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The Proteus Group of Organisms with Special Reference to Agglutination and Fermentation Reactions and to Classification

A DISSERTATION

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Department of Hygiene and Bacteriology

BY

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THE PROTEUS GROUP OF ORGANISMS WITH SPECIAL REFERENCE TO AGGLUTINATION AND FERMENTATION REACTIONS AND TO CLASSIFICATION

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INTRODUCTION

The proteus group, of which Proteus vulgaris may be considered the type species, is frequently referred to in the literature, although its members are often indefinitely characterized. The term "proteus group" has been loosely used to include a number of organisms which are distantly related.

Members of the proteus group are of interest on account of their occurrence under widely different conditions. They are usually thought of as saprophytic in nature, but the list of pathologic processes in which they are present, occasionally as the primary cause of disease and frequently as secondary agents enumerated by Meyerhof, justifies their characterization by this author as facultative parasites.

The recent work of certain French authors, including the studies of Metchnikoff and Berthelot on the relation of Proteus vulgaris to infantile diarrhea, and later that of Horowitz, who describes this organism as the causal agent in an epidemic of gastro-enteritis simulating dysentery, as well as the accounts of food poisoning epidemics attributed to this organism, are of special interest.

The rôle of Proteus vulgaris as a secondary agent in pathological processes in which admittedly pathogenic organisms are concerned is of importance. In the literature an increase in the virulence of B. diphtheriae associated with Proteus vulgaris has been noted. The presence of Proteus vulgaris in infections with pneumococcus, streptococcus and staphylococcus has been observed by several authors. It has been shown experimentally that the virulence of cultures of these cocci may be increased in the presence of Proteus vulgaris or its metabolic products.

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Proteus vulgaris (associated with streptococci, B. coli, B. lactis-aerogenes, B. welchii, and other organisms) has been frequently observed as an accompanying agent in wound infections. Swan and Goadby in their recent work in vaccine therapy in septic gunshot wounds recommend that cases of septic wounds receive an initial dose of a polyvalent vaccine of streptococcus and proteus strains. The rôle of Proteus vulgaris and other organisms in symbiosis with pathogenic anaerobes is worthy of consideration. In this connection Douglas, Fleming and Colebrook have recently determined experimentally that B. perfringens, B. edematis maligni and B. hibler in association with Proteus vulgaris and other aerobes commonly occurring in wounds multiply much more rapidly than when alone.

The Weil-Felix reaction in the diagnosis of typhus exanthematicus is a recent development. While the organism is not connected etiologically with this disease, it has been found that the serum of patients suffering from this disease agglutinates regularly certain strains of Proteus vulgaris originally isolated by Weil and Felix from the urine and stools of such patients, in dilutions of 1 : 100 to 1 : 2000 or higher.

These facts justify a study of the proteus group, from the standpoint of classification, since many widely different organisms have been classified here, and also from the standpoint of pathogenicity. The present paper embodies the results obtained in a study of the cultural and agglutination properties of a number of organisms isolated from various sources. An attempt has been made to determine the characteristics of the group as a whole as well as the differential characters of the various members, by a review of the more important literature as well as by the study of a limited number of cultures.

The cultures studied include those shown in Table 1.

All cultures were planted in plain broth three successive times, then plated on gelatin or Endo medium and colonies fished and subjected to preliminary tests consisting of planting in dextrose, lactose and saccharose broth fermentation tubes and in gelatin. In most cases several cultures from the same source were studied, and if their behavior was uniform in the various mediums used, the duplicate cultures were discarded and the results not included.

In the following discussion, all of the cultures included in Table 1, with the exception of the two designated as B. zopfii and B. proteus zenkeri, and the cultures of Pseudomonas protea, will be considered first as a whole. While not all of the cultures correspond to Proteus vulgaris it has been found convenient to discuss them together, pointing out differences as the occasion arises. The discussion of the literature in this section will in general refer to the vulgaris type.

TABLE 1

DESCRIPTION OF CULTURES STUDIED

No. of Culture	Source
	(a) Feces
{11	
{13	Typhoid feces 53-3
{17	
{18	Typhoid feces 601
20	Typhoid feces 611
{29	Feces in food poisoning epidemic case, tho not proven to be causal agent.
{32	
{54	Old typhoid feces B5 (examination of feces for typhoid carriers)
{55	
68	Culture from child's stool, Northwestern University Medical School
96	Normal feces 41HT
98	Normal feces Fe. 106
99	Normal feces Fe. 100
103	Normal feces 48HT
108	Normal feces Fe. 97
115	Normal feces Fe. 93
	(b) Meat
{34	Chopped meat
{35	
{36	
{43	Chopped meat
{45	
63	Chopped meat
78	Putrifying meat, Sheffield Scientific School
113	Chopped meat
114	Sausage
	(c) Water
{24	Water (filtered ?)
{27	
79	Stagnant water. Sheffield Scientific School
110	Sewage 7634
111	Sewage 7634
112	Sewage 7641
	(d) Necropsied animals
51	Peritoneal fluid. Guinea-pig.
53	Peritoneal fluid. Guinea-pig
94	Heart's blood. Mouse
	(e) Blood
69	Blood culture of H.
	(f) Air ?
{47	Contamination of B. coli growing in phenol solution
{49	
	(g) Wound bandage
75	Wound bandage, Sheffield Scientific School
	(h) Saliva
76	Dog's saliva, Sheffield Scientific School
	(i) Laboratory cultures from various sources and cultures of unknown origin
1	Proteus vulgaris I (University of Chicago)
2	Proteus vulgaris II (University of Chicago)
3	Proteus vulgaris Kral (University of Chicago)
4	Proteus mirabilis (University of Chicago)
64	Proteus vulgaris (University of Minnesota)
65	Proteus vulgaris (University of Minnesota)
67	Proteus vulgaris (University of Minnesota)
70	226 B. proteus, laboratory culture, American Museum Natural History
71	142 B. proteus vulgaris, laboratory culture, American Museum of Natural History
77	Proteus vulgaris, University of Pennsylvania. Sheffield Scientific School
80	Proteus vulgaris, Novy, Mich. Sheffield Scientific School
92	B. proteus vulgaris. A. I. K.
	(j) Proteus zopfii and zenkeri
73	B. proteus zopfii, laboratory culture, American Museum of Natural History
85	B. zenkeri, laboratory culture, Hygienic Laboratory
	(k) Pseudomonas protea
86	Ps. protea 362t. Hygienic Laboratory. Isolated from filtered water (W. H. Frost)
87	Ps. protea 366. Hygienic Laboratory. Isolated from filtered water (W. H. Frost)

METHODS OF ISOLATION

A number of different methods have been recommended for the isolation of members of the proteus group. In my work cultures were isolated by several of the methods described below, the majority however, by growing in dextrose broth fermentation tubes, plating in gelatin or streaking on agar or Endo plates, and fishing characteristic colonies. Direct streaking on Endo medium was also used in the isolation of Proteus strains from normal stools.

Metchnikoff and Bertrand used the method of Choukévitch in the isolation of Proteus vulgaris from stools. This consisted in planting the material on the surface of tubes of gelatin, retaining at a temperature of 22 C. for one day, and if liquefied, transplanting a loopful to the condensation water of slant agar tubes. If Proteus vulgaris is present it grows quickly to the top of the slant, and by making several successive transfers, a pure culture may be obtained. Jordan, in a study of bacteria from river water, isolated proteus strains by the gelatin plate method and also by planting in dextrose broth fermentation tubes and then plating.

Mediums containing lactose have been used by several authors, including Feltz and Horowitz. The former used litmus lactose agar plates in isolating Proteus vulgaris from stools, fishing colonies which did not give a characteristic B. coli reaction into peptone water, and then testing this for indol, as he considered a positive indol test particularly characteristic of Proteus vulgaris. Horowitz, in part of his work, used a gelatin litmus lactose medium. He also used a preliminary enrichment medium, consisting of broth or peptone water and bile, after which he plated on solid mediums. Drigalski-Conradi medium has also been used by several authors.

MORPHOLOGY

The morphology of the cultures studied was determined from 24-hour-old cultures grown on agar slants at 37 C. A small amount of the drier portion of the cultures was transferred to a drop of salt solution on a slide, which after drying and fixing was stained with methyl violet for one minute. All were rods, which in general were small, varying from 0.3-0.5 by 0.8-3 mikrons. Pseudomonas cultures were somwhat larger, while the B. zopfii cultures showed organisms ranging from 4 mikrons to long filaments. The pleomorphic forms described by a number of authors were not present in all of the cultures. Certain of the cultures, however, especially those of more recent isolation showed this characteristic, some exhibiting gradations from coccoid forms to bacilli, 5-8 mikrons in length. Swollen forms were present in only one or two instances. Hauser[1] considered the pleomorphic feature of this organism to be one of its distinguishing characteristics, and Feltz also states that it is one of the most polymorphic of all organisms. Spiral forms are described by this author. The occurrence of the organism in pairs and chains including up to 20 elements has been noted by several authors. Of the cultures in this study, Nos. 78, 96, 98, 103 showed long chains of strepto-bacilli. Pfuhl notes the fact that no involution forms were present in cultures 4 months old which he examined.

PRESENCE OF FLAGELLA AND SPORES

Flagella.—The presence of peritrichic flagella is generally conceded to be characteristic of Proteus vulgaris. Silberschmidt describes long flagella all around the bacillus, of which there were 4, 8 12 or more. Wesenberg found up to 20 or more flagella in cultures of Proteus vulgaris isolated in an epidemic

of meat poisoning. Pfuhl, Meyerhof, Cantu and Levy also demonstrated numerous flagella. Frost, who isolated an organism from filtered water, which he designated as Pseudomonas protea, calls attention to the fact that the descriptions of Proteus vulgaris cited in the literature are not sufficient to differentiate between it and the organism which he isolated. In my work several cultures of Ps. protea isolated by Frost have been included, in order to determine by cultural and agglutination reactions their relation to Proteus vulgaris.

Culture 4 in this study, which was typical in all respects of Proteus vulgaris, when stained by the Loeffler method showed numerous flagella surrounding the entire organism, which in comparison with the typhoid bacillus were much more numerous and much finer. Culture 24 showed polar flagella corresponding to Ps. protea as described by Frost.

Spores.—The absence of spores has been demonstrated by a number of authors and is generally accepted as characteristic of Proteus vulgaris.

GRAM STAIN

There is some disagreement among various authors in regard to the gram staining properties of Proteus vulgaris, but the concensus of opinion is in favor of a gram-negative stain. Feltz states that fresh cultures stained with anilin gentian violet made up at the time of using are always gram-positive. Horowitz found results with the Gram stain not always clear cut, but the majority of organisms gram-negative. Van Loghem also states that in young cultures, there are many gram-positive forms, but that in 24-hour-old cultures they are mostly gram-negative. The majority of authors, however, describe proteus as gram-negative. It is probable that some of the earlier authors who described gram-positive cultures were not describing Proteus vulgaris forms.

The following technic was used in determining the gram-staining properties of the cultures in this study. Smears were made from 24-hour-old agar slants and stained as follows:

1. Fixed by heat.
2. Methyl violet applied 1 minute, and shaken off slide.
3. Gram's iodin solution applied 1 minute and shaken off slide.
4. Slide rinsed with absolute alcohol until no color was observable in the rinsings.
5. Washed with tap water.
6. Stained with 0.1% basic fuchsin 10 seconds.

By this method all the cultures were found to be gram-negative (with the exception of the zopfii and zenkeri strains).

MOTILITY

Motility was studied by the usual hanging drop method, using 24-hour cultures from agar slants, grown at incubator temperature, a small amount of the growth being transferred to a drop of salt solution on a cover-slip. The various cultures showed motility in varying degrees, and not all could be described as exhibiting active motility. The movement in most cases was both rotatory and progressive, in a zigzag direction. One of the cultures under proteus species, No. 63, and the cultures B. zopfii showed only slight motility.

Cantu found motility to be at a maximum in 12-40 hours at incubator temperature, after which time it diminished, so that in 3 days the organisms were almost immotile.

TABLE 2

CULTURAL CHARACTERISTICS

No. of Culture	Rods > 1.5 μ	Pleomorphism	Motility	Rapidly Spreading Growth on Agar	Pigment Production in Broth	Gelatin Liquefaction		Brom-cresol-purple Milk				Indol	Reduction of Nitrates		Voges-Proskauer Reaction	Gas Production		
						3 da.	30 da.	Acid	Coag.	Pep.	Alk.		Nitrites	Ammonia		Dextrose Broth	Lactose Broth	Saccharose Broth
							Mm.									%		%
Proteus vulgaris																		
Feces 11	—	—	+	+	+ 19 YO-Y	+	26	+	+	+	+	—	+	+	—	+ 30	—	+ 15
17	—	—	+	+	++ 17′ O-Y	+	25	+	+	+	+	—	+	+	—	+ 35	—	+ 12
29	—	—	+	+	++ 17′ O-Y	+	30	+	+	+	+	—	+	+	—	+ 40	—	+ 10
55	—	—	+	+	+++ 17 O-Yk	+	20	+	+	+	+	—	+	+	—	+ 30	—	+ 8
96	—	+	+	+	++ 17′ O-Y	+	25	+	+	+	+	—	+	+	—	+ 35	—	+ 10
98	—	—	+	+	+++ 17′ O-Yi	+	20	+	+	+	+	—	+	+	—	+ 15	± (bubble)	+ 15
99	—	—	+	+	+++ 17′ O-Yi	+	30	+	+	+	+	—	+	+	—	+ 20	± (bubble)	+ 10
103	—	—	+	+	++ 17′ O-Y	+	25	+	+	+	+	—	+	+	—	+ 25	—	+ 5
108	—	+	+	+	+++ 17′ O-Yi	+	30	+	+	+	+	—	+	+	—	+ 20	—	+ 8
115	—	+	+	+	+++ 17′ O-Yi	+	45	+	+	+	+	—	+	+	—	+	—	+
Meat 34	—	—	+	+	—	+	25	+	+	+	+	+	+	+	—	+ 25	—	+ 3
78	+	+	+	+	± 19 YO-Y	+	29	+	+	+	+	++	+	+	—	+ 25	—	+ 4
113	—	+	+	+	+++ 17 O-Yi	+	50	+	+	+	+	+	+	+	—	+ 20	—	+
114	+	+	+	+	+ 19 YO-Y	+	28	+	+	+	+	+	+	+	—	+ 15	± (bubble)	+
Water 79	—	—	+	+	+++ 17 O-Yi	+	22	+	+	+	+	++	+	+	—	+ 30	—	+ 8
110	+	+	+	+	+++ 17 O-Yi	+	30	+	+	+	+	—	±	+	—	+ 25	± (bubble)	+
111	+	+	+	+	+++ 17 O-Yi	+	32	+	+	+	+	—	+	+	—	+ 25	± (bubble)	± (bubble)
112	+	+	+	+	+++ 17 O-Yi	+	36	+	+	+	+	—	+	+	—	+ 20	—	+
Necropsied animals																		
53	..	..	+	+	± 19 YO-Y	+	16	+	+	+	+	++	+	+	—	+ 25	—	+ 10
94	+	+	+	..	+++ 17 O-Yi	+	16	+	+	+	+	+	+	+	—	+ 5	± (bubble)	—
Wound bandage 75	+	—	+	+	+++ 17 O-Yi	+	30	+	+	+	+	—	+	+	—	+ 20	—	+ 5
Dog's saliva 76	—	—	+	+	+ 19 YO-Y	+	30	+	+	+	+	—	+	+	—	+ 30	—	+ 10

Lab. culture 1.....	+	+	+	+	+++	17 O-Yi	+	22	+	+	+	+	—	+	+	—	+ 30	—	+ 10
2.....	+	+	+	+	+++	17 O-Yi	+	18	+	+	+	+	—	+	+	–	+ 30	—	± (bubble)
3.....	+	+	+	+	++	17′ O-Y	+	30	+	+	+	+	+	+	+	—	+ 28	—	+ 10
4.....	+	+	+	+	++	17′ O-Y	+	22	+	+	+	+	—	+	+	—	+ 30	—	+ 8
64.....	—	—	+	+	±	21′ O-YY	+	30	+	+	+	+	—	++	+	—	+ 40	—	+ 5
65.....	+	+	±	+	++	17′ O-Y	+	21	+	+	+	+	+	+	+	—	+ 30	—	+ 10
67.....	—	—	+	+	+++	17 O-Yi	—	11	+	+	+	+	—	+	+	—	+ 35	—	+ 18
70.....	—	—	+	+	+++	17 O-Yi	+	30	+	+	+	+	—	+	+	—	+ 25	—	+ 5
71.....	—	—	+	+	++	17′ O-Y	—	—*	+	+	+	+	—	+	+	—	+ 30	—	+ 10
77.....	+	+	+	+	+++	17 O-Yk	+	28	+	+	+	+	+	+	+	—	+ 10	± (bubble)	+ 10
92.....	+	+	+	—	+++	17 O-Yi	+	15	+	+	+	+	trace —	+	+	—	+ 30	—	+ 5
Proteus species																			
Feces 20.....	—	—	+	—	—		—	2	—	—	—	+	+	±	±	—	+ 50	± (bubble)	± (bubble)
Blood 69.....	—	—	..	—	+	19 YO-Y	+?	10	++	+	—	—	+	++	++	—	+ 40	± (bubble)	+ 2
Air 49.....	—	—	+	—	+?	21′ O-YY	+	28	++	+	—	—	±	++	++	+	+ 40	—	+ 2
Meat 63.....	+	—	±	—	—		+	22	++	+	—	—	—	++	+	—	+ 3	—	+ 12
Lab. culture 80.....	+	+	+	—	++	17′ O-Y	+	33	+	+	+	+	+	+	++	—	+ 15	—	+ 2
B. Cloacae																			
Necropsied animals 53.....	—	—	+	—	—		—	8	++	+	—	—	—	+	+	++	+ 65	—	+ 60
Feces 68.....	—	—	+	—	—		—	12	++	+	—	—	—	+	+	++	+ 80	+ 10†	+ 80
Meat 45.....	+	—	+	—	—		+	21	++	+	—	—	—	++	++	—	+ 5	+ 20†	+ 18
B. zopfii																			
Lab. culture 73..... (B. proteus zopfii)	+	+	+	—	+	19 YO-Y	—	..	—	—	—	—	—	—	—	—	—	—	—
Lab. culture 85..... (Proteus zenkeri)	+	+	±	—	—		—	..	—	—	—	—	—	—	—	—	—	—	—
Pseudomonas protea																			
Filtered water 86....	+	+	+	—	±	19 YO-Y	+	12	++	+	—	—	+	++	++	—	+ 30	± (bubble)	+ 2
87....	+	+	+	—	+	19 YO-Y	+	12	++	+	—	—	+	++	++	—	+ 20	—	+ 2
Water (filtered?) 24.	+	+	+	—	±	19 YO-Y	+	25	++	+	—	—	+	++	++	—	+ 50	± (bubble)	+ 25

* 15 mm. in 2 months. † Per cent.

GROWTH ON AGAR

A characteristic of Proteus vulgaris not emphasized by some authors as much as it deserves, is the distinctive growth of the organism on agar slants, namely, its tendency to spread very rapidly. This has been observed by French authors and has been taken advantage of in its isolation from mixed cultures. Metchnikoff, who studied 204 cultures from stools, found that only five failed to rise rapidly to the top of the slant when planted in the lower part of the tube.

In this study one culture (92) which was typical in other respects failed to reach the top of the tube in from 6 to 24 hours when planted at the bottom of the slant. The growth of cultures of Proteus vulgaris may be described as effuse, moist and moderately luxuriant.

None of the cultures included under the headings Proteus species, B. cloacae, B. zopfii, and Pseudomonas protea showed the characteristic of rapidly spreading growth of Proteus vulgaris.

PIGMENT PRODUCTION

The production of brown pigment is considered by some authors to be characteristic of proteus strains. The presence of a brown coloration was observed by Jordan in the case of strains of Proteus varieties isolated from river water.

Pigment production in cultures of Proteus vulgaris appears to be as much an effect in the medium as in the growth itself. It is therefore evident that observations on the pigment production of the surface growth on agar slants may not be a true index of the pigment producing powers of cultures. A distinct difference of color in broth cultures of typical Proteus vulgaris strains and other strains was noticed, and it was determined to make tests of pigment production, by growing the cultures in plain broth for 14 days and noting change of color. The tests were made by removing a sufficient amount of the culture to a flat bottomed vial of a diameter of about 1 cm., so that a layer 1 cm. in depth was obtained, placing this on a white background, and comparing with the color standards (Ridgway's) by looking downward through the fluid. A culture which had not perceptibly darkened the medium in comparison with an uninoculated tube was used as a control, as readings of the tubes showing pigment production were made more easily in comparison with a turbid than a clear medium.

The cultures which were typical in all respects, with one or two exceptions, produced pigment varying from light cadmium to antique brown (19YO-Y to 170-Yk). The control culture was recorded as straw yellow (21'0-YYd).

Cultures 34 and 78 from meat which were typical of Proteus vulgaris in cultural and agglutination reactions produced no pigment. Cultures in this study which are classified as Proteus species, B. cloacae and Ps. protea, in general produced no pigment, or very slight. These include 20 and 68 (feces), 45, 63, 24, 86, 87 (water), 51 (necropsied animal), 49 (air).

BROTH

Growth in broth showed nothing characteristic except the production of pigment as noted above. A turbid growth without pellicle formation was produced in 24 hours. At the end of 7 days, all of the cultures showed more or less precipitate at the bottom of the tube, and some a slight pellicle.

GELATIN

The power of liquefying gelatin has usually been considered to be one of the most characteristic properties of the proteus group. Hauser's original classification of the group into three species was based on differences in

liquefying power, the vulgaris type liquefying gelatin rapidly, mirabilis more slowly and zenkeri not at all. This classification was withdrawn by Hauser on observing that zenkeri did sometimes liquefy gelatin after long cultivation, and the two types, mirabilis and zenkeri, were considered by this author to be attenuated forms of vulgaris. It has been shown by several authors that the power of liquefying gelatin varies in the same culture with growth on artificial mediums. Heim states that the property of liquefying gelatin may be lost and later return.

In the cultures studied, liquefaction of gelatin was determined by inoculating the surface of gelatin stabs, and noting the amount of liquefaction in millimeters after periods of 3 days and 30 days. All of the cultures corresponding to typical Proteus vulgaris, with one exception, No. 71, liquefied gelatin in 3 days, and showed liquefaction varying from 10-35 mm. in 30 days. Culture 71 was retained for a longer period and in 2 months was liquefied to a depth of 15 mm. This culture was typical in all other respects, furnishing evidence that delay in the liquefaction of gelatin should not necessarily be considered a sufficient reason for classifying in different species providing the culture is otherwise typical. Cultures 20 under Proteus species, 68 (feces) and 51 (necropsied animal) under B. cloacae did not liquefy in 3 days, but showed a certain amount of liquefaction in 30 days. Cultures 45, 63 (meat), 69 (blood), 47, 49 (air), differing in certain other respects from typical Proteus vulgaris, liquefied gelatin promptly.

GELATIN COLONIES

The appearance of the colonies of Proteus vulgaris on gelatin plates is a matter to which considerable attention has been paid. "Swarming" as a criterion for classification in the proteus group, has been emphasized by a number of authors. Swarm colonies have been described by Hauser, Pfuhl, Weber, Silberschmidt and others. That this property is not constant, however, has been admitted by Hauser and others. Herter and Ten Broeck in a critical study of two strains of Proteus vulgaris state that swarming was observed in only one instance, and that in a culture which had been rapidly passed through milk tubes and then placed on glucose gelatin. Five per cent. gelatin which is recommended as the best medium for the formation of swarm colonies, however, was not used by this author.

Berthelot and Horowitz do not emphasize characteristic colonies on gelatin. Horowitz found that characteristic colonies, with tortuous prolongations were sometimes produced on gelatin plates and at other times the colonies were simply round, and that the same strain often showed both kinds of colonies. The best results were obtained by slowly cooling the plates.

It is probably true that characteristic colonies are often produced by strains when freshly isolated, but that cultures soon lose this property. Boehnke, who studied the subject of swarm colonies in detail, at the instigation of Hauser, attempted to determine under what conditions swarming takes place. He points out that certain authors have not been justified in classifying organisms in the proteus group, inasmuch as swarming has not been described. He states that "swarming" is the only sure criterion for proteus, and that if one observes threadlike prolongations and islands in active motion under the microscope, or corkscrew projections extending outward from the colony in 5% gelatin medium it is a certain demonstration of the organism. He adds, however, that this property is inconstant, and sought to explain this discrepancy by growing cultures under different conditions of temperature, concentration of medium and difference of oxygen tension, but was not able to determine the favorable conditions.

In view of the observations of the above investigators, it appears that the property of forming swarm colonies may identify the organism in question as a member of the proteus group, but nonformation of swarm colonies does not necessarily exclude an organism from the proteus group, if it is typical in other respects.

MILK

The behavior of Proteus vulgaris in litmus milk has been variously described by different authors. Jordan notes acid reaction followed by curdling and digestion of casein. Cantu, Wesenbery, Weber and others report preliminary acid reaction followed by curdling, digestion of casein and alkalin reaction in typical cultures. Larson and Bell[1] and Berthelot do not observe change of reaction, but found milk curdled and then peptonized. Horowitz reports that cultures coagulated milk readily at the end of 2 days, without formation of acid, which was followed by pronounced peptonization. Archibald describes acid formation without curdling, followed by an alkaline reaction 3 days later. Herter and Ten Broeck found that one culture showed no visible change in 12 days, at which time the reaction was + 1.5. In the other culture, a soft curd was present in 3 days, which showed signs of digestion. Cantu calls attention to the fact that Proteus vulgaris derived from animal sources coagulates milk more strongly than strains derived from other sources.

It is probable that the difference of expression of results obtained by various workers is partly due to the fact that cultures of Proteus vulgaris actively reduce litmus, and it is difficult to determine reaction. In order to overcome this difficulty I decided in my work to substitute for litmus milk, milk containing brom-cresol-purple as recommended by Clark and Lubs.* With this medium it was possible to distinguish readily changes in reaction, and at the end of 14 days the differences in the typical and nontypical forms were very marked.

In 24 hours all of the Proteus vulgaris cultures (except 71) including 11, 17, 29, 55, 96, 98, 99, 103, 108, 115 (feces), 34, 78, 113, 114 (meat), 79, 110, 111, 112 (water), 53, 94, (necropsied animals), 75 (wound bandage), 76 (dog's saliva), 1-4, 64, 65, 67, 70, 77, 92 (laboratory cultures) were slightly acid, but showed no curdling. The majority of these showed curdling in 3 days, and beginning peptonization. Nos. 67, 94, 110 and 115 showed an alkaline reaction without curdling in 3 days, but in 7 days showed curdling with partial peptonization. Culture 71 was markedly acid in 24 hours, then became decidedly alkaline and showed no peptonization in 14 days.

Of the remaining cultures, included under proteus species, and B. cloacae, Nos. 68 (feces), 46, 63 (meat), 47, 49 (air), 51 (necropsied animal), 69 (blood), 80 (laboratory culture) were acid at the end of 24 hours, and in the case of Cultures 45, 47, 49, sufficient acid was produced to cause coagulation of the casein. These cultures showed increased acidity in 3 days with coagulation in all except 68.

Culture 20 (feces) was alkaline in 24 hours and showed increased alkalinity in 3 days.

In 14 days the Proteus vulgaris cultures all showed a purple color (alkaline reaction) while the remaining cultures were a decided yellow, with hard curd (except 20, alkaline).

* The medium is made by adding 0.005% of the sodium salt of dibromo-ortho-cresol-sulfonphthalein to milk and sterilizing for 20 minutes at a pressure of 15 lbs. (Clark and Lubs: Jour. Agr. Research, 1917, 10, p. 105.)

The behavior in brom-cresol-purple milk is of value in the identification of Proteus vulgaris, correlating with rapidly spreading growth on agar, gelatin liquefaction, fermentation and agglutination tests.

The reaction of the cultures of Ps. protea in brom-cresol-purple milk contrasted strongly with that of typical Proteus vulgaris. More acid was produced in 24 hours, and a hard curd was produced in 3 days, which remained undigested with a strongly acid reaction at the end of 14 days.

Endo Plates.—Tests were made by streaking broth cultures on Endo plates to determine the appearance of colonies on this medium. The appearance was typical in most cases in the Proteus vulgaris cultures. A pink or reddish spreading colony was produced in most cases in 24 hours, but in a few cases spreading was not evident until after 48 hours, the colony first appearing as round, raised and moist. In some cases spreading and nonspreading colonies were present on the same plate, particularly if the colonies were numerous. Several cultures, including 3, 65, 77, 78, and 92, failed to show spreading in 48 hours, the colonies being simply round.

None of the cultures classed as proteus species, B. cloacae or Pseudomonas protea showed the spreading growth characteristic of Proteus vulgaris. Cultures 43 and 80 showed colonies with the metallic luster characteristic of B. coli.

FERMENTATION REACTIONS

The use of fermentation reactions in various carbohydrates and related substances has been extensively applied in the classification of certain groups of organisms, particularly the B. coli and paratyphoid groups and the streptococci. To a certain extent, correlation of fermentation reactions with source and pathogenicity has been demonstrated, but there are also many discrepancies in such correlations.

In rating the value of fermentation reactions as a basis for classification certain factors have to be taken into consideration. The kind of medium, the age of the cultures, which involves variations, and the length of the incubation period are of importance.

In this work it has been found that the presence or absence of meat extract in the carbohydrate medium influences to a great extent carbohydrate metabolism, particularly in the case of saccharose. Most authors describe incompletely or not at all the composition of the mediums used in the study of fermentation reactions. Presumably most of these are broth mediums containing a certain percentage of the carbohydrate.

Berthelot used as a medium peptone solution containing the various carbohydrates, in the proportion of 3% of the total volume. Tubes of peptone solution without carbohydrate were inoculated with the cultures as controls, the test for acid production being made by adding litmus solution to both carbohydrate and control tubes at the end of a certain period of incubation, and comparing results in the two tubes. The advantage of this method obviously lies in the fact that reduction of the litmus is avoided, which interferes with determination of acid production.

The age of the culture in the case of Proteus vulgaris apparently is involved in the problem of carbohydrate metabolism. A number of instances are quoted in the literature of variation in carbohydrate metabolism by cultures which had been kept under cultivation for a certain length of time. Theobald Smith[1] considered fermentation reactions in dextrose, saccharose and lactose more stable than other cultural reactions of Proteus vulgaris. In the case of the disaccharid maltose, however, several well authenticated cases of the loss of

power to ferment this sugar by cultures which originally did so are recorded. Horowitz found that cultures which at the time of isolation fermented maltose, failed to do so after having been retained in broth for one month. Thjøtta also found that cultures from diarrheal stools, which originally fermented dextrose, saccharose, maltose and mannite, failed to ferment maltose and mannite in 1½ months, and fermented dextrose and saccharose in a less degree.

The period of incubation is a factor involved in the study of carbohydrate fermentations. Action on certain carbohydrates by Proteus vulgaris cultures is prompt, particularly in the case of dextrose, while in the case of others, such as saccharose, action may be very much delayed. This may partially account for variable results obtained with this carbohydrate by different authors.

The cultures made use of in my study were subjected to preliminary tests in dextrose, lactose and saccharose broth and for liquefaction of gelatin, and in general those cultures included which fermented dextrose and saccharose and which liquefied gelatin. This was in accordance with the suggestions of Jordan, Theobald Smith, Herter and Ten Broeck and others.

Theobald Smith, who studied the fermentation reactions of several strains of proteus, found that cultures kept under cultivation for a year showed a diminished or complete loss of power of liquefying gelatin, while the fermentation reactions in dextrose, lactose and saccharose remained the same. He therefore recommends the fermentation tube test in classifying organisms in this group, considering as proteus those varieties of organisms which ferment dextrose and saccharose but not lactose.

Jordan in a study of bacteria found in river water has included in the proteus group, organisms which ferment dextrose and saccharose, and rarely lactose, which are actively proteolytic, liquefying gelatin, and blood serum and precipitating, then digesting casein. Three subdivsions are considered in the group: (1) Proteus vulgaris; (2) proteus varieties; (3) B. cloacae.

Herter and Ten Broeck who studied two proteus strains, one of which was derived from putrid material, found that the only constant cultural properties exhibited by the two cultures were in the matter of fermentation reactions in dextrose, lactose and saccharose, though the two cultures produced the same chemical products and their pathogenic properties were the same.

In my study the amount of gas present in dextrose and saccharose broth fermentation tubes (Smith tubes) was recorded in these preliminary tests. Gas was nearly always present in dextrose in 24 hours, and in 7 days, the amount varied from 15-40% in the Proteus vulgaris cultures, the majority showing about 30% of gas. The cultures classified as proteus species varied from 3-50% in gas production. Two cultures classified as B. cloacae produced 65-80% of gas and another produced 5%.

Production of gas in saccharose broth was delayed and was considerably less in amount than in dextrose broth. Most of the Proteus vulgaris cultures showed a very small amount or no gas in two days, and not above 15% in 7 days. Culture 94, typical in other respects, produced no gas in saccharose broth in 14 days. The cultures classified as proteus species in general produced small amounts of gas in saccharose broth, and B. cloacae large amounts.

Most authors are in accord regarding fermentation of dextrose and non-fermentation of lactose by Proteus vulgaris, but fermentation of saccharose has given variable results in the hands of different workers. Berthelot, who studied a number of strains of Proteus vulgaris derived for the most part from cases of infantile diarrhea, and Horowitz who studied strains isolated from feces in an epidemic of gastro-enteritis found the reaction variable in saccharose mediums. Larson and Bell,[1] observing a number of strains derived

from pathologic sources noted that saccharose was not acted on by most of the strains. Bahr and Thomsen, in a study of proteus strains isolated from feces, classified them in three types, two of which produced acid and gas in saccharose and one which did not — the majority of the cultures, however, belonging to the first class. Archibald describes a strain isolated from a case of choleraic diarrhea, which had no action on saccharose.

Tests on the cultures studied in my work were also made by the hydrogen-ion concentration method, using the following: Monosaccharid, dextrose; disaccharids, lactose, saccharose and maltose; trisaccharid, raffinose; and alcohol, mannite (Table 3).

The medium adopted was one without meat extract and consisted of peptone water (1% Witte's peptone) to which was added 1% of the various fermentable substances. The medium was tubed in Durham fermentation tubes, so the gas production could be observed as well as acid production. The mediums were sterilized by the fractional method. The length of the period of incubation was 5 days at a temperature of 37 C.

In selecting the medium, it was decided to use as simple a medium as possible without meat and without the use of a regulator (dibasic potassium phosphate). On the supposition that carbohydrate may be utilized first and then protein it was thought the results obtained in this medium would show best the results of carbohydrate metabolism. However, as will develop later, the results obtained did not altogether bear out this hypothesis.

Standards for determination of H-ion concentration were prepared as recommended by Clark and Lubs,[1] and the appropriate indicators covering the range P_H 8 to 4.4 used.

Referring to Table 3, it will be noted that the Proteus vulgaris cultures from the feces behave uniformly as regards fermentation reactions. Dextrose was the only substance fermented with acid and gas production in 5 days in the medium used. Other cultures showing the same fermentation reactions include 110, 111, 112 from water, 75, wound bandage, 76, dog's saliva, and laboratory cultures 1, 4, 64, 67, 70, 71, 92.

Another type of fermentation reactions includes those in which saccharose and maltose were fermented in addition to dextrose. In this group are included all of the Proteus vulgaris cultures from meat, 34, 78, 113, 114; one culture from water, 79; two cultures from necropsied animals, 53 and 94; 3 laboratory cultures, 2, 3 and 65. In this group may also be included stock culture 77, which showed a reading of P_H 5.6 in saccharose and 5.8 in maltose without production of gas in either. Gas production in dextrose consisted of only a bubble of gas, indicating that this strain was a feeble gas producer.

None of the typical Proteus vulgaris cultures fermented lactose, mannite or raffinose.

In general the cultures derived from meat and decomposing animal matter acted on carbohydrates more vigorously than strains from fecal sources. This is in accord with the observations of Cantu.

The behavior of the cultures classified as proteus species and B. cloacae in carbohydrate mediums was variable, and as a rule these cultures were more active in the breaking down of carbohydrate substances than Proteus vulgaris. Culture 51 from the peritoneal fluid of a guinea-pig and Culture 68 isolated from a child's stool were both very active as to gas production from carbohydrates. They correspond throughout in regard to acid and gas production with the exception of lactose, in which no gas was produced by Culture 51, while a small amount was produced by Culture 68 — the P_H value being the same, 5.4 in both cases. These cultures were similar in other respects

TABLE 3

FERMENTATION REACTIONS

No. of Culture	Dextrose		Lactose		Saccharose		Maltose		Mannite		Raffinose	
	pH	Gas	pH	Gas	pH	Gas	pH	Gas	pH	Gas	pH	Gas
Proteus vulgaris												
Feces 11	4.9	+	7.6	—	7.4	—	7.3	—	7.4	—	7.6	—
17	5.0	+	7.7	—	7.6	—	7.4	—	7.4	—	7.6	—
29	5.1	+	7.8	—	7.6	—	7.5	—	7.4	—	7.6	—
55	4.8	+	7.5	—	7.6	—	7.4	—	7.5	—	7.6	—
96	5.5	+	7.6	—	7.3	—	7.4	—	7.6	—	7.4	—
98	5.3	+	7.5	—	7.6	—	7.6	—	7.6	—	7.5	—
99	5.0	+	7.4	—	7.5	—	7.5	—	7.5	—	7.4	—
103	5.0	+	7.6	—	7.3	—	7.3	—	7.5	—	7.5	—
108	5.0	+	7.3	—	7.5	—	7.4	—	7.4	—	7.6	—
115	5.0	+	7.4	—	7.4	—	7.6	—	7.7	—	7.6	—
Meat 34	5.1	+	7.8	—	5.3	+	5.2	+	7.5	—	7.5	—
78	5.3	+	7.6	—	5.3	+	5.3	+	7.5	—	7.5	—
113	5.1	+	7.4	—	5.3	+	5.2	+	7.7	—	7.7	—
114	5.3	+	7.5	—	5.1	+	5.2	+	7.8	—	7.6	—
Water 79	5.3	+	7.8	—	5.4	+	5.5	+	7.5	—	7.6	—
110	4.8	+	7.4	—	7.3	—	7.3	—	7.8	—	7.5	—
111	4.8	+	7.4	—	7.3	—	7.6	—	7.6	—	7.6	—
112	4.8	+	7.3	—	7.5	—	7.6	—	7.6	—	7.7	—
Necropsied animals												
53	5.3	+	7.8	—	5.3	+	5.7	+	7.5	—	7.6	—
94	5.2	+	7.6	—	5.3	+	5.1	+	7.6	—	7.6	—
Wound bandage 75	5.1	+	7.7	—	7.5	—	7.5	—	7.5	—	7.5	—
Dog's saliva 76	4.9	+	7.6	—	7.5	—	7.5	—	7.5	—	7.6	—
Lab. cultures 1	4.9	+	7.6	—	7.4	—	7.4	—	7.7	—	7.6	—
2	4.8	+	7.6	—	5.3	—	5.7	—	7.5	—	7.5	—
3	5.4	+	7.6	—	6.7	+	5.9	+	7.5	—	7.7	—
4	5.0	+	7.5	—	7.4	—	6.9	—	7.4	—	7.6	—
64	5.1	+	7.7	—	7.6	—	7.4	—	7.6	—	7.6	—
65	5.1	+	7.6	—	5.3	+	5.3	+	7.5	—	7.5	—
67	4.7	+	7.6	—	7.5	—	7.4	—	7.5	—	7.5	—
70	5.2	+	7.0	—	7.6	—	7.4	—	7.5	—	7.6	—
71	4.8	+	7.6	—	7.1	—	7.6	—	7.7	—	7.6	—
77	5.3	+	7.5	—	5.6	—	5.8	—	7.4	—	7.4	—
92	4.8	+	7.5	—	7.3	—	7.4	—	7.5	—	7.5	—
Proteus species												
Feces 20	4.8	+	7.5	—	7.6	—	7.6	—	7.7	—	7.8	—
Blood 69	5.1	+	7.8	—	7.6	—	5.3	+	5.4	+	7.6	—
Air 49	4.8	—	7.1	—	5.1	—	5.2	—	5.3	—	7.5	—
Meat 63	4.8	+	7.6	—	4.9	+	5.1	+	4.9	+	6.2	± (bubble)
Lab. culture 80	5.4	—	7.2	—	5.7	+	6.4	+	7.3	—	7.5	—
B. cloacae												
Necropsied animal 51	5.3	+++	5.4	—	5.3	+++	5.1	+++	5.1	+++	5.0	+++
Feces 68	5.5	+++	5.4	+	5.0	+++	5.3	+++	5.1	+++	5.1	++
Meat 45	4.8	+	4.9	+	4.9	+	5.0	+	5.0	+	4.9	±
B. zopfii												
B. proteus zopfii 73	7.3	—	7.4	—	7.1	—	7.4	—	7.5	—	7.5	—
Proteus zenkeri 85	7.3	—	7.0	—	7.1	—	7.3	—	7.5	—	7.5	—
Pseudomonas protea												
Filtered water												
Ps. protea 86	5.3	++	7.7	—	7.7	—	5.3	+	5.3	+	7.7	—
87	5.0	++	7.7	—	7.7	—	5.2	+	5.4	+	7.7	—
Water (filtered ?) 24	5.1	++	7.8	—	5.1	+	5.5	+	5.5	+	7.6	—

culturally and in agglutination tests and correspond to the type B. cloacae. They both showed a strong positive Voges-Proskauer reaction and produced no indol. Culture 45 should perhaps also be classed here. This organism is included in the study inasmuch as in the preliminary tests made just after isolation this organism failed to produced an appreciable amount of gas in lactose and corresponded in general to typical Proteus vulgaris. At the time H-ion tests were made, after the organism had been under cultivation a considerable length of time, gas production was quite pronounced in lactose broth. Two cultures from the same source showed this variation, though but one is included in this study.

The remaining cultures—20 (feces), 63 (meat), 69 (blood), 49 (air?) and 80 (laboratory culture)—are irregular in their fermentation reactions, and probably represent different species. Cultures 20 and 80 agreed with the typical fecal Proteus vulgaris cultures in fermentation of carbohydrates, but did not conform in all other respects. Culture 63 (meat) fermented all of the carbohydrates tested except lactose and Culture 69 (blood) fermented maltose and mannite but failed to ferment saccharose. Culture 49 produced no gas in any of the carbohydrates tested, but produced acid in dextrose, saccharose, maltose and mannite. This culture, however, produced gas in dextrose and saccharose broth in the preliminary tests. These cultures differed in other respects also from the typical Proteus vulgaris cultures.

DISCUSSION OF FERMENTATION REACTIONS

A survey of the fermentation reactions by Proteus vulgaris strains in the peptone carbohydrate mediums shows that dextrose is fermented by all; lactose, raffinose, and mannite by none, and saccharose and maltose are variable.

It should be emphasized that the results obtained in saccharose broth are not identical with those obtained in the peptone medium. It is probable that the meat extract used in broth mediums contains substances (hormones?) which increase the metabolic activities of certain strains as regards carbohydrate substances. This may apply also to maltose. Burton and Rettger in an investigation of the fermentation properties of high-ratio cultures of the aerogenes-cloacae type, found that when mediums containing Witte's peptone without meat extract was used the sugar utilization was never complete, but when Eimer and Amend peptone was used the sugar disappeared without the aid of meat extract. They refer to the variable amino-acid content of the two peptones as the reason for this difference. Witte's peptone was made use of in this study, and whether different results would have been obtained with other peptones was not determined.

As indicated by the preliminary tests all of the typical cultures produced a certain amount of gas in saccharose broth, though this was not always evident in the first two days, as in the case of dextrose, in most of the cultures. All, however, showed gas in 7 days. In the hydrogen-ion concentration tests in the peptone mediums only the cultures from decomposing meat and animal matter, one from water and several stock cultures produced acid and gas.

CORRELATION WITH RESULTS OBTAINED BY OTHER WORKERS

Berthelot, who has probably investigated most extensively the proteus group, also used a peptone solution as a basis for carbohydrate mediums. All of his strains (principally from diarrheic material) produced acid from dextrose, and none from lactose and mannite. Fermentation of saccharose was determined

at the end of 2, 5, 10 and 15 days, and while some strains showed a neutral reaction in 5 days, all were acid in 10-15 days. Maltose was variable in its behavior.

Horowitz, in a study of cultures isolated in an epidemic of gastro-enteritis, found glucose fermented by all strains with acid and gas production, maltose by all except one and mannite by all except one, though loss of power to ferment maltose was observed in some cultures after they had been kept under cultivation for some time. Saccharose was attacked by 7 out of 24 cultures. The author does not state whether the medium used contained meat extract or not. Three strains isolated from river water, which were agglutinated by a serum obtained by immunizing rabbits with one of the fecal cultures, failed to ferment maltose and mannite.

Larson and Bell found that pathogenic strains from various sources produced acid and gas in maltose.

Thjøtta studied a culture isolated from stools of a child ill with dysentery-like symptoms and found that the organism fermented glucose, saccharose, maltose and mannite at the time of isolation, though it lost its power of fermenting maltose and mannite after a short time.

Archibald isolated a strain from a case of choleraic diarrhea which fermented maltose and mannite, but not saccharose.

From the evidence previously given it seems probable that fecal strains often ferment maltose, saccharose and mannite when freshly isolated and later lose the power to a greater or less extent and sometimes completely.

In my study opportunity was not afforded for testing the cultures immediately on isolation, since a number of the strains were collected in the routine examination of feces for other purposes, and the study as a whole not attempted until a number of cultures had been obtained. It is thus possible that the cultural characteristics may have been altered considerably since the time of their isolation. According to the results obtained by Horowitz the power to ferment certain substances may be lost in as short a period as four weeks.

A much more extended investigation immediately on isolation of strains isolated from feces and from meat and water would be of value in throwing more light on the changes in metabolic activities as regards various carbohydrates.

Fermentation tests with various other carbohydrates are recorded in the literature. Glenn found that the monosaccharids, mannose and galactose, were fermented in addition to the carbohydrates previously referred to. Berthelot used galactose and levulose as test substances, and found that all cultures produced acid from galactose, but results were variable with levulose.

Archibald records acid and gas in glucose, mannite, levulose, maltose, galactose and dextrin, and no change in lactose, saccharose, dulcite, adonite, inulin and raffinose by a strain derived from a case of choleraic diarrhea.

Horowitz records no action on dulcite by the culture which he observed.

In my study several of the strains collected at the beginning of the work were tested in a large number of carbohydrate and related substances (Table 4). A broth medium neutral to litmus and containing 1% of the test substances and litmus and tubed in Durham fermentation tubes was used. The 7-day readings are recorded. It was found that the reduction of the litmus interfered considerably with the readings of acid production. Table 4 is included as being of a certain value, though the use of a number of the test substances was discontinued in a further study of the group. The results obtained indicate that levulose, galactose, glycerin and xylose, in addition to dextrose and saccharose, are fermented by all strains of Proteus vulgaris.

TABLE 4

RESULTS OBTAINED IN THE STUDY OF STRAINS TESTED IN A LARGE NUMBER OF CARBOHYDRATES AND RELATED SUBSTANCES

	Monosaccharids				Disaccharids			Trisaccharids	Polysaccharids		Alcohols						Tetraoxyaldehyds			Hydraoxybenzene	Glucosid
	Dextrose	Levulose	Galactose	Mannose	Saccharose	Lactose	Maltose	Raffinose	Dextrin	Inulin	Glycerin	Erythrite	Adonite	Dulcite	Mannite	Sorbite	xylose	Arabinose	Isodulcite	Inosite	Salicin
Proteus vulgaris																					
Feces 11	+ a	+ r	+ r	— a	+ r	— a	— r	— r	— r	— r	+ r	— r	— r	— r	— a	— r	+ a	— r	— r	— r	— r
17	+ a	+ r	+ r	— a	+ r	— a	— r	— r	— r	— r	+ r	— r	— r	— r	— a	— r	+ r	— r	— r	— r	— r
29	+ a	+ r	+ r	— r	+ r	— r	— r	— r	— r	— r	+ a	— r	— r	— r	— r	— r	+ r	— r	— r	— r	— r
Meat 34	+ a	+ r	+ r	+ r	+ a	— r	+ r	— r	— r	— r	+ r	— r	— r	— r	— r	— r	+ r	— r	— r	+ r	+ r
Lab. cultures 1	+ a	+ r	+ r	— r	+ r	— a	— r	+ r	— r	— r	+ r	— r	— r	— r	— r	— r	+ r	— a	— r	— r	— r
2	+ a	+ r	+ r	— r	+ a	— r	+ r	+ r	— r	— r	+ r	— r	— r	— r	— r	— r	+ a	— r	— r	— r	— r
3	+ a	+ r	+ r	— r	+ a	— a	+ a	— r	— r	— r	+ a	— r	— r	— r	— a	— r	+ a	— r	— r	— a	— r
4	+ a	+ r	+ r	— r	+ r	— a	— r	— r	— r	— r	+ r	— r	— r	— r	— a	— r	+ r	— r	— r	— r	— r
Proteus species 20	+ a	+ a	+ r	+ a	+ r	—	—	— r	— a	—	— r	— r	—	—	—	—	+ r	— r	— r	— a	—
47	+ r	+ a	— r	— r	+ a	— r	— r	— r	— r	— r	— a	— r	— r	— r	+ a	+ r	— a	— a	— r	— a	— a
B. cloacae 45	+ a	+ a	+ r	+ a	+ a	— a	+ a	+ a	— r	— r	+ r	— r	—	—	— a	+ r	+ a	+ a	— r	+ a	— a
Ps. protea 24	+ a	+ a	+ r	+ a	+ a	— a	+ a	— r	— r	— r	+ a	— r	— r	— r	+ r	— r	— a	— r	— r	— r	—

+ = gas; — = no gas; a = acid; r = litmus reduced.

VOGES-PROSKAUER REACTION

The Voges-Proskauer reaction as a test for the identification of Proteus vulgaris is referred to by few authors. Archibald found that the organism isolated by him in a case of choleraic diarrhea gave a positive Voges-Proskauer reaction. This organism was typical in other respects, except that it produced a greenish fluorescence on agar, and rendered milk acid, and then alkaline, without curdling. The medium used was a dextrose peptone solution. He classifies the organism as B. Proteus fluorescens. This author refers to the statement of Orr that organisms of the proteus group frequently give a positive Voges-Proskauer reaction. Kligler,[2] in a study of 6 laboratory strains, found that all gave a negative Voges-Proskauer reaction.

In making the Voges-Proskauer test on my cultures, the same medium was made use of as in testing for hydrogen-ion concentration, namely 1% peptone solution containing 1% of dextrose. After an incubation period of 5 days at 37 C., tests were made by rendering the solution alkaline by the addition of 10% solution of potassium hydroxid and observations made at the end of 6 and 24 hours. None of the Proteus vulgaris cultures gave a positive test. Of the cultures classed as proteus species one culture, 49 (air) showed a positive test. Cultures 51 and 68 classed as B. cloacae gave decided positive tests. The Pseudomonas protea gave negative tests.

INDOL

Tests for the production of indol were made in accordance with the method recommended in the Standard Methods for Water Analysis of the American Public Health Association (1917). Tryptophan not being available, tests were made on various peptones to determine which gave the strongest tryptophan reaction by the bromin test. Armour's peptone was selected as being most favorable and the medium made up with 5% of this peptone, and 0.5% of dipotassium hydrogen phosphate in 1,000 c c of distilled water. After 2 days' incubation at 37 C., tests were made using paradimethyl-amino-benzaldehyde and concentrated hydrochloric acid.

Among Proteus vulgaris cultures the four isolated from meat (34, 78, 113, 114), one from water (79), the two from necropsied animals (53, 94), and laboratory cultures 3, 65 and 77 gave positive indol test and the remaining were all negative. This correlates almost perfectly with the fermentation reactions in saccharose and maltose, the cultures recorded as producing a positive indol reaction consistently fermenting these carbohydrates. Culture 77 was recorded as giving a trace of indol, and this culture though producing no gas from saccharose or maltose peptone solution, showed a H-ion concentration represented by a P_H value of 5.6 and 5.8 in the respective sugars.

The cultures classified under proteus species except 63 and the Pseudomonas protea cultures gave positive tests for indol, while the B. cloacae and B. zopfii cultures were negative.

Cantu calls attention to the fact that the production of indol is more marked in cultures derived from animal sources which is borne out by the results obtained.

Horowitz comments on the fact that strains which gave a positive indol test as a rule fermented saccharose.

The results obtained by various workers in testing for indol are not in agreement. Feltz, who first made a careful study of the indol reaction, carried out tests with a number of different varieties of peptone and obtained very divergent results, some giving good positive reactions, some moderately good

and some negative with the same strains. He demonstrated that the presence of dextrose delayed the production of indol and also that acid interfered with the reaction, which was confirmed by Glenn. As a result of his tests, however, he considers the production of indol one of the distinguishing characteristics of Proteus vulgaris. In doubtful cases he recommends distillation, indol distilling very readily in the presence of watery vapor.

Herter and Ten Broeck found indol present in moderate amounts in the case of cultures derived from putrefying material. Indol-acetic acid was present in cultures 4 days old. Larson and Bell report indol present in perceptible proportions by strains from pathologic sources.

Kligler,[1] in studying the cultural characteristics of 5 laboratory strains of Proteus vulgaris, found that 3 produced indol (glycerin negative) and 2 produced no indol (glycerin positive).

Van Loghem and van Loghem-Pouw found that a strain of Proteus isolated from the urine of a diabetic patient was indol negative, and the same organism was later isolated by them from an intestinal abscess. These two strains produced a reddish violet color on testing for indol by the nitrite test, but this color was shown to be different from the color of nitrosoindol, by spectrum analysis. Other indol tests with these organisms were negative. These investigators further isolated 3 strains among 30 proteus strains derived from intestinal contents and feces, which showed the same reaction. A new species, B. proteus anindologenes, is proposed by these authors to cover this group of organisms. Absence of interagglutination of the two types is cited as added evidence of basic differences.

Berthelot, who made a detailed and accurate study of the indol reaction of strains derived principally from cases of infantile diarrhea, demonstrated that the non-indol producing strains acting in a tryptophan medium produced at least indol-3-acetic acid, and on this basis a separation should not be made into the two species Proteus vulgaris and B. proteus anindologenes.

Horowitz found that 7 out of 24 strains gave a strongly positive reaction for indol in 24 hours, and the remaining 17 did not give a positive reaction even in 6-7 days. The medium used and method of testing for indol are not described.

Thjøtta tested a strain of Proteus vulgaris at the time of isolation and found it to be indol positive, but a month and a half later it failed to produce indol in either broth or peptone water, at the same time exhibiting loss of power of fermenting maltose and mannite.

In my study no tests were made for the presence of indol-acetic acid, nor was distillation resorted to in order to determine indol. It is possible that some of the strains tested immediately on isolation would have shown a postive indol test. The correlation between the indol positive and the maltose and saccharose positive organisms is so striking that it seems certain that the two reactions are closely related in cultures of Proteus vulgaris.

REDUCTION OF NITRATES

Tests were made on the cultures studied for reduction of nitrates, by the methods recommended in the Standard Methods of Water Analysis of the American Public Health Association (1912), the medium consisting of 0.1% peptone medium containing 0.02% nitrite free potassium nitrate. The incubation period was for 4 days at 37 C. Tests were made with sulphanilic acid solution and a-amidonaphthelene acetate solution. Tests for ammonia were made with the same medium by adding a few drops of Nessler's solution. All

of the cultures tested with the exception of the cultures of Proteus zenkeri gave positive tests for nitrites and ammonia.

UREA DECOMPOSITION

Tests were not made on the cultures studied for decomposition of urea, but for the sake of completeness, reference is made to the work of Brodmeier, who records the active decomposition of urea by Proteus vulgaris in neutral and alkaline solutions, and to Horowitz, who found that fecal strains transformed urea energetically with the evolution of ammonia in large amounts.

HYDROGEN SULPHID

The production of hydrogen sulphid by Proteus vulgaris cultures in peptone mediums containing lead acetate has been recorded by Horowitz. This was previously demonstrated by several other authors.

AGGLUTINATION OF PROTEUS CULTURES BY PROTEUS SERUM

There is little agreement among various workers in regard to the matter of agglutination of Proteus vulgaris by immune serum, some authors finding that only the strain used for immunizing is agglutinated, while others have been able to show that an immune serum produced by one strain may also influence other strains. Klieneberger[1] has reviewed the literature on this subject.

Rodella tested an immune serum produced from a strain isolated by Silberschmidt in a food poisoning epidemic against seven other strains. The strains used are not described except for the statement that all belonged to the variety Proteus vulgaris, and one was isolated from a wound and another from the stool of a child ill with enteritis. The latter culture was the only one which gave a positive result, and that occurred in as high a dilution as the homologous organism.

Grossmann found that an organism isolated in a case of cystitis was agglutinated by an immune serum produced from a strain isolated in a case of peritonitis in a dilution of 1:750, while the immune serum from the cystitis organism agglutinated the other in 1:400. Immune serums produced by Proteus vulgaris isolated from meat agglutinated the pathogenic strain from cystitis in a dilution of only 1:20.

Weber studied the agglutination of three strains derived from decaying meat and found interagglutination in dilutions of 1:10 to 1:50, but no agglutination except with the homologus organism in dilutions above 1:50.

Klieneberger[1] studied the agglutination reactions of a number of strains from different sources, including several from cystitis urine, one from brain abscess, two from meat and two which he classified as mirabilis from urine and meningitis pus. The immune serums from four strains from pathologic processes agglutinated the strain used for immunizing and also other strains from pathologic sources. As a result of his work this author considers proteus strains as forming a biological entity in the matter of agglutination just as B. typhosus and B. paratyphosus. He found that as a rule strains derived from meat were not influenced by serums derived from pathogenic sources, but that serums derived from meat strains did influence pathogenic strains. He considers this behavior as indicative of differences between saprophytic and parasitic forms and of gradual adaptation of saprophytic forms to the animal body.

Glaser and Hachla tested the agglutination reactions of a number of strains derived from sausage and meat, and also several laboratory strains including Proteus vulgaris, Proteus mirabilis Kral, Proteus zenkeri and B. piscicidus versicolor. These authors concluded from the results obtained that the action was individualistic like that of B. coli.

Cantu tested a number of strains derived from various sources, including normal and diarrheic human stools, spoiled meat, vegetables, etc., and succeeded in obtaining agglutination with the homologous organism in a dilution of only 1:10, while other strains were not at all agglutinated except in one instance.

Horowitz studied the agglutination reactions of a number of strains derived from diarrheic stools and separated them into five groups by the method of testing all the strains with a serum derived from one strain, then testing the remaining strains with a serum derived from one of the strains not agglutinated by the first serum and so on, until a serum had been found for all the different strains.

Agglutination tests in my work were carried out with immune serums obtained by inoculating rabbits with suspensions of several different cultures. Cultures representing different habitats were selected. These included No. 29 (feces), 34 (putrefying meat), 75 (wound bandage), 4 (laboratory strain), and 27 Ps. protea (water).

Inoculations for the production of immune serums were carried out as follows:

First inoculation: 1 c c of suspension in salt solution of killed 24-hour culture subcutaneously.

Second inoculation, 5 days later: 1 to 2 c c of suspension of live culture of organism subcutaneously.

Third inoculation, 5 days later: 2 c c of suspension of live culture of organism subcutaneously.

Fourth inoculation, 5 days later: 2 c c of suspension of live culture of organism intraperitoneally.

Bled 10-12 days after the last injection.

A serum of titer 1:20,000 from Culture 29 was obtained, 1:1,000 from 34, 1:10,000 from 75, 1:2,000 from 27 and 1:30,000 from 4.

In performing the agglutination tests a 2-hour incubation period at 37 C. was used. The tubes were then kept at a temperature of 15 C. overnight and the readings made 18-24 hours after the test was begun.

The suspensions of organisms used in testing the serums for agglutinins were prepared as follows: 24-hour cultures grown on agar slants were washed off with 0.85% salt solution containing 0.1% of formalin. The suspensions were kept at cold room temperature for 3 days with daily shakings. The suspensions were then diluted by the addition of salt solution containing formalin as above to contain about 1½ billion organisms per c c in the final test, the standardization being made by comparison with a typhoid-paratyphoid vaccine this number of organisms.

Readings were made as follows:

++++ = Organisms all precipitated, supernatant fluid perfectly clear.
+++ = Organisms nearly all precipitated, supernatant fluid slightly cloudy.
++ = Organisms partially precipitated, supernatant fluid more cloudy than preceding.
+ = Precipitate of organisms perceptible, supernatant fluid cloudy.
— = No precipitate or clearing (like control without serum).

TABLE 5

AGGLUTINATION TESTS OF CULTURES WITH PROTEUS VULGARIS IMMUNE SERUMS

	Serum 4 (Titer 1:30,000)	Serum 29 (Titer 1:20,000)	Serum 75 (Titer 1:10,000)	Serum 34 (Titer 1:1,000)	Serum 27 (Ps. protea) (Titer 1:2,000)
Proteus vulgaris					
Feces 11	1:2,000	1:2,000	1:5,000	1:500	0
17	1:10,000	1:2,000	1:1,000	1:100	0
29	1:200	1:20,000	1:500	0	0
55	1:500	1:20,000	1:5,000	0	0
96	1:5,000	1:10,000	1:2,000	1:1,000	0
98	1:100	1:10,000	1:2,000	no test	no test
99	0	1:5,000	1:500	1:1,000	0
103	1:5,000	1:20,000	1:2,000	1:200	0
108	1:100	1:2,000	1:500	1:200	0
115	1:100	1:5,000	1:1,000	1:500	no test
Meat 34	1:30,000	1:20	1:10,000	1:1,000	0
78	0	0	0	0	0
113	0	0	0	0	no test
114	0	1:2,000	1:500	1:200	0
Water 79	1:5,000	1:5,000	1:200	1:1,000	0
110	1:100	1:10,000	1:500	0	0
111	1:200	1:10,000	1:500	0	0
112	1:500	1:500	1:200	0	0
Necropsied animals 53	1:5,000	0	1:200	no test	0
94	1:20,000	0	1:5,000	1:1,000	0
Wound bandage 75	1:10,000	0	1:10,000	1:1,000	0
Dog's saliva 76	1:5,000	0	1:5,000	1:1,000	0
Laboratory cultures 1	1:500	0	1:5,000	1:50	1:50
2	1:30,000	0	1:10,000	1:500	0
3	1:1,000	1:1,000	1:2,000	1:200	0
4	1:30,000	0	1:5,000	1:500	0
64	1:1,000	1:200	1:5,000	1:500	0
65	1:5,000	0	1:10,000	1:1,000	0
67	1:200	1:1,000	1:5,000	0	0
70	1:5,000	0	1:2,000	1:200	0
71	1:5,000	0	1:2,000	1:200	0
77	1:500	1:1,000	1:1,000	1:1,000	1:20
92	1:10,000	1:20,000	1:2,000	1:200	no test
Proteus species					
Feces 20	1:200	0	0	0	0
Blood 69	0	0	0	0	1:40
Air 49	0	0	0	0	0
Meat 63	0	0	0	0	0
Laboratory culture 80	0	0	0	0	0
B. cloacae					
Necropsied animals 51	0	0	0	0	0
Feces 68	0	0	0	0	0
Meat 43	0	0	0	0	0
B. zopfii					
B. proteus zopfii 73	1:100	0	0	0	0
Proteus zenkeri 85	0	0	0	0	0
Ps. protea					
Filtered water Ps. protea 86	0	0	0	0	1:400
87	Spontan.	Spontan.	Spontan.	Spontan.	1:100
Water (filtered ?) 24	1:200	1:40	0	0	1:2,000

In Table 5 the highest dilution in which agglutination occurred with the various organisms is recorded. These readings correspond to +, in case the next higher reading was ++, or to ++ if the next lower was —.

Preliminary tests of all cultures were made with the various serums in a dilution of 1:20, and if a positive result was obtained tests were made in higher dilutions: 1:50, 1:100, 1:200, 1:500, 1:1,000, 1:2,000, 1:5,000, etc., the highest dilution used depending on the titer of the serum.

A review of the results obtained with the 5 different immune serums and the various cultures as antigens brings out the following points:

Serums 4 (laboratory culture) and 75 (wound bandage) in general agglutinate the same cultures, there being only a few exceptions. Serum 34 (meat) which had a comparatively low titer (34, 1:1,000; 4, 1:30,000; 75, 1:20,000) agglutinated most of the cultures agglutinated by Serums 4 and 75, but in correspondingly lower dilutions. In many cases cultures were agglutinated by this serum in exactly 1/10th the dilution in which the same cultures were agglutinated by Serums 4 and 75 (cf. 11, 17, 3, 4, 64).

Cultures 4, 34 and 75 interagglutinated with the respective serums to the titer limit, except that 4 was agglutinated by Serum 34 in a dilution 1:500 instead of 1:1,000.

Serum 29 (feces) did not agglutinate 4, 34 or 75, but its homologous organism was agglutinated by Serums 4 and 75 in low dilutions (4, 1:200; 75, 1:500). Apparently the agglutinins of Serum 29 produced no receptors for 4, 34 or 75, but the agglutinins of Serums 4 and 75 did produce receptors for 29. It is to be expected, therefore, that strains agglutinated by Serum 29 should also be agglutinated by Serums 4, 34 and 75, and such proves to be the case with a few exceptions.

The agglutination of all the fecal cultures by Serum 29 is striking. This serum also agglutinated Culture 114 derived from meat, 79, 110, 111, 112 from water, and laboratory cultures 3, 64, 67, 77, and 92, though several of these were agglutinated in comparatively low dilutions (112, 64). Of the fecal cultures, Nos. 96, 98, 99, 103, 108, 115 were of recent isolation at the time of testing, while Cultures 11, 17, 29, 55 had been carried on artificial mediums for a considerable time. Apparently the length of time of artificial cultivation was not a factor in agglutination, since agglutination in equally high dilutions was obtained with old and with freshly isolated cultures.

All of the Proteus vulgaris cultures were agglutinated by Serum 75 in dilutions varying from 1:200 to 1:10,000, with the exception of 78 and 113 (cultures derived from meat). Most of these cultures were also agglutinated by Serum 4, though results were not always consistent, in some cases Serum 75 agglutinating in higher dilutions than 4 and vice versa.

The general deduction may be drawn from the results obtained with the cultures studied that two or more types may be established on the basis of agglutination reactions. Cultures 29, 110 and others may be cited as examples of one type, that in which agglutination was obtained in high dilutions with a serum produced by a culture derived from feces. Cultures 34, 94, 4 and others are examples of another type which is not agglutinated by such a serum. On this basis Cultures 78 and 113, which were not agglutinated by any serum, belong to still other types.

Whether this division into types is justifiable cannot, however, be determined without further evidence. Horowitz subdivided a number of cultures derived from feces, river water and meat into 5 different groups on the basis of agglutination reactions, all of which 5 groups contained cultures of fecal

origin. This author suggests variation in agglutinability of cultures kept under laboratory condtions.

The results obtained, while not exhibiting as clear cut a division into types as might be desired, nevertheless demonstrate conclusively that agglutination by an immune serum derived from one culture is not limited to the homologous culture. Variable degrees of adaptation to the animal body as suggested by Klieneberger[1] offers a possible explanation of the diversity of results obtained. Variability may also have a bearing on the matter. Cultures from different sources which show practically the same agglutinations with the 5 serums used may be selected from Table 5 (34 from meat and 94 from necropsied animal), (29 from feces and 11 from water), (75 from wound bandage and 76 from dog's saliva), but on the other hand, there are numerous discrepancies as regards agglutination with the various serums used among cultures derived from the same source.

CORRELATION OF FERMENTATION AND AGGLUTINATION TESTS

A comparison of the results obtained in the fermentation tests and the agglutination tests shows that correlation is present to a limited extent. Cultures derived from feces which were alike in fermentation reactions were agglutinated somewhat similarly. The cultures derived from meat, however, which had the same fermentation reactions throughout, showed three different sets of agglutination reactions, Culture 34 agglutinated in high dilutions by Serums 4, 34 and 75, but not by 29; Cultures 78 and 113 agglutinated by none of the serums used, and Culture 114 agglutinated in the highest dilution by Serum 29.

Of the cultures isolated from water, 79 corresponded to the cultures isolated from meat in fermentation reactions and 110, 111 and 112 corresponded to the fecal types. The three latter cultures were like the fecal type in agglutination reactions, and 79 was also probably more closely related to this type than the other.

Cultures 53 and 94 (necropsied animals) agreed with the cultures derived from meat in fermentation reactions, while 75 (wound bandage) and 76 (dog's saliva) were like the fecal type, but all showed similar agglutination reactions.

The above results confirm those obtained by Horowitz, who found similar variations among cultures derived for the most part from feces. The immune serums from strains which failed to ferment saccharose and maltose and gave a negative indol reaction agglutinated strains which fermented saccharose and maltose and gave a positive indol reaction, and strains which were alike in fermentaton reactions exhibited different agglutination propertes. Apparently strains may be closely related and yet differ in their fermentation reactions or agglutination properties. It is problematical as to what extent agglutination and fermentation reactions are correlated in this as in other groups of organisms. Hefferan found that a high degree of interaction as regards agglutination properties existed in a group of organisms more or less like B. prodigiosus which showed the same fermentation reactions.

The results obtained in the case of the organisms grouped under proteus species, B. cloacae, B. zopfii and Ps, protea when tested with immune serums derived from the strains of Proteus vulgaris used, are conspicuous by being negative throughout with few exceptions. (Immune Serum 4 agglutinated Culture 20 derived from feces in a dilution of 1:200 and B. proteus zopfii in 1:100.) These results correlate with cultural differences between these groups and Proteus vulgaris. The serum derived from Ps. protea 27 agglutinated very few of the Proteus vulgaris cultures, and these in low dilutions (Culture 1

in 1:50, 77 in 1:20, and 69 in 1:40). Ps. proteus 86 was agglutinated in a dilution of 1:400, and Culture 24 derived from the same source as 27 in a dilution of 1:2,000.

In addition to the above experimental work on the agglutination reactions of immune serums with the strains used in this study, further consideration of agglutination behavior of Proteus vulgaris as recorded in the literature is presented in the following pages.

AGGLUTINATION OF PROTEUS VULGARIS BY THE SERUM OF PATIENTS INFECTED WITH PROTEUS STRAINS

Agglutination of the organism concerned in cases of suppurative infections, cystitis, pyelonephritis and others are recorded in the literature. Klieneberger[1] and Frost have reviewed the works of Pfaundler, who demonstrated positive agglutination of Proteus vulgaris in intestinal catarrh, Wolf in cystitis and suppurative process of gonorrheal origin, Grassberger in suppurative infection, Lubowski and Steinberg in mastoid infection, Jochmann in suppurative mastoid infection, Haim in typhoid fever, Doerng in a case of food poisoning.

The more recent literature includes the work of Klieneberger,[2] who found cultures of Proteus vulgaris isolated in cases of urinary infection agglutinated by the serum of the patients in dilutions of 1:80 and 1:320. In a case of cystopyelitis the infecting strain was agglutinated by the serum of the patient in dilutions of 1:1,280 and 1:2,560. The high agglutination he considered probably due to general infection. The same author reports a case of sinus thrombosis due to Proteus vulgaris in which the organism was agglutinated in a dilution of 1:320.

Flinzer describes a case of rib abscess in which Proteus vulgaris was isolated, and in which the serum of the patient agglutinated the organism in a dilution of 1:6000.

Maymone cites the case of a general septicemia following a gunshot wound in which Proteus vulgaris was isolated from the blood and which was agglutinated by the serum of the patient in a dilution of 1:2,500. A case of proteus meningitis and proteus sepsis in a new-born child is described by Goebel, in which Proteus vulgaris in pure culture was isolated from the blood and spinal fluid and which was agglutinated in a dilution of 1:60.

Thjøtta reports a case of pyemia with abscesses of the supra- and infraclavicularis in which Proteus vulgaris isolated from the pus was agglutinated in a dilution of 1:80. By the complement fixation test an amount as low as 0.0063 of the patient's serum produced strong fixation, while a normal serum required 0.1.

Agglutination of the serum of patients with intestinal infections with proteus strains are described by several authors, though others have obtained negative results in such cases.

Archibald isolated an organism in a case of choleraic diarrhea classified by him as B. proteus fluorescens which was agglutinated by the patient's serum in low dilutions. Mandel describes an organism in a food poisoning epidemic which he identifies as Proteus vulgaris, but concerning the classification of which there seems to be some question, which was agglutinated by the serum of patients in a dilution of 1:25.

Bertrand, who studied organisms of the proteus group isolated from cases of infantile diarrhea, was unsuccessful in demonstrating clearly agglutination of the organisms by the blood of infants from whom the organisms were isolated. He was only able to obtain and then only irregularly an agglutination as high as 1:20.

Horowitz in a study of Proteus vulgaris concerned in an epidemic simulating dysentery, was unable to show agglutination of the organism even in a dilution of 1:25, in explanation of which fact he thinks the presence of the organism in the intestine may have been of such short duration, that there was not sufficient time for the elaboration of specific antibodies.

Thjøtta isolated a culture of Proteus vulgaris in a case of diarrhea resembling dysentery which was not agglutinated in a dilution even as low as 1:10 by the serum of another patient suffering with pyemia due to proteus infection.

AGGLUTINATION OF PROTEUS STRAINS BY THE SERUM OF PATIENTS SUFFERING FROM WEIL'S DISEASE

Jaeger has ascribed to an organism classified by him as Bacillus proteus fluorescens resembling Proteus vulgaris except in the matter of greenish pigment, an etiologic rôle in Weil's disease. This organism was isolated from the urine, blood and organs of patients dead of the disease. Altho not now generally accepted as the causal agent, agglutination of Proteus fluorescens and Proteus vulgaris by the serum of patients has been reported by several authors. The works of Lüdke and Pfaundler in this connection are referred to by Frost, both of these authors finding the serum of patients agglutinating the organism in dilutions of 1:20 or higher. Brüning isolated an organism which he describes as Bacillus proteus fluorescens from the urine and feces of a patient with infectious icterus (Weil's disease) in which the organism was scarcely agglutinated in a dilution of 1:50. The serum of the patent, however, agglutinated B. typhosus strongly in a dilution of 1:50. Abeles tested human icterus serum and did not obtain agglutination in dilutions higher than 1:40.

AGGLUTINATION OF PROTEUS CULTURES BY THE SERUM OF TYPHOID FEVER PATIENTS

A review of the literature dealing with agglutination of proteus cultures by the serum of patients with typhoid fever as well as the agglutination of the typhoid bacillus by the serum of patients with proteus infection has been included by Frost in his work on the agglutination of Ps. protea by the serum of typhoid fever patients.

Abeles also investigated the action of human typhoid serum on 6 proteus strains derived from enteritis stools. Seventeen cases were studied, and in only 2 cases was an agglutination as high as 1:40 obtained, while in the others agglutination rarely exceeded 1:10.

In my work a number of tests were made on specimens of serums obtained from the various Marine hospitals in this country. These were tested for agglutination reactions against B. typhosus Rawling, and those serums which gave positive results were then tested against a number of strains of Proteus vulgaris and a strain of Ps. protea. Several of the strains not agglutinated by B. typhosus and therefore considered as normal in this respect, were also tested against Proteus vulgaris cultures.

The cultures used in the first set of tests included the laboratory strains 1, 2, 3, 4, and Culture 11, isolated from feces. These were all tested against the same serums. Of these, Cultures 3 and 11 showed no agglutination with the serums used, except that 11 was agglutinated in a dilution of 1:10 by one of the serums. Cultures 1, 2, 4 showed agglutination in varying degrees, as indicated by Table 6.

Agglutinations were recorded at the end of 1 hour and in 24 hours, a 1-hour incubation period being used. The tubes were then placed at cold room tem-

TABLE 6

AGGLUTINATION TESTS OF TYPHOID-AGGLUTINATING SERUMS WITH PROTEUS VULGARIS AND PSEUDOMONAS PROTEA

Serum	B. typhosus (Rawling)						Culture 86 (Ps. protea 362t)						Culture 1 (Proteus vulgaris I)						Culture 2 (Proteus vulgaris II)						Culture 4 (Proteus mirabilis)					
	1:10		1:20		1:40		1:10		1:20		1:40		1:10		1:20		1:40		1:10		1:20		1:40		1:10		1:20		1:40	
	1 hr.	24 hr.	1 hr.	24 hr.	1 hr.	24 hr.	1 hr.	24 hr.	1 hr.	24 hr.	1 hr.	24 hr.	1 hr.	24 hr.	1 hr.	24 hr.	1 hr.	24 hr.	1 hr.	24 hr.	1 hr.	24 hr.	1 hr.	24 hr.	1 hr.	24 hr.	1 hr.	24 hr.	1 hr.	24 hr.
7051	4+	4+	4+	4+	4+	4+	2+	3+	+	2+	—	+	—	4+	—	3+	—	2+	—	—	—	—	—	—	—	—	—	—	—	—
7052	3+	4+	2+	2+	—	+	+?	3+	—	2+	—	+?	—	4+	—	2+	—	+	4+	4+	—	2+	—	+	+	2+	+?	+	—	+?
7053	—	4+	—	2+	—	+	2+	4+	+	3+	—	2+	+?	4+	—	3+	—	2+	—	—	—	—	—	—	—	—	—	—	—	—
7086*	4+	4+	4+	4+	4+	4+	3+	4+	2+	3+	+?	2+	+	4+	+?	4+	+?	4+	—	—	—	—	—	—	—	—	—	—	—	—
7103	+?	3+	+	2+	—	+	2+	4+	—	2+	—	+	—	2+	—	+	—	+?	—	—	—	—	—	—	—	—	—	—	—	—
7106†	2+	4+	2+	4+	2+	4+	3+	4+	+±	3+	+?	3+	—	2+	—	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—
7107	+	4+	—	2+	—	+	+?	3+	—	2+	—	+	—	3+	—	2+	—	+	—	—	—	—	—	—	—	—	—	—	—	—
7112	2+	3+	+?	2+	—	+	3+	4+	3+	4+	+	3+	—	3+	—	2+	—	+	—	—	—	—	—	—	—	+?	—	—	—	—
7118	3+	4+	3+	4+	2+	4+	3+	4+	+	4+	—	2+	+	4+	+?	3+	—	2+	—	2+	—	+	—	+?	—	2+	—	+	—	+?
Control No serum	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

1:80 1:160 1:320

* Patient received one inoculation of typhoid vaccine one week before blood was drawn.

† 7106 + 2+ — 2+ — +

perature over night. Tests in this series were made only in dilutions of 1:10, 1:20, and 1:40. The record of agglutination in the highest dilution, 1:40, furnished a comparison of the relative agglutinating powers of the various sera.

The serums which agglutinated B. typhosus strongly, 7051, 7086, 7106 and 7118, agglutinated Ps. protea somewhat less strongly (Table 6). Proteus vulgaris 1 was agglutinated by these serums also less strongly than B. typhosus, corresponding more closely to Ps. protea, with the difference that practically no agglutination of the Proteus vulgaris culture was evident in 1 hour, though in 24 hours the reaction was marked. Proteus vulgaris Cultures 2 and 4 were not agglutinated by any of these serums except by 7118, the reactions being less marked than with Culture 1.

TABLE 7

SHOWING RESULTS OBTAINED WITH TYPHOID AGGLUTINATING SERUMS AND STRAINS OF PROTEUS VULGARIS AND PSEUDOMONAS PROTEA

	Serum 7245 (1:160)						Serum 7254 (1:320)							
	1:10		1:20		1:40		1:10		1:20		1:40		Control	
	2 hr.	24 hr.	2 hr.	24 hr.	2 hr.	24 hr.	2 hr.	24 hr.	2 hr.	24 hr.	2 hr.	24 hr.	2 hr.	24 hr.
Proteus vulgaris														
Feces 11	—	—	—	—	—	—	—	—	—	—	—	—	—	—
17	—	—	—	—	—	—	—	—	—	—	—	—	—	—
29	.....		.....		.....		—	—	—	—	—	—	—	—
55	.....		.....		.....		—	—	—	—	—	—	—	—
Meat 34	.....		.....		.....		—	—	—	—	—	—	—	—
35	.....		.....		.....		—	—	—	—	—	—	—	—
Laboratory cultures 1*	—	++	—	++	—	++	—	+	—	+	—	+	—	—
2	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—	—	—	—	—	—	—
64	.....		.....		.....		—	—	—	—	—	—	—	—
65	.....		.....		.....		—	—	—	—	—	—	—	—
67	.....		.....		.....		—	—	—	—	—	—	—	—
Proteus species														
Feces 20	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Air 49	.....		.....		.....		.....		—	—	—	—	—	—
Meat 63	.....		.....		.....		—	—	—	—	—	—	—	—
B. cloacea														
Feces 68	.....		.....		.....		—	—	—	—	—	—	—	—
Ps. protea														
Water 24	.....		.....		.....		+	++++	—	+++	—	+	—	—

* Laboratory culture 1: 1:80 — ++ 1:160 — +

The remaining serums, 7052, 7053, 7103, 7107 and 7112, agglutinated B. typhosus in lower dilutions than the preceding serums. The results obtained with the Ps. protea culture and these serums were as high as those with B. typhosus, and in the case of Serum 7112 higher. The results with Proteus vulgaris 1 corresponded in general with those obtained with the B. typhosus and Ps. protea cultures, but as before no agglutination was evident in 1 hour. Cultures 2 and 4 were agglutinated by Serum 7052, but by none of the others.

A larger number of cultures were then tested with two serums which agglutinated B. typhosus in comparatively high dilutions (Serum 7245 in 1:160 and 7254 in 1:320). None of the cultures tested except Proteus vulgaris 1 and No. 24 (Ps. protea) gave positive results (Table 7). The results obtained with the latter culture confirm the results obtained by Frost.

AGGLUTINATION OF PROTEUS VULGARIS CULTURES BY NORMAL HUMAN SERUM

Agglutination of Proteus vulgaris by normal serums has been considered by several authors. Lannelongue and Achard found in only one case tested by them strong agglutination of Proteus vulgaris by normal serum. Klieneberger[1] tested 6 normal serums against 22 strains of Proteus and found none positive except some strains of B. zopfii and Proteus zenkeri. Abeles tested serums of patients with bronchitis, tuberculosis, gastro-enteritis, and paralysis, classing these under normal human serums, against 6 proteus strains and found none agglutinated in higher dilutions than 1:40.

In my study serums which were known to be normal as regards typhoid agglutination, namely, showing negative agglutination with B. typhosus, were tested with a culture of Ps. protea and Cultures 1, 2, 3, 4, and 11 of Proteus vulgaris (Table 8). Positive agglutinations in dilutions of 1:20 to 1:40 (with one exception, Serum 749) were obtained with the culture of Ps. protea and Proteus vulgaris 1, though as before agglutination of the proteus culture was not evident before 24 hours. A comparison with the results obtained with the serums agglutinating B. typhosus shows that agglutination took place in higher dilutions with the typhoid serums than with the normal serums. Proteus cultures 2, 4 and 11 were not agglutinated by any of the serums except with 7050 (dilution 1:20).' This corresponds in general with the behavior of these cultures with typhoid serums.

The results of the tests made indicate that occasional cultures of Proteus vulgaris may be agglutinated by the serum of typhoid fever patients, but agglutination takes place, in general, in lower dilutions and is not as characteristic as with the homologous organism or even as with Ps. protea. It is not apparent why certain other cultures of Proteus vulgaris which are alike as regards cultural behavior and agglutination by Proteus immune serums do not behave similarly.

The same culture (Proteus vulgaris 1) which was agglutinated by typhoid serums also showed a tendency to be agglutinated by serums which were normal as regards typhoid agglutination, positive results being obtained in somewhat lower dilutions.

AGGLUTINATION OF TYPHUS SERUM WITH PROTEUS VULGARIS, THE SO-CALLED WEIL-FELIX REACTION

The agglutination of certain strains of Proteus vulgaris by the serum of patients suffering from typhus exanthematicus has recently been described by Weil and Felix. These organisms were isolated from the urine of patients suffering from the disease. Agglutination occurs regularly in dilutions of 1:100 to 1:2,000. These strains are described as differing from the saprophytic strains of Proteus vulgaris, though such strains may be agglutinated by a serum artificially produced by the strains in question. Epstein and Morawetz reported agglutination in dilutions as high as 1:10,000 occasionally. Dietrich tested the serum of 81 typhus fever patients and 100 normal persons and found that in the former class the serums of all agglutinated the organism in dilutions of 1:100 to 1:6,400, while in the latter class all failed to agglutinate in dilutions up to 1:50 and 1:100. Paneth found the agglutinins present in the serum of typhus fever patients to be transitory.

The high dilutions in which agglutination occurs in typhus fever cases is noteworthy, being higher than in cases reported of infection with Proteus vulgaris itself or in cases of typhoid fever or in Weil's disease.

TABLE 8

AGGLUTINATING TESTS OF NON-TYPHOID-AGGLUTINATING SERA WITH PROTEUS VULGARIS AND PSEUDOMONAS PROTEA

Serum	B. typhosus (Rawling)						Culture 86 (Ps. protea 362t)						Culture 1 (Proteus vulgaris I)						Culture 2 (Proteus vulgaris II)						Culture 4 (Proteus mirabilis)					
	1:10		1:20		1:40		1:10		1:20		1:40		1:10		1:20		1:40		1:10		1:20		1:40		1:10		1:20		1:40	
	1 hr.	24 hr.	1 hr.	24 hr.	1 hr.	24 hr.	1 hr.	24 hr.	1 hr.	24 hr.	1 hr.	24 hr.	1 hr.	24 hr.	1 hr.	24 hr.	1 hr.	24 hr.	1 hr.	24 hr.	1 hr.	24 hr.	1 hr.	24 hr.	1 hr.	24 hr.	1 hr.	24 hr.	1 hr.	24 hr.
7049	—	—	—	—	—	—	—	+?	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
7050	—	—	—	—	—	—	2+	3+	+	2+	+?	+	—	2+	—	+	—	+?	+	2+	+?	+?	—	—	+	2+	+?	+	—	—
7054	—	—	—	—	—	—	+	3+	—	2+	—	+?	—	+	—	+?	—	—	—	—	—	—	—	—	—	—	—	—	—	—
7088	—	—	—	—	—	—	2+	3+	+?	2+	—	+?	+?	3+	—	3+	—	+	—	—	—	—	—	—	—	—	—	—	—	—
7090	—	—	—	—	—	—			3+	4+	+?	2+			—	2+	—	+			—	—	—	—			—	—	—	—
7114	—	—	—	—	—	—	—	2+	—	+	—	—	—	2+	—	+	—	+?	—	—	—	—	—	—	—	—	—	—	—	—
7119	—	—	—	—	—	—	3+	4+	—	2+	—	+	+?	4+	—	2+	—	+?	—	—	—	—	—	—	—	—	—	—	—	—
7120	—	+	—	—	—	—	2+	4+	—	2+	—	—	—	2+	—	+	—	+?	—	—	—	—	—	—	—	—	—	—	—	—
7122	—	+	—	—	—	—	3+	4+	2+	4+	—	2+	—	2+	—	+	—	+?	—	—	—	—	—	—	—	—	—	—	—	—
Control No serum	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

PROTEIN METABOLISM

Putrefaction and the proteolytic enzyme of Proteus vulgaris have been the object of study of a number of investigators, including Feltz, Herter and Ten Broeck; Glenn; Drummond; Kendall and Walker; Sperry and Rettger; Rettger, Berman and Sturges; Rettger and Newell; Tissier and Martelly; Berthelot, and others.

It has been common to consider Proteus vulgaris among the true putrefactive bacteria, or those which decompose native albumin. Rettger, Berman and Sturges, however, have shown that the decomposition of complex proteins like egg albumin, as well as of albumoses and peptones by certain organisms with proteolytic and putrefactive properties including Proteus vulgaris, is due primarily to the proteolytic enzyme elaborated by such organisms, and not to the direct action of the bacteria themselves. Mediums containing purified egg albumin and dialyzed proteoses were not attacked if the inoculations were made with very few organisms or from cultures less than 24 hours old in which there had not been sufficient time for the elaboration of the proteolytic enzyme.

Drummond states that peptone is necessary in the medium for the elaboration of the enzyme, though the results obtained by Rettger and associates indicate that the amino acids contained in peptone are utilized for this purpose. If these are available for furnishing the nitrogen necessary for bacterial development, the enzyme elaborated may bring about complete hydrolysis of native proteins.

Feltz demonstrated that the presence of glucose in mediums inhibited the production of indol, an index of proteolytic action. Glenn has shown that the presence of acid in mediums produced as the result of the breaking down of the carbohydrate was the factor concerned. Liquefaction of gelatin likewise was inhibited by the presence of carbohydrates including dextrose, saccharose, levulose, mannose, galactose, maltose and raffinose. Rettger, Berman and Sturges also showed that the presence of 1% of glucose in peptone mediums inoculated with gelatin-liquefying bacteria prevented reduction in the amounts of proteoses and peptones for a period of two weeks.

Kendall and Walker studied the action of the bacteria-free enzyme, obtained by filtration of plain broth or gelatin cultures through unglazed porcelain, on dextrose gelatin and found that the liquefaction of gelatin by the proteolytic enzyme was not inhibited by the presence of the dextrose, and that moderate amounts of organic acids also did not interfere.

Tissier and Martelly and Glenn describe the enzyme as tryptic. Glenn considers the enzyme of B. cloacae, on the other hand, to be peptic in its action.

Herter and Ten Broeck demonstrated the fact that the curdling of milk was due to the enzyme elaborated by the organism and not to acid production. By neutralizing the acid formed with $CaCO_3$, they found that coagulation still took place. Kendall, Day and Walker showed that the action of proteus on milk proteins was very vigorous, about 6% of the total nitrogen content being decomposed in 3 weeks.

OCCURRENCE

Proteus vulgaris is usually considered to be very widespread in its distribution, but whether it is ubiquitous to the extent that certain authors consider it, has not been definitely established. Decomposing organic matter, particularly of animal origin, appears to be the habitat by preference of this organism. Its occurrence in water, in the soil, in the digestive tract is probably not as frequent as is commonly supposed.

Burton and Rettger in a study of coli-like organisms isolated from 1,000 samples of soil, leaves, twigs, flowers, bark of trees, berries, snow, etc., comment on the fact that no recognizable members of the proteus group were encountered, and conclude that their habitat is elsewhere than in the soil. These authors used the method of inoculating all samples into glucose broth tubes, which should favor the development of Proteus vulgaris.

Rogers, Clark and Evans in a study of 166 coli-like organisms from grains, including corn, barley, wheat and oats, include a group of 7 cultures, of which 6 appear to correspond to the Proteus vulgaris type. These authors also made use of glucose broth in the isolation of their organisms. The results obtained indicate that Proteus vulgaris is relatively much less frequent than the B. coli or Lactis aerogenes types of organisms on grains.

A number of authors have observed the relatively infrequent occurrence of Proteus vulgaris in normal feces. This matter has been considered at greater length in this paper under the heading Pathological Occurrence.

Meat.—The presence of Proteus vulgaris in partially decomposed meat has been observed on numerous occasions. Cantu tested 22 specimens of such meat and found Proteus vulgaris present in all. A number of food poisoning epidemics have been ascribed to the presence of Proteus vulgaris in meat products. These are considered under a separate heading. The occurrence of Proteus vulgaris in sausage and chopped meat which had not yet reached the stage of noticeable decomposition has been noted by a number of authors. Cantu found the organism present in 33.3% of samples of raw sausages. Sacquépée and Loygue found bacteria belonging to the proteus group present in 36% of samples of sausages and other meat products, B. paratyphosus being present only once, and Cary found Proteus vulgaris present in 33% of sausages tested. Zweifel reports the occurrence of 23 organisms which resembled paratyphoid B, but which he states in reality belonged to the proteus group in 248 samples of raw chopped meat tested.

Water.—Jordan in a study of 543 organisms isolated from river water, includes 23 which correspond to Proteus vulgaris type. Cantu tested 80 samples of potable water and 1.25% gave positive results for the organism.

Digestive Tract of Animals.—Berthelot reports Proteus vulgaris always present in the feces of normal rats. Choukévitch showed it to be present in the intestinal contents of horses in 25% of tests made. The organisms were present, however, in relatively small numbers, and it was necessary to use a considerable amount of material which was allowed to putrefy to permit of the development of putrefactive organisms. Cantu found Proteus vulgaris present in the excrement of 4% of hens whose feeding was normal and in 66.6% of those which were fed with meat. Jordan[2] reports the occurrence of proteus vulgaris in swine feces in the proportion of 41 out of 725 colonies isolated from 58 hogs. Most of these were isolated by a preliminary growth in brilliant green broth, followed by plating on Endo medium.

Soil.—Though Burton and Rettger consider the occurrence of Proteus vulgaris rare in soil, Cantu reports 44.2% samples of soil positive for the organism. The presence of decaying organic matter of animal origin in soil has a bearing on the subject.

Miscellaneous Food Substances.—Cantu investigated the presence of Proteus vulgaris in a number of food substances and found that the organism occurred on celery, melons, bananas, in cheese and other foods. Two hundred samples of milk were tested and 3.5% contained the organism.

Mouth Secretions, etc.—Proteus vulgaris has been shown to be present in the mouth secretions by Feltz, and Cantu. The latter author also found the organism present in a small percentage of tests of the skin.

OCCURRENCE, PATHOLOGICAL

The occurrence of Proteus vulgaris in pathologic conditions has been very completely covered in the review of the literature of the proteus group up to the year 1898 by Meyerhof. This includes the occurrence of the organism in mixed infections, infection with the organism alone, local and general. Mixed infections with streptococci are described in suppuratve phlegmons, parametritis, puerperal fever and meningitis; with staphylococci in parametritis, osteomyeltis, meningitis, brain abscess; with pneumococci in pneumonia and gangrene of the lungs; with B. coli in cystitis. Pure infection is described in proteus cystitis, pyelonephritis, abscesses, pleuritis and peritonitis. Among general infections are included infections due to meat poisoning, as well as Weil's disease, though in the latter case, the etiology has recently been established on another basis.

In the more recent literature a number of infections both primary and secondary are described. The following is a partial résumé of such infections.

Eye Infections.—These are described by Hanke and Tertsch, and Wirtz, the case described by the former being an infection with an unusually virulent strain of Proteus vulgaris, and the latter a case of infection with B. tetani and several other organisms including Proteus vulgaris as secondary agents. Larson and Bell[1] in a study of Proteus vulgaris derived from pathologic sources include one from an eye infection following cataract operation.

Ear Infections.—Klieneberger[2] describes an infection by Proteus vulgaris in sinusthrombosis in which the serum of the patient agglutinated the organism in a dilution of 1 : 320. Lauffs found Proteus vulgaris present 6 times out of 26 in mastoid abscesses and their complications, 2 times in pure culture and once with streptococci and diplococci. Urbantschitsch isolated Proteus vulgaris in a perisinus abscess.

Other Suppurative Processes.—Proteus vulgaris in brain abscess is described by Leutert, in sublingual abscess by Ware, in rib abscess by Flinzer, in pustule simulating anthrax by T. Orr, in periurethral abscesses by Bertelsmann and Mau, in uterine abscess by Broughton-Alcock, in a laparotomy wound by Larson and Bell.[1] Ungermann found Proteus vulgaris in 37% of 38 diseased appendices, but also in 2 normal, and always with other organisms, and therefore considered it only of secondary importance. The presence of Proteus vulgaris in purulent war wounds in association with other organisms has been described by Doyen and Yamanouchi, Tissier, Goadby and Stewart.

Infections of the urinary tract are described by Klieneberger,[2] Jeffreys, van Loghem, and Saathof. The latter author considers mixed infection of B. coli and Proteus vulgaris as more severe than of B. coli alone. Geraghty finds organisms of the proteus group usually associated with alkaline cystitis.

Osteo-periostitis of the inferior maxillary caused by Proteus vulgaris is recorded by Domínguez.

Grossmann describes a case of general peritonitis, in which Proteus vulgaris of high virulence was isolated.

A case of gas gangrene in which the bacillus of malignant edema was associated with Proteus vulgaris is described by Heyde. Larson and Bell[2] refer to a culture of Proteus vulgaris isolated in a case of gas gangrene of the lungs.

The presence of Proteus vulgaris in the circulating blood has been recorded by several authors. Jochmann isolated Proteus vulgaris and streptococci from the blood of a patient with a suppurative mastoid infection. Bertelsmann and Mau demonstrated the presence of Proteus vulgaris in large numbers in the blood in a patient suffering from periurethral abscess. Libman and Celler in a study of a number of cases of otitis media found the organism once in blood cultures. Maymone describes a case of septicemia due to proteus infection following a gunshot wound. Pauly describes a wound infection with Proteus vulgaris. Following an operation pleural infection was evident and the organism in pure culture was isolated from the resulting empyema. This author considers it possible, in the light of the above case and from a review of the literature, that a local tissue necrosis in a patient whose condition otherwise is bad may increase the virulence of Proteus vulgaris and allow its passage into the blood. Larson and Bell describe Proteus vulgaris isolated from the heart's blood of a patient who had died from peritonitis following a gunshot wound of the intestines.

OCCURRENCE, PATHOLOGICAL — DIGESTIVE TRACT

The occurrence of Proteus vulgaris in the digestive tract is a matter which has received a considerable amount of attention. For the sake of convenience, in this discussion the occurrence of the organism in the normal digestive tract will be considered together with its pathologic occurrence, although strictly it should not perhaps be here included.

Certain authors state that Proteus vulgaris is a habitual inhabitant of the intestinal tract just as B. coli. This statement, however, is not borne out by the investigations of several recent workers. It seems certain that numerically Proteus vulgaris is much less abundant than B. coli in normal feces. In my work this organism was isolated only 5 times in 80 tests made by streaking pooled specimens of feces from several subjects, directly on Endo plates, Endo medium having been shown to be a favorable medium for the growth of Proteus vulgaris; B. coli, on the other hand, was present in practically all specimens.

Cantu found Proteus vulgaris in 30% of normal human stools. Feltz as a result of his researches decided that Proteus vulgaris in feces occurs only rarely. In 12 healthy subjects he isolated it in only one case. He ascribes the rare occurrence of the organism in feces to the fact that the gastric juice plays an antiseptic rôle with organisms introduced by means of food.

In an investigation of several thousand samples of feces from dysentery convalescents, Stewart found Proteus vulgaris present less than a dozen times, and considers proteus uncommon as an inhabitant of the intestinal tract.

A number of authors have considered the occurrence of Proteus vulgaris in the stools of normal infants. Bertrand studied 24 specimens from healthy infants and found the organism twice. Metchnikoff considers normal infants in whose stools the organism is present as carriers of Proteus vulgaris. He reports two such "carriers" among 6 normal infants. In an institution 18, or 57%, of 33 children proved to be "carriers." This percentage has been considerably lower than among infants suffering from gastro-enteritis, of which 96% were found by this author to have the organisms in their stools. Bahr examined the feces of 27 normal children and found Proteus vulgaris in two cases. Horowitz examined the dejecta of 40 infants and was unable to isolate the organism a single time.

The occurrence of Proteus vulgaris in diarrheic stools, on the other hand, has been frequently noted, especially in infants. Cantu found it in 40% of diarrheic stools. Horowitz, who investigated an epidemic which was of the

nature of dysentery, being characterized by bloody stools, demonstrated Proteus vulgaris in 24 out of 63 cases, or 38%. Dysentery, pseudo-dysentery or typhoid organisms could not be isolated from the stools and the serum of patients failed to agglutinate the dysentery bacillus. As a result of his studies this author believes Proteus vulgaris penetrating into the intestinal tract, either with contaminated water or food, may provoke gastro-enteritis

Infantile Diarrhea.—Proteus vulgaris as concerned in infantile diarrhea in this country has been described only occasionally. Booker as a result of his investigations of the subject decided that not a single organism was responsible, but that streptococci and Proteus vulgaris were of most frequent occurrence. On the other hand, a number of European workers report the frequent occurrence of Proteus vulgaris in diarrheic stools of infants.

Bertrand studied the stools of 55 infants suffering from infantile diarrhea (in London) and was able to demonstrate Proteus vulgaris in all. Metchnikoff studied 217 cases of infantile diarrhea covering a period of four years and isolated the organism in 204, or 96%. He attributes the infection of infants to carriers and flies. Gildemeister and Baerthlein studied infantile diarrhea in Berlin and isolated Proteus vulgaris in 31% of 70 cases. Tsiklinsky investigated infantile diarrhea in Moscow and Paris, including 70 cases in the former locality and 8 in the latter, during a period of four years. Proteus vulgaris was isolated in 65% of the cases studied, sometimes in almost pure culture. This author also isolated it in 20% of normal feces, but found these cultures not virulent for rabbits, in comparison with the cultures isolated in the diarrheic cases, ¼-½ c c of which invariably killed rabbits and guinea-pigs when inoculated subcutaneously. Thjøtta isolated a culture of Proteus vulgaris from the stools of a child with dysentery symptoms.

The matter of symbiosis of Proteus vulgaris with other organisms in infantile diarrhea has been considered by several authors. Berthelot studied the association of Proteus vulgaris with B. aminophilus intestinalis, an organism belonging to the lactis aerogenes group, and found that either organism alone produced no ill effect in rats on an exclusive milk diet, but when administered together, diarrheic symptoms were produced in 6-8 days. Metchnikoff found that nursing rabbits receiving by mouth mixed cultures of the two organisms, Proteus vulgaris and B. welchii, developed symptoms similar to experimental cholera. Tsiklinsky isolated B. perfringens in 10% of 78 cases (Proteus vulgaris in 65%) and considers symbiosis of these organisms plays an important rôle in infantile diarrhea.

PROTEUS VULGARIS IN FOOD POISONING EPIDEMICS

The relation of proteus to food poisoning epidemics has been discussed by various authors. Epidemics ascribed to this organism have been of very much less frequent occurrence than similar epidemics ascribed to members of the paratyphoid group.

Food poisoning epidemics, in which this organism has been considered to bear a causal relationship, described in the literature are recorded in Table 9. In all of these epidemics with two exceptions meat products have been concerned. Dieudonné reports an epidemic in which infected potato salad was incriminated, and Ohlmacher attributes one to the eating of infected oatmeal.

In certain of these epidemics the organism was isolated from the food product and an effort was made to establish its causative relation by feeding experiments with the culture as well as by feeding the suspected food. In several other epidemics the organism was isolated from the stools of patients, and tests made on animals with the culture thus obtained.

In the epidemic described by Levy, pure cultures of the organism were obtained on gelatin plates planted with vomitus and stools of a patient who later died. Animals injected died of hemorrhagic diarrhea. The same organism was isolated from slime in the bottom of an ice chest in which the meat in question was considered to have become infected. The blood of animals tested was sterile and the author considers the effects produced to have been due to the production of the toxic substance, sepsin, in the decomposed meat rather than to infection.

Wesenberg isolated an organism which corresponded to Hauser's Proteus vulgaris, except that he states it was more virulent for animals and failed to produce indol. He believes the causal relationship of the organism to the epidemic was established by the fact that the organism was found so exclusively in the meat tested, and that broth cultures in 0.2 c c amounts injected subcutaneously killed mice in the same way as the infected meat. However, no tests were made on the vomitus and stools of the patients.

Ohlmacher describes an epidemic which he ascribes to the eating of oatmeal which he believed had been infected with Proteus vulgaris. The oatmeal prepared the evening before and warmed the following morning had been probably contaminated with dust from falling plaster. None of the suspected food or the vomitus of patients was available for examination, but the organism was isolated from the plaster dust.

Silberschmidt sought to establish the relationship of the organism in the epidemic investigated by him by bacteriologic examination of the sausage in question and of samples of "good" sausages as controls, and by feeding and inoculation experiments with the meat and with the organism isolated. Proteus vulgaris was not isolated a single time from the control sausages plated directly on gelatin, and only once from such sausages planted first in broth and then on gelatin. The organism was present in large numbers in the suspected sausages. The suspected sausages in the dry state fed to mice and cats produced no ill effects, but if incubated in broth for several days and fed in the moist state caused death of mice and guinea-pigs in 2 days. Broth cultures of the organism in the suspected sausages injected subcutaneously caused the death of white mice in 7-24 hours, while injection of control cultures from the "good" sausages sometimes, but not always, caused death. Guinea-pigs and rabbits died in 5-9 days when injected subcutaneously with the cultures from the suspected sausages. Proteus vulgaris was recovered in several cases from the peritoneal fluid and from the heart blood of the injected animals. The author also attempted to determine the presence of toxic substances in the meat by extraction, and injecting animals with the extract, but obtained inconclusive results. He considers the harmful effects due to the multiplication of the organism in the intestines, with the formation of toxic substances; in other words, he believes that infection is accompanied by intoxication, but that infection is of primary importance.

Glücksmann reports cases of food poisoning supposedly caused by the ingestion of smoked meat which had come from a sick hog, one patient succumbing. The organs from the body were received for bacteriologic examination, but were in such a state of decomposition that nothing definite could be established from their examination. Portions of the meat were also examined, and from these Proteus vulgaris was isolated, almost in pure culture. Mice injected with broth cultures in amounts varying from 0.1-1 c c died in 18 hours to 4 days, and showed symptoms of diarrhea, while guinea-pigs receiving (subcutaneously) 0.5-2 c c died in 7 days. Postmortem examination

TABLE 9

FOOD POISONING EPIDEMICS ASCRIBED TO PROTEUS VULGARIS

Author	Place	Date	No. of Persons Affected	Organism	Suspected Source of Infection	Symptoms	Convalescence or Deaths	Animal Tests
Johne.....	Chemnitz, Germany	1886	About 160	Proteus mirabilis	Sausages.....	Aching of limbs, vomiting, malaise, diarrhea, headache, fever, dizziness	.....	No effect on animals
Levy, E.....	Strassburg, Germany	1893	18	Proteus	Meat kept in ice box which was unclean	Bloody vomitus and stools, great weakness, slight fever	1 death; others convalescent 2-4 weeks	Animals (inoculated?) died with symptoms of hemorrh diarrhea
Wesenberg, G. ..	Mansfeld, Germany	1897	63	.....	Chopped meat from sick cow. Eaten raw or not well cooked. Meat had been kept in damp, musty cellar	Diarrhea, headache, pain in limbs, muscular weakness, dizziness, faintness	1 death (child); others convalescent 5-7 days	Mice and guinea-pigs inoculated with the suspected meat in 18 hours to 3 days; 0.2 c c broth culture killed mice, than 0.2 c c caused weakness
Ohlmacher, A. P.	Gallipolis, Ohio	1897	218	Proteus vulgaris	Oatmeal, which was cooked, allowed to stand over night and reheated. Contaminated by dust	Chills, aching of limbs, severe headache, nausea and vomiting, pain in abdomen, profuse diarrhea, dizziness, pulse 100-120, temperature 102.5-105	Convalescence in 4 days to 2 weeks	Suspected food not available. Oatmeal was artificially taminated with cultures of organism and treated by ex tion method. Extract caused septic peritonitis and deat 3 guinea-pigs (½-1 c c injected intraperitoneally)
Silberschmidt, W.	K. & St. G., Switzerland	1898	43	Proteus vulgaris	Sausages.....	Aching of limbs, chills, fever, vomiting, diarrhea, headache, thirst	1 death (18 yrs. old), some convalescent in 2-5 days, others 7-30 days	Feeding: Feeding suspected sausages did not affect mice or cats Feeding suspected sausages in moist state after incub in broth caused death of white mice and guinea-pig 2 days Inoculation: Cultures of suspected sausages caused death of mice, guinea-pigs, rabbits, by subcutaneous, intraperitoneal and intravenous inoculations Cultures of recovered organism injected subcutaneously caused death of mice, also killed guinea-pigs injected intraperitoneally. Extract of sausages was toxic for rabbits, especialy when injected intravenously
Glücksmann, S. ..	St. Gallen, Switzerland	1899	2	Proteus vulgaris	Smoked, uncooked meat of sick hog	Acute gastro-enteritis (fever, vomiting, diarrhea) collapse, rapid pulse, palpable abdomen	1 death; 1 convalescent after 8 days	Broth cultures of organism caused death of mice with symptoms of diarrhea in 0.1 to 0.5 c c amounts injected subcutaneously; 0.5 to 2 c c of broth cultures caused death of guinea-pigs in 6-7 days when injected subcutaneously
Pfuhl, A.	Germany	1900	81	"Proteus mirabilis" (but fermented lactose more vigorously than dextrose (B. cloacae?)	Sausages.....	Vomiting, diarrhea, loss of appetite, weakness	Convalescent in 12 hours	Feeding: Meat fed to mice caused death Inoculation: Rabbit injected with 4 c c broth cultures died the following day. Broth cultures heated to 65 C. injected into mice in 0.5 c c amounts produced no effect. Filtrates of old live cultures caused only temporary effect. Filtrates of young live cultures had slightly toxic effect. Residue of filtered cultures caused death of animals
Schumburg.....	Hanover, Germany	1901	34	Proteus	Sausages.....	Malaise, diarrhea, weakness, vomiting	Convalescent in 12 hours	Feeding: Rats and mice fed with sausage died Inoculation: Filtrate of broth culture injected subcutaneously into animals (0.1 to 0.5 c c) caused death
Dieudonné, A.	Hammelburg, Germany	1903	150–180	Proteus vulgaris	Potato salad.....	Headache, dizziness, weakness, vomiting, collapse, aching of limbs, colic-like pain, pulse 88-92	Majority convalescent in a few hours, a few weak for several days	Feeding: Mice fed with potato salad died in 24 hours Mice fed with artificially infected potatoes incubated 12 hours at 20–37 C. died in 24–48 hours Broth cultures of organism fed to mice on bread had no effect Inoculation: Mice and guinea-pigs injected subcutaneously with suspensions of agar cultures were slightly sick, but recovered. Subcutaneous injection of filtrate of cultures in mice and guinea-pigs had no effect
Mayer, ... Mandel, H.	Germany	1912	46	Proteus vulgaris	Fish kept in unclean box	Chills, headache, aching of limbs, weakness, loss of appetite, vomiting, diarrhea, temp. 38-39.3 C., pulse 120, in one case herpes facialis, in three cases trace of albumin in urine	Early recovery	Feeding experiments not conclusive

showed enlarged spleen, congested intestines and adrenals. The organism was recovered in 2 animals out of 9. The author considered the effects produced as due to infection with the organism and intoxication by its metabolic products.

Pfuhl attributes an epidemic of food poisoning to the eating of infected sausages. in which a slowly liquefying organism producing swarm colonies in gelatin and which he classifies as Proteus mirabilis was isolated. He states, however, that the organism isolated did not correspond in all respects to Hauser's description of Proteus mirabilis. It produced gas in dextrose and lactose, but more actively in lactose than dextrose. No digestion of casein is described in milk. The organism appears to have been more closely related to B. cloacae than to Proteus vulgaris. In this case also the causal relationship was considered to have been established by the isolation of the organism from the suspected meat and by feeding and inoculation experiments, though the organism was not isolated from the stools of the patients. The author considered the harmful effects due rather to a toxin than to infection.

Schumberg, who investigated an epidemic involving 34 persons and due to eating infected sausages, isolated the organism from the suspected food. The sausage fed to rats and mice caused death in 24 hours with congestion of the intestines and enlargement of the spleen and liver. Only a few organisms were found in the blood, and the author considers the effects produced were due to toxic substances. The author examined several other kinds of sausages and was not able to isolate Proteus vulgaris.

Dieudonné recovered Proteus vulgaris from potato salad, which had caused an epidemic affecting 150-180 persons. In this case mice fed with the salad died in 24 hours, and Proteus vulgaris was isolated from the spleen and kidneys, while mice inoculated remained well. The author artificially contaminated potatoes with broth cultures of the organism isolated and allowed them to stand at various temperatures for 12 hours and then fed them to animals. Animals fed with potatoes which had been incubated at temperatures above 18 C. died. Meat was infected in the same way and similar results were obtained. Bread artificially contaminated in the same way had no effect on animals. The author therefore concludes that the organism itself was not pathogenic for animals, but that toxic substances were produced in the potatoes and meat which caused death.

Mayer described an epidemic due to the eating of spoiled fish. In this case stools of the patients were examined and Proteus vulgaris was isolated, which was agglutinated by the serums of the patients in a dilution of 1:25. This author also considers the harmful effects produced to have been due to toxic substances produced in the meat by the multiplication of the organism.

Mandel has reported the same epidemic and isolated an organism from the stools of patients which he states was undoubtedly Proteus vulgaris. The cultural characteristics of a number of the strains described appear to be somewhat variable. No gas production in dextrose, no curdling of milk and no liquefaction is recorded of some of the cultures, but laboratory strains of Proteus vulgaris used as controls show the same discrepancies and it is difficult to judge as to the significance of the results obtained.

In both accounts of the above epidemic the appearance of Proteus vulgaris in the stools was followed by that of members of the paratyphoid-enteritidis group. Mandel agrees with Mayer in considering the bacteria themselves much less harmful than the toxic substances produced by them in the food. The short incubation period and quick recovery point to an intoxication rather than to an infection.

A definite proof of the causal relationship of the organisms isolated to the illness is lacking in all of the above epidemics. The isolation of identical organisms from the suspected food and from the stools and vomitus of patients has not been reported in any case. The real cause of the effects produced in alleged cases of food poisoning by Proteus vulgaris has not been satisfactorily explained, some of the investigators attributing it to a multiplication of the organism itself in the body, and others to the toxic substances produced by it, either before or after entering the intestine. Levy, Pfuhl, Schumburg, Dieudonné, Mayer and Mandel incline to the view that toxic substances are produced in the food before its entrance into the body, and that these cause the injurious effects. Wesenberg considers multiplication of the organism in the body the cause of harm. Silberschmidt thinks the multiplication of the organism in the body and the formation of toxic substances in the intestine following this multiplication are both factors to be considered though infection is the more important. Glückmann considers that these injurious effects are due to infection and intoxication by the metabolic products which have been elaborated in the food substance before it enters the body.

The assumption that injurious effects are produced by the presence of toxic substances in the food, before it enters the intestine, is open to the criticism that partially decomposed meat in which it is probable that Proteus vulgaris is often present, since meat seems to be one of the natural habitats of Proteus vulgaris, has often been shown to produce no harmful effects. Levy considers that toxic substances are produced at only certain stages of decomposition. The short duration of illness and quick recovery in most cases, however, would seem to point to an intoxication either by substances produced before or after the food substance reaches the intestine, and most investigators incline to this view. Dieudonné found organisms in the blood, spleen and liver of animals fed with potatoes and meat artificially contaminated with broth cultures of Proteus vulgaris and kept 12 hours at temperatures above 18 C., but the organisms were few in number, and he considers infection as playing a minor rôle. Schumburg also found very few organisms in the blood and spleen of animals fed with meat artificially infected and incubated for 24 hours before feeding.

The presence of the organism in large numbers in the digestive tract after feeding has been demonstrated on the other hand by several authors, including Silberschmidt and Metchnikoff. The latter performed feeding experiments on chimpanzees and nursing rabbits. Proteus vulgaris was absent in the normal feces of chimpanzees, but chimpanzees fed with diarrheic material showed large numbers of the organism in the feces. The contents of the jejunum, ileus and cecum of one animal which died showed abundant cultures of the organism at necropsy. The same results were obtained with young rabbits. Chimpanzees and rabbits fed with pure cultures of Proteus vulgaris isolated in cases of infantile diarrhea died and large numbers of the bacilli were present in all parts of the digestive tract of the chimpanzee. Cultures of the organism administered with B. welchii provoked infections similar to cholera. Negative results as regards harmful effects produced by feeding with broth cultures have been reported by Archibald, Feltz, Herter and Ten Broeck, Meyerhof and Bahr. Further evidence is needed to establish the etiological rôle of Proteus vulgaris in cases of food poisoning.

Toxin.—Levy isolated organisms from putrefying beer yeast which he identified as Proteus vulgaris. By treating a liquefied gelatin culture of the organism with absolute alcohol, and precipitating with calcium chlorid and

drying, the toxic substance "sepsin" was obtained. This produced the same effect as living cultures of the bacteria, causing the death of dogs injected intravenously with 1 gm. of the sterile powder and of rabbits and guinea-pigs injected intravenously, intraperitoneally or subcutaneously with 0.2-0.3 gm. Symptoms of vomiting, bloody diarrhea and rise of temperature were present, and hemorrhagic infiltration of the intestines and enlargement of the spleen were present on necropsy.

Fornet and Heubner also investigated putrefying beer yeast and isolated an organism differing from Proteus vulgaris which produced sepsin and killed dogs, which they designated as Bact. sepsinogenes. By artificially contaminating putrefying beer yeast with cultures of Proteus vulgaris isolated from meat and human feces, these authors were not able to produce the symptoms in dogs described by Levy.

Meyerhof in studying the effects of cultures of Proteus vulgaris and of the filtrates of cultures on rabbits, mice and guinea-pigs, came to the conclusion that infection was concerned in the effects produced, since the organism was present in the blood and organs of the animals, but also that a sort of toxemia was produced as in tetanus, diphtheria, and botulism, the minimal lethal dose for mice corresponding to 0.1 of live culture, 0.5 of killed culture and 2 c c of filtrate.

Herter and Ten Broeck precipitated cultures of Proteus vulgaris with alcohol, centrifugalized and dried in vacuo over H_2SO_4, dissolved in sterile salt solution and inoculated intraperitoneally into guinea-pigs and found the lethal dose to be 8.2-11.5 mg. per 100 gm. body weight. The toxin of B. coli prepared in the same way, however, was stronger, the minimal lethal dose being 4 mg. per 100 gm. body weight. The authors were not able to establish an immunity to the toxin.

Berthelot made an extensive series of experiments to ascertain the toxic properties of cultures of Proteus vulgaris isolated in cases of infantile diarrhea, and also of a culture isolated from putrefying material. Seven-day cultures grown in different kinds of mediums and sterilized by means of ether were tested on 500 gm. guinea-pigs, injected intravenously and intraperitoneally and the fatal dose determined. In general, the amount of culture required to produce death in 6-18 hours varied from 1-5 c c, and the culture from putrefying material as far as tested was not much less toxic than the one from the case of infantile diarrhea. The filtrate from cultures was tested in the same way and the fatal dose varied from 2½ c c to more than 12 c c. The toxic action of dried bacterial cells was also tested. The results expressed in milligrams of bacterial cells before drying varied from 10-50 mg. injected intravenously producing death in 15-20 hours, and 25-300 mg. injected intraperitoneally producing death in less than 24 hours.

The results obtained by these writers indicate that Proteus vulgaris produces a very weak soluble toxin as compared with tetanus or diphtheria. It is more comparable with that produced by B. paratyphosus, recently shown by Ecker to be toxic to the extent that amount of 1 to 5 c c of filtrate of broth cultures injected intravenously caused the death of young rabbits.

PATHOGENICITY

The pathogenicity of Proteus vulgaris has been discussed by a number of workers. Though the organism is apparently often saprophytic in its nature, it has been shown also on numerous occasions to be parasitic. Stewart, who isolated Proteus vulgaris from septic wounds in 24% of cases studied, states

that "as a pathogenic agent this organism is now well recognized." The enumeration of pathologic processes by Meyerhof and by Klieneberger[1] in which Proteus vulgaris is concerned as a factor as well as a number of more recent reports of proteus infections referred to in this paper attest to the pathogenic properties of the organism under certain conditions.

A number of authors have differentiated between virulent and nonvirulent strains of Proteus vulgaris. Tsiklinsky states that cultures isolated in infantile diarrhea exhibited a high degree of virulence, killing guinea-pigs and rabbits in 24-36 hours when ¼-½ c c was injected subcutaneously, while proteus isolated from normal feces had no effect on rabbits. Larson and Bell[1] found that freshly isolated cultures of proteus from human lesions were pathogenic for rabbits, rats and guinea-pigs, producing abscesses or granulomatous types of lesions, but that after being kept under laboratory conditions, such cultures produced no lesions. Large doses of these cultures, however, showed toxic properties when injected into laboratory animals. Cultures of Proteus vulgaris from decaying protein matter were not found to be pathogenic by these authors. They conclude that strains of Proteus vulgaris pathogenic for rabbits, rats and guinea-pigs are also pathogenic for man. Nonpathogenic strains could be rendered pathogenic by inoculation into the anterior chamber of the eye of a rabbit. Pauly, as referred to above, considers that a local tissue necrosis may increase the virulence of proteus.

Numerous tests on animals for pathogenicity are recorded in the literature by other workers. In general, the results obtained indicate that strains freshly isolated from pathologic sources, or such strains which have lost their virulence and been subjected to animal passage for increase of virulence may produce definite lesions including abscesses, enlargement of spleen, hemorrhage of the intestine and a diarrheic condition. Strains which are not pathogenic in the sense of producing lesions as noted above may in large doses produce toxic symptoms.

In my work, virulence tests were carried out by injecting mice with 24-hour broth cultures in 1 c c and 0.1 c c amounts to determine whether strains from certain sources were more virulent for mice than strains from other sources. In general, 1 c c of broth culture injected subcutaneously caused death of mice within 24 hours, and 0.1 c c produced no ill effects, regardless of source. The following cultures of Proteus vulgaris killed in 1 c c amounts but not in 0.1 c c: 17, 29, 55, 96 (feces); 78 (meat); 79 (water); 75 (wound bandage); 76 (dog's saliva); 2, 3, 4, 64, 67, 70, 71, 92 (laboratory cultures). Culture 108 from feces caused death in a 0.1 c c amount. The following cultures did not cause death in 1 c c amounts: 11 (feces); 34 (meat); 53 and 94 (necropsied animals); 65, 77, 80 (laboratory cultures). The cultures classified as B. cloacae 51, 68 and 45; and B. zopfii 73 and Proteus zenkeri 85 had no effect on animals in 1 c c amounts. Cultures 47 (air?), 63 (meat) and 69 (blood) classified as proteus species killed in 1 c c amounts but not in 0.1 c c. The cultures classified as Ps. protea, 86, 87 and 24 also were virulent in 1 c c amounts but not in 0.1 c c.

As far as investigated no definite lesions were present, and it is probable that the effects produced were due to toxicity of the cultures. Death of several rabbits injected with killed cultures for the production of immune serums ensued within 24 hours after injection, probably due to toxic action. Abscesses were present at the site of inoculation.

The results obtained by various workers who have tested cultures of Proteus vulgaris isolated in cases of food poisoning or from cases of infantile diarrhea, indicate that such cultures were not markedly pathogenic in com-

parison with the cultures used in this study. Glücksmann who caused death of mice in 18 hours to 3 days by the injection of 0.1-0.5 c c of broth cultures of an organism isolated in a food poisoning epidemic considers the organism to have been very pathogenic for animals. Levy, Schumburg and Silberschmidt used 0.1-0.5 c c of cultures to produce death. Wesenberg states that mice were killed with 0.2 c c of a 24-hour broth culture, and that less than 0.2 c c caused weakness and loss of appetite.

On the other hand, cultures of Proteus vulgaris isolated from certain other pathologic processes and tested immediately on isolation sometimes exhibit a high degree of virulence. Grossmann reports a culture isolated from a case of peritonitis which killed mice in amounts of 0.005 c c.

CLASSIFICATION

The criteria to be used in the classification of organisms in the proteus group do not seem to be well established in some cases. Kruse, whose classification consists of a number of groups, includes among these the proteus group, members of which are characterized as aerobes or facultative anaerobes of medium size decolorized or irregularly stained by Gram's stain. Spores are absent and colonies have a tendency to spread and occur with raylike extensions or with stellate outgrowths away from the colony, sometimes with the separation of daughter colonies. Decomposition of protein substances is accompanied by putrefactive odor. Morphologically, the organisms occur as varying from pleomorphic coccoid forms to long filaments. A supplementary group consists of liquefying pathogenic forms. This classification would exclude all gram-positive forms and spore-bearers, but not nonliquefying forms.

Klieneberger,[1] who studied strains derived from pathologic sources and from meat recommends the marked growth energy as the differential group characteristic and the putrefactive odor, peptonizing power and gram-staining reaction as characteristic of the different species. However, his study did not cover the different species described in the literature, and the characteristic of unusual growth energy as exhibited by rapidly spreading growth on agar, is not recorded of a number of species classified by other authors as proteus. This property is a characteristic of the species vulgaris, but not necessarily of others.

In the proposed classification of the committee of the Society of American Bacteriologists, Proteus Hauser may properly be considered as a genus under Family VI: "Bacteriaceae, rod-shaped organisms without endospores, gram-negative, flagella when present peritrichic, metabolism complex, amino-acids being utilized, and generally carbohydrates."

The proteus group may be considered to include those species which occur as rods varying from short coccoid forms to filaments, which are gram-negative, without endospores, flagella when present peritrichic, which are aerobes and facultative anaerobes, which liquefy gelatin, often producing characteristic stellate colonies, which often exhibit a marked rapidity of growth, which utilize amino-acids and generally carbohydrates which may be saprophytic or parasitic in their nature.

The type species of the proteus group may be considered to be the organism described in the literature as Proteus vulgaris Hauser. The type Proteus mirabilis first considered by Hauser to be a different species was later regarded by him (Hauser[2]) as an attenuated form of vulgaris and should probably be

considered identical with the latter. The third type, Proteus zenkeri, originally considered as a species distinct from vulgaris and later as an attenuated form of vulgaris is really a distinct species and inasmuch as it is unquestionably gram-positive and differs in all important points from Proteus vulgaris, it should not be included in the proteus group. It is probably identical with B. zopfii.

The characterization of Proteus vulgaris has been well established by the work of recent authors, including Berthelot, Horowitz, and others. It may be described as a gram-negative rod, which may exhibit pleomorphism, which is nonspore-forming, motile, noncapsulated, with peritrichic flagella, liquefying gelatin, often with the formation of characteristic colonies, fermenting dextrose with the formation of acid and gas, often fermenting saccharose, maltose and mannite but never lactose, precipitating then dissolving casein, producing a putrefactive odor from protein substances, usually forming indol, reducing nitrates and producing H_2S. In the present study rapidly spreading growth on agar, brown pigment production in broth, negative Voges-Proskauer reaction in peptone mediums, agglutination of related strains by immune serums, have been shown to be practically constant.

In addition to the type species Proteus vulgaris there are doubtless many more or less closely related forms which may be grouped as proteus species, as discussed by Jordan, which show the same fermentation reactions, but vary as regards proteolytic power as well as in certain other respects. A more complete study of such forms is needed to throw light on the relationship to Proteus vulgaris and other species of the proteus group.

An attempt has been made by a study of the literature to bring together species which have been classified under proteus and to determine as far as possible the validity of such classification. The earlier descriptions, however, are lacking in many of the essential points necessary to determine with accuracy the true nature of the organism in question and of its relation to other organisms classified in the same group. Such a classification must necessarily be considered tentative.

Genus Proteus Hauser
- Proteus vulgaris Hauser
  - Syn. Bacillus vulgaris (Hauser) Mig. Macé.
    - Bacterium vulgare (Hauser) Lehmann-Neumann
    - B. vulgaris (Hauser) Chester
    - Bacillus proteus vulgaris Kruse
  - Identical or closely related forms
    - Proteus mirabilis (Hauser)
      - Syn. B. mirabilis (Hauser) Trev. Mig.
    - Proteus sulfureus Lindenborn, Holschewnikoff
      - Syn. Bacillus sulfureus Mig.
    - Bacillus murisepticus pleomorphus (Karlinski)
      - Syn. Proteus of Karlinski
    - Bacillus proteus anindologenes van Loghem and Loghem-Pouw
- Proteus septicus Babes
  - Syn. B. septicus Babes Chester
    - B. proteus septicus (Babes) Kruse
- Bacillus fetidus ozenae Hajek
  - Syn. Bacillus ozenae (Hajek)
- Bacillus septicus putidus Roger
- Bacillus ranicida Ernst
- Bacillus proteus fluorescens Jaeger
  - Syn. B. urinae Chester
    - Pseudomonas jaegeri Mig.
    - Proteus fluorescents Macé
- Proteus piscicidus versicolor Babes and Riegler
  - Syn. Bac. piscicidus versicolor
- Bacillus cloacae Jordan

The following species do not conform to the description of the genus Proteus in one or more respects:

Proteus hominis capsulatus Bordoni-Uffreduzzi
Syn. Proteus capsulatus septicus Banti
Bacterium proteus Migula
Bacillus capsulatus septicus Kruse
Proteus zenkeri Hauser
Syn. Bacillus zenkeri Hauser
Bacillus zopfii Kurth
Syn. Proteus zopfii
Bacterium zopfii
Proteus lethalis
Bacillus albus cadaveris (Strassmann-Strecker)
Syn. Bacillus cadaveris (Strassmann and Strecker)
Bacillus proteus ruber (Fortinau and Soubrane)

Proteus mirabilis Hauser, first described as a separate species by Hauser,[1, 2] was later considered by this author and others an attenuated form of Proteus vulgaris and not a separate species.

Proteus sulfureus Lindenborn, Holschewnikoff as described by Holschewnikoff, is identical with Proteus vulgaris in morphology, pleomorphism, motility, nonspore-formation, appearance of colonies on gelatin, liquefaction of gelatin, appearance on agar and in milk, rapidity of growth, oxygen requirement. Formation of H_2S is emphasized, but Proteus vulgaris is known to produce large amounts of H_2S from peptone. The organism is described as occurring in water.

Proteus murisepticus pleomorphus Karlinski, an organism isolated from pus, as far as described by Karlinski, is apparently identical with Proteus vulgaris, occurring as a pleomorphic organism sometimes in the form of short rods, with rounded ends, sometimes as long rods and spirillum forms. The organisms were gram-negative and very motile. Spores were not observed. Gelatin colonies were surrounded by concentric rings and sinuous prolongations extending into the medium. Liquefaction of the medium followed with the emission of a butyric acidlike odor. Growth on agar was abundant and white, and broth showed a white sediment and emitted a strong odor. On blood serum growth was grayish white and thin, and the medium was rapidly liquefied. The organism was pathogenic for white mice, a loopful of a broth culture causing death in 24 hours, but gray mice were more resistant. Proteus vulgaris has often been shown to be pathogenic for mice.

Bacillus proteus anindologenes van Loghem and van Loghem-Pouw. Van Loghem and van Loghem-Pouw describe a number of organisms isolated by them from intestinal contents, feces, urine and intestinal abscess which varied from Proteus vulgaris in the nonproduction of indol. Berthelot has found that typical cultures which do not yield a positive indol reaction by the usual tests produce at least indol-3-acetic acid, and on this basis nonindol-producing strains should not be classified as a separate species.

Proteus septicus Babes.[2] This organism is described as a gram-negative, pleomorphic, motile bacillus, showing forms of varying lengths and 0.4 mikrons in width and without spores. A putrefactive odor was noticeable in cultures. The organism liquefied gelatin very energetically when first isolated, produced a spreading growth on agar and liquefied blood serum. The organism which killed mice and is described by the author as being very pathogenic was isolated from the blood and organs of a child dead of septicemia.

The author states that the organism is very similar to Hauser's Proteus vulgaris. The only discrepancy as far as described lies in the fact that the organism is stated to have stained gram-positively in the tissues.

Bacillus fetidus ozenae Hajek. The organism described by Hajek as occurring in the secretion of ozena coincides with Proteus vulgaris except in the matter of growth on gelatin plates, which is described as sometimes occurring with the formation of gas bubbles before liquefaction, which soon disappear, and the colony extends short projections into the medium, following which liquefaction takes place. The organism was a short gram-negative bacillus with a tendency to form in pairs or chains, was actively motile and nonspore-forming. Agar slants showed a slimy moist spreading growth outward from the line of inoculation, and an unpleasant putrefactive odor was noticeable. Blood serum showed a whitish growth covering the entire surface. Growth took place rapidly aerobically or anaerobically. The organism was pathogenic for mice, several drops of a gelatin culture injected subcutaneously causing death in 5 days.

Ward, who has recently made a study of the bacteriology of ozena isolated Perez' bacillus in 44% of 50 cases and proteus in 40%. Perez' bacillus according to this author is distinguished by slow fermentation of glucose, less luxuriant growth and less vigorous action on protein substances than Proteus vulgaris and a characteristic pigment in gelatin. Injections of Perez' bacillus and Proteus vulgaris in rabbits produced the same condition at necropsy, that is, increased nasal discharge and increase of temperature.

Bacillus septicus putidus Roger isolated from the liver and spinal fluid of a cholera patient who died with meningeal symptoms appears to be identical with Proteus vulgaris in staining properties, oxygen requirements, putrefactive odor, reactions in milk and carbohydrate mediums, liquefaction of gelatin and blood serum. Appearance of growth on agar is described as a thick, white, creamy growth along the line of inoculation, with semi-transparent separated colonies on the remainder of the surface. The author considers the organism as differing from Proteus vulgaris in not showing pleomorphism, in its more rapid liquefaction of gelatin, more marked turbidity in broth and different appearance on potato. The organism was not highly pathogenic, a rabbit receiving 1 c c intravenously succumbing after 2 or 3 days. He states the two organisms may be two different species or two strains of one species.

B. ranicida described by Ernst in a fish epidemic is stated by Babes and Riegler to have been indistinguishable from Proteus vulgaris except for a slight bluish opalescence on agar.

Bacillus proteus fluorescens Jaeger. This organism described by Jaeger as the cause of Weil's disease, was isolated from the urine during life, and from the blood and organs of patients dead of this disease. Though the etiology of this disease has been recently established on another basis, namely, that of Spirocheta icterohemorrhagiae as the causal agent, the isolation of a Proteus vulgaris-like organism from the urine has been reported by Pfaundler, Brüning, and others. Reports of the agglutination of the serum of patients suffering from this disease, with the typhoid bacillus are described by Eckhardt, Zupnik, Brüning and others, and of B. typhosus and Proteus vulgaris both by Lüdke.

The organism as described by Jaeger corresponds in general with Proteus vulgaris in its morphologic aspects, motility, lack of spores, staining properties, putrefactive odor and cultural behavior as far as recorded, except that growth on agar and in gelatin was characterized by a greenish fluorescence. Intraperitoneal or subcutaneous injection of mice caused death in a few days to two weeks. Migula classifies the organism as a pseudomonas though Jaeger describes peritrichic flagella. It seems proper to classify as a species in the proteus group.

Archibald has classified as B. proteus fluorescens an organism which he isolated from a case of choleraic diarrhea, which produced a greenish-yellow fluorescence on Drigalski-Conradi medium, which gave positive indol and Voges-Proskauer tests, produced acid and gas in dextrose, mannite, levulose, maltose, galactose and dextrin, but failed to ferment lactose, saccharose, dulcite, adonite, inulin and raffinose, produced acid in milk, without curdling, then alkali, liquefied blood serum and was pathogenic for guinea-pigs.

Bataillon described an organism isolated in a fish epidemic which was distinguished from Proteus vulgaris by the production of a greenish sheen in gelatin.

Proteus piscicidus versicolor Babes and Riegler. This organism was described by Babes and Riegler in a fish epidemic and classed as a member of the proteus group, but differing from Proteus vulgaris in certain respects.

Colonies on agar were yellowish with thin transparent border. A noticeable putrefactive odor was produced. Growth was best at 20 C. Gas production was vigorous in sugar mediums. Characteristic proteus-like colonies were produced on gelatin plates. Glycerin potato showed a color display characteristic of the organism, the upper part being reddish-brown and the colors varying from top to bottom through brown, green, flesh-color and yellow. Gelatin stabs also showed similar color changes. Milk was coagulated with the formation of a brick-red scum. The organism was a gram-negative rod with peritrichic flagella. The organism was found to be pathogenic for fish, mice and rabbits. Proteus vulgaris which had also been isolated from the organs of the dead fish was found to be nonpathogenic for fish. The blood of the infected fish agglutinated the organism in dilutions of 1 : 50, but failed to agglutinate Proteus vulgaris.

Glaser and Hachla tested this organism for agglutination properties and found it to be agglutinated only by the strain used for immunizing, but not by serums obtained by immunizing with Proteus vulgaris.

Bacillus cloacae Jordan. This organism as described by Jordan is characterized by an inverted gas formula, namely, CO_2 in excess of H. In a number of strains isolated from water, all fermented dextrose and saccharose, and the majority lactose, though sometimes slowly. Most of the cultures liquefied gelatin, though some very slowly, and milk was acidified and curdled, the casein being dissolved in some cases. The indol reactions were variable. The organisms of this group were in general less actively proteolytic than Proteus vulgaris. Gas production was much more vigorous in dextrose than was the case with B. coli.

Levine classifies B. cloacae with Aerogenes-cloacae group under aerobic nonspore-forming bacteria which ferment lactose with gas formation, and describes B. cloacae as follows:

"Motile, gelatin liquefied (often very slowly); indol, dulcitol, glycerol, inulin and starch usually negative (rarely positive); dextrin occasionally positive; sucrose, raffinose, salicin and mannitol positive (rarely negative)." The group reactions include "Voges-Proskauer reaction positive; . . . reaction to methyl red alkaline, or if acid at first it reverts to a distinct alkaline reaction after long incubation (7 days); indol, usually negative, polysaccharids, starch, inulin and dextrin, negative or positive."

FOOTNOTE.—In Kruse's classification are included several organisms, Bacillus b (Vignal), Bacillus havaniensis liquefaciens (Sternberg), Bacillus albus putidus (Maschak) which as far as described may be included in the proteus group, while several others differ in regard to Gram's stain, non-liquefaction of gelatin and other respects, which would exclude them from this group. In his addenda to the proteus group Kruse includes pathogenic liquefying organisms, a number of which as far as described might also possibly be included in the proteus group: Bacillus dysenteriæ liquefaciens, Bacillus leucaemiae canis, Bacillus septicus ulceris gangraenosi (Sternberg), Bacillus pyogenes liquefaciens, Bacillus pyogenes gingivae (Miller), Bacillus pneumonicus agilis (Flügge), Bacillus leporis letalis (Sternberg).

Glenn noted that B. cloacae differed from Proteus vulgaris in its ability to liquefy gelatin in the presence of carbohydrates, and concluded that the enzyme of proteus was more resistant to acids than that of Proteus vulgaris.

In this study Cultures 51 (from necropsied animal) and 68 (feces) correspond to the above descriptions of B. cloacae. They are characterized by very vigorous gas production in dextrose, saccharose, maltose and mannite, with less gas in raffinose and lactose in the case of 68 and with a large amount of gas in raffinose in the case of 51, but no gas in lactose in 5 days. Lactose was, however, fermented by this culture as indicated by a hydrogen-ion concentration represented by a P_H value of 5.4 in this medium. Neither culture liquefied gelatin in 3 days, but both showed a small amount of liquefaction in 30 days. Both yielded a positive Voges-Proskauer reaction and neither produced indol. Growth on agar was not spreading and the brown pigment production characteristic of Proteus vulgaris was absent. Milk was rendered acid and coagulated without peptonization.

Culture 45 has also been classified in this group, though not characteristic in all respects. Gas production was less vigorous in the carbohydrates above noted than was the case with Cultures 51 and 68. This culture presented one of the few cases of variation observed in the study. Two cultures of the same organism were tested as to carbohydrate reactions in the early part of the work, and gas production in lactose was recorded as a bubble, which in the case of one of the cultures disappeared in 7 days. In a test made over a year later, 15% of gas was recorded in lactose broth in 24 hours, and 20% in 7 days. This culture also presented the anomaly of fermenting dextrose much less actively than other carbohydrates. Liquefaction of gelatin by this culture took place more rapidly than in the case of Cultures 51 and 68. The Voges-Proskauer reaction was negative, differing in this respect also from 51 and 68.

The question as to whether B. cloacae should be classified in the proteus group or with B. coli has been considered by a number of authors. It is intermediate between the two, resembling B. coli in certain respects and Proteus vulgaris in others. The results obtained with the three cultures classified in this group illustrate the relationships to the above groups. Culture 45 in gelatin liquefaction, negative indol reaction, and negative Voges-Proskauer reaction seems more closely related to Proteus vulgaris than to B. coli. Culture 51 is identical with Culture 68 except that no gas was produced in lactose in 7 days, tho a certain amount of lactose was utilized as indicated by the hydrogen-ion concentration test. This culture on the basis of nonproduction of gas in lactose and liquefaction of gelatin would incline toward Proteus vulgaris, but it is undoubtedly very closely related to 68, which on the basis of gas production in lactose seems more like B. coli. A further study of liquefying lactose fermenters is needed to elucidate the relationship between these groups of organisms.

Proteus zenkeri Hauser
  Syn. Bacillus zenkeri Hauser
Bacillus zopfii Kurth
  Syn. Bacterium zopfii
    Proteus zopfii

The organism classified as Proteus zenkeri by Hauser[1] was originally described as a distinct species, but later was considered by this author and others as an attenuated form of Proteus vulgaris on the basis of positive gelatin liquefaction on long continued cultivation.

The organism is a thin rod, often appearing in the form of long filaments. Spore formation has not been observed. It retains the stain of Gram's method, is motile and shows characteristic growth on gelatin plates. The growth may be described as feathery or mycelium-like and occurs for the most part below the surface of the medium. No liquefaction of gelatin is reported in most of the descriptions. Growth on agar is a thin, transparent film. Macé describes a strong putrefactive odor in broth and negative indol formation.

B. zopfii (Kurth) is apparently identical with Proteus zenkeri. It is reported as found in long matted filaments or balls in old cultures. Klieneberger considers the two organisms identical on the basis of agglutination reactions.

In the cultural and agglutination tests made on the two members of this group, 73 B. proteus zopfii and 85 Proteus zenkeri, the behavior differed from that of Proteus vulgaris in nearly all respects. While the latter organism acts vigorously on carbohydrate and protein mediums, B. proteus zopfii and Proteus zenkeri were very inert. Growth on agar was nonspreading and showed filaments extending into the medium. Litmus milk was not curdled and showed no change of reaction or was very slightly alkaline after 14 days. Gelatin was not liquefied, tho the culture of zopfii was retained 4 months. No growth was obtained on Endo plates. No acid or gas production was observed in Russell's medium or other carbohydrate mediums. Indol production was negative, as was reduction of nitrates. A slight turbidity was produced in broth, but no pigment was noticeable. In the hanging drop the organisms, which are considerably larger than Proteus vulgaris and sometimes assume the form of long filaments, show a slight motility. Neither of the cultures was virulent for mice in 1 c c amounts.

In the tests with Proteus vulgaris immune serums no positive results were obtained except in the case of Serum 4 which agglutinated zopfii in a dilution of 0.01. Proteus zenkeri showed a tendency to agglutinate spontaneously throughout the tests.

The question of agglutination of Proteus zenkeri by human normal and typhoid serums was considered. Klieneberger[1] found both B. proteus zopfii and Proteus zenkeri agglutinated by human normal serums and therefore considers them identical.

The following results were obtained in my work with six serums tested:

		1:10	1:20	1:40
Normal serums:	7049	++++	+++	+
	7050	++++	++++	+++
	7054	++++	++++	++
Typhoid serums:	7051	++++	++++	++
	7052	++++	+++	++
	7053	++++	++++	+++
Control (no serum)		+		

The results obtained indicate that both normal and typhoid serums agglutinate B. proteus zopfii in dilutions of 1:40 or higher.

The two organisms which show identical reactions throughout and are apparently the same organism, are so distantly related to Proteus vulgaris that they should not properly be classified in the proteus group nor considered as attenuated forms of Proteus vulgaris. Gram-positive staining as well as cultural and agglutination reactions clearly differentiate them from the latter organism. It is recommended that these two forms be designated as B. zopfii and not considered as belonging to the proteus group.

The organism described by Bordoni-Uffreduzzi as Proteus hominis capsulatus in 3 cases of "Hadern-Krankheit," as Proteus capsulatus septicus by Banti, as Bacillus capsulatus septicus by Kruse should not properly be included in the Proteus group. The organism is described by Bordoni-Uffreduzzi as a gram-positive organism, showing a capsule in cultures. Gelatin was not liquefied. Macé calls attention to the resemblance of this organism to the pneumobacillus of Friedländer.

Proteus lethalis isolated by Babes[2] from the spleen and gangrenous lung of a patient who died of septicemia is described as a gram-positive, nonliquefying motile bacillus, pathogenic for mice and rabbits. This organism also is improperly included in the proteus group.

Bacillus albus cadaveris (Strassmann and Strecker) originally described as occurring in the blood of patients 4 days after death agrees with Proteus vulgaris in respect to appearance of gelatin colonies and gelatin liquefaction, putrefactive odor, nonspore-formation, active motility, fairly rapid growth on agar, pathogenicity for mice, but it is described as gram-positive. Whether this organism or the true Proteus vulgaris is a usual postmortem invader is apparently not established. Flexner, who isolated Proteus vulgaris in a case of peritonitis 12 hours after death, questions the frequent occurrence of proteus, since in his experience in routine necropsies proteus has been encountered rarely.

Bacillus proteus ruber isolated from river water by Fortineau and Soubrane is a gram-positive organism producing red growth on agar, and should probably not be included in the proteus group. The organism is described as liquefying gelatin slowly, and coagulating milk and as being pleomorphic in morphology.

SUMMARY AND CONCLUSIONS

The proteus group as described in the literature includes one well defined species, Proteus vulgaris, and a number of other species more or less distantly related, some of which clearly should not be thus classified.

As distinguishing characteristics of the group the following may be considered: Rods, varying from short coccoid forms to filaments, which are gram-negative, without endospores, with flagella when present, peritrichic, which are aerobes or facultative anaerobes, which liquefy gelatin, often producing characteristic stellate colonies, which utilize amino-acids and generally carbohydrates in their metabolism, which may be saprophytic or parasitic in their nature.

Proteus vulgaris may be described as a gram-negative rod, which may exhibit pleomorphism, which is nonspore-forming, motile, noncapsulated, with peritrichic flagella, which liquefies gelatin, often with the formation of characteristic colonies, which shows a rapidly spreading growth on agar, which ferments dextrose, with acid and gas formation, which often ferments saccharose, maltose, and mannite, but never lactose, which precipitates, then dissolves casein, which pro-

duces a putrefactive odor from protein substances, which usually produces indol and H_2S from peptone mediums, which reduces nitrates, which usually shows a negative Voges-Proskauer reaction in peptone mediums, which often produces brown pigment in mediums, which may be saprophytic or pathogenic, which is often agglutinated by immune serums derived from the same or related strains.

There are probably a number of related forms which are similar to Proteus vulgaris in fermentation reactions and in the property of liquefying gelatin, but which vary in proteolytic power and agglutination properties and other respects, which may be tentatively grouped as proteus species until further work may establish definite types.

A number of different species described in the literature may properly be considered as belonging to the genus proteus as listed under the heading Classification in this work. Several others, however, have been incorrectly classified here, including the organism designated as Proteus zenkeri which is a gram-positive organism and which differs in almost every particular from the other members of the group.

Ps. protea described by Frost, though closely related to Proteus vulgaris in cultural reactions, by reason of absence of peritrichic flagella would be unfortunately separated from the latter, in following the proposed classification based on morphologic characteristics. It is quite possible that other species classified as members of the proteus group do not possess peritrichic flagella, as descriptions are not sufficient to determine this point.

Proteus vulgaris possesses unusual agglutination properties. Somewhat discordant results have been obtained by different workers, but the results obtained in most of those in which true Proteus vulgaris has been considered, indicate that a relationship may often be established between different strains on the basis of agglutination reactions. It seems probable that strains from pathologic sources inter-agglutinate and that antibodies against the infecting organism are often produced, especially in suppurative processes, general infections and urinary infections. Such immunity seems to be lacking in cases of invasion of the digestive tract, however, except that a few cases of agglutination in low dilutions have been reported.

The results obtained in agglutination tests in this study point to the existence of a number of types. All of the strains derived from feces were agglutinated in comparatively high dilutions by a serum derived from a strain of fecal origin. This serum also agglutinated

in high dilutions 3 strains derived from water, 1 from meat, and several laboratory cultures of unknown history. An immune serum derived from a culture isolated from a wound bandage agglutinated the cultures derived from feces, but usually in lower dilutions than was the case with the preceding serum, and also agglutinated several cultures not agglutinated by the serum derived from the fecal culture. These included 1 from meat, 2 from necropsied animals and 1 from dog's saliva as well as 6 laboratory cultures. The results seem to indicate a type which is characteristic for cultures from fecal sources and another one perhaps from putrefactive sources outside of the body as well, as other types, though a final decision on the grouping cannot be established without further investigation.

Fermentation reactions did not necessarily correlate with the agglutination reactions in the tests made. It seems probable that closely related strains may sometimes vary either in respect to fermentation or agglutination reactions.

The agglutination of Proteus vulgaris cultures by the serum of patients with Weil's disease has been demonstrated only in low dilutions, and in some cases the organism cannot be identified with Proteus vulgaris. The agglutination of Proteus vulgaris by the serum of patients with typhus fever, on the other hand, is established on a better basis, agglutination by such serum having been reported as occurring regularly in high dilutions.

A number of cases are recorded of the agglutination of B. typhosus by the serum of patients with proteus infection, altho always in lower dilutions than with the homologous organism. Agglutination of proteus by the serum of patients with typhoid fever has been reported by some workers, while others have obtained negative results. In this work, a number of tests were carried out with serums which agglutinated B. typhosus in dilutions of 1 : 40 or higher, testing them against various cultures of Proteus vulgaris and with a culture of Ps. protea, which was found by Frost to be agglutinated by typhoid serums. Only one out of 13 cultures of Proteus vulgaris tested was regularly agglutinated by these serums, and this not as characteristically as was B. typhosus or the culture of Ps. protea. Agglutination of this culture was not evident after an incubation period of 1 hour, as was the case with B. typhosus and Ps. protea, but only after being retained at a temperature of 15 C. over night was there marked agglutination. One or two other proteus cultures were irregularly agglutinated by typhoid sera.

Proteus vulgaris is probably most frequently associated with decomposing organic matter of animal origin and the extent of its occurrence in water and soil probably bears a relation to the amount of such organic matter present. The occurrence of proteus in normal feces is apparently not as frequent as commonly stated.

Proteus vulgaris may be saprophytic or parasitic in nature. A pathogenic rôle has been ascribed to it by certain French workers in infantile diarrhea. Proteus has been associated with certain food poisoning epidemics as a possible causal agent, but such epidemics are relatively few in comparison with similar epidemics ascribed to B. paratyphosus B, and are not established on as firm a basis. As in the case of the latter organism the harmful effects produced may be due to the multiplication of the organism as well as to the formation of toxic substances, these toxins being very low in potency as contrasted with those produced by tetanus and diphtheria organisms.

A number of local infections including wound infections, in which Proteus vulgaris has been concerned as the primary agent or as secondary agent are described in the literature. Infections of the urinary tract due to this organism have been noted a number of times. Occasional general infections are also described.

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