pushed into the bottle, its stem is cut off flush with the rim, and the cap is screwed down. Stuart gives figures showing that by the use of these methods the gonococcus will remain capable of sub-culture up to two, three, or four days, and there is no tendency for the other organisms to grow out: they are at least as much restrained as the gonococcus.

The results of culture and gonococcal complement fixation tests during this period depended mainly on the work of my colleagues Miss Bertha Wheatley and Messrs. K. I. Johnstone, K. Zinnemann, and D. Dolby, to whom I wish to express my indebtedness.

REFERENCES

Christiansen, W., and Becker, H. (1938). *Munch med. Wschr.*, **85**, 990.
Ellingworth, S.. Gordon, J., and McLeod, J. W. (1929). *J. Path. Bact.*, **32**, 173.
Gordon, J., and McLeod, J. W. (1928). *Ibid.*, **31**, 185.
Harrison, L. W. (1937). *Brit. J. vener. Dis.*, **13**, 116.
Leahy, A. D., and Carpenter, C. M. (1936). *Amer. J. Syph.*, **20**, 347.
Loele, W. (1928). *Virchows Arch.*, **267**, 733.
——(1929). *C. F. Bakt. Orig. I.*, **111**, 325.
McLeod, J. W., Coates, J. C., Happold, F. C., Priestley, D. P., and Wheatley, B. (1934). *J. Path. Bact.*, **39**, 221.
Neumann, H. (1938). *Derm. Wschr.*, **106**, 325.
Price, I. N. Orpwood (1932). *J. Path. Bact.*, **35**, 635.
——(1933). "The Complement Fixation Test for Gonorrhœa." Published by the L.C.C., London.
——(1929). *Brit med. J.*, **1**, 199.
Reymann, F. (1941). Thesis for Doctorate. Copenhagen. Einar Munksgaand.
——(1943). *Acta Derm.-vener.*, **24**, 130.
Sanderson, E. S., and Allison, G. G. (1942). *Amer. J. Syph.*, **26**, 196.
Stuart, R. D. (1946). *Glasgow med., J.* **27**, 131.
Thompson, L. (1937). *J. infect. Dis.*, **61**, 129.
Tulloch, W. J. (1923). *J. R.A.M.C.*, **41**, 334.
——(1924). *Ibid.*, **42**, 20.
——(1925). *Brit. J. vener. Dis.*, **1**, 31.
Wortman, M. S., Gronau, A., Deakin, R., and Love, F. (1941). *Vener. Dis. Inform.*, **22**, 195.

DISCUSSION ON THE PRECEDING PAPER

DR. G. L. M. MCELLIGOTT (the President) was in favour of cultures as against smears. He had been brought up to the idea that the gonococcus hated the cold, and to know that it could be frozen with impunity was reassuring. He asked what Prof. McLeod thought of portable incubators for use in country clinics where the choice of the best method of transporting the gonococcus from clinic to laboratory was important. Dr. Stuart's work sounded extremely interesting. To a question about how long one kept the gonococcus in the semi-fluid agar, Prof. McLeod replied that it was a matter of preservation: it was kept there until it could be cultivated.

DR. T. E. OSMOND said he had always taught that the gonococcus did not mind the cold; in fact when he had it growing rapidly if he could not sub-culture for a few days he put it in the ice chest during the interval. Prof. McLeod replied that the question of cold might not be so familiar to clinicians as to bacteriologists. Oddly enough the accepted method to-day of preserving all bacteria was to dry them from the frozen state. Dr. Osmond asked whether Prof. McLeod had adopted the method for identifying the gonococcus recommended by Thomson, that is, solubility in weak alkali. In his experience the gonococcus colonies were much more clearly visible on a colourless medium—where one could see the details, the shape, the scalloped edge, and the striations—than on a blood medium. He had used the medium described by Thomson, which contained the usual ingredients plus hydrocele fluid, and also the chocolate medium agar; though the gonococcus might grow as well on the latter, it was much more difficult to see the nature of the colonies. Prof. McLeod said he had not tried Thomson's test. The best colour contrast was largely a question of what one was accustomed to. A chocolate background was quite useful, but he noticed that Reymann on going into this in detail had chosen a chocolate medium reinforced with ascitic fluid. He preferred Dr. Osmond's suggestion of hydrocele fluid. Noguchi, in cultivation of spirochætes, had great difficulty because of the varying qualities of different samples of fluid, and that would probably apply also to the gonococcus. Dr. Osmond asked whether Prof. McLeod considered hæmoglobin an essential or an advantage, and Prof. McLeod replied that the blood pigment appeared to be an important part of the blood in this connexion.

DR. LEES said that formerly he had been sceptical of the superiority of cultures over smears in diagnosing gonorrhœa, but since using the excellent service provided in Leeds he had become convinced that many cases of chronic and latent gonorrhœa, especially in females, could be detected only by efficient cultural examination. It had been a source of surprise and disappointment that so few Army and civilian laboratories could grow gonococci, even in cases where direct smears from the case showed the organisms to be abundant. His former colleague, Dr. Betty Walker, had recently examined a series of 140 consecutive cases in which a diagnosis of gonorrhœa had been made,

and in 138 of these patients positive cultures had been obtained. The remaining two had negative cultures, but Gram-negative diplococci were found in smears, and the patients' consorts suffered from gonorrhœa. The failure of the cultures in these cases might be due to errors of technique, but in this series there were 85 cases with negative smears and positive cultures.

The technique of collection of the specimen was important and should avoid contamination as much as possible. He preferred to use a platinum loop rather than a dressed probe, and a plate should be used rather than a sloped tube or bottle, as it facilitated examination and picking off colonies. The freshness of the medium was important, and he collected the culture plates as far as possible on the day they were to be used, and stored them in cool conditions. They were placed in the incubator at once after inoculation, and, if not to be sent to the laboratory within 2 hours, they were placed in a special container and gassed with the mixture of 8 per cent. CO_2 and air. It was helpful to place damp cotton wool in the incubator and container so that the plates did not dry readily, and also to have the temperature of the incubator checked regularly by a responsible person: otherwise the temperature might fluctuate widely, and any rise above 37° F. was associated with indifferent diagnostic results. He usually used one plate per patient, one half being inoculated with urethral secretion, and the other half with either prostatic or cervical secretion.

He employed cultures as a routine for the diagnosis and tests of cure in females, tests being made once per week for 4 weeks after treatment, and afterwards during the last day of three successive menstrual periods, or once a month for 3 months if the patient was not menstruating. In male cases he relied on smear diagnosis in early acute cases unless there were some medico-legal indication for more complete identification of the organism. But tests of cure in the male required cultural technique just as much as tests in the female, and he usually made tests of the urethral and prostatic fluid once a month for 3 months after treatment, and at any intermediate time if the clinical signs indicated a relapse.

Prof. McLeod, he thought, had dealt very little with organisms such as might be called pseudo-gonococci, or with the occurrence of meningococci. These organisms presented difficulties in some cases of ophthalmia or vulvovaginitis of children. He recalled one case where Major Hughes had isolated and fully identified meningococci in a case of ophthalmia in a young soldier. This soldier was stationed at a very isolated site, and infection with gonorrhœa was considered highly improbable.

While he was convinced of the value of cultures in diagnosis of gonorrhœa, a series of negative results was possible in certain cases of chronic infection with a focus of infection shut off from the surface. In such cases the gonococcus complement fixation test was of value. These cases were usually suffering from metastatic lesions such as iritis or arthritis, and the focus of infection was in a fibrosed seminal vesicle or Fallopian tube.

He asked for advice on the viability of the gonococcus in urine, and whether such specimens were worth sending to the laboratory for diagnosis of gonorrhœa.

To this Prof. McLeod replied that his own experience of examination of urine had been very disappointing. It was usually a long time in coming to the laboratory. He had noticed that the Americans, who were enthusiastic about freezing methods, recorded many results where the urine was frozen; they spun it down immediately, and then froze the sediment and sent it for examination. This method appeared to have worked almost as well as immediate culture.

DR. I. N. ORPWOOD PRICE thought it could be said in general terms that the results obtained from smears and cultures depended upon the stage of the disease at which these specimens were taken. Thus, in the acute stages of gonorrhœa an equal number of positive results would be obtained by either method. In the later stages of the disease, and in treated cases, culture methods gave better results. At the same time it should be more clearly realized that culture methods depended on two factors, the quality of the medium, and the method of inoculation. Apart from its essential growing qualities, three things were necessary in a medium: (1) a firm surface; (2) that the pH should not be below 7·3 or above 7·6; and (3) a moist atmosphere. Even when inocula were made from early acute cases the results obtained by different operators varied enormously. The most potent cause of failure was the omission of the preliminary cleansing by spirit of the external mucous membrane from the surface of which the inocula were made.

The production of gonococcal antigen for use in the complement fixation test depended on the type of medium used to grow the organism, and Dr. Price recommended the use of egg-albumen agar.

MR. A. J. KING felt that Prof. McLeod did less than justice to those working in venereal disease control when he said that there was a delay of six years before the oxidase test was applied to the identification of gonococcal cultures. Dr. Price published his work on the subject in 1929, and the test was in routine use at the Whitechapel Clinic from 1930 onwards. Prof. McLeod replied that he was thinking of the wider use; there was a gap of six or seven years outside this country.

Mr. King then asked whether Prof. McLeod would be prepared to swear in a Court of Law that he had grown gonococci in culture if he were unable to apply sugar fermentation tests in sub-culture. In fact, would he accept as the gonococcus an organism which grew like the gonococcus, which looked like that organism, and which gave a positive oxidase reaction? To this Prof. McLeod replied that if he knew he was going into a Court of Law he would not stop short of isolating the organism in pure culture and determining its sugar fermentations; but for everyday diagnosis the appearance of the colony, which was very characteristic, and the facts that

it gave a sharp oxidase reaction and that it was morphologically a frank diplococcus, were quite adequate for the purpose of diagnosis. A percentage of strains was always examined for sugar fermentations just to make sure that no errors were being made.

Asked what were his experience and views on the substitution of acetone for alcohol in the modified Gram stain, Prof. McLeod replied that he could make no comparison as he always decolorized with aniline-xylol.

DR. HEYWOOD: In September last, while in Copenhagen on holiday, Dr. Heywood had met Dr. Reymann, who showed him the diagnostic and therapeutic routine used in Danish venereal disease clinics. The diagnosis of gonorrhœa by cultural methods interested him clinically, and he had gone to some pains to find out how the Danes carried out this procedure. The two clinics he visited took specimens on dressed probes, and these were placed in an ordinary test tube containing about 1·0 cm. of turbid fluid. He had asked what it was, but owing to the language difficulty was not able to understand the reply fully: he thought it must be something like the medium Dr. Stuart was using in Glasgow. The specimen in the test tube was sent by post to the State Serum Institute, which carried out the cultural diagnosis of gonococci for the whole of Denmark. Dr. Reymann had for a number of years been in charge of the cultural work at the State Serum Institute, but he had recently relinquished it to Dr. Alice Reyn. The specimens on arrival were plated in aluminium Petri dishes, which he had not seen before, and he wondered whether they were obtainable here, because they would have a longer life. The dishes contained Reymann's modification of Prof. McLeod's medium. They were incubated in tall glass jars in which carbon dioxide was generated from a mixture of sodium carbonate and sulphuric acid, and at the end of 16 to 24 hours they were subjected to the oxidase test and the positive colonies were Gram-stained, fermented, and sub-cultured. This answered one of Mr. King's questions as to what would be sworn to in a Court of Law. In Denmark the sub-culture was grown at 22° C. to eliminate the confusion of the gonococcus with the micrococcus catarrhalis. The primary culture was continued for a further day in ordinary atmospheric conditions because, according to Dr. Reyn, the carbon dioxide culture for 24 hours gave only 80 per cent. of possible positive results, and the further 24 hours' incubation gave better results.

Prof. McLeod was interested to hear what Dr. Heywood had said about Dr. Reymann's work. He was in Copenhagen at an earlier period and knew the laboratory and the general line of work. They had used the aluminium plates for many years, particularly in the diagnosis of whooping cough, but his experience of aluminium plates was disappointing; the metal corroded, but it might be a question of getting a suitable preparation of aluminium.

DR. W. NEVILLE MASCALL said that although he was a clinician he had always been interested in this subject and about 1934 published a paper dealing with the pathological diagnosis of female gonorrhœa; in it he tried to point out that a large number of cases would not have been diagnosed correctly if cultures had been omitted. Since then he had been an advocate of culture methods, but he would like to see a greater use of plates instead of slopes. While working at the Whitechapel Clinic a plate was inoculated from the posterior fornix of the vagina as a routine, and it was amazing how often that plate proved to be positive even when the other cultures were negative. He felt that most probably it was due to the fact that the greater surface area allowed the gonococcus to breathe more freely. The gonococcus did not like to work with a lot of other organisms present, and part of the difficulty of diagnosis in females was undoubtedly due to the fact that there was always a heavy secondary infection. He felt that if plates were used they would get even better results with the cultures than they did at present.

Recently he wrote a paper and was criticized for his method of Gram-staining because he used acetone in place of alcohol for decolorization. He was told that his diagnosis would not be so accurate as with alcohol. In view of this he had been doing a series of parallel stainings, using alcohol and acetone, and so far there was no appreciable difference between the two.

In the future planning of a medical service a central venereal disease laboratory should be established where specimens from doubtful cases could be sent for verification.

DR. LAIRD did his own cultures and the results were reasonably encouraging. He was able to consider the different aspects of the case, the clinical side, the smear side, and finally the cultures, and in that way he believed that in a good proportion of cases a fairly accurate diagnosis could be reached.

The question of conjunctivitis due to the meningococcus rather than the gonococcus was a problem which he came across very early in the war. In conjunction with an eye specialist he published in the *Journal of the Royal Army Medical Corps* in about 1940 an account of two such cases. This possibility should be kept in mind in cases in which there was no other evidence of gonococcal infection.

DR. MCELLIGOTT said that meningococcal conjunctivitis could not be such a rarity, because he had seen the condition in a medical student. This student had been examining a case of secondary syphilis when the patient coughed into his face and some saliva fell on to the student's conjunctiva. The eye was disinfected within an hour or two, but nevertheless conjunctivitis developed 10 or 12 days later. The original patient later came to the speaker's clinic, where the diagnosis of secondary syphilis was confirmed. Gram-negative diplococci were grown in culture from the syphilitic patient's throat and from the student's conjunctiva; the organism was found to be the meningococcus.