Reports of the special Committee upon the Standardisation of Pathological Methods. No. 2, The laboratory diagnosis of gonococcal infections. No. 3, Methods for the detection of spirochaetes.

Contributors

Great Britain. Medical Research Committee. Committee upon the Standardisation of Pathological Methods.

Publication/Creation

London : H.M.S.O., 1918.

Persistent URL

https://wellcomecollection.org/works/nzhhb5ns

License and attribution

This work has been identified as being free of known restrictions under copyright law, including all related and neighbouring rights and is being made available under the Creative Commons, Public Domain Mark.

You can copy, modify, distribute and perform the work, even for commercial purposes, without asking permission.

wellcome collection

Wellcome Collection
183 Euston Road
London NW1 2BE UK
T +44 (0)20 7611 8722
E library@wellcomecollection.org
https://wellcomecollection.org

Special Report Series, No. 19.

NATIONAL HEALTH INSURANCE.

MEDICAL RESEARCH COMMITTEE.

Reports of the Special Committee upon the Standardisation of Pathological Methods.

THE LABORATORY DIAGNOSIS OF GONOCOCCAL INFECTIONS.

METHODS FOR THE DETECTION OF SPIROCHAETES.

LONDON:
PUBLISHED BY HIS MAJESTY'S STATIONERY OFFICE.
To be purchased through any Bookseller or directly from
H.M. STATIONERY OFFICE at the following addresses:
IMPERIAL HOUSE, KINGSWAY, LONDON, W.C.2, and
23, ABINGDON STREET, LONDON, S.W.1;
37, PETER STREET, MANCHESTER;
1, ST. ANDREW'S CRESCENT, CARDIFF;
23, FORTH STREET, EDINBURGH;
or from E. PONSONBY, LTD., 116, GRAFTON STREET, DUBLIN.

1918.

Price 1s. *Net.*

NATIONAL HEALTH INSURANCE.

MEDICAL RESEARCH COMMITTEE.

The following publications relating to the work of the Medical Research Committee can be purchased through any bookseller, or directly from H.M. Stationery Office, at the following addresses: Imperial House, Kingsway, London, W.C. 2, and 28, Abingdon Street, London, S.W. 1; 37, Peter Street, Manchester; 1, St. Andrew's Crescent, Cardiff; 23, Forth Street, Edinburgh; or from E. Ponsonby, Ltd., 116, Grafton Street, Dublin.

First Annual Report of the Medical Research Committee, 1914–1915. [Cd. 8101.] Price 3*d*., post free 4*d*.

Second Annual Report of the Medical Research Committee, 1915–1916. [Cd. 8399.] Price 3½*d*., post free 5*d*.

Third Annual Report of the Medical Research Committee. [Cd. 8825.] Price 6*d*., post free 7*d*.

Special Report Series.

No. 1. First Report of the Special Investigation Committee upon the Incidence of Phthisis in relation to Occupations.—The Boot and Shoe Trade. Price 3*d*., post free 4*d*.

No. 2. Report of the Special Advisory Committee upon Bacteriological Studies of Cerebro-spinal Fever during the Epidemic of 1915. Price 6*d*., post free 7½*d*.

No. 3. Bacteriological Studies in the Pathology and Preventive Control of Cerebro-spinal Fever among the Forces during 1915 and 1916. Price 1*s*. 6*d*., post free 1*s*. 8½*d*.

No. 4. Reports upon Investigations in the United Kingdom of Dysentery cases received from the Eastern Mediterranean.
I. Amœbic Dysentery and the Protozoological Investigation of Cases and Carriers. Price 1*s*., post free 1*s*. 2*d*.

No. 5. II. Report upon 878 Cases of Bacillary Enteritis. Price 1*s*. 6*d*., post free 1*s*. 8*d*.

No. 6. III. Report upon recovered Cases of Intestinal Disease in the Royal Naval Hospital, Haslar, 1915–16.
IV. Report upon combined Clinical and Bacteriological Studies of Dysentery Cases from the Mediterranean. Price 1*s*. 6*d*., post free 1*s*. 8*d*.

No. 7. V. Report upon 2,360 Enteritis "Convalescents" received at Liverpool from various Expeditionary Forces. Price 2*s*., post free 2*s*. 2½*d*.

22501971925

MEDICAL RESEARCH COMMITTEE.

REPORTS

OF THE

SPECIAL COMMITTEE UPON THE STANDARDISATION OF PATHOLOGICAL METHODS.

No. 2. THE LABORATORY DIAGNOSIS OF GONOCOCCAL INFECTIONS.

No. 3. METHODS FOR THE DETECTION OF SPIROCHAETES.

WELLCOME INSTITUTE LIBRARY
Coll. WellCMac
Coll. pam
No. WC150
1918
G78r

Approved for publication by the Medical Research Committee, 7th June, 1918.

-32301

Medical Research Committee.

(National Health Insurance.)

The Hon. WALDORF ASTOR, M.P. (*Chairman*).

The Right Hon. CHRISTOPHER ADDISON, M.D., M.P.

The VISCOUNT GOSCHEN, C.B.E. (*Treasurer*).

C. J. BOND, C.M.G., F.R.C.S. (Hon. Colonel).

A. K. CHALMERS, M.D., D.P.H. (Lieut.-Colonel R.A.M.C. (T.)).

PROFESSOR WILLIAM BULLOCH, M.D., F.R.S.

PROFESSOR F. G. HOPKINS, D.Sc., F.R.S.

COLONEL SIR WILLIAM LEISHMAN, K.C.M.G., C.B., F.R.S.

PROFESSOR G. R. MURRAY, M.D., F.R.C.P. (Major R.A.M.C. (T.)).

SIR WALTER M. FLETCHER, K.B.E., M.D., F.R.S. (*Secretary*).

In April, 1917, the Medical Research Committee invited the following special Committee to consider how far it may be practicable and desirable to obtain the standardisation of routine pathological methods, and to report to them. The special Committee were empowered to co-opt additional members for particular divisions of their subject.

LIEUT.-COLONEL J. G. ADAMI, M.D., F.R.S., C.A.M.C.

MAJOR F. W. ANDREWES, M.D., F.R.S., R.A.M.C., T.

PROFESSOR WILLIAM BULLOCH, M.D., F.R.S.

For the consideration of the subjects dealt with in the two Reports now presented the Special Committee invited the co-operation of COLONEL L. W. HARRISON, D.S.O., K.H.P., who by permission joined the Committee for this purpose.

REPORTS OF THE SPECIAL COMMITTEE UPON THE STANDARDISATION OF PATHOLOGICAL METHODS.

The Special Committee beg leave to present to the Medical Research Committee two reports upon subjects of great importance in the recognition of venereal disease—the laboratory diagnosis of gonococcal infections and the methods for the detection of spirochaetes. They have again to acknowledge gratefully the assistance given to them by Colonel L. W. Harrison, D.S.O., K.H.P., throughout their consideration of these subjects and the preparation of the reports. To other workers they are also greatly indebted for help in particular directions, as will appear in the text of the reports, and to all these they would express their gratitude here.

No. 2.—THE LABORATORY DIAGNOSIS OF GONOCOCCAL INFECTIONS.

CONTENTS.

Introductory.

The Committee desire to call attention to the special difficulties associated with the diagnosis of gonococcal infections, and to recommend the employment of methods whereby the chances of incorrect diagnosis may be reduced to a minimum. Their action is prompted by a recognition of the increased call made upon the bacteriological laboratories of this country under the provisions of the Venereal Diseases Act. A wrong positive diagnosis may be the means of inflicting grave injury upon the individual; a wrong negative diagnosis is liable to injure the family of the individual and the community to an even greater extent.

The stage has not yet been reached at which a diagnosis can be made with certainty in all cases by bacteriological methods alone. There still remain cases of latent gonorrhœa, both in the male and in the female, which fail to react to any of the bacteriological tests at present at our disposal. In this as in any other infectious disease bacteriological evidence cannot always replace clinical. It is an adjunct, the value of which

(5/18.) (18277r.) Wt. 14927—92. 3000. 8/18. D & S. G. 2. A 2

is augmented to a remarkable degree when the diagnosis is entrusted to a thoroughly trained bacteriologist employing the best methods. It is timely, therefore, to direct attention to what are the best methods and what are their limitations.

Cases presenting themselves for examination for the detection or elimination of gonococcal infection are to be divided into:—

(1) Cases in the acute stage, actual or suspected.

(2) Cases in the late or chronic stage, with or without a history of previous acute gonococcal infection.

In the male the history of an acute attack is usually obtainable; in the female this history of the acute stage is commonly wanting.

The bacteriological diagnosis of gonorrhœa is based in the first place upon the recognition of the gonococcus under the microscope. The picture presented in films, made from the discharge obtained in a frank case of acute gonorrhœa, is characteristic as regards the intracellular position of the gonococci, their morphology and staining, and these ordinary cases present no difficulty. In those, however, that are clinically doubtful or suspicious, the bacteriologist can render valuable aid. In these cases the picture presented is apt to be atypical and the routine bacteriological technique must be such as to reduce to a minimum the likelihood of false diagnosis.

The Recognition of Gonococci in Films.

The Committee are of opinion that a positive diagnosis may be justified from microscopic examination of films alone:—

(i) When the clinical history and appearances are those of an *acute* gonorrhœa.

(ii) When the proper technique has been employed.

(iii) When the observer is so thoroughly familiar with the appearance of the gonococcus in stained films as to be beyond the danger of confusing other micrococci with it.

As an aid and a reminder, recognising that the danger of this confusion is very real, the Committee have considered it advisable to include in this report the accompanying plate of accurate coloured reproductions of gonococci as seen in films, and of other micro-organisms which are apt to be present in discharges from the genito-urinary passages (*see* Plates I and II). For these reproductions they are indebted to Colonel Harrison, D.S.O., who selected and provided the material, and to Mr. S. A. Sewell who made the drawings.

Where, in the acute discharge, microscopical examination gives negative results, where the clinical history and appearances are doubtful, and where chronic gonorrhœa is indicated or suspected, microscopical examination alone is inadequate for diagnostic purposes, and its results must be confirmed by the cultural and other methods detailed below. It also has to be recognised that the gonococci, when apparently eliminated from the primary focus or foci of infection, may still persist in the tissues; that, in fact, there may be systemic without local discharge; cases of arthritis that may develop months, and it may be years, after the primary infection, at a time when for long there has been no discharge. In this order of cases neither microscopical nor cultural investigations of the external genito-urinary passages give positive results, and an indirect method is required such as complement fixation.

I.—The localities to be examined for the presence of Gonococci.

For diagnostic purposes the acute stage of gonorrhœa with its abundant discharge, whether from the genito-urinary passages or the conjunctiva (in ophthalmia) presents little or no difficulty in this connection. All that is necessary is to spread a film and make cultures of the abundant secretion which is usually available for the purpose.

In cases where there is little or no secretion the procedure is more difficult and the Committee are indebted to Dr. G. T. Western, of the London Hospital, who has a peculiarly large experience in cases of this kind, for a description of his method.

A. In the *Male* the gonococci may persist for long periods in:—

i. A deep follicle in the anterior urethra, a para-urethral canal, Tyson's duct or in Cowper's glands.
ii. Behind a small, incomplete stricture.
iii. In the prostate or seminal vesicles.
iv. More rarely in the bladder or kidney.

Persistence in the first three regions may lead to the development of a chronic gleet, although chronic prostatic or vesicular infection may be present without any visible gleet.

The routine procedure should be as follows:—

(*a*) The patient is to be instructed to present himself when he has held his water for 4 to 6 hours.
(*b*) The meatus should first be cleansed with an alcohol swab applied for about a minute, and an attempt then made to express secretion from the deeper part of the urethra. If this be present films should be made.
(*c*) Massage of the prostate, seminal vesicles and Cowper's glands, films being made of any secretion which appears at the meatus,
(*d*) Urine should then be passed, and the first ounce collected in a sterile tube. This is centrifuged, and the deposit examined, smears and cultures being made.
(*e*) Gonorrhœal infection of the bladder or either kidney demand the co-operation of a trained cystoscopist to procure samples of urine from the bladder and both ureters respectively, for the purpose of comparative study by films and cultures.

B. In the *Female* the gonococcus may be harboured for long periods in:—

i. The urethra and para-urethral ducts.
ii. The glands of Bartholin.
iii. The cervix.
iv. The uterus and tubes.

Merely to secure a smear from the vulva is (save in the vulvo-vaginitis of children) of absolutely no value in demonstrating the absence of gonorrhœal infection.

Films and cultures should be obtained by the following procedures:—

(*a*) The urethra should be examined for any discharge. After cleansing the meatus the urethral canal should be massaged forwards with the loop end of a fine hairpin through the anterior vaginal wall, or material may be secured by introducing the looped end of the hairpin into the urethra itself.

(*b*) The cervix should be exposed with a speculum. After cleansing with a swab, a platinum loop should be passed into the cervical canal. Menge's tube is useful for obtaining secretion from the cavity of the uterus as well as from the cervical canal.

(*c*) The contents of Bartholin's glands should be expressed.

There is evidence that in chronic cases positive results are more often obtained immediately after the menstrual period.

It is admitted that the above procedure, thorough and conscientious as it is, is of diagnostic value only when a positive result is obtained. A negative result does not eliminate the possibility that the gonococci are still in existence in other regions of the body, nay, even in the regions examined by the bacteriologist. The gonococci, it is known, may lie latent in epithelial cells, and while present may not be actively discharged at the period when the examination is made.

II.—Methods for the Identification of the Gonococcus in film preparations.

i. *Diagnosis cannot be based simply on the presence or absence of intracellular diplococci*, even if of characteristic bean shape, and this because—

(*a*) The diplococci of gonorrhœa need not be intracellular. All or almost all may be extracellular as in the very acute disease and in some cases of chronic gonorrhœa.

(*b*) The leucocytes escaping into discharges in the genito-urinary passages can take up other organisms which by single stain methods are not distinguishable from gonococci.

The common practice of diagnosis by single stain methods alone is, therefore, not permissible.

ii. The gonococcus is a Gram-negative organism. The majority of micro-organisms which may be confused with it are Gram-positive. The only way to exclude these Gram-positive organisms is by the routine employment of the original Gram's method, or a reliable modification of it. For *official purposes microscopical diagnosis of the gonococcus must be by Gram's method*. The limitations of this method are discussed below.

Gram's Method.

In the course of years considerable variation has developed in different laboratories in carrying out the technique of staining by Gram's method.

The original recommendation of Gram[1] consisted in staining with Ehrlich's anilin water gentian violet for 1 to 3 minutes, treatment with Lugol's solution (iodine 1 part, potassium iodide 2 parts, water 300 parts) for 1 to 3 minutes, decolorisation in absolute alcohol and counterstaining with Bismarck brown or vesuvin.

It has been the experience of every bacteriologist that this original method is replete with possibilities of error, and demands constant super-

---

(1) *Fortschritte der Medizin*, 1884, 2, 185.

vision of the reagents and a carefully regulated technique. It will be serviceable to call attention to these possibilities of error.

(1) *Thickness of Film.*—If the film be thick or irregular in its thickness, decolorisation becomes difficult, and Gram-negative organisms may in parts retain the stain.

(2) *Drying Over the Flame.*—If this be accomplished too rapidly, and the film be subjected to too great a heat, ambiguous results are obtained.

(3) *Anilin Water Gentian Violet.*—This has to be fresh; old solutions fail to afford a differential stain.

(4) *Iodine Solution* (Lugol's Solution).—To a slighter extent the same is true regarding this. With age and exposure it loses its differential properties.

(5) *Alcohol.*—This *must* be absolute, *i.e.*, 98 per cent. or over. Weak alcohol decolorises Gram-positive organisms. There is gradual decolorisation with absolute alcohol, but only after a length of treatment that is not employed in ordinary routine. It should never be used for more than two minutes.

(6) *Counterstain.*—Gram-positive organisms, which successfully resist exposure to absolute alcohol for two minutes may be decolorised, the gentian violet being masked—(*a*) if the counterstain be in too concentrated a solution, (*b*) if it be made up in a weak alcoholic solution, or (*c*) if when properly prepared its action be prolonged. As a result Gram-positive organisms may appear to be Gram-negative.

To obviate these fallacies numerous modifications have been proposed (Weigert, Unna, Günther, Nicolle). The most unsatisfactory step in the process is the employment of the unstable anilin water gentian violet combination. This has been replaced by carbolic acid gentian violet. Recently W. Jensen(1), of Copenhagen, has shown that the intensifying action of either anilin-water or carbolic acid on gentian-violet is quite unnecessary,(2) and as his method removes the greatest stumbling-block in the way of successful technique, and gives excellent and consistent results, the Committee recommend that it be employed to the exclusion of the original method. Briefly, Jensen (1) discards the anilin water, (2) increases the concentration of the iodine solution, and (3) counterstains by neutral red.

The exact technique of Jensen's method may be described as follows:—

1. Make the film thin and evenly distributed.
2. Fix in the flame, taking care to avoid overheating.
   (In laboratories dealing with much routine examination, it is to-day almost universal to make films on the slide and not on the cover glass. Overheating is guarded against by testing the heated slide against the back of the hand.)
3. *Let the preparation become cool.*
4. Stain with a 0·5 per cent. aqueous solution of methyl violet (6 B.) for one quarter to half a minute.*
5. Pour off the mass of methyl violet solution and wash away the remainder with a drop or two of *strong* Lugol's solution (iodine 1 part, potassium iodide 2 parts, distilled water 100 parts). Do not wash off with water.

---

(1) *Berl. klin. Wchnschr.*, 1912, **49**, 1663.

(2) Unna and others have shown that the essence of this process lies in the reaction between one of the para-rosaniline series of dyes (gentian violet, methyl violet and Victoria blue) and iodine in the presence of the bacterial body.

* This solution remains stable for months.

6. Pour on a fresh quantity of strong Lugol's solution and leave for one half to one minute.
7. Wash off the iodine solution with *absolute* alcohol (not with water).
8. Pour on fresh absolute alcohol (98 per cent.) moving the slide from side to side as in developing a photographic film. A third quantum of absolute alcohol may be required to complete the decolorisation.
9. Finally rinse with a few drops of absolute alcohol followed immediately, without any intervening washing in water, by a solution of neutral red.† Let this act for fifteen seconds to one minute.
10. Wash in water; dry with blotting paper and in air, and mount in the usual way.

To test the individual technique it is recommended that there be kept at hand pure cultures of a known Gram-positive organism, such as the *Micrococcus* (*Staphylococcus*) *aureus*, and of a Gram-negative organism (e.g., *B. coli*) and that parallel films of the two be made and stained side by side with the material under examination.

On the limitations of Gram's method in relationship to the diagnosis of gonorrhœa.

It deserves note:—

(*a*) That Gram-positive organisms when taken up and digested by leucocytes may lose their Gram-positive character.

(*b*) That more than one observer has noted, in cases of chronic gonorrhœa in which the material is such that a satisfactory film is difficult to obtain, that organisms determined by culture to be gonococci retain the violet stain with unusual obstinacy and appear thus to be Gram-positive.

(*c*) That the stain as such does not distinguish between gonococci and other Gram-negative, but non-pathogenic diplococci which may be present in both male and female genito-urinary tracts.

The Committee are of opinion that these limitations do not seriously affect the diagnosis of the one order of cases in which, in their view, diagnosis by microscopical examination alone gives consistent results, namely the active acute cases of the disease; and that with the figures provided in Plate I. the danger of confusion with other actual or apparent Gram-negative organisms is reduced to a minimum.

III.—The Cultivation of the Gonococcus.

Since the gonococcus was first successfully cultivated by Bumm in 1885 many different media have been devised for the culture of this micro-organism. The numbers and diversity of the media employed appear to be an index of the difficulty of cultivating the organism and the dissatisfaction which the various authors have felt

† Make up the solution in the proportion of—

Neutral red	1 grm.
Distilled water	1,000 ccm.
1% glacial acetic acid	2 ccm.

If this be found too weak, a somewhat stronger solution of neutral red may be employed.

with the media devised by others. Possibly this dissatisfaction may be due to the fact of different strains of gonococci varying in their adaptability to an existence on artificial media. More probably it is due to the omission from the various authors' descriptions of details of preparation which are apparently unimportant, with the result that the copies have been less successful than the originals. As an example, the influence of over-heating the medium designed for the growth of the gonococcus seems rarely to have been mentioned, though it has proved to be a factor of considerable importance, an over-heated medium producing a growth which is considerably feebler than one which has been sterilised with the minimum of heat.

The various media which have been devised for the growth of the gonococcus may be divided into three categories:—

(*a*) Those which contain human blood, blood-serum, or serous exudates, such as ascitic, pleuritic, or hydrocele fluid.

(*b*) Those containing animal blood-serum.

(*c*) Those which contain none of these but, in their place, artificially prepared enriching substitutes.

Media which contain human blood serum undoubtedly give the best results, and serum derived from shed blood is better than that of exudates, the latter being apt to vary in their constitution and prove disappointing. Animal serum is often good, that of horse, pig, rabbit and guinea-pig being perhaps the most successful, but the bactericidal action of sera derived from the same species of animal varies, and this has been found by some workers to be a cause of variability in the media prepared from them. Of the artificially prepared enriching substitutes, many have recently been prepared which have proved very successful in the isolation and sub-culture of the gonococcus. It is true that the growth obtained on them may not be so good as some which may occasionally be obtained on media of class (*b*) above, but they are not so inconstant as these and are therefore preferable for routine work.

In consequence of the diversity of opinions as to the best culture medium, a series of comparative tests has been carried out by Captain David Thomson, R.A.M.C., at the Rochester Row Military Hospital, and his results have been communicated to the Committee. Three media proved themselves eminently satisfactory: (i) Thomson's human plasma-glucose agar, (ii) Cole's tryptic blood agar, and (iii) Gordon and Hine's trypsinised pea extract agar, each of these having been prepared by its originator. The last named was prepared for the cultivation of the meningococcus, and it is quite probable that if the reaction of the medium were made + 6 (Eyre's scale) instead of + 1, it would enhance its value as a medium for gonococcus.

The method of preparing each of these is given in Appendix I.

IV.—Methods for the Production of a Focal Gonococcal Reaction

Long years before the gonococcus was discovered a common method of testing whether cure had been effected was by observing whether alcoholic or venereal indulgence brought about a return of the gleet. Since the causative agent has been known it has been found that the gonococci reappear in the discharge which is so induced. This renewal of the inflammation and discharge is most directly and most effectively

brought about by adopting the principle which Koch introduced with tuberculin. If, that is, there be a focus in which the gonococci are still present, by inoculating or vaccinating the individual with a suitable dose of a suspension of dead gonococci* a focal reaction may be set up which is frequently accompanied by an active discharge containing gonococci. In cases of metastatic gonococcal infection the reaction is manifested by increased pain in, or tenderness of, the affected part. In either case it may be accompanied by a definite rise of temperature with or without malaise.

This procedure is to be adopted with caution; there is danger, for instance, that, if present, a latent iritis or latent salpingitis may be lighted up, with serious results.

An alternative and perhaps less desirable method of inducing active discharge in cases of doubtful urethritis is the introduction of a 0·5 per cent. solution of silver nitrate into the urethra.

V.—The Complement Fixation Test.

It is clear from the foregoing statement of procedure that the conscientious examination of a case of suspected chronic gonorrhœa may develop into a lengthy process, demanding repeated appearance of the patient for examination, and, what is more, examination by trained specialists.

Its obvious difficulties suggest the desirability of some test which will more simply indicate the presence of the gonococcus in chronic disease and in this connection it is necessary to discuss the complement fixation test.

During the last seven years a technique has been developed and tested which to a certain extent fulfils the needs of the case, a test which is on similar principles to those of the Wassermann test. Like the Wassermann test its technique is delicate and precise, and to ensure satisfactory results it must be performed by those with adequate training in serological methods. Its value is so much appreciated in the United States that at one large hospital at least it is now the practice to test each blood sample obtained both for syphilis and for gonorrhœa by the complement fixation method.

As it is little employed in Great Britain the main data as to its development need to be set down.

Müller and Oppenheim† in Austria were the first to publish on this subject in 1906. They studied a case of gonococcal arthritis and employed as antigen a suspension of a culture of gonococci in salt solution. Bruck‡ reported favourably on the reaction the same year. In 1907 Meakins,§ of Montreal, working at Johns Hopkins Hospital, reported a series of cases of arthritis tested by this method. The findings of these and other earlier workers were somewhat contradictory; some cases afforded positive results, others, with a yet more definite history of gonorrhœal infection and progressive gonorrhœal disease, gave negative findings. The failure of these earlier workers to establish a satisfactory

---

* A suitable dose is usually 50 to 100 millions of an autolysed culture, or 100 to 200 millions of a suspension of the cocci killed by heat. The correct dose for any freshly isolated strain must be determined by cautious trial beginning with a small dose.

† Mueller u. Oppenheim, *Wien. klin. Wchnschr.*, 1906, **19**, 894.

‡ Bruck, *Deutsche med. Wchnschr.*, 1906, **32**, 1368.

§ Meakins, *Johns Hopkins Hosp. Bull.*, Balt., 1907, **18**, 525. See also Vannod, *Centralbl. f. Bakteriol. u. Parasitenk.*, Jena, 1907. Abt. I, Orig., **44**, 10.

technique was shown by Teague and Torrey(1), whose work was confirmed and extended by Wollstein(2) and Watabiki(3), to be due to the fact that there exist numerous strains of gonococci, which are so distinct that cultures obtained from one case and employed as antigen in the complement fixation test upon another clinically well-defined case of the disease, may yield a negative result. Torrey recognised as many as 14 different strains of gonococcus.

To guard against this defect Schwartz and McNeil(4) (1911) introduced and employed a polyvalent antigen, formed by pooling together several cultures known to be serologically distinct. They found that an antigen composed of six pooled strains failed to detect cases of clinically determined gonorrhœa which were detected by an antigen composed of twelve. To-day there is upon the market, provided by a well known American firm, an antigen containing all the 14 strains described and isolated by Torrey. The multiplicity of strains required to produce an efficient gonococcus antigen is a matter which demands further study.

Here it should be added that blood serum from one other disease only has thus far been recorded as occasionally affording a positive result when tested with gonococcus antigen, namely, cerebrospinal meningitis(5), and here the observations are conflicting. The symptoms of the two diseases are so distinct that if there be this cross fixation it introduces no danger of false diagnosis.

It is this test, as modified by the employment of a polyvalent antigen, that has been extensively employed in the United States, so that there is a considerable literature upon the subject, and on the whole a striking uniformity in the results obtained. Confirmatory reports have been published by Major (now Brevet Colonel) Harrison(6) in this country, and by Ower(7) in Canada.

The Advantages, Disadvantages and Limitations of the Complement Fixation Test.

The obvious advantages of this indirect method of diagnosis are that:—

i. It requires no preparatory directions to the patient or action on his part.

ii. It may replace examination of the genito-urinary passages by removal of blood from one of the extremities, and may thus be conducted without a knowledge on the part of the examinee as to the nature of the investigation.

---

(1) Teague and Torrey, *J. Med. Research*, Bost., 1907, **17**, 223. See also Torrey, *ibid.*, 1910, **22**, 95.

(2) Wollstein, *J. Exper. M.*, N.Y., 1907, **9**, 588.

(3) Watabiki, *J. Infect. Dis.*, Chicago, 1910, **7**, 159.

(4) Schwartz and McNeil, *Am. J. M. Sc.*, Phila., 1911, **141**, 693 and 1912, **144**, 815 and Schwartz, *ibid.*, 1912, **144**, 369.

(5) Wollstein (1907) obtained crossed fixation between gonococcus antigen and anti-meningococcus serum and *vice versa*. Vannod (1907) obtained none. Schwartz and McNeil obtained positive results with Flexner's anti-meningococcus serum, but none with sera from cases of cerebrospinal fever. Major Bowman, C.A.M.C., reports to this Committee that at Folkestone in the late epidemic the serum of two cases of cerebrospinal fever was found negative when tested against gonococcus antigen. It is in fact eminently unlikely that anti-bodies should appear in the blood after acute disease of but a few days' duration, and quite likely that they should make their appearance after repeated injections such as are necessary for the production of an anti-meningococcus serum. Admittedly the gonococcus and the meningococcus are nearly related. (*See also* p. 33.)

(6) Harrison, *J. Roy. Army Med. Corps*, Lond., 1914, **22**, 125.

(7) Ower, *Canadian Med. Ass. J.*, 1914, N.S. **4**, 1074.

iii. It demands the co-operation of no specialist beyond the pathologist.

iv. Where a positive result is obtained the time consumed in arriving at a diagnosis is very much less than that demanded by the method of films and cultures.

The disadvantages hitherto reported are that:—

i. *It very frequently fails in acute gonorrhœa.* Antibodies do not accumulate in the blood in sufficient amount to bring about a positive reaction until the disease has progressed for some three or four weeks.(1)

ii. Even where the disease has become chronic, negative results are obtained in those cases in which the disease has remained narrowly localised. Infection limited to the anterior urethra and fossa navicularis in the male gives no positive results; in the female it is generally accepted that the reaction does not show itself when the disease is confined to the urethra or vagina, but only when the cervix uteri has become involved, and the disease has ascended to the level of the uterus, and this in cases in which the gonococci can be demonstrated bacteriologically.

iii. Where gonococcal vaccines are employed as a method of cure the complement fixation test is inadmissible. Of late, active treatment by injection of vaccines has become increasingly common.

iv. The blood serum of patients who have been treated with vaccine may continue to give the reaction for four months. Animal experiments indicate that once antibodies are developed in amounts sufficient to afford the reaction they continue to be produced for some weeks after the gonococci have disappeared from the system. Thus Torrey (*loc. cit.*) found that when the process of immunization of animals against the gonococcus is suspended no change in the complement fixation test is noticeable for ten days; at the end of this period the antibodies in the blood begin to be eliminated rapidly, though they persist for some weeks afterwards. It follows from these observations that positive reactions are obtainable for some weeks after complete cure. From a public health point of view it is open to question how far this is to be regarded as telling against the method, how far as a distinct advantage as a safeguard.

v. It may give non-specific results. Uhle and Mackinney(2) had the sera of 141 individuals tested by each of four competent serologists. Of these 141, fifteen were what they regarded as normal controls, believed never to have been infected with gonorrhœa. Only one of the four laboratories reported all the fifteen as negative, two of the laboratories reported one of the fifteen as positive, the fourth found two of them positive. Similar discrepancies occurred with the 37 sera of patients suffering from other diseases who denied having had gonorrhœa. One observer found all these negative, another observer reported as many as five (13·5 per cent.) with positive complement fixation test. But these observations are weakened by the fact that the authors merely state that the observers were competent without giving any indication of the extent of their experience, nor are we advised regarding the technique employed in each of the four laboratories, or as to the status of the laboratories. Admittedly even competent serologists need considerable experience with the method before consistent results are ensured. The observations may thus be on a par with the known discrepancies which occur in connection with the Wassermann test when in the hands of those without extensive training and experience in the performance of that test. Workers in the best American laboratories who

(1) Thomas and Ivy (*Arch. Int. Med.*, Chicago, 1914, **13**, 143) note that in the absence of any complication they failed to obtain positive results within six weeks.

(2) *N. York M.J.* [*etc.*], 1915, **102**. 737.

are careful to describe their technique, agree with singular accord in laying down that a positive complement fixation reaction is most reliable, more specific in fact than is a positive Wassermann test. It is asserted that, contrary to what is the case in syphilis, there is no non-gonococcal infection in which the blood serum gives a positive reaction. Reference has already been made (on p. 11) to the case of cerebrospinal fever with its allied micro-organism. The observations of Thomas, Ivy & Beardsall(1) that out of 180 cases of possible gonorrhœa, 5 gave a positive reaction with an antigen formed of 3 strains of *Micrococcus catarrhalis* has no direct bearing.(2) What is necessary, and to our knowledge has not yet been afforded, is the demonstration whether or no infections with the *Micrococcus catarrhalis* afford positive reactions with gonococcus antigens.

It will be seen from the above that while it cannot replace other methods of diagnosis, the complement fixation test may, in certain cases, be the only laboratory means by which a gonococcal infection may be diagnosed. It is most likely to be of value in cases of metastatic infection where direct demonstration of the gonococcus may be difficult, if not impossible, and it is in these that the highest proportion of positive results is obtained.

As for the Wassermann reaction, and for the same reasons, the Committee cannot at present recommend any one standard method of complement fixation. They can at most append the methods of four careful observers, each of which has been well tested, and invite workers in this country to report their results with one or other method and suggest improvements.

They recommend that such steps be taken as are necessary to secure the provision of an official standardised polyvalent antigen. It would be an advantage if in addition the polyvalent gonorrhœal antigen supplied by one or other firm were issued under Government control, *i.e.*, that each batch of such antigen be tested officially and issued with date of preparation, serial number, and certificate that it has passed the official test.

They recommend that in tests made for official returns the method of complement fixation employed be clearly stated.

They are of opinion that the laboratories in which gonorrhœal complement fixation tests are performed for the public services should be few rather than many, the laboratories selected for the performance of Wassermann tests being utilised for this purpose.

The fact that the complement fixation test for gonorrhœa has not, so far, with rare exceptions, been employed in Great Britain, makes it possible to recommend that reports upon this test be accepted only from pathologists who have been thoroughly trained in the performance of the test and of the control observations which are necessary for it. In respect to payments for this test, it should be placed upon the same footing as the Wassermann reaction.

---

(1) *Arch. Int. Med.*, Chicago, 1915, **15**, 265.

(2) In four out of these five the same serum gave a stronger positive reaction with polyvalent gonococcus antigen, the remaining one was negative with the polyvalent gonococcus antigen. *See also* the same authors in Surgery, Gynaecology and Obstetrics, 1914, **19**, 390.

VI.—The Keeping of Records.

For statistical and departmental purposes it is important that each venereal clinic should keep a card index or other accurate record of all cases of gonorrhœa presenting themselves for diagnosis. Each card or form should afford the following data:—

Particulars of Case of Suspected Gonorrhœa.

Identification number or letter of patient.....Date.....

Age.....Sex.....Name of clinic.....

Character of lesion.....

Number of previous attacks.....

Probability of present attack being a relapse.....

Length of time disease has existed.....

Complications { Local..... / Metastatic.....

Nature of treatment previously given.....

Nature of any co-existing venereal disease.....

Diagnosis now made, *positive or negative* (*delete word not applicable*).

Methods of diagnosis employed, as below (*underline which*)—

Clinical signs and symptoms only.

Detection of gonococci in secretion from (state which part)..... { *Obtained direct.* / ,, *after provocative injection of vaccine.* / ,, *after local irritation.*

Complement fixation.

Co-existence of other micro-organisms.

Nature of treatment employed.....

Final result.....

Nature of tests employed to determine the final result should be clearly stated.

Each laboratory performing tests for venereal disease should forward to the proper authority a return of the results obtained at least once a quarter. In the opinion of the Committee, this would allow a steady supervision of the laboratory results and of the accuracy of laboratory routine, at periods not too far apart to permit serious defects to remain long unnoticed.

It is recommended that such returns, for cases of gonorrhœa, actual or suspected, should be made on the following form :—

LABORATORY REPORT ON EXAMINATIONS FOR GONOCOCCAL INFECTIONS.

Quarter ending.....

A.—Microscopical Examination of Stained Specimens.

Sources of Specimens.	Total Number of Examinations.	No. which contained Gonococci only.	No. which contained Gonococci and also other Micro-organisms.	No. which contained no Gonococci but other Micro-organisms.	No. which contained no Micro-organisms at all.
Male Urethra					
Female Urethra					
Cervix Uteri.....					
Bartholin's Glands					
Other parts as under :—					
.....					
.....					
.....					
.....					
.....					

B.—Cultures. Medium usually employed.....

Male Urethra					
Female Urethra					
Cervix Uteri.....					
Bartholin's Glands					
Other parts as under :—					
.....					
.....					
.....					
.....					
.....					

C.—Complement Fixation Tests. Method employed.....

Nature of Affection.	Total Number Tested.		Number Positive.		Number Negative.		Remarks.
	Males.	Females.	Males.	Females.	Males.	Females.	
Acute uncomplicated Gonorrhœa							
Chronic uncomplicated Gonorrhœa							

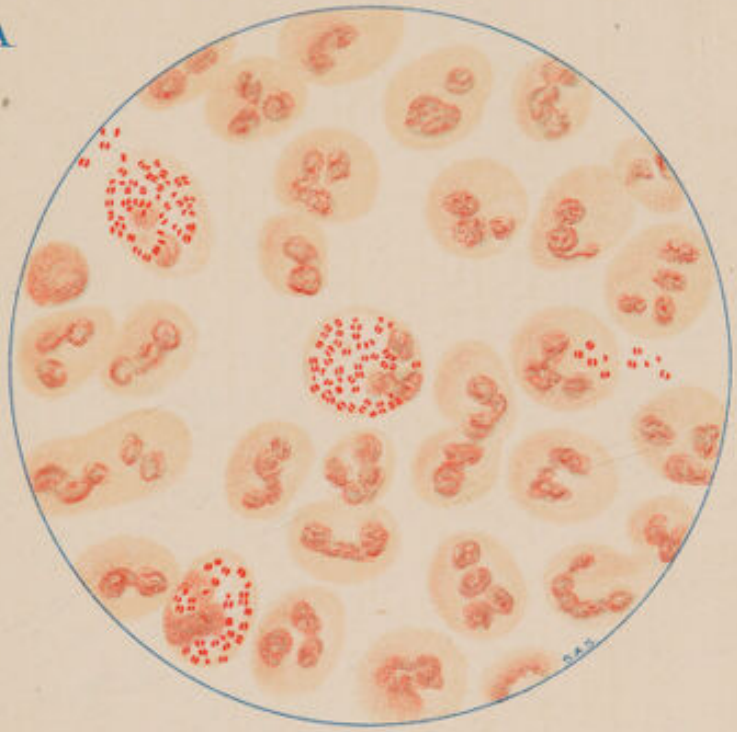
C.—COMPLEMENT FIXATION TESTS—*cont.*

Nature of Affection.	Total Number Tested.		Number Positive.		Number Negative.		Remarks.
	Males.	Females.	Males.	Females.	Males.	Females.	
Gonorrhœa complicated as under :—							
Cowperitis	.....	.....	.....	.....	.....	.....	.....
Prostatic Abscess ...	.....	.....	.....	.....	.....	.....	.....
Chronic Prostatitis...	.....	.....	.....	.....	.....	.....	.....
Epididymitis	.....	.....	.....	.....	.....	.....	.....
Ophthalmia	.....	.....	.....	.....	.....	.....	.....
Salpingitis	.....	.....	.....	.....	.....	.....	.....
Peritonitis	.....	.....	.....	.....	.....	.....	.....
Arthritis	.....	.....	.....	.....	.....	.....	.....
Teno-Synovitis	.....	.....	.....	.....	.....	.....	.....
Bursitis	.....	.....	.....	.....	.....	.....	.....
Iritis.....	.....	.....	.....	.....	.....	.....	.....
Endocarditis	.....	.....	.....	.....	.....	.....	.....
Other Metastatic Gonococcal Affections as under :—							
.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....
Non-Gonococcal Affections as under :—							
.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....

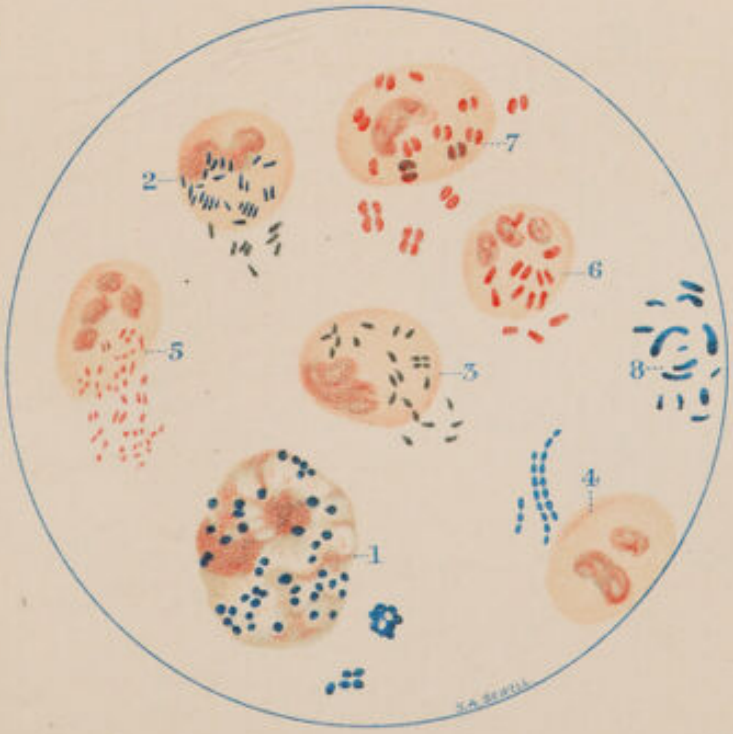
NOTE.—In the report on complement fixation tests do not include one specimen under more than one heading. In choosing the heading, whenever applicable, include under a metastatic complication rather than under one which is due to transfer of the gonococci by other means than the blood stream.

Plate I.

A



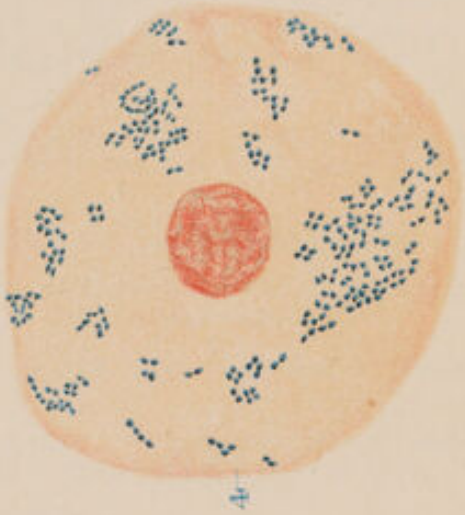
B



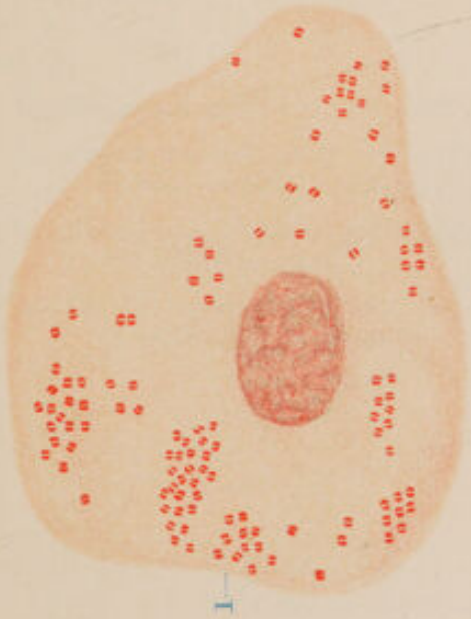
7806. 14927/92. 3000. 7. 18.

Plate II.

2



4



1

3

1836.

EXPLANATION OF PLATES I. and II.

All represent drawings from gonorrhœal urethral smears, stained by Gram's method (Jensen's modification). Magnification, about 1,000 diameters.

Plate I.

A.—Shows pus cells containing intracellular gonococci, also some lying extracellularly. Note that they are Gram-negative and kidney shaped.

B.—This is a composite painting made up from various urethral smears to show the different varieties of secondary organisms which frequently occur in gonorrhœal pus, more especially in chronic cases. All these secondary organisms may occur within pus and epithelial cells, but more commonly they lie extracellularly. Nos. 1 to 6 are painted from the actual specimens. Nos. 7 and 8 are somewhat diagrammatic. Nos. 1, 2, 3 and 5 are the most common types found.

1.—Pus cell showing *Staphylococcus albus*—Gram-positive. Note that some are lying partially digested in vacuoles and have lost their gram-positive character.

2.—Pus cell showing a diphtheroid bacillus (Xerosis type)—Gram-positive.

3.—Pus cell showing a short Gram-positive *Diplobacillus* with pointed ends, indistinguishable morphologically from the *Pneumococcus*.

4.—Shows a similar organism to No. 3, except that it occurs in chains, resembling a *Streptopneumococcus*.

5.—Shows a small *Diplobacillus*—Gram-negative. It assumes various shapes and coccoid forms; the latter are not kidney-shaped but lanceolate.

6.—Pus cell with a large Gram-negative bacillus.

7.—Shows the *Diplococcus magnus* of Rosenthal (?) or "*Pseudogonococcus*." It is weakly Gram-negative, and is four times as large as the *Gonococcus*. It occurs rarely in the urethra, more commonly in conjunctivitis.

8.—Shows a diphtheroid bacillus with clubbing and involution forms—Gram-positive.

Plate II.

1.—Shows an epithelial cell containing numerous *Gonococci*.

2.—Shows an epithelial cell containing many *Staphylococci* and a few diphtheroid bacilli (Xerosis type).

3.—Shows an epithelial cell containing diphtheroid bacilli (Xerosis type) and a few *Staphylococci*.

4.—Shows an epithelial cell containing numerous Gram-positive *Diplobacilli* resembling *Pneumococci*.

APPENDIX I.

THE PREPARATION OF MEDIA FOR GROWTH OF THE GONOCOCCUS.

The Committee are indebted to the authors of the media referred to in the text, namely, to Captain David Thomson, R.A.M.C., to Lieut.-Colonel M. H. Gordon, C.M.G., R.A.M.C. and Major T. G. M. Hine, R.A.M.C., and to Mr. Sydney W. Cole, M.A., for the following detailed descriptions of the methods of preparation of their media:—

I.—Human Plasma Glucose-Agar (Thomson).

One of the most important points in the preparation of this medium is avoidance of prolonged heating in any of the stages where heating is necessary.

A.—Preparation of Double-strength Meat Extract.

Take a bullock's heart and cut away all the fat. Cut the heart into small cubes and mince them. Weigh the mince and place it in a pan with as many c.c. distilled water as there are grammes of heart. (2) Heat the mixture gently, stirring meanwhile with a glass rod until a temperature of 40° C. is reached. Keep at this temperature for 20 minutes. (3) Raise to boiling point and keep boiling for 10 minutes. (4) Strain through a perforated enamelled iron strainer and four thicknesses of butter muslin. (*See* Fig. 1).

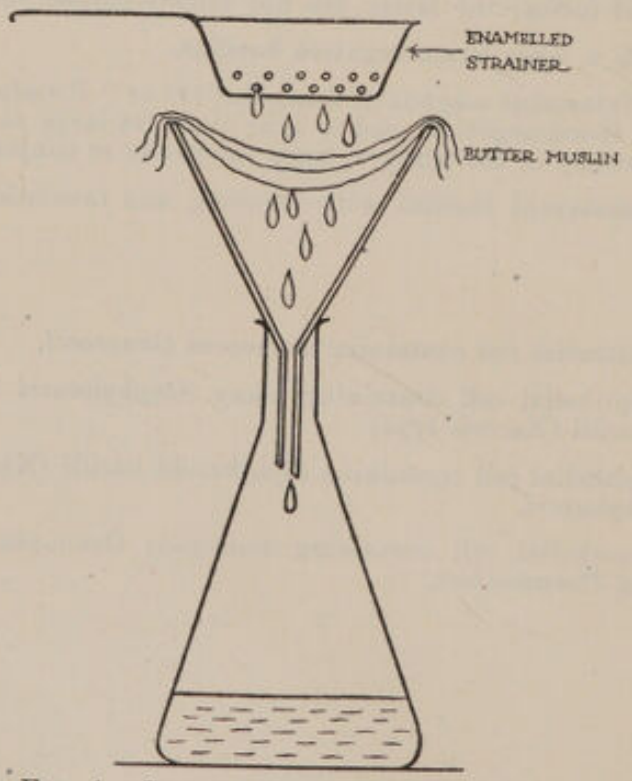


Fig. 1. Straining of Meat Extract.

B.—Preparation of Disodium Hydrogen Phosphate Solution.

Dissolve 10·00 grammes disodium hydrogen phosphate (Na_2HPO_4) in one litre of sterile distilled water.

C.—Preparation of Nutrient Disodium Hydrogen Phosphate Bouillon.

(1) Measure the double-strength meat extract into a sterile flask and add to it an equal volume of the disodium hydrogen phosphate solution.

(2) Weigh out as much good commercial peptone as will make a 1 per cent. solution when mixed with the whole of the extract prepared as under (1). Take about a quarter of this extract, heat it to 60° C. and, in a mortar, mix it with the peptone so as to make a smooth paste.

(3) Add this emulsion to the extract in the flask and steam in the Koch steamer for 45 minutes, in order to complete the solution and stabilise its acidity.

D.—Preparation of Nutrient Agar from the above.

Weigh out sufficient agar-agar to make with the amount of nutrient bouillon prepared as above a 3 per cent. jelly. Mix this into a smooth paste with about one-third of the product of C (3), which must be cold. Add the mixture to the remainder of the nutrient bouillon in the flask and steam in the Koch steamer until the agar is dissolved. This should occupy not more than 60 minutes in the case of powdered, and 90 minutes in the case of shredded, agar.

(2) Allow the agar to cool to 60° C., whip the white of one egg for every 300 c.c. of the agar solution and add the froth to the latter. Replace in the steamer and steam for 30 minutes.

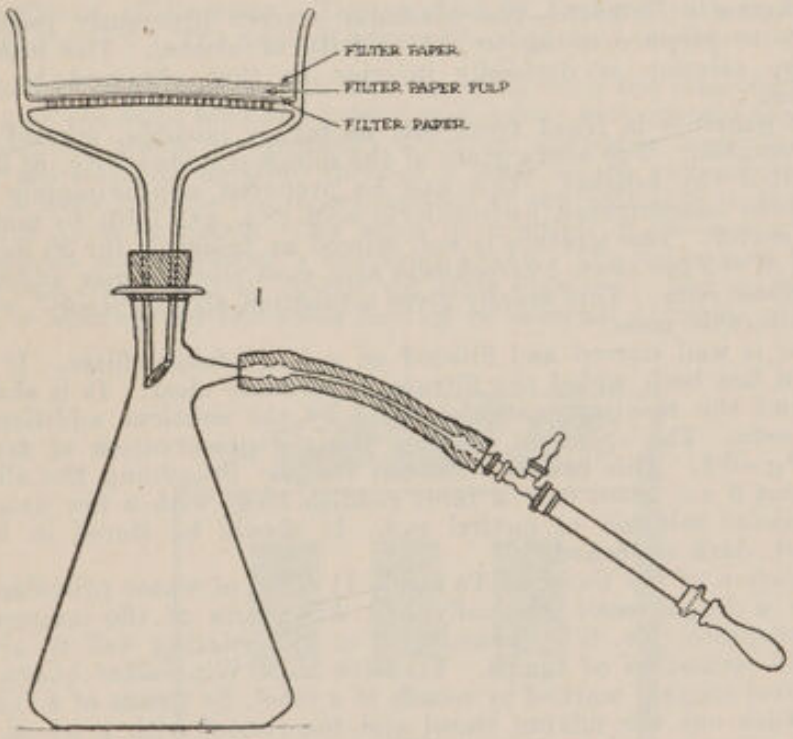


FIG. 2. METHOD OF FILTERING MEDIUM.

(3) Filter in the steamer through a layer of cotton wool laid in a funnel, or outside the steamer through paper pulp laid on a Buchner funnel fitted into a vacuum flask (as shown in Fig. 2). Filtration should be as rapid as possible, as the medium deteriorates if it is kept long in the steamer.

(4) Titrate carefully, using phenolphthalein as the indicator, and make the final product + 6 (Eyre's scale).

(5) Add to the agar solution sufficient glucose powder to make of it a 2·5 per cent. solution of glucose.

(6) Replace in the steamer and steam for 20 minutes. It is better to attempt sterilisation with one steaming than to steam on two or three successive days, and, with care over the aseptic technique of the preceding steps, this is usually successful.

(7) Tube the agar in sterile test-tubes, placing about 4 c.c. in each. Store the tubes of agar in the ice-chest.

E.—Addition of Human Plasma.

Provide the following:—

Apparatus for the drawing off of blood from a vein into sterile test-tubes.

Sterile 10 c.c. centrifuge tubes, each containing 2 c.c. of a sterile 2 per cent. solution of sodium citrate and plugged with cotton wool.

Corks or rubber bungs in alcohol to fit the centrifuge tubes.
Sterile 10 c.c. pipettes.

From any volunteer draw off about 20 c.c. of blood into a sterile test-tube and fill up centrifuge tubes at once with the freshly drawn blood. Cork the latter with one of the corks provided, having previously flamed off the alcohol, and spin in the centrifugal machine until the cells are deposited. Melt the required number of agar tubes, prepared as described above, and reduce their temperature to 60° C. Pipette off the supernatant plasma in the centrifuge tubes and add 0·5 to 1·0 c.c. of it to each of the agar tubes. Mix the agar and plasma by rolling the tube between the hands, and slope in the usual manner. As a rule the yield from each centrifuge tube is about 6 c.c., or about enough for 10 tubes.

Incubate for 24 hours to make sure the medium is sterile.

II.—Tryptamine Media (Cole).

To facilitate and improve the preparation of these media the descriptions given below deviate in some details from those afforded in two previous reports to the Medical Research Committee.(1)

A.—Tryptamine-Blood-Extract Agar ("T.B.E. Agar").

(i) *The Pancreatic Extract.*—The alcoholic extract previously recommended is now expensive to prepare owing to the scarcity of alcohol. The stability of the trypsin in the solution is decidedly inferior to that obtained by the method described below.

Fresh pig's pancreas is freed from fat, as far as possible, minced finely in a machine and weighed. For every gram of the mince is added 3 c.c. of 0·5 per cent. hydrochloric acid (by weight). This can be prepared approximately by diluting 13·7 c.c. of pure concentrated hydrochloric acid (Sp. gr. 1·16) to make 1000 c.c. with distilled water. The mixture is well stirred at intervals for 30 minutes. For every 100 c.c. of 0·5 per cent. hydrochloric acid used there is now added 6·4 c.c. of 5 per cent. caustic soda. This usually gives a reaction about P_H=4·7, which results in a readily filterable mass.

The mixture is well stirred and filtered on a large folded filter. If the correct amount of acid has been added the filtrate is perfectly clear. It is shaken with a little toluol and the reaction made less acid by the cautious addition of 10 per cent. caustic soda. The optimum reaction for the preservation of trypsin seems to be about P_H=5·5. This can be obtained roughly by adding the alkali until a portion of about 3 c.c. gives only a faint reddish tinge with a few drops of a 0·02 per cent. alcoholic solution of methyl red. It should be stored in a stoppered bottle in a cool, dark cupboard.

(ii) *The digestion of the Casein.*—To about 1½ litres of water (distilled, if readily procurable) in a large vessel gradually add 200 grams of the commercial casein known as "lait-proto, No. 6(2)," dusting it in and stirring well to avoid, as far as possible, the formation of lumps. Transfer to a Winchester quart, which has the 2000 c.c. level roughly marked by means of a label, by means of a funnel with a wide neck. Wash out the mixing vessel and the funnel with a jet of hot water, adding the washings to the main bulk. Make the volume up to 2 litres. Shake well to break up any lumps. As the amount of alkali in the casein is not quite constant it is advisable to test the reaction of small portions of the mixture with cresol red and with phenol phthalein. The optimum reaction for the tryptic digestion of casein is about P_H=8·1, at which reaction cresol red gives a reddish violet colour and phenol phthalein remains colourless. Should cresol red give a yellowish colour it is necessary to add 10 per cent. caustic soda in 5 c.c. portions to the bulk until the desired point is reached. The mixture must be well shaken after each addition of soda. If the original solution gives a reddish tinge with phenol phthalein it is not necessary to adjust the reaction by the addition of acid.

To the alkaline solution thus prepared add 120 c.c. of the pancreatic extract and 10 c.c. of toluol as a preservative. Stopper with a cork, shake thoroughly and incubate at 38 to 40 C. Shake the bottle well the next day to break up any lumps that may still remain. Allow the digestion to proceed for 10 days, shaking occasionally to keep the fluid saturated with toluol, a few c.c. more of which may be added if necessary. At the end of the digestion the mixture is transferred to a 3 litre flask, treated with 15 c.c. of concentrated hydrochloric acid diluted to 200 c.c. with water, shaken, steamed for 20 minutes and filtered. To the filtrate

---

(1) Cole, S. W., and Lloyd (Miss), D. J. H., *Journ. of Pathol. and Bacteriol.*, 1917, 21, 267.
Cole, S. W., and Onslow, H. *Lancet*, 1916, ii. 9.

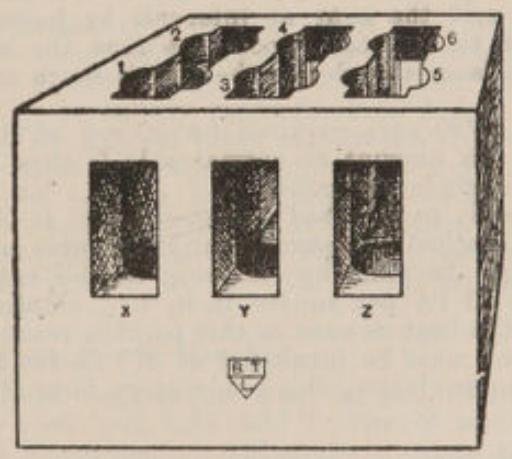
(2) This is manufactured by Casein, Ltd., Culvert Works, Battersea, S.W.

5 per cent. caustic soda is added until the reaction is nearly neutral to litmus, but it is important that it should not be alkaline to this indicator. The fluid thus obtained is known as " stock tryptic broth " or " Tryptamine."(1)

(iii) *Preparation of the Agar.*—To 1 volume of the above broth add 2 volumes of tap water, and, if necessary, render the mixture distinctly acid to litmus by the cautious addition of strong hydrochloric acid. Add agar (powdered or fibre) to make 2 per cent. Dissolve by steaming and filter according to the usual laboratory procedure. To every 500 c.c. of the agar at the temperature of 65° to 70° C. add 50 c.c. of defibrinated sheep's blood and one egg-white, well beaten. The addition of the egg-white is found to assist in the subsequent formation of the clot and materially to improve the final yield. The mixture should be transferred to (Erlenmeyer) flasks, and must be steamed for 45 to 60 minutes. The best yield is obtained by steaming in volumes not greater than 1 litre. The bulk of the clot can be very rapidly removed by straining through a fine wire sieve, the clot being squeezed by a glass plate or clock glass. The final clearing is effected by straining through a mat of glass wool supported on a perforated filtering disc in an ordinary funnel, the whole being kept hot by means of a hot water funnel. *On no account must filter paper or cotton wool be employed.*

(iv) *Adjustment of Reaction.*—The reaction is brought to a hydrogen-ion concentration of $P_H = 7{\cdot}6$. The method adopted is that used by Cole and Onslow.(2)

The method is based on the fact that solutions with the same hydrogen-ion concentration give the same tone and intensity of colour with certain special indicators. As bacteriological media are generally coloured, allowance must be made for this by viewing the standard solution through a layer of the medium employed. It is important to keep the relative concentrations of the indicator in the standard and test solutions quite constant. The effect of dilution of bacteriological media on the hydrogen-ion concentration is relatively slight. The tubes used for the method must be of thin clear glass, of exactly the same diameter. The stand figured below is adapted to hold special test-tubes having an external diameter of five-eighths of an inch.(3)



Stand to hold six test-tubes for colorimetric estimation.

In the method described the comparison is made with tubes containing phosphate solutions of P_H 7·5 and 7·7, it being desired to obtain a tint intermediate between these. If preferred a single tube containing a phosphate solution of P_H 7·6 can be employed. This is left to the choice of the worker, some preferring the single tube, whilst others find it easier to get between two limiting tubes.

The standard solutions required are best prepared by adding standard soda to a standard solution of acid potassium phosphate (KH_2PO_4).

0·2 *M. Acid Potassium Phosphate.*—Dissolve 27·231 gm. in distilled water and make up to 1 litre.

---

(1) A concentrated solution of Tryptamine can be obtained from Messrs. Baird & Tatlock (London).

(2) Loc. cit.

(3) The colorimeter stand and special test tubes can be obtained from Messrs. Baird & Tatlock (London).

0·2 *N. Sodium Hydroxide.*—This can be prepared by diluting accurately standardised normal soda with four times its volume of distilled water. To get exact results the soda should be free from carbonate. This can be obtained by the method given by Clark and Lubs,(1) but for this purpose the ordinary soda gives a sufficient approximation.

Standard Solution $P_H = 7{\cdot}5$.—To 50 c.c. of the acid potassium phosphate add 41·2 c.c. of the 0·2 N. soda and dilute with distilled water to make 200 c.c.

Standard Solution $P_H = 7{\cdot}6$.—To 50 c.c. of the acid potassium phosphate add 42·8 c.c. of the 0·2 N. soda and dilute with distilled water to make 200 c.c.

Standard Solution $P_H = 7{\cdot}7$.—To 50 c.c. of the acid potassium phosphate add 44·2 c.c. of the 0·2 N. soda and dilute with distilled water to make 200 c.c.

Indicator, 0·02 per cent. solution of phenol-sulphone-phthalein (" phenol red "). This can be obtained(2) in phials containing 2 c.c. of a 0·6 per cent. solution, sufficient to prepare 60 c.c. of the dilute indicator required.

Into tube (1) measure 5 c.c. of phosphate solution. $P_H = 7{\cdot}5$, and 0·5 c.c. of phenol red.

Into tube (5) measure 5 c.c. of phosphate solution. $P_H = 7{\cdot}7$ and 0·5 c.c. of phenol red.

To tube (4) add about 5 c.c. of water.

Measure exactly 10 c.c. of the hot agar into 40 c.c. of cold distilled water and mix well. Before it has set measure 10 c.c. into tube (3) and add 1 c.c. of phenol red.

To tubes (2) and (6) add about 5 c.c. of the diluted agar.

Titrate the diluted agar in tube (3) with N/20 soda, preferably from a microburette, mixing well by closing with the thumb and inverting two or three times. If a microburette is not available the titration can be done by means of a 1 c.c. pipette graduated in 1/100ths c.c. Continue the addition of the soda until the colour tint seen by holding the box to the light and viewing through Y approaches that seen through X. Note the amount of soda used and add half this volume of distilled water to each of the tubes (1) and (5), the purpose being to keep the indicator at the same concentration. Continue to add the N/20 soda to tube (3) until the tint seen through Y is intermediate between those seen through X and Z, distilled water being added to tubes (1) and (5) as the titration proceeds. It is generally necessary to melt the agar at intervals by immersing the tube (3) in boiling water. It is of the utmost importance that the solution be well cooled before the colour comparison is made, as the tint is much affected by temperature variations.

The total amount of N/20 soda required for 10 c.c. of the 1 in 5 agar is read off. Twenty-five times this amount of normal soda is then added to each litre of the melted agar, and the whole well mixed.

The medium is now ready to be tubed and sterilised. It is probably better to do this by the intermittent method of steaming for 15 minutes on three successive days. For most purposes it can be done by steaming in the autoclave for 5 minutes, raising the pressure to 10 lb. per square inch, *i.e.*, obtaining a temperature of 115° C. and cutting off the heat as soon as this point is reached.

After sloping the tubes must be incubated at 37° C. for 24 hours. If they are kept several days before inoculation, this preparatory incubation may be omitted.

B.—Tryptamine-Blood-Extract Broth (" *T.B.E. Broth* ").

Dissolve 15 grms. of gelatine in 333 c.c. of tap water. Add 167 c.c. of tryptamine, 50 c.c. of defibrinated sheep's blood, and one egg-white, well beaten. Steam for an hour. Strain off the clot by means of a fine wire sieve, squeezing the clot well by means of a glass plate. Cool to 45° and measure. To every 100 c.c. add 2 c.c. of the pancreatic extract described in A (i), or of any other suitable tryptic preparation. Place the mixture in a tall cylinder and allow it to stand at room temperature over night. Siphon off the clear fluid, which must not be filtered through filter paper. The liquefaction of the gelatine can also be effected by incubation for two or three hours at 37° C., but the resultant broth obtained by this method is apt to be cloudy.

The medium must now be adjusted to $P_H = 7{\cdot}6$. This is done in a manner similar to that described in A (iv), except that in tubes (2), (3) and (6) are measured 5 c.c. of the broth. To tube (3) is added 0·5 c.c. of phenol red. This is titrated with N/20 soda from a burette until the tint seen through Y is intermediate between

---

(1) Clark, W. M., and Lubs. *J. Bact.*, Balt., 1917, **2**, 22.

(2) This and other valuable indicators, are manufactured by the Cooper Laboratory of Economic Research, Watford.

that seen through X and Z. In this case the volume of water that has to be added to (1) and (5) is the same as the volume of soda employed.

For every 0·1 c.c. of N/20 alkali required for the 5 c.c. of broth, 0·1 c.c. of normal soda is added for 100 c.c. of broth. Sterilise by steaming or autoclaving as described above.

This medium is mainly used for testing fermentation reactions. Glucose, maltose and sucrose are employed in 1 per cent. concentration. The best indicator is phenol-sulphone-phthalein, 4 c.c. of a 0·06 per cent. solution being added to every 100 c.c. of the sugar broth.

C.—The Preparation of Tryptamine-Spleen Broth.

Remove the capsule from a bullock's spleen (milt). Mince the pulp and weigh. For every 100 grms. of pulp take 200 c.c. of water and 9 grms. of gelatine. Dissolve the gelatine in the water, cool to 40° C. and add it to the pulp. Incubate for two hours at 37° C. For every 1,000 grms. of spleen pulp taken add 20 c.c. of defibrinated sheep's blood. Add also one egg-white for every 500 c.c. of the total mixture. Steam for an hour. Strain through a fine wire sieve. Cool to 45° C. and measure. To every 100 c.c. add 2 c.c. of one of the pancreatic extracts mentioned above. Place the mixture in a tall cylinder and allow to stand at room temperature over night. Siphon off the clear fluid and measure. To every 100 c.c. add 50 c.c. of tryptamine broth. Adjust the reaction to $P_H = 7·6$ as described for the T.B.E. broth. Tube and sterilise by the intermittent method.

D.—The Preparation of Tryptamine-Spleen-Blood Agar.

Prepare tryptamine-blood-extract agar, as described above, except that 2·5 per cent. agar is employed. Adjust the reaction to $P_H = 7·6$, as described in A (iv).

To 200 c.c. of this add 100 c.c. of the tryptamine-spleen-broth, also adjusted to $P_H = 7·6$. Tube and sterilise.

III.—Trypsinized Pea-Extract Agar ("Trypagar") (Gordon and Hine).

A.—Preparation of Saline Pea Extract.

Take 100 grammes of peaflour (Pearce Duff's) and add 1 litre of distilled water with 100 grammes of salt. Mix and steam for half-an-hour, stirring occasionally. Allow to settle and filter, then sterilize and label "Saline Pea Extract." This pea-flour extract should preferably be freshly made for each batch of agar. The filtering should be done through an English make of Chardin's filter paper, which may be obtained from Messrs. Baird & Tatlock; ordinary filter paper or wool must not be used. The quality of the peaflour is of great importance. The extract may be tested by adding one drop of concentrated HNO_3, when a heavy precipitate forms which should dissolve in excess of the acid. This test, however, is not of very much value; the only real test is that of "growth" when the extract is made up with the medium.

B.—Preparation of Trypsin Broth (Douglas).

Take some fresh bullocks' hearts, free from fat and vessels, mince the meat very finely and weigh. To each half kilo. add 1 litre of water and make distinctly alkaline to litmus with 20 per cent. KOH solution. Heat this slowly to 75°–80° C. for five minutes. Cool to 37° C. and add 1 per cent. of liquor trypsinae co. (Allen & Hanbury's) and keep it at 37° C. for three hours. (The trypsin preparation mentioned should be used, as several others have not proved satisfactory.) When trypsinising is finished, test for peptone in the following manner:—Take 5 c.cm. of broth, add 0·1 c.cm. of 5 per cent. solution of $CuSO_4$; mix, and then add 5 c.cm. normal NaOH; a true pink colour indicates that trypsinisation is sufficient; a bluish-purple shade that it is incomplete. Then render slightly acid to litmus with glacial acetic acid and bring slowly to the boil for a quarter of an hour. Leave covered overnight in a cool place and siphon off the clear liquid in the morning. Make this broth distinctly alkaline to litmus and (if not to be used at once) sterilise in an autoclave at 118° C. for one hour on each of two days. The necessary addition of acids and alkalis must be carefully made, little by little, so that there shall be no excess of either.

C.—Preparation of Fibre Agar.

Weigh out the required quantity of fibre agar, cut up small with scissors, place in a large flask or enamel pail and wash twice quickly in water. Drain thoroughly, add water just to cover and put in glacial acetic acid to 0·25 per cent. Mix well and leave for a quarter of an hour. Pour off the liquid and wash *thoroughly* four or five times to make sure that all the acetic acid is washed out. Drain carefully.

D.—To Make Trypagar.

Take a measured quantity of the trypsinised broth, add 1·75 per cent. agar fibre prepared as above, and 0·125 gramme of calcium chloride per litre. Autoclave at 118° C. for three quarters of an hour to dissolve the agar. Titrate 20 c.c. of the medium diluted with 20 c.c. of water with $\frac{N}{10}$ NaOH while boiling, using α-naphtholphthalein (0·04 per cent. in 60 per cent. alcohol) as an indicator, adding with a glass rod a drop of the medium to a drop of the indicator at a time on a white tile till the first transient flush of bluish-green occurs; read off the amount used from the burette. Then go on with the titration again, adding 0·5 c.c. of 1 per cent. phenolphthalein in 99·8 per cent. alcohol to the medium and continuing until the first faint pink colour appears; take this reading also. The mean of these two readings gives the point to which the medium must be adjusted, and the requisite amount of 10/N NaOH (dekanormal) is then added to the bulk of the medium. This medium should now prove alkaline to α-naphtholphthalein and acid to phenolphthalein. Cool to 60° C., add white of egg (2 to a litre), beaten up well; autoclave again at 118° C. for 75 minutes (or in the steamer for two hours). Filter again through the Chardin's filter paper supplied by Baird & Tatlock, add to the filtrate 5 per cent. of the sterile pea extract and sterilise in the ordinary way. This medium must not be steamed more than necessary, as it gives off ammonia in steaming, which alters the reaction if done too often.

For the cultivation of both the meningococcus and the gonococcus, it is advisable to add 2 per cent. of a sterile 5 per cent. hæmolysed solution of rabbit's blood in normal saline, etherised by the addition of 10 per cent. of ether.

APPENDIX II.

COMPLEMENT FIXATION TESTS FOR GONORRHŒA.

I.—Method of Schwartz and McNeil.

Given in "The Complement Fixation Test in the Diagnosis of Gonococcic Infections," by H. J. Schwartz, M.D., and Archibald McNeil, M.D. (From the Department of Clinical Pathology of the Cornell University Medical School, New York.) American Journal of Medical Sciences, 1911, N.S. **141,** 693.

"In our work we have used both the antisheep and antihuman hæmolytic systems, and have followed the technique as laid down in the well-known Wassermann test for syphilis, and in Noguchi's modification of the Wassermann test. We would, however, say that in employing the antihuman system we have invariably used *inactivated* patients' serum. The preparation and titration of the amboceptor, complement, and red blood cell suspension for the two systems has been so frequently described that we do not think it necessary to enter into the details here.

Preparation of the Antigen.—1. In the preparation of the antigen, from nine to twelve different strains of gonococci have been used at various times.

2. To cultures of gonococci from 24 to 48-hours old grown on Thalmann's medium,(1) containing 2 per cent. of glucose, 2 c.c. of sterile 0·85 per cent. saline solution to each tube was added and the growth of cocci scraped off the surface of the media with a platinum loop.

3. The resulting suspension of cocci in saline was then decanted into a sterile bottle and placed in an inactivating oven at a temperature of 56° C. for 30 minutes; it was then placed in a shaker and agitated for 24 hours, after which it was centri-

(1) This alkaline agar medium can well be replaced by any of the media described in the Appendix (pp. 18–24).

fugated; the supernatant fluid was then pipetted off and placed in a tightly corked sterile bottle, a capillary drop of lysol being added as a preservative. This was placed in the ice chest until required for tests.

Some recent experiences have led us to believe that possibly a better antigen might be obtained by allowing the suspension of cocci in the saline to remain for several hours without shaking, at a temperature of about 37° C., as a more thorough extraction seems to take place by so doing.

For the titration of the antigen we have used as a positive control either the serum of a patient who has been treated with injections of dead gonococci, or else Torrey's antigonococcic serum, as prepared by Parke, Davis & Co. This is serum from an animal which has been immunized against many different strains of gonococci. The quantity of antigen to be used is the quantity that will completely inhibit hæmolysis of the given suspension of red blood cells in the presence of definite quantities of positive serum and complement; provided that double this amount does not interfere with complete hæmolysis of the cells, using a normal serum and complement. Our daily routine is as follows:—

1. *Titration of the Complement* with the given quantity of red blood cell suspension and given quantity of an amboceptor of known hæmolytic strength, the object of this being to determine the exact complement value of the guinea-pig serum used. Careful estimation of the exact quantities of amboceptor and complement to be used is of the greatest importance if accurate and reliable results are to be obtained.

2. *Titration of antigen* against a positive serum. This procedure gives us known quantities of amboceptor, complement, antigen, and red blood cell suspension.

The technique of the day may be graphically depicted as follows, using the anti-sheep hæmolytic system in a positive case."

TABLE I.

Patient's Serum.	Antigen.	Complement 10 per cent.	Amboceptor.	Sheep's red blood cells 5 per cent.	Hæmolysis.
0·15 c.c.	0	0·05 c.c.	0·05 c.c.	0·05 c.c.	Complete.
0·10 c.c.	0	0·05 c.c.	0·05 c.c.	0·05 c.c.	Complete.
0·05 c.c.	0	0·05 c.c.	0·05 c.c.	0·05 c.c.	Complete.
0	Double titrated quantity.	0·05 c.c.	0·05 c.c.	0·05 c.c.	Complete.
0	0	0·05 c.c.	0·05 c.c.	0·05 c.c.	Complete
0	0	0	0·05 c.c.	0·05 c.c.	None.
0	0	0·05 c.c.	0	0·05 c.c.	None.
0·15 c.c.	Titrated quantity	0·05 c.c.	0·05 c.c.	0·05 c.c.	None
0·10 c.c.	,,	0·05 c.c.	0·05 c.c.	0·05 c.c.	None.
0·05 c.c. Pos. serum.	,,	0·05 c.c.	0·05 c.c.	0·05 c.c.	None.
0·10 c.c.	,,	0·05 c.c.	0·05 c.c.	0·05 c.c.	None.
0·10 c.c.	0	0·05 c.c.	0·05 c.c.	0·05 c.c.	Complete.

II.—Ower's Method.

Abstracted by permission from the Article by Major J. J. Ower, C.A.M.C., Canadian Medical Association Journal, 1914, N.S. **4**, 1074.

The technique is given here in detail inasmuch as careful preparation of the reagents is essential for the best results.

"*Complement.*—A guinea-pig is bled 12 hours before the tests by cutting the carotid arteries. The blood is allowed to clot, put on ice, and immediately before use the serum is withdrawn. It is cleared by centrifugation if any red cells are present.

Amboceptor.—Four or five injections of from 2 to 5 c.c. of a 50 per cent. solution of fresh sheep red blood cells in normal saline (0·85 per cent.) are made into the ear vein of a rabbit at intervals of five days. Ten days after the last injection the serum is titrated to determine its value as an amboceptor. If suitable, the rabbit is bled, the blood is allowed to clot, the serum withdrawn, then inactivated by heating at 56° C. for 30 minutes to destroy its natural complement, placed in ampoules and stored on ice.

Red Blood Cells.—Fresh sheep blood cells are washed with saline and centrifugated several times until the supernatant fluid is absolutely clear.

Serum to be Tested.—Five c.c. of blood is drawn from the vein of the patient, allowed to clot, and the serum withdrawn. The serum is cleared by centrifugation and is inactivated by heating at 56° C. for 30 minutes in order to destroy the complement present.

Antigen.—Cultures of gonococci are grown on hydrocele fluid dextrose agar for 24 hours and washed off with sterile distilled water. The suspension is centrifugated, mixed with sterile distilled water, and the supernatant fluid removed. This is repeated three or four times, to remove extraneous material derived from the media; 50 c.c. of sterile distilled water is then added to each 0·5 gm. of sediment and the suspension enclosed in sealed tubes and placed in water at 56° C. for half an hour. It is kept in a warm place (incubator) for ten days, being shaken for about ten minutes daily in a vaccine shaker. It is then centrifugated and the supernatant fluid withdrawn to be used as antigen. A drop of carbolic acid or lysol is added to prevent contamination. To make a polyvalent antigen several monovalent antigens are mixed.

Titrations of the reagents are necessary to establish their value. Complement has been found to vary frequently and is therefore titrated before every series of tests. Amboceptor and antigen as a rule, when kept on ice, do not tend to lose their properties, but as a precaution should be titrated frequently.

In the system usually employed the tests and titrations are made on a basis of a total quantity of 2·5 c.c. in each tube. In both the quantity of red cells used is 0·5 c.c. of a 2·5 per cent. emulsion of fresh red cells in normal saline. In the tests alone the amount of amboceptor and of complement required in each tube is made up to 0·5 c.c. with saline in each case for convenience in handling the pipettes.

Titration of Complement.—Ten per cent. complement and saline is used in this titration. The complement value of guinea-pig serum has been found to vary considerably especially in young pigs. In the system here employed, the unit has been found to vary between 0·3 c.c. and 0·15 c.c. of a 10 per cent. dilution; therefore these limits must be exceeded in the titration. In the titration of complement which is made every day before the tests proper the unit of amboceptor used on the previous day is taken as the standard. The following is a table of the titration:—

Tubes	I.	II.	III.	IV.	V.	VI.	VII.
Amboceptor ...	1 unit in each tube.						
Complement, 10 %	0·5 c.c.	0·4 c.c.	0·3 c.c.	0·25 c.c.	0·2 c.c.	0·15	0·1
Red Blood Cells ...	0·5 c.c. in each tube.						
Saline	Add sufficient to make the total quantity 2·5 c.c. in each tube.						

Incubate in a water bath at 37° C. for thirty minutes. The least amount of complement causing complete hæmolysis is to be taken as the unit of complement.

Titration of Amboceptor.—With this system it has been found that using a 1 to 150 or 1 to 200 dilution of amboceptor in saline with 1 unit of complement the least amount causing complete hæmolysis is usually in the neighbourhood of 0·1 c.c.; although not necessarily so, as it depends entirely on the strength of the amboceptor. The actual titration in the case of an amboceptor known to be approximately this strength would be as follows:—

Tubes	I.	II.	III.	IV.	V.	VI.	VII.
Amboceptor, 1–150	0·3 c.c.	0·25 c.c.	0·2 c.c.	0·15 c.c.	0·1 c.c.	0·075	0·05.
Complement, 10 %	1 unit in each tube.						
Red Blood Cells (Sheep) 2·5 %.	0·5 c.c. in each tube.						
Saline	Add sufficient to make the total quantity 2·5 c.c. in each tube.						

Incubate in a water bath at 37·5° C. for 30 minutes. The unit of amboceptor to be used in the test is represented by the tube containing the least amount which causes complete hæmolysis.

Titration of Antigen.—This is necessary to determine the antigenic value of the extract and also to detect the presence of anti-complementary properties. In a suitable antigen there should be a wide margin between these two. Anti-complementary properties should not be present in less than twice the amount of antigen used in the tests otherwise there would be the danger of false positive reactions. A 20 per cent. solution of antigen in normal saline is usually used in preliminary determination of its antigenic properties, although this may be found

to be too concentrated, in which case a weaker solution is used. The following table gives the usual routine titration:—

Tubes ...	I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.
Antigen, 20%,	0·05 c.c.	0·1 c.c.	0·2 c.c.	0·4 c.c.	0·6 c.c.	0·8 c.c.	1 c.c.	1·2 c.c.	1·4 c.c.
Complement 10 %, 2 units in each tube									
Known Positive Serum	0·2 c.c in each tube.								
Saline ...	Add to 2 c.c. in each tube								

Incubate in water bath at 37·5° C. for thirty minutes, then add:—

Amboceptor	2 units in each tube.
Red Blood Cells 2·5 % ...	0·5 c.c. in each tube.

The presence of antigenic qualities is determined by the presence of inhibition of hæmolysis, and in a good antigen this should be present in the second or third tube.

To determine the anti-complementary properties the above test is repeated, omitting the known positive serum. This is an important titration, and in a suitable antigen hæmolysis should be complete in the tube representing double the unit of antigen chosen for the test.

The routine usually followed each day when tests are made is:—

1. Titration of complement.
2. Titration of antigen.
3. Tests proper. Here three tubes are used—one as a control without antigen and the other two with different quantities of antigen. The unit of antigen used is usually one-half the greatest amount showing complete hæmolysis in the anticomplementary titration. A known positive and a known negative serum are included in each series of tests as additional controls.

The following is a table of the test:—

Tubes	I.	II.	III.
Suspected Serum ...	0·2 c.c. in each tube.		
Complement ...	2 units made up to 0·5 c.c. in each tube.		
Antigen	None.	½ unit.	1 unit.
Saline	Add to make total quantity 1·5 c.c. in each tube.		

Incubate in water bath at 37·5° c.c. for thirty minutes and then add:—

Amboceptor ...	2 units made up to 0·5 c.c. in each tube.
Red Blood Cells 2·5%	0·5 c.c. in each tube.

Return to water bath at 37·5° C. for thirty minutes.

Instead of the last step in the above table the red cells may be sensitised synchronously with the first part of the test by adding equal quantities of amboceptor (two units made up to 0·5 c.c. with saline) and red cells. The mixture is incubated in the water bath at 37·5° C. for half an hour. One c.c. is then added to each tube and the tubes returned to the water bath. The sensitising of the red cells has the effect of hastening the reaction.

The method of reading the results is of importance. This is done when hæmolysis is complete in the controls. A strong positive reaction consists in complete inhibition of hæmolysis. For diagnostic purposes it is doubtful if it is wise to return a report of a positive reaction if there is less than 50 per cent. inhibition of hæmolysis, which one would term a weak positive reaction."

III.—Kolmer's Method.

Method employed by John A. Kolmer, M.D., D.P.H., Instructor of Experimental Pathology, University of Pennsylvania; Pathologist to the Philadelphia Hospital for Contagious Diseases, abstracted by permission from his "Practical Textbook of Infection, Immunity and Specific Therapy," Philadelphia and London. W. B. Saunders Company, 1917, pp. 503 *et seq.*

"*Hæmolytic System.*—As a rule, the antisheep hæmolytic system is employed; the various ingredients may be used in one-half the quantity employed in the original Wassermann reaction, . . . or one-tenth the quantity employed in the original Wassermann technic may be employed. I prefer to employ the larger amounts because the readings are usually easier to interpret.

Fresh guinea-pig complement serum is diluted 1:20 and used in dose of 1 c.c. (=0·05 c.c. serum); sheep's corpuscles are made up in a 2½ per cent. suspension and used in dose of 1 c.c.; antisheep amboceptor is titrated and used in an amount equal to 2 hæmolytic doses in conducting the antigen titration and in the test proper.

Kolmer and Brown have compared the practical value of the antisheep and antihuman hæmolytic systems in the examination of a number of serums. When the latter were used, some of the reactions were somewhat stronger and yielded slightly better results, showing the influence, probably, of natural antisheep amboceptor present in a large proportion of human serums.

Antigen.—This constitutes the most important ingredient of the test. As Teague and Torrey and Schwartz and McNeil have emphasized, the antigen should be prepared of many different strains of gonococci. The difficulty of isolating this organism and the constant care required in subculturing and keeping a large number of strains alive render it practically impossible for many persons to prepare a gonococcus antigen. Therefore, until simpler methods are devised, this antigen is best prepared in large central laboratories, where the cultures are handled and preserved by specially trained persons.

The gonococci are well grown on a salt-free veal agar, neutral in reaction to phenolphthalein, to which a few drops of sterile hydrocele fluid may be added. After culturing for from 24 to 48 hours, the growths are washed off with distilled water, and the emulsion is heated in a water-bath for two hours at 56° C. It is then heated to 80° C. for 1 hour, filtered through paper pulp or centrifugalized and passed through a Berkefeld filter which is reserved for this purpose alone and is neutral in reaction. A small amount of preservative, as, *e.g.*, 0·1 c.c. of a 1:100 dilution of phenol to each cubic centimetre of antigen, may be added. The antigen is then well preserved in small amounts in ampoules that are sealed and heated to 56° C. for half an hour on three successive days. Just before being used the antigen is made isotonic by adding one part of a 10 per cent. salt solution to nine parts of antigen. I preserve the antigen in ampoules containing 1 c.c., and after removing the antigen from the ampoule to a large test-tube, add 1 c.c. of 10 per cent. salt solution, and dilute the whole 1:10 with the addition of 8 c.c. of normal salt solution, after which the anticomplementary titration is made.

In this method of preparing antigen the endotoxins constitute the main antigenic principle. Kolmer and Brown, after an experimental study of the various antigens, found that a simple suspension of gonococci in salt solution yielded slightly better results. The various strains are grown for from 48 to 72 hours, and are then washed off with sterile saline solution, observing particular care not to include portions of the culture-medium. The suspension is then shaken to break up clumps, and heated to 56° C. for an hour. A small amount of preservative is now added, and the antigen stored in 1 c.c. ampoules. Before using it is diluted 1:10 or 1:20, and titrated for the anticomplementary dose. Warden has reported good results with a new lipoid antigen.

Alcoholic extracts of gonococci have very little practical value, as alcohol is not satisfactory for extracting the antigenic principle of bacteria.

The anticomplementary dose of the antigen should be determined, and one-half or one-quarter this amount should be used in conducting the main test. An antigenic titration may also be conducted with an antigonococcus serum, to determine the antigenic value of the antigen, but in practice it is sufficient to use one-half

the anticomplementary dose. This *titration should be conducted and the antigen standardized before the main tests are adjusted.*

In the following table the results of an anticomplementary titration of a gonococcus antigen are given, the approximate dose having been ascertained in previous titrations:—

Anticomplementary Titration of a Gonococcus Antigen.

Tube.	Antigen 1:10 c.c.	Complement, 1:20 c.c.	Saline solution in each tube to 2 c.c.; tubes are shaken and incubated for 1 hour at 37° C.	Anti-sheep Amboceptor, Units.	Sheep's Corpuscles (2·5 %) c.c.	Tubes are shaken and incubated for 1 hour at 37° C.	Results. Hæmolysis.
1	0·2	1		2	1		Complete hæmolysis.
2	0·4	1		2	1		Complete hæmolysis.
3	0·6	1		2	1		Complete hæmolysis.
4	0·8	1		2	1		Slight inhibition of hæmolysis.
5	1·0	1		2	1		Marked inhibition of hæmolysis.
6	0	1		2	1		Complete hæmolysis.

If the antigen is new and the anticomplementary dose is entirely unknown, it may be necessary, in making this titration, to use a different dilution, with higher and lower doses. In conducting the main test the foregoing antigen could be used in dose of 0·2 or 0·4 c.c. of this dilution.

The Test.—The serums should be fresh and clear, and heated to 56° C. for one-half hour. For each serum use four test-tubes (12 by 1 cm.), arranged in a row. Into each of the first three place the dose of antigen and increasing doses of serum—0·05 c.c., 0·1 c.c., 0·2 c.c.; the fourth tube is the serum control, and into this is placed the maximum dose of serum (0·2 c.c.) but no antigen; 1 c.c. of complement diluted 1:20 is added to each tube. The following controls are included:—

1. A positive control with an antigonococcus serum or with the serum of a patient who reacted positively on a former occasion.

2. A negative control with the serum of a healthy person.

Both of these controls may be set up with but the maximum dose of serum (0·2 c.c.).

3. The serum control of each serum is conducted in the fourth tube of each series. At the completion of the test this tube should show complete hæmolysis and thereby indicate that the serum was not anticomplementary.

4. The antigen control at this time includes the dose of antigen and complement.

5. The hæmolytic system control at this time receives the dose of complement.

6. The corpuscle control receives 1 c.c. of the corpuscle suspension.

To each tube sufficient saline solution is added to bring the total volume up to about 2 c.c. The tubes are shaken and incubated for one hour at 37° C. in the thermostat or in a water bath (not less than 1 hour), when 2 units of antisheep amboceptor and 1 c.c. of sheep corpuscle suspension are added to each tube except the corpuscle control. The tubes are gently shaken again and re-incubated for an hour or longer, depending upon the hæmolysis of the controls, after which the results are recorded. This secondary incubation may be omitted and the tubes placed in a refrigerator overnight and the results read the next morning. Under these conditions hæmolysis occurs slowly, and according to some workers in this field the reaction becomes more delicate. *Conducting the primary incubation by placing the tubes in a refrigerator at 8° C. for 4 hours or overnight after the method of McNeal, followed by the addition of 2 units of hæmolysin and 1 c.c. of corpuscle suspension to each tube and incubation in a water bath at 38° C. for ½ to 1 hour yields particularly delicate reactions and intensifies the degree of specific fixation of complement.*

The following table is an example of a gonococcus fixation test with the serum of a case of gonorrhœal arthritis:—

Gonococcus Complement—Fixation Test.

Patient's Serum. c.c.	Antigen 1:10 c.c.	Complement, 1:20 c.c.		Antisheep Amboceptor Units.	Sheep's Corpuscles (2·5 %) c.c.	Results, after one and a half hours incubation.
0·05	0·2	1	Saline solution q.s. 2 c.c.; tubes are gently shaken and incubated for an hour at 37° C.	2	1	Slight inhibition of hæmolysis.
0·1	0·2	1		2	1	Marked inhibition of hæmolysis.
0·2	0·2	1		2	1	Complete inhibition of hæmolysis.
0·2	0	1		2	1	Serum control: hæmolysis.
Pos. Serum.						
0·2	0·2	1		2	1	Complete inhibition of hæmolysis.
0·2	0	1		2	1	Serum control: hæmolysis.
Neg. Serum.						
0·2	0·2	1		2	1	Hæmolysis.
0·2	0	1		2	1	Hæmolysis.
0	0·2	1		2	1	Antigen control: hæmolysis.
0	0	1		2	1	Hæmolytic control: hæmolysis.
0	0	0		0	1	Corpuscle control: no hæmolysis.

In reading the results the controls are first examined and should show complete hæmolysis; the test is reported as negative if all the tubes are hæmolysed, weakly positive if the largest dose only of serum (0·2 c.c.) shows inhibition of hæmolysis, moderately positive if the 0·1 and 0·2 c.c. doses of serum show inhibition of hæmolysis and strongly positive if the 0·05, 0·1 and 0·2 c.c. doses react positively.

The test may be conducted with but one dose of serum, namely, 0·2 c.c. with the antigen and 0·2 or 0·3 c.c. in the serum control tube, after the manner of the original Wassermann reaction; in this case the readings are made after the usual +, + +, + + +, + + + + method.

Gonococcus Fixation Test, Using One-tenth the Usual Amounts.

This technic is employed for purposes of economy, especially since the antigen is likely to be expensive. Otherwise the method is less desirable than the preceding one, as the results are more difficult to read.

Complement serum is diluted 1 : 10 and used in dose of 0·1 c.c.; corpuscles are made up in a 10 per cent. suspension and used in dose of 0·1 c.c., the amboceptor is titrated with these amounts of complement and corpuscles, and used in dose equal to two units. Each day, before the main tests are undertaken, the anticomplementary dose of antigen is determined by placing increasing doses of diluted antigen with complement and salt solution in a series of tubes, incubating for an hour, adding two units of amboceptor and the corpuscles, followed by incubation for another hour. One-half or one-quarter of the anticomplementary dose is used in making the main test. The serums are inactivated and used in three ascending doses—0·005 c.c., 0·01 c.c. and 0·02 c.c.—equivalent respectively to 0·5 c.c., 1 c.c. and 2 c.c. of a 1 : 100 dilution (0·1 c.c. serum, 9·9 c.c. salt solution). The fourth tube of each series contains the maximum dose of serum without antigen, and is the serum control. The other controls, general technic, and readings of the reaction are the same as those previously described."

IV.—Thomson's Method.

A new method of preparing Gonococcus Antigen and a new technique for the Complement Deviation Test.

Captain David Thomson, R.A.M.C., M.B., Ch.B. (Edin.), D.P.H. (Camb.) has been good enough to communicate for the purposes of this Report the following preliminary description of some new methods. His work was carried out in the laboratory of the Military Hospital, Rochester Row, London, S.W., under the direction of Colonel L. W. Harrison, D.S.O., K.H.P.

"*Introduction*—The complement deviation test in gonorrhœa is destined to be of great importance. It is well known that it is extremely difficult to determine, by clinical and microscopic methods, when a patient is definitely cured of this disease. This is especially true in the case of women, and it is a great responsibility for a physician to state that a given case is free from infection. The test will come to be of great value in this respect. It will prove a great source of help to the gynæcologist and will aid considerably in the differential diagnosis of many obscure cases of joint disease.

Researches which led to the new method of preparing the Gonococcus antigen.—The gonococcus is a germ which autolyses or breaks up very readily. For this reason a vaccine of the organism does not keep well, but rapidly disintegrates and eventually becomes an autolysate. An investigation was carried out with a view to finding the cause of this autolysis, so that a more stable vaccine might be prepared. I eventually ascertained that weak acids prevented autolysis, but that weak alkalis increased it. In consequence we now put up the vaccine in a weak acid solution by using 0·6 per cent. acid sodium phosphate instead of ordinary saline. In the course of this research I found that the gonococcus was extremely soluble in alkali, so much so, that if a profuse growth of the germ is shaken up with beads in an $\frac{N}{10}$ or $\frac{N}{20}$ solution of sodium hydrate, a clear solution is formed almost instantaneously. If sufficient acid be added to this alkaline solution to render it acid, a white precipitate is immediately formed, which I take to be a precipitate of fatty acids. So it would appear that the gonococcus consists largely of a fatty substance, which is immediately saponified by the addition of an alkali. The meningococcus is similarly soluble in alkali but apparently not quite so soluble as the gonococcus and a faint milky tint is left in the solution. A strain of micrococcus catarrhalis on the other hand is not soluble in alkali, so that a permanent opaque white emulsion remains after shaking up a growth of this organism in $\frac{N}{20}$ sodium hydrate. It seems to me that this might be a useful method of distinguishing between these three allied species of organisms.

Method of preparing the Gonococcus antigen.—Colonel Harrison asked me to try to work up the complement deviation test for gonorrhœa. He had used the ordinary gonococcus vaccine as antigen with successful results in 1914. In consequence I employed at first our stock vaccine and found that it gave successful results when used in a strength of about 100 millions per c.c. More concentrated vaccines were too anticomplementary for use. It then occurred to me that an antigen prepared by dissolving the gonococci in alkali and then rendered neutral, might be suitable, and indeed it proved to be superior to the vaccine itself, being less anticomplementary and at the same time more powerful as an antigen in the blood test.

I make up this dissolved antigen in the following manner:—

(1) Prepare a standard emulsion of gonococci such that 1 c.c. contains 1,000 millions per c.c. and place this in a sealed test tube about 1 inch in diameter. (Emulsion in 0·5 per cent. NaH_2PO_4 + 0·5 per cent carbolic acid.)

(2) Shake up a profuse growth of gonococci (24 hours incubation) in a test tube containing beads and about 6 c.c. of saline.

(3) Add one half of this concentrated emulsion to a test tube (A) of the same diameter as that which contains the standard, and add the other half to a similar test tube (B).

(4) Dilute the emulsion in tube (A) until it exactly matches the standard containing 1,000 millions per c.c.

(5) To the other tube (B) add a few c.c. of $\frac{N}{10}$ sodium hydrate to dissolve the gonococci; a clear alkaline solution will be immediately obtained. Now add $\frac{N}{10}$ hydrochloric acid gradually until it is just neutral or very faintly alkaline to litmus. We now have a nearly neutral solution of gonococci. Add to this 0·85 per cent. saline containing 0·5 per cent. carbolic acid until the volume is exactly the same as that in tube (A)

prepared as in (4) to match the standard. This gives us a solution containing 1,000 millions of dissolved gonococci per c.c. This should be stored in the ice chest as the stock antigen. It appears to keep well and it does not become infected because of the weak carbolic it contains.

(6) For use this antigen should be diluted ten times with 0·85 per cent saline, so as to bring it to a strength corresponding to 100 millions per c.c. When used in the blood test, it will be found that this antigen will absorb as a rule much less than one minimum hæmolytic dose of complement.

Antigens of many strains of gonococci have been prepared as above and are now being used in this research. So far I have tested the sera of 30 cases, with 12 separate antigens, as well as with a mixed antigen composed of all the 12 strains together. This has shown clearly that a compound antigen is better than the antigen prepared from a single strain. A single antigen may only give a very weakly positive test with a given serum while the single antigen of a different strain will give a strongly positive test, a compound antigen, however, gives a good positive where a single antigen may only give a very indefinite positive. It was also considered advisable to use a meningococcus antigen simultaneously with the gonococcus strains in my experiments, and a culture of this organism was kindly given by Major Hine, R.A.M.C. So far my experiments have shown that the meningococcus antigen almost invariably gives a weakly positive result with a strongly positive gonococcal serum; but a weakly positive serum gives a negative result with the meningococcus antigen. This shows that the meningococcus is a very closely allied organism.

Parke Davis's compound gonococcus antigen was tested against a compound gonococcus antigen prepared by the new method described above. The latter proved to be superior in two respects. It was less anticomplementary and more powerful antigenically, and sera which gave a weak positive with the Parke Davis antigen, gave a strong positive with that prepared by the new method. It would appear therefore that a definite advance in the preparation of the antigen has been achieved.

The new technique employed in carrying out the blood test.—For this new and highly satisfactory technique I am indebted to Lieut-Colonel Harrison. He advised me at the commencement of my experiments to use a well-diluted serum, and to allow for thorough fixation of the complement by placing the tube in the ice-chest overnight, previous to the addition of the sensitised cells, according to the method investigated by Drs. Griffith and Scott,. Local Government Board, Bacteriological Laboratory, as a result of their researches on the Wassermann test (unpublished).

(1) *Inactivation of the serum.*—The serum to be tested is inactivated by heating to 55° C. for ten minutes in a water bath, before dilution.

(2) *Standardisation of the complement.*—The complement should be titrated against the stock antigen diluted 1 in 10 with 0·85 per cent. saline as used in the test. To twelve Wassermann tubes add 0·1 c.c. of guineapig's serum (complement) diluted as follows:—

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
$\frac{1}{10}$	$\frac{1}{20}$	$\frac{1}{30}$	$\frac{1}{40}$	$\frac{1}{50}$	$\frac{1}{60}$	$\frac{1}{70}$	$\frac{1}{80}$	$\frac{1}{90}$	$\frac{1}{100}$	$\frac{1}{110}$	$\frac{1}{120}$

To each add 0·1 c.c. of the diluted antigen (1 in 10). Shake the tubes and place the tray in the ice-chest for one hour. Then put tray in water-bath at 37° C. for half an hour. Add to each tube 0·1 c.c. of fully-sensitised sheep's corpuscles (3 per cent. suspension). Replace tray in water-bath at 37° C. and take the readings after 15 minutes. The lowest dilution of complement which produces hæmolysis in the tubes should be taken as one minimum hæmolytic dose.

(3) *The test.*—Four tubes, A, B, C and D, are employed.

(*a*) To A, B and C add 0·1 c.c. of the inactivated serum (diluted 1 in 20). To D add 0·1 c.c. of the inactivated serum diluted 1 in 5.

(*b*) To A add complement 0·1 c.c. containing $3\frac{1}{2}$ minimum hæmolytic doses.
" B " " " " " 3 " " "
" C " " " " " $2\frac{1}{2}$ " " "
" D " " " " " $2\frac{1}{2}$ " " "

(*c*) To A, B and C add 0·1 c.c. compound antigen (diluted 1 in 10). Tube D acts as the control.

(*d*) Place the tubes in the ice-chest overnight and next morning add to each 0·1 c.c. of a 3 per cent. emulsion of fully-sensitised corpuscles. Then place the tubes in the water bath at 37° C. and take the reading after

15 minutes. The control tube D should show complete hæmolysis. Hæmolysis in A, B and C indicates a negative reaction. No hæmolysis in A, B and C indicates a strong positive. No hæmolysis in C alone indicates a weak positive.

An antigen control containing no serum but an equivalent amount of saline and 2½ M.H.D. is necessary, since the antigen absorbs a considerable amount of complement during the long period of fixation in the ice-chest. It is not practicable to use the long fixation method in the process of standardising the complement, since the complement deteriorates from day to day. Known positive and negative sera should always be included in the series, and all the tests rejected in the event of these controls being unsatisfactory.

The results obtained have been very satisfactory, and this is due, no doubt, to the superiority of the antigen as well as to the superiority of the long method of complement fixation in the ice-chest. In 25 cases the sera of patients whose gonorrhœal discharge commenced less than one week previously 15 positive reactions were obtained. In 348 cases of gonorrhœa, complicated and uncomplicated, the reaction was positive 322 times. In addition to this, out of 28 sera of clinically cured gonorrhœa cases, 18 gave a positive reaction. Six out of 46 cases of syphilis, where gonorrhœa was denied, gave a positive reaction. In these cases there was no opportunity of testing the truth of the denial. In no case was a reaction obtained where gonorrhœa could clearly be excluded except in cerebro-spinal meningitis which gave five positive reactions out of five specimens tested.

The test also indicates the presence of the disease long after the disappearance of the gonococci according to the microscopic and cultural tests. A case which had had no discharge for a month gave a strong positive test and he relapsed shortly afterwards.

I wish to thank Dr. Allport for his assistance in supplying me with cases and the histories thereof from his clinic at the hospital. I am especially indebted to Colonel Harrison, D.S.O., for giving me the full credit of this work, since the success obtained has been so largely due to technique which he advised."

References:

HARRISON, L. W. (1914).—"Complement Deviation in the Diagnosis of Gonococcal Infections." *Jour. Roy. Army Med. Corps*, Feb., Vol. 22, No. 2.

THOMSON, D. (1918).—"Researches on the Cause of Autolysis in the Gonococcus, with special reference to the preparation of Gonococcal vaccine." *Journ. Path. and Bact.* (in the Press).

No. 3.—METHODS FOR THE DETECTION OF SPIROCHAETES.

CONTENTS.

The Committee in their first report dealt with the diagnosis of syphilis by means of the Wassermann Test. They have now brought together the conclusions reached as to the most satisfactory methods for the microscopic detection of *Spironema pallidum*.* They desire to express their great indebtedness to Mr. J. Edwin Barnard, President of the Royal Microscopical Society, for the service which he has rendered in describing in detail the technique of dark ground illumination. The Committee know of no such authoritative description elsewhere, and they have embodied it in the text of the Report.

Too much emphasis cannot be laid upon the importance of the detection of the *Spironema* as affording the earliest means of diagnosis of syphilis, and this at a period when clinically it is not possible to arrive otherwise at a definite decision. *Treatment undertaken in the early primary stage is relatively simple and assured, and is a matter of weeks; after the infection has become generalised, with development of a positive Wassermann reaction or secondary lesions, it is prolonged, uncertain and a matter of months.* It is bad practice to defer diagnosis and treatment until the typical Hunterian chancre has developed. The duty of the medical man is to suspect the syphilitic nature of every localised lesion upon the organs of generation, however small and atypical, and to remember that a simple papule with tendency to ulcerate may show itself even within a few days after infection with syphilis. *It should be the routine practice to examine every such eruption for spirochaetes,* and if these are not found at first, examination should be repeated on subsequent occasions. The most reliable methods of detection are so simple and expeditious that there is no excuse for neglect to utilise them.

I.—Collection of Material.

The methods about to be detailed are specially applicable to superficial lesions. They may also be used for examination of the juices of solid organs.

To obtain good results it is, in the first place, necessary to employ *not the contaminated liquid which has collected on the surface, but fluid freshly expressed from the tissue of the lesion.*

The prospect of success is diminished by any previous local antiseptic treatment of the lesion. If any such treatment has been applied the material is to be obtained from the deepest portion of the lesion, or by puncture, with a syringe, of the nearest enlarged gland.

* In regard to the name of this organism the customary usage of pathologists is not uniform, while among morphologists there have been misunderstandings and some anarchy in terminology. The Committee are indebted to Mr. Clifford Dobell, F.R.S., for a statement of the recognised international conventions of systematists as applied to the nomenclature of this group of organisms which is given in Appendix II on page 47. In accordance with the view he expresses, they propose to use *Spironema* as the name of the genus including the organism of syphilis. As a popular group name for ordinary use the English word "spirochaete" will be used for all the members of the group.

(7284). Wt. 14927. 3000. 8/18. T. & B. G. 36.

Further, it has to be borne in mind that salvarsan and its substitutes introduced intravenously in the proper dose have a very rapid action upon the spirochaetes. Within four hours the majority of those present in a cutaneous lesion may be dead and dying, and have lost their characteristic wavy curvature. Microscopical diagnosis must therefore be made prior to arsenical treatment.

Directions.

(a) *Superficial lesions.*

1. Cleanse the sore or the surface of the lesion of all *debris* with a piece of cotton wool moistened with saline or tap water.
2. Squeeze the sore firmly (preferably this is done by the patient himself) until drops of serum are expressed on the surface; or the sore may be lightly scraped with the edge of a spud or scarifier.
3. If bleeding has been caused by the manipulation or cleaning of the sore it is necessary to wait until it has stopped before collecting the sample, as the presence of blood lessens the chance of finding the spirochaetes.
4. Take up the serum in a pipette (Pasteur) drawn out to a capillary end: it is sufficient merely to place the end of the pipette in the exuded serum, when it will be drawn in by capillary attraction.

(b) *Lesions of the mouth.*

Lesions of the mouth are dealt with on the principles outlined above. It is important to avoid the inclusion of saliva in the specimen as this may contain spirochaetes which are difficult to distinguish from *Spironema pallidum*. The specimen may be removed from the tonsillar region, after scraping, with the help of a capillary pipette.

(c) *Skin lesions.*

Papules.—The superficial layers of epithelium are removed by scraping with a scarifier, and squeezing or dry-cupping the papule.

Macules.—These may be examined by the application of a blister about the size of a threepenny-bit, removal of the blister fluid and examination of the serum expressed from the raw area.

(d) *Lymph Glands.*

Fix the gland so that it bulges out the skin. Run a fairly stout hollow needle of about 56 gauge into the centre of the gland from one pole. Inject into the gland about five minims of sterile salt solution (0.85 per cent.) with a hypodermic syringe which fits the needle. Massage the gland, fit the syringe to the needle, and apply suction with the piston. Withdraw the needle with the syringe attached and force the aspirated material (which usually just covers the bottom of the syringe) on to the surface of a slide. Collect into a capillary tube if the specimen has to be sent to a laboratory.

II.—Demonstration of the Spirochaetes.

1.—*Dark ground illumination.*

Undoubtedly the ideal method for demonstrating the *S. pallidum* is by the dark ground condenser. Plates III., IV., V. illustrate spirochaetes demonstrated in this way, and show the main points by which *S. pallidum* can be distinguished from other spirochaetes.

The method of illuminating microscopic objects known as "dark-ground illumination" is of English origin, but in recent years it has

been reintroduced from Germany, and has become a comparatively common means of observation. It is interesting to note that an immersion paraboloid was designed by Edmunds about 1875, and an example of the appliance is to be found in the collection of historical instruments in the possession of the Royal Microscopical Society. In 1879 J. W. Stephenson described a "Catoptric Immersion Illuminator" (Journal of the Royal Microscopic Society, 1879, p. 37), which is a complete anticipation of the dark-ground illuminator introduced by Reichert, and which embodies the main principles of the modern spherical surface dark-ground illuminators which are erroneously regarded as of purely German origin. It must be admitted that dark-ground illumination has not made such headway as might be expected of it, in view of its undoubted merits and advantages. This is perhaps entirely due to the fact that, at least in medical circles, there has been little effort to master the technique of the subject, simple though it is both in principle and practice. In principle the method consists in rendering a microscopic object self-luminous, so that no preliminary treatment such as fixing or staining is necessary, any suitable material being examined in the living state. The conditions necessary to achieve self-luminosity are that the structure or organism should have a refractive index differing substantially from that of the medium in which it lies, and that it should be illuminated in such a manner that only the light refracted or reflected by the object can reach the eye of the observer. Under these conditions greater visibility is secured, and the object is seen as a bright image on a dark ground. The appliance used to achieve this result is sometimes referred to as an "ultra-microscope," but this use of the term is inaccurate. The term "ultra-microscopic" should only be applied to those methods which enable objects to be made visible that are beyond the limits of resolution by direct methods of microscopic observation. (See Appendix.) The following are the essential parts of an outfit for dark-ground illumination :—

The Microscope.—Any instrument suited to bacteriological work may be utilised, but it should be provided with rack-work or lateral screw for adjusting the sub-stage illuminating apparatus and with centreing screws for adjusting the condenser. (Fig. 1.) The mirror

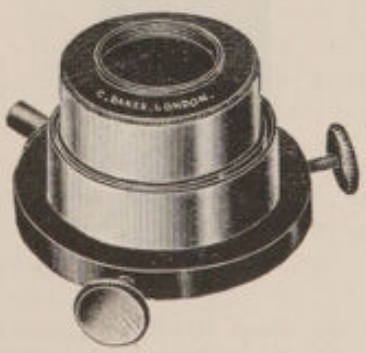


FIG. 1.

should preferably be mounted separately from the sub-stage condenser, so that the light having once been adjusted in relation to the mirror, no change in the position of the latter occurs when the sub-stage rack-work is moved. A mechanical stage gives an advantage for searching purposes, but it must be of such construction that the sub-stage illuminator can be racked up until the top of the latter is level with the stage. In other

FIG. 2.

respects any instrument suitable for bacteriological or pathological work can be utilized. Even the absence of centreing screws from the sub-stage may be remedied by having a centreing nose-piece fitted to the microscope (Fig. 2), so that the objective in use may be centred to the dark-ground illuminator. This arrangement should only be regarded as an expedient; it is not so satisfactory as the use of a centreing sub-stage.

Objectives.—The objectives may be any of the following :—

8 mm. Apochromatic Dry.
4 mm. Apochromatic Dry with correction collar.
3 mm. Apochromatic Oil Immersion.
2 mm. do. do.
¼ inch Achromatic.
1/6th inch do.
1/12th inch do. Oil Immersion.

The lenses here enumerated are given in order of preference. It cannot be too strongly insisted that, for dark-ground work, both from the point of view of optical theory and of practical experience, an oil immersion objective is not essential. For instance, with the 8 mm. objective and an 18 compensating ocular, spirochaetes can be seen and differentiated with ease and certainty. If this combination of objective and ocular is available, nothing further is necessary for an observer of moderate experience. The 4 mm. Apochromat does not need such a high ocular, although it is quite permissible to use it.

The numerical aperture (N.A.) of any objective for the purpose in view must not exceed 0.95. The N.A. of the 4 mm. Apo. is therefore the utmost permissible, and with most dark-ground illuminators, even this aperture must be reduced. This is best achieved by placing a funnel stop in the

FIG. 3.

objective (Fig. 3), so that the stop itself is almost in contact with the back lens. The funnel stop is simply a short tube with a constricted end which drops into the back of the objective. This is necessary to reduce the N.A. of the objective effectively. A stop of any description, such, for instance, as an iris diaphragm, which is operative at any appreciable distance from the back lens of the objective, is useless, and should be avoided. All oil immersion objectives must be fitted with a funnel stop, and in cases where this is not already provided it can easily be obtained from an optician. It should be remembered that the funnel stop must be removed for ordinary observational purposes. The permissible N.A. is also to some extent limited by the light source used. Relatively weak illuminants allow of a larger N.A. being utilized, but this point will be further referred to when discussing the subject of illumination.

When a dry lens is used, the principal adjustment necessary is tube-length. All makers specify a certain length of tube for which their objectives are corrected, usually 160 or 170 m/m., but this is dependent on the thickness of cover glass used. The thickness usually allowed for is from

0.15 to 0.18 mm. If the cover glass is thicker than this, tube-length should be reduced, if thinner the tube must be lengthened. The effect of this is easily seen, particularly with strong illuminants and high-power eye pieces. If the cover glass is not of the correct thickness or the tube length inaccurate, the image has a very definite haze surrounding it, which does not entirely disappear even when the object is in focus. The remedy is to adjust the tube-length until the image appears bright and clear with no surrounding nebulosity. This adjustment is quite easy to perform with dark-ground illumination. An alternative method is to observe any small bright particle in the field of view. When tube-length is accurate this appears as a disc of light equal in form and intensity whether the objective is above or below the focus, that is, it goes in or out of focus with the same appearance in whichever direction the fine adjustment screw is moved. If tube-length is inaccurate, it has a nebulous or misty appearance in one direction. If this nebulosity appears below the focus, that is when the tube moves downwards, tube-length must be reduced. The opposite conditions indicate that the tube requires lengthening. No objective of poor quality is satisfactory for dark-ground work, as no method of observation so accentuates faults or imperfect correction. It is necessary therefore to make sure by ordinary testing methods, or by submitting the lens to a competent observer that it is of sufficiently good quality for the purpose in view.

It is recognised, however, that in most bacteriological outfits the 1/12th inch oil immersion objective is the only one likely to be usable, few 1/6th inch objectives being of sufficiently good quality. By the use of a low-power ocular (×6 or ×8) careful attention to centration, and the provision of a suitable stop, this may be satisfactorily utilised for the purpose in view.

A list of Fellows of the Royal Microscopical Society is now being prepared, to whom reference may be made for assistance in this matter or for help in setting up and adjusting microscopic apparatus. Enquiries may be addressed to the Medical Research Committee or to the Secretary of the Royal Microscopical Society, 20, Hanover Square, London, W.

Concise instructions for the use of any available apparatus may be obtained from Mr. J. E. Barnard, Lecturer in Microscopy, University of London, King's College, or demonstrations may be arranged by appointment.

Oculars.—If Apochromatic objectives are used, the ocular should be of the compensating type, otherwise ordinary oculars will suffice. Two oculars at least should be provided, a low-power one (circa × 4) for adjusting and centreing the dark-ground illuminator, and a high power one (× 8 to × 18) for observational purposes.

Cover Glasses and Slides.—The cover glasses used must be of good quality free from blemishes or scratches. At the present time these are not easy to obtain, but to secure the best results with any save immersion lenses a cover glass which is free from surface defects is essential. Examination of the surface with a low power objective, say a 2/3 inch, will demonstrate its suitability or otherwise at once. The glass surface should be illuminated with incident light at an angle of about 45° to the normal. If gross defects are present they will at once become evident. The slides should be of the thickness specified by the maker of the dark-ground illuminator. The limits of error should not exceed 0.1 mm. Slides of 1 mm. are the thinnest usable, and 1.2 mm. is about the limit of thickness. When an oil-immersion objective is used, thickness of cover glass is unimportant provided that it is not too great for the objective to focus through; neither is it so essential to ensure that the cover glass is free from defects, as many of these consist of irregularities in the glass itself, which are not so evident when the objective is immersed. In any case it is important that both slides and covers should

be well cleaned, as otherwise it is difficult to make a thin even preparation, free from air-bubbles, on which success largely depends. The cleaning may be effected by boiling in a 5 per cent. solution of strong sulphuric acid in a saturated solution of bichromate of potash or in 10 per cent. chromic acid. After boiling for about five minutes, they should be well washed in running water, rinsed in distilled water, and put into alcohol to which a few drops of strong ammonia have been added. They may be kept in the latter until required, and only need wiping with a clean rag or with rice-paper before use. Alternatively, the cover glasses may be cleaned with absolute alcohol, using a piece of well washed rag or an old piece of silk for the cleaning operation. In any case, the amount of rubbing should be the least possible, as in the event of any small particle of grit or dirt being on the cloth, some abrasion of the cover glass surface is sure to occur, and this becomes evident in use.

The Dark-Ground Illuminator.—There are three types of illuminator suitable for the purpose in view. They are as follows:—

(1) An immersion achromatic condenser with N.A. 1.35 to 1.4, to which a stop may be applied in the sub-stage stop carrier usually provided for that purpose. (Fig. 4.)

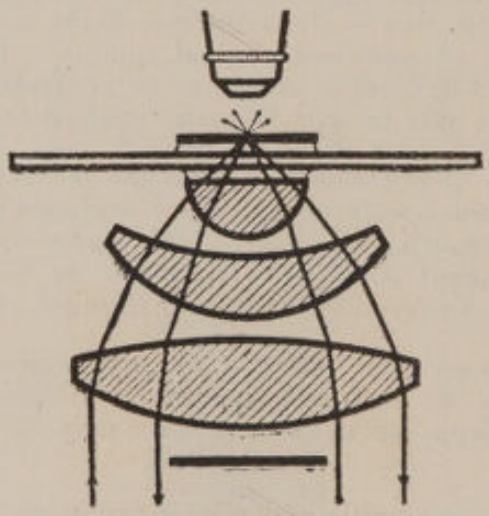


FIG. 4.

(2) A paraboloidal immersion condenser of the type designed in England many years ago and more recently re-introduced by Zeiss. (Fig. 5.)

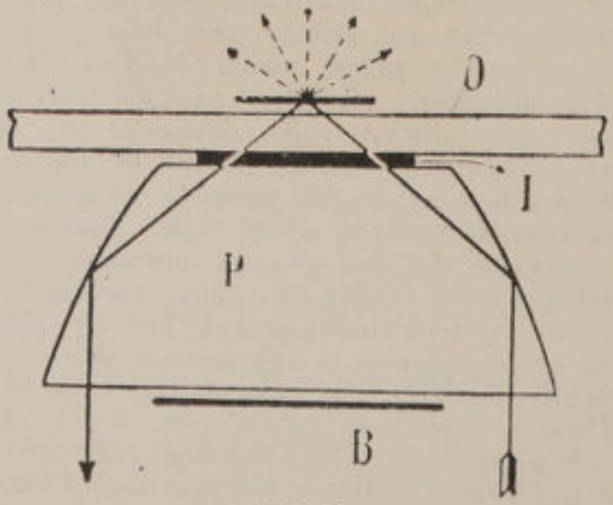


FIG. 5.

(3) The spherical surface reflecting condenser as introduced by Leitz. (Fig. 6.)

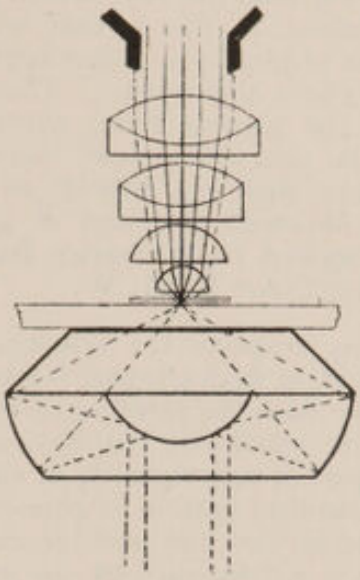


FIG. 6.

The relative advantages of the three types are not particularly well marked, as any one may be so used as to produce a satisfactory result. In the first type, however, it is essential that the condenser should be one of good quality with a large aplanatic cone, that is, the condenser must be as free as practicable from chromatic or spherical aberration. Such condensers are made by Swift or Watson, and yield good results, but it is important to see that the appliance for carrying the stop is accurately

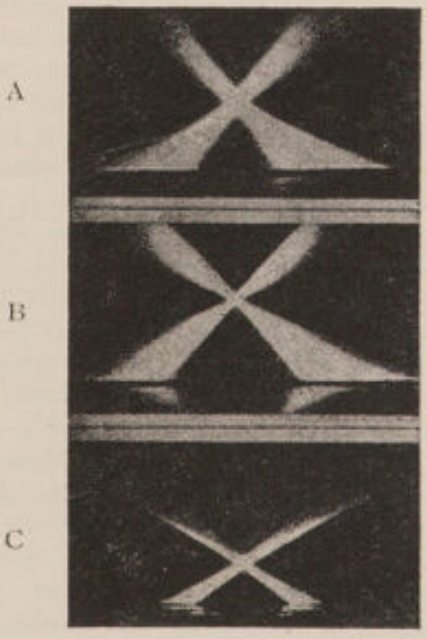


FIG. 7.

Showing Path of Rays in Dark Ground Illuminators.

A. Result of Stop in Achromatic Condenser.
B. Spherical Surface Illuminator.
C. Paraboloidal Illuminator.

centred to the optic axis. The size of the stop necessary is dependent on the N.A. of the objective used, but in practice it is perhaps simplest to procure a set of stops of various sizes and to use the smallest one which gives the dark-ground effect. The second type of illuminator is rather better

suited for illuminants of large size, such as an inverted incandescent gas burner. It has the advantage that if provided with an iris diaphragm, the latter so acts on contraction that the rays which are least refracted are cut off first. The obvious effect of this is that where the illuminant is too intense or the N.A. of the objective is rather high, the central rays are cut off and the dark-ground effect improved. The third type includes those most generally in use at the present time, which as they are almost free from spherical or chromatic aberration, lend themselves particularly well to the work in view, and are also less difficult to adjust. They are more suited for light sources of small size and of greater intrinsic brilliancy. This type can now be obtained from Charles Baker, of High Holborn, or J. Swift & Son, Tottenham Court Road, W.

Illuminants.—In many respects the selection of a suitable illuminant is the most important point in dark-ground work. The one usually recommended is a small arc lamp with the positive carbon in a horizontal position. This lamp is supplied with small size carbons, so that it can be run with a current of about 4 amperes; consequently it can be used on an ordinary lighting circuit. The horizontal carbon is connected with the positive lead on the main, the result being that the positive crater remains in a constant position when the carbon is fed forward by the mechanism provided for the purpose. This source of light is, however, almost invariably too intense for observational purposes, as nearly all objects are sufficiently refractile to cause considerable irradiation, with the result that the finer details become invisible.

The most satisfactory electric illuminant is that made by the Ediswan Company, known as the "Pointolite" lamp. In this the light source is a small sphere of tungsten, which by an ingenious mechanism forms a tungsten arc in a gas filled bulb, the result being a light source of high intensity and small area. For almost any optical purpose, where the light is sufficiently intense, it is ideal, as it is the nearest approximation to a point source of light at present known. For dark-ground illumination of blood or pus, it is somewhat too powerful, but by interposing a pale-blue colour screen, the light may be subdued sufficiently and then yields an exceedingly good result. The lamp is only suitable for continuous current, so that it cannot be used where alternating current only is available. Any electric lamp of the filament type is unsatisfactory, as even if there is sufficient intensity, the light source is too irregular, and the resulting image in the microscope suffers, even apart from the lack of intrinsic brilliancy. If, however, such a light source is the only possible one, then two alternatives are available. One is the small lamp of the pocket type which can be obtained from Messrs. Hearson, which fits directly beneath the microscope sub-stage, the mirror not being used. This requires storage batteries for satisfactory running, the ordinary dry cells supplied commercially not maintaining a constant voltage for any length of time. The other lamp is one of the half-watt type of 100 candle power. These can be obtained from most dealers in any voltage to suit the supply available. The tungsten filament in these is run at a very high temperature, consequently the luminosity is great. It is necessary, however, to interpose a piece of fine ground glass between the lamp and the microscope mirror, otherwise the narrow filament does not illuminate the object equally in all directions. Fairly good results may be obtained with an inverted incandescent gas burner, but in general the quantity of light available per unit area of the incandescent mantle is not sufficiently high. An acetylene bicycle lamp may be made to do service where no other light is available, but in this case again the brilliancy of the light is not quite high enough for satisfactory results, although the actual amount of light emitted is considerable.

A new form of incandescent light which may be used either with ordinary gas or with a high pressure methylated spirit burner will shortly be available and will be obtainable from Messrs.

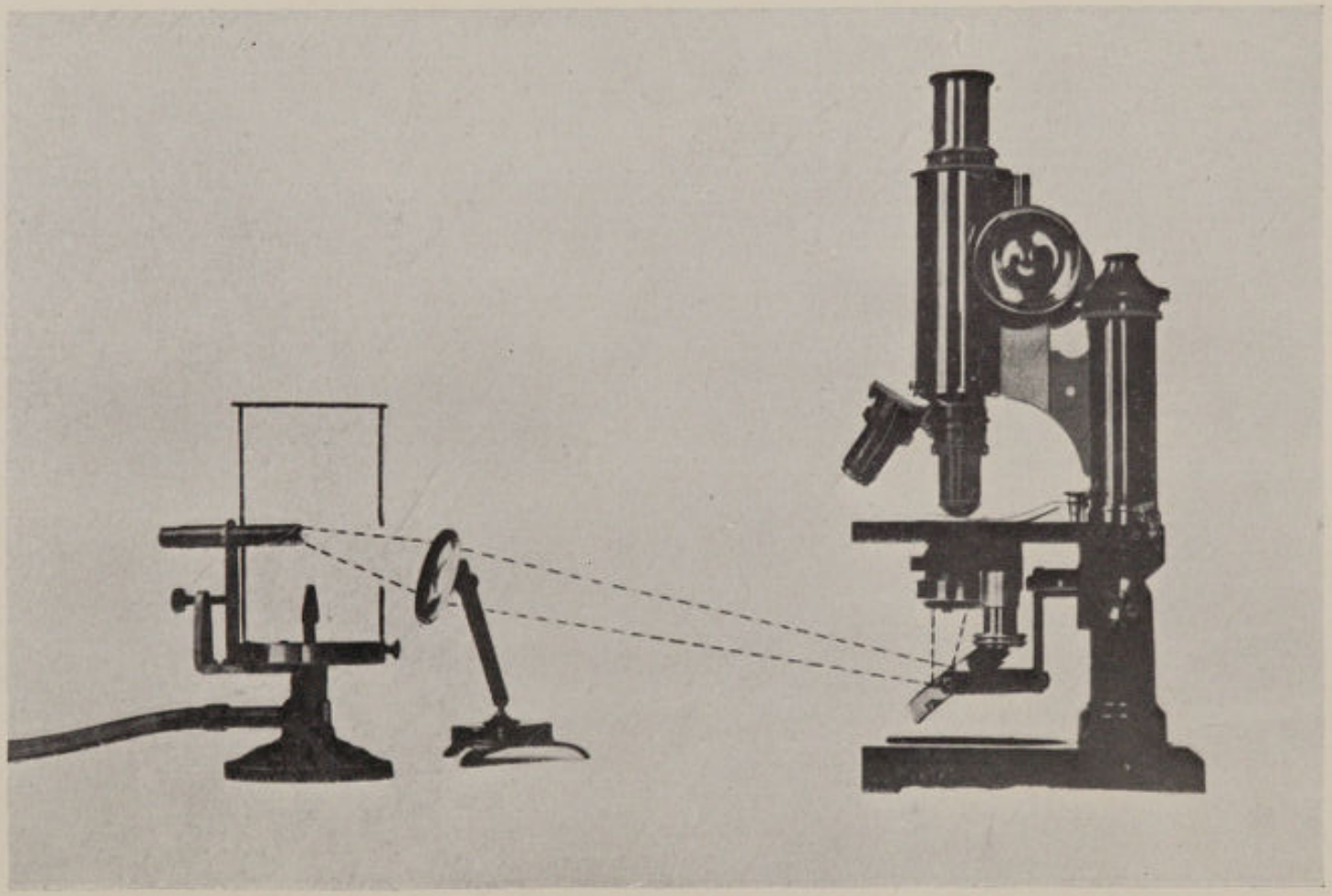


FIG. 8.

H. F. Angus & Co., of Wigmore Street, London, W. This embodies a somewhat new but very simple method. It consists of a specially made incandescent mantle which gives an intense uniform source of light of about 5 mm. across. It is sufficiently intense for seeing *Spironema pallidum*, and for differentiating it from other similar organisms. The simplest method of arranging the light for use is to set it up at one end of a board which may be about 21 inches long by 9 or 10 inches broad. In front of the light is placed the bull's eye or condensing lens (*Vide* Figure 8). The position of the latter should be such that the image of the radiant is projected about 7 to 9 inches from the condensing lens. The microscope is then put at the other end of the board, so that this image of the radiant falls exactly in the centre of the mirror. The choice of type of condensing lens is not important; either the ordinary bull's eye or a bi-convex lens, not exceeding five inches in focal length, may be employed. The arrangement of the apparatus is, therefore, that the light is in a position furthest from the observer, the condensing lens being between the light and the microscope. The lens must be placed with its surface at right angles to a line joining the centre of the illuminant with the centre of the microscope mirror. In the case of paraboloidal illuminators it is important to see that the light as projected is completely filling the back of the condenser, and to ensure that a large image of the radiant is being projected the bull's eye must be fairly close to the radiant. With spherical surface condensers, the bull's eye may be somewhat further away, with the result that a smaller image of the radiant reaches the mirror. An arrangement to facilitate the adjustment of these subsidiary parts will also be supplied by Messrs. Angus & Co. in connection with the new source of light. In this the radiant and the bull's eye are mounted correctly in relation to each other. A fixed mirror is provided on the base-board in such a position that a vertical beam is projected from the mirror surface. It is then only necessary to remove the mirror from the microscope, and having placed the latter in a vertical position, to stand it centrally over the fixed mirror and satisfactory illumination results without any further adjustment of the subsidiary apparatus.

Manipulation.—There are many methods of adjusting dark-ground illuminators, but the following has the merit of simplicity, and if carried out with reasonable care is sufficiently correct for any purpose. The illuminant is first set up in the manner already described with its bull's eye condenser in correct relation to the mirror of the microscope. No optical appliance is put into the microscope itself with the exception of a low-power ocular. The light is then directed up the tube so that the eye piece is evenly and completely filled with light. That this has been accomplished may be ascertained in one of two ways. If the observer places his eye exactly over the ocular and at a distance of from 8 to 10 inches from it, he will see, if the adjustment is correct, a perfectly even disc of light. A more exact method is, if the illuminant is sufficiently powerful, to project a disc of light on to the ceiling; if that is impracticable—on to a piece of paper held two or three feet above the microscope. If there is any lack of centration, due either to the beam not being projected along the optic axis of the microscope or to the image of the source of light not falling in the centre of the mirror, it can at once be ascertained and corrected by manipulation of the mirror or alteration of the position of the light. If the board with a fixed mirror, as already described, is used, it is then only necessary to shift the microscope about until satisfactory illumination is obtained, that is, until the beam passes centrally up the microscope body tube. This adjustment having been effected the next step is to slip the dark-ground illuminator into the sub-stage. Place a low-power objective in the microscope and the object on the stage, having previously put a large drop of cedar-wood oil or glycerine and water on the top of the dark-ground illuminator; carefully rack up the latter so that the under side of the object slide is immersed and is nearly in contact with the top of the dark-ground illuminator. Now get the object into view by suitable adjustment of the objective. On observing, it will be seen that the object

is illuminated by a disc of light. By means of the sub-stage rackwork, the illuminator should be moved up or down until the smallest possible area of the object in the field of view is illuminated. It will probably now be found that the illuminated disc is not central, and this must be corrected by the centreing screws of the sub-stage, or by the fitting on the nose piece of the microscope, if the latter alternative is adopted. Under no circumstances should the relation of the light to the microscope be interfered with after the preliminary adjustment described has been carried out. Any correction necessary to ensure centration must be effected entirely by the centreing screws, either of the sub-stage or the nose-piece. The higher power dry or immersion objective which is to be used for searching purposes is now substituted for the low power one and the object again brought into view. It may be found that some want of centration is apparent, due to the optic axes of the objectives not being co-incident. This again may be corrected by the centreing screws. A higher power ocular may now be substituted, and the apparatus should be in perfect adjustment. It should be remembered that when using a dry lens any alteration of tube-length necessary to correct for different thicknesses of cover glass must be effected, and this should be the final operation.

It is well worth while to procure a simple micrometer gauge and to calibrate both cover glasses and slides before taking into use. By the use of such a gauge, the trouble incidental to correction by means of alteration of tube-length is avoided. These can be obtained from any optician, but a thoroughly satisfactory substitute is that made for engineering purposes and sold by most tool dealers. These read to 1/100th of a millimetre with accuracy, and are preferable to the cheap form of gauge with a long lever arm which is sold for optical purposes, and which is very easily put out of adjustment should the arm be knocked or bent.

Absolute cleanliness is one of the main conditions for successful manipulation. The cover glasses and slides must be dealt with in the manner already described, and both objectives and oculars should be kept free from dust. If operations are carried out in a room where dust is prevalent then it is advisable, if a dry lens is used for observational work, occasionally to dust the cover glass with a small camel-hair brush. This class of microscopic work is best carried out in a room where the light is at least subdued. Absolute darkness is not essential, in fact it is doubtful whether it is advisable. In no case should the microscope be placed opposite a lighted window or in any position where a strong light falls upon the eye of the observer.

The immersion fluid used with any illuminator must be free from air-bubbles. A method of avoiding this is to place a large drop of oil or glycerine and water on the top surface of the illuminator. Also place a small drop of the fluid on the *under* side of the object slide. See that the illuminator is low enough down to avoid contact of the drops when the slide is placed on the stage. Now carefully raise the illuminator until the two drops touch. Owing to the convexity of the contact surfaces of these drops, there is no tendency for air to be enclosed, and the result invariably is that air-free immersion is secured.

2.—*Stained Films.*

In the first few years which followed the discovery of the *S. pallidum* stained films were employed almost exclusively for diagnostic purposes. They have now been largely replaced by other methods.

To obtain good results it is important in the first place that the moment the film has dried it be properly fixed, preferably by absolute alcohol, methyl alcohol or osmic acid. The film should be fixed *as soon as dried.*

It is the experience of the Committee that the Romanowsky staining in one or other of its modifications affords the best results, more particularly Giemsa's long method, although Leishman's and Homer Wright's modifica-

tions are not far behind. By these methods *Spironema pallidum* is coloured a rose pink, while other spirochaetes are blue in colour.

Methods.

(1) *Giemsa's ordinary or long method.*—Staining for 12 hours with a 1-in-10 to 15 dilution in tap water.

(2) *Giemsa's rapid method.*—In this method the same dilution of stain is used, only the stain is poured on the slide and the slide held over a Bunsen burner until steam rises. The process is repeated 3 or 4 times, the final application lasting 2 minutes.

(3) *Leishman's method.*—As recommended by the author.

3.—*Indian Ink and Congo Red Methods.*

Of all the methods devised for the demonstration of the *Spironema pallidum* these are certainly the simplest in that no special apparatus or complicated stain is necessary. The advantages, as will be pointed out later, are more than counterbalanced by certain inherent defects.

Indian Ink Method (Burri).—For use, a drop of the fluid to be examined is mixed with one drop of the Indian ink on an ordinary microscopic slide and spread in a thin film, as in the making of a blood film. The film is then dried in the air without heat, and when dry examined under the 1/12 oil immersion.

The *Spironema pallidum* by this method appears as a white undulating thread against a dark (i.e., yellowish or dark brown) background.

Congo Red Method (Benians).—Here 2 per cent. Congo Red solution replaces the Indian ink. When the film has dried, it is flooded with acid alcohol, whereby the background is converted into a semi-opaque bluish purple, against which the clear unstained spirochaetes stand out in sharp contrast

Advantages and disadvantages.—These methods, although very useful for demonstrating other spirochaetes, cannot be recommended for the detection of *Spironema pallidum*, and this because :—

(1) The width of the spirochaete varies according to the thickness of the film; in a thick film it is seen as a fine filament, in a thin film it appears thick.

(2) There is a liability to great distortion of the spirochaetes.

(3) The characteristic movement of the spirochaetes cannot be observed.

The only advantage of the methods is their great simplicity. It has, however, to be confessed that in the hands of inexperienced workers their use leads to inaccurate diagnoses.

4.—*Silver Nitrate Methods.*

From time to time different writers have recommended silver nitrate solutions for staining the *S. pallidum*, but after a few trials they were mostly found to be unreliable. Recently, however, Fontana* has introduced a method which gives much more consistent results. The preparation after fixing is mordanted with a solution containing tannic acid, washed in water and stained with an ammoniacal solution of silver nitrate.

The following are the details of the method as modified and improved by Tribondeau †:—

* Fontana, A., *Dermat. Wchnschr.*, Leipz. u. Hamb., 1912, **55,** 1003.

† Tribondeau, *Bull. Soc. franç. de dermat. et syph.*, Par., 1912, **23,** 474. *Ibid. Ann. de l'Inst. Pasteur*, Par., 1917, **31,** 425.

Three solutions are used in the process, viz. (1) a fixing solution, (2) a mordant, (3) silver solution. It is of the utmost importance that all glass ware employed in the process should be chemically clean.

(1) Formol-acetic acid (Ruge's solution): Acetic acid pure, 1c.c.; commercial formalin (40 per cent.), 2c.c.; distilled water, 100 c.c.

(2) Tannin (alcoholic or ethereal), 1 grm.; distilled water, 20c.c. Dissolve the tannin in hot distilled water and preserve it from mould contaminations in a well-stoppered bottle with a small piece of camphor.

(3) Silver solution (Fontana's solution): Silver nitrate, 1 grm.; distilled water, 20 c.c. Dissolve the silver nitrate in the cold. After solution is complete a quantity is placed in a perfectly clean stoppered measure, and by means of a pipette ammonia is added drop by drop, the mixture being frequently shaken. At first a brownish precipitate appears, gradually becoming darker as more ammonia is added. A point comes when the precipitate begins to dissolve; then the ammonia, preferably diluted, should be added very cautiously, and it is necessary to stop while the fluid is still very faintly opalescent. If this stage has been inadvertently passed a small quantity of the silver nitrate solution should be added until the desired opalescence is reached and maintained on shaking.

Technique.—Films are made in the usual way, and dried in air. Fix in the formol-acetic solution, which is poured on and off once or twice, a fresh quantity being left to act for 1-5 minutes. It is of the greatest importance that haemoglobin which may be present in blood corpuscles in the film should be completely dissolved out by the solution. In the case of secretion of syphilitic ulcers the films should be washed with absolute alcohol. Smears from organs should have any fat removed by alcohol, ether, and finally alcohol. After treatment by the formol-acetic solution the preparations are then covered with the tannin mordant, and heated gently till vapour rises (not boiled). The mordant is left on for 30 seconds.

Wash thoroughly under the tap for 30 seconds, and follow by a wash in distilled water.

Pour on the silver solution and allow it to act for a few seconds in the cold. Pour off and add a fresh quantity of silver solution and heat gently till vapour arises. Leave the silver solution to act for a further 15 seconds. By this time the film is of a maroon colour.

Wash in distilled (not tap) water for a few seconds; dry with blotting paper.

Spirochaetes are stained yellowish brown or blackish, and stand out very sharply.

They may be examined directly in cedar oil, but this should not be left on as the oil destroys the preparation. The film can be safely mounted in neutral glycerine under a cover glass (Tribondeau) or in Canada balsam dissolved in xylol (Fontana).

Advantages and disadvantages of staining methods.—Admittedly, well-stained preparations afford characteristic appearances; where, that is, the results are positive they cannot be questioned. On the other hand, the *Spironema pallidum* has a notable lack of affinity for most staining reagents. As a result, material which by other methods yields numerous spirochaetes, may fail to yield them by staining methods, or but an occasional individual may be made out, this affording an erroneous idea of their frequency. So also the distortion caused by drying, and absence of the characteristic movement, are distinct disadvantages.

III.—Soft Chancre and Balanitis.

The Committee find no sufficient evidence that what is clinically known as "soft chancre" or "soft sore" is a specific disease induced by a single species of micro-organism.

In these circumstances they recommend that a diagnosis of soft chancre be founded on clinical evidence, and this only after syphilis has been excluded by observation extending over a period of twelve weeks. A similar recommendation is made with respect to conditions of balanitis.

APPENDIX I.—*On the Resolving Power of the Microscope.*

It is necessary to appreciate that increased visibility is secured by means of dark-ground illumination, but that resolution, that factor in microscopic observation on which the elucidation of fine structure depends, is reduced. When an object is seen as a bright object on a dark ground it is possible to see much smaller particles than by any other means. If a single molecule in fact could be isolated and illuminated by a sufficiently intense source of light, it would be visible. The image seen would, however, bear no relation to the form or size of the object, it would be a bright circular point of light surrounded by one or more bright rings.

In all dark-ground illumination work such luminous particles are seen and these may be regarded as ultra-microscopic. The number visible depends almost entirely on the intensity of the illuminant, and this constitutes still another reason for avoiding too intense light sources where the object is to demonstrate the presence of an organism which is within the limits of resolution of the microscopic objective employed.

Resolving power is dependent on two factors, the numerical aperture (N.A.) of the objective and the wave-length of the light used.

It is therefore evident that increased resolution can only be secured by increasing the N.A. of our objectives or by decreasing the wave-length of the light employed. In the present state of knowledge there is no probability of objectives being produced of greater N.A. than those now available, but there is a very wide field for the use of light of shorter wave-length, such as ultra-violet light, or other invisible radiations. In dark-ground work, bacteria and similar translucent objects, which require to be stained to be visible by ordinary methods of observation, are easily shown, but these are well within the limits of resolution. An object which is less than 0.2 μ. in diameter, is beyond the limits of resolution by transmitted light of average wave-length. As it is impossible to utilize the full N.A. of an oil-immersion objective by dark-ground methods, the limit is reached with a considerably larger object by the latter method.

APPENDIX II.—*On the Nomenclature of the Spirochaete of Syphilis.*

By Clifford Dobell, F.R.S.

The organism was discovered and first named by Schaudinn (1905), who called it *Spirochaete pallida*. [The name was proposed *by Schaudinn*, in a joint paper with E. Hoffmann (1905). The authority for the name is therefore Schaudinn—not Schaudinn and Hoffmann, as often incorrectly stated]. The specific name of the organism is therefore *pallida* Schaudinn, 1905, and has never been in dispute.

The generic name *Spirochaete*, used by Schaudinn, is a mistake [probably first committed by Cohn], the genus intended being *Spirochaeta* Ehrenberg, 1834. Reinvestigation of the type species of this genus (*S. plicatilis* Ehrbg.), especially by Zülzer (1910, 1911), has shown that it is morphologically so unlike the syphilis spirochaete that the latter cannot be placed in the same genus.

As the syphilis spirochaete cannot be placed in the genus *Spirochaeta* Ehrenberg, another generic name must be assigned to it. The early proposals to place it in the genus *Trypanosoma* or *Spirillum* were founded upon misconceptions, and need not be considered further here. [*Trypanosoma* Gruby is a flagellate protozoon; and *Spirillum* Ehrenberg is, by definition, only applicable to bacteria of spiral form which are *rigid*, and not flexible]. The other generic names proposed for the syphilis spirochaete are:—

(1) *Spironema*, proposed by Vuillemin in June, 1905.
(2) *Treponema*, ,, ,, Schaudinn in October, 1905.
(3) *Microspironema*, proposed by Stiles and Pfender in December, 1905.

(1) The name *Spironema* thus has priority over the others, and is therefore the correct generic name unless it can be shown to be unavailable.—It was proposed by Vuillemin, to replace *Spirochaeta*, on the grounds that the latter is a genus of plants (Schizophyta), whereas the syphilis organism—according to Schaudinn's original interpretation—was a flagellate protozoon related to the trypanosomes. It is now known, however, that the

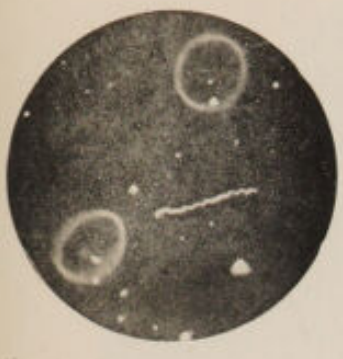


Fig1.

Fig.2.

Fig.3.

Fig.4.

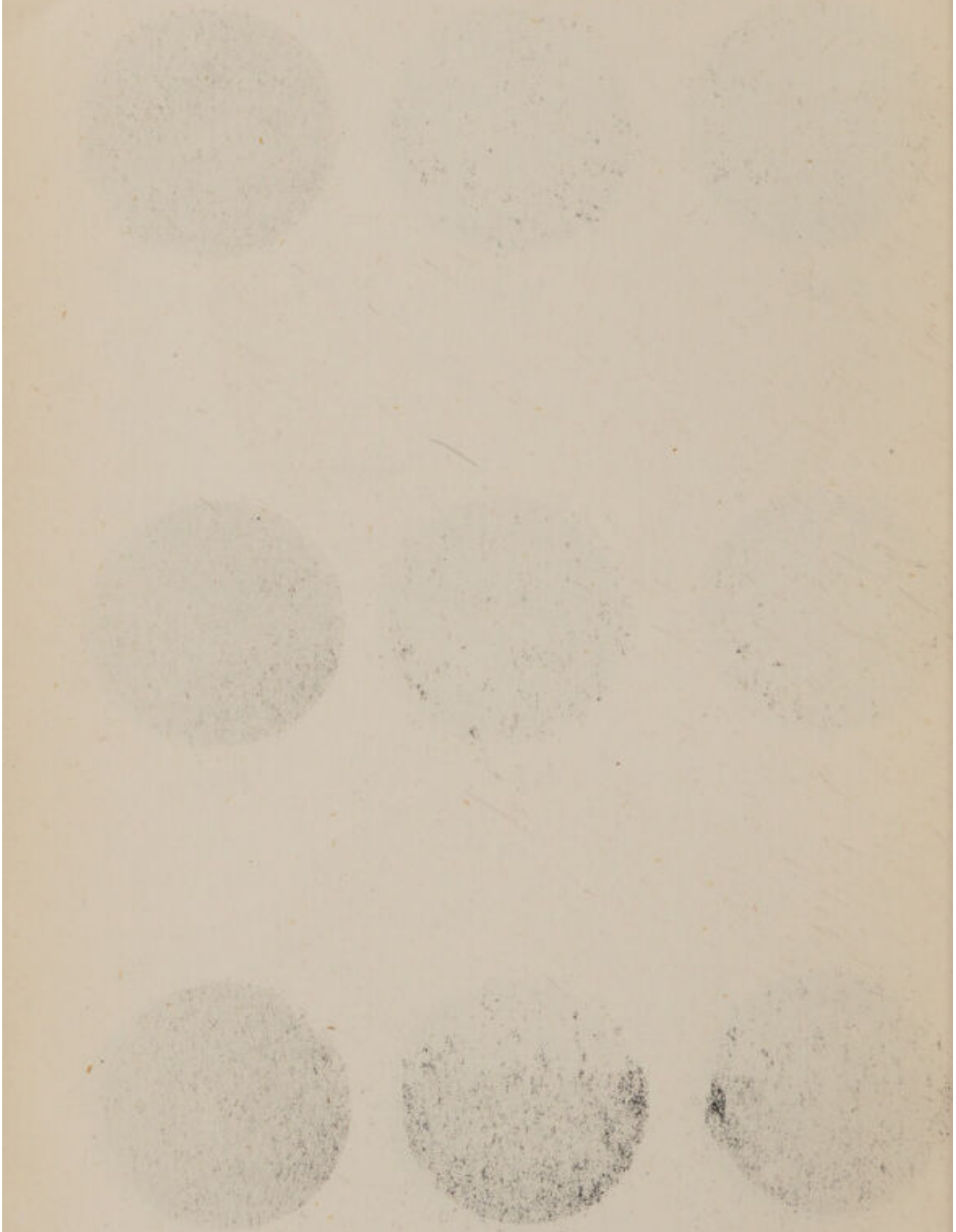
Fig.5.

Fig.6.

Fig.7.

Fig.8.

Fig.9.



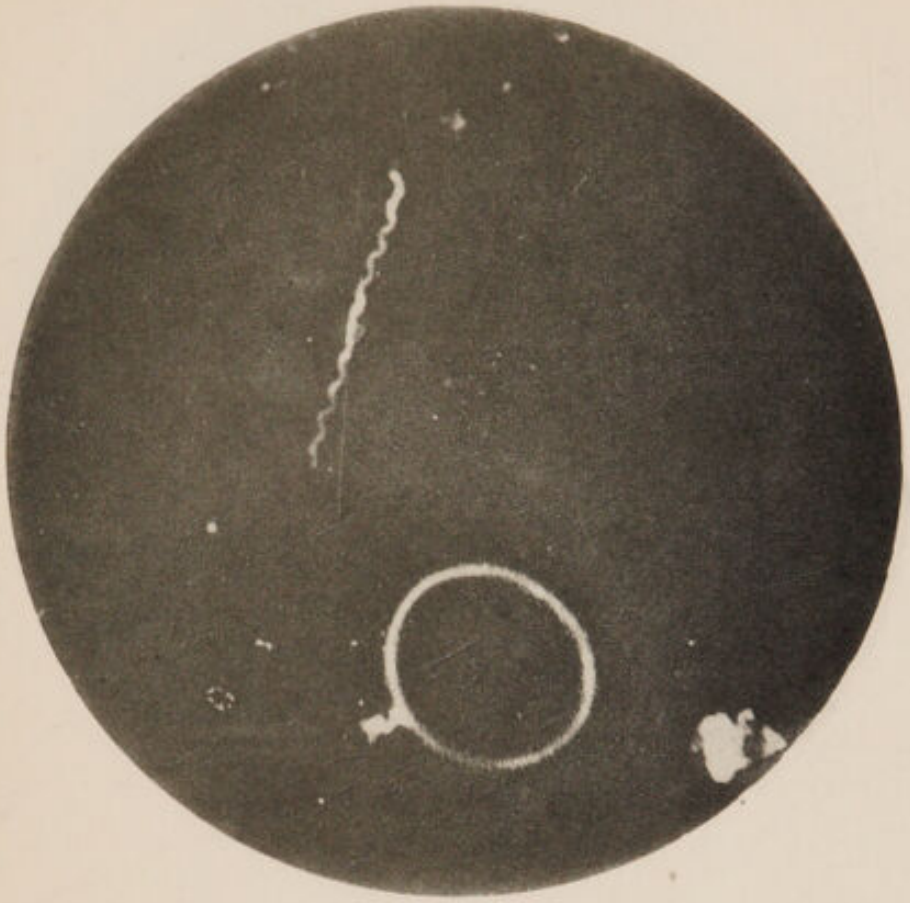


Fig.1.

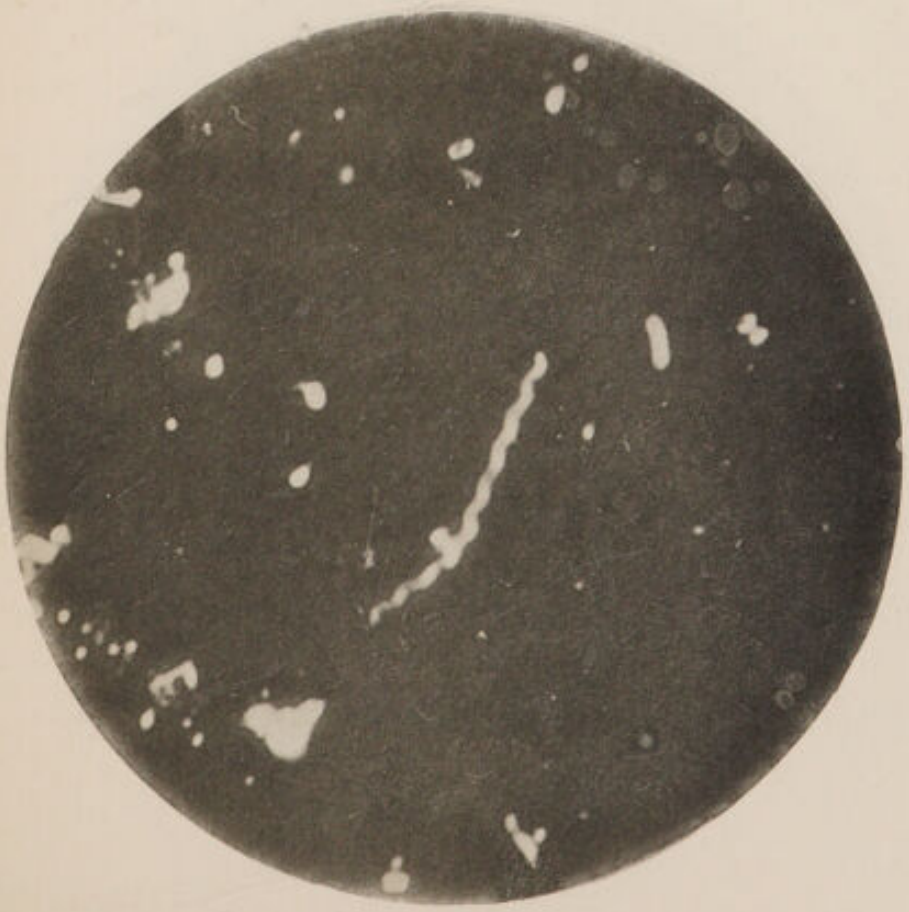
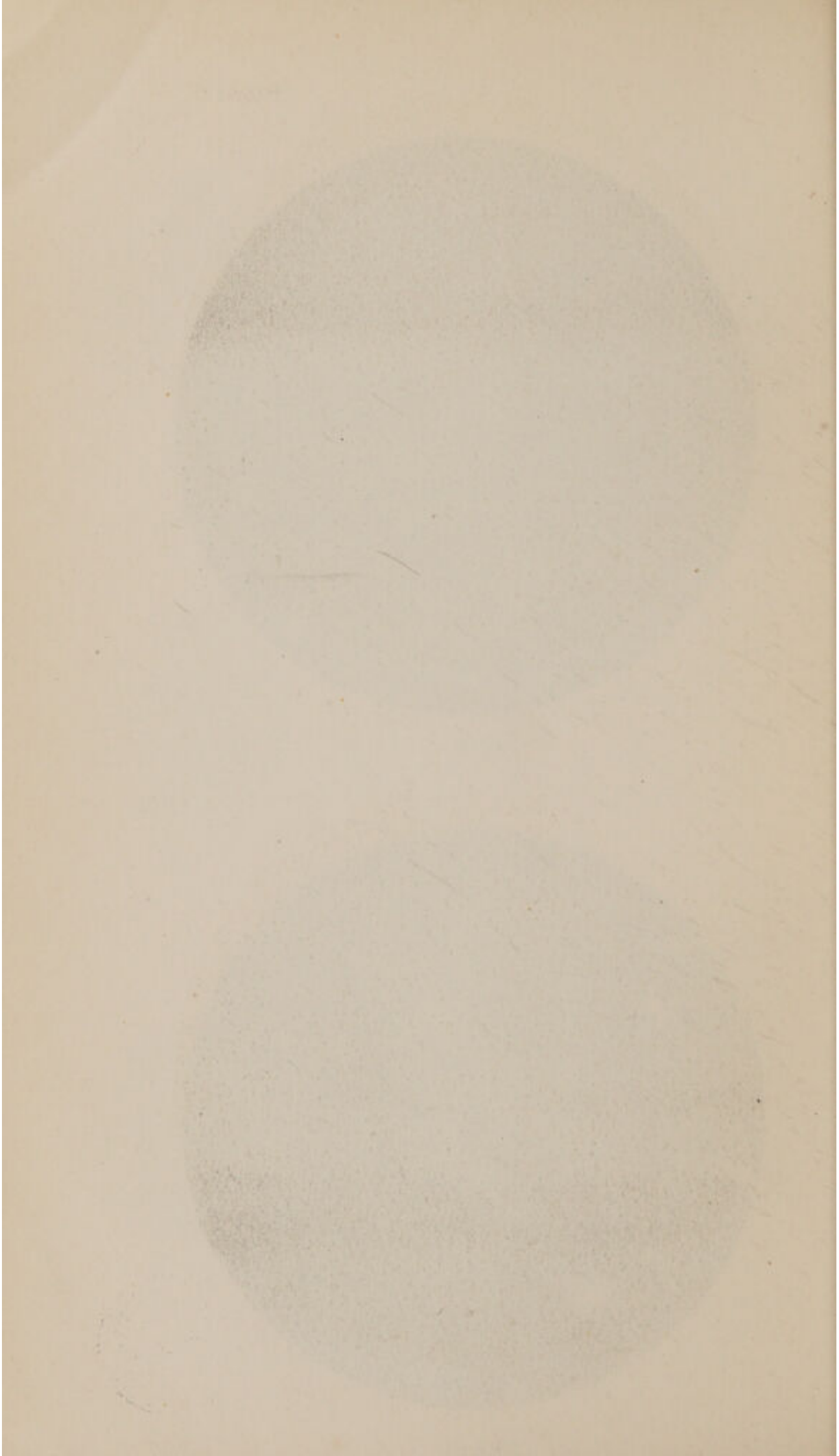


Fig.2.



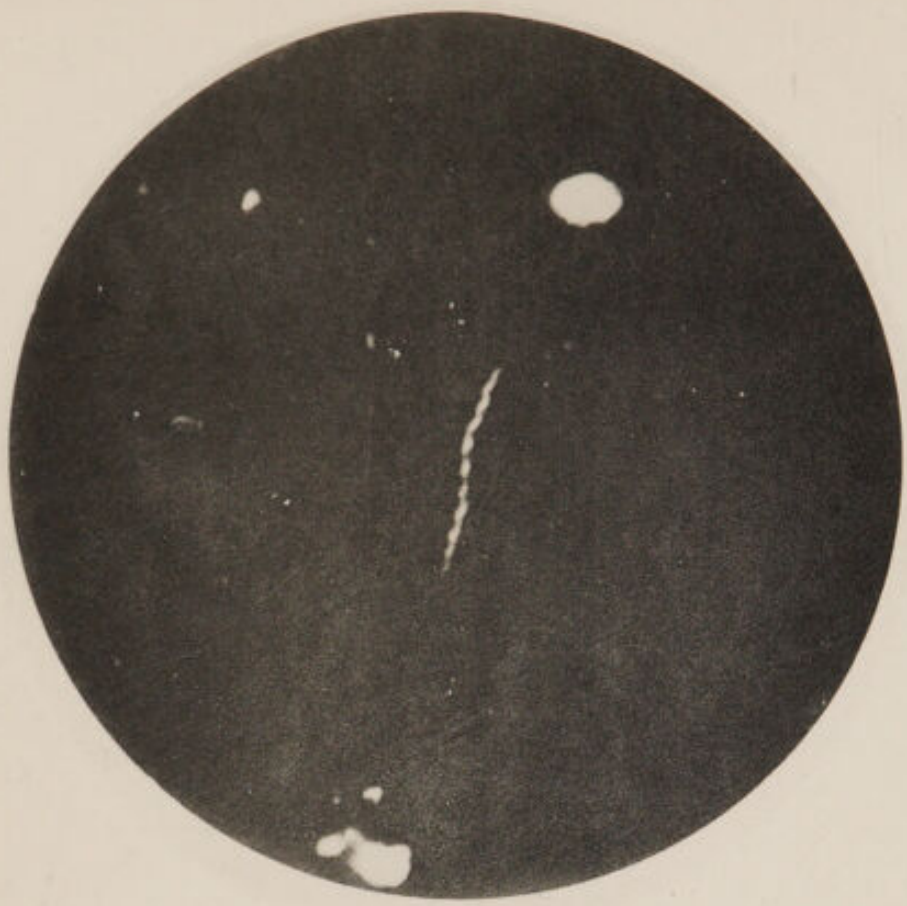
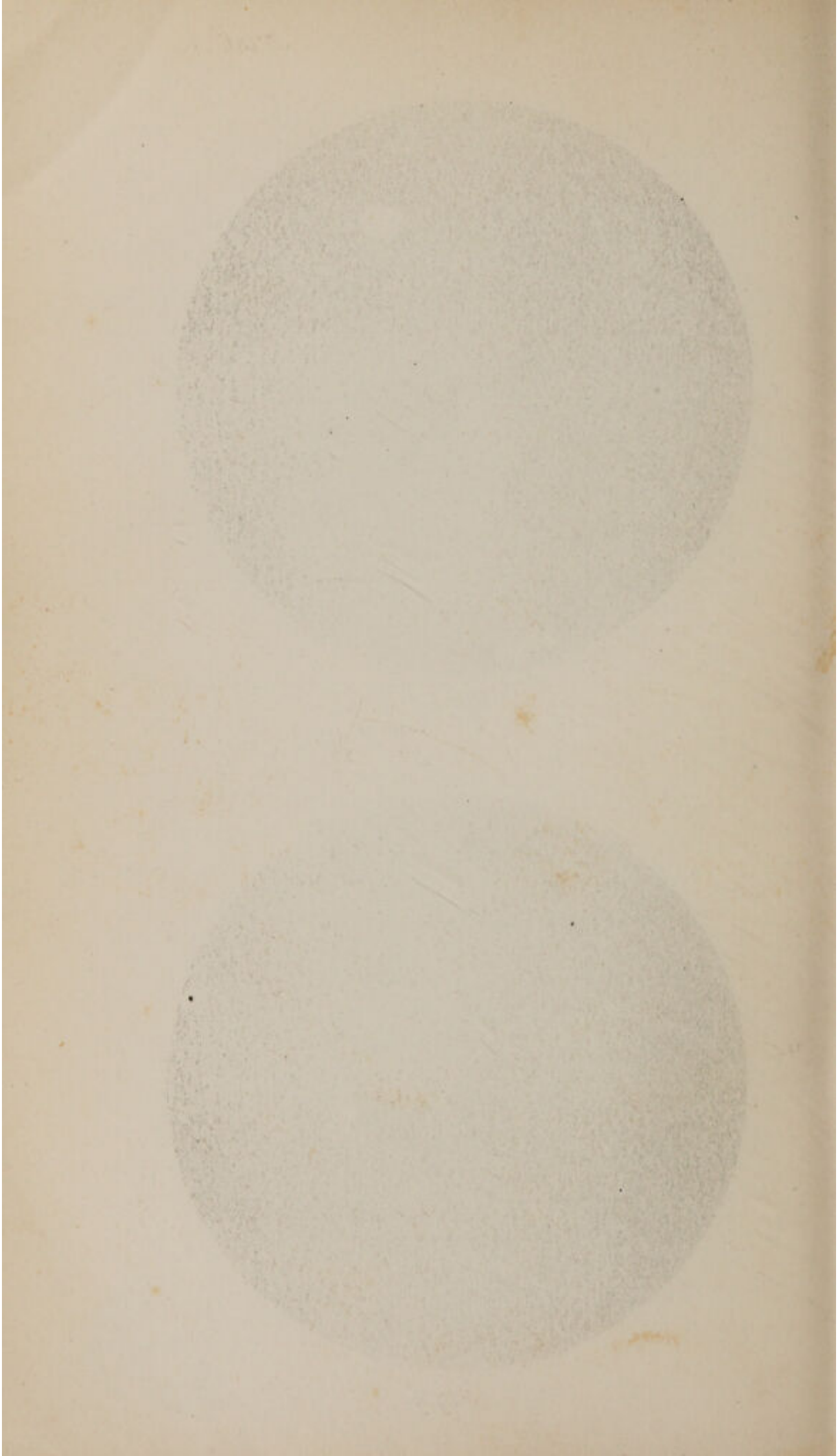


Fig.1.

Fig.2.

WELLCOME LIBRARY INSTIT



Special Report Series—*continued.*

No. 8. Report upon Soldiers returned as Cases of "Disordered Action of the Heart" (D.A.H.), or "Valvular Disease of the Heart" (V.D.H.). Price 1*s.*, post free 1*s.* 1½*d.*

No. 9. A Report upon the use of Atropine as a Diagnostic Agent in Typhoid Infections. Price 1*s.*, post free 1*s.* 1½*d.*

No. 10. The Mortalities of Birth, Infancy and Childhood. Price 1*s.* 6*d.*, post free 1*s.* 8*d.*

No. 11. The Causation and Prevention of Tri-nitro-toluene (T.N.T.) Poisoning. Price 1*s.*, post free 1*s.* 2*d.*

No. 12. The Classification and Study of the Anaerobic Bacteria of War Wounds. Price 2*s.*, post free 2*s.* 2½*d.*

No. 13. An Enquiry into the Composition of Dietaries, with Special Reference to the Dietaries of Munition Workers. Price 3*d.*, post free 4½*d.*

No. 14. Reports of the Special Committee upon the Standardization of Pathological Methods.

No. 1. The Wassermann Test. Price 3*d.*, post free 4½*d.*

No. 15. A Study of 1,300 Convalescent Cases of Dysentery from Home Hospitals: with special reference to the Incidence and Treatment of Amœbic Dysentery Carriers. Price 6*d.*, post free 7*d.*

No. 16. A Report on the Causes of Wastage of Labour in Munition Factories. Price 1*s.* 6*d.*, post free 1*s.* 7½*d.*

No. 17. I. A Report upon the Seasonal Outbreak of Cerebro-spinal Fever in the Navy at Portsmouth, 1916–1917.

II. The Treatment of Cerebro-spinal Meningitis by Anti-meningococcus Serum at the Royal Naval Hospital, Haslar, 1915–16–17. Price 2*s.* 6*d.*, post free 2*s.* 9*d.*

No. 18. An Investigation into the Epidemiology of Phthisis Pulmonalis in Great Britain and Ireland. Price 1*s.* 3*d.*, post free 1*s.* 5*d.*

No. 19. Reports of the Special Committee upon the Standardization of Pathological Methods.

No. 2. The Laboratory Diagnosis of Gonococcal Infections.

No. 3. Methods for the Detection of Spirochaetes. Price 1*s.*, post free 1*s.* 1½*d.*

The following is published under the direction of the Medical Research Committee by Messrs. Longmans, Green & Co., 39, Paternoster Row, London, E.C. 4.

Milk and its Hygienic Relations, by Janet E. Lane-Claypon, M.D., D.Sc. (Lond.) Price 7*s.* 6*d.* net.

Printed under the authority of His Majesty's Stationery Office
By Darling & Son, Ltd., Bacon Street, E.2.