THE BIOCHEMICAL AND SEROLOGICAL RELATIONSHIPS OF THE ORGANISMS OF THE GENUS *PROTEUS*

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There has been marked confusion and disagreement in definitions and limitations of the genus *Proteus* since it was established by Hauser in 1885. Our review of the literature will be limited to those reports which contributed or should have contributed much to the modern concept of this genus. Hauser (1885) noted the swarming characteristics of these organisms and named two species *Proteus vulgaris* and *Proteus mirabilis*, the former liquefying gelatin rapidly and the latter more slowly. Theobald Smith (1893), who was the first to study the action of *P. vulgaris* in glucose, lactose, and sucrose, emphasized two important characteristics of this organism. First, its powers of gas production were "peculiar" since a much smaller gas volume was formed in glucose in 24 hours by *P. vulgaris* than by "*B. coli-communis*." His tables show *P. vulgaris* produced a gas volume in 24 hours of only 4 per cent, whereas several strains of "*B. coli*" isolated from different sources produced from 28 to 46 per cent volumes in the same time. Moreover, he showed that certain species now classed as *Salmonella* produced gas volumes comparable to *Escherichia coli*, whereas species of *Aerobacter* yielded even greater volumes of gas. Second, he noted that the fermentative powers of "*B. coli-communis*" differed from those of *P. vulgaris* in that the former produced acid and gas in glucose, lactose, and sucrose, whereas the latter failed to attack lactose. In 1894 Smith, finding that old *Proteus* cultures showed a diminished or complete loss of power to liquefy gelatin, cited the fermentation of glucose and sucrose and the absence of lactose fermentation as a particular characteristic of *Proteus* organisms.

Most unfortunately, subsequent investigators, almost without exception, established as a cardinal character of the genus *Proteus* the fermentative reaction recommended by Smith (1894) without, however, reference to the small gas volume produced by such organisms (Smith, 1893). In the present investigation of 454 *Proteus* cultures studied in detail and about 200 additional cultures differentiated routinely in this and neighboring laboratories, none has produced more than from a bubble to approximately 15 per cent gas in 48 hours in any fermentable carbohydrate. Shell vials, 7 mm in inside diameter by 28 mm long, were used in fermentation tubes.

Wenner and Rettger (1919) studied 73 *Proteus* strains with a fairly wide range of biochemical reactions. Their cultures swarmed and liquefied gelatin. They pointed out, however, that the liquefying property of a culture might be entirely lost. The 73 cultures fermented glucose, sucrose, and occasionally maltose, but not lactose. All cultures produced hydrogen sulphide, and 69 digested casein. No strain produced any pigment on agar. This work, which

was taken as a point of departure in the American system of classification, divided *Proteus* into two distinct groups. Strains attacking glucose, sucrose, and maltose readily were *P. vulgaris*, and those strains attacking glucose rapidly, sucrose slowly, and not fermenting maltose were called *P. mirabilis*.

In 1927 Moltke made a comprehensive study of 194 *Proteus* cultures. In addition to the characteristics established by Wenner and Rettger (1919), Moltke demonstrated that all his cultures attacked urea rapidly and many fermented salicin. It is curious that considerable controversy arose about the work of Wenner and Rettger (1919) and Moltke (1927) despite the fact that where the same tests were used, their results (except for indole production) agreed very closely.¹ Taylor (1928) studied 53 *Proteus* cultures isolated principally from urinary and wound infections and feces. All cultures swarmed, liquefied gelatin, and peptonized milk. Lactose and mannitol were not attacked by any culture. In glucose "acid and a small quantity of gas were produced in 24 hours and no change occurred subsequently. This reaction was uniform

¹ Wenner and Rettger (1919) stated: "Of 73 strains studied, 25 showed an immediate and 48 a delayed reaction (in sucrose). In correlating these results with the action in other carbohydrates, it soon became apparent that the strains which fermented sucrose readily also fermented maltose, while those which showed a delayed action on sucrose did not attack maltose." The authors conclude their paper with the statement, "For the species fermenting this sugar (maltose) the name *Proteus vulgaris* is suggested and for the species failing to attack it the name *Proteus mirabilis*."

In view of the foregoing quotations it is difficult to understand two statements made by Moltke (1927) in referring to the work of Wenner and Rettger (1919): "Twenty-five (of the 73) strains fermented maltose at once, none later on. It is not stated whether these 25 strains are the same as those fermenting saccharose at once." And, "In the maltose fermentation Wenner and Rettger see a possibility of sub-grouping, but they do not attempt such."

Thirty-seven of Moltke's 194 strains fermented maltose and 157 did not. The maltose-positive strains fermented sucrose rapidly and the maltose-negative ones slowly. In Moltke's summary under "Fermentations by *Bact. proteus* . . .," it is stated: "Their action on maltose divides the *Proteus* bacilli into two sharply distinguished fermentation groups. . . . The maltose fermenting strains ferment saccharose at once; the non-maltose fermenting strains ferment saccharose but slowly." Nevertheless in Bergey *et al.* (5th ed., 1939) and elsewhere is found, "However, Moltke . . . and others do not recognize this description (Wenner and Rettger's) and state that most *Proteus vulgaris* strains are maltose negative." This, we believe, arose from a misinterpretation of Moltke's writing. The term "*Bact. proteus vulgaris*" appears in the title of Moltke's paper and seldom thereafter. The term "*Proteus mirabilis*" appears only once and then in a direct reference to Hauser's work. "*Bact. proteus*" and "*Proteus* bacilli" are used almost exclusively throughout the paper, giving us the impression that he considered "*Bact. vulgaris*" or the "*Proteus* bacilli" as a group rather than a species. To assume, therefore, that the species *P. vulgaris* does not ordinarily ferment maltose because 157 of Moltke's 194 strains of *Proteus* bacilli did not ferment maltose does not seem to be what this author meant to imply.

One reason is apparent to explain Moltke's failure to support Wenner and Rettger's division of the genus into two groups according to the fermentation of sucrose and maltose. Wenner and Rettger (1919) found no correlation between fermentation reactions and indole production. Moltke, on the other hand, found almost perfect correlation between the two reactions. All but one of Moltke's 37 maltose-positive strains (*P. vulgaris*) were indole-positive whereas the 157 maltose-negative strains (*P. mirabilis*) were indole-negative. The present investigation is in entire agreement with Moltke with respect to indole production.

in every strain." Sucrose was fermented by the 53 cultures. In a majority of cases this reaction was delayed. Three cultures fermented maltose and these were the only indole-positive strains. Of Taylor's 53 cultures 3, or 5.7 per cent, were *P. vulgaris*, and 50, or 94.3 per cent, were *P. mirabilis*. The low ratio of *P. vulgaris* isolates to *P. mirabilis* isolates is a characteristic distribution of these two species.

Rauss (1936) examined 48 cultures of Morgan's bacillus no. 1 and concluded that this organism belonged to the genus *Proteus*. He found that such cultures swarmed under suitable conditions, formed indole, and fermented glucose consistently and sucrose occasionally. Both the American (Bergey *et al.*, 5th ed.) and British (Topley and Wilson, 1936) systems classify this organism as *Proteus morganii*.

In 1904 Rettger isolated from chickens during a choleralike epidemic a previously undescribed organism. Some years later this culture together with others was sent to Hadley who was working on the paratyphoid and related organisms (Rettger, personal communication). Hadley *et al.* (1918) named the unidentified culture "*Bacterium Rettgeri*." Since that time this organism has undergone the following taxonomic changes: *Bacillus* (St. John-Brooks and Rhodes, 1923), *Eberthella* (Bergey, 1st ed.), *Shigella* (Bergey, 5th ed.), and finally *Proteus* (Rustigian and Stuart, 1943a). Edwards (1942, personal communication) found it to be motile. Rustigian and Stuart (1943a) confirmed the motility of this species; showed that it hydrolyzed urea strongly and rapidly, and that some strains swarmed under suitable conditions; and proposed that the organism be classified as *Proteus rettgeri*. Even in this laboratory, however, independently isolated strains of this organism were called "anaerogenic paracolon type 33111" (Stuart *et al.*, 1943a), then "*P. entericus*" (Rustigian and Stuart, 1943b) before our strains finally were identified as *P. rettgeri*.

Prior to the division of the genus *Proteus* into two species by Wenner and Rettger (1919) there was little agreement concerning the antigenic relationships among *Proteus* organisms. Some workers found that only the homologous strain agglutinated in an antiserum, whereas others found an antiserum agglutinated a number of heterologous strains to high titers. Wenner and Rettger showed that of 73 cultures of *P. vulgaris* and *P. mirabilis* only 19 failed to agglutinate in one or another of a total of 7 antisera prepared against the two species. All except 1 antiserum agglutinated heterologous cultures.

Moltke (1927) studied the H and O antigens of his cultures. By direct agglutination tests 3 main groups were established, whereas adsorption tests divided the 3 groups into a number of subgroups. The nonswarming strains lacked H antigens and exhibited a variety of O antigens. Rauss (1936) found that the H antigens of *P. morganii* had group specific and the O antigens type specific tendencies. He established 7 different H and 17 different O antigens in his 48 strains. An H antigen common to *P. morganii* and *P. vulgaris* was discovered. The first cultures of *P. rettgeri* studied showed marked serological homogeneity (Stuart, Wheeler, *et al.*, 1943, anaerogenic paracolon type 33111), but as more strains were studied antigenic heterogeneity was encountered.

Strains of this species were found which failed to agglutinate in an antiserum, but upon adsorption completely removed all agglutinins. Similar findings for other species of *Proteus* have been reported by Taylor (1928) and by Welch and Poole (1934).

Sources of Cultures

A total of 454 cultures of *P. vulgaris*, *P. mirabilis*, *P. morganii*, and *P. rettgeri* was studied. Of 69 *P. vulgaris* strains, 29 were isolated from feces, 6 from urine, 2 from osteomyelitis cases, and 32 were received from stock cultures of many different laboratories. Of 205 *P. mirabilis* strains, 118 were isolated from feces, 32 from urine, 34 from such sources as ureter, blood, lung, kidney, bladder, chest fluid, ileum, peritoneum, intestinal mucosa, wounds, and burns, and 21 were received from stock culture collections. One hundred and two *P. morganii* cultures were studied; 65 from feces, 7 from urine, 17 from such sources as stomach, kidney, scalp, and wounds, and 13 were obtained from culture collections. Of 78 *P. rettgeri* strains, 77 were isolated from fecal specimens and one from blood.

During the 4-year period of this investigation 190 cultures of *P. vulgaris* and *P. mirabilis* were isolated or received by us shortly after isolation from human sources. Of the 190 cultures, 14.3 per cent were *P. vulgaris* and 85.7 per cent were *P. mirabilis*. The marked difference in the relative incidence of the two species agrees with Levine (1942) and with Dammin and Belling (1942) who found that 85 to 90 per cent of the *Proteus* bacteria isolated from humans were *P. mirabilis*.

To determine the frequency of *Proteus* in normal feces, 110 specimens were examined. Specimens were plated directly on eosin methylene blue (EMB) agar and on *Salmonella* and *Shigella* ((SS) agar, and also inoculated into either selenite or tetrathionate enrichment broth. After 24 to 36 hours' incubation the enrichment broths were plated on EMB and SS agar. On direct plating 10 per cent of the specimens yielded *Proteus*, 8.2 per cent *P. mirabilis* and 1.8 per cent *P. morganii*. From preliminary enrichment broth 36.3 per cent of the specimens were positive; 23.6 per cent *P. mirabilis*, 10 per cent *P. morganii*, and 2.7 per cent *P. vulgaris*. Hynes (1942) examined 353 fecal specimens from typhoid and paratyphoid patients. With direct plating on desoxycholate agar 10 per cent of the specimens yielded *Proteus*, whereas with tetrathionate broth 25 per cent were positive.

Cultural Characteristics

The 454 cultures of *Proteus* grew readily on standard agar and other mediums at 30 to 37 C. On EMB agar (1.3 per cent agar) *P. vulgaris* and *P. mirabilis* colonies possessed white, pink, or blue centers with the spreading growth a white to whitish blue color in 24 to 48 hours at 37 C. On SS agar (1.35 per cent agar) swarming was completely inhibited, even with moist surfaces. Well-isolated colonies of *P. vulgaris* and *P. mirabilis* had dark brown centers with white entire edges in 24 hours at 37 C. At 48 hours the centers became jet black,

but some isolated colonies failed to develop black centers in 3 to 4 days. Where colonies were crowded, 3 to 4 days elapsed before an occasional colony developed black centers. A strong odor of hydrogen sulphide was noted upon removal of the plate covers, and the removal of black-centered colonies revealed a black precipitate in the medium. On EMB agar *P. morganii* and *P. rettgeri* failed to spread in 24 hours at 37 C, but after an additional 24 hours some *P. morganii* strains showed spreading. Both species on EMB agar produced white, pink, or blue colonies. On SS agar colonies were colorless and opaque, and showed no spreading.

Almost without exception descriptions of the morphology of *Proteus* start with the phrase "highly pleomorphic rods." Most authors, however, fail to point out that pleomorphism in *Proteus* cultures is a transient characteristic and for the most part confined to actively swarming cultures. In smears from young cultures (5 to 7 hours' incubation) and young colonies (10 to 12 hours) pleomorphism is marked, but smears from the same culture or colony after 24 to 48 hours show little or no more morphological variation than comparable smears from the average coliform culture. On the other hand, smears, particularly from the periphery, of young colonies of an occasional coliform or paracolon culture may show pleomorphism almost as marked as does *Proteus*. In using pleomorphism as a criterion of *Proteus*, the age, motility, and swarming characteristics of the culture in question should be carefully recorded.

We have only one thing to add to the swarming characteristics of *Proteus* organisms as described by Hauser (1885), Moltke (1927), and others. On 1 per cent agar slants with water of expression in the base of the tube, straight line inoculations of *P. vulgaris*, *P. mirabilis*, and *P. morganii* behave similarly at 25 C. Growth along the line of inoculation is first established, then lateral, straight, or curved branches spread over the surface; often these branches reach the edge of the slant before the interstices are filled in. In such cases the line of inoculation can be distinguished easily from the swarm portion of growth. Frequently, however, the swarm growth is so thin that a needle is required to detect its presence. With *P. vulgaris* and *P. mirabilis*, especially the former, as the concentration of agar increases, the character of swarming is not markedly altered until it ceases. With *P. morganii*, as the concentration of agar increases, lateral branchings decrease in length until they disappear. The culture may continue to spread in a continuous wave outward from the line of inoculation to cover the entire surface of the slant. As the wave spreads, growth thickens until it is difficult to detect the line of inoculation. On very fresh 1 per cent agar slants a few strains of *P. rettgeri* show short lateral branches, but for the most part the culture spreads like *P. morganii* on higher concentrations of agar. On 1 per cent agar plates an occasional strain of *P. rettgeri* may swarm in a manner quite indistinguishable from *P. vulgaris*.

It is evident that the swarming of *P. vulgaris* and the spreading of *P. rettgeri* are quite different processes, but that they have a common origin is shown by the following fact. In experiments involving the growth curve of *P. vulgaris* several nonmotile strains were used. Occasionally one colony on several plates

used for counts showed some evidence of spreading. From a well-defined center an even ring of growth extended outward for several millimeters. No branches were observed from the edge of the colony. Transplants from the edge of the colony to 1.2 per cent agar slants exhibited spreading as described for *P. rettgeri*. With subsequent transplants spreading either ceased or by a series of transitional stages progressed to the characteristic swarming of *P. vulgaris*. Of the 69 *P. vulgaris* strains, 12, for the most part old stock cultures, were nonmotile. Three fresh isolates and one old stock culture were the only nonmotile strains of the 205 *P. mirabilis* strains studied. Of the 102 *P.morganii* strains, 2 fecal and 3 wound isolates were nonmotile. All *P. rettgeri* strains were motile, but many strains received by us were labeled "nonmotile." The motility of *P.morganii*, and particularly *P. rettgeri*, cultures is poor, or is completely inhibited, at 37 C.

Biochemical Reactions

As previously pointed out, the 454 *Proteus* cultures used in this investigation, and over 200 additional *Proteus* cultures routinely recorded in this and neighboring diagnostic laboratories, produced acid only or from a bubble to 15 per cent gas in Durham fermentation tubes with any fermentable carbohydrate in 48 hours. With very few exceptions gas volumes were not appreciably increased on prolonged incubation. Of the 454 cultures studied in detail, only 3 strains of *P. mirabilis* produced as much as 20 to 30 per cent gas in sucrose in 3 to 5 weeks. Hereafter "the production of acid and gas" or a "+" reaction of *Proteus* in any carbohydrate will signify slight gas production, a bubble to 15 per cent gas. Gas production, especially in cultures from stock collections, is variable in sucrose, salicin, and maltose.

Biochemical reactions, characteristic of the different species, are given in table 1 and will be discussed for each species.

Proteus vulgaris. Of the 69 strains, 67 produced acid and gas in glucose in 24 to 48 hours. Two cultures gave acid only in 24 hours and no gas in subsequent tests after prolonged incubation. All strains produced acid or acid and gas in sucrose and maltose in 24 to 48 hours, though on occasions the reaction, particularly in maltose, was weak. Twenty-seven strains fermented salicin rapidly (24 to 48 hours), and 6 strains slowly (7 to 21 days). One strain fermented lactose and mannitol slowly. All strains hydrolyzed urea in 8 to 12 hours, produced hydrogen sulphide, and failed to produce acetylmethylcarbinol. Five failed to liquefy gelatin, 4 were indole-negative, and 35 were citrate-positive.

Recently isolated *P. vulgaris* strains differed considerably from those obtained from stock collections. Of 37 recent isolates, 4 per cent and, of 32 stock cultures, 87.5 per cent produced acid only in one or more of the carbohydrates, sucrose, salicin, and maltose. Nine recent isolates fermented salicin (3 rapidly and 6 slowly) for a total of 24.3 per cent, whereas 75 per cent of the stock cultures fermented salicin rapidly. Recent isolates liquefied gelatin in 1 to 3 days, 28 stock strains from 7 to 24 days, and 4 such strains were negative after 2 months. One recent isolate and 3 stock strains were indole-negative. (Contrary to the

statement [Bergey *et al.*, 5th ed.] that indole formation by *P. vulgaris* is slight, the positive strains gave an immediate and strong reaction with Kovac's reagent.) Twenty-five of the 35 citrate-positive strains were from stock collections and 5 of the 10 recent isolates required varying periods of time to grow on the medium. One stock culture produced acid in lactose and mannitol. Welch and Poole (1934) reported a variant of an X19 strain which fermented lactose and mannitol.

Proteus mirabilis. All 205 strains produced acid and gas rapidly in glucose. Acid or acid and gas was produced in sucrose slowly by 197 strains, and 19 of these strains also fermented salicin slowly. One otherwise normal strain produced acid slowly in lactose, and another otherwise normal strain produced acid and gas in maltose. The type of growth of *P. mirabilis* in sucrose broth fre-

TABLE 1
Biochemical reactions characteristic of the different species of the genus Proteus

	P. VULGARIS	P. MIRABILIS	P. MORGANII	P. RETTGERI
Strains studied	69	205	102	78
Glucose	+	+	+	A+
Lactose	-	-	-	-
Sucrose	+	+s	-	As
Salicin	-+	-	-	-A+
Maltose	+	-	-	-
Mannitol	-	-	-	A+
Urea	+r	+r	+s	+r
Hydrogen sulphide	+	+	-	-
Gelatin	+	+	-	-
Indole	+	-	+	+
Citrate	-+	+	-	+
Voges-Proskauer	-	+ -	-	-

s = slow.

r = rapid.

+ = acid and small gas volume in carbohydrates.

A = acid.

-+ indicates that a substantial number of strains fall in either category. For minor exceptions see section on biochemical reactions.

quently was a presumptive criterion for this species. It was characterized by a surface scum with marked sedimentation and decolorization of the brom cresol purple indicator after several days. All strains hydrolyzed urea rapidly. The Malay-Kingsbury HXK strain, though consistently positive, produced a nonmotile variant that was urea-negative. The ability of the variant to hydrolyze urea was restored after serial transplants in urea medium for 9 months. Two of the 205 strains failed to produce hydrogen sulphide. Recent isolates liquefied gelatin in 1 to 3 days except for 2 strains that required 10 to 14 days, whereas strains from stock collections, when positive, usually required 14 to 21 days. Seven stock strains, including 4 XK strains, failed to liquefy gelatin. Indole was formed by only 3 strains and one of these gave a weak reaction. Acetylmethylcarbinol was produced by 119 strains. The Voges-Proskauer

reaction was never strong, occasionally moderate, usually weak, and sometimes to be described as a trace. Furthermore, in a subsequent test 2 to 3 years later, 10 strains previously positive were negative. Nine strains failed to grow on citrate agar.

Proteus morganii. The biochemical reactions of this species were uniform. The 102 strains produced acid and gas rapidly from glucose. Sucrose was fermented slowly by 11 strains. Lactose, salicin, maltose, and mannitol were not attacked. One otherwise normal strain failed to form indole. Hydrogen sulphide and acetylmethylcarbinol were not produced, gelatin was not liquefied, and growth did not occur on citrate agar. All strains hydrolyzed urea in 24 to 48 hours. Levine *et al.* (1925), Rauss (1936), and others had reported *P. morganii* as producing hydrogen sulphide. Sevin and Battiaux (1939) observed only slight blackening on lead acetate with some strains in 4 to 6 days, whereas other strains showed no blackening. The discrepancy between our findings and those cited above is probably due to the fact that only definite blackening with diffusion into the medium was called a positive reaction in this work.

The growth of all strains of *P. morganii* in tryptone broth (distilled water containing 1 per cent bacto-tryptone) in 4 to 6 hours yielded a strong fetid odor; in 18 to 24 hours the odor was considerably diminished or even absent. Tribondeau and Fichet (1916) noted a fecal odor with this species in peptone broth. The rapid development of the odor proved to be of marked differential value for *P. morganii*.

Proteus rettgeri. Seventy-one of the 78 strains produced acid, and 7 produced acid and gas rapidly in glucose. All but 3 strains produced acid in 7 to 40 days in sucrose. Twenty-six strains produced acid and one acid and gas in salicin in 1 to 21 days. Seventy-one produced acid and 7 acid and gas rapidly in mannitol. Lactose and maltose were not attacked. Three strains failed to hydrolyze urea rapidly. All strains were indole-positive and grew on citrate agar. Hydrogen sulphide and acetylmethylcarbinol were not produced, and gelatin was not liquefied.

There remain to be described briefly 74 cultures, without taxonomic status, which biochemically resemble *Proteus*. Twenty-three of these cultures were previously described as type 29911 (Stuart, Wheeler, *et al.*, 1943). Seventy-one of the cultures were isolated from feces and 3 from urine. Acid or acid and gas was produced rapidly in glucose by all strains. Gas, when present, was limited to a small bubble. All strains produced acid in sucrose in 5 to 15 days and a weak acid reaction in maltose in 14 to 30 days. Lactose, salicin, and mannitol were not attacked. Sixty-seven strains did not hydrolyze urea. Seven strains hydrolyzed urea weakly, raising the pH to 7.2 or 7.4 in 48 hours without further increase even after 5 days (Rustigian and Stuart, 1941). All strains were indole- and citrate-positive, and gelatin-, acetylmethylcarbinol-, and hydrogen-sulphide-negative.

Serological Reactions

Final detailed antigenic analyses of *P. vulgaris* must await further investigation. By H and O agglutination and adsorption test in antisera prepared

from 6 *P. vulgaris* strains, however, 42 of the 57 strains were divided into 5 groups, which were further subdivided into from 1 to 5 types in each group on the basis of possessing 2 or more major antigens in common. Of the remaining 15 strains, 13 showed only one major or minor antigen, and 2 had no antigens in common with any of the 5 groups. (Group as used in this report does not signify a number of antigenically identical cultures but rather a number of cultures with the same antigenic pattern when tested in two or more antisera.) Antigenically identical strains were relatively common among the X strains, but very uncommon among the others. The fact that further work is contemplated on this species does not imply that we hope to devise a practical system for

TABLE 2

Representative antigenic groups of P. mirabilis as determined by cross-agglutination reactions of 138 strains of this species in four P. mirabilis antisera

GROUP	STRAINS	ANTISERUMS			
		48	94	225	248
1	49	2,560	2,560	20,480	20,480
2	9	2,560	2,560	20,480	2,560
3	8	20,480	20,480	20,480	20,480
4	11	20,480	2,560	2,560	20,480
6	5	20,480	20,480	2,560	2,560
9	3		2,560	2,560	2,560
10	3				2,560
12	2	2,560	2,560	2,560	160
13	3		2,560	2,560	10,240
14	4	640	640	10,240	640
15	1	640		10,240	2,560
17	1		640		2,560
21	1			640	10,240
23	1	10,240	2,560		2,560
24	1			10,240	2,560
26	1	640	640	640	640
27	1	640	640		2,560
33	1	20,480	20,480	10,240	
38	2				

antigenically typing strains of *P. vulgaris*, for we are firmly convinced that this is impracticable.

To determine the serological relationships of *P. mirabilis* within the species, 138 strains were tested in 4 *P. mirabilis* antisera. Because of the number of tests involved, agglutination tests were set up with saline suspension of living organisms in antiserum dilutions of 160, 640, 2,560, 10,240, and 20,480. The 4 antisera possessed titers of 20,480. Adsorption experiments were done in a 160-dilution of the antisera. Only 2 of the 138 strains failed to agglutinate in any of the 4 antisera. The 138 strains could be divided into 38 groups on the basis of cross-agglutination reactions, the largest group containing 49 strains and the smallest 1 strain. Representative groups are shown in table 2.

Cultures agglutinating to titer or within one tube of titer in one or more antisera were used for adsorption.

Antiserum 48 was adsorbed with 25 strains, antiserum 94 with 23 strains, antiserum 225 with 85, and antiserum 248 with 80 strains. Four cultures reduced the titer of antiserum 48 from 20,480 to 1,280, 5 to 5,120, and 16 failed to reduce the titer. One culture completely adsorbed antiserum 94, 17 strains reduced the titer from 20,480 to 2,560 or 5,120, and 5 failed to alter the titer. No significant reduction in titer was caused by the 85 strains employed to adsorb antiserum 225. Two cultures completely adsorbed antiserum 248, two reduced the titer from 20,480 to 1,280, ten to 5,120, and sixty-six failed to reduce the titer. With a total of 213 adsorptions only 3 cultures were found homologous to one or another of the 4 cultures used to produce antisera.

TABLE 3
*Antigenic groups of P. morganii as determined by cross agglutination
of 75 strains of this species in four P. morganii antisera*

GROUP	STRAINS	ANTISERUMS			
		213	278	305	312
1	8	20,480	20,480	640	
2	3	20,480	20,480	640	20,480
3	15	20,480	20,480	20,480	20,480
4	2	160	10,240	40,960	10,240
5	1	40	640	20,480	10,240
6	36		1,280	40,960	40,960
7	1	20,480	20,480	20,480	
8	1	640	1,280	10,240	5,120
9	1		10,240		160
10	1			10,240	5,120
11	1				20,480
12	1				2,560
13	1	640	160		160
14	1			640	160
15	2				

One of the 49 cultures in group 1, table 2, was used to prepare an antiserum. Of the 48 group 1 strains (exclusive of the strain homologous to the antiserum), 47 cultures agglutinated to titer, 40,960, or to within one tube of titer, and one to 640. Adsorption of the antiserum with the 48 cultures failed to reveal any culture identical with that used to produce the antiserum. Eight strains reduced the titer to 320 to 640, 18 strains to 1,280, 19 strains to 2,560 to 5,120, and 3 strains failed to reduce the titer. These and other experiments not herein recorded show the great antigenic complexity of *P. mirabilis*.

P. morganii, like *P. vulgaris* and *P. mirabilis*, lacked serological homogeneity. Seventy-five strains were tested in antisera prepared for 4 *P. morganii* strains, 213, 278, 305, and 312. The titers of these antisera were 20,480, 20,480, 40,960, and 40,960, respectively. Table 3 reveals 15 antigenic types as shown

by cross-agglutination titers in the 4 antisera. All strains agglutinating to titer or within one dilution of titer were used to adsorb the one or more antisera in which this reaction occurred. Antiserum 213 was adsorbed with 27 strains. Four group 1 strains (exclusive of 213) and three group 2 cultures completely removed the homologous agglutinins from antiserum 213. However, the group 2 strains and 213 were not identical since the former agglutinated to a titer of 20,480 and the latter failed to agglutinate in antiserum 312 (table 3). Some of the adsorbing strains reduced the titer of 213 antiserum to varying titers, whereas others did not alter the titer. One group 3 strain, exclusive of 278, completely removed the homologous agglutinins from antiserum 278, whereas 29 other adsorbing strains gave varying results. Of 20 used to adsorb 305 antiserum, one group 4 culture, exclusive of 305, completely removed homologous agglutinins, and the others varied in their reactions. Antiserum 312 was adsorbed with 42 strains; varying reactions were obtained, but none completely removed the homologous agglutinins.

The adsorption experiments above and others, such as the adsorption of antisera 305 and 312 with one of the 36 strains of group 6 and the testing of the 35 remaining strains, revealed a minimum of 30 major and minor antigens in the 75 *P. morganii* strains studied.

As with *P. vulgaris*, detailed antigenic analysis of *P. rettgeri* must await further investigation. Rustigian and Stuart (1943a) pointed out the serological heterogeneity of *P. rettgeri*. This was further confirmed in the present work by the study of 53 strains in 4 *P. rettgeri* antisera. A recent development, although not altering our concept of the antigenic heterogeneity of *P. rettgeri*, may modify the extent of heterogeneity within the species. In the early work on *P. rettgeri* (then called "anaerogenic paracolon, type 33111," Stuart, Wheeler, *et al.*, 1943) it was found that a strain not agglutinating at all in an antiserum could completely remove all agglutinins from the antisera upon adsorption. As the number of strains and antisera increased, this fact was ignored and the conventional procedure of testing *P. rettgeri* in the different antisera and adsorbing only with strains agglutinating to high titers was carried out. At the end of the present work the number of stock strains of *P. rettgeri* was reduced to 10 and the number of antisera to 3. Recently when heating several strains of *Shigella alcalescens* for rapid tube agglutination tests (Stuart, Rustigian, *et al.*, 1943) 4 cultures of *P. rettgeri* not agglutinating in the 3 antisera were also heated to 85 C for 1 hour. One strain agglutinated to titer in one antiserum and to partial titer in another, 2 strains agglutinated to partial titer in one or more antisera, and the other strain failed to agglutinate in any antiserum. The 3 antisera were adsorbed with the 4 cultures. The one culture agglutinating to titer completely removed the agglutinins from the serum, whereas the titer was not altered by any other strain upon adsorption. Tests in the 3 antisera of the 10 stock strains and 5 additional isolates after heating revealed, even with this small number of cultures and antisera, too great an antigenic heterogeneity for the practical typing of the species.

Of the type 29911 cultures, 7 were chosen to prepare antisera. One culture

previously reported (Stuart, Wheeler, *et al.*, 1943) agglutinated to titer in one antiserum and upon adsorption reduced the homologous titer from 20,480 to 320. Twenty-four cultures agglutinated to titers of 40 to 1,280 in one or another of the 7 antisera, whereas 41 failed to agglutinate in any antiserum. Varying numbers of *P. vulgaris*, *P. mirabilis*, *P. morganii*, and *P. rettgeri* cultures selected at random were tested for cross agglutination in the antisera of the other 3 species. Between *P. vulgaris* and *P. mirabilis*, and between *P. vulgaris* and *P. morganii*, common major and minor antigens were encountered in a large majority of the tests. On the other hand, major or minor antigens common to *P. mirabilis* and *P. morganii* were found in a minority of the tests. For example, of 132 *P. mirabilis* strains tested in 4 *P. vulgaris* antisera, every culture agglutinated to titers of 40 to 5,120 in one or another of the antisera, whereas of 100 of the same cultures, 74 failed to agglutinate in 3 *P. morganii* antisera, 12 agglutinated to a titer of 40, and 14 to a titer of 1,280 in one or another of the antisera. Twenty-two *P. rettgeri* cultures were tested in 4 *P. vulgaris*, 2 *P. mirabilis*, and 4 *P. morganii* antisera. Three strains agglutinated to 640 in one *P. vulgaris* serum, and to 1,280 in one *P. mirabilis* serum; 14 other strains to titers of 160 to 320 in three of the four *P. morganii* antisera. Of 36 type 29911 strains tested in 4 antisera each of *P. vulgaris*, *P. mirabilis*, and *P. morganii*, 19 agglutinated to titers of 80 to 1,280 in one or another of the 12 antisera.

Antigenic relationships between coliform, paracolon, and *Salmonella* cultures have been found by Schiff *et al.* (1941), Stuart, Wheeler, *et al.* (1943), Wheeler *et al.* (1943), and others. Bornstein *et al.* (1941) reported antigens common to *Salmonella* and *Shigella* cultures. *Proteus* cultures with antigens in common with *Shigella* have been discovered. A culture of *P. morganii* received in this laboratory agglutinated strongly in pooled *Salmonella paratyphenteriae* antiserum. Wheeler (personal communication) found that this culture possessed the specific antigen of *S. paratyphenteriae* type III. Other tests in this laboratory and in the Bureau of Laboratories of the Connecticut State Department of Health have revealed several minor antigens common to *Proteus* and *Shigella* cultures.

DISCUSSION

Despite the fact that as early as 1893 Smith recognized the "peculiar" nature of gas production by *Proteus* cultures most investigators have used as the principal test for inclusion in this genus fermentation reactions in glucose, lactose, and sucrose without reference to gas volume. This oversight has permitted strains of paracolon bacteria to be classified as members of the genus *Proteus*. Moreover, failure to recognize that sucrose fermentation by *P. mirabilis* may be slow and that exceptions may occur in the biochemical reactions of almost any species of bacteria has led to giving specific rank to variants of one species.

St. John-Brooks and Rhodes (1939), Reed and Tonner (1942), Rustigian and Stuart (1941, 1943a), Thornton (1944), and others would exclude *Proteus hydrophilus* and *Proteus ichthyosmii* from the genus *Proteus*. Rustigian and Stuart (1941), except for gelatin liquefaction, failed to find any characteristics in common

between *Proteus bombycis* and the genus *Proteus*. This was further confirmed in the present investigation on five strains each of *P. hydrophilus* and *P. ichthyosmius* and one of *P. bombycis*. All the foregoing investigators who have worked on *Proteus americanus* and *Proteus ammoniae* agree that these are variants of *P. mirabilis*. Of special interest is the work of De Assis (1939) who demonstrated increasing acid and gas production with serial transplants of *P. americanus* in sucrose medium. A strain of *Proteus pseudovaleriei* from the American Type Culture Collection possessed few of the characters noted for this organism in Bergey *et al.*, 5th ed., but did possess the characteristics of *P. mirabilis*. A culture of this organism received from De Assis agreed with the description in Bergey *et al.*, 5th ed., but failed to swarm under any conditions, produced relatively large gas volumes in all fermentable carbohydrates except lactose, and did not attack urea. This culture had all the characteristics of a paracolon organism.

The careful and detailed analyses of *Proteus melanovogenes* made by Miles and Halnan (1937) reveals that by present-day standards this organism should not be in the genus *Proteus*. While actively motile, it is monotrichate. Attempts to produce the peritrichous condition by twice-daily transplants for 3 weeks in 0.3 per cent semisolid agar failed. *P. melanovogenes* produced acid and gas "vigorously" in glucose, sucrose, and maltose in one day, and in mannitol in one or two days. Some strains attack lactose slowly and weakly, and some strains attack salicin in the same way. This organism did not swarm, gelatin liquefaction was slow, and urease was not produced. In fairness to these investigators it should be pointed out that they recognized the limitations of their cultures and the organism was "tentatively" classed in the genus *Proteus*.

Steinhaus (1941) classified 3 organisms isolated from insects in the genus *Proteus*. An attempt to obtain these cultures failed since by the time the paper was published the cultures had been discarded. The criteria for the genus *Proteus* for these organisms appears to have been no action on lactose, liquefaction of gelatin, and "slight production of hydrogen sulfide" for 2, and gelatin liquefaction for the other. The 3 cultures, although motile, did not swarm or spread according to the description of agar colonies. Two cultures were indole-negative and fermented both maltose and mannitol, which would exclude them from any recognized species in the genus. Moreover, the fermentation of both mannose and rhamnose would exclude them from the genus (Moltke, 1927). The remaining culture was indole-negative and also fermented maltose. Only 4 carbohydrate reactions were recorded for this culture. Urease production was not determined. It is highly probable that all 3 cultures in question were members of the intermediate group of the paracolon since some strains of the group are indole-negative, liquefy gelatin, and produce hydrogen sulphide (Stuart, Wheeler, *et al.*, 1943).

Lobik (1915) described *Proteus nadsonii* as a peritrichous rod, liquefying gelatin, producing hydrogen sulphide, not forming indole, coagulating but not peptonizing litmus milk, and having a variable gram stain. No carbohydrate reactions were given. As this organism was indole-negative, if in the genus

Proteus it would have to be *P. mirabilis* (or by the British system *P. vulgaris*), but both these species peptonize milk readily (Bergey *et al.*, 5th ed.) Moreover, the variable gram stain would seem to exclude this organism from the genus *Proteus*.

Three organisms labeled with the epithets *diffluens*, *metadiffluens*, *paradiffluens* were described by Castellani and Chalmers (1919) as species of the genus *Proteus*, primarily on the basis of gelatin liquefaction. *P. metadiffluens* is clearly not in the genus *Proteus* as it does not ferment glucose, sucrose, or maltose. *P. diffluens* differs from *P. mirabilis* in its failure to ferment sucrose and peptonize litmus milk. Litmus milk was not used, but it was found that one strain of *P. mirabilis* required 5 weeks to ferment sucrose, and 2 sucrose-negative strains were encountered. *P. paradiffluens* appears to be a normal *P. mirabilis* including fermentation of sucrose and peptonization of milk. The *Proteus pseudomorganii* of Castellani and Chalmers (1919) is a normal *P. morganii*, whereas their *P. morganii* is an O form of the same species.

The *Proteus sp. nov.* reported by Warren and Lamb (1924) was isolated from blood of a patient eleven days before death. The organism produced acid and gas in glucose, sucrose, maltose, and mannitol, and acid only in salicin. Although motile, colonies of the organism did not "spread," gelatin was liquefied, indole was positive, and milk was coagulated and peptonized. There was "slight browning in lead acetate." Since fermentation reactions were observed for only 7 days, this organism, fermenting both maltose and mannitol, could have been a slow lactose-fermenting paracolon.

Proteus photuris (Brown, 1927) was isolated from the luminous organs of the firefly (*Photuris pennsylvanicus*). The organism was a "minute," nonmotile, gram-negative, "bipolar," staining rod. Thirty-day-old colonies were "very irregular, amoeboid, slimy growths, white at the margin and yellowish to light brown in the middle." Gelatin was liquefied but there was no digestion of milk. Hydrogen sulphide was not produced. Acid and gas were formed in glucose, sucrose, and mannitol. Gas volumes in these carbohydrates could have been moderate to large since it is stated that gas was weakly produced in inulin. Aberrant coliforms, slow or nonfermenting lactose strains, with the colonial and biochemical characteristics of *P. photuris*, have been studied in this laboratory. These cultures were isolated from water, soil, and cereal and were classed as aberrant *Aerobacter* (Stuart, Mickle, and Borman, 1940).

Reference must be made also to the organisms described as *P. morganii* types by Waaler (1931) and others based on the work of Morgan and Ledingham (1909). With difficulty 4 cultures tentatively classified as *P. morganii* types XII and XIV were obtained. On the basis of relatively large gas volumes, inability to swarm though motile, and inability to produce urease, these organisms were considered to be paracolon cultures. Waaler's (1931) five *P. morganii* type XII strains, from their characteristics, appear to be members of the paracolon group. Since the term *P. morganii* is now used to designate a species, it is recommended that it no longer be employed for other organisms. Such practices connote a close relationship to *P. morganii* and add confusion to the concept of the genus *Proteus*.

The four species of the genus *Proteus* described in the present investigation constitute a fairly compact group of organisms. Urea hydrolysis and failure to ferment lactose appear to be the best criteria for the genus. More than 600 *Proteus* cultures have been tested in the buffered urea medium described by Rustigian and Stuart (1941). All cultures were positive except 5: one was a urea-negative variant of a urea-positive parent; one strain of *P. mirabilis* from a stock collection, although positive when received, was negative 4 years later; one *P. rettgeri* strain was completely negative; and 2 others were only moderately positive (pH 7.4 to 7.8). A subsequent publication will show that in the family *Enterobacteriaceae* utilization of urea is not restricted to *Proteus* organisms even though evidence of hydrolysis in the buffered medium cited is limited to the genus *Proteus*. Gas production is an excellent criterion for 3 species of the genus. Relatively recent isolates of *P. vulgaris*, *P. mirabilis*, and *P. morganii* almost without exception produce small gas volumes in all fermentable carbohydrates even after prolonged incubation. Many old stock cultures of these species were found to be anaerogenic. Most strains of *P. rettgeri* were anaerogenic; an occasional strain produced a small amount of gas. Swarming under suitable conditions is an excellent criterion for H forms of 3 species, but is without value for the O forms of these 3 species and for some strains of *P. rettgeri*. Gelatin liquefaction can no longer be accepted as a characteristic of the genus though it may serve to differentiate species within the genus. This also holds true for production of indole and hydrogen sulphide and for growth on citrate agar.

From an academic viewpoint there is adequate evidence for differentiating *P. vulgaris* and *P. mirabilis*. The former characteristically acts on glucose, sucrose, and maltose rapidly and produces indole; whereas *P. mirabilis* characteristically acts on glucose rapidly, sucrose slowly, and not at all on maltose, is indole-negative, and produces acetylmethylcarbinol in many instances. Furthermore, *P. vulgaris* is Eijkman negative, whereas *P. mirabilis* is positive (Stuart, Zimmerman, *et al.*, 1942; Stuart, Van Stratum, and Rustigian, 1944). On the other hand, in the British system these 2 species are united under *P. vulgaris*. The swarming characteristics and the ability to liquefy gelatin, to produce hydrogen sulphide, to grow on citrate agar, and to produce urease rapidly show the close relationship of these two "types." The fact that only 10 to 20 per cent of the isolates of the two "types" are *P. vulgaris* may indicate that the minority group are variants of the majority group. Moreover, an occasional strain is isolated possessing characteristics intermediate between the two "types." The present authors are not in perfect agreement on this point. One of us (Rustigian) is inclined to give specific rank to both *P. vulgaris* and *P. mirabilis*. The other (Stuart) is inclined to group the two "types" into one species, *P. vulgaris*, for the purpose of taxonomic simplification (Borman *et al.*, 1944).

P. morganii seems clearly established as a member of the genus *Proteus*. It utilizes urea more slowly than the other species in the genus. *P. rettgeri* also seems clearly established in the genus despite its borderline characteristics. As previously pointed out, many strains spread on moist 1 per cent agar plates but only an occasional strain covers the entire surface of the agar. Two strains

attacked urea only moderately and one not at all. All strains reduced trimethylamine oxide weakly. Two strains were negative on one test but were weakly positive on subsequent tests. Other *Proteus* strains reduce the compound strongly and rapidly (Wood and Baird, 1943; Wood, personal communication). Recently, Wheeler tested 250 *Proteus* cultures for trimethylamine oxide reduction and found that 6 cultures were unable to reduce this compound (personal communication).

Biochemically and antigenically, organisms of the genus *Proteus* present a much more compact picture than do those of the coliform group. Common antigens are relatively infrequent among coliform bacteria as a whole (Stuart, Baker *et al.*, 1940). In *Aerobacter* antisera 16.9 per cent of *Aerobacter* strains agglutinated to an average titer of 1,148; in intermediate antiserum 7.4 per cent of the intermediate strains reacted to an average titer of 1,572; and in *Escherichia* antisera 38.4 per cent of the *Escherichia* strains agglutinated to an average titer of 1,261. With *Proteus* cultures, each in its own antiserum, 66.5 per cent of *P. vulgaris*, 92.6 per cent of *P. mirabilis*, 78 per cent of *P. morganii*, and 59 per cent of *P. rettgeri* strains agglutinated to average titers of 13,726, 7,804, 12,779, and 4,725, respectively. (A higher percentage of cultures agglutinating and a higher average titer would probably have resulted from the use of heated antigens with *P. rettgeri*.) The marked antigenic continuity of antigens in the species of *Proteus* is not indicative of great numbers of antigenically identical strains within the different species. Except for X strains, antigenically identical strains are relatively infrequent within each species. Common antigens among the different species are frequently encountered. Antigenic typing as with the *Salmonella* group would be a tremendous task incommensurable with the practical importance of the genus *Proteus*.

CONCLUSIONS

The genus *Proteus* is a relatively homogeneous group biochemically, and on this basis can be divided into four species—*P. vulgaris*, *P. mirabilis*, *P. morganii*, and *P. rettgeri*.

The first two species could be combined into one, *P. vulgaris*, as in the British system for taxonomic simplification.

Urease production for all species and small gas volumes, except with *P. rettgeri* which usually produces acid only, are two cardinal physiological criteria of the genus. Gelatin liquefaction and fermentation of glucose and sucrose but not lactose do not satisfactorily delineate the genus *Proteus*.

The genus *Proteus* is serologically heterogeneous, although common antigens are frequently encountered among the different species.

It is recommended that *Proteus ammoniae* and *Proteus americanus* be considered as variants of *P. mirabilis*, and that *Proteus hydrophilus*, *Proteus ichthyosmius*, *Proteus pseudovaleriei*, and *Proteus bombycis* be excluded from the genus *Proteus*.

It is recommended that the epithet "morganii" be employed solely for *Proteus morganii* (Morgan's bacillus number 1) as described by Morgan and Ledingham.

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