tivity of this series of compounds was determined using the same test organisms and was found that there is no appreciable difference in the respective compounds with the exception of the nicotinic amide derivative.

(4) The substitution of beta hydrogen in the pyridine ring by $-\overset{\overset{O}{\|}}{C}NH_2$ reduces the bactericidal potency of the pyridinium derivative.

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Taxonomic Relationships in the Genus Proteus.

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Recently Cope and Kilander[1] described 83 strains isolated from gastroenteritis patients, contacts and foodhandlers with suggestive or definite history of enteric disorders as "atypical enteric organisms of the *Shigella* group." Stuart *et al.*[2] reported 19 comparable strains tentatively classed as "anaerogenic paracolon" cultures which were isolated in this laboratory or received from the laboratories of several states. To date the number of strains studied by us has increased to 48, including 5 strains kindly furnished by Dr. E. J. Cope. Forty-three of the strains were isolated from fecal specimens of gastroenteritis patients or foodhandlers, one from the blood of a patient and 4 from apparently normal contacts. To facilitate discussion the cultures studied in this laboratory will be called, type 33111.

Cultural Characteristics. Type 33111 cultures grew readily on eosin methylene blue agar and on Salmonella Shigella agar forming transparent and colorless colonies. All strains isolated in this laboratory were motile in semisolid, tryptone agar (0.25% agar) at 25°C. Several strains received from different laboratories as nonmotile on isolation were motile under the conditions specified. The motility of type 33111 cultures was usually poor and sometimes negative at 37°C. On agar slants an occasional strain showed slight spreading. On nutrient agar plates (1.0% agar) inoculated by touching the center of the plate with a needle, the growth of some strains covered the entire surface of the plate, others partially, others spread slightly while a few showed no tendency to spread after 48 hours at 25°C. The swarming characteristic of some strains was suggestive of the genus *Proteus* and flagella stains were made on representative strains of each species in this genus. *Pr. vulgaris, Pr. mirabilis, Pr. morganii* and type 33111 cultures showed peritrichous flagellation. *Pr. hydrophilus* and *Pr. ichthyosmius* possessed a single polar flagellum. Kulp and Borden[3] and Guthrie and Hitchner[4] found *Pr. hydrophilus* to be monotrichate.

Biochemical reactions. Type 33111 cultures fermented glucose and mannitol in 24 to 48 hours and sucrose in 14 to 21 days. Nineteen strains fermented salicin in 24 to 48 hours. Lactose and maltose were not attacked. Six strains produced a bubble to 10% gas in glucose and mannitol and one of these a bubble of gas in salicin but no gas was produced in sucrose by any culture. The remaining strains were anaerogenic in all fermentable carbohydrates.

Using a new urea medium Rustigian and Stuart[5] showed that the production of urease by *Pr. vulgaris* and *Pr. mirabilis* could be de-

1 Cope, J. E., and Kilander, K., *Am. J. Pub. Health*, 1942, **32**, 352.

2 Stuart, C. A., Wheeler, K. M., Rustigian, R., and Zimmerman, A., *J. Bact.*, 1943, **45**, 101.

3 Kulp, W. L., and Borden, D. G., *J. Bact.*, 1942, **44**, 673.

4 Guthrie, R., and Hitchner, E. R., *J. Bact.*, 1943, **45**, 52.

5 Rustigian, R., and Stuart, C. A., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 108.

tected in 4 to 5 hours while strong reactions were obtained in 10 to 12 hours. *Pr. morganii* attacked urea strongly but more slowly. To further study urea decomposition as a limiting characteristic for *Proteus* 689 cultures representing all genera of the family *Enterobacteriaceae* have been tested. None of 13 *Serratia*, 24 *Erwinia*, 40 coliform intermediates, 32 *Salmonella* and 33 *Shigella* cultures attacked urea. Of 217 *Escherichia*, 169 paracolon and 161 *Aerobacter* cultures 4, 15 and 51 strains respectively gave weakly positive reactions, pH 7.0 to 7.2 in 5 days. Forty-seven of the 48 type 33111 cultures decomposed urea rapidly and strongly. One urea negative strain of *Proteus* has been reported.[5] All type 33111 cultures produced indole and grew on citrate agar in 24 hours. Gelatin was not liquefied and neither hydrogen sulfide nor acetyl-methyl-carbinol was produced by any strain.

Except for 2 strains Wood *et al.*[6] found that type 33111 cultures possessed the ability to reduce trimethylamine oxide and it was found in this laboratory that these cultures were incapable of growing in glucose broth at 45° C.

Serological Reactions. The antigenic relationships of type 33111 strains to one another is not clear. The fairly good antigenic homogeneity of the first 19 cultures studied[2] was not maintained as additional strains were isolated. In the first antiserum prepared against a type 33111 culture several strains which upon isolation agglutinated to titer and completely adsorbed or markedly reduced the homologous titer later either failed to agglutinate or did not reduce the titer of the same antiserum upon adsorption. Moreover cultures not agglutinating in this antiserum but capable of markedly reducing the titer for the homologous strain were encountered. A similar condition has been observed for *Proteus* in this laboratory[7] and by Taylor.[8] Agglutination and adsorption tests with additional strains and antiserums showed type 33111 cultures to be antigenically heterogeneous.

Selected type 33111 strains were tested in pooled *Shigella paradysenteriae*, in *Sh. sonnei*, *Sh. alkalescens* and 12 *Proteus* antiserums. Negative reactions were obtained in the *Shigella* antiserums. Three cultures agglutinated to 640 in one *Pr. vulgaris* antiserum with a homologous titer of 20480 and to 1280 in one *Pr. mirabilis* antiserum with a homologous titer of 20480. Fourteen cultures agglutinated to 160 to 320 in 3 *Pr. morganii* antiserums (homologous titers 20480 to 40960). The reactions in each instance were due to flagellar antigens.

Because of their cultural, biochemical and serological reactions Rustigian and Stuart[9] suggested that type 33111 cultures be tentatively called, *Proteus* entericus.

Recently Wood and Baird[10] found that the less restricted *Shigella* species, *sonnei, alkalescens, dispar* and *ceylonensis* were able to reduce trimethylamine oxide. Stuart and Rustigian[11] found that these same species grew readily and 97% of 190 cultures tested produced a strong acid reaction in glucose broth at 45° C. A culture of *Sh. rettgeri*, however, reduced trimethylamine oxide but failed to grow at 45° C. A review of the literature revealed that St. John-Brooks and Rhodes[12] found *Sh. rettgeri* indole positive and Edwards[13] found this organism to be actively motile under suitable conditions. The biochemical and IMViC reactions of Dr. Wood's strain and of the A. T. C. C strain of *Sh. rettgeri* were identical with those of type 33111 cultures, including rapid and strong hydrolysis of urea. Furthermore the 2 strains of *Sh. rettgeri* agglutinated but not to titer in 2 of 3 Type 33111 antiserums.

Discussion. Neter[14] suggested that *Sh.*

6 Wood, A. J., Baird, E. A., and Keeping, F. E., *Can. J. Res.*, in press.

7 Rustigian, R., and Stuart, C. A., manuscript in preparation.

8 Taylor, J. F., *J. Path. and Bact.*, 1928, **31**, 897.

9 Rustigian, R., and Stuart, C. A., *J. Bact.*, 1943, **45**, 198.

10 Wood, A. J., and Baird, E. A., *J. Bact.*, in press.

11 Stuart, C. A., and Rustigian, R., *J. Bact.*, in press.

12 St. John-Brooks, R., and Rhodes, M., *J. Path. and Bact.*, 1923, **26**, 433.

13 Edwards, P. R., personal communication.

14 Neter, E., *Bact. Rev.*, 1942, **6**, 1.

rettgeri should be eliminated from the genus *Shigella*. In our opinion *Sh. rettgeri* and type 33111 cultures are the same organism and should be redescribed as *Proteus rettgeri* in the genus *Proteus* or in the appendix of that genus. Because the majority of *Pr. rettgeri* strains were anaerogenic and all strains fermented mannitol some investigators will question the feasibility of placing this organism in the *Proteus* group. *Pr. vulgaris*, *Pr. mirabilis* and *Pr. morganii* produce small gas volumes and old cultures of *Pr. vulgaris*[7,15] not infrequently become anaerogenic and since in the present study some of the strains of *Pr. rettgeri* were aerogenic actual gas production could be considered a flexible criterion for *Proteus*. Despite the fact that Topley and Wilson[16] and St. John-Brooks and Rhodes[17] consider the inability to ferment mannitol a genus characteristic it would seem that ability to attack urea and to swarm are more important genus characteristics. The cultural, biochemical and serological reactions of *Pr. rettgeri* illustrate the difficulty in drawing strict lines of division between related groups of bacteria.

Pr. rettgeri has been described vicariously as a *Bacterium*,[18] *Bacillus*,[12] *Eberthella*,[19] and *Shigella*.[20] This sequence of events in our opinion was not due to carelessness but was brought about by increases in the delicacy and particularly in the number of tests used to differentiate species of bacteria since the isolation of *Pr. rettgeri* in 1909. It seems strange, however, that this relatively rare organism originally isolated from a cholera-like epidemic among fowls should have been isolated from more than 100 gastroenteritis patients or foodhandlers in the past 3 or 4 years.

The taxonomic position of *Pr. hydrophilus* and *Pr. ichthyosmius* has already been questioned.[4,5,17,21] These organisms do not decompose urea, are monotrichate, do not swarm and some strains produce from 20 to 100% gas volumes at 25°C from fermentable sugars, and produce acid and gas slowly from lactose at this temperature. A culture of *Pr. pseudovaleriei* received from Dr. de Assis of Brazil while peritrichate did not attack urea, produced approximately 30% gas in fermentable carbohydrates and appeared to possess all the characteristics of a paracolon organism. For these reasons it is recommended that *Pr. hydrophilus*, *Pr. ichthyosmius* and *Pr. pseudovaleriei* be dropped from the genus *Proteus*.

15 Welch, H., and Poole, A. K., *J. Bact.*, 1934, **28**, 523.

16 Topley, W. W. C., and Wilson, G. S., *The Principles of Bacteriology and Immunity*, 1936, second edition.

17 St. John-Brooks, R., and Rhodes, M., Third International Congress for Microbiology, Report of Proceedings, 1939, 167.

18 Hadley, P., Elkins, M. W., and Caldwell, D. W., Agr. Exp. Sta. Rhode Island State College, 1918, Bull. No. 174.

19 Bergey, *et al.*, *Manual*, 1st ed., 1923, 232.

20 Bergey, *et al.*, *Manual*, 5th ed., 1939, 476.

21 Reed, G. B., and Toner, C. G., *Can. J. Res.*, 1941, **19** D, 139.

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Effect of Temperature on Bacteriostatic Action of Sulfathiazole and Other Drugs. I. *Escherichia coli*.

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As a possible means of throwing light on the mode of action of the sulfonamides, the bacteriostatic effect of sulfathiazole, alone and with other drugs, has been studied as a function of temperature. Other such studies have given revealing information concerning the interaction of these substances with luminous bacteria, as well as isolated enzyme systems.[1]

1 Johnson, Frank H., *Science*, 1942, **95**, 104.