

The variability of sensitivity of humans to the Capsicum pungency test is 50:1, according to Crosbie and Munch (5), who found it necessary to standardize the test animal against piperine or capsaicin.

It is, accordingly, evident that owing to the great variability of sensitivity of individuals to the present U. S. P. pungency test, this test is not reliable as a means for evaluating Capsicum.

When powdered Tabasco and La. Sport Capsicum were tasted by the tip of the tongue by 12 non-standardized individuals they appeared as pungent as powdered African Capsicum.

It would seem urgent that either the U. S. P. method be revised by first standardizing humans or that a chemical test probably based on the capsaicin content be devised as a standard of assay for this drug.

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THE EFFECT OF PEPTONE ON THE RESISTANCE OF STAPHYLOCOCCUS AUREUS.*

BY GEORGE F. REDDISH¹ AND ELLA M. BURLINGAME.

It is a well-known fact that the different brands of commercial peptone vary as to their chemical composition. Different batches of the same brand of peptone will also vary chemically, dependent, probably, on the degree of digestion. We have some evidence to the effect that there is a correlation between the state of digestion and the nutritive value of peptone, but exactly what chemical factors are responsible for these differences is not known at this time.

The *growth-promoting* value of the different peptones is not the only factor to be considered. Different peptones may give equally luxuriant growth of certain bacteria and yet the condition of these organisms will vary considerably. In making diphtheria toxin, tetanus toxin, etc., certain peptones will give good growth of the organisms in broth, but it is usually found that one certain peptone will give toxin of a higher potency than the others. For this reason, we cannot be guided entirely by the growth-promoting property of peptones in choosing the brand to be used in bacteriological work. For plate counting in milk and water examination, however, the peptone chosen must possess maximum growth-promoting properties and give the maximum number of colonies. The condition of the organisms after they have grown on the plates is of no importance so long as the colonies are large enough to be easily counted.

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¹ St. Louis College of Pharmacy, St. Louis, Mo.

In testing disinfectants and antiseptics, however, the *condition* of the test organisms, their vigor and resistance is of paramount importance. The peptone used in the media in this work should give good growth, of course, but the media must be such that the resistance of these bacteria will be retained and conserved. The vigor of the test organisms must be maintained at a level equal to their resistance when freshly isolated.

It is for this reason that so much emphasis is placed on the character of the media used in the bacteriological testing of disinfectants and antiseptics. Comparative tests using different peptones have been made during recent years and the specific brand of peptone for use in this work is now specified. In the original Hygienic Laboratory Method for disinfectant standardization (1) published in 1912, however, the peptone was not specified. It was simply stated that the broth to be used should be "in exact accordance with the standard methods adopted by the American Public Health Association for water analysis." It was soon recognized that the peptone found suitable for use in water analysis would not necessarily be applicable for use in broth for disinfectant testing. Consequently, in the 1921 revision of the Hygienic Laboratory Method (2), this error was corrected and a peptone suitable for disinfectant testing was specified. The peptone specified is that made by Armour and Company.

This peptone has been found satisfactory for sixteen years and is still recognized as being better suited for work of this kind than other brands on the market. In the methods of testing disinfectants and antiseptics published by Reddish (3), (4), Armour's peptone is specified for the broth and agar to be used in these tests. In spite of the fact that this peptone is specified in the American standard methods for testing antiseptics and disinfectants, many laboratories throughout the country have ignored this important specification. The result is that widely different results have been obtained by these laboratories. Some of these laboratories have reported results by standard methods of test and in giving the details have even stated that a peptone was used which is different from that specified in these methods. To make matters worse, the substituted peptone often is one which actually weakens the test organisms so that the results obtained indicate that the products tested are much more germicidal than they really are. Tests in my own laboratory have shown that one such peptone will render *Staphylococcus aureus* quite weak within 48 hours, that is, when transferred twice at 24-hour intervals in broth made with this particular brand of peptone. Such a weakened culture is obviously not fit for use as a test organism. *Staphylococcus aureus* is the standard test organism used in testing antiseptics and is employed by the U. S. Food and Drug Administration in their control work on disinfectants and antiseptics (5). The normal resistance of this organism has been thoroughly studied by Reddish (6) and a phenol standard for it has been established.

In order to demonstrate again the effect of different commercial brands of peptone on the resistance of this organism, ten brands of peptone were used in the present study.¹ The following brands were employed: Wilson, Witte, Merck, Stearns, Parke, Davis and Company, Fairchild, Bacto, Bacto-Proteose, Bacto-Tryptophane, and Armour. Broth of the following composition was made from each of these peptones: 1% peptone, 0.5% beef extract and 0.5% NaCl, dis-

¹ Part of a study conducted in cooperation with Emil G. Klarmann, Lehn and Fink, Inc., Bloomfield, N. J., and Burton G. Philbrick, Skinner and Sherman, Inc., Boston, Mass.

solved in water and adjusted to pH 6.8. These ten broths were inoculated with *Staphylococcus aureus* from an agar slant culture and transfers made from each broth culture at intervals of 24 hours and incubated at 37° C. At the end of the third, seventh and tenth day, these ten cultures of *Staphylococcus aureus* were tested for resistance against phenol and an antiseptic similar to Liquor Antisepticus.

Staphylococcus aureus by standard F. D. A. test (using Armour's Peptone) is not killed by 1-60 phenol in 5 minutes nor by 1-70 phenol in 15 minutes at 20° C. and by 1-80 phenol in 5 minutes and 1-90 in 10 minutes at 37° C. The time periods, 5, 10 and 15 minutes, are rather broad for detecting small differences in resistance of these cultures. The average results from four sets of such tests indicate that these ten cultures (*Staphylococcus aureus* grown in broth made with ten different brands of peptone) are of approximately equal resistance. While there was a difference in the character of the culture, apparently this would not affect materially the resistance to phenol as determined in 5-, 10- and 15-minute time periods. The results obtained with each peptone on the third, seventh and tenth days were often approximately the same as that given by the Armour's peptone culture. However, the growth and resistance of the Armour's peptone culture was always uniform, whereas some of the other cultures were granular and clumpy and varied in resistance. Five of these ten cultures gave growth in broth which was very granular with large clumps, whereas the other five gave a more uniform growth with only slight clumping. While this is a factor in obtaining uniform results in short time periods, there was not always sufficient difference in the cultures to show a marked difference in resistance in the 5-, 10- and 15-minute time periods.

Since the time periods used in the phenol tests were 5, 10 and 15 minutes, the differences in resistance of these ten cultures were not so accurately demonstrated. In order to bring out differences in resistance it was necessary to prepare a special antiseptic which would not kill the Armour peptone broth culture in 2 minutes, but would kill in 3 minutes by F. D. A. test at 37° C. When the resistance of the other cultures was lowered, they were killed in time periods ranging from 30 seconds to 2 minutes. Tests with this antiseptic were found to be more accurate and show differences in resistance of these cultures which were not revealed with phenol at 5-, 10- and 15-minute time periods. In this report this solution will be designated as Antiseptic "A."

TABLE I.—F. D. A. METHOD, STAPHYLOCOCCUS AUREUS—0.5 Cc. OF BROTH CULTURE TO 5.0 Cc. OF ANTISEPTIC "A" AT 37° C.

| Brand of Peptone. | 3rd Day. | | | | 7th Day. | | | | 10th Day. | | | |
|--------------------|----------|-----|-----|-----|----------|-----|-----|-----|-----------|-----|-----|-----|
| | 30". | 1". | 2". | 3". | 30". | 1". | 2". | 3". | 30". | 1". | 2". | 3". |
| Wilson | + | + | 0 | 0 | + | + | 0 | 0 | + | + | 0 | 0 |
| Witte | + | + | 0 | 0 | + | + | 0 | 0 | + | + | 0 | 0 |
| Merck | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | 0 | 0 | 0 |
| Stearns | + | + | 0 | 0 | + | + | 0 | 0 | + | + | 0 | 0 |
| Parke, Davis & Co. | + | + | 0 | 0 | + | 0 | 0 | 0 | + | 0 | 0 | 0 |
| Fairchild | + | 0 | 0 | 0 | + | 0 | 0 | 0 | + | 0 | 0 | 0 |
| Bacto | + | 0 | 0 | 0 | + | 0 | 0 | 0 | + | 0 | 0 | 0 |
| Bacto-Proteose | + | + | 0 | 0 | + | + | 0 | 0 | + | + | 0 | 0 |
| Bacto-Tryptophane | + | + | 0 | 0 | + | + | 0 | 0 | + | + | 0 | 0 |
| Armour | + | + | + | 0 | + | + | + | 0 | + | + | + | 0 |

When Antiseptic "A" was used at time periods of 30 seconds, 1, 2 and 3 minutes, it was possible to show differences in resistance between these ten cultures. Considering Armour's peptone as a standard for comparison, since this is the one specified in the American standard methods for testing antiseptics and disinfectants, we found that the resistance of *Staphylococcus*

aureus was reduced by the other peptones used. Four of these peptones were found to have a very definite weakening effect on *Staphylococcus aureus*, reducing its resistance so that it was killed within 1 minute, and in one case 30 seconds, whereas the culture grown in Armour's peptone was not killed within 2 minutes. The results of these tests are given in Table I.

It is shown in Table I that whereas the culture of *Staphylococcus aureus* grown in broth made with Armour's peptone was not killed by Antiseptic "A" within 2 minutes, the cultures in broth made from the other peptones were killed within 2 minutes and four of them were killed within 1 minute. While these differences do not seem great, it is shown that Armour's peptone broth is best suited for retaining the resistance of this test organism. The very fact that a peptone will weaken a test organism at all, regardless of how much, is sufficient reason for excluding its use in media employed for this purpose. Since Armour's peptone assures uniform cultures of *Staphylococcus aureus* in broth and will maintain normal resistance of this test organism, this peptone only should be employed in the testing of disinfectants and antiseptics for germicidal activity against this standard test organism.

SUMMARY.

It is shown in the present study that Armour's peptone is best suited for use in media employed for growing *Staphylococcus aureus* for use in testing antiseptics and disinfectants. The resistance of this standard test organism is maintained consistently and uniformly when broth made with this peptone is used, whereas when other peptones are employed the resistance of this organism is reduced to a significant degree. For this reason Armour's peptone should continue to be specified for use in media employed in the testing of antiseptics and disinfectants for germicidal activity.

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