ROUTINE USE OF LIQUID UREA MEDIUM FOR IDENTIFYING SALMONELLA AND SHIGELLA ORGANISMS

BY

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In the routine examination of faeces for salmonella and shigella organisms many of the non-lactose-fermenting colonies isolated are found after further investigation to belong to the *Proteus* and paracolon groups. If these organisms could be rapidly detected and eliminated there would be a considerable saving of time and media.

Literature

The ability of the *Proteus* group to decompose urea with the formation of free ammonia and the production of alkalinity in the medium has been employed by various investigators in the identification of this group. Rustigian and Stuart (1941, 1943, 1945), and Stuart, Van Stratum, and Rustigian (1945) used a strongly buffered urea broth. In this medium *Proteus* group organisms produced enough ammonia to give a reaction more alkaline than pH 8.1 after 12–48 hours' incubation at 37° C. These investigators pointed out that a large number of organisms other than those of the *Proteus* group are capable of urease production and will give a positive reaction in weakly buffered media. By decreasing the amount of buffer to one-tenth or one-hundredth that of the original concentration the time of incubation in the identification of the *Proteus* group can be decreased from 12–48 hours to 2–4 hours.

Rustigian and Stuart (1941, 1945) used the decomposition of urea in the differentiation of *Proteus* strains from other intestinal organisms. Ferguson and Hook (1943) confirmed that this method was an excellent means of differentiating the *Proteus* group from the *Salmonella* and *Shigella* groups. They examined 75 strains of the *Salmonella* and *Shigella* groups and found that none of them produced urease.

Christensen (1946) thought that the well-buffered liquid urea medium described by Rustigian and Stuart was suited only for the identification of the *Proteus* group, as other organisms capable of decomposing urea could not do so in this medium because of the presence of the large amount of buffer and the small amount of nutritive material. In order to overcome these limitations Christensen devised a urea-agar medium in which he included peptone and dextrose and reduced the buffer content. This medium supported a more vigorous growth of many of the Gram-negative enteric bacilli and readily permitted observation of urease production by the *Proteus* group and by some members of the paracolon group.

Cook (1948) studied the production of urease by organisms of the *Proteus* group as well as some organisms of the paracolon group. For this study he used two media. One was Christensen's solid medium and the other was a solid modification of the highly buffered medium of Rustigian and Stuart. Cook showed the value of the use of Christensen's urea medium in the routine investigation of non-lactose-fermenting organisms isolated from faeces.

Elek (1948) introduced a "rapid test" for the detection of urease production. In this method the ammonia produced by urea decomposition is detected directly by nesslerization and not indirectly by means of an indicator to detect the production of alkalinity. There are several practical disadvantages in using such a method. As Nessler's reagent detects traces of ammonia, the substrate has to be kept in a bottle with a glass stopper smeared with petroleum jelly and kept in a refrigerator. The substrate has to be renewed each

month. Owing also to the sensitivity of Nessler's reagent, positive and negative controls have to be used in the test. With this method the production of ammonia cannot be detected until the Nessler's reagent is added at the end of three hours. The addition of the reagent renders the substrate useless for subinoculation or for further incubation to test for late urease production.

The medium devised by Christensen has a solid agar base. In use, although an alkaline reaction can be detected after two to five hours' incubation at 37° C., there may not be enough growth to inoculate further test media with ease. I therefore conceived the idea of modifying Christensen's formula (by omitting the agar) and using it as a liquid medium. By placing small amounts (1 ml.) of this modified Christensen's medium into 5-ml. screw-capped bottles ("bijou" type) it was found that a rapid growth ensued and that a clear-cut alkaline reaction could be observed within two to five hours. As the urea medium is liquid it can also be used to inoculate "sugars" from those cultures producing no alkaline reaction. This procedure has proved satisfactory in the routine examination of faeces and rectal swabs; it has saved much time and media, and has reduced delay in reporting the results of examinations.

Preparation of Medium

Modified Christensen's Urea Medium Base

Difco bacto protease peptone	..	..	1 g.
Dextrose	..	..	1 g.
Potassium dihydrogen phosphate	..	..	..
(KH ₂ PO ₄)	..	..	2 g.
Sodium chloride	..	..	5 g.
Phenol red (0.04%)	..	..	10 ml.
Distilled water	..	..	1,000 ml.

Steam until dissolved (10 minutes) and adjust to pH 6.8–7. Bottle in 180-ml. amounts in 250-ml. screw-capped bottles, and sterilize by autoclaving for 10 minutes at 15 lb. (1 kg. per cm.²) pressure. Allow the medium to cool. Prepare a 20% solution of urea and sterilize by passing through a Seitz filter. Add 10 ml. of urea solution to every 90 ml. of base. Under sterile conditions pipette 1-ml. amounts into sterile bijou bottles. Incubate at 37° C. for 24 hours as a sterility test.

Method of Use

The following method for the routine examination of faeces and rectal swabs is used in this laboratory. Rectal swabs are plated on desoxycholate citrate medium (Hynes, 1942). Faecal specimens are plated on desoxycholate citrate medium and also inoculated into selenite-F medium. These are incubated for 24 hours. The culture in the selenite-F medium is then plated on desoxycholate citrate medium and incubated for 24 hours. Careful plating to ensure well-isolated colonies is essential. Plates showing no non-lactose-fermenting colonies are reported as "no non-lactose fermenters isolated." With a plate microscope at least four non-lactose-fermenting colonies are picked and inoculated into the urea medium. This was found to be a practicable number for routine work, but in special cases—for example, convalescent cases or carriers—the number of colonies thus screened should be increased.

The bottles of urea medium are incubated at 37° C. for five to six hours. It has been found that a higher proportion of positive reactions are obtained within five hours if the medium is incubated in a water bath at 37° C. All organisms producing an alkaline reaction (pink) are regarded as not belonging to the *Salmonella* or *Shigella* groups and are discarded, and the specimens are reported as "no salmonella or shigella isolated." Those organisms producing no alkaline reaction (colourless) are examined further. The medium is inspected with the naked eye to see that growth has occurred, and then, using a Pasteur pipette, one drop is inoculated into each of the following media: plain peptone water, four peptone water "sugars" (dextrose, lactose, mannitol, and saccharose), and a nutrient-agar slope. These together with the remaining urea medium are incubated until the next morning. If the urea medium now shows an alka-

line reaction no further examination is necessary other than observing the "sugar" reactions as a check. If there is no reaction in the urea medium and "sugar" reactions are suggestive, further tests for motility and indole production are performed on the peptone water culture. If necessary, slide agglutinations are carried out on the nutritive-agar culture.

Results

The method described above has been used as a routine in this laboratory since January, 1949. During the first 19 months 10,065 faecal specimens and rectal swabs have been investigated for salmonella and shigella organisms. These specimens yielded 260 strains of *Salmonella* and 270 strains of *Shigella*. All were urease-negative after 24 hours' incubation at 37° C.

Discussion

As the medium is weakly buffered, paracolon organisms of the aerogenes and intermediate types may produce alkalinity in it (Christensen, 1946). However, if any organism produces a considerable alkaline reaction within three to five hours it is most likely to belong to the *Proteus* group. Most urease-producing paracolon organisms (and some strains of *Proteus morganii*) only produce the required alkalinity after incubation overnight. Except for *Shigella alkalescens* and *Shigella equirulis* (Stuart, Van Stratum, and Rustigian, 1945), salmonella and shigella organisms have not been reported to produce urease (Ferguson and Hook, 1943).

The object of this report is to draw the attention of laboratory workers to the advantages of the urease test as a screening method in the isolation of intestinal pathogens. The method is rapid, simple, and inexpensive. It enables large numbers of colonies to be investigated with ease should the occasion demand, and therefore increases the chances of isolating intestinal pathogens. The urease test can be read within five hours; this allows test media to be inoculated on the same day and be ready for reading the next morning.

The advantages of using urea in a fluid rather than a solid form (as described by Christensen) are: (1) peptone water and peptone water "sugars" can be inoculated with a Pasteur pipette, thus saving time; (2) it is easier to check that growth has occurred after five hours' incubation; (3) it is easier to detect any contamination arising during storage; and (4) the medium is more easily bottled from stock.

Summary

The use of a liquid modification of Christensen's urea medium is described. It has proved satisfactory in the routine examination of faeces and rectal swabs for organisms of the *Salmonella* and *Shigella* groups. The advantages claimed for the medium are its simplicity and economy of time and media.

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The insecticidal power of D.D.T. on walls can nearly always be prolonged by previous spraying with a phosphate solution, according to a letter in *Nature* (August 23, p. 328) from a writer in the Belgian Congo. Investigation of why, when sprayed on the mud walls of certain African houses, D.D.T. rapidly lost its insecticidal properties suggested that this was connected with the presence of ferric ions in the mud. The phosphate solution is thought to protect the D.D.T. by forming chemical complexes with the ferric ions.

Medical Memoranda

A Treatment of Cardiac Arrest

The treatment of cardiac arrest during the course of an operation under anaesthesia is a problem which most anaesthetists of experience will have had to tackle at some stage of their careers. The recognized lines of treatment are usually put into action under such unfortunate circumstances that one wonders whether at the time of crisis the plan of campaign is not sometimes disorderly, the really effective steps of resuscitation being delayed until the patient is beyond benefiting from them. The following case brought this very much to mind recently.

CASE REPORT

A baby only 48 hours old was admitted to St. Bartholomew's Hospital suffering from what seemed to be a congenital lesion of the bowel, resulting in complete obstruction. The child was in no worse condition than might have been expected, and after suitable preparation (Ryle's tube into stomach) and premedication (atropine, 1/200 gr. (0.32 mg.) subcutaneously) was taken to the theatre.

The operation proceeded under ether-oxygen anaesthesia, with a small amount of cyclopropane to aid induction. The smallest endotracheal tube (size 00) was passed to help prevent any subsequent inhalation of regurgitated material. Unfortunately the obstruction was found to be due to gross meconium blockage in the lower jejunum, the impacted material being so thick and adherent that multiple incisions into the gut had to be made in an effort to establish a normal passage along the ileum. Even with these measures it was not possible to remove all the obstructing material, and grave doubts about the prognosis were expressed when the abdomen was finally closed, well over an hour after the skin incision had been made.

During the early part of the operation the child's condition seemed fairly satisfactory, but later the pulse became very thready, and at one stage it was thought, but difficult to be certain, that ventricular fibrillation had set in. This was followed by some improvement, however, and it was not until the dressings were being applied that the child suddenly collapsed, the apex beat disappearing completely, followed closely by respiratory failure. Immediately 1 ml. of 1% procaine hydrochloride was injected into the left side of the heart, and almost instantaneously pulsation was felt, the beat being regular and of better force than at any stage before or during the operation. The general condition rapidly improved, and within a short time the child was fit enough to be returned to the ward in an oxygen tent. Unfortunately, as it had been impossible to relieve the obstruction completely, the child died four days later, having by this time developed a superadded peritonitis. It died, however, without having shown any further effects from its temporary cardiac arrest in the theatre. Post-mortem examination showed the heart to be normal and evidence of a terminal bronchopneumonia.

COMMENT

The question which arises is concerning the mechanism of the startling recovery of the child while it was still on the operating table. C. A. Smith (1951) tells us that the capacity of the heart at birth is 3-4 ml. At the same time we learn very early in our medical careers that the blood flow through the coronary arteries varies with the mean aortic pressure (Macleod, 1930; Samson Wright, 1945). It seems reasonable, therefore, when faced with cardiac failure in a tiny child, that a small quantity of fluid injected rapidly into the left heart might so raise the aortic pressure that blood is forced into the coronaries, thus revitalizing the arrested heart. The fact that in this particular case procaine hydrochloride 1% was injected was, we feel, probably immaterial, and we believe that the response would have been similar had isotonic saline been used instead.

In the light of this we attempted to find some information on the subject from the literature. There was no great