A SYSTEMATIC STUDY OF THE PROTEUS GROUP OF BACTERIA

JOHN J. WENNER AND LEO F. RETTGER

From the Sheffield Laboratory of Bacteriology and Hygiene, Yale University

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The term *Proteus* signifies changeability of form, as personified in the Homeric poems in Proteus, "the old man of the sea," who tends the sealflocks of Poseidon and has the gift of endless transformation. The first use of this term in bacteriological nomenclature was made by Hauser (1885) who described under this term three types of organisms which he isolated from putrefied meat. Variations in form and size and in cultural characters were the basis of his classification. Other investigators have since applied the name *Proteus* to specific organisms which they isolated from various sources. Of these organisms some show close similarities and in many instances are identical with the types described by Hauser. Some do not appear, however, to have sufficient common properties to justify inclusion in the *Proteus* group, and attempts to place them here tend to further obscure the poorly defined limits of this group.

The three species which Hauser described under the genus *Proteus* were as follows: *Proteus vulgaris*, which liquefied gelatin and formed zooglea in this medium, and which was very active in its various physiological properties; *Proteus mirabilis* which likewise liquefied gelatin and formed zooglea, but which was less active; and *Proteus zenkeri*, which was unable to liquefy gelatin and which was relatively inactive. The main basis for distinguishing the three species appears to be their action on gelatin.

Babes (1889) isolated an organism from a case of lung gangrene in man which he called *Proteus lethalis*, and another from the

organs of a child which died with symptoms of septicemia, *Proteud septicus*. The same bacterium was later described by Kruse (1896). These organisms appear to be very closely related to one or two of Hauser's types, if indeed they are not identical with them.

Jaeger (1892) isolated a fluorescent organism which, due to some points of similarity with the *Proteus* group, he called *Proteus fluoresecens*. It was described as the causative factor in several cases of Weil's disease. It has since been isolated from similar cases by Bar and Renon (1895), by Conradi and Vogt (1901), and by Bruning (1904). While this organism in a general way bears some resemblance to the *Proteus* group, it is so atypical that its inclusion in this division may be seriously questioned. A more natural grouping would place it in the genus *Pseudomonas*.

Fuller and Johnson (1899) describe two spore-forming organisms as *Proteus*. The property of forming proteus-like colonies on gelatin seems to be their sole basis of classification, hence these organisms may be eliminated from further consideration, especially because no other spore-producing bacteria have been referred to the *Proteus* group.

Prior to the work of Hauser an organism was described by Kurth (1883) which on account of its marked resemblance to Hauser's *Proteus zenkeri* deserves mention here. Kurth's *Bacterium zopfii* was isolated from the intestine of fowls, and has since been observed by others on numerous occasions.

The purpose of the present investigation was to determine the exact relation of the *Proteus* group to other groups of organisms, and to point out more clearly than has been done heretofore the specific properties which serve to distinguish the members of what has so generally been termed the "*Proteus* group."

For this purpose 84 strains were obtained from different sources. Of these 58 were procured from other laboratories, and were labelled as follows:

B. proteus-vulgaris	27
B. proteus-mirabilis	8
B. proteus-zenkeri	4
B. proteus	13
B. zopfii	5
B. proteus-viridis	1

Twenty-six different strains were isolated in this laboratory, of which 25 were of the *Proteus vulgaris* or *Proteus mirabilis*, and one of the *B. zopfii* or *Proteus zenkeri* type.

A morphological and cultural study of the organisms of this collection showed that it could be divided into the three following divisions:

Group I comprising *Proteus vulgaris, Proteus mirabilis*, and *Bacillus proteus.*

Group II comprising *Proteus zenkeri* and *Bacterium zopfii.*

Group III comprising *Proteus fluorescens.*

The members of group I are Gram negative and very actively motile, and on agar show a peculiar spreading growth. They usually exert proteolytic action on gelatin and in milk, and to some extent attack carbohydrates, and protein material in general. Furthermore, they grow luxuriantly on all of the ordinary media, and are not limited to any specific temperature range.

The two types which comprise group II are distinctly Gram-positive. They possess no proteolytic action and do not attack carbohydrates; neither do they produce a luxuriant spreading growth on moist agar, as do the members of group I. They develop very poorly in liquid media.

The one available strain of group III differed markedly from the organisms of the other two groups. Fluorescent pigment production, together with its other properties so characteristic of the fluorescent group, should naturally place this strain within the genus *Pseudomonas.*

While Hauser at first described the *Proteus* group as being composed of three distinct species, that is *P. vulgaris, P. mirabilis*, and *P. zenkeri*, he later thought that the last two species might be only varieties of *Proteus vulgaris.* His latter conclusion seems to have been accepted by most investigators, though little evi-

dence can be found to substantiate it. Kendall (1916) states "that it is now recognized that cultures of *B. proteus* may gradually lose their gelatin-liquefying power after prolonged cultivation, so that a cultural transition from *B. proteus* to *B. zenkeri* may be observed in the laboratory." While we have observed loss of ability to liquefy gelatin in certain strains, we have never noted other changes in *Proteus vulgaris* which would tend to give it the characters of *Proteus zenkeri.*

In the present study the strains of *Proteus* which were labelled *Proteus vulgaris* and *Proteus mirabilis* when received were found to be practically identical in all of their characters. Both liquefied gelatin with the same rapidity. Although in each species variability in proteolytic action was noted in a few instances, no other changes accompanied the partial or complete loss of gelalatin-liquefying power, and the strains did not in the least assume the characters of Hauser's *Proteus zenkeri.* Thus it appears that the classification of Hauser holds only in so far as the separation of his species *Proteus zenkeri* from the other two is concerned.

Kruse (1896) and Chester (1909) noted a similarity between Hauser's *Proteus zenkeri* and *Bacillus zopfii* of Kurth (1883). We have found the two to be identical, and hence would classify them as one and the same genus under the generic name of Zopfius.

The types which were labelled *Proteus vulgaris, Proteus mirabilis* and *Bacillus proteus* have been reduced by us to two species, namely *Proteus vulgaris* and *Proteus mirabilis, as B. proteus* is but another name applied to either or both of the others. The *Proteus* group as a whole is sometimes referred to as *Bacillus proteus;* but the use of this name should be discontinued.

In the present investigation the original *Proteus* group of Hauser has been split, therefore, into two distinct genera, namely *Proteus* and *Zopfius.* In the former are included *P. vulgaris* and *P. mirabilis* of Hauser, together with the strains in our collection labelled *Bacillus proteus,* and under the genus *Zopfius, Bacterium zopfii* of Kurth and *Proteus zenkeri* of Hauser. The basis for this classification will be brought out further in the data and discussions which follow.

GENUS PROTEUS

This genus may be defined as comprising organisms which in form are small coli-like rods with rounded ends and occurring singly, in pairs or in chains; they are Gram negative, form neither spores not capsules, and are actively motile by means of peritrichiate flagella. Gelatin is usually liquefied rapidly, though this property may be entirely lost. When inoculated into the condensation fluid of slant agar tubes a rapidly spreading growth is produced over the entire surface of the agar. The strains ferment, with acid and gas production, glucose, levulose, galactose, sucrose and glycerol and occasionally maltose. Alkalinity is usually produced in litmus milk, followed by decoloration of the litmus and digestion of the casein. At times there is slight coagulation or precipitation of casein with subsequent re-solution or digestion.

Organisms of this genus are widely distributed in nature, and have been isolated from numerous sources. Their presence in soil appears to depend largely upon recent contamination with animal excreta or putrefactive organic matter of animal origin. Cantu (1911) was able to isolate organisms of this genus from 23 out of 52 samples of garden soil.

Members of this genus are often present in stagnant pools, sluggish streams and other contaminated waters. We have obtained them from stagnant pools, aquaria and street washings. Ward (1899) isolated several strains from the Thames River, and Jordan (1903) from the waters of the upper Mississippi. Horowitz (1916) made several isolations from snow water.

Proteus organisms may be said to be present in practically all sewage, for here there is a constant source of contamination and a favorable medium for development.

The presence of this genus in the intestinal tract of man is by many authorities regarded as an indication of intestinal trouble or some other pathological condition. Ford (1901) claims to have isolated it from various parts of the intestine, but as some of his organisms were fermenters of lactose, there is some doubt as to whether all were *Proteus*. Stewart (1917) be-

lieves that *Proteus* members found in war wounds are of non-fecal origin. In the examination of several thousand samples of feces from dysentery convalescents he found this genus to be a very uncommon inhabitant of the colon of man. It may be obtained from the intestinal tract of lower animals, as for example guinea pigs. Jensen (1903) observed this genus to be present in large numbers in calves affected with a form of dysentery. Its presence in similar conditions in man and animals may in part account for its wide distribution in nature.

The most favorable habitat of the genus *Proteus* is decomposing organic matter of animal origin. In such material it is almost invariably present. Cantu (1911) was able at will to isolate it from putrefied meat, as have many other investigators. Wyss (1898) obtained a strain of *Proteus* from dead fish, and Shrank (1888) from spoiled eggs. Isolations have been made also from human cadavers, where this organism was found in large numbers by Hauser (1885), Hofmeister (1893), Haegler (1892) and Kuhn (1891). We were able to obtain it from meat which had been allowed to undergo decomposition, and from the partly decomposed bodies of dead rabbits and guinea-pigs.

Method of isolation

Until quite recently the usual gelatin and agar plate methods of solation have been employed for this group. As these were very faulty for this type of bacteria, many efforts resulted in failure. The newer methods have rendered valuable service, however. In the present work the procedure of Cantu (1911) was at first adopted. Gelatin tubes are inoculated directly with the material in question. After incubation at 20°C. for several days transfers are made from tubes in which liquefaction has taken place to the condensation fluid of new slant agar tubes. If *Proteus* organisms are present a rapidly spreading growth occurs in twelve to twenty-four hours at 30° to 37°C. This growth is quite characteristic and usually spreads over the entire surface. From the uppermost portion of the surface growth inoculations are made in the condensation fluid of a second agar tube, and the process repeated until a pure culture is obtained.

It soon became apparent in the present investigation that the materials for study could be inoculated directly into the condensation water of the sloped agar tube, and the period in which isolation is effected very much shortened. This modification in no way detracts from the merits of the Cantu procedure. As a rule very little effort is required to effect complete isolation of the *Proteus* genus, owing to its peculiar property of overspreading agar rapidly and leaving associated organisms behind in the condensation fluid. Fresh agar is necessary, however, and the results are greatly facilitated by washing the agar surface with the condensation water just before inoculation.

General characters of the Genus Proteus

The salient features of this genus have already been defined. The following is an elaboration of the different characters, in so far as Journal space will permit.[1]

The individual cells are usually short Coli-like rods with rounded ends, varying in dimensions from 0.4 to 0.6μ by 1.2 to 2.5μ, though occasionally much longer cells are seen. The rods may be grouped singly, in pairs or in short chains. They are actively motile, possessing peritrichous flagella. The unstained cells appear homogeneous in structure. Neither spores nor capsules have been observed. All strains are at all times Gram-negative. Young cultures are readily stained with methylene blue, fuchsin and other common basic dyes.

Members of the *Proteus* genus grow luxuriantly on the usual solid and liquid laboratory media. They are capable of growing within a wide range of temperature, and within reasonable limits development is not materially affected by change in hydrogen ion concentration.

Various ranges of temperature have been reported as most favorable. Hauser (1885) gives 20° to 34°C. as the optimum. Kendall (1916) places it at about 25°. Berthelot (1914), Cantu (1911), and Glenn (1911) grew the organisms successfully at 37°. Levy

[1] For more complete descriptions and discussions the reader is referred to the doctorate thesis (J. J. Wenner) in the Yale University Library.

(1894) showed that the group develops slowly at a temperature as low as 0° and as high as 43° to 45°C. We have invariably obtained maximum growth at 34° to 37°. Good growth was obtained also at 20°, though longer incubation was required, as shown for example in glucose broth culture in which maximum acidity was attained in twenty-four hours at 37°, as against forty-eight hours at 20°, and maximum gas production in twenty-four hours at 37°, as against one hundred and twenty hours at 20°C.

Growth on plain agar

The most characteristic growth of *Proteus* is obtained on slant agar. This was pointed out by Cantu (1911) when he showed that inoculation in the condensation fluid of fresh sloped agar resulted in a uniform growth over the entire surface. This growth may be homogeneous, or of a more or less peculiarly modeled character. It is of a butyrous consistency. If the surface of the inoculated agar is dry a streak inoculation results in a pronounced growth which spreads very irregularly, with a more or less lacerated margin. The extent of the spreading depends on the amount of moisture on the agar.

Colony growth on plate agar may be at times characteristic, that is, of ameboid appearance, or in the form of large colonies which are more or less rosette-like, with very irregular borders. Again, the colonies may be small and with entire margin, resembling those of *B. coli.*

Action on gelatin

Much interest has centered around the property of gelatin liquefaction of this genus. Hauser in his original work laid special emphasis on it and used it as the chief basis for his distinction of types. Since then marked variations in individual strains have been observed by different investigators. Smith (1894) was able by selection to transform a liquefying *Proteus vulgaris* into a non-liquefying strain. Herter and Broeck (1911) showed that a liquefying strain of *Proteus vulgaris* which had

lost its liquefying properties, but remained typical in other respects, could have the lost function restored by passage through a mouse.

Of the 73 strains studied in this investigation, 3 lost the property of liquefying gelatin while in other ways they remained typical. Two of these organisms were old laboratory strains labeled *Proteus vulgaris;* the third was isolated from putrefying meat. We were unable to restore the liquefying function by a single passage of one of these strains through a white rat.

On gelatin plates of *Proteus* small colonies are noticeable in eighteen to twenty-four hours. They show an entire margin at first, but as they increase in size irregular spreading may occur. Liquefaction soon takes place and the colonies assume a dewdrop appearance. Radiating filaments extend from the liquefied zone into the surrounding gelatin. The colonies increase in size until the entire plate is liquefied. Hauser employed 5 per cent gelatin, and describes the occurrence of wandering ameboid colonies, that is, irregular masses of cells which constantly underwent changes in form and position, and sometimes separated from the mother colony. In order to obtain colonies that are at all characteristic gelatin of rather soft consistency is required. On the usual 10 per cent gelatin the colonies are often entire and without distinguishing marks.

In gelatin stab cultures liquefaction begins at the surface, soon becomes stratiform and eventually involves the entire tube. The rate depends on the temperature and an abundance of free oxygen. Liquefaction may be completely inhibited by a layer of oil over the surface of the gelatin. The oxygen is essential in the production of the proteolytic enzyme.

Growth in bouillion

Marked turbidity is rapidly produced, reaching its maximum in from three to five days at 30 to 37°C. Young cultures usually show no surface film, while older tubes gradually develop a thin brittle pellicle which is easily broken up. As broth cultures present few if any features which are characteristic and of special

interest no further comments are necessary. Nitrite is formed in nitrate broth.

Action of the genus Proteus in milk

As a rule vigorous development occurs in milk, and a marked change may be brought about in the appearance of this medium in twenty-four to thirty-six hours at 37°C., the litmus being reduced and coagulation or digestion of the casein taking place. The rate of transformation varies with different strains, some of them completely digesting the casein in three to five days. On the other hand, other strains appear to have lost this proteolytic power completely. The usual change observed in this study of 73 strains was an initial alkalinity which gradually became more intense and was followed by decolorization of the litmus and digestion of the casein. Some strains (3) showed slight acid production at first. Casein was digested by 69 strains.

The ability of organisms to digest casein was demonstrated definitely by growing them in a medium containing, besides 0.5 per cent meat extract and 0.5 per cent sodium chloride, 0.2 per cent of purified casein, and observing the loss of the protein by means of the biuret method of Vernon (1903), or by precipitation with acetic acid.

Action on carbohydrates, glycerol, etc.

Fermentation is limited to glucose, levulose, galactose, sucrose maltose and glycerol. The glucose, levulose, galactose and glycerol were attacked more or less uniformly by all strains, sucrose readily by some and slowly by others, and maltose only by some of the strains. Fermentation in all cases comprises both acid and gas production. The medium employed in the fermentation experiments was plain sugar-free broth to which 1 per cent of the carbohydrate in question was added. Other agents used were lactose, inulin, dulcitol, mannitol, sorbitol, salicin, raffinose, arabinose, adonitol, dextrin and starch. The results with these were negative.

Glucose

This is one of the most favorable sources of energy for the organisms of the *Proteus* genus. Its presence in a medium considerably hastens growth. From 25 to 30 per cent of gas, and from 2.5 to 3 per cent of acid in terms of N/20, with phenolphthalein as an indicator, are produced. These results agree with those of other investigators.

Sucrose

Smith (1893) was the first to show that the action of this group on sucrose was practically the same as on glucose. Similar results have been obtained since by other investigators, though Glenn (1911) found several indifferent strains among his stock cultures, and Horowitz (1916) reports a positive reaction in only 7 out of a total of 24 strains.

In the present investigation a variation in the action of *Proteus* on this sugar was noted, some strains producing the maximum amounts of acid and gas in twenty-four to seventy-two hours, while others required twelve to fifteen days. The delayed action of the latter (8 or 9 days) was at first overlooked, but it was observed that when the period of delayed action was passed the fermentation was as pronounced as with the strains which attacked the sucrose immediately. Of the 73 strains studied, 25 showed an immediate, and 48 a delayed action. In correlating these results with the action on other carbohydrates, it soon became apparent that the strains which fermented sucrose readily also fermented maltose, while those which showed delayed action on sucrose did not attack the maltose.

Maltose

Maltose appears to be the only carbohydrate that is of any value as a means of subdividing the *Proteus* group. Berthelot (1914) noted a variation in the action of different strains on this sugar. Horowitz (1916) found that 23 out of 24 strains fermented it with the production of acid and gas; and Stewart (1917) observed 2 out of 29 having this property. Of the 73 strains in the present collection, 25 showed distinct acid and gas production. No

delayed action on the sugar could be detected, as in the case of sucrose.

Galactose, levulose and glycerol

While these agents are fermented by this group the action is not so marked and does not occur as readily as with the sugars just mentioned. The amount of gas produced may vary from a mere bubble to 20 per cent, and the acid from 1 to 2 cc.

Lactose

Conflicting results have been reported. While most investigators have claimed that lactose is not attacked by the *Proteus* group, others have observed fermentation with acid and gas production. In the light of our own experiments these conflicting results may be explained by the presence of an available carbohydrate as an impurity in the lactose. When absolutely pure lactose was employed no fermentation could be detected under either aerobic or anaerobic conditions.

Growth on potato

On cooked potato prepared in the usual way very luxuriant growth is produced. It appears within twenty-four hours along the line of inoculation and gradually spreads over the surface irregularly. It is of a butyrous consistency and of a dirty brown color which quickly diffuses through the potato. A characteristic fish brine odor is produced in this medium.

Browning of lead acetate medium

All of the 73 strains of *Proteus* used in this investigation caused a distinct browning of a medium consisting of 0.5 per cent nitrate agar, 0.05 to 0.1 per cent lead acetate, and 0.2 per cent glucose.

Hemolytic action

This genus is unable to hemolyze red blood cells. Different strains were tested both in suspensions of washed erythrocytes and on plates of sterile blood agar.

Growth in synthetic media

Development in Uschinsky and similar synthetic media is limited. It becomes more marked, however, when glucose is substituted for glycerol in the medium. In the phthalate medium of Clark and Lubs (1917) growth is likewise limited.

Chromogenesis

With the exception of a few investigators (Ward, 1899, and Jordan, 1903) the *Proteus* group is considered as non-pigment producing. In the present work no color production was noted in any of the media except the brownish growth on potato and the gradual browning of the potato itself.

Changes in hydrogen ion concentration

In plain bouillon prepared from Liebig's beef extract and Witte's peptone no change in titratable acidity was noted, while hydrogen ion determination by the newer colorimetric method showed slight alkali production. In plain bouillon containing an available carbohydrate sufficient acid is produced to bring the H ion concentration to about 5 on the colorimetric scale. Similar results were obtained in the special peptone medium of Clark and Lubs (1917). Little acid production occurs, however, in their phthalate medium owing to the limited growth of the organisms.

Indol production

Indol production by this genus has been pointed out by many investigators. Variations in this property have been noticed also. Steensma (1906) studied several strains which failed to produce indol. Van Loghem and Van Loghem-Pouw (1912) made two subdivisions out of the strains under observation, namely *B. proteus-anindologènes* and *B. proteus-indologenes*. Berthelot (1914) found that 24 out of a total of 61 strains formed indol; Horowitz (1916), 7 out of 24; and Stewart (1917) 1 out of his collection of 29.

In the present work results were obtained which varied with the methods employed. Dunham's solution, sugar-free broth, and a 1 per cent solution of predigested casein were used. Both the Salkowski and the Ehrlich aldehyde method were employed. Of the 73 strains all gave a positive reaction with the sulphuric acid and nitrite test of Salkowski, while 46 gave a reddish color on the addition of the acid alone. With the Ehrlich method 33 of the 73 strains gave a strongly positive, 36 a slightly positive and 4 a negative reaction. These variations were obtained in each of the 3 media.

Hydrogen sulphide and mercaptan

All of the strains formed hydrogen sulphide in appreciable amounts. On the other hand, little if any mercaptan could be detected. Mercaptan production has been the subject of investigation on previous occasions. It has been assumed by many that this is a common product of *Proteus*, because this genus is so constantly present in organic matter undergoing putrefactive decomposition, though it is not itself a strictly putrefactive organism. Rettger (1906) found no mercaptan in anaerobic cultures of *Proteus vulgaris* in egg-meat mixture. Herter and Broeck (1911) also were unable to detect it in plain bouillon cultures, even when cystin was added. Ward (1916) claims, however, that he obtained marked mercaptan production with 4 different strains which he grew in plain bouillon.

Nine strains were tested for the property of mercaptan production by the method formerly employed by Rettger, and involving the use of isatin-sulphuric acid and of mercuric cyanide. In some instances a slight change in the color of the test solutions could be detected, but as control flasks gave a similar change in color, little, if indeed any, mercaptan was present in the culture flasks. Contamination of such flasks with a putrefactive anaerobe, however, soon resulted in abundant mercaptan production.

Putrefaction

The experiments of Hauser (1885), Emmerling (1896) and others, demonstrating putrefactive changes in what appeared

to be pure cultures of *Proteus* organisms, as well as the frequent assertions that members of the *Proteus* group are always present in organic matter that is undergoing putrefaction, has led to the assumption that this group has distinct putrefactive properties. Rettger and Newell (1912) have shown more recently that no decomposition of protein material takes place under anaerobic conditions when pure cultures of *Proteus* are used. Similar experiments were conducted in the present investigation, and the results of Rettger and Newell corroborated. No changes in the character of protein material could be brought about by pure cultures of *Proteus vulgaris* in the absence of atmospheric oxygen, whether in milk, egg-meat mixture, or other protein-containing medium. There was no reduction in the volume of the solid matter in the egg-meat medium, nor could any of the foul smelling products of putrefaction be detected. Furthermore, there was very little, if indeed any mercaptan present in the medium. Under aerobic conditions, however, the ordinary non-putrefactive products of protein decomposition are produced.

Agglutination

Several attempts have been made in the past to employ agglutination as a basis for subdividing the *Proteus* group. Cantu (1911) showed that the blood serum of animals which had been injected with heated suspensions of these organisms had agglutinating properties which, barring some exceptions, were specific for the strains injected. He concluded that this method can not be employed for subdividing different strains. Van Loghem and Van Loghem-Pouw (1912) claimed that indol-producing strains could be distinguished from those which do not form indol, by their agglutination properties. Horowitz (1916) obtained cross agglutinations among homologous strains, and thereby was able to split the *Proteus* group into 5 subdivisions, the members of each having specific properties, as regarded indol production and carbohydrate fermentation.

In the present work several rabbits were immunized against specific strains of *Proteus vulgaris* and *Proteus mirabilis*. Killed

suspensions were injected at first, followed by at least one or two suspensions of living organisms grown on slant agar and washed off with saline solution. After each injection the animals showed some loss in weight which was very soon regained. At the site of inoculation a large abscess was formed which disappeared only after several months. The production of agglutinins could be demonstrated very soon after the first injection. After the last injection agglutination in as high as 1:100,000 dilution took place.

The different strains of *Proteus* were tested by the macroscopic method in dilutions of 1:50, 1:100, 1:500, 1:1000, and 1:5000. Seven different sera were prepared with as many strains of *Proteus*. Table 1 shows the number of strains agglutinated by each serum. With one exception, all of the sera agglutinated other strains besides those employed in their preparation. Some strains were agglutinated by more than one serum. Nineteen of the strains used in the agglutination tests failed to be agglutinated by any of the sera. It would appear, on the whole, that the *Proteus* group is more or less heterogeneous, like the *Streptococcus* and *B. dysenteriae* group. While the agglutination method may be of some value, in identifying members of the *Proteus* group, negative results do not necessarily exclude an organism from this group.

Pathogenicity

The occurrence of the genus *Proteus*, either in pure culture or in association with other organisms, in pathological conditions, has been reported by various investigators. Foa and Bonome (1889) isolated it from a case of volvulus, Schnitzler (1890) and Krogius (1890) from cases of cystitis, Flexner (1893) from a patient having peritonitis, and Reed (1894) in croupous pneumonia, associated with a pneumococcus. Booker (1897) and Metchnikoff (1909) made *Proteus* isolations from cases of diarrhea in children, Vincent (1909) from typhoid fever patients, and Horowitz (1916) from persons suffering with gastro-enteritis. Larson and Bell (1915) recovered *Proteus* organisms from a

laparotomy wound, infected eye and finger, from the heart's blood of a fatal case of peritonitis, and from one of gangrene of the lung. Ward (1916) obtained it from supposed diphtheria subjects and from typical cases of atrophic rhinitis.

Dudgeon, Gardner and Bantree (1915) found typical *Proteus* in 5 per cent and atypical *Proteus* in 2 per cent of a total of 100 cases of war wounds. Goadby (1916) encountered *Proteus* in 47 per cent of the 200 wounds studied bacteriologically. Distaso

TABLE 1

Number of strains agglutinated by each serum or combination of sera

NUMBER OF STRAINS	SERA*						
	A	B	C	D	E	F	G
1	+	0	0	0	0	0	0
1	+	+	0	0	0	0	0
1	0	0	0	0	0	+	0
8	+	+	0	0	+	0	0
3	0	+	0	+	0	0	0
1	0	+	+	+	0	+	0
1	0	0	+	+	0	0	0
23	0	0	+	+	0	+	0
11	0	0	0	+	0	0	0
3	0	0	0	+	+	0	0
1	0	0	0	0	0	0	+
19	0	0	0	0	0	0	0
Total, 73	10	13	25	42	11	25	1

* Sera A, B, C, D, E and F were prepared with stock strains of which the first five were labeled *Proteus vulgaris* Hauser and the last *Proteus mirabilis* Hauser. Serum G was prepared with a strain isolated by us from putrefied meat.

(1916) found coliform organisms including *Proteus* predominating in the first stages of wound infection. He suggests the use of *Proteus* vaccine along with others in the treatment of war wounds. Stewart (1917) isolated 29 strains of the *Proteus* genus from infected war wounds, or a case rate of 24 per cent.

While this genus is ordinarily regarded as non-pathogenic, there is ample evidence to show that it may assume a pathogenic rôle, and thus occupy a position analogous to the pyogenic micrococci. The pathogenicity varies in experimental animals,

some strains causing death in 16 to 24 hours, while others cause no apparent ill effects. For example, Kühnau (1897) found that strains from several cases of diphtheria, and Larson and Bell (1915) that some strains of *Proteus* obtained from human lesions, were decidedly pathogenic for rabbits, guinea-pigs and rats. In general the virulence of a strain is shown by the production of local pathological conditions or by symptoms of intoxication.

In the present investigation both virulent and non-virulent strains were met with. One of the most pathogenic was an old stock culture of unknown origin, this indicating that virulence may be mantained indefinitely.

Hauser, in his original work on the *Proteus* group, found that boullion and gelatin cultures were toxic and produced fatal results when injected into animals. Other investigators have obtained similar results. The nature of the toxicity is not known, although the effects are apparently those of real toxemia.

The toxicity of several strains of the *Proteus* genus was demonstrated by the writers by injecting 2 cc. of saline suspensions from 24-hour agar cultures. Subcutaneous injections in rabbits produced abscesses and inflammatory conditions which lasted several months, usually accompanied by loss of weight, weakness and lessened appetite. In white rats the results varied with the strains, some causing symptoms of toxemia and killing the animals in eighteen to twenty-four hours, when injected by the subcutaneous route. Others caused no apparent ill effects even when the injections were intraperitoneal. One strain caused the formation of an abscess in one rabbit, and definite symptoms of toxemia and death in another. In the fatal cases the organisms could be isolated from the blood and internal organs. Killed suspensions when injected into rabbits caused definite lesions at the site of inoculation.

Classification of species

Since Hauser's classification several investigators have attempted to group the various strains of the genus *Proteus* on properties other than gelatin liquefaction. Ford (1901) defines

the *Proteus* group as consisting of alkali-producing non-chromogenic, non-sporing bacilli capable of liquefying gelatin, casein and blood serum. He made a further division, on the basis of motility and carbohydrate fermentation, into six varieties, two of which fermented lactose.

In his study of bacteria found in river water, Jordan (1903) divided the *Proteus* group into two subdivisions, namely the *Proteus vulgaris* type and *Proteus* varieties. The first of these he described as always fermenting glucose and sucrose, with gas production, but never lactose; liquefying gelatin, casein and blood serum, and curdling milk, with acid production. The second subdivision differed from the first mainly in its proteolytic action. Cantu (1911) in a study of 184 strains isolated from various sources was unable to subdivide them. Van Loghem and Van Loghem-Pouw (1912) were able to divide a series of strains obtained mostly from intestinal contents into two groups on the basis of their indol-producing function. The strains belonging to one or the other group were similar in their agglutinating properties. Horowitz (1916) divided 24 strains into 5 subgroups, on the basis of agglutination. Stewart (1917) found 27 strains isolated from war wounds to differ in their action on maltose and litmus, and in their motility and idol production.

The present investigation has shown that attempts of others to divide the *Proteus* group into two or more subdivisions are unsound. The classification of Hauser on the basis of gelatin liquefaction is of little value, since this property is too irregular and inconsistent. In their agglutination power the members of the *Proteus* genus are heterogeneous in character, so that no distinct separation into species is possible on this basis. Indol production is also very unsatisfactory as a distinguishing character. The only property which appears to us to be of value in making subdivisions of the genus *Proteus* is that of carbohydrate fermentation. Several investigators have noted a difference in the action of individual strains on maltose. Of the 73 strains employed by us 25 fermented this sugar, while the remaining 48 failed to do so. A definite correlation existed between the property of attacking maltose and

the rapidity with which sucrose was fermented with gas production. All of the strains which fermented maltose, with both acid and gas production, also fermented sucrose readily, while all of those which failed to attack maltose showed a delayed action on sucrose and brought about visible acid and gas production only after the expiration of eight to nine days.

While no other property could be correlated with this action on the sugar, it lends itself as a definite basis for dividing the *Proteus* genus into two species, the one fermenting maltose with acid and gas production, and the other being unable to attack this disaccharide. For the former the name *Proteus vulgaris* may be retained, while for the other *Proteus mirabilis* is here suggested. By retaining these names the nomenclature would be simplified. The differentiating characters of Hauser must be set aside, however, in order to avoid confusion.

GENUS ZOPFIUS

Under this genus the types formerly known as *B. zopfii* and *Proteus zenkeri* will be described. Very few strains of these organisms are kept in stock, as only 4 strains of *Proteus zenkeri* and 5 strains of *B. zopfii* could be obtained by a canvass of 40 bacteriological laboratories. To these 9 strains one was added which we were able to isolate from putrefied meat. All of these strains were practically identical.

The individual cells are rod-shaped, usually about 0.8μ by 3.5μ in size, have somewhat rounded ends, and in young cultures occur in long evenly-curved chains. They stain well and are Gram-positive. The organisms are motile, having peritrichous flagella, but do not form spores or capsules. They are facultative anaerobes and grow well on the surface or directly beneath the surface of agar and gelatin. In gelatin stab tubes an arborescent growth results which is most luxuriant at the top of the stab. In plain bouillon growth is slow and moderate, while in litmus milk it is very scant and produces no visible change. Gelatin is not liquefied, and none of the carbohydrates are attacked. On potato the growth is moderate with subsequent darkening of

the medium. The most favorable temperature for this genus is about 25°C. Good growth also occurs at 20° and at 30°, while at 37°C. the growth is very poor. No distinguishable odor was noted on any of the cultures. Hydrogen sulfide was not produced and growth in egg-meat medium was poor, resulting in no visible changes. On slant-agar and in agar and gelatin plates a more or less characteristic spider web growth often develops, but inoculations in the condensation water of slant agar do not cause a spreading over the surface. A division of the various strains into species did not seem possible on account of the few differentiating properties of these organisms.

SUMMARY AND CONCLUSIONS

The *Proteus* group has been known to include various types of organisms some of which have few common properties.

The types *Proteus vulgaris* Hauser, *Proteus mirabilis* Hauser, and *B. proteus* are, with a few exceptions, identical. The genus *Proteus* should be limited to organisms of this group.

Proteus zenkeri is identical with *B. zopfii* and therefore should not be grouped with the *Proteus* genus but rather with *B. zopfii*, the organisms of this type forming a genus to be known as *Zopfius*.

The organism *Proteus fluorescens* Jaeger does not resemble the *Proteus* genus, but rather the fluorescent group (genus *Pseudomonas*), and should not be known by the name *Proteus*.

The *Proteus* genus comprises a large group of organisms which can be subdivided on the basis of their action on maltose into two distinct species. For the species fermenting this sugar the name *Proteus vulgaris* is suggested, and for the species failing to attack it the name *Proteus mirabilis*. The genus cannot be subdivided satisfactorily on the basis of proteolytic action, indol production, or agglutinating properties.

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