As has already been pointed out, bacteria grown on various culture media may be employed in the filter paper strip test, which has the advantage of being simple and rapid (Bachrach and Wormser, 1959). The test is very sensitive, and pure culture is not required. Furthermore, the filter paper strip, which is easily prepared at low cost, may be kept for a long period without deterioration.

The use of filter papers for the identification of bacteria has already been suggested by different authors. Knox (1949) placed filter paper strips impregnated with carbohydrates on inoculated agar plates and noted the subsequent change of the indicator. Paper discs containing culture media were employed by Snyder (1954) for the differentiation of bacteria. Similar techniques were described by Sanders, Faber, and Cook (1957) and Cook and Pelczar (1958).

Summary

A filter paper strip impregnated with urea, phenolphthalein, and tryptophan was used in the identification of bacteria of the *Proteus-Providence* group.

Bacterial cells were suspended in 0.1 ml. saline, and the impregnated filter paper strip inserted into the suspension. After one to two hours of incubation at 37° C., urease activity was detected by a change of the colour of the indicator to pink.

Tryptophan deaminase activity was demonstrated by the addition of one drop of hydrochloric acid and ferric chloride solutions. A brown coloration indicated a positive reaction.

Of all *Enterobacteriaceae* tested, only *Proteus-Providence* strains gave positive results.

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A New Method of Demonstrating Capsulated *Bacillus anthracis*

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In order to show the capsules of anthrax bacilli, Heim (1901) and M'Fadyean (1903) stained them by 1% aqueous solution of methylene blue. According to M'Fadyean (1903, 1909) films made from blood or tissue of an animal which died from anthrax should be gently heated, or preferably only dried in order not to destroy the capsule. Such films, stained by 1% methylene blue for about three to five minutes and examined microscopically, showed blue-stained bacilli, surrounded by a deposit of granular or amorphous material scattered among the bacilli coloured reddish purple, representing the debris of imperfectly fixed capsules.

The slight fixation by heat results of course in a danger of infection from the slide. Despite the precautions normally taken to prevent infection with *Bacillus anthracis*, a laboratory worker sometimes becomes infected from slides. Soltys (1948) reported anthrax in a laboratory worker infected from a stained film. He showed that ordinary fixation and staining of films of anthrax bacilli have no bactericidal effect on anthrax bacilli. Even films containing non-sporulating forms, made from the infected tissue, after being only gently heated to preserve the capsule are still dangerous, as such films left for some time will sporulate.

In order to kill the organism, but at the same time to preserve the capsule, various chemicals which kill spores of *B. anthracis* were tested. Of all chemical compounds tested, Zenker's solution used for the fixation of tissue for histological studies provided the best results. It was found that once the preparation was fixed with Zenker's solution it could be stained by any available stain at any time. The organism takes on the colour of the stain, while the capsule remains as a well-defined clear zone. If films fixed by Zenker's solution are stained by methylene blue, the capsule is stained faintly purple. Films should be washed with water after being fixed by Zenker's solution. This method has been used by the writer since 1948 in his classes for students and also for routine diagnostic work, and has proved to be far superior to the old method recommended by M'Fadyean. In addition to being a much better method it is completely safe, as anthrax bacilli are killed.

In order to show that slides containing both capsulated bacilli and sporulating bacilli were safe

they were washed with wet swabs and suspended in 1 ml. of nutrient broth and inoculated on blood agar media and into mice. All tested slides failed to produce either culture or infection.

A similar experiment was performed with the same type of slides from which mercuric chloride was removed. Both the medium and mice failed to show the presence of *B. anthracis*.

Because unstained areas round the bacteria may simply be the space between a lightly stained surrounding medium, which retracted from the cells on drying and bacteria, the best way to demonstrate capsules is actually to stain them by some procedure which differentiates them from the cell itself. A modification of Leifson's method (1930) for staining flagella accomplishes this in the case of *B. anthracis*.

Staining Procedure

Fix the films in Zenker's fluid for one to two minutes and wash in water. Then flood slides with the solution A for 10 minutes. Wash with tap water and stain with solution B for five to 10 minutes. Wash in tap water, dry, and examine.

Solution A

$\text{NH}_4\text{Al}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$ (sat. aqueous solution)	..	20 ml.
Tannic acid (20% aqueous solution)	..	10 "
Distilled water	..	10 "
Ethyl alcohol 95%	..	15 "
Basic fuchsin (sat. solution in 95% ethyl alcohol)	..	3 "

The stain deteriorates after a week and should be freshly prepared.

Solution B

Methylene blue in powder	..	0.1 g.
Borax	..	1 "
Distilled water	..	100 ml.

Anthrax bacilli stained by the second method appear as dark blue bacilli surrounded by pale blue capsules. Background and red cells are stained red.

The first method (films are fixed in Zenker's solution for three to five minutes, washed with water, stained with methylene blue, and washed in water) is recommended for routine diagnostic work in the field by a veterinary surgeon or medical practitioner, but the second method by which capsules as well as bacteria are stained is the better for laboratory and teaching purposes.

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A Simplified Method for the Quantitative Bacterial Culture of Urine

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The value of quantitative bacterial culture of freshly voided clean mid-stream urine in assessing infection of the urinary tract is established (Kass, 1955; Boshell and Sanford, 1958; Monzon, Ory, Dobson, Carter, and Yow, 1958). However, the technique of serial dilution recommended is time consuming and requires a large number of petri dishes and pipettes, so that its use on a large scale in a clinical laboratory is impractical. It was therefore decided to compare the colony counts obtained by the dilution technique with those obtained by culture of a "standard" loopful of urine. The results with the latter are sufficiently accurate for the purpose of assessing urinary tract infection in clinical practice.

Collection of Specimens

The urine was collected in the course of an investigation into the prevalence of urinary tract infection in diabetic and general populations. There were 100 urines in all, from 39 men and 61 women. A high proportion of the subjects were middle-aged and elderly.

In males the glans penis was swabbed with 1:1,000 benzalkonium hydrochloride ("roccal") and a mid-stream specimen of urine collected. Female patients were prepared in the dorsal lithotomy position as for catheterization with the same antiseptic. The labia were separated, the patient was then encouraged to void urine and the specimen was collected from the mid portion of the urinary stream into a sterile container. Specimens were dealt with immediately after collection; if this was not possible they were stored at -20°C .

Method

The number of viable organisms per millilitre of urine was determined by the same observer with each specimen of urine as follows:

Dilution Technique.—Serial tenfold dilutions of urine from 1 in 10 to 1 in 1,000,000 were made in sterile normal saline and 1 ml. of each dilution mixed with 10 ml. of molten nutrient agar kept at 50°C ., then poured into a petri dish and allowed to set. The six plates were incubated aerobically at 37°C . for 48 hours. The plate containing approximately 50–200 colonies was counted, and the result multiplied by the dilution gave the number of viable organisms per ml.