geqslant$ 0. 08 µg/ml. , comparison of our data with those of other Dutch authors gives the following picture (Table VIII)

The percentage of strains relatively resistant to penicillin has not changed appreciably in recent years among the civilian population. In general, the percentages among seafaring personnel are more difficult to interpret as these patients form a less homogeneous group in comparison to civilians. However, when the figures here are considered, there is no real

evidence of an increase in the percentage of strains relatively resistant to penicillin in the MS group.

Our percentages of relatively resistant MC and FC strains are very similar to those found by Lynn, Nicol, Ridley, Rimmer, Symonds, and Warren, (1970) and Leigh, Le Franc, and Turnbull (1969) in London, and by Gundersen, Ødegaard, and Gjessing (1969) in Norway, viz. 35, 39, and 34. 5 per cent. respectively (relative resistance defined as MIC $\geqslant$ 0. 05 µg. /ml. by Lynn and others, $\geqslant$ 0. 06 µg. /ml. by Leigh and others, and $\geqslant$ 0. 075 µg. /ml. by Gundersen and others).

If we define relative resistance to tetracycline HCl for the strains investigated by us as MIC $\geqslant$ 1. 28 µg. /ml. , we find that 17 per cent. of the MC, 12. 2 per cent. of the FC, and 38. 8 per cent. of the MS strains are relatively resistant. In the Netherlands, Heimans (1967) found that 14 per cent. of his FC strains were relatively resistant to tetracycline (MIC $\geqslant$ 1. 00 µg. /ml.); this result agrees very closely with ours.

If we define relative resistance to ampicillin for the strains investigated by us as MIC $\geqslant$ 0. 16 µg. /ml. , then 40. 3 per cent. of the MC, 34. 8 per cent. of the FC, and 68. 6 per cent. of the MS strains are relatively resistant. The agreement between these percentages and those found for strains relatively resistant to penicillin is striking.

The correlation between the pairs of antibiotics, expressed in terms of the rank correlation coefficient r for the MCU, FCC and MSU groups of gonococci (Table VII), was found to be consistently highest for penicillin-ampicillin, and consistently lowest for tetracycline-ampicillin.

The correlation between the sensitivities of gonococci for tetracycline and penicillin has been described previously. Reyn and Bentzon (1968) stated that they had always found a positive correlation between tetracycline and penicillin. In the Netherlands, Heimans (1967) pointed out that there was a marked positive correlation between strains relatively resistant to penicillin and tetracycline. Verhagen, van der Ham, Heimans, Kranendonk,

and Maina (1971) found a highly positive correlation between sensitivities to penicillin and tetracycline in 736 strains isolated in Kenya (P < 0.01; r = 0.7273).

A positive correlation between the sensitivities for penicillin and ampicillin has also been described previously, e. g. Reyn and Bentzon (1968) and Jokipii and Renkonen (1970). Ødegaard (1962), Reyn and Bentzon (1968), and Jokipi and Renkonen(1970) showed that penicillin G was more effective in vitro than ampicillin for the sensitive strains, while the reverse was found for the relatively resistant strains. This pattern was also found in our series (Table IV).

The positive correlation between tetracycline and ampicillin would be expected on the basis of the two correlations mentioned above.

Summary.

In the period between October 18, 1971, and July 1, 1972, quantitative sensitivity determinations for ampicillin, penicillin, and tetracycline were carried out on gonococcal strains isolated in the outpatient department for Dermatology and Venereal Diseases of the University Hospital, Rotterdam. MIC distributions are given for 959 strains isolated from the urethra, prostatic fluid, rectum or tonsils/oropharynx in men, and from the cervix, urethra, rectum, vagina or tonsils/oropharynx in women.

The patients consisted of male and female civilians(MC and FC) and male and female seafaring personnel (MS and FS).

A representative picture of the MIC distributions for ampicillin, penicillin, and tetracycline in the largest patient groups (MC, FC, and MS) could be obtained by considering only the strains from the urethra in men and the cervix in women. In total, 258 urethral strains from male civilians (MC), 172 cervical strains from female civilians (FC), and 134 urethral strains from male seafaring personnel (MS) were tested for sensitivity.

40. 3 per cent. of the MC, 34. 8 per cent of the FC, and 68. 6 per

cent. of the MS strains were relatively resistant to ampicillin (MIC $\geqslant$ 0.16 µg. /ml.).

38.4 per cent. of the MC, 34.9 per cent. of the FC, and 67.2 per cent. of the MS strains were relatively resistant to penicillin (MIC $\geqslant$ 0.08 µg. /ml.).

17 per cent. of the MC, 12.2 per cent. of the FC, and 38.8 per cent. of the MS strains were relatively resistant to tetracycline (MIC $\geqslant$ 1.28 µg. /ml.).

Statistical analysis showed a strong positive rank correlation between the sensitivities to the three antibiotics.

References.

Bakker, P.G., Esseveld, H., and Leiker, D.L. (1960)
Ned. T. Geneesk., 104, 65.

Ericsson, H.M., and Sherris, J.C. (1971).
Acta path. microbiol. scand., Section B 1971, Suppl. 217.

Gundersen, T., Ødegaard, K., and Gjessing, H.C. (1969).
Brit. J. vener. Dis. 45, 235.

Heimans, A.L., (1967).
'Diagnosis and Treatment of Gonorrhoea in Women '(Thesis).

Jokipii, A.M.M., and Renkonen, O.V. (1970).
Chemotherapy, 15, 317.

Leigh, D.A., Le Franc, J., and Turnbull, A.R. (1969).
Brit. J. vener. Dis. 45, 151.

Lynn, R., Nicol, C.S., Ridley, M., Rimmer, D., Symonds, M.A.E.
and Warren, C. (1970).
Ibid., 46, 404.

Ødegaard , K. (1962).

T. norske Laegeforen, 82, 55.

Reyn, A. , and Bentzon, M. W. (1968).

Brit. J. vener. Dis. , 44, 140.

Thayer, J.D. , and Martin, J. E. (1966).

Publ. Hlth. Rep. (Wash.), 81, 559.

Verhagen, A. R. , van der Ham, M. , Heimans, A. L. , Kranendonk, O. ,

and Maina., A. N. (1971).

Bull. Wld. Hlth. Org. , 45, 707.

Vermeer, D. J. H. , and Schaap, G. J. P. (1962).

Ned. T. Geneesk. 106, 1025.

Wols-van der Wielen, A. (1971).

Brit. J. vener. Dis. , 47, 190.

Table 1.

Distribution of positive cultures (total) and cultures for which sensitivity
was determined (figures between brackets), by site of isolation and population
group.

Site	Civilians			Seafarers			Total
	MC		FC	MS		FS	
Rectum (R)	11	(10)	98 (92)	4 (4)			113 (106)
Urethra (U)	277	(258)[a]	156 (147)	137 (134)[a]		1 (1)	571 (540)
Cervix (C)			183 (172)[a]			2 (2)	185 (174)
Vagina (V)			93 (91)			2 (2)	95 (93)
Prostatic fluid (P)	35	(32)		10 (8)			45 (40)
Tonsils/oro pharynx (T)	2	(1)	4 (4)	1 (1)			7 (6)
Total	325	(301)	534 (506)	152 (147)		5 (5)	1016 (959)

MC = male civilians MS = male seafaring personnel

FC = female civilians FS = female seafaring personnel

[a] For these groups of strains, the correlation between the MIC's for penicillin and ampicillin,
tetracycline and ampicillin, and tetracycline and penicillin was determined (Tables IV to VII).

Table II. Distribution of MIC's (expressed as per cent. of total) for ampicillin, penicillin, and tetracycline in the MCU, FCC, MSU, MCP, FCR, FCU, and FCV strains. Number of strains tested shown in brackets.

MIC (μg/ml.)	MCU[a] (258)	FCC[a] (172)	MSU[a] (134)	MCP (32)	FCR (92)	FCU (147)	FCV (91)
Ampicillin							
0.005	0.4						
0.01	5.8	7.0	2.2	6.3	4.3	4.1	5.5
0.02	19.8	22.7	9.0	37.5	20.7	24.5	30.8
0.04	25.6	25.0	10.4	25.0	28.3	23.1	16.5
0.08	8.1	10.5	9.7	6.3	12.0	12.2	12.1
0.16	9.3	8.1	13.4	9.4	5.4	5.4	8.8
0.32	16.3	11.6	24.6	3.1	17.4	19.0	19.8
≥ 0.64	14.7	15.1	30.6	12.5	12.0	11.6	6.6
Penicillin							
0.0025	7.0	5.2	3.0	18.8	7.6	7.0	3.3
0.005	21.3	26.2	7.5	40.6	25.0	21.3	24.2
0.01	21.7	26.2	9.7	15.6	23.9	21.7	33.0
0.02	6.2	4.7	6.0	--	5.4	6.2	3.3
0.04	5.4	2.9	6.7	--	4.3	5.4	1.1
0.08	8.1	6.4	11.2	3.1	6.5	8.1	4.4
0.16	8.9	11.6	6.0	3.1	13.0	8.9	13.2
0.32	10.1	9.3	15.7	9.4	9.8	10.1	7.7
0.64	6.6	6.4	26.1	6.3	3.3	6.6	8.8
≥ 1.28	4.7	1.2	8.2	3.1	1.1	4.7	1.1
Tetracycline							
0.04	0.8	--	0.7	--	1.1	--	--
0.08	5.4	7.0	1.5	3.1	6.1	6.8	2.2
0.16	43.8	46.5	17.2	59.4	52.5	44.9	44.0
0.32	16.3	18.6	14.9	12.5	16.3	17.7	20.9
0.64	16.7	15.7	26.9	15.6	14.1	18.4	17.6
1.28	15.1	10.5	32.8	9.4	8.8	10.2	13.2
≥ 2.56	1.9	1.7	6.0	--	1.1	2.2	2.2

MCU = male civilians-urethra   MSU = male seafaring personnel-urethra

FCC = female civilians-cervix   MCP = male civilians-prostatic fluid

FCR = female civilians-rectum   FCU = female civilians-urethra

FCV = female civilians-vagina.

a
 For these groups of strains, the correlation between the MIC's for penicillin and ampicillin, tetracycline and ampicillin, and tetracycline and penicillin has been calculated (Tables IV to VII).

Table III Contribution to detection of gonorrhoea in our series from cultures taken from sites other than urethra in men and cervix in women.

Positive cultures				MC	MS	Positive cultures					FC
U+				277	137	C+					183
U-	P+			1	0	C-	U+				3
U-	P-	R+		9	4	C-	U-	R+			5
U-	P-	R-	T+	1	0	C-	U-	R-	V+		0
						C-	U-	R-	V-	T+	0
Total patients				288	141	Total patients					191

U = urethra	MC = male civilians	C = cervix	+ = positive
P = prostatic fluid	MS = male seafaring personnel	V = vagina	- = negative
R = rectum	FC = female civilians		

Table IV Correlation of MIC's for penicillin and ampicillin in MCU (male-civilians-urethra) strains.

	MIC (μg/ml)	Penicillin										
		0.0025	0.005	0.01	0.02	0.04	0.08	0.16	0.32	0.64	≥1.28	Total
Ampicillin	0.005	1	0	0	0	0	0	0	0	0	0	1
	0.01	7	7	1	0	0	0	0	0	0	0	15
	0.02	10	23	15	1	1	0	1	0	0	0	51
	0.04	0	25	34	7	0	0	0	0	0	0	66
	0.08	0	0	5	7	5	3	1	0	0	0	21
	0.16	0	0	1	1	8	9	1	4	0	0	24
	0.32	0	0	0	0	0	9	16	10	5	2	42
	≥ 0.64	0	0	0	0	0	0	4	12	12	10	38
	Total	18	55	56	16	14	21	23	26	17	12	258

r Rank correlation coefficient = 0.89821.

Table V

Correlation of MIC's for tetracycline and ampicillin in MCU (male civilians-urethra) strains

	MIC (µg./ml.)	Tetracycline							
		0.04	0.08	0.16	0.32	0.64	1.28	≥ 2.56	Total
Ampicillin	0.005	0	1	0	0	0	0	0	1
	0.01	1	0	14	0	0	0	0	15
	0.02	1	6	34	8	2	0	0	51
	0.04	0	6	43	13	3	1	0	66
	0.08	0	1	6	8	5	1	0	21
	0.16	0	0	7	7	9	1	0	24
	0.32	0	0	7	4	16	13	2	42
	≥ 0.64	0.	0.	2	2	8	23	3	38
	Total	2	14	113	42	43	39	5	258

r = Rank correlation coefficient = 0.71155

Table VI

Correlation of MIC's for tetracycline and penicillin in MCU (male civilians-urethra) strains

	MIC (µg/ml.)	Tetracycline							
		0.04	0.08	0.16	0.32	0.64	1.28	≥ 2.56	Total
Penicillin	0.0025	1	4	13	0	0	0	0	18
	0.005	1	7	37	10	0	0	0	55
	0.01	0	3	42	10	1	0	0	56
	0.02	0	0	4	4	6	2	0	16
	0.04	0	0	2	8	4	0	0	14
	0.08	0	0	5	6	9	1	0	21
	0.16	0	0	7	3	11	2	0	23
	0.32	0	0	3	1	10	12	0	26
	0.64	0	0	0	0	2	11	4	17
	≥ 1.28	0	0	0	0	0	11	1	12
	Total	2	14	113	42	43	39	5	258

r · Rank Correlation coefficient = 0.77159.

Table VII

Coefficients of rank correlation between MIC's for penicillin and
ampicillin, tetracycline and ampicillin, and tetracycline and penicillin
in the gonococcal strains MCU (male civilians-urethra), FCC (female
civilians-cervix), and MSU (male seafaring personnel-urethra).

Antiobiotics	Strains		
	MCU	FCC	MSU
Penicillin-ampicillin	0.89821	0.88296	0.83959
Tetracycline-ampicillin	0.71155	0.60167	0.65547
Tetracycline-penicillin	0.77159	0.67219	0.79328

Table VIII

Comparison of percentages of relatively resistant strains to penicillin isolated
from male civilians (MC), female civilians (FC), and male seafaring personnel
(MS), with percentages reported by other Dutch workers.

Authors	Town	Year of report	Percentage relatively resistant to penicillin			Criterion
			MC	FC	MS	
Vermeer and Schaap	Amsterdam	1962			57.0	$\geq$ 0.06 µg./ml
Heimans	Amsterdam	1967		37.0	78.2	$\geq$ 0.06 µg./ml.
Wols-v. d. Wielen	Rotterdam	1971	35.0	35.0	70.0	> 0.06 µg./ml.
Present study	Rotterdam	1974	38.4	34.9	67.2	$\geq$ 0.08 µg./ml.

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Chapter X[1]

IN VITRO ACTIVITY OF EIGHT ANTIMICROBIAL AGENTS AGAINST NEIS-
SERIA GONORRHOEAE.

The occurrence of gonococcal strains which are (relatively)
resistant to penicillin and other antibiotics has gradually come to be an im-
portant practical problem during the past fifteen years. It is striking that
the percentages of penicillin relatively resistant (P RR) strains found in
Europe are always lower than those found in other parts of the world, being
less than 40% in practically all investigations. Of recent years, there have
even been reports of a drop of the percentage of P RR strains in Europe.
Alarmingly high (70%) percentages of P RR strains have been found in Afri-
ca, the Far East and the Pacific.

The percentages of P RR strains among the strains investi-
gated in India, the USA and Canada lie between those found in Europe and
in Africa, the Far East and the Pacific (Willcox, 1970, 1972; Wigfield,
1973). The favourable situation in Europe can in principle be unfavourably
influenced by the import of P RR strains. One of the groups which could be
responsible for such import is that of sailors (Wols- van der Wielen, 1971).

Strong positive rank correlations have been found between the
distributions of the sensitivity of gonococcal strains to antibiotics with such
widely differing chemical structures and modes of operation as penicillin,
tetracycline, erythromycin, chloramphenicol, streptomycin, fusidic acid
and rifampicin (Reyn, 1961; Reyn and Bentzon, 1968, 1969; Phillips, Rimmer,
Ridly, Lynn and Warren, 1970; and Maness and Sparling, 1973). Reyn and
Bentzon (1969) stated that these correlations were strongest at the highest
levels of (relative) resistance observed for each drug.

[1]
Stolz, E. , Michel, M.F. and Zwart, H.G.F. (to be published).

Only partial or relative resistance has been observed for all antibiotics with the exception of streptomycin and spectinomycin.

This study describes quantitative determinations of the sensitivity of four groups of gonococcal strains to ampicillin (Am), penicillin (P), tetracycline (T), rifampicin (Ri), spectinomycin (Sp), sulphamethoxazole (Su), trimethoprim (Tr) and a combination of Su and Tr in the ratio 5:1 (Su/Tr 5:1). The various groups of strains were isolated in Rotterdam (R) in 1972 from male civilians (MC), female civilians (FC) and sailors (S) by the authors, and in Amsterdam (A) in 1967 by Heimans from FC. This made it possible to compare the S-R strains (strains isolated from sailors in Rotterdam), which may be regarded as largely imported, with the MC-R and FC-R strains which may be regarded as mainly of Dutch origin. Further, comparison of the FC-A and FC-R strains will show up any changes in the frequency of (relative) resistance to the antibiotics investigated in the Netherlands in the period from 1967 to 1972. Finally, rank correlations between the sensitivity distributions for all pairs of antibiotics are calculated for all groups of strains and discussed.

Material and methods

Quantitative sensitivity determinations for Am, P, T, Ri, Sp, Su, Tr and Su/Tr 5:1 were carried out from 15 May to 28 July 1972 on 248 strains of N. gonorrhoeae isolated in the out-patient department for Dermatology and Venereal Diseases of the University Hospital, Rotterdam and stored by low-temperature method as described in Chapter II. Of the 248 strains, isolated from 248 patients, 114 were isolated from MC, 90 from FC and 44 from S. The majority of these strains, viz those isolated from 15 May to 30 June, were also included in an earlier study on sensitivity testing for Am, P and T

(Stolz, Zwart and Michel, 1974).

In addition, quantitative sensitivity determinations in all the above-mentioned antibiotics were performed on 94 freeze-dried FC-A strains of N.gonorrhoeae isolated in 1967 by Heimans.

Sensitivity was expressed as the minimum inhibitory concentration (MIC) in μg/ml. Sensitivity testing was performed using the agar-dilution method (Ericsson and Sherris, 1971). The medium used for sensitivity testing for Am, P, T, Ri and Sp was: Bacto GC medium base (Difco) + Bacto haemoglobin (Difco) + Isovitalex TM enrichment (BBL). The medium used for sensitivity testing for Su, Tr and Su/Tr 5:1 was DST agar (Oxoid) + 7.5% haemolysed horse blood. A series of plates with increasing concentrations of the antibiotic and one controle plate without an antibiotic were prepared. The concentrations of Am were in the range 0.005 - 1.28μg/ml, those of P 0.0025 - 1.28μg/ml, those of T 0.04 - 5.12μg/ml, those of Ri 0.0025 - 0.64μg/ml, those of Sp 7.5 - 25.0μg/ml, those of Su 0.16 - 20.48μg/ml, those of Tr 2.56 - 40.96μg/ml and those of Su/Tr 5:1 between 0.10 and 12.29 μg/ml. The organisms to be tested were freshly suspended in trypticase soya broth (T.S.B.) (BBL) to a density of 10^6 - 10^7 v.u./ml. These suspensions were inoculated on the plate series with a multipoint replicator, resulting in spot inoculas covering a circle of 4-6 mm diameter and containing 10^3 - 10^4 v.u. In each run three gonococcal strains and one Staphylococcus aureus (strain Oxford) with known sensitivity for Am, P and T were tested simultaneously. After incubation (18-20 hours) the MIC was determined by reading off the lowest concentration of antibiotic at which bacterial growth was completely or almost completely inhibited, as judged by the naked eye. A haze of growth or a single colony was disregarded.

The results were recorded on optically readable forms, which

were converted into punched cards with the aid of an IBM 1232 mark page reader and of a coupled IBM 534 card punch. An IBM 2780 terminal connected via a permanent telephone line to an IBM 360 (model 65) computer system was used, with the assistance of the System Development Department of the Medical Faculty.

Results

The distributions of the MIC's for the eight antibiotics investigated are given for the MC-R, FC-R, S-R and FC-A strains as absolute values in Tables I-IV, and as percentages of the total number of strains investigated in Tables I-IV and in Fig. 1-4. The Figures are discussed here in semi-qualitative terms; for more exact information, the reader is referred to the Tables. Fig. 1 gives the MIC distributions for Am, P, T and Ri, Fig. 2 for Sp, Fig. 3 for Su and Tr and Fig. 4 for Su/Tr 5:1.

The MIC distributions for Am and P (Fig. 1) are bimodal, the left peak being more pronounced than the right one for the MC-R, FC-R and FC-A strains. The S-R strains give a bimodal distribution with the right peak more pronounced than the left one.

The MIC distributions for T (Fig. 1) for the MC-R, FC-R and FC-A strains are characterized by a peak at the left-hand end of the histogram, while the S-R strains give a bimodal distribution with the left peak practically as high as the right one.

The MIC distributions for Ri (Fig. 1) for the MC-R and FC-R strains show a peak sloping off stepwise to the right, with a maximum at 0.08 μg/ml; that for the FC-A strains has a peak sloping off stepwise to both left and right, with a maximum at the MIC's 0.08 and 0.16 μg/ml; while the peak for the S-R strains is block-shaped and covers the MIC's 0.08, 0.16 and 0.32 μg/ml.

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The MIC distributions for Sp (Fig. 2) are U-shaped for the

MC-R and FC-R strains, covering MIC's between 12.5 and 22.5 µg/ml; that for

the S-R strains shows a peak which slopes off step-wise to left and right, with

a maximum at 17.5 µg/ml; and that for the FC-A strains has a peak sloping off

step-wise to the left with a maximum at 20 µg/ml. Only one (FC-A) strain had

an MIC greater than 25 µg/ml (Table II).

The MIC distributions for Su (Fig. 3) for the MC-R, FC-R

and S-R strains are characterized by peaks of various forms, covering MIC

values from 1.28 to 10.24 µg/ml. The peak for the FC-A strains extends fur-

ther left in the diagram.

The MIC distributions for Tr (Fig. 3) for the MC-R, FC-R

and FC-A strains are characterized by peaks of various forms, covering the

MIC range from 7.68 to 20.48 µg/ml. The distribution for the S-R strains

shows a peak at MIC $\geq$ 40.96 µg/ml in addition to one at MIC 20.48 µg/ml.

The MIC distribution for Su/Tr (Fig. 4) is characterized

by peaks at 1.54 and 3.07 µg/ml for all strains. For the MC-R, FC-R and

S-R strains, there are roughly the same number of strains to the left and

right of the peak, while with the FC-A strains there appear to be more strains

on the left.

The percentages of the MC-R, FC-R, S-R and FC-A strains

which are relatively resistant to Am, P, T and Ri are presented in Table V.

The S-R groups gave the highest percentages of strains relati-

vely resistant to Am, P, T and Ri. The percentages of strains relatively re-

sistant to Am, P, T and Ri were compared with the aid of the chi-square test

(degree of freedom 1; $\alpha = 0.05$) on the observed frequencies for the strains

MC-R and FC-R, MC-R and S-R, FC-R and S-R and FC-R and FC-A.

Significant differences were found only for Am, P and T between the MC-R

and S-R strains, and for T and Ri between the FC-R and S-R strains.

For Sp, Su, Tr and Su/Tr 5:1, the position of the boundary between sensitive and (relatively) resistant strains is not known. Moreover, the X^2 test represents a very rough approximation when applied to the distribution of sensitivity and relative resistance to Am, P, T and Ri in the various strains.

For this reason, we used the Yates- Cochran test with continuity correction to test for significant differences in the MIC distributions for the various antibiotics between the strains MC-R and FC-R, MC-R and S-R, FC-R and S-R and FC-R and FC-A. Significant differences were found between the MC-R and S-R strains and the FC-R and S-R strains for Am, P, T, Ri and Tr. In all these cases, the S-R strains were the least sensitive. (see Table VI).

Further, the FC-A strains were significantly more sensitive to Su/Tr 5:1 than the FC-R strains. Means $\overline{X}_1$ = 4.0 and $\overline{X}_2$ = 4.5, standard value T = 2.6 and two-sided significance P = 0.009.

Table VII gives Spearman's rank correlation coefficient $\underline{r}$ for the MIC distributions of the various pairs of the antibiotics investigated, for the strains MC-R, FC-R, S-R and FC-A.
For the sake of simplicity, we shall refer briefly to the $\underline{r's}$ between two antibiotics from now on, instead of to the $\underline{r's}$ of the MIC distributions for the pair of antibiotics in question.

The value of $\underline{r}$ between Am and P, Am and T and P and T was found to be > 0.50 for all groups of strains. Between Am and Ri, P and Ri and Am and Tr, $\underline{r} \geqslant 0.50$ was found only for the S-R strains. In addition, the

value of $\underline{r}$ between Ri and T, Tr and P, Tr and T and Tr and Ri was higher for the S-R strains than for the MC-R and FC-R strains. Appreciably higher values of $\underline{r}$ (though not > 0.50) were found between Am and Sp, P and Sp, T and Sp and Ri and Sp for the FC-A strains than for the other groups of strains. For the MC-R and S-R strains, we found $\underline{r}$ > 0.50 between Su and Tr, Su and Su/Tr 5:1 and Tr and Su/Tr 5:1. For the FC-R strains, $\underline{r}$ between Su and Tr was 0.35, and that between Su and Su/Tr 5:1 and Tr and Su/Tr 5:1 was > 0.50, while for the FC-A strains we found $\underline{r}$ > 0.50 between Su and Su/Tr 5:1 only; the $\underline{r's}$ between Su and Tr and between Tr and Su/Tr 5:1 were low (0.11 and 0.14 respectively).

Discussion

Reyn, Korner and Bentzon (1958) compared the distributions of the sensitivities to P of gonococcal strains isolated in 1944 and 1957. The strains isolated in 1944 showed a unimodal distribution of sensitive strains, while those isolated in 1957 showed a bimodal distribution, with one peak for sensitive strains and one for relatively resistant strains.

We also found bimodal distributions for the MIC's for Am and P (Fig. 1). Comparison of the S-R strains with the MC-R and FC-R reveals the more pronounced right-hand peak in the case of S-R. The (relative) resistance to T and Tr is not as marked in the MC-R and FC-R strains as that to Am and P; however, the S-R strains show a clear bimodal distribution of T-sensitive and T relatively resistant strains. Some indication of a second (right-hand) peak is also found in the distribution of the MIC's for Tr in the S-R strains (Fig. 3). The higher percentage of Ri RR strains in the histogram for the S-R strains is reflected not in a bimodal distribution but in an overall shift of the whole curve to the right (Fig. 1). The shape of the MIC distri-

butions for Am, P, T, Ri and Tr in the S-R strains indicate the direction in which

the (relative) resistance might develop in the MC-R and FC-R strains (Fig. 1

and 2). It was shown with the aid of the Yates-Cochran test with continuity cor-

rection that the S-R strains are significantly less sensitive to Am, P, T, Ri and

Tr than the MC-R and FC-R strains. This finding (insofar as Am, P and T

are concerned) is in agreement with previous observations (Stolz, Zwart and

Michel, 1974).

Comparison of the MIC distributions for Am, P, T, Ri, Sp,

Su and Tr in the FC-A strains (isolated in 1967) and the FC-R strains (iso-

lated in 1972) revealed no marked changes (see Fig. 1-3). However, one

Sp-resistant strain was found in the FC-A groups, with an MIC>168 µg/ml

(Table II). Reyn, Schmidt, Trier and Bentzon (1973) have described three

Sp-resistant strains, with an IC_{50} > 480 µg/ml.

The only marked difference between the FC-A and FC-R

strains was found in the MIC distributions for Su/Tr 5:1 (Table IV). It was

shown with the aid of the Yates-Cochran test with continuity correction that

the FC-A strains were significantly more sensitive to Su/Tr 5:1 than the

FC-R strains. This is surprising, in view of the fact that no increase in the

(relative) resistance to the other antibiotics investigated (in particular to Su

and Tr) was found among Dutch FC strains in the period from 1967 to 1972.

The potentiation of Su by Tr and hence the MIC for Su/Tr

5:1 is mainly determined by the ratio of the MIC for Su to that for Tr. This

potentiation is absent or slight for strains in which this ratio is less than 1,

is greater for strains in which the ratio is unity, and is greatest for the

strains with a ratio > 1. (Chapter XI). The difference found between

the FC-A and FC-R strains might be ascribed to the difference between

the combinations of the MIC for Su and the MIC for Tr for the various gonococ-

cal strains, and hence to the difference in the distributions of the ratio of MIC for Su to MIC for Tr. The fact that the value of r between Su and Tr is greater (0.35) for the FC-R strains than for the FC-A strains (0.11) also might point in this direction.

It may be seen from Table VII that high values of r are observed between Su and Tr, Su and Su/Tr 5:1 and Tr and Su/Tr 5:1 among the MC-R, FC-R and S-R strains. In the FC-A strains, on the other hand, a high r is only found between Su and Su/Tr 5:1; the r's between Su and Tr and between Tr and Su/Tr 5:1 are low. The higher degree of correlation among the Rotterdam strains might be due to the use of drugs containing Su and Tr in the years between 1967 and 1972.

In all groups of strains, r's > 0.50 were found between Am and P, Am and T and P and T. The correlations found are in agreement with the results of an earlier study (Stolz, Zwart and Michel, 1974). The values of r between Am and Ri, Am and Tr, P and Ri, P and Tr, T and Ri, T and Tr and Ri and Tr for the S-R strains were higher than those for the MC-R and FC-R strains. This is in agreement with the observation reported by Reyn and Bentzon (1969) that the r's are highest at the highest levels of observed (relative) resistance for each drug.

Although the r's between Sp and the other antibiotics were highest in the FC-A strains and much lower in the R strains (in particular the S-R strains), little importance can be attached to this fact since with one exception all strains were sensitive to Sp, with MIC's between 7.5 and 25 μg/ml. The fact that none of the strains isolated in Rotterdam in 1972 were resistant to Sp indicates that Sp can be regarded as a reliable substitute for the drugs generally used at present for gonorrhoea therapy.

It follows from this study that, with the exception of the increased resistance to Su/Tr 5:1, there has been no significant change in the incidence of (relative) resistance to the antibiotics investigated among the Dutch C population in the period from 1967 to 1972. If the S-R strains (which are less sensitive to Am, P, T, Ri and Tr than the MC-R and FC-R strains) had been able to spread freely among the civilian population, there would have been an increase in the number of strains resistant to Am, P, T, Ri and Tr among the C strains. It seems likely, however, that the S strains are mainly spread throughout the world via sailors and sailors' prostitutes. Spread of the S strains to the civilian population only occurs incidentally. Apart from this factor, the efficient administration and right dosage of the therapy for gonorrhoea most probably plays an important role in keeping the percentage of strains (relatively) resistant to P and other antibiotics among the C strains low in the Netherlands and other European countries.

Summary

The sensitivity of four groups of gonococcal strains to ampicillin (Am), penicillin (P), tetracycline (T), rifampicin (Ri), spectinomycin (Sp), sulphamethoxazole (Su), trimethoprim (Tr) and a combination of Su and Tr in the ratio 5:1 (Su/Tr 5:1) has been determined. The various groups of strains were isolated from male civilians (MC), female civilians (FC) and sailors (S) in Rotterdam (R) in 1972, and from FC in Amsterdam (A) in 1967. The S-R strains, which may be regarded as mainly imported, were compared with MC-R and FC-R (which may be considered as mainly of Dutch origin). The authors also investigated whether the incidence of (relative) resistance to the antibiotics investigated among Dutch strains has changed in the period from 1967 to 1972. For this purpose, the FC-A and FC-R strains were compared.

The MC-R and FC-R strains were also compared with one another. Finally, Spearman's rank correlation coefficients r were calculated between the sensitivity distributions for each pair of antibiotics investigated, for all strains.

The S-R strains were significantly less sensitive to Am, P, T, Ri and Tr than the MC-R and FC-R strains. Comparison of the FC-A and FC-R strains revealed that the FC-R strains were only significantly less sensitive to Su/Tr 5:1. A possible explanation for this finding is given.

With the exception of one FC-A strain , all gonococcal strains were sensitive to Sp.

High values of r (> 0.50) were found between Am and P, Am and T and P and T for all groups of strains. The values of r between any pair of the antibiotics Am, P, T, Ri and Tr (with the exception of the pair Am - P) were always highest for the S-R strains.

High values of r (> 0.50) were found between Su and Su/Tr 5:1 for all groups of strains. The FC-A strains, unlike the R strains, gave low values of r between Su and Tr and between Tr and Su/Tr 5:1. A possible explanation for this is given.

Finally, a hypothesis is put forward to explain the fact that no significant changes were found in the sensitivity of Dutch gonococcal strains to Am, P, T, Ri and Tr in the period from 1967 to 1972, while the S strains (which may be regarded as imported) showed a significantly higher percentage of strains relatively resistant to Am, P, T, Ri and Tr.

References

Ericsson, H. M. and Sherris, J.C. (1971)

Acta path. microbiol. scand. , section B 1971, suppl. no. 217.

Heimans, A. L. (1967)

"Diagnosis and treatment of gonorrhoea in women"

(Thesis)

Maness, M.J. and Sparling, P.F. (1973)

J. Infect. Dis. , 128, 321.

Phillips, I. , Rimmer, D. , Ridly, M. , Lynn, R and Warren, C. (1970)

Lancet 1, 263.

Reyn, A. , Korner, B. and Bentzon, M.W. (1958)

Brit. J. vener. Dis. , 34, 227.

Reyn, A. (1961)

Brit. J. vener. Dis. , 37, 145.

Reyn, A. and Bentzon, M.W. (1968)

Brit. J. vener. Dis. , 44, 140.

Reyn, A. and Bentzon, M.W. (1969)

Brit. J. vener. Dis. , 45, 223.

Reyn, A. , Schmidt, H. , Trier, M. and Bentzon, M.W. (1973)

Brit. J. vener. Dis. , 49, 54.

Stolz, E. , Zwart, H. G.F. and Michel, M.F. (1974)

Brit.J. vener. Dis. , 50, 202.

Verhagen, A.R. , van der Ham, M. , Heimans, A. L. , Kranendonk, O. and

Maina, A. N. (1971)

Bull. W. H. O. , 45, 707.

130

Wigfield, A.S., Selkon, J.B. and Rich, G.E. (1973)

Brit. J. vener. Dis., $\underline{49}$, 277.

Willcox, R.R. (1970)

Brit. J. vener. Dis., $\underline{46}$, 217.

Willcox, R.R. (1972)

Brit. J. vener. Dis., $\underline{48}$, 163.
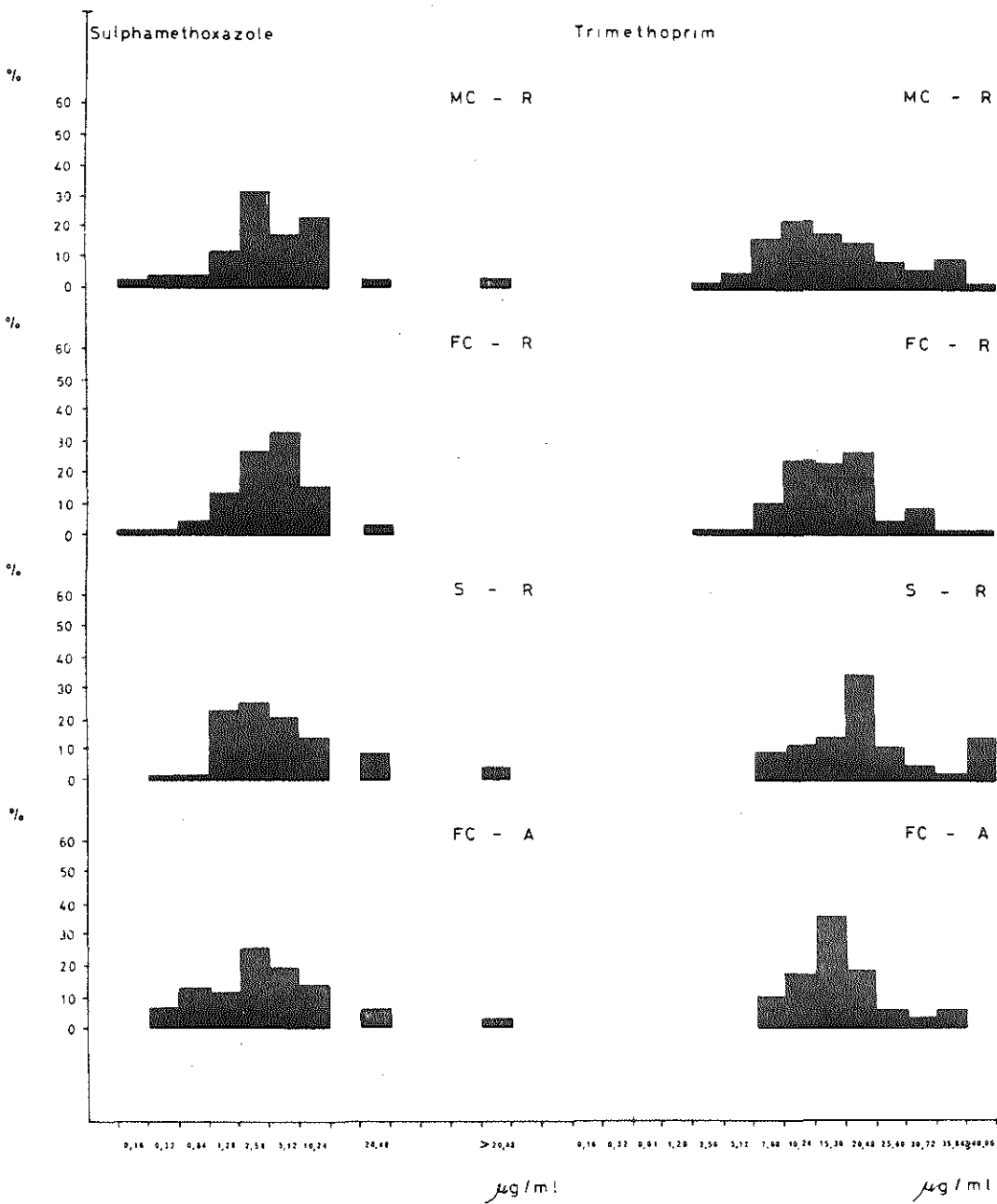
Wols-van der Wielen, A. (1971)

Brit. J. vener. Dis., $\underline{47}$, 190.

Figure 1

The distribution of the MIC's, expressed as percentages of the total number

of strains investigated, for ampicillin (Am), penicillin (P), tetracycline (T)

and rifampicin (Ri) of strains of gonococci isolated in 1972 from male civilians

(MC), female civilians (FC) and sailors (S) in Rotterdam (R) and in 1967 from

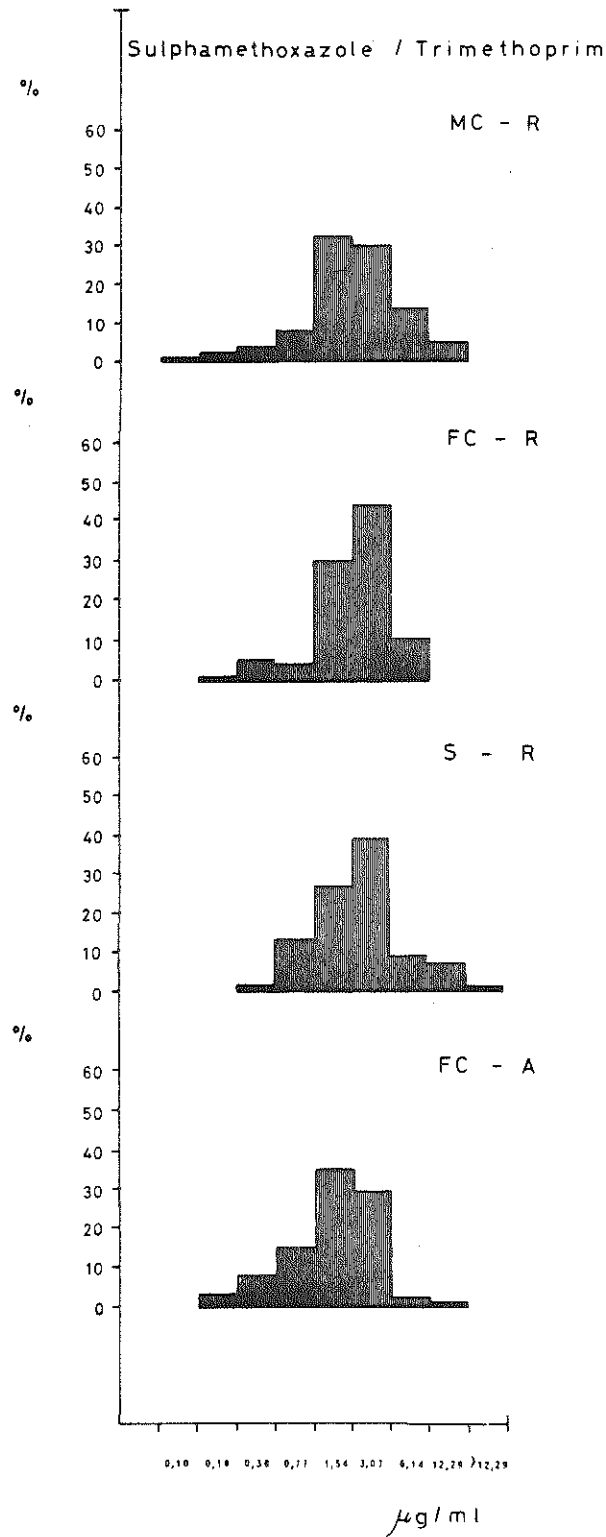female civilians (FC) in Amsterdam (A).
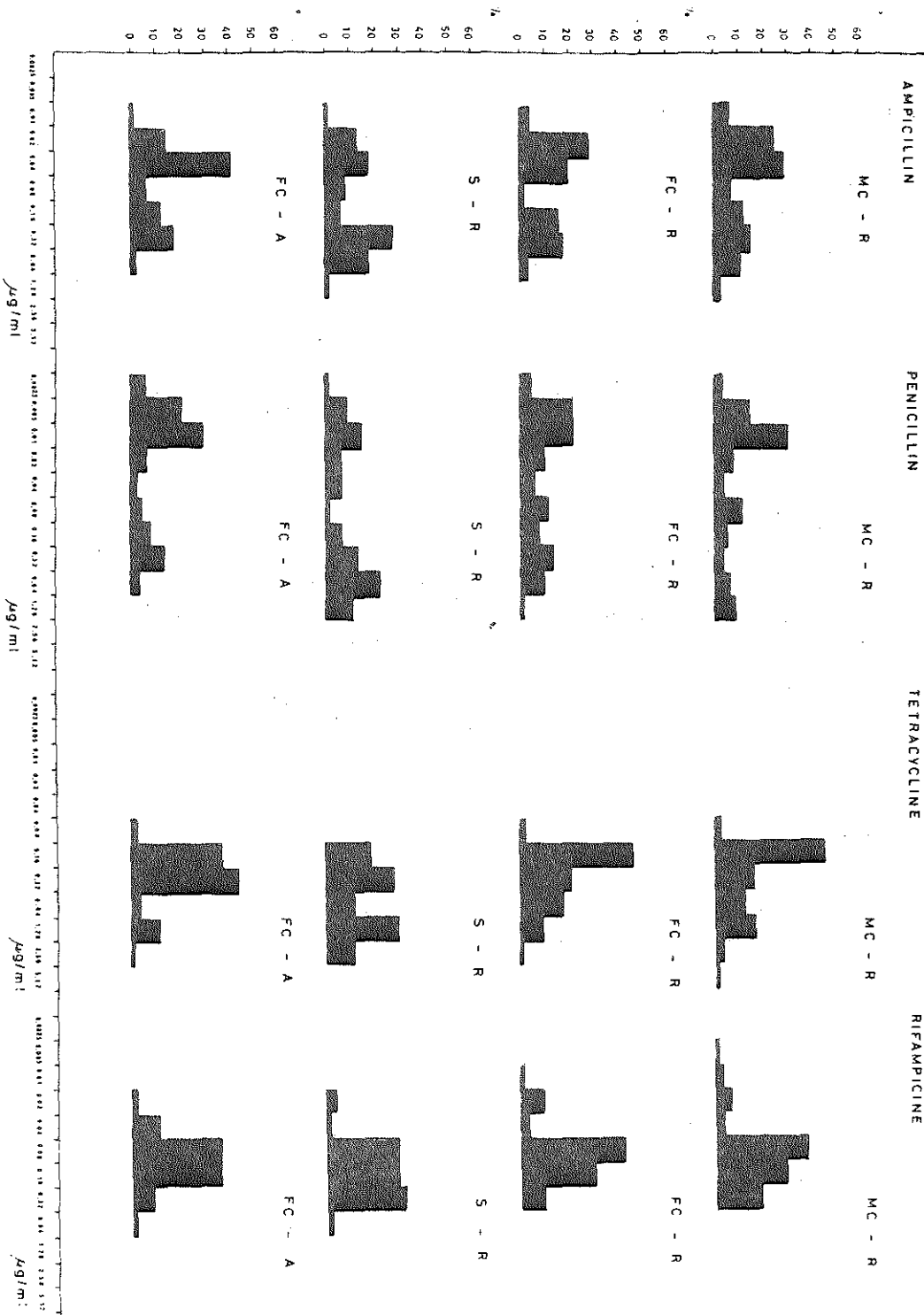
Figure 2

The distribution of the MIC's expressed as percentages of the total number of

strains investigated for spectinomycin of strains of gonococci isolated in

1972 from male civilians (MC), female civilians (FC) and sailors (S) in Rot-

terdam (R) and in 1967 from female civilians (FC) in Amsterdam (A).

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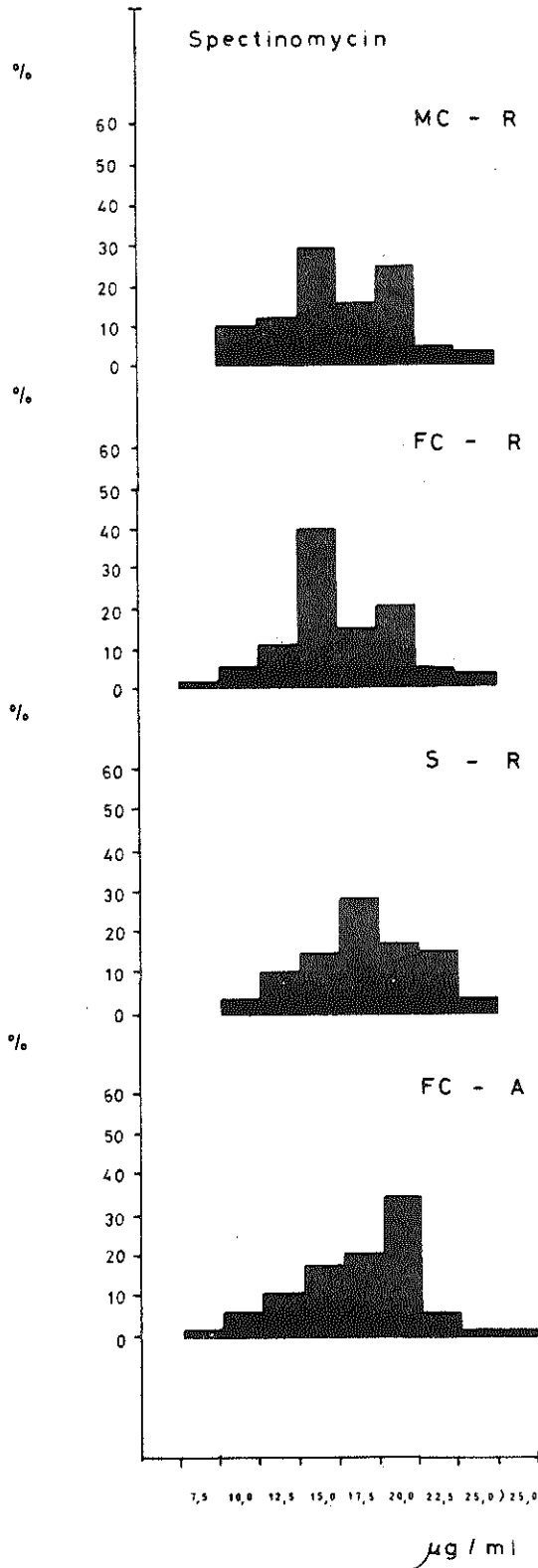
Figure 3

The distribution of the MIC's expressed as percentages of the total number of strains investigated for sulphamethoxazole (Su) and trimethoprim (Tr) of strains of gonococci isolated in 1972 from male civilians (MC), female civilians (FC) and sailors (S) in Rotterdam (R) and in 1967 from female civilians (FC) in Amsterdam (A).

Figure 4

The distribution of the MIC's, expressed as percentages of the total number of strains investigated for the combination of sulphamethoxazole (Su) and trimethoprim (Tr) in a proportion of 5:1 (Su/Tr 5:1) of strains of gonococci isolated in 1972 from male civilians (MC), female civilians (FC) and sailors (S) in Rotterdam (R) and in 1967 from female civilians (FC) in Amsterdam (A).

Table I

The distribution of the MIC's for ampicillin (Am), penicillin (P), tetracycline (T) and rifampicin (Rf) of strains of gonococci isolated in 1972 from male civilians (MC), female civilians (FC) and sailors (S) in Rotterdam (R) and in 1967 from female civilians (FC) in Amsterdam (A).

			not performed	0.0025	0.005	0.01	0.02	0.04	0.08	0.16	0.32	0.64	1.28	2.56	5.12
Am	MC-R 114 strains	N[1]	2	–	–	7	27	31	7	12	16	11	1	–	–
		P[2]	–	–	–	6.3	24.1	27.7	6.3	10.7	14.3	9.8	0.9	–	–
	FC-R 80 strains	N	1	–	–	4	23	17	2	14	15	4	–	–	–
		P	–	–	–	5.1	29.1	21.5	2.5	17.7	19.0	5.1	–	–	–
	S-R 44 strains	N	1	–	–	1	6	8	4	3	12	8	1	–	–
		P	–	–	–	2.3	14.0	18.6	9.3	7.0	27.9	18.6	2.3	–	–
	FC-A 94 strains	N	–	–	–		2	14	39	7	12	17	3	–	–	–
		P	–	–	–		2.1	14.9	41.5	7.4	12.8	18.1	3.2	–	–	–
P	MC-R 114 strains	N	2	4	17	35	9	5	13	6	5	8	10	–	–
		P	–	3.6	15.2	31.3	8.0	4.5	11.6	5.4	4.5	7.1	8.9	–	–
	FC-R 80 strains	N	1	3	17	17	8	3	8	5	11	6	1	–	–
		P	–	3.8	21.5	21.5	10.1	3.8	10.1	6.3	13.9	7.6	1.3	–	–
	S-R 44 strains	N	1	1	4	7	3	3	1	3	6	10	5	–	–
		P	–	2.3	9.3	16.3	7.0	7.0	2.3	7.0	14.0	23.3	11.6	–	–
	FC-A 94 strains	N	–	6	20	28	7	3	5	8	13	4	–	–	–
		P	–	6.4	21.3	29.8	7.4	3.2	5.3	8.5	13.8	4.3	–	–	–
T	MC-R 114 strains	N	2	–	–	–	–	–	3	52	19	14	19	4	1
		P	–	–	–	–	–	–	2.7	46.4	17.0	12.5	17.0	3.6	0.9
	FC-R 80 strains	N	1	–	–	–	–	–	2	37	17	14	8	1	–
		P	–	–	–	–	–	–	2.5	46.8	21.5	17.7	10.1	1.3	–
	S-R 44 strains	N	1	–	–	–	–	–	–	8	12	5	13	5	–
		P	–	–	–	–	–	–	–	18.6	27.9	11.6	30.2	11.6	–
	FC-A 94 strains	N	–	–	–	–	–	–	2	35	41	4	11	1	–
		P	–	–	–	–	–	–	2.1	37.2	43.6	4.3	11.7	1.1	–
Ri	MC-R 114 strains	N	–	–	1	3	7	5	43	33	22	–	–	–	–
		P	–	–	0.9	2.6	6.1	4.4	37.7	28.9	19.3	–	–	–	–
	FC-R 80 strains	N	3	–	–	1	8	3	33	24	8	–	–	–	–
		P	–	–	–	1.3	10.4	3.9	42.9	31.2	10.4	–	–	–	–
	S-R 44 strains	N	–	–	–	–	2	1	13	13	14	1	–	–	–
		P	–	–	–	–	4.5	2.3	29.5	29.5	31.8	2.3	–	–	–
	FC-A 94 strains	N	5	–	–	–	2	12	33	33	8	1	–	–	–
		P	–	–	–	–	2.2	13.5	37.1	37.1	9.0	1.1	–	–	–

1 Number   2 Percentage

Table II

The distribution of the MIC's for spectinomycin of strains of gonococci isolated in 1972 from male civilians (MC), female civilians (FC) and sailors (S) in Rotterdam (R) and in 1967 from female civilians (FC) in Amsterdam (A).

		not performed	7.5	10.0	12.5	15.0	17.5	20.0	22.5	25.0	>25.0
MC-R 114 strains	N[1]	1		11	13	33	17	28	6	5	–
	P[2]			9.7	11.5	29.2	15.0	24.8	5.3	4.4	–
FC-R 80 strains	N	4	1	4	8	30	11	15	4	3	–
	P	–	1.3	5.3	10.5	39.5	14.5	19.7	5.3	3.9	–
S-R 44 strains	N	–	–	2	5	7	13	8	7	2	–
	P	–	–	4.5	11.4	15.9	29.5	18.2	15.9	4.5	–
FC-A 94 strains	N	6	1	5	10	16	18	31	5	1	1[3]
	P	–	1.1	5.7	11.4	18.2	20.5	35.2	5.7	1.1	1.1

1 = Number

2 = Percentage

3 = MIC > 168 µg/ml

Table III

The distribution of the MIC's for sulphamethoxazole (Su) and trimethoprim (Tr) of strains of gonococci isolated in 1972 from male civilians (MC), female civilians (FC) and sailors (S) in Rotterdam (R) and in 1967 from female civilians (FC) in Amsterdam (A).

		not performed	0.16	0.32	0.64	1.28	2.56	5.12	7.68	10.24	15.36	20.48	25.60	30.72	35.84	≥40.96 (>20.48 SuMIC)
MC-R	N[1]	4	3	4	4	13	35	19	–	26	–	3	–	–	–	3
114 strains	P[2]	–	2.7	3.6	3.6	11.8	31.8	17.3	–	23.6	–	2.7	–	–	–	2.7
FC-R	N	2	1	1	4	11	21	26	–	12		2	–	–	–	–
80 strains	P	–	1.3	1.3	5.1	14.1	26.9	33.3	–	15.4		2.6	–	–	–	–
S-R	N	–	–	1	1	10	11	9	–	6	–	4	–	–	–	2
44 strains	P	–	–	2.3	2.3	22.7	25.0	20.5	–	13.6	–	9.1	–	–	–	4.5
FC-A	N	4	–	6	12	11	23	17	–	13	–	5	–	–	–	3
94 strains	P	–	–	6.7	13.3	12.2	25.6	18.9	–	14.4	–	5.6	–	–	–	3.3
MC-R	N	4	–	–	–	–	2	5	18	24	19	16	9	6	10	1
114 strains	P	–	–	–	–	–	1.8	4.5	16.4	21.8	17.3	14.5	8.2	5.5	9.1	0.9
FC-R	N	2	–	–	–	–	1	1	8	19	18	20	3	6	1	1
80 strains	P	–	–	–	–	–	1.3	1.3	10.3	24.4	23.1	25.6	3.8	7.7	1.3	1.3
S-R	N	–	–	–	–	–	–	–	4	5	6	15	5	2	1	6
44 strains	P	–	–	–	–	–	–	–	9.1	11.4	13.6	34.1	11.4	4.5	2.3	13.6
FC-A	N	4	–	–	–	–	–	–	11	16	32	17	5	4	5	–
94 strains	P	–	–	–	–	–	–	–	12.2	17.8	35.6	18.9	5.6	4.4	5.6	–

[1] = Number

[2] = Percentage

Table IV

The distribution of the MIC's for the combination of sulphamethoxazole (Su) and trimethoprim (Tr) in a proportion of 5:1 (Su/Tr 5:1) of strains of gonococci isolated in 1972 from male civilians (MC), female civilians (FC) and sailors (S) in Rotterdam (R) and in 1967 from female civilians (FC) in Amsterdam (A).

		not performed	0.10	0.19	0.38	0.77	1.54	3.07	6.14	12.29	>12.29
MC-R	N[1]	4	1	3	5	10	36	33	16	6	–
114 strains	P[2]	–	0.9	2.7	4.5	9.1	32.7	30.0	14.5	5.5	–
FC-R	N	2	–	1	5	4	24	35	9	–	–
80 strains	P	–	–	1.3	6.4	5.1	30.8	44.9	11.5	–	–
S-R	N	–	–	–	1	6	12	17	4	3	1
44 strains	P	–	–	–	2.3	13.6	27.3	38.6	9.1	6.8	2.3
FC-A	N	4	–	4	8	14	32	27	3	2	–
94 strains	P	–	–	4.4	8.9	15.6	35.6	30.0	3.3	2.2	–

1 = Number

2 = Percentage

Table V

Percentages of strains relatively resistant (RR) to ampicillin (Am), penicillin (P), tetracycline (T) and rifampicin (Ri) among strains of gonococci isolated in 1972 from male civilians (MC), sailors (S) and female civilians (FC) in Rotterdam (R) and in 1967 from FC in Amsterdam (A).

	MC-R	S-R	FC-R	FC-A
RR to Am/MIC ≥ 0.16 ug/ml	35.7 -	55.8	41.8	34.1
RR to P/MIC≥0.08 ug/ml	37.5 -	58.2	39.2	31.9
RR to T (MIC≥1.28 ug/ml)	21.5 -	41.8 -	11.4	12.8
RR to Ri (MIC ≥ 0.32 ug/ml)	19.3	34.1 -	10.4	10.1

- = significant difference (X^2 test, degree of freedom 1, α = 0.05)

Table VI

Significant differences in the distribution of the MIC's for ampicillin,
penicillin, tetracycline, rifampicin and trimethoprim between strains
of gonococci isolated in 1972 in Rotterdam (R) from male civilians
(MC) and sailors (S) and between gonococcal strains isolated in 1972
in Rotterdam from female civilians (FC) and sailors (S) (Yates-Cochran
test with continuity correction).

Antibiotic	Comparison between strains isolated from	$\bar{X}^1$ [a]	$\bar{X}^2$ [a]	T [a]	Two-sided significance
Ampicillin	MC-R and S-R	2.8	3.7	2.8	0.006
	FC-R and S-R	2.8	3.7	2.7	0.007
Penicillin	MC-R and S-R	3.8	5.3	3.0	0.003
	FC-R and S-R	3.7	5.3	3.1	0.002
Tetracycline	MC-R and S-R	3.2	3.9	2.9	0.004
	FC-R and S-R	2.9	3.9	3.9	< 0.001
Rifampicin	MC-R and S-R	5.4	5.9	2.2	0.03
	FC-R and S-R	5.2	5.9	2.9	0.004
Trimethoprim	MC-R and S-R	4.1	5.2	2.8	0.006
	FC-R and S-R	4.1	5.2	2.9	0.003

$\bar{X}^1$ = mean of observations for first group of strains in column 1

$\bar{X}^2$ mean of observations for second group of strains in column 2

T = standard value

a = figures are rounded off

The difference between $\bar{X}^1$ and $\bar{X}^2$ is considered to be significant when the
two-sided significance (last column) is ≤ 0.05

Table VII

Rank correlation coefficients between the MIC distributions for the various pairs of antibiotics investigated for strains isolated from male civilians in Rotterdam (MC-R), female civilians in Rotterdam (FC-R), sailors in Rotterdam (S-R) and female civilians in Amsterdam (FC-A).

		Ampicillin	Penicillin	Tetracycline	Rifampicin	Spectinomycin	Sulphamethoxazole	Trimethoprim
Penicillin	MC-R	0.91						
	FC-R	0.94						
	S-R	0.91						
	FC-A	0.87						
Tetracycline	MC-R	0.63	0.70					
	FC-R	0.58	0.62					
	S-R	0.72	0.81					
	FC-A	0.55	0.61					
Rifampicin	MC-R	0.27	0.27	0.23				
	FC-R	0.13	0.15	0.13				
	S-R	0.62	0.59	0.49				
	FC-A	0.38	0.43	0.38				
Spectinomycin	MC-R	0.25	0.19	0.26	0.24			
	FC-R	0.13	0.08	0.06	0.10			
	S-R	0.07	0.05	0.04	0.14			
	FC-A	0.43	0.43	0.49	0.43			
Sulphamethoxazole	MC-R	0.08	0.13	0.15	0.05	0.09		
	FC-R	0.09	0.10	0.002	0.12	0.04		
	S-R	0.25	0.23	0.10	0.09	0.06		
	FC-A	0.04	0.02	0.02	0.12	0.27		
Trimethoprim	MC-R	0.21	0.17	0.12	0.36	0.12	0.56	
	FC-R	0.26	0.30	0.26	0.23	0.03	0.35	
	S-R	0.50	0.44	0.37	0.49	0.09	0.52	
	FC-A	0.26	0.24	0.01	0.14	0.16	0.11	
Sulphamethoxazole/Trimethoprim	MC-R	0.03	0.03	0.09	0.20	0.05	0.83	0.63
	FC-R	0.19	0.19	0.05	0.20	0.07	0.82	0.55
	S-R	0.15	0.15	0.10	0.06	0.15	0.78	0.53
	FC-A	0.07	0.17	0.10	0.05	0.24	0.84	0.14

POTENTIATION OF SULPHAMETHOXAZOLE BY TRIMETHOPRIM IN GONO-COCCUS STRAINS.

Sulphonamides and trimethoprim have a strong synergistic effect. They interfere with the last two successive stages of the folic acid synthesis in bacteria in the following way.

Fig. 1

Interference of sulphonamides and trimethoprim with the last two successive stages of the folic acid synthesis in bacteria.
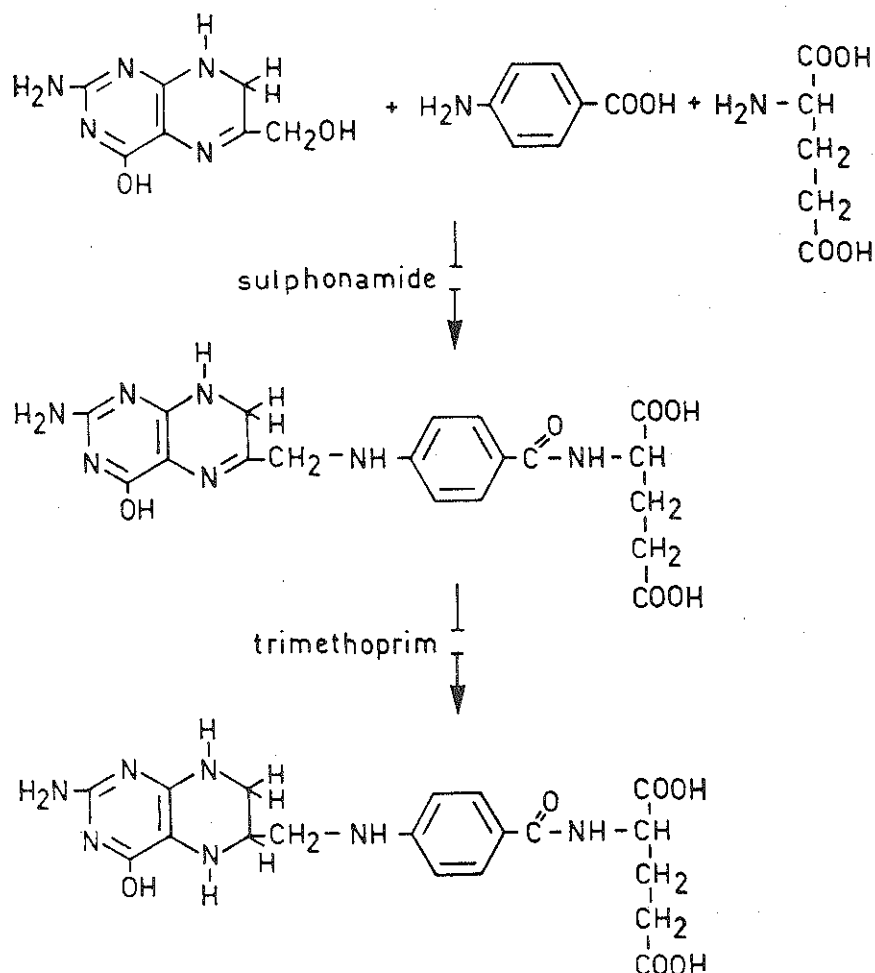
[1] Stolz, E., Michel, M.F. and Zwart, H.G.F. (to be published)

The condensation of para-aminobenzoic acid,dihydropteroate and glutamic acid to dihydrofolic acid can be disturbed by supplying substances related to para-aminobenzoic acid, with a high affinity for dihydrofolic acid synthetase, to this enzyme. As a result, no folic acid or a non-active folic acid will be synthesized. The sulphonamides are related to para-aminobenzoic acid, and produce their effect as described above. Trimethoprim retards the action of dihydrofolic acid reductase. The sulphonamides alone have a bacteriostatic effect and so does trimethoprim. When used together they have a synergistic effect and are then bactericidal.

In this study quantitative sensitivity determinations for sulphamethoxazole (Su), trimethoprim (Tr) and a combination of sulphamethoxazole and trimethoprim in a ratio of 5:1 (Su/Tr 5:1) were performed on 322 gonococcus strains isolated from 322 patients.Further, data are given on the potentiation of Su by Tr. Finally, the sensitivity of seven gonococcus strains (with known sensitivity for Su and Tr) was determined for combinations of Su and Tr in the ratios of 20:1, 10:1, 5:1, 2:1, 1:1, 1:2, 1:5, 1:10 and 1:20. On the basis of these data, rules are given for the variation of the potentiation of Su by Tr in gonococci.

Materials and methods

The sensitivity to Su, Tr and the various combinations of Su and Tr was determined by the agar dilution method (Ericsson and Sherris, 1971). The sensitivity was expressed as the minimum inhibitory concentration (MIC) in ug/ml. The medium used for the sensitivity determinations consisted of DST agar (Oxford) and 7.5% haemolysed horse blood. A series of plates with increasing concentrations of the antibiotic under investigation and a control plate without antibiotic were prepared. The concentrations of Su varied between 0.16 and 20-48 μg/ml, those of Tr between 2.56 and 40.96 μg/ml and those of

Su/Tr 5:1 between 0.10 and 12.29 μg/ml.

Organisms from overnight cultures were suspended in phosphate-buffered physiological saline pH 7.2 at a density of $10^6 - 10^7$ viable units (v.u.) per ml. The suspensions were inoculated on a series of plates with the aid of a multipoint replicator. The inoculated points were 4 - 6 mm in diameter and contained $10^3 - 10^4$ v.u. each. In each sensitivity determination 3 gonococcal strains and one Staphylococcus aureus (Oxford) strain with known sensitivities to Su, Tr and Su/Tr 5:1 were investigated at the same time.

After incubation for 18-20 hours, the MIC was determined by reading off the lowest antibiotic concentration at which no, or practically no bacterial growth was visible. A haze of growth or a single colony was regarded as practically no growth.

Results

The distribution, the median values and the geometric means of the MIC's for Su (MIC-Su), for Tr (MIC-Tr) and for Su/Tr 5:1 (MIC-Su/Tr 5:1) are given in Table I.

The potentiation of Su by Tr was calculated by dividing MIC-Su by the concentration of Su in MIC-Su:Tr 5:1. The distribution of the potentiation of Su by Tr is given in

Table II

It may be seen from Table II that no potentiation of Su by Tr was found in 23% of the strains. A two fold potentiation of Su by Tr was found in 51.6% and a fourfold potentiation in 18.7% of the strains.

Table III

        The sensitivities to Su and the potentiation of Su by Tr are com-
pared in Table III. It may be seen from this table that as the sensitivity to Su
decreases, the potentiation of Su by Tr increases. The X^2 test after dicho-
tomy at the medians gave $P \leq 0.001$.

        In order to uncouple the conclusion from Table III that decrea-
sing sensitivity to Su is associated with increasing potentiation of Su by Tr from
the sensitivity to Tr, all gonococcal strains were redistributed in groups ac-
cording to their sensitivity to Tr. The sensitivity to Su and the potentiation
of Su by Tr were then compared for each group separately. In order to save
space, we give only one of the resulting tables here; however, this is repre-
sentative of the other. This table (Table IV) gives the sensitivities to Su
and the potentiation of Su by Tr for all gonococcal strains with MIC-Tr
of 10.24 µg/ml.

Table IV

        It may be seen from Table IV that gonococcal strains with
the same sensitivity to Tr also show increasing potentiation of Su by Tr as
the sensitivity to S decreases. The X^2 test after dichotomy at the medians
gave $P \leq 0.001$.

        In order to investigate whether the MIC-Tr is one of the fac-
tors influencing the potentiation of Su by Tr, the gonococcal strains were divi-
ded into groups according to their sensitivity to Su. The sensitivity to Tr and
the potentiation of Su by Tr was then determined for each group. Once again,
only one representative table is shown.

Table V

This gives the sensitivity for Tr and the potentiation of Su by Tr for all gonococcal strains with an MIC-Su of 10.24 μg/ml.

It follows from Table V that gonococcal strains with the same sensitivity to Su show increasing potentiation of Su by Tr as the sensitivity to Tr increases. The X^2 test after dichotomy at the medians gave P = 0.005.

It follows further from Tables IV and V that the potentiation of Su by Tr for a group of strains with an identical MIC-Su and an identical MIC-Tr lies within narrow limits, and that the potentiation of Su by Tr is predictable.

In Table VI the values of MIC-Su, MIC-Tr, the quotients MIC-Su/MIC-Tr and the potentiation of Su by Tr for combinations of Su and Tr in ratios varying from 20:1 to 1:20 are given for seven gonococcal strains with known sensitivities to Su and Tr.

The gonococcal strains are arranged according to decreasing sensitivity to Su, and with the same sensitivity to Su according to decreasing sensitivity to Tr. The quotient MIC-Su/MIC-Tr gives the relation between the sensitivity to Su and that to Tr. This quotient is greater than 1 for strain 7, is equal to 1 for strains 3 and 6 and is less than 1 for strains 1,2, 4 and 5.

Reading this table horizontally, we see that for strains with an MIC-Su/MIC-Tr quotient of ≥ 1, potentiation of Su by Tr already occurs at an Su/Tr ratio of 20:1. For the strains with an MIC-Su/MIC-Tr quotient of < 1, the amount of Tr in the combination of Su and Tr has to be larger before potentiation of Su by Tr is found. From the Su/Tr ratio at which potentiation is first observed, the potentiation of Su by Tr increases with an increasing amount of Tr in the combination of Su and Tr. The only exception to this is the value 1 in column Su/Tr 1:1 against strain 1.

Reading Table VI vertically, we see that for each Su/Tr

ratio the strain with the MIC-Su/MIC-Tr ratio>1 gives stronger potentiation of Su by Tr than the strains with an MIC-Su/MIC-Tr ratio = 1. The latter strains, on the other hand, show stronger potentiation of Su by Tr than the strains with an MIC-Su/MIC-Tr ratio < 1.

In the columns Su/Tr 5:1 no potentiation of Su by Tr is found for the three strains with the smallest MIC-Su/MIC-Tr quotient.

The synergistic effect of Su and Tr can also be assessed with the aid of the FIC value and the FIC index. The FIC value is the MIC of the drug in question in the presence of the other drug, expressed as a fraction (F) of the MIC for the first drug when used alone. The FIC index is the sum of the FIC values for the two drugs in question (Bushby and Hitchings, 1968). The FIC index for Su and Tr is thus the sum of the FIC value for Su and that for Tr. The smaller the FIC index, the stronger the synergistic effect of Su and Tr.

Table VII

Table VII gives the FIC indices for five of the seven strains with known sensitivities to Su and Tr, for combinations of Su and Tr with Su/Tr ratios ranging from 20:1 to 1:20.

It will be seen from Table VII that the smallest FIC indices are found at lower Su/Tr ratios for strains with an MIC-Su/MIC-Tr quotient < 1, and at higher Su/Tr ratios for strains with an MIC-Su/MIC-Tr ratio = 1. There is an optimum Su/Tr ratio for each strain. However, these optimum Su/Tr ratios are always lower than 5:1.

Further, the very lowest FIC indices are found for the strains with an MIC-Su/MIC-Tr quotient = 1, while the lowest FIC index increases as the quotient MIC-Su/MIC-Tr decreases. The Su-sensitive strains with very low MIC-Su/MIC-Tr quotients show little synergism of Su and Tr. Their lowest FIC indices are quite high.

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Discussion

Gonococci, unlike most other bacteria, are generally more sensitive to Su than to Tr (Bushby and Barnett, 1967; Darrell, Garrod and Waterworth, 1968; Garrod and Waterworth, 1968; Sutherland, 1970). In vitro determinations of the sensitivity of the gonococci have shown that Su is 5 to 10 times more active than Tr (Sutherland, 1970). The gonococci investigated by us were about five times more sensitive to Su than to Tr (Table I).

Other micro-organisms, on the other hand, are from twenty to a hundred times less sensitive to Su than to Tr.

Use of the combination Su/Tr 5:1 showed that the gonococci are on the average 3 times more sensitive to this combination than to Su alone (Sutherland, 1970). The gonococci investigated by us were less than 2 times more sensitive to Su/Tr 5:1 than to Su alone (Table I). The synergism achieved for gonococci with the combination Su/Tr 5:1 is thus slight. A number of au-. thors (Bushby and Barnett, 1967; Bushby and Hitchings, 1968; Darrell, Garrod and Waterworth, 1968; Garrod and Waterworth, 1968; Böhni, 1969; Phillips, Ridley, Rimmer, Lynn and Warren, 1970; Reeves, 1971) have suggested that the optimum Su/Tr ratio for gonococci must differ from that for other micro-organisms. In general, more trimethoprim is needed in the Su-Tr combination which is optimal for gonococci. Our investigation has shown that each gono-coccus strain has its own optimum Su/Tr ratio, which is however always lower than 5:1. For strains which are Su-sensitive and therefore generally have a low MIC-Su/MIC-Tr quotient, the synergism of Su and Tr is slight and is in fact completely lacking at an Su/Tr ratio of 5:1.

Reeves (1971) has reported that administration of tablets of a

combined preparation with an Su/Tr ratio of 5:1 gave an Su/Tr ratio of 20:1 in the blood of the test subjects. In the tissues, on the other hand, this situation was again reversed to an extent which depended strongly on the nature of the tissue involved, bringing the Su/Tr ratio finally nearer the optimum values for gonococci. It is therefore hardly possible, on the basis of in vitro sensitivity determinations for Su, Tr and Su-Tr combinations alone, to propose a better Su/Tr ratio than 5:1 for combined preparations. On the other hand, it may be stated that for Su-sensitive strains, which make up a large part of all gonococcus strains, the presence of Tr in the combined preparations is not strictly necessary.

Summary

Quantitative determinations of the sensitivity of 322 gonococcus strains to sulphamethoxazole (Su), trimethoprim (Tr) and a combination of Su and Tr in a ratio of 5:1 (Su/Tr 5:1) are described. Data are also given on the potentiation of Su by Tr. The sensitivity to combinations of Su and Tr in various ratios was determined for seven gonococcal strains with known sensitivity to Su and Tr. On the basis of these data, the rules according to which Su is potentiated by Tr could be formulated.

The synergistic effect of Su and Tr was further assessed for the above-mentioned seven gonococcus strains with the aid of the FIC index. It was striking that the Su-sensitive gonococcal strains showed no potentiation of Su by Tr and no synergism of Su and Tr when an Su/Tr ratio of 5:1 is used.

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References

Böhni, E. (1969)

Chemotherapy Suppl. ad. vol. 14, 1.

Bushby, S.R.M. and Barnett, M. (1967)

Proc. 5th. Int. Congr. of Chemotherapy, Vienna

Ed. K.H. Spitzy and H. Hascek, Vol. 1/2. Chemotherapy, 753.

Bushby, S.R.M. and Hitchings, G.H. (1968)

Br. J. Pharmac. Chemother. , 33, 72.

Darrell, J.H. , Garrod, L.P. and Waterworth, P.M. (1968)

J. Clin. Path. , 21, 202.

Ericsson, H.M. and Sherris, J.C. (1971)

Acta path. microbiol. scand. , Section B 1971, suppl. no. 217.

Garrod, L.P. and Waterworth, P.M. (1968)

Brit. J. vener. Dis. , 44, 75.

Phillips, I. , Ridly, M. , Rimmer, D. , Lynn, R. and Warren, C. (1970)

The Lancet, 1, 263.

Reeves, D.S. (1971)

J. Clin. Path. , 24, 430.

Sutherland, R. (1970)

Personal communication.

Table I

The distribution, the median values and the geometric means of the MIC's for sulphamethoxazole (Su), trimethoprim (Tr) and a combination of Su and Tr in the ratio 5:1 (Su/Tr 5:1).

MIC's in μg/ml		Su		Tr		Su/Tr 5:1	
		No. of strains	%	No. of strains	%	No. of strains	%
0.08	(0.10)					1	0.3
0.16	(0.19)	4	1.2			8	2.5
0.32	(0.38)	12	3.7			19	5.9
0.64	(0.77)	21	6.5			34	10.6
1.28	(1.54)	45	14.0			104	32.3
2.56	(3.07)	90	28.0	3	0.9	112	34.8
5.12	(6.14)	71	22.0	6	1.9	32	9.9
7.68				41	12.7		
10.24	(12.29)	57	17.7	64	19.9	11	3.4
15.36				75	23.3		
20.48	(>12.29)xx	14	4.3	68	21.1	1	0.3
25.60				22	6.8		
30.72				18	5.6		
35.84				17	5.3		
40.96x		8	2.5	8	2.5		
Median valuexxx		2.36 μg/ml		13.22 μg/ml		1.49 μg/ml	
Geometric Meanxxx		3.26 μg/ml		15.31 μg/ml		1.95 μg/ml	

( )   MIC's for Su/Tr 5:1

x   ≥ 40.96 μg/ml for Tr, but > 20.48 μg/ml for Su.

xx   (> 12.29 μg/ml) arbitrarily grouped with 20.48 μg/ml for Su and Tr.

xxx   For the calculation of the median value and the geometric mean, the strains with an MIC for Su > 20.48 are arbitrarily taken together with the strains with an MIC for Su = 20.48 μg/ml; the strains with the MIC for Su/Tr 5:1 > 12.29 μg/ml are arbitrarily grouped together with the strains with MIC = 12.29 μg/ml; and the strains with an MIC for Tr ≥ 40.96 μg/ml are arbitrarily considered as strains with an MIC 40.96 μg/ml.

Table II

Distribution of the potentiation of sulphamethoxazole (Su) by trimethoprim (Tr).

	Potentiation of Su by Tr									
	0.25	0.50	1	2	4	8	≥ 2	?xx	Total	
No. of strains	2	3	74	166	60	9	7x	1	322	
Percentage	0.6	0.9	23.0	51.6	18.7	2.8	2.1	0.3	100.0	

x   = 2 strains ≥ 2; 4 strains ≥ 8; 1 strain ≥ 64.

xx   = cannot be calculated.

Table III

Sensitivity of gonococci to sulphamethoxazole (Su) and potentiation of sulphamethoxazole by trimethoprim (Tr).

MIC-Su in µg/ml	No. of strains with a potentiation of Su by Tr of:							
	0.25	0.50	1	2	4	8	≥ 2	$?^{xx}$
0.16		1	2	1				
0.32	1		5	6				
0.64		1	12	8				
1.28	1	1	25	15	3			
2.56			20	62	6	2		
5.12			7	53	11			
10.24			3	15	35	4		
20.48				6	5	3		
> 20.48							7^{x}	1

x = 2 strains $\geq$ 2; 4 strains $\geq$ 8; 1 strain $\geq$ 64.

xx = cannot be calculated.

Table IV

Potentiation of sulphamethoxazole (Su) by trimethoprim (Tr) in gonococcus strains with the same value of MIC-Tr (10.24 µg/ml) and increasing values of MIC-Su (0.32 -> 20.48 µg/ml).

MIC-Su in µg/ml	No. of strains with a potentiation of Su by Tr of:				
	1	2	4	8	> 8
0.32	2	1			
0.64	3	2			
1.28	3	3			
2.56	2	21	2		
5.12		8	4		
10.24		2	9	1	
> 20.48					1

Table V

Potentiation of sulphamethoxazole (Su) by trimethoprim (Tr) in gonococcus strains with the same value of MIC-Su (10.24 µg/ml) and increasing values of MIC-Tr (7.68 ->40.96 µg/ml).

MIC-Tr in µg/ml	No. of strains with a potentiation of Su by Tr of:			
	1	2	4	8
7.68			3	1
10.24		2	8	1
15.36			8	
20.48		5	9	1
25.60		3	2	1
30.72		4	3	
35.84	2	2	1	
≥ 40.96	1			

Table VI

MIC's for sulphamethoxazole (MIC-S) and trimethoprim (MIC-Tr), quotients of MIC-Su and MIC-Tr (MIC-Su/MIC Tr) and potentiation of sulphamethoxazole (Su) by trimethoprim (Tr) for combinations of sulphamethoxazole and trimethoprim (Su/Tr) in the ratios 20:1, 10:1, 5:1, 2:1, 1:1, 1:2, 1:5, 1:10 and 1:20 for seven gonococcus strains.

				Potentiation of Su by Tr								
Strain no.	MIC-Su	MIC-Tr	MIC-Su MIC-Tr	Su/Tr 20:1	Su/Tr 10:1	Su/Tr 5:1	Su/Tr 2:1	Su/Tr 1:1	Su/Tr 1:2	Su/Tr 1:5	Su/Tr 1:10	Su/Tr 1:20
1	0.64	5.12	1/8	1	1	1	2	1	2	2.5	5.0	5.0
2	0.64	7.68	1/12	1	1	1	1	1	1	1.25	2.5	2.5
3	5.12	5.12	1	4	4	4	8	16	16	20	40	40
4	5.12	25.60	1/5	1	1	2	2	4	4	5	10	20
5	5.12	>40.96	<1/8	1	1	1	2	2	2	5	5	20
6	40.96	40.96	1	2	4	4	8	8	8	20	20	40
7	>40.96	10.24	>4	>4	>8	>8	>16	>16	>32	>40	>80	>160

Note:

When making the dilutions of Su for the determination of the MIC's for the combinations of Su and Tr with Su/Tr ratios of 1:5, 1:10 and 1:20, the dilution series mentioned under "Materials and methods" was replaced by the geometric dilution series from 0.13 to 32.8 μg/ml .

Table VII

FIC indices of strains 1, 2, 3, 4 and 6 from table VI[x] for combinations of sulphamethoxazole (Su) and trimethoprim (Tr) with Su/Tr ratios of 20:1, 10:1, 5:1, 2:1, 1:1, 1:2, 1:5, 1:10 and 1:20.

				FIC index								
Strain no.	MIC-Su	MIC-Tr	MIC-Su MIC-Tr	Su/Tr 20:1	Su/Tr 10:1	Su/Tr 5:1	Su/Tr 2:1	Su/Tr 1:1	Su/Tr 1:2	Su/Tr 1:5	Su/Tr 1:10	Su/Tr 1:20
1	0.64	5.12	1/8	1.00	1.01	1.03	0.53	1.13	0.63	0.65	0.45	0.70
2	0.64	7.68	1/12	1.00	1.00	1.02	1.04	1.08	1.17	1.13	0.73	1.07
3	5.12	5.12	1	0.26	0.28	0.30	0.19	0.13	0.19	0.30	0.28	0.53
4	5.12	25.60	1/5	1.01	1.02	0.52	0.55	0.30	0.35	0.40	0.30	0.25
6	40.96	40.96	1	0.53	0.28	0.30	0.19	0.25	0.38	0.30	0.55	0.53

x = The FIC indices for strains 5 and 7 could not be calculated

_ = lowest FIC index for the strain in question.

TREATMENT OF GONORRHOEA USING A COMBINATION OF INTRAMUS-CULAR AND ORAL AMPICILLIN.

Recent studies have proved that the treatment of uncomplica-ted gonorrhoea with oral ampicillin, administered either in a single dose of 2 g. plus 1 g. probenecid or 2 g. divided into two doses at an interval of 5 hrs. is an effective alternative to conventional treatment with a single intramuscular injection of 1. 2 m.u. procaine penicillin plus 1 m.u. sodium penicillin G (Gundersen, Ødegaard and Gjessing, 1969; Ericsson, 1970 a, b, 1971). Other regimens of ampicillin treatment are reviewed by Willcox, Woodcock, Latto, John, Redmond, Parker, Rees and Cobbold (1973).

Ampicillin given by intramuscular injection is less frequent-ly used in the treatment of gonorrhoea. Kercull (1968) reported a primary cure rate of 95 per cent. in acute gonorrhoea in military patients in Vietnam, using 1 g. ampicillin daily for two or three days. In the Far East, Keys, Halverson, and Clarke (1969) reported a failure rate of only 1 per cent. with an intramus-cular dose of 2 g. ampicillin together with 1 g. probenecid orally.

In this study we have tested the efficacy of 1 g. ampicillin intramuscularly in combination with an oral dose of 2 g. ampicillin taken 4 hrs. later.

In order to find out if there was a correlation between thera-peutic failures and the minimum inhibitory concentrations (MIC's) for ampi-cillin of the strains of N. gonorrhoeae isolated, the MIC's for ampicillin were estimated before and, in the cases of therapeutic failure, also after treat-ment. In addition, ampicillin serum levels were measured in six male patients.

Patients and methods.

The investigation was carried out between 18 October 1971 and 1 July 1972.

The study included 619 outpatients with uncomplicated uro-genital or rectal gonorrhoea; 289 were male civilians, 204 were female ci-

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1. Stolz, E. and Kerkkamp, H.J.J. (1974) Brit. J. vener. Dis. to be published.

vilians, and 126 were sailors.

These numbers differ from those given in chapter IV(which refer to the same patient population) because 12 MC, 9 FC and 19 MS were not given our standard ampicillin treatment, and because 15 MC and 14 FC who consulted our clinic 3 months or more after an earlier (cured) infection were regarded as new patients and in connection with a reinfection, included in the data sets as such.

Six of these patients were excluded. One developed an urticarial rash and two had a drop in blood pressure (but not real shock) after the ampicillin injection. These three patients were not given oral ampicillin. Three other patients admitted that they forgot to take the oral ampicillin. Of the remaining 613 patients, 286 were male civilians, 202 were female civilians, and 125 were sailors;they all received 1 g. ampicillin intramuscularly and took 2 g. ampicillin by mouth 4 hrs later.

Diagnosis was based on the results of Gram-stained smears and cultures from the urethra in males and from the cervix and urethra in females. Culture was performed on material from the rectum in all females and in eight homosexual males. Specimens were cultured on a selective medium (Thayer and Martin, 1966). Sensitivity tests for ampicillin using the agar-dilution method (Ericsson and Sherris, 1971) were performed on all positive cultures. Strains with MIC's of ampicillin ≥ 0.16 ug/ml. were defined as relatively resistant.

Female patients were asked to return after 1, 2, 6, and 12 weeks, and male patients after 1 and 2 weeks. Smears and cultures were taken at each visit. The criterion for a satisfactory result was two consecutive negative examinations in the first 2 weeks after treatment.

Any patient with a recurrence within 2 weeks who admitted re-exposure to risk of infection, was considered to have been re-infected. Any patient with a recurrence after 2 weeks was also considered to have been re-infected.

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In six male volunteers, all with gonococcal urethritis, specimens of serum were assayed by means of the cylinder plate method using Sarcina lutea NCTC 8340 as test organism. (Michel, van Waardhuizen and Kerrebijn, 1973).

The volunteers satisfied the following conditions: Age between 20 and 40 years, weight between 75 and 80 kg., height between 170 and 190 cm., serum creatinine within the normal range and no history of renal or gastrointestinal disease.

Specimens of serum were taken just before ampicillin treatment started and 15 min. ,30 min. and 1, 2 and 4 hours after the injection. After the oral treatment further samples were taken after 30 min. and 1, 2, 4 and 6 hours.

Results

The follow-up achieved is presented in Table 1.
Of the 613 patients treated, 142 (23.2 percent) did not return for follow-up within 2 weeks, whereas 54 (8.8 percent) returned after a longer interval than 2 weeks, but within 3 months. Sailors who did not return for any follow-up within 2 weeks (56 percent) were not considered to be genuine defaulters. In most cases their ships had to leave Rotterdam and so it was impossible for them to return. The percentages of patients who returned within 2 weeks for follow-up was highest among female civilians (85.1 percent); however, a considerable disparity was observed between female and male civilian patients also (85.1 percent and 67.1 percent, respectively).

Strains of N.gonorrhoeae could not be tested for sensitivity in all patients. In some cases the culture was negative and the Gram-stained smear positive. In other cases cultures were positive, but sensitivity testing could not be carried out because the strains did not survive.

In 388 (93.0 percent) of the 417 patients who returned within 2 weeks, the strains of N.gonorrhoeae had been isolated before treatment and could be tested for sensitivity. The percentages for male civilians, female civilians, and sailors were 91.1, 95.3 and 92.5 percent, respectively.

MIC's of ampicillin for all strains of N.gonorrhoeae tested were in the range of 0.005 to 0.64 µg/ml.

Table II shows the number and percentages of strains relatively resistant (RR) to ampicillin (MIC≥ 0.16 µg/ml) among strains of N.gonorrhoeae isolated in the three patient groups.

The percentage of strains relatively resistant to ampicillin was lowest among those isolated from patients who returned within 2 weeks and, with the exception of the male civilians, highest among those isolated from patients who did not return for follow-up. The greatest difference was found between strains isolated from sailors who returned within 2 weeks. (53.1 per cent. RR to ampicillin) and strains isolated from sailors who did not return at all (73.9 per cent. RR to ampicillin). No satisfactory explanation could be found for this difference. A higher percentage of strains relatively resistant to ampicillin was found in sailors than in male and female civilians. This finding has been noted in previous studies (Stolz, Zwart, and Michel, 1974; Wols- van der Wielen, 1971).

In Table III, relapses and re-infections in patients who returned for follow-up within two weeks after treatment are recorded. Relapses were found in only four male civilians. The relapse rate was 2.1 per cent. in male civilians and 1.0 per cent. in all patients. Thus the cure rate was 97.9 per cent. in male civilians and 99.0 per cent. in all patients. Re-infections were found in five male civilians, four female civilians, and one sailor. Re-infection rates in male civilians, female civilians, sailors and in all patients were 2.6, 2.3, 1.9 and 2.4 per cent. respectively. The total recurrence rate within 2 weeks (relapse rate + re-infection rate) was therefore 4.7 per cent. in male civilians, 2.3 per cent in female civilians, 1.9 per cent. in sailors, and 3.4 per cent. in all patients.

In all four patients with relapses, strains relatively resistant to ampicillin (MIC ≥ 0.16 µg/ml) were isolated. In three the MIC was 0.64 µg/ml and in one it was 0.16 µg/ml. Of the ten patients with re-infections eight had sensitive strains and two relatively resistant strains (MIC of 0.64

μg/ml in both). In the latter two patients the strains isolated after treatment were highly sensitive to ampicillin. In all other patients with relapses and re-infections the sensitivities to ampicillin of the strains isolated before and after treatment did not differ by more than one dilution.

All four patients with relapses were succesfully treated with tetracycline HCl, 2 g. daily for 5 days.

In the follow-up period after 2 weeks within 3 months, 65 patients returned with gonococcal infections. In fifty of these patients, the recognition of the gonococcal infection was preceded by one or more negative examinations. In only fifteen patients (eleven male civilians and four female civilians) had no previous examination taken place. All 65 patients admitted re-exposure to risk of infection. Together with the ten re-infections seen within the first 2 weeks, there was thus a total of 75 patients with re-infections in the follow-up period within three months (33 male civilians, 37 female civilians, and 5 sailors). The total re-infection rate for the patients follow-up within 3 months after treatment amounted to 14.6 percent in male civilians, 19.6 percent in female civilians, 9.1 percent in sailors, and 15.9 percent in all patients. All 75 patients with re-infections were retreated with our standard ampicillin treatment; 39 returned at least once within 2 weeks of re-treatment and cultures were negative in all.

During the 3 months after treatment, out of 227 male civilians 42 (18.5 percent) and out of 55 sailors 8 (14.5 percent) patients were treated for a post-gonococcal urethritis with tetracycline HCl 2 g. daily for 5 days.

Ampicillin serum levels in six male patients (A, B, C, D, E, and F) treated with 1 g. ampicillin intramuscularly, followed by 2 g. ampicillin 4 hrs later are presented in Figures 1 and 2. Three patients (A, B and C) had fasted overnight before the start of treatment and were not allowed to take any food or drink until 10 hrs after the start of treatment. Their ampicillin serum levels are presented in Fig. 1.

The other three patients (D, E, and F) had also fasted overnight before the start of treatment, but were allowed to take a light breakfast

15 min. before the oral dose. Their ampicillin serum levels are presented

in Fig. 2.

Appreciable variations in ampicillin serum values were found.

Two patients (A and E) showed high levels after the injection of 1 g. ampi-

cillin. Peak levels after injection were noticed after 1 hour in patients A, B,

C, E and F and after 2 hours in patient D. Peak levels after the oral dose

of 2 g. given 4 hrs after the injection were noticed after 1 hour in patient B,

after 2 hrs in patients A, C, D and F, and after 4 hrs in patientE. Patient

E absorbed the orally administered ampicillin very slowly. The light break-

fast did not seem to have any significant effect on the serum levels achieved

(Fig. 2).

The MIC's of ampicillin for the strains of N.gonorrhoeae iso-

lated in the six patients A, B, C, D, E and F were respectively: 0.64, 0.16,

0.005, 0.64, 0.01 and 0.01 µg/ml. All these patients were cured.

Discussion

In spite of intensive case-holding a high defaulter rate for male

civilians (20.6 percent compared with 6.4 percent for female civilians) was

found in the civilian population in the Rotterdam outpatient department. The

higher percentage of defaulters in males can partially be explained by the fact

that males without symptoms after treatment often assume that they are cured

and no further examination is necessary, while females, frequently symptom-

less before treatment, are anxious to know whether they are cured or not. High

re-infection rates within 3 months (14.6 percent in male civilians and 19.6 per-

cent in female civilians) are, like high defaulter rates, a common characteris-

tic of large venereal diseases clinics. In patients treated for gonorrhoea,

Willcox (1963) reported a re-infection rate within 3 months of 7 to 11 percent.

(St. Mary's Hospital, London). In our study the majority of patients with re-infec-

tions were re-infected by their regular consorts. Some of these consorts were

not named by the patients in the first interview and others refused to visit a

physician. These findings imply that, in the Rotterdam outpatient department, intensified contact tracing is of the utmost importance to reduce the re-infection rate.

A high cure rate of 99 percent was found in all patients (97.9 percent in male civilians, 100 percent in female civilians and sailors) who returned within 2 weeks. These results are comparable with those of the other ampicillin treatment schedules reported by Keys and others(1969), Gundersen and others (1969) and Eriksson (1971).

All four patients with relapses harboured strains of N.gonorrhoeae relatively resistant to ampicillin. The majority (8 out of 10) of strains isolated in patients with re-infections within 2 weeks was , on the contrary, sensitive to ampicillin. Of 388 strains from patients who returned within 2 weeks, 247 strains (63.7 percent) were sensitive and 141 strains (36.3 percent) were relatively resistant to ampicillin. For 175 strains from male civilians, the corresponding numbers were 113 strains (64.6 percent) sensitive and 62 strains (35.4 percent) relatively resistant to ampicillin (Table II).

According to these findings, male civilian patients with strains of N.gonorrhoeae relatively resistant to ampicillin might be more prone to relapses than male civilian patients with strains sensitive to ampicillin. The difference is statistically significant ($X^2 = 4.85$; $P < 0.05$); however, it does not consider the defaulter cases. Eriksson and Wallmark (1972) observed therapeutic failures with oral ampicillin treatment both when the strains were sensitive and when they were relatively resistant to the antibiotic.

Eickhoff, Kislak and Finland (1965) and Klein and Finland (1963) ascertained ampicillin serum levels by the agar diffusion method using Sarcina lutea (described by Grove and Randall, 1955) after a single intramuscular injection of 1 g. ampicillin in ten normal young men. Average ampicil-

lin serum levels in blood samples drawn ½, 1, 2 and 4 hrs after injection were

9.27, 10.10, 7.28 and 2.52 µg/ml respectively (Eickhoff and others, 1963).

These values are apparently lower than those obtained in our six patients.

The difference might be explained by the fact that our six

patients were confined to bed during the trial. Eriksson (1971) recorded a

personal communication of Wahlqvist and Lönell that in comparison with

healthy volunteers in patients confined to bed serum concentrations twice as

high and more were found. Eriksson (1971) ascertained blood serum levels,

using the method described by Grove and Randall (1955), after the oral ad-

ministration of 2 g. ampicillin in one dose in twelve healthy male volunteers.

Average mean ampicillin serum levels and standard errors after 2, 3, 5 and

8 hrs were 8.85 ± 0.93, 7.50 ± 0.71, 2.42 ± 0.35 and 0.29 ± 0.049 µg/ml

respectively.

In our patients, oral treatment was given 4 hrs after injec-

tion and therefore the results are not comparable. We found wide variations

in ampicillin serum values (Fig. 1 and 2). Eriksson (1973) also stressed the

fact that these variations can be found with both oral and intramuscular treat-

ment. However, with the exception of patient E, ampicillin serum levels were

high over a period of 8 hrs in comparison with the MIC's of ampicillin for

the strains of N.gonorrhoeae in our patients ($0.005\,\mu g/ml \leqslant MIC \leqslant 0.64\,\mu g/ml$).

Eriksson (1971) estimated that a minimum effective duration of 5 to 12 hrs,

and most likely 7 to 8 hrs, and a minimum ratio between maximum serum

concentration and MIC of two to five times, was required for succesful am-

picillin therapy. The treatment described in this paper satisfied both condi-

tions.

Summary

   In a series of 613 patients (286 male civilians, 202 female civilians and 125 sailors) 417 patients (192 male civilians, 172 female civilians and 53 sailors) returned for follow-up within two weeks after our standard ampicillin treatment. Using 1 g. ampicillin intramuscularly followed by an oral dose of 2 g. ampicillin 4 hrs later a cure rate of 99 percent was obtained in the 417 patients mentioned above. In the four patients with relapses, strains of N.gonorrhoeae relatively resistant to ampicillin (MIC ≥ 0.16 µg/ml) were isolated. Ampicillin serum levels were measured in six of the male patients.

References.

Eickhoff, C. , Kislak, J.W. , and Finland, M. (1965)
Amer. J. med. Sci. , 249, 163.

Ericsson, H. M. , and Sherris, J. C. (1971).
Acta path. microbiol. scand. , Section B, Suppl. 217.

Eriksson, G. (1970a) Acta derm. venereol. (Stockh.), 50, 451.
---(1970b) Ibid. , 50, 461.
---(1971) Ibid. , 51, 305
---(1973) Brit. J. vener. Dis. , 49, 353
---and Wallmark, G. (1972) Acta derm. -venereol
    (Stockh.), 52, 419.

Grove, D. C. , and Randall, W. A. (1955) Assay of Methods of Antibiotics,
p. 14. Medical Encyclopedia Inc. , New York

Gundersen, T. , Ødegaard, K. , and Gjessing, H. C. (1969)
Brit. J. vener. Dis. 45, 235.

Kercull, R. G. (1968) Milit. Med. (Wash.), 133, 985

Keys, T. F. , Halverson, C. W. , and Clarke, E. J. (1969)
J. Amer. med. Ass. , 210, 857

Klein, J. O. , and Finland, M. (1963) Amer. J. med. Sci. 245, 544.

Michel, M. F. , van Waardhuizen, J. P. and Kerrebijn, K. F. (1973)
Chemotherapy, 18, 77

Stolz, E. , Zwart, H. G. F. , and Michel, M. F. (1974)
Brit. J. vener. Dis. 50, 202

Thayer, J. D. , and Martin, J. E. (1966) Publ. Hlth. Rep. (Wash.) 81, 559

Willcox, R. R. (1963) Curr. Med. Drugs, 3, no. 12, p. 3
---, Woodcock, K. R. , Latto, D. , John, J. , Redmond, A. , Parker, R. B. ,
Rees, G. D. , and Cobbold, R. J. C. (1973) Brit. J. vener. Dis. , 49, 263.

Wols-van der Wielen, A. (1971) Ibid. , 47, 190

Fig.1

Ampicillin serum levels in three patients (A, B and C), who had fasted

overnight before the start of treatment and who were not allowed to take

any food or drink until 10 hours after the start of treatment. Treatment

consisted of 1 g. ampicillin intramuscularly followed by 2 g. ampicillin

orally 4 hours later.

Fig. 2

Ampicillin serum levels in three patients (D, E and F) who had fasted

overnight before the start of treatment and who were allowed a light

breakfast 15 minutes before the oral dose of ampicillin.

Treatment consisted of 1 g. ampicillin intramuscularly followed by

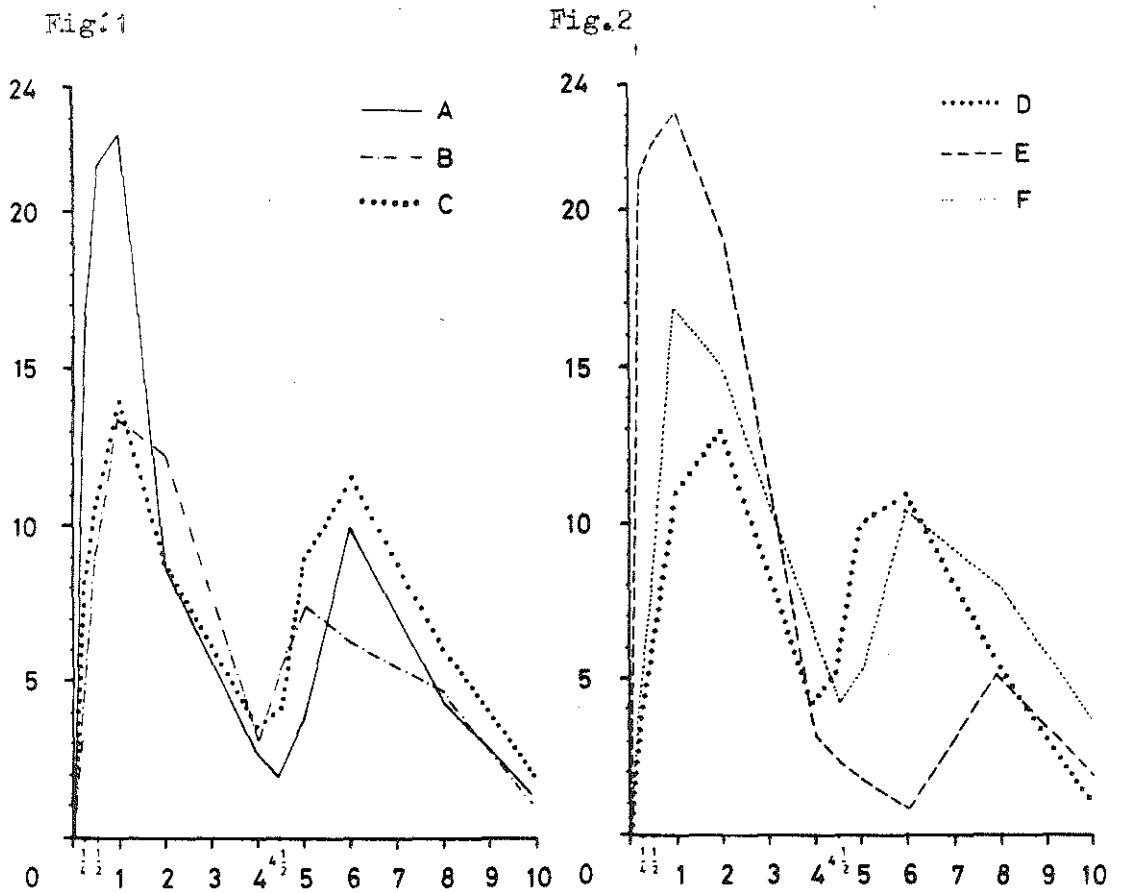2 g. ampicillin orally 4 hours later.

Table I Follow-up of ampicillin-treated patients

Follow-up	Male civilians		Female civilians		Sailors		Total	
	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.
Within 2 wks	192	67.1	172	85.1	53	42.4	417	68.0
Later than 2 wks but within 3 mths	35	12.2	17	8.4	2	1.6	54	8.8
No follow-up	59	20.6	13	6.4	70	56.0	142	23.2
Total	286	99.9	202	99.9	125	100.0	613	100.0

Table II Strains relatively resistant (RR) to ampicillin (MIC > 0.16 µg./ml.) among strains of N. gonorrhoeae isolated in the three patient groups.

Follow-up	MC[a] strains RR to ampicillin		FC[b] strains RR to ampicillin		S[c] strains RR to ampicillin		Total strains RR to ampicillin	
	No.	Per cent. of all MC strains	No.	Per cent. of all FC strains	No.	Per cent. of all S strains	No.	Per cent of all strains
Within 2 wks	62	35.4	53	32.3	26	53.1	141	36.3
Within 3 mths but later than 2 wks	16	50.0	6	35.3	1	50.0	23	45.1
No follow-up	21	42.0	5	38.5	51	73.9	77	58.4
Total	99	38.5	64	32.9	78	65.0	241	42.3

[a]MC = male civilian   [b]FC = female civilian   [c]S = sailor

Table III Relapses and re-infections among patients who returned for follow-up within 2 weeks

Result	Male civilians		Female civilians		Sailors		Total	
	No.	Per cent.	No.	Per cent.	No.	Per cent	No.	Per cent
Relapses	4	2.1	--	--	--	--	4	1.0
Re-infections	5	2.6	4	2.3	1	1.9	10	2.4
Relapses and re-infections	9	4.7	4	2.3	1	1.9	14	3.4
Total number of patients examined within 2 wks.	192	100.0	172	100.0	53	100.0	417	100.0

A FEMALE PATIENT WITH GONOCOCCAL TONSILLITIS

Few publications concerning gonococcal infections of the mucous membranes other than those of the urogenital tract, the rectum and the conjunctivae have appeared in the literature. Bronson (1919) surveyed 12 cases of gonococcal stomatitis described in literature. In one of these cases, the diagnosis was reached on culture of gonococci. Schmidt, Hjörting -Hansen and Philipsen (1961) described one other patient with this complaint. Gonococcal pharyngitis was described by Metzger (1970) and Fiumara, Wise and Many (1967). Fiumara, Wise and Many (1967) came across this complaint three times during an explosion of gonorrhoea in a group of homosexual men; the diagnosis was again based on the culture of gonococci. Only in one case could fermentation tests be made. In routine screening of 505 military subjects by means of gonococcal culture Thatcher ,Mc Craney , Kellog and Whaley (1969) found no gonorrhoea in the urethra nor in the rectum, but in one case the culture from the pharynx was positive. Glossitis gonorrhoica was described by Cowan (1969) in a female patient suffering from gonococcal cervicitis and urethritis. The diagnosis was reached with the aid of culture and fermentation tests. A questionable case of gonococcal tonsillitis was described by Iqbal (1971).

In a series of 200 patients suffering from gonorrhoea Hellgren (1971) found gonococci on tonsils in one woman out of thirty and in one man out of twenty,who admitted to their last sexual encounter having been orogenital (mouth patient-genitals partner). Bro-Jørgensen and Jensen (1971) found gonococci on tonsils in 12 of 161 non-selected Danish patients suffering from urogenital gonorrhoea. Eleven patients stated that their last sexual contact had been orogenital.

Of 49 foreign men, only in one gave a positive pharyngeal culture. By means

[1]Schuller, J. L. and Stolz, E. (1972)

Ned. T. Geneesk. , 116, 2216.

of a questionaire submitted to 71 men and 62 women of Danish nationality, these authors found, that 57 men (80%) and 38 women (61%) had orogenital contact, mostly with their permanent partner.

Case history

Patient A, a 25-year-old married women, visited our clinic on January 10th, 1972 in connection with a genito-genital contact with a foreign gonorrhoea patient on 12 December 1971. Gonorrhoea was ascertained in her and her husband. Both were treated with our "standard" therapy of 1 g. ampicillin intramuscularly (i.m.), followed by 2 g. ampicillin orally, 4 hours after the injection. The patient had regular sexual contact with her husband; on 26 December 1971 she had orogenital contact with him. On 17 January 1972 she stated during a check-up visit that her throat had been hurting her since around New Year's eve. She ran a temperature (39.2^{o}C), felt generally ill, had swollen and painful neck lymphglands and one day later pain in the left elbow. The family doctor confined her to bed and treated her with ampicillin (1 g. ampicillin per day during five days), after which only the throat complaint continued.

After treatment of the gonorrhoea on 10 January 1972, the throat complaints vanished for five days; then they recurred and the neck lymphglands increased in size and sensitivity. At the age of six the patient had undergone tonsillectomy. In 1970 she had suffered from Pfeiffer's disease.

During a general physical examination on 17 January 1972 we found inflamed tonsillary residues without any specific coating. There were multiple, painful lymphglands in front of the sternocleidomasteoid muscle on both sides of the neck. No abnormalities of the skin or the joints were ascertained. The temperature was 37.4^{o}C. In view of the case history and the complaints, a culture

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was taken from the tonsillary recesses and tested for gonococci which were indeed found. On 20 January 1972 we started treatment with Bicillin[2] (4.8 mega-units daily) for nine days. On 24 January 1972 the patient stated at a check-up that the sore throat had improved; there had been no more fever; however, the left elbow was still painful; the pain in the left elbow had vanished three days previously; the lymph glands in the neck had shrunk and no longer hurt. On that day the patient left for a holiday.

Bacteriological investigation

The existence of gonococci was demonstrated with the aid of culture on selective media according to Thayer and Martin (VCN), by microscopic examination of direct Gram-stained smears and by delayed immunofluorescence. The suspected gonococcal colonies were tested for oxidase activity and to determine whether they fermented only glucose in the sugar fermentation tests. Moreover, six colonies from all positive plates were used for ampicillin, penicillin and tetracycline sensitivity tests by the dilution method, the minimum inhibitory concentration (MIC) found being expressed in μg/ml. Furthermore, on serum from the patient the Gonococcal Complement Fixation Test (GCFT) was made and serological test for syphilis were performed by the National Institute for Public Health at Bilthoven.

Table I shows the results of the examinations on the various days.

Table I

No haemolytic streptococci or other pathogenic organisms could be cultured from the pharynx. The blood picture on that day was: Hb 7.9 mmol/l; the ESR 12 mm in the initial hour; leucocytes 4000/mm³.

The first note to Table I gives details of the identical outcome of the sensiti-

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[2]A combination of procaine penicillin G and sodium penicillin G in a ratio of 3:1.

vity tests on the gonococcal cultures taken from the patient and her husband at various sites. These data can be regarded as confirmation of the case history obtained by questioning of the patient (genitogenital and orogenital contact with her husband after genito genital contact with a strange partner).

Discussion

Our findings with this patient led us to question 178 consecutive gonorrhoea patients, visiting our clinic in the period from 18 October 1971 to 31 December 1971, about their manner of sexual contact during recent sexual encounters. We considered the following possible modes:

genito-genital (genitals patient in contact with genitals partner);

genito-oral (genitals patient in contact with mouth partner);

oro-genital (mouth patient in contact with genitals partner);

genito-anal (genitals patients in contact with rectum partner); and

ano-genital (rectum patient in contact with genitals partner).

The results are summarized in Table II.

Of the 178 patients, a total of 19 admitted to orogenital and a further 22 to genito-oral contact. The percentages of genito-oral contact within each group of patients were:

20% for the Dutch women; 22% for the Dutch men; none for the foreign men (non-sailors); 3% for the sailors.

The percentages of oro-genital contact were:

25% for the Dutch women; 7% for the Dutch men; none for the foreign men (non-sailors); 6% for the sailors. It seems reasonable to expect from these figures that the incidence of gonorrheal stomatitis, tonsillitis and pharyngitis was appreciable in the patients visiting our clinic, especially among the Dutch civilians.

It is a well known fact that urogenital gonococcal infections can lead to such complications as salpingitis, prostatitis and epididymitis. Of recent years, more frequent incidence of other complications in the shape of gonococcal sepsis, arthritis and dermatitis has been reported, particularly in cases where the gonorrhoea was not recognized. Metzger (1970) described a patient suffering from gonococcal pharyngitis, sepsis and polyarthritis. Our female patient also had gonococcal tonsillitis, lymphadenitis and possibly sepsis and a touch of arthritis while suffering from urogenital and rectal gonorrhoea. Various different therapies for gonococcal tonsillitis have been reported in the literature. Moreover, only occasionally is the sensitivity of the bacteria to the antibiotic used reported. The large number of failures after treatment with the standard therapies is conspicious.

Bro-Jørgensen and Jensen (1971) initially treated their 12 patients, suffering from gonococcal tonsillitis with one dose of 2 g. ampicillin and 1 g. probenecid orally. In 7 of these patients, 3 consecutive gonococcal cultures from the tonsils were negative after this treatment. All 7 patients were asymptomatic. After treatment the oropharyngeal cultures from the other 5 patients continued to be positive; 2 of the 5 patients had no complaints, however. One patient of the group of 5 then gave 3 negative cultures after one intramuscular injection of 5 mega-units penicillin in combination with 1 g. probenecid. Three of the 5 patients were treated with tetracycline 250 mg four times daily for 1 week. During the check-up positive gonococcal cultures were found in 2 of these 3 patients; in both, the gonococcal colonies were relatively resistant to penicillin and tetracycline, unlike the strains in the other cases. The relatively resistant gonococci remained in the tonsils for 8 weeks. As our female patient had already been treated with ampicillin by her family doctor and since she had not reacted to our standard treatment, while

suffering from a number of other possible gonorrheal complications, we decided to treat her with the above-mentioned therapy of daily 4.8 mega-units Bicillin[2] i. m. for 9 days. The failure of our standard therapy for the gonococcal tonsillitis cannot be ascribed to the observed ampicillin sensitivity of the isolated gonococcus strain.

Postscript

Since we started taking tonsil swabs to culture for gonococci as part of the routine examination of each patient visiting our clinic, we frequently find gonococci carriers, who are usually asymptomatic. More extensive study of a larger group of patients would be necessary to work out all the epidemiological, clinical and therapeutic consequences of this finding.

Summary

A patient with gonorrheal tonsillitis - The case is reported of a married woman aged 25 years who suffered from gonorrheal tonsillitis.

The diagnosis was made after a course of ampicillin treatment by the family physician for an angina with an atypical evolution, lymphadenitis and arthritis, and a course of ampicillin treatment administered by the authors for gonorrheal cervicitis, urethritis and proctitis.

The literature on gonorrheal tonsillitis pharyngitis and stomatitis is reviewed. The problems of the treatment of this disease are also considered.

References

Bro-Jørgensen, A. and Jensen, T. (1971)

Brit. med. J. IV, 660.

Cowan, L. (1969)

Brit. J. vener. Dis., 45, 228.

Fiumara, N. J., Wise, H. M. and Many, M. (1967)

New Engl. J. Med., 276, 1248.

Hellgren, L. (1971)

Läkortidningen, 68, 569.

Iqbal, Y. (1971)

Brit. J. vener. Dis., 47, 144.

Metzger, A. L. (1970)

Ann. intern. Med., 73, 267.

Schmidt, H., Hjörtingh-Hansen, E. and Philipsen, H. P. (1961)

Acta derm. -venereol. (Stockh.), 41, 324.

Thatcher, R. W., Mc Craney, W. T., Kellogg, D. S. and Whaley, W. H. (1969)

J. Amer. med. Ass., 210, 315.

Table I

Results of the examination of patient A on various days.

	Date (January 1972)					
	10	17	20	24	26	28
Culture						
Cervix	+[1]	-		-		
Urethra	+[1]	-		-		
Rectum	+[1]	-		-		
Tonsils		+[1]	+[1]	-	-	-
Delayed direct immunofluorescence						
Cervix	+	-		-		
Urethra	-	-		-		
Rectum	-	-		-		
Tonsils		+		-	-	-
Gram-smears						
Cervix	+	-		-		
Urethra	+	-		-		
Rectum						
Tonsils			+[2]			
Gonococcal Complement Fixation Test (GCFT)	1:2			1:4		
Serologycal reactions[3] on syphilis	-			-		

[1] The MIC for ampicillin was 0.04 µg/ml, for penicillin 0.005 µg/ml and for tetracycline 0.32 µg/ml.

[2] The same values were found for the colonies isolated from the husband on 10 January. The smear preparation showed: squamous cells, no leukocytes and miscellaneous flora such as Gram-negative diplococci.

[3] Kolmer reaction, VDRL, RPCF, TPI and FTA/ABS negative.

Table II

Type of sexual contact in different groups of patients.

Type of contact patient-partner	Inhabitants of Rotterdam and surroundings and other non-sailors.						Sailors Dutch and foreign	
	Dutch				Foreign			
	Women		Men		Men		Men	
	No. (56)	Per cent. (100)	No. (46)	Per cent. (100)	No. (44)	Per cent. (100)	No. (32)	per cent. (100)
Genital-genital	51	91	40	87	44	100	31	97
Genital-oral	11	20	10	22	–	–	1	3
Oral-genital	14	25	3	7	–	–	2	6
Genital-anal	–	–	3	7	–	–	–	–
Anal-genital	2	4	7	15	1	2	–	–

GONOCOCCAL ORO- AND NASOPHARYNGEAL INFECTION.

Although known to occur in the past, it is only in recent years that the incidence and importance of oral infection by the gonococcus has come to be assessed. The first case of gonococcal pharyngitis was described by Fiumara, Wise, and Many (1967). Thatcher, McCraney, Kellogg, and Whaley (1969) found no gonococci in the urethra and rectum in a routine screening by culture of 505 military subjects; in one case, they obtained a positive culture from the pharynx. Gonococcal ulceration of the tongue was described by Cowan (1969) in a female patient with gonococcal cervicitis and urethritis. Metzger (1970) described one patient with gonococcal pharyngitis, septicaemia, and polyarthritis. A possible case of gonococcal tonsillitis was described by Iqbal (1971).

In a series of fifty patients with gonorrhoea who admitted recent orogenital contact, Hellgren (1971) isolated gonococci from the tonsils in one out of twenty women and one out of thirty men. Bro Jørgensen and Jensen (1971), investigating unselected Danish patients with gonorrhoea, found gonococci in the tonsils in six (6 per cent.) of 95 Danish men and in six (9 per cent.) of 66 women. Among 49 foreign men with gonorrhoea, the incidence was 2 per cent. Of 71 Danish men 57 (80 per cent.), and of 62 Danish women 38 (61 per cent.), stated that they had had orogenital contact during their last sexual experience; it was much more commonly practised with regular partners than with casual contacts.

Owen and Hill (1972) reported eleven positive cultures from the pharynx in an investigation of 79 homosexual men in the U. S. A. Rodin, Monteiro, and Scrimgeour (1972), in London, found only one positive culture in 73 throat swabs from 65 male homosexuals, 33 of whom had urethral or

1. Stolz, E. and Schuller, J. L. (1974) Brit. J. vener. Dis.,50, 104.

rectal gonorrhoea; half of these 65 patients admitted recent orogenital contact. Ratnatunga (1972) described three gonococcal infections of the pharynx in male homosexuals. These three cases were diagnosed in a V. D. clinic where 113 men with gonorrhoea (including 23 homosexuals) were diagnosed in the same year.

Schuller and Stolz (1972) described a female patient with gonococcal tonsillitis and polyarthritis.

Wiesner, Tronca, Bonin, Pedersen, and Holmes (1973) found a frequency of 5-6 per cent. in an investigation of 2224 patients in a V. D. clinic. Among the patients with gonorrhoea, infection of the oropharynx was found in 20. 9 per cent. of the homosexual men, 10. 3 per cent. of the women, and 3. 2 per cent. of the heterosexual men. Treatment with procaine penicillin G (4. 8 m. u.) and tetracycline HCL (2 g. daily for 5 days) was succesful, while treatment with 4 g. spectinomycin in a single dose was often unsuccesful.

We now report a series of patients with positive cultures of material from the oropharyngeal and tonsillar (OP/T) areas. Special attention is paid to the epidemiology, bacteriology, and results of treatment of gonococcal infection at this site.

Material and methods.

The patients consisted of males and females from the civilian population, and male sailors. The investigation was carried out in three periods:

(1) After the recognition of a female patient with gonococcal tonsillitis, we took OP/T specimens in the period from January 20 to August 1, 1972, from some of those patients who admitted recent orogenital contact.

(2) From August 1,1972, to January 1, 1973, OP/T specimens were taken from all patients at each visit. The average number of swabs taken was 2. 6 for males, 3. 4 for females,1. 6 for sailors, and 2. 4 for the group as a whole.

(3) It is known that the nasopharynx can harbour <u>Neisseria meningitidis;</u> in order to investigate whether it can also harbour <u>Neisseria gonorrhoeae</u> gonococcal cultures were made from the nasopharynx (NP) as well as from the OP/T during the last two months of 1972. Special flexible nasopharyngeal swabs were passed via the nares.

During all the investigation periods, culture material was also taken from the cervix (C), urethra (U), and rectum (R) in the cases of women, and from the urethra (U) in men.

If indicated, prostatic fluid (P) and material from the rectum (R) were also investigated in men.

The sampling was carried out with carbon-impregnated swabs, which were then placed in Stuart's transport medium. The material was inoculated within 4 hrs on to a selective medium like that described by Thayer and Martin (1966). The medium consisted of GC Base (Difco) with haemoglobin (Difco) and isovitalex (BBL).

The following antibiotic mixture was added:

3 μg/ml.vancomycin

7.5μg/ml. Na-colistimethate

12.5 i.u/ml. nystatin

Incubation was carried out at 37°C. in a CO_2-rich atmosphere. If suspect colonies were observed after 48 hrs, Gram preparations were made, the oxidase test applied, and sugar fermentations carried out. The strains isolated from the OP/T and from the NP, like those isolated from the urogenital/rectal region, were tested for sensitivity to ampicillin, penicillin, and tetracycline by the plate-dilution method, using GC base, haemoglobin (Difco), and isovitalex (BBL). Apart from the gonococcal strains from the patients, three gonococcal strains of a known sensitivity pattern and one Oxford staphylococcus were inoculated as controls. The minimum inhibitory concentration (MIC) was estimated using two-fold dilutions:

between 0. 64 and 0. 005 μg/ml. for ampicillin

between 1. 28 and 0. 0025 μg/ml. for penicillin

between 2. 56 and 0. 04 μg/ml. for tetracycline

Results.

Frequency of positive OP/T and NP cultures.

During the first investigation period, seven patients had positive OP/T

cultures. The numbers of these patients according to age and sex are

shown in brackets in Table I.

In the second investigation period, genococci were found

in the OP/T cultures in 23 patients. The number of cases diagnosed in

this second investigation period will be used for the frequency calculations.

In the third investigation period, out of 160 patients with

gonorrhoea, we found two with gonococci in the NP; one of the two also

had a positive OP/T culture, and was thus classified with the 23 patients

from the second investigation period. Both these patients with positive

NP cultures were from a group of 452 new patients.

The thirty positive patients from the first (in brackets) and

the second investigation periods were divided as follows among the various

groups:

Male civilians 9 + (2); Females 11 + (4); Male sailors 3 + (1)

The number of individuals with gonococci in the OP/T cul-

tures, taken as a percentage of all the patients with gonorrhoea in the second

investigation period was as follows:

Male civilians 3. 6 per cent. (9 out of 250);

Females 8.5 per cent. (11 out of 130);

Male sailors 3. 4 per cent (3 out of 87);

Total 4. 9 per cent. (23 out of 467)

When the percentage of individuals with gonococci in the OP/T was cal-
culated for all new patients seen in the second period, the following
figures were found:

Male civilians 1. 3 per cent. (6 out of 455);

Females 3.4 per cent. (9 out of 268);

Male sailors 0. 7 per cent. (3 out of 410);

Total 1. 6 per cent. (18 out of 1,133)

The age distribution of the thirty patients from both inves-
tigation periods with positive OP/T cultures is given in Table I (numbers
in brackets are those from the first period). The youngest patient was a
16-year -old girl; the oldest a man aged 57.

Of the eleven male civilians, five + (2) were Dutch, two
were from Surinam, one from the Dutch Antilles, and one from Pakistan.
Of these eleven men, four +(2) were single, four married, and one divorced,
and one + (1) was homosexual. Two heterosexual single men lived with their
partner.

Of the fifteen females, nine + (4) were Dutch, one was from
Surinam, and one from England; seven + (2) were single, one + (2) married,
and three divorced.
The latter and one of the single women lived with their partner.

Of the four sailors, two were West German, one was Finnish,
and one a Portuguese from the Cape Verde Islands; two + (1) were single and
one married.

Information about the sites where gonococci were isolated in
the patients with positive OP/T cultures and their partners with gonorrhoea
is given in Table II, which also shows the common minimum inhibitory con-
centrations (MIC's) for ampicillin, penicillin, and tetracycline of the gono-
coccal strains isolated in these patients and their partners, and whether

they admitted orogenital contact.

Of the thirty patients, nineteen had both urogenital gonorrhoea and positive OP/T cultures. In nine patients a positive OP/T culture was found in a follow-up check after ampicillin treatment of urogenital gonorrhoea. An isolated OP/T culture was found in two patients, in one of whom this culture was followed by a positive urethral and OP/T culture; the gonococci had the same sensitivity pattern in all tests on this patient.

Symptoms.

Of the thirty patients, 25 were asymptomatic. Three complained spontaneously of a slight sore throat. Two women had a severe sore throat; one of these had a raised temperature but a blood culture for the gonococcus was negative; the other had regional lymphadenitis and arthritis, probably gonorrheal in origin (Schuller and Stolz, 1972).

Treatment.

All patients with uncomplicated gonorrhoea were given the standard treatment of 1 g. ampicillin intramuscularly followed by 2 g. ampicillin by mouth 4 hrs later.

If this treatment was not suitable (e. g. because of hypersensitivity to penicillin), the patient was given 2 g. tetracycline HCL daily for 5 days.

Patients with a positive OP/T culture after ampicillin treatment and those known to have a positive OP/T culture before treatment was started were given 4. 8 m. u. Bicillin (3 parts procaine penicillin G + 1 part sodium penicillin G) intramuscularly daily for 10 days.

Follow-up tests were planned for 1 and 2 weeks after treatment and in the case of females again at 6 and 12 weeks.

In order to compare the results of various therapies, we considered only those cases in which the OP/T culture was positive at the time of treatment and in which at least two successive OP/T cultures were negative after treatment.

Of the nineteen patients with positive urogenital and OP/T
cultures fourteen were given ampicillin with ten faillures and four were
given tetracycline with one failure; four of the failures defaulted, seven
were re-treated with Bicillin and five of the latter who attended for follow-
up were all cured. One sailor (Case 28) was treated with 1.2 m.u. procaine
penicillin daily for 14 days because he also had secondary syphilis but the
OP/T culture remained positive.

Of the nine patients who had positive OP/T cultures only
after ampicillin treatment for urogenital gonorrhoea, two defaulted and
seven were given Bicillin; the five of the latter who attended for follow-
up were all cured. The two patients with positive OP/T cultures who had
not had urogenital gonorrhoea defaulted without receiving any treatment .

Discussion.

The frequency of gonococci in the OP/T in our patients with
gonorrhoea (3.6 per cent. for male civilians and 8.5 per cent. for fema-
les) is similar to that found by Bro Jørgensen and Jensen (1971), viz.
6 and 9 per cent. Like them we also found a lower incidence among fo-
reigners; of the 75 foreign male civilians only one (1.3 per cent.) had a
positive OP/T culture.

In a former study (Schuller and Stolz, 1972), we found on
routine questioning of a series of patients at the VD clinic that 25 per cent.
of the women admitted orogenital contact. The possibility of OP/T infec-
tion is clearly apparent from Table III.

The distribution by age, nationality, marital status, and
MIC for ampicillin and tetracycline among those with positive OP/T cultures
could not be compared with the overall distribution among all the gonor-
rhoea patients and new patients, because of the small numbers involved.
However, it is striking that in only two patients (Cases 12 and 30) was a
difference found in the MIC patterns for ampicillin, penicillin, and tetra-
cycline between the urogenital and OP/T strains. In all other cases, the

sensitivity patterns agreed within one dilution.

Of the twenty partners investigated, only one (the female contact No. 8 who was also Case 12) was found to have markedly different MIC's for ampicillin, penicillin, and tetracycline for the strain cultured from the OP/T. In the nineteen other partners, there was close agreement between their sensitivity patterns and those of the partner's strain. Case 12 (=partner 8) admitted to both orogenital and genito-genital contact with her partner; she stated that the last orogenital contact with a person other than her present partner had occurred 4 years previously.

Recent orogenital contact was not admitted in several cases (see Table II); one man who denied such contact explained the presence of gonococci in his oropharynx by fellatio followed by kissing.

The fact that nine patients showed a positive OP/T culture only at a follow-up check after treatment of urogenital gonorrhoea is not surprising. OP/T cultures from patients in our series who received no treatment between the taking of several specimens also did not give constantly positive results. It is likely that at least three cultures are needed to detect all possible cases of OP/T infection. Bro Jørgensen and Jensen (1971) have pointed out that the treatment normally given in cases of urogenital gonorrhoea was often not effective against OP/T infection. This has been confirmed by our study.

Summary.

The epidemiology, bacteriology, and therapy of gonococcal pharyngitis are discussed with reference to 31 patients.

The incidence of pharyngeal infection among 467 consecutive patients with urogenital gonorrhoea was 4.9 per cent. (3.6 per cent. for male civilians, 8.5 per cent. for females, and 3.4 per cent. for sailors). Sensitivity patterns to ampicillin, penicillin, and tetracycline of the strains isolated from both sites were compared in each patient and also with the strain isolated from the partner. Routine therapy (1 g. ampicillin intra-

muscularly followed by 2 g orally 4 hours later) usually failed to eradicate gonococci from the pharynx. However, the administration of a combination of 3.6 m.u. procaine penicillin and 1.2 m.u. sodium penicillin intramuscularly daily for 10 days was succesful in all cases.

References

Bro-Jørgensen , A. and Jensen, T. (1971)

Brit. med. J. , 4, 660.

Cowan, L. (1969)

Brit. J. vener. Dis. , 45, 228.

Fiumara, N. J, Wise, H. M. and Many, M. (1967)

New Engl. J. Med. , 276, 1248.

Hellgren, L. (1971)

Läkartidningen, 68, 569.

Iqbal, Y. (1971)

Brit. J. vener. Dis. , 47, 144.

Metzger, A. L. (1970)

Ann. intern. Med. , 73, 267.

Owen, R. L. and Hill, J. L. (1972)

J. Amer. med. Ass. , 220, 1315.

Ratnatunga, C. S. (1972)

Brit. J. vener. Dis. , 48, 184.

Rodin, P. , Monteiro, G. E. and Scrimgeour, G. (1962)

Brit. J. vener. Dis. , 48, 182.

Schuller, J. L. and Stolz, E. (1972)

Ned. T. Geneesk. , 116, 2216.

Thatcher, R. W. , McCraney, W. T. , Kellogg, D. S. and Whaley, W. H. (1969)

J. Amer. med. Ass. , 210, 315.

Thayer, J.D. and Martin, J.E. (1966)

Publ. Hlth. Rep. (Wash.), 81, 559.

Wiesner, P.J., Tronca, E., Bonin, P., Pedersen, A.H.B. and Holmes, K.K. (1973)

New Engl. J. Med., 288, 181.

Table I.

Sex and age distribution of patients with positive OP/T cultures
during the first and second investigation period.

Sex	Age (yrs)				
	16 – 20	21-25	26-30	31-35	>36
Male civilians	--	2	4 + (1)	2	1 + (1)
Females	2 + (2)	3 + (1)	5	--	1 + (1)
Male sailors	1 + (1)	--	2	--	--
Total	3 + (3)	5 + (1)	11 + (1)	2	2 + (2)

Figures in brackets indicate patients of the first investigation period.

Table II Sites of gonococcal infection in patients with positive OP/T cultures and in the partners with gonorrhoea, common MIC's of strains isolated for ampicillin, penicillin, and tetracycline, and incidence of orogenital contact.

Patients with positive oropharynx tonsils (OP T) cultures			Corresponding partners with gonorrhoea			Common MIC's			Orogenital contact admitted
Group	Case no.	Other positive sites	Partner no.	Sex	Positive sites	Ampicillin (µg/ml.)	Penicillin (µg/ml.)	Tetracycline (µg/ml.)	
Males	1	U	1	Female	CUR	0.64	0.16	0.64	+
	2H	U	-			0.64	0.64	1.28	+
	3	U	2	Female	CU	0.04	0.01	0.08	-
	4	U	-			0.32	0.64	2.56	-
	5	U	3	Female	CU	0.64	1.28	1.28	+
	6	U/PF	4	Female	CUR	0.02	0.01	0.32	+
			5	Female	CUR/OP/T	0.04	0.01	0.32	
	7	U	6	Female	CU	0.04	0.02	0.16	+
			7	Female	CU	0.04	0.02	0.16	
	8	U	8	Female	CU/OP/T *1	0.64	0.64	1.28	+
	9	U	-			0.04	0.005	0.16	+
	10 H	R	9 H	Male	U	0.04	0.01	0.16	+
	11	U	10	Female	CU	0.08	0.04	0.32	+
Females	12	CU*1	11	Male	U/OP/T	0.64	0.64	1.28	+
	13	C	12	Male	U	0.02	0.04	0.32	-
	14	CU	13	Male	U	0.04	0.0025	0.16	+
	15	CUR	14	Male	U	0.04	0.01	0.16	+
	16	CU	-			0.04	0.02	0.16	+
	17	CUR	15	Male	U	0.32	1.28	1.28	+
	18	C	16	Male	U	0.08	0.04	0.64	+
	19	CU	-			0.02	0.005	0.16	+
	20	CUR	17	Male	U/PF/OP/T	0.02	0.01	0.32	-
	21	CU	18	Male	U	0.16	0.08	0.64	+
	22	CUR	-			0.16	0.08	0.64	-
	23	CU	19	Male	U	0.32	0.16	0.64	-
	24	CU	-			0.64	0.32	0.16	+
	25	CU	-			0.04	0.01	0.16	-
	26	CUR	20	Male	U	0.32	1.28	1.28	+
Sailors	27	U	-			0.02	0.01	1.28	-
	28	U	-			0.32	0.64	1.28	?
	29	NP	-			0.16	0.08	0.64	?
	30	U*2	-			0.64	1.28	2.56	-

H = homosexual

U = urethra   Patient 6 = partner 17   *1 and *2 OP/T isolated showed different MIC's
PF = prostatic fluid   Patient 8 = partner 11   *1 0.02   0.01   0.16   ? not asked
R = rectum   Patient 12= partner 8   *2 0.16   0.08   0.16
C = cervix   Patient 20=partner 5
NP = nasopharynx
OP/T=oropharynx/tonsils

Table III

Type of sexual contact in different groups of patients.

Type of contact patient-partner	Inhabitants of Rotterdam surroundings and other non-sailors						Sailors	
	Dutch				Foreign		Dutch and foreign	
	Women		Men		Men		Men	
	No. (56)	Per cent. (100)	No. 46	Per cent. (100)	No. 44	Per cent. (100)	No. 32	Per cent. (100)
Genital-genital	51	91	40	87	44	100	31	97
Genital-oral	11	20	10	22	--	---	1	3
Oral-genital	14	25	3	7	--	--	2	6
Genital-anal	--	--	3	7	--	--	--	--
Anal-genital	2	4	7	15	1	2	--	--

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Chapter XV[1]

A FEMALE PATIENT WITH THE SEPTIC GONOCOCCAL DERMATITIS SYNDROME.

Skin complaints as a complication of urogenital gonorrhoea was first described by Vidal in 1893. In 1903 Silvestrini described a syndrome consisting of fever, arthritis and skin lesions in a patient suffering from gonococcal sepsis. Before the discovery of antibiotics, this syndrome was frequently reported.

For gonococcal sepsis, a distinction has been made between a fairly benign variant and one with a violent course terminating in a fatal endocarditis (Keil, 1938). After the introduction of penicillin, it was not until 1963, that the first publication concerning the syndrome described by Silvestrini (1903) appeared. (Abu-Nassar, Hill, Fred and Yow). Since then, various authors have reported cases of patients with the septic gonococcal dermatitis syndrome (Ackerman, Miller and Shapiro, 1965; Björnberg and Gisslén, 1965; Fred, Eiband, Martincheck and Yow, 1965; Danielsson and Michaelsson, 1966; Kahn and Danielsson, 1969; Björnberg, 1970; Ackerman, 1970; Barr and Danielsson, 1971; Forström, Mustakallio, Sivonen and Kousa, 1972; Laugier and Orusco, 1972; Shapiro, Teisch and Brownstein, 1973). Over a hundred patients with septic gonococcal dermatitis have been described in the various count ries of the world. Over 50% of these patients have been found in Sweden. Data on the frequency of occurrence of this syndrome in Sweden have been published by various authors. For example Björnberg and Gisslén (1965) give a frequency of 1-2% for patients with gonorrhoea. Barr and Danielsson (1971) found a percentage of 1.9% (3% in women and 0.7% in men).

Case history

Female patient A, divorced, 36 years of age, had been referred

by her family doctor to the Clinic for Rheumatic Diseases on 17 January 1973

in connection with painful joints in various areas. The fact that said patient

had recently had sexual contact with a friend, who after this encounter had

been treated for gonococcal urethritis, and the clinical presentation pointed

towards gonococcal arthritis.

Therefore on 18 January 1973 she was examined in the Clinic for Venereal

Diseases. On 14 January 1973 she had been struck by acute pain, swelling and

restriction of the movement of the left wrist. She could not move her fingers

on the left side. Some days previously there had been some slight trouble in

the left wrist and elbow; the ankle and knee on the left side had been painful

and swollen. At the same time pustules had appeard above the joints. A soli-

tary pustule was discernable at another site. Although the patient had clear-

ly noticed the pustules, she did not believe them to be of importance and did

not report them to the doctors of her own accord.

The patient had suffered from vaginal discharge for a long time. Otherwise

she had no symptoms to report. Until 29 October 1972 she had had regular

sexual contact with a Yugoslavian friend. After that date she had sexual con-

tact with a Dutch partner, the last coitus having occurred on 11 January

1973. On 15 January 1973 this partner consulted a dermatologist in Rotter-

dam on account of a gonococcal urethritis.

Patient A's joints showed the following symptoms. The left wrist joint was

slightly swollen and there was minor restriction of the dorsal and volar flexion.

The joint was painful in the ultimate position. There was a minor swelling un-

der the left malleolus medialis. Whitish-yellow pustules of the size of a

barley grain, surrounded by a livid erythema (see photographs), werd found

scatterd over the body, especially on the right wrist, the lower parts of the

legs and on the right ankle. A total of 12 pustules were counted.

Furthermore a white, cream-coloured discharge was observed at the ure-

thral orifice and in the specula; the rectal temperature was 38°C.

<u>Laboratory tests</u>

A.   Examination for gonorrhoea

Gram-stained smears from the cervix and urethra were examined under the

microscope. Both smears were negative. The Gram-stained smear from the

pustule showed objects which might pass for deformed Gram-negative diplo-

cocci and for remains of Gram-negative cocci.

The selective medium according to Thayer and Martin (1966) was used for the

culture of gonococci. Material for the cultures was obtained from the cervix,

the urethra, the rectum, the oropharynx and from a pustule. A blood cultu-

re was made at the same time. Growth of suspicious-looking colonies was

found only on the plates with material from the cervix and the urethra. The

identification of the bacteria as gonococci was carried out as follows. The

plate yielded gram-negative diplococci. The colonies were oxidase-positive.

The bacteria isolated fermented glucose only in the sugar fermentation tests.

A dried immunofluorescent preparation of material from the pustule was made

with the aid of a non-absorbed fluorescent anti-gonococcal serum, prepared

in our own laboratory. In this preparation fluorescent objects were discer-

nable which might pass for deformed diplococci or remains of cocci. It was

thought that this result might be due to the presence of gonococci in the skin.

However, a deparaffinized skin section treated with the same antigonococcal se-

rum showed no fluorescent cocci, possibly owing to strong concomitant fluo-

rescence of tissue cells. The Gonococcal Complement Fixation Test (GCFT)

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performed in the National Institute for Public Health (Rijks instituut voor Volks-

gezondheid - RIV) at Bilthoven, was negative.

B.   Histopathological examination

Examination of a pustule (see photographs)

The sections to be examined by direct microscopy were embedded in para-

plast, and were stained as follows: hematoxyline-azofloxine, PAS, PTAH,

Lendrum, Giemsa and Gram. Histopathological changes were present both

in the epidermis and in all layers of the dermis. The most spectacular change

in the epidermis consisted of a relatively small intraepidermally, unilocu-

larly, subcorneally implanted pustule.

The pustule contained numerous neutrophilic polymorphonuclear leukocytes,

nuclear debris, necrotic epidermal debris and a few erythrocytes. The

adjoining epidermis showed evidence of some slight acanthosis and spongio-

sis. The Giemsa-stained and Gram-stained preparations showed no bacteria

within and around the pustule. Three morphological characteristics were pre-

sent in the dermis, viz changes of the blood vessels, inflammatory infil-

trates and oedema. The vascular changes were found predominantly in the

upper and middle layer of the dermis and referred to capillaries, minor

arterioles and venulae. Severe endothelial swelling and invasion of the ar-

teriole walls was clearly apparent. In a single case a lumen was found which

had been blocked off by thrombotic material and fibrinoid necrosis of the

arteriole wall as demonstrated by PTAH and Lendrumstaining. A cellular

infiltration consisting of lymphocytes, hisitocytes and a number of poly-

morphonuclear cells and cell debris was present in the dermis, particu-

larly round the blood vessels. Moreover small infiltrates of inflammation

cells and erythrocytes were to be observed scattered over the middle and lower

layer of the dermis. The upper part of the dermis clearly displayed oedema.
This oedema diminished away from the surface.

C.   Other laboratory investigations

ESR 50 mm in the initial hour. Hb 7.7 mmol/l, leukocytes $7500/mm^3$.

Differentiation: Bas.1, Eos. 1, Bars. 2,Neutr. 62, Lymph. 32, Mon. 2.

Serological tests for syphilis (Kolmer, VDRL, RPCF, TPI, FTA/ABS)per-
formed in the RIV at Bilthoven, were negative.

AST: 125 U. Throat swab: normal throat flora.

D.   X-ray tests

X-ray photographs of the wrists, elbows, ankles and knees showed no abnor-
malities.

E.   Complementary investigation.

Because of the substantial clinical and bacteriological indications of a septic
gonococcal dermatitis syndrome, a report of the case history of the patient, to-
gether with diapositive slides, smears and sections of the pustules, was sent
for verification to Dr.D.Danielsson, Department of Clinical Bacteriology,
Regional Hospital, Ørebro, Sweden. In one of the two smears he found a few
gonococci by direct immunofluorescence. The skin section showed the typi-
cal histological picture of a pustule due to septic gonococcal dermatitis. Gono-
cocci were observed in one skin section by direct immunofluorescence. Da-
nielsson considered on the basis of these findings, the case history and the
clinical picture that septic gonococcal dermatitis could be diagnosed with cer-
tainty.

Treatment and course of the complaint.

          The patient was treated with intramuscular penicillin for 10

days (4.8 mega-units of Bicillin[2] daily). The pustules and the pains in the

joints disappeared after a few days. On 26 January 1973 all gonococcal cul-

tures were negative. On 14 February 1973 the patient was referred back

to the Clinic for Rheumatic Diseaesss.The ESR amounted to 17 mm. On 6

March 1973 the patient was examined again at the Clinic for Venereal Diseases.

No complaints pertaining to painful joints were found on that occasion.

Discussion

The skin lesions found in the septic gonococcal dermatitis

syndrome are generally described as maculous, papulous, haemorrhagic,

vesiculo-pustulous and bullous. The lesions, varying in diameter from 1 mm

to 2 cm may have an erythematous halo. They are often situated above

the joints, especially on the distal part of the extremities. (Keil, 1938;

Abu-Nassar, Hill, Fred and Yow, 1963;Ackerman, 1970; Bjørnberg,

1970).

Whenever one observes the trias of fever, rheumatic com-

plaints and typical skin lesions, one should consider the possibility of sep-

sis and look for gonococci by urogenital/rectal and pharyngeal examination.

(Keil, 1938;Abu-Nassar, Hill, Fred and Yow, 1963; Björnberg, 1970; Wies-

ner, Tronca, Bonin, Pedersen and Holmes, 1973). Furthermore, the blood

and the skin should also be examined for the presence of gonococci. Attempts

to prove the presence of gonococci in the blood generally give disappointing

results. For instance, Björnberg (1970) and Barr and Danielsson (1971) re-

ported that positive blood cultures were found in only 20% of the cases.

It is also difficult to demonstrate the presence of gonococci

[2]Bicillin=procaine penicillin G + sodium penicillin G in a ratio of 3:1

in skin lesions with the aid of Gram-stained smears, gonococcal cultures and histological methods. Additional support for the diagnosis may be expected from immunofluorescence techniques. The first succesful result with this procedure was described by Danielsson and Michaëlsson (1966). Kahn and Danielsson (1969) indicate that this method yields better results than the gonococcal culture.

Histopathological examination of a gonococcal pustule may reveal a combination of features, supporting the diagnosis (Shapiro, Teisch and Brownstein, 1973). Only in extremely rare cases will gonococci be discovered in skin sections of a pustule (Bruusgaard and Thjøtta, 1925; Ackerman, Miller and Shapiro, 1965). Moreover the diagnosis can be backed up by the demonstration of the presence of complement fixating antibodies for gonococci in serum of the patient. (Danielsson, Thyresson, Falk and Barr, 1972). Application of a fluorescent antigonococcal serum to a skin section may make it possible to prove the presence of gonococci in the pustules (Kahn and Danielsson, 1969).

The clinical picture of the septic gonococcal dermatitis syndrome could also be due to meningococcal sepsis. Bacteriological tests for this disorder should be performed to exclude this possibility. Moreover, it should be remembered that gonococcal endocarditis can be accompanied by similar skin efflorescences. As was mentioned in the introduction, gonococcal endocarditis may lead to a severe case of gonococcal sepsis.
Other complaints, such as bacterial endocarditis, typhus, miliary tuberculosis and acute rheuma should be differentiated on grounds of the case history, the clinical picture and in particular the morphology of the skin lesions.
(Keil, 1938; Abu-Nassar, Hill, Fred and Yow, 1963).
A variety of treatments are described in the literature for septic gonococcal

dermatitis syndrome. For instance, Ackerman, Miller and Shapiro (1965) administered 2.4 mega-units of penicillin G intramuscularly (i.m.) for 10 days. Barr and Danielsson (1971) gave their patients 4.5 mega-units of penicillin G i.m. for 2 days and subsequently 3-4 mega-units of penicillin V per day orally for 10 days.

Summary

A woman with the septic gonococcal dermatitis syndrome. – The case is reported of a woman aged 36 years with a septic gonococcal dermatitis syndrome; with reference to this case, problems concerning the diagnosis and treatment of this condition, which in only little known in the Netherlands, are described.

It can be concluded from the literature that the diagnosis may be made on the basis of the clinical symptoms, fever, arthritic phenomena and typical skin lesions, in combination with demonstration of a gonorrhoic focus and of the presence of gonococci in skin lesions and in the blood.

References

Abu-Nassar, H. , Hill, N. , Fred, H. L. and Yow, E. M. (1963)

Arch. intern. Med. , 112, 731.

Ackerman, A. B. (1970)

New Engl. J. Med. , 282, 793.

Ackerman, A. B. , Miller, R. C. and Shapiro, L. (1965)

Arch. Derm. , 91, 227.

Barr, J. and Danielsson, D. (1971)

Brit. med. J. I, 482.

Björnberg, A. (1970)

Acta derm. -venereol. (Stockh.), 50, 114.

Björnberg, A. and Gisslén, H. (1965)

Nord Med., 73, 338.

Bruusgaard, E. and Thjøtta, Th. (1925)

Acta derm. -venereol. (Stock.), 6, 262.

Danielsson, D. and Michaëlsson, G. (1966)

Acta derm. -venereol. (Stockh.), 46, 257.

Danielsson, D. , Thyresson, N. , Falk, V. and Barr, J. (1972)

Acta derm. -venereol. (Stockh.), 52, 467.

Forstrøm, L. , Mustakallio, K. K. , Sivonen, A. and Kousa, M. (1972)

Ann. clin. Derm. , 4, 49.

Fred, H. J. , Eiband, J. M. , Martincheck, L. A. and Yow, E. M. (1965)

Arch. intern. Med. , 115, 191.

Kahn, G and Danielsson, D. (1969)

Arch.Derm., 99, 421.

Keil, H. (1938)

Quart.J.Med., 7, 1.

Laugier, P. and Orusco, M. (1972)

Bull.Soc.franc.Derm.Syph., 70, 112.

Shapiro, L., Teisch, J.A. and Brownstein, M.H. (1973)

Arch.Derm., 107, 403.

Silvestrini, R. (1903)

Riv.crit.Clin.med., 4, 385, 401, 417.

Thayer, J.D. and Martin, J.E. (1966)

Publ.Hlth.Rep. (Wash.), 80, 559.

Vidal, E. (1893)

Ann.Derm.Syph. (Paris), 4, 3.

Wiesner, P.J., Tronca, E., Bonin, P, Pedersen, H.B. and Holmes, K.K. (1973)

New Engl.J.Med., 228, 181.
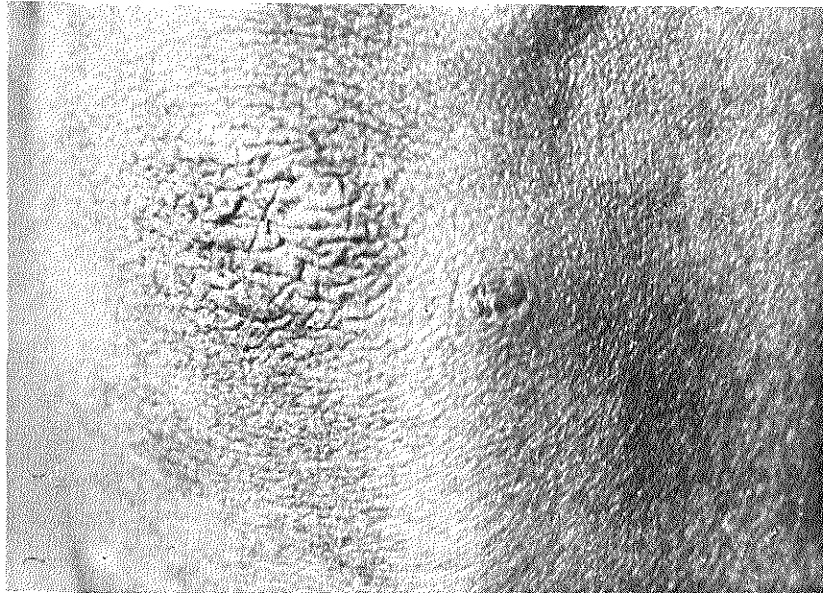
Photograph 1

Gonococcal pustule on elbow
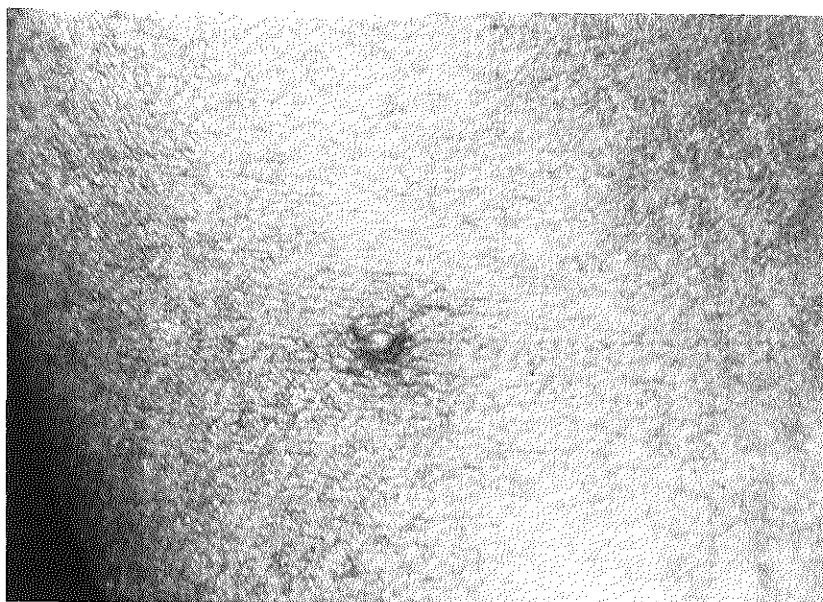
Photograph 2

Close-up gonococcal pustule
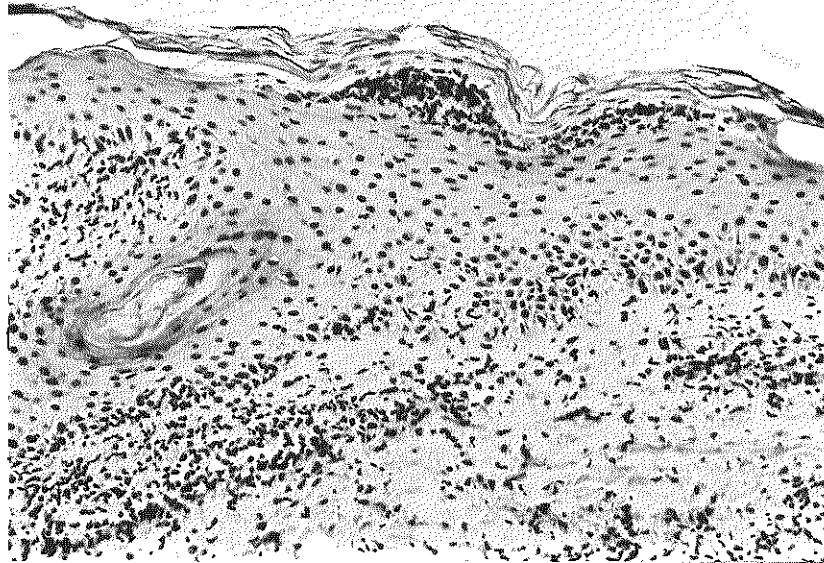
Photograph 3

Histopathology-gonococcal pustule in epidermis, oedema and infiltration in dermis.

HE-staining, magn. 285 x.
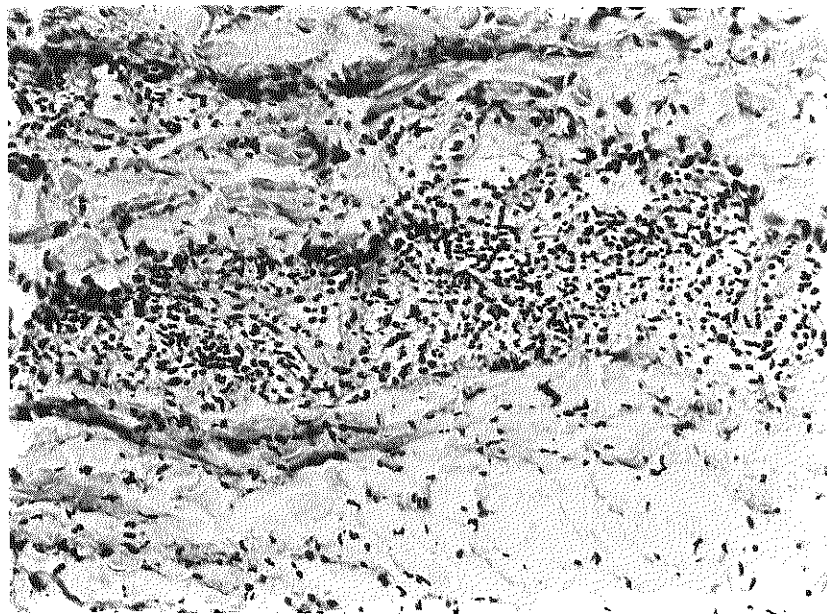
Photograph 4

Histopathology, vasculitis in the dermis.

HE staining, magn. 460 x.

SAMENVATTING

In de eerste drie Hoofdstukken wordt een beschrijving van methoden van onderzoek, behandeling en nacontrole (Hoofdstuk I), bakteriologische onderzoekmethoden (Hoofdstuk II) en de registratie en verwerking van gegevens (Hoofdstuk III) gegeven.

In Hoofdstuk IV worden enkele epidemiologische gegevens van patienten met gonorroe uit de periode 18 oktober 1971 tot 1 juli 1972 verstrekt en in het kort besproken.

De diagnostische aspekten van gonorroe die in dit proefschrift worden behandeld hebben betrekking op de optimale herkenning van de gonorrhoische infektie (Hoofdstukken V - VIII), de gevoeligheid van de gonokokken voor verschillende antibiotika (Hoofdstukken IX - XI), de antigonorrhoische behandeling in relatie tot de gevoeligheid van de gonokokken voor het gebruikte antibiotikum (Hoofdstuk XII) en ten slotte de wijze van herkennen van verschillende extragenitale vormen van gonorroe (Hoofdstukken XIII - XV).

In Hoofdstuk V wordt een vergelijkend onderzoek naar de groeikwaliteit voor gonokokken van verschillend samengestelde gonokokken-media beschreven.
Vier basismedia (Bacto GC (Gonococcal Culture) medium base, GC agar base (BBL), Columbia agar base (BBL) en Mueller-Hinton agar (Oxoid)) werden met behulp van groeiscores op hun geschiktheid als gonokokken-voedings-bodem vergeleken.
Bacto GC medium base bleek het beste en GC agar base het één na beste basis medium. Vervolgens werden kombinaties van deze twee basis media met twee supplementen (Bacto supplement A en Isovitalex TM (Thayer Martin) enrichment (BBL)) met elkaar vergeleken. De beste resultaten werden met Bacto GC medium base en Isovitalex TM enrichment verkregen. Het beste

niet selektieve medium in dit onderzoek bestond uit Bacto GC medium base,

Bacto haemoglobin en Isovitalex TM enrichment. Vergelijking van dit niet

selektieve medium met een zelfde medium waaraan VCN (vancomycine, coli-

stimethaat en nystatine) was toegevoegd gaf signifikant hogere scores voor het

selektieve medium te zien. Bij vergelijking van dit selektieve medium met

een zelfde medium waaraan trimethoprim (T) was toegevoegd werden geen

signifikante verschillen in de verdeling der scores waargenomen. Wel werd

minder overgroei door Proteus op de VCNT platen waargenomen. Het VCN

medium werd voor routine-onderzoek van patienten in onze kliniek verkozen.

Wanneer overgroei door Proteus werd waargenomen, werd het VCNT medium

bij een volgend onderzoek gebruikt.

In Hoofdstuk VI wordt een vergelijking van de waarde van de

plaats van afname van gonokokkenkweken voor het herkennen van gonorroe

bij vrouwen beschreven.

Op grond van gegevens uit eigen onderzoek en uit de literatuur werd de waarde

van de afname van gonokokkenkweken uit de cervix, de urethra, het rektum

en de vagina voor het herkennen van urogenitale gonorroe bij vrouwen bepaald.

Tevens werd de waarde van de gonokokkenkweek uit de orofarynx voor het her-

kennen van gonorroe bij vrouwen bepaald.

Meer dan 90 procent van alle vrouwen met urogenitale/rektale gonorroe kon-

den met een geisoleerd   afgenomen gonokokkenkweek uit de cervix worden her-

kend. Voor een optimale herkenning van urogenitale/rektale gonorroe bij

vrouwen zijn echter gonokokkenkweken uit de cervix, de urethra en het rek-

tum nodig. Bij gebruik maken van gonokokkenkweken uit de cervix, de urethra

en het rektum is de gonokokkenkweek uit de vagina overbodig. Bij gebruik

maken van gonokokkenkweken uit de cervix, de urethra en het rektum dragen

gonokokkenkweken uit de orofarynx slechts weinig bij tot het herkennen van

gonorroe bij vrouwen. Het belang van het uitvoeren van gonokokkenkweken uit de orofarynx is gelegen in het feit dat soms een "septic gonococcal dermatitis" syndroom als gevolg van een niet herkende orofaryngeale gonorroe kan ontstaan. Het is raadzaam bij het onderzoek van vrouwen op gonorroe gonokokkenkweken uit de cervix, de urethra, het rektum en de orofarynx af te nemen.

In Hoofdstuk VII wordt een vergelijkend onderzoek naar de waarde van het methyleenblauw-preparaat (MBP), het Gram-preparaat (GP) en de selektieve gonokokkenkweek (SGK) voor het herkennen van urethrale gonorroe bij mannen en urogenitale/rektale gonorroe bij vrouwen beschreven. Naar onze mening hoort het MBP op grond van het grote aantal vals positieve uitslagen niet voor de herkenning van bovengenoemde vormen van gonorroe te worden gebruikt. In ons materiaal waren het GP en de SGK van gelijke waarde voor het herkennen van urethrale gonorroe bij mannen; gebruik van beide methoden leidde tot een geringe stijging van het herkenningspercentage van gonorroe. Voor een optimale herkenning van asymptomatische urethrale gonorroe bij mannen bleek de SGK noodzakelijk. De SGK is verder van essentieel belang voor het herkennen van urogenitale/rektale gonorroe bij vrouwen. Het GP is voor dit doel minder geschikt, in het bijzonder voor onderzoek van materiaal uit het rektum.

Wanneer de SGK wordt gebruikt, doet het GP voor het herkennen van urethrale gonorroe bij mannen en van urogenitale gonorroe bij vrouwen het herkenningspercentage van gonorroe slechts met enkele procenten stijgen. Deze bijdrage is waarschijnlijk terug te voeren op patienten die vancomycine-gevoelige stammen bij zich dragen. Deze stammen groeien namelijk niet op de vancomycine-bevattende SGK.

Naar onze mening is het raadzaam voor het herkennen van urethrale gonorroe bij mannen en van urogenitale gonorroe bij vrouwen zowel de SGK als het GP

te gebruiken

Voor de herkenning van rektale gonorroe bij vrouwen hoort de SGK te worden gebruikt.

In Hoofdstuk VIII wordt een vergelijkend onderzoek naar de waarde van de uitgestelde direkte immunofluorescentie (UDIF) en de selektieve gonokokkenkweek (SGK) voor het herkennen van urethrale gonorroe bij mannen en urogenitale gonorroe bij vrouwen beschreven. Met behulp van de SGK werden meer mannen met urethrale en vrouwen met urogenitale/rektale gonorroe gediagnostiseerd dan met de UDIF.

Bij vrouwen werd ook per plaats van afname vaker gonorroe met de SGK dan met de UDIF vastgesteld. Van de mannen met een urethrale gonorroe, waarbij de kombinatie positieve SGK en negatieve UDIF werd gevonden, hadden 6 (66. 7 procent) een asymptomatische urethrale gonorroe. In bijna alle gevallen waarin een positieve SGK en negatieve UDIF werd vastgesteld, waren slechts enkele gonokokkenkolonies op de voedingsbodems waargenomen.

In Hoofdstuk IX worden de resultaten van kwantitatieve gevoeligheidsbepalingen van gonokokkenstammen voor ampicilline, penicilline en tetracycline besproken. Van 959 bij mannen uit de urethra, prostaatvocht, rektum of tonsillen/orofarynx en bij vrouwen uit de cervix, urethra, rektum, vagina of tonsillen/orofarynx geisoleerde gonokokkenstammen worden minimale remmingsconcentratie (MRC) – verdelingen voor de genoemde antibiotika verstrekt. Het patientenmateriaal bestond uit mannen burgers (MB) en vrouwen burgers (VB), en mannen zeelieden (MZ) en vrouwen zeelieden (VZ).

Het bleek dat een representatief beeld van de MRC verdelingen voor ampicilline, penicilline en tetracycline voor de grootste patientengroepen (MB,

VB en MZ) kon worden verkregen door bij mannen alleen de stammen uit de urethra en bij vrouwen alleen de stammen uit de cervix in beschouwing te nemen. In totaal werden bij 258 MB stammen uit de urethra, 172 VB stammen uit de cervix en 134 MZ stammen uit de urethra gevoeligheidsbepalingen verricht. Bij de MB stammen waren 40.3 procent, bij de VB stammen 34.8 procent en bij de MZ stammen 68.6 procent van de stammen relatief resistent voor ampicilline (MRC $\geq$ 0.16 µg/ml).

Bij de MB stammen waren 38.4 procent, bij de VB stammen 34.9 procent en bij de MZ stammen 67.2 procent van de stammen relatief resistent voor penicilline (MRC $\geq$ 0.08 µg/ml).

Bij de MB stammen waren 17 procent, bij de VB stammen 12.2 procent en bij de MZ stammen 38.8 procent van de stammen relatief resistent voor tetracycline (MRC $\geq$ 1.28 µg/ml).

Met behulp van statistische analyse werd een sterk positieve rangcorrelatie tussen de gevoeligheden van de drie antibiotika aangetoond.

In Hoofdstuk X wordt de _in vitro_ aktiviteit van acht antimicrobiële middelen tegen _Neisseria gonorrhoeae_ beschreven.

Van vier groepen gonokokkenstammen werd de gevoeligheid voor ampicilline (Am), penicilline (P), tetracycline (T), rifampicine (Ri), spectinomycine (Sp), sulphamethoxazole (Su), trimethoprim (Tr)en een kombinatie van Su en Tr in een verhouding van 5:1 (Su/Tr 5:1) bepaald. De verschillende groepen stammen waren in 1972 bij mannen burgers (MB), vrouwen burgers (VB) en mannen zeelieden (MZ) in Rotterdam (R) en in 1967 bij VB in Amsterdam (A) geisoleerd. De MZ-R stammen, die in hoofdzaak als importstammen kunnen worden beschouwd, werden met de in hoofdzaak als nederlandse stammen te beschouwen MB-R en VB-R stammen vergeleken. Verder werd nagegaan of

de (relatieve) resistentie voor de bovengenoemde antibiotika onder nederland-
se stammen in de periode 1967 tot 1972 was toegenomen. Hiertoe werden de
VB-A en VB-R stammen met elkaar vergeleken. Ook de MB-R en VB-R
stammen werden vergeleken. Tenslotte werden voor de gevoeligheidsver-
delingen van telkens twee onderzochte antibiotika voor alle groepen stammen
Spearman's rangcorrelatiecoefficienten r berekend.

De MZ-R stammen waren signifikant minder gevoelig voor A, P, T, Ri en
Tr dan de MB-R en VB-R stammen. Vergelijking van de VB-A en VB-R stam-
men liet zien dat de VB-R stammen alleen signifikant minder gevoelig voor
Su/Tr 5:1 waren. Een mogelijke verklaring voor deze bevinding wordt gege-
ven.

Met uitzondering van één VB-Astam waren alle stammen gevoelig voor Sp.
Hoge r waarden (> 0.50) werden bij alle groepen stammen tussen Am en P,
Am en T en P en T waargenomen; met uitzondering van de r waarde van Am
en P waren de r waarden tussen telkens twee van de volgende antibiotika,
Am, P, T, Ri en Tr, steeds het hoogst voor de MZ-R stammen.

Hoge r waarden (> 0.50) werden ook voor alle groepen stammen tussen Su en
Su/Tr 5:1 gevonden. In tegenstelling tot de R groepen stammen hadden de
VB-A stammen lage r waarden voor Su en Tr, en voor Tr en Su/Tr 5:1.
Een mogelijke verklaring voor deze bevinding wordt gegeven.

Tot slot wordt een hypothese gegeven om het feit te verklaren dat onder neder-
landse gonokokkenstammen in de periode 1967 - 1972 geen signifikante veran-
deringen in de gevoeligheid voor Am, P, T, Ri, en Tr werden gezien,
terwijl onder de als importstammen te beschouwen MZ-R stammen toch een
signifikant hoger percentage voor Am, P, T, Ri en Tr relatief resistente
stammen werd waargenomen.

In Hoofdstuk XI wordt de potentiering van sulphamethoxazole door trimethoprim bij gonokokkenstammen besproken.

Kwantitatieve gevoeligheidsbepalingen voor sulphamethoxazole (Su), trimethoprim (Tr) en een kombinatie van Su en Tr in een verhouding van 5:1 (Su/Tr 5:1) worden voor 322 gonokokkenstammen vermeld. Ook worden gegevens van de potentiering van Su door Tr verstrekt. Voor zeven gonokokkenstammen met bekende gevoeligheid voor Su en Tr werden gevoeligheidsbepalingen voor kombinaties van Su en Tr in verschillende verhoudingen bepaald. Op grond van deze gegevens werd geformuleerd volgens welke regels Su door Tr wordt gepotentieerd.

Met behulp van de FRC (fraktionele remmingsconcentratie) index werd verder het synergistisch effekt van Su en Tr bij de 7 eerder genoemde stammen bepaald. Het was opvallend, dat, wanneer een kombinatie van Su/Tr 5:1 werd gebruikt, voor de Su-gevoelige gonokokkenstammen geen potentiering van Su door Tr en geen synergistische werking van Su en Tr wordt waargenomen.

In Hoofdstuk XII worden de resultaten van een gonorroebehandeling bestaande uit intramusculair en oraal toegediend ampicilline verstrekt. Een serie van 613 patienten (286 mannen burgers, 202 vrouwen burgers en 125 mannen zeelieden) werd behandeld met 1 gram ampicilline intramusculair gevolgd door 2 gram ampicilline oraal. Van deze patienten kwamen 417 patienten (192 mannen burgers, 172 vrouwen burgers en 53 mannen zeelieden) binnen twee weken na behandeling terug voor controle. Het genezingspercentage voor deze 417 patienten bedroeg 99%. Bij 4 patienten met recidieven werden voor ampicilline relatief resistente gonokokkenstammen (MRC $\geq$ 0.16 µg/ml) geisoleerd. Bij 6 manlijke patienten werden ampicilline serumspiegels bepaald.

In Hoofdstuk XIII wordt de eerste in onze kliniek gediagnostiseerde patiente met orofaryngeale gonorroe beschreven.

De ziektegeschiedenis van een 25-jarige gehuwde vrouw met tonsillitis gonorrhoica wordt vermeld. Na een ampicilline-behandeling door de huisarts in verband met een atypisch verlopende angina, lymphadenitis en arthritis en na een ampicilline-behandeling door ons wegens urogenitale/rektale gonorroe werd de diagnose gesteld. Er wordt een overzicht uit de literatuur gegeven over tonsillitis, faryngitis en stomatitis gonorrhoica. Verder wordt ingegaan op de therapeutische problemen bij deze aandoening.

In Hoofdstuk XIV worden de epidemiologie, bakteriologie en therapie van de gonorrhoische faryngitis besproken aan de hand van 31 patienten.

De frekwentie van faryngeale gonorroe onder 467 opeenvolgend onderzochte patienten met urogenitale/rektale gonorroe bedroeg 4. 9 procent (3. 6 procent voor mannen burgers, 8. 5 procent voor vrouwen burgers en 3. 4 procent voor mannen zeelieden). Voor iedere patient werden gevoeligheden voor ampicilline, penicilline en tetracycline van de uit de orofarynx en uit het urogenitaal/rektaal gebied geisoleerde stammen vergeleken. Op dezelfde wijze werden stammen van de patient met die van de partner vergeleken.

In het algemeen lukte het niet met de routine therapie (1 gram ampicilline intramusculair gevolgd door 2 gram ampicilline oraal 4 uur later) gonokokken uit de orofarynx te elimineren. Dagelijkse toediening van een kombinatie van 3. 6 mega-U procaine penicilline en 1. 2 mega-U natrium penicilline intramusculair gedurende 10 dagen bleek in alle gevallen succesvol.

In Hoofdstuk XV wordt de eerste in onze kliniek gediagnostiseerde patiente met het "septic gonococcal dermatitis" syndroom beschreven.

Naar aanleiding van een 36-jarige vrouw met een "septic gonococcal derma-
titis" syndroom worden problemen betreffende de diagnostiek en de therapie
van dit in Nederland weinig bekende ziektebeeld beschreven.

Uit de literatuur blijkt, dat de diagnose kan worden gesteld op grond van kli-
nische verschijnselen, koorts, arthritisklachten en typische huidverschijn-
selen in kombinatie met het aantonen van een gonorrhoische haard en het
aantonen van gonokokken in huidlaesies en in het bloed.

LIST OF PUBLICATIONS ON GONORRHOEA.

- Stolz, E.

  Een onderzoek naar de waarde van de plaats van afname van gonokokken-

  kweken voor de herkenning van gonorroe bij vrouwen.

  (to be published, accepted by Ned. T. Geneesk. (Chapter VI)).

- Stolz, E. , Zwart, H. G. F. and Michel, M. F. (1974)

  Sensitivity to ampicillin, penicillin and tetracycline

  of gonococci in Rotterdam.

  Brit. J. vener. Dis. , 50, 202.(Chapter IX).

- Stolz, E. and Kerkkamp, H. J. J.

  Treatment of gonorrhoea using a combination of intramuscular

  and oral ampicillin.

  (to be published, accepted by Brit. J. vener. Dis.) (Chapter XII).

- Schuller, J. L. and Stolz, E. (1972)

  Een patiente met tonsillitis gonorrhoica.

  Ned. T. Geneesk. , 116, 2216 (Chapter XIII).

- Stolz, E. and Schuller, J. L. (1974)

  Gonococcal oro- and nasopharyngeal infection.

  Brit. J. vener. Dis. , 50, 104.(Chapter XIV).

- Stolz, E. , van Kampen, W. J. , Vuzevski, V. and van IJzerloo, J. A. G.

  (1974)

  Een patiente met het "septic gonococcal dermatitis"-syndroom.

  Ned. T. Geneesk. , 118,618. (Chapter XV).

LIST OF PUBLISHED SUMMARIES OF LECTURES ON GONORRHOEA.

.   Stolz, E. (1973)

        Kwantitatieve gevoeligheidsbepalingen voor ampicilline, penicilline

        en tetracycline van in Rotterdam geisoleerde gonokokkenstammen.

        Ned. T. Geneesk., 117, 1914.

.   Stolz, E., Diederen, J. en Verdonk, A. (1973)

        Een ecologisch onderzoek betreffende patienten met gonorroe.

        Ned. T. Geneesk., 117, 1912.

.   Stolz, E. and Schuller, J. L. (1973)

        Patienten met gonokokken in oro- en nasopharynx.

        Ned. T. Geneesk., 117, 1914.

.   Stolz, E. and Schuller, J. L. (1974)

        Patienten met gonokokken in oro- en nasopharynx.

        Ned. T. Geneesk., 118, 120.

.   Stolz, E., Michel, M.F. and Zwart, H.G.F. (1974)

        Sensitivity to different antibiotics of strains of Neisseria gonorrhoeae

        isolated in Rotterdam (1972) and Amsterdam (1967).

        Antonie van Leeuwenhoek, 40, 323.

CURRICULUM VITAE

Schrijver van dit proefschrift werd op 23 augustus 1939 te Soerabaja geboren. Hij behaalde in 1957 het eindexamen Gymnasium β aan het Gemeentelijk Lyceum te Utrecht. Vervolgens studeerde hij Geneeskunde te Utrecht, waar hij in 1964 het doctoraal examen aflegde. De opleiding tot arts werd aan het Hoger Klinisch Onderwijs te Rotterdam voortgezet. Op 26 april 1967 werd hij tot arts bevorderd. Na het vervullen van zijn militaire dienstplicht begon hij zijn opleiding tot dermatoloog in november 1968 in Rotterdam. Op 1 november 1972 werd hij in het specialisten register ingeschreven. Gedurende de laatste jaren is hij werkzaam als hoofd van de polikliniek geslachtsziekten van de Afdeling Dermatologie van het Academisch Ziekenhuis Rotterdam en als zelfstandig praktizerend dermatoloog in het Gemeente-Ziekenhuis Schiedam.