UREA DECOMPOSITION AS A MEANS OF DIFFERENTIATING
PROTEUS AND PARACOLON CULTURES FROM EACH OTHER
AND FROM SALMONELLA AND SHIGELLA TYPES

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Bacterial decomposition of urea has been especially useful in distinguishing between members of the Proteus group and other fecal organisms encountered in stool examinations. Originally, the reactions of these organisms on urea were determined by the use of sterile urine as a culture medium, and only recently have media of known composition been employed (Rustigian and Stuart, 1941). Of the media of known composition, those of Rustigian and Stuart and of Ferguson and Hook (1943) have been found suitable to differentiate between Proteus and other organisms believed to be urease-negative.

In considering the formulae of the two media mentioned, it is obvious that the medium of Ferguson and Hook (urea, 2 per cent; phosphate buffer; NaCl, 0.5 per cent; and ethyl alcohol, 1.0 per cent) is of such a nature that only an organism capable of utilizing urea as a sole source of nitrogen would grow. The possibility therefore arises that this medium would not detect organisms which could hydrolyze urea but which could not utilize the liberated ammonia as a source of nitrogen. In such a case, no growth would occur, and the organism would be considered urease-negative.

The medium of Rustigian and Stuart (urea, 2 per cent; yeast extract, 0.01 per cent; and phosphate buffer), because of the extremely small amount of nutritive material present, also is subject to the same theoretical limitations, particularly in view of the fact that an organism incapable of utilizing ammonia is probably forced to use the yeast extract not only as a nitrogen source but also as a carbon source. If these materials are exhausted before the organism shows appreciable growth, its ability to hydrolyze urea cannot be adequately determined. The high buffer capacity of the medium would also mask slight urease activity if such an organism were capable of initial growth in the medium.

These two media, however, as stated by the authors, were designed for the detection of Proteus organisms, and for this purpose they are well suited. It was thought possible, however, that if a medium were devised which eliminated the necessity of an organism's utilizing ammonia as a sole source of nitrogen, other organisms of the fecal group could be detected which would hydrolyze urea.

MEDIUM AND METHODS OF TESTING

The medium devised consists of urea, peptone, sodium chloride, monopotassium phosphate, glucose, phenol red, and agar in proper proportions in distilled

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water. Urea hydrolysis in this medium is shown by a change in color from the pale yellow of the fresh medium to an intense red-violet color.

Preliminary experiments showed that if the quantities of peptone ordinarily used in media were employed (0.5 per cent), the false reactions described by Ferguson and Hook in Ashworth's medium were encountered. However, it was thought that by decreasing the quantity of peptone used, and by adding glucose, the false reactions might be eliminated. The acidity from glucose fermentation would thus counteract the alkalinity produced by peptone decomposition, and the glucose would in addition furnish a readily available source of energy, further protecting the peptone from decomposition. To test this medium for false positive reactions, and to determine whether or not the acid produced from glucose would lead to false negative results, three controls were used, together with the final medium, as follows:

I. Control (Base)

<table>
<thead>
<tr>
<th>component</th>
<th>quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone, bacto</td>
<td>1 g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5 g</td>
</tr>
<tr>
<td>Monopotassium phosphate</td>
<td>2 g</td>
</tr>
<tr>
<td>Phenol red</td>
<td>0.012 g</td>
</tr>
<tr>
<td>Agar</td>
<td>20 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1,000 ml</td>
</tr>
</tbody>
</table>

II. Control

Base + 0.1 per cent glucose

III. Control

Base + 2 per cent urea

IV. Final medium

Base + 0.1 per cent glucose + 2 per cent urea

The base medium (I) and the base medium with glucose (II) were adjusted to pH 6.8 to 6.9, tubed, and sterilized at 15 pounds' pressure for 20 minutes. A 20 per cent solution of urea (Mallinckrodt, analytical reagent) was sterilized by passage through a Seitz filter, and enough was added to tubes of I and II, after they were cooled to approximately 50°C, to make up III and IV with a final concentration of 2 per cent urea.

A rough estimation of the rate of urease activity in Proteus and other coliform organisms was made in the following manner: Media were tubed in approximately 5-ml quantities, and the tubes were slanted so as to leave a butt of about 1 inch in depth with a slant of about 1.5 inches in length. The media were inoculated heavily by spreading a bit of growth from a positive Kligler agar culture over the entire slant. Organisms which hydrolyzed urea produced the characteristic red-violet color on the slant, and, as incubation proceeded, the color extended toward the bottom of the tube. The extent of color penetration into the medium was taken as a measure of urease activity and was recorded as plus and minus signs, as follows: If the color had developed just beneath the surface of the slant but had not penetrated further into the medium, the reaction
was designated $+ - -$. If it had penetrated to the junction of the bottom of the slant with the butt, it was designated $+$. Further penetration into the butt was designated $++$, $+++$, and $+++++$, the latter symbol being used when the color had reached the bottom of the tube.

ORGANISMS USED AND RESULTS

In addition to the various Proteus species, members of the paracolon, Salmonella, and Shigella groups were tested on the media. The paracolon cultures were classified as paracolon Aerobacter, paracolon intermediates, or paracolon Escherichia on the basis of biochemical characteristics. All the cultures tested were freshly isolated from stool specimens in connection with another investigation.

Cultural reactions of the test organisms on the urea medium and the controls are shown in table 1. All media were inoculated as described and incubated at 37°C. Observations were recorded at the end of 6 and 24 hours, and every
day thereafter for 6 days. For the sake of brevity, only the 6- and 24-hour and the 6-day periods of incubation are given in the table. Reactions are recorded as letters which represent the colors of the media at the incubation periods shown. Tubes of uninoculated media were incubated at the same time. Inoculated controls were observed to go through all or part of the following color changes: Y \rightarrow N \rightarrow YO \rightarrow O \rightarrow OP. The meaning of these letters is given under table 1. Uninoculated media showed no appreciable color change after 6 days' incubation.

The letter R is used to represent the red-violet color produced by definite urea hydrolysis. The difference between this color and the deepest color (OP) produced on the control media is striking. Furthermore, in only one culture of the 45 urease-positive cultures tested was this color developed gradually over the 6-day period. In all other cultures it was produced abruptly (overnight) from a negative slant, then it penetrated either rapidly or slowly into the body of the medium, depending on the rate of urea hydrolysis.

The data in table 1 show that the moderate alkaline reaction produced by the test organisms on the basal medium (I), even though not of sufficient intensity to be confusing, is effectively counteracted by the addition of glucose (medium II). On the other hand, the presence of glucose not only fails to give false negative reactions through acid production, but actually stimulates urease activity in the slow urea-splitting paracolon organisms (medium IV). In no case was the characteristic red-violet color developed by the latter organisms on medium III, though there was a greater color development after 6 days (OP) than was shown by urease-negative organisms. Hence, glucose apparently increases the sensitivity of the medium in detecting weak urea-splitting organisms. The stimulating action is probably due to the increased rate of metabolism and cell reproduction accompanying such a readily available source of energy. The fact that the stimulating effect is slight in the Proteus group suggests that the urease in that group is perhaps a strong constitutive enzyme of considerable importance in the normal metabolism of the cell.

The new medium demonstrates definite urease activity in the paracolon Aerobacter and paracolon intermediate organisms tested. This is not surprising in view of the fact that normal Aerobacter and intermediate organisms are known to be able to utilize urea as a sole source of nitrogen (Vaughn and Levine, 1942). It might be expected that though these organisms in their evolutionary development might lose their capacity to utilize urea ammonia as a sole source of nitrogen, they could retain or quickly regain their ability to hydrolyze urea.

There is a marked difference between Proteus and the urease-positive paracolon organisms in the rate of urea hydrolysis as determined by this medium. The majority of Proteus organisms within 1 hour after inoculation gave R+ -- reactions, and within 6 hours the color had penetrated deeply into the medium. All Proteus species gave R+ + + + reactions after 24 hours. Members of the urease-positive paracolon organisms varied from a few which gave only a R+ reaction after 6 days, indicating delayed ability to hydrolyze the urea, to those which gave R+ -- reactions in 6 hours, developing slowly to R+ + + + reactions after 3 to 5 days.
Within the genus Proteus itself no marked difference between the various species in rates of urea hydrolysis was detected. Rustigian and Stuart (1941) have reported that Proteus morganii was distinctly slower in hydrolyzing urea than were other species of the group. Although comparatively few strains of this species were tested in the present study, it appears that, under the more suitable conditions of growth offered by the new medium, the organism hydrolyzes urea as readily as do other members of the group.

The paracolon Escherichia cultures tested did not hydrolyze urea. It would not be surprising, however, in view of the cultural variations among paracolon organisms, to find occasional paracolon Escherichia cultures capable of hydrolyzing urea, and conversely to find occasional paracolon Aerobacter and paracolon intermediate cultures which fail to hydrolyze the compound. The data presented here, however, indicate that most paracolon Aerobacter and paracolon intermediate cultures are urease-positive, and that paracolon Escherichia cultures are urease-negative.

The Salmonella and Shigella species tested in this investigation and in subsequent routine use of the medium over a period of 6 months were never found to produce even a trace reaction on the medium described. Ewing (1946) subsequently used the medium routinely in the examination of thousands of feces specimens and confirmed these findings.

It is believed that the medium described is of distinct value as a routine aid in enteric bacteriology. Its usefulness lies in its convenience, dispensing as it does with further manipulation after it has been inoculated, and in its ability to demonstrate hydrolysis of urea in Proteus organisms within 1 to 6 hours and in paracolon Aerobacter and paracolon intermediate organisms usually within 24 hours. These organisms can thus be eliminated from subsequent routine serological investigations without further time- and material-consuming tests.

The medium, however, cannot be used to determine absolute rates of urease activity, nor, it is believed, can any medium thus far described. The fact that most of the urease-positive paracolon organisms tested in this study hydrolyzed urea within 24 hours, and then proceeded slowly to decompose the urea further, as shown by the slow penetration of the alkaline color of the indicator into the medium, may be merely an indication of lack of tolerance of the organisms to increased alkalinity, with subsequent depression of urease activity. Final word on absolute rates must therefore await further investigation.

SUMMARY

A urea medium is described which eliminates the necessity of an organism's utilizing urea ammonia as a sole source of nitrogen, and which is therefore capable of demonstrating urease activity in organisms somewhat more fastidious in their nitrogen requirements. The medium shows that, in addition to Proteus, paracolon Aerobacter and paracolon intermediates are definitely urease-positive. Paracolon Escherichia as well as Salmonella and Shigella species are urease-negative.

The medium is recommended as an aid in routine enteric bacteriology because
of the convenience of its use, the rapidity with which it detects paracolon *Aerobacter* and paracolon intermediate organisms, as well as *Proteus*, making it possible to eliminate these cultures from further serological tests. Theoretical aspects of urea decomposition as shown by this medium are discussed.

REFERENCES


