BIOCHEMICAL INVESTIGATION OF PROVIDENCE STRAINS AND THEIR RELATIONSHIP TO THE *PROTEUS* GROUP*

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The search for the possible causative agents of acute infective diarrhoea in infants and children has brought to light a great number of groups and strains of enteric bacteria other than *Salmonella* and *Shigella*. None of them can yet be incriminated with certainty as being pathogenic. The problem of potential or conditioned pathogenicity cannot be solved without a more comprehensive knowledge of the biological and serological properties of the different groups of enteric bacteria and their different types. One such group of bacteria—the Providence group—has not been definitely investigated, but 'biochemically appears to be intermediate between the *Proteus* and *Shigella* groups and at present they can be classified only as a transitional form between these two groups and given neither genus nor species rank' (Stuart, Wheeler & McGann, 1946).

Stuart, Wheeler, Rustigian & Zimmerman (1943), in Providence, reported twenty-three strains, calling them anaerogenic paracolon type 29911. Formerly, Sachs (1943) designated such strains as mannitol negative types B 81 and B 105 of the *Shigella* group. Sachs investigated 15,000 specimens of stools of healthy and ill people and found the B 81 and B 105 only in persons with a history of diarrhoea. Similar organisms were listed by Bergey (1945) as *Bacterium wakefield*, related to the *Shigella* group. Rustigian & Stuart (1945) studied fifty-one strains of type 29911, three of which were isolated from urines. Stuart *et al.* (1946), who investigated a further 109 cultures, believed that 'considerable evidence has been accumulated to show that the type 29911 organism causes gastro-enteritis and diarrhoea'. Ewing & Graratti (1947), in a study of *Shigella* types in the Mediterranean area, isolated several motile cultures identical with Sachs's B 81 and B 105 and similar to the anaerogenic paracolon type 29911 of Stuart *et al.* (1946).

Galton, Hess & Collins (1947) isolated 158 types of 29911 from routine specimens received from thirty-one different cities in Florida. They could not prove their significance in relation to enteric diseases. Plass (1947), in an outbreak of diarrhoea, found an anaerogenic paracolon 29911 as the predominating organism in the food and in the stools of thirteen out of sixteen hospitalized patients. Buttiaux, Tacquet & Kesteloot (1949) isolated anaerogenic paracolon from the stools of some children during an epidemic of infantile diarrhoea. Later they found 1·5% of these types in 1000 stool cultures. Kauffmann (1951) designated the paracolon type 29911 as 'Providence' group for the first time. Brooke (1951) investigated the

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biochemical properties of thirty-five urinary strains of the Providence group. Edwards & Ewing (1952) mention that, according to unpublished data of Ewing, 50 'O' groups were established.

SOURCES AND FREQUENCY OF ISOLATION

In a group of 116 infants under 1 year suffering from non-specific infectious diarrhoea, strains of the Providence group were isolated from 10 % of the investigated stools. In a control group of 337 healthy babies examined in baby welfare centres and in closed baby homes the Providence strains were isolated from 2·3 % of the investigated stools. In both groups only one stool per infant was examined. In a second series of thirty infants with diarrhoea, from four consecutive stool examinations made per infant, organisms of the Providence group were isolated in 11·6 %, and from thirty healthy control children in 3·3 %. In another group of infants and children with *Shigella* infection organisms of the Providence group were isolated from 5·2 %, and in a series of sixty cases of *Salmonella* infection in children the corresponding incidence was 6·4 %. On the whole, strains of the Providence group were isolated from 3·25 % in a series of 5400 specimens from children with diarrhoea of all types. In a corresponding series of 500 adults with diarrhoea an incidence of only 0·6 % was found.

MATERIAL

The material investigated in this paper consists of 121 anaerogenic strains. Eighty-six belonged to the Providence group, and of those, 74 were urease negative, 12 weakly urease positive; there were 5 anaerogenic *Proteus* species and 30 *Proteus rettgeri* strains. Six of the strains initially isolated were sent to Dr C. A. Stuart, Brown University, Providence. Dr M. Greenstein identified three of them as belonging to the anaerogenic paracolon 29911 group and the other three to the *Proteus rettgeri* subgroup. Antisera were prepared from 10 strains of the Providence group and 5 strains of *P. rettgeri* for O-agglutination. Twenty further strains of Providence, *rettgeri* and *Proteus* species were identified at the Communicable Disease Center, Chamblee, Georgia, by Dr W. H. Ewing. Only their biochemical properties will be dealt with here.

Source of 86 Providence strains investigated:

	Urine	Stool	Pharynx	Post-mortem colon	Total
Children under 1 year with diarrhoea	—	17	2	2	21
Healthy children under 1 year	—	8	—	—	8
Children up to 13 years with diarrhoea	2	16	—	—	18
Children up to 13 years without diarrhoea	1	8	—	—	9
Hospitalized adults with diarrhoea	1	10	—	—	11
Hospitalized adults without diarrhoea	1	10	—	—	11
Healthy personnel of the hospital	—	8	—	—	8
	5	77	2	2	86

METHODS

The technique and methods used in the biochemical examination of the strains were those of Kauffmann (1951).

The following media were used: brom-thymol blue peptone broth containing: arabinose, rhamnose, glucose, galactose, lactose, maltose, saccharose, trehalose, raffinose, starch, dextrin, inulin, glycerol, adonitol, dulcitol, mannitol, sorbitol, inositol salicin, aesculin, cellobiose. Gas was detected only in glucose, mannitol and glycerol (with Durham's fermentation tubes). Caseolysate broth was employed for indole production, glucose-phosphate broth with 1 % glucose for the determination of the methyl red reaction and acetylmethylcarbinol formation, ferrochloride gelatin for gelatin liquefaction and H_2S production; nitrate media for nitrate reduction. Bacto-peptone solution containing dextro-tartrate, sodium citrate and mucic acid for organic acid utilization. In addition, the following media were used: Simmon's citrate agar for citrate utilization, urea agar (Christensen, 1946) and fluid urea medium (Singer, 1950) for the demonstration of urease, semisolid agar 0·25 % in U tubes for motility, 1 % agar plate incubated for 48 hr. at 37° C. or at room temperature for swarming or spreading.

The sodium citrate media, urea Christensen agar and nitrate reduction tests were observed for 4 days; the 'sugars' for 30 days, ferro chloride gelatin for at least 60 days, organic acid utilization for 14 days. The phenylpyruvic acid (P.P.A. reaction) was tested according to Henriksen's method (1950) and the result read after 4 hr. O antisera were prepared with 15 strains heated for $2\frac{1}{2}$ hr. at 100° C. The strains and sera were tested for α- and β-antigens with strains and sera received from Dr R. Mushin, Australia.

RESULTS

The properties common to the 86 Providence strains, 5 anaerogenic *Proteus* species and 30 *P. rettgeri* strains were: Gram-negative, non-sporing rods. The majority of the strains showed swarming on 1 % agar plates. Most of the strains were motile; all the strains had a characteristic smell. All the strains formed indole, were methyl-red positive, Voges-Proskauer negative, grew on Simmon's citrate agar, reduced nitrate to nitrite, did not liquefy gelatin and did not produce H_2S. Sodium citrate in bacto-peptone was decomposed, *d*-tartrate and mucate were not attacked. All strains gave strongly positive phenylpyruvic acid reaction.

Differential properties

(*a*) *Seventy-four urease negative Providence strains.* 'Sugars'. The strains can be divided into two subgroups according to mannitol fermentation: (1) anaerogenic mannitol fermenting (50 strains); (2) non-mannitol fermenting (24 strains). All strains fermented glucose, glycerol, galactose and saccharose without forming gas; all except three strains fermented also inositol and trehalose. The following substrates were not fermented by any of the strains: dulcitol, arabinose, rhamnose, lactose, maltose, raffinose, inulin, adonitol, sorbitol, xylose, starch, dextrin, salicin,

aesculin, cellobiose, except three strains which fermented one or the other of these substrates.

Table 1. *Biochemical behaviour of* 121 *anaerogenic strains*

No. of strains ...	Providence 74		Providence 12		*Proteus rettgeri* 30		Anaerogenic *Proteus* species 5	
Urea broth	—	74−	—	12−	30+	—	5+	—
Urea agar	—	74−	12±	—	30++	—	5++	—
Phenylpyruvic acid	74++	—	12++	—	30++	—	5++	—
Indol	74+	—	12+	—	30+	—	5+	—
Methyl red	74+	—	12+	—	30+	—	5+	—
Voges Proskauer	—	74−	—	12−	—	30−	—	5−
Simmon's citrate	74+	—	12+	—	30+	—	5+	—
KNO_3	74+	—	12+	—	30+	—	5+	—
Gelatin	—	74−	—	12−	—	30−	—	5−
H_2S	—	74−	—	12−	—	30−	—	5−
Smell	74+	—	12+	—	30+	—	5+	—
d-tartrate	—	74−	—	12−	—	30−	—	5−
Na-Citrate	74+	—	12+	—	30+	—	5+	—
Mucate	—	74−	—	12−	—	30−	—	5−
Mannitol	50+−	24−−	9+−	3−−	29+−	1−−	2+	3−
Glucose	74+−	—	12+−	—	30+	—	5+	—
Galactose	74+	—	12+	—	30+	—	5+	—
Saccharose	74+	—	11+	1−	27+	3−	5+	—
Glycerine	74+−	—	12+−	—	29+	1−	5+	—
Inositol	73+	1−	12+	—	30+	—	5+	—
Trehalose	73+	1−	11+	1−	8+	22−	2+	3−
Adonitol	2+	72−	—	12−	29+	1−	2+	3−
Dulcitol	—	74−	—	12−	—	30−	—	5−
Sorbitol	1+	73−	1+	11−	—	30−	—	5−
Arabinose	—	74−	1+	11−	6−	24−	—	5−
Rhamnose	—	74−	1+	11−	5+	25−	1+	4−
Xylose	1+	73−	—	12−	6+	24−	1+	4−
Lactose	—	74−	—	12−	1−	29−	—	5−
Maltose	—	74−	—	12−	—	30−	—	5−
Raffinose	—	74−	—	12−	—	30−	—	5−
Starch	1+	73−	—	12−	—	30−	—	5−
Dextrin	1+	73−	—	12−	2+	28−	—	5−
Inulin	—	74−	—	12−	—	30−	—	5−
Salicin	3+	71−	1+	11−	15+	15−	2+	3−
Aesculin	2+	72−	1+	11−	15+	15−	2+	3−
Cellobiose	3+	71−	1+	11−	—	30−	1+	4−

Key. 'Sugar': + = fermentation; − = no fermentation even after 30 days' incubation; +− = acid without gas. Gelatin: − = no liquefaction after 60 days. Smell: + = characteristic smell. Utilization tests: + = utilization after the first day of incubation; − = no utilization even after 4 days' incubation. Organic acids: + = fermentation after 2 days; − = no fermentation after 14 days' incubation. Urea-agar Christensen: ± = purple colour corresponding to slope but not reaching bottom of butt; ++ = purple colour reaching to bottom of butt. Phenylpyruvic acid: ++ = green after 4 hr.

(*b*) *Twelve weakly urease positive strains.* The biochemical behaviour of this group was identical with that of the urease negative Providence strains, except that they did not utilize urea in liquid medium but did so in urea agar Christensen medium.

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(*c*) *Five anaerogenic* Proteus *species.* All strains fermented glucose, galactose, saccharose, glycerol, inositol. No strains fermented dulcitol, sorbitol, arabinose, lactose, maltose, raffinose, starch, dextrin, inulin. Only two strains fermented mannitol, trehalose, adonitol, and only one strain fermented rhamnose, xylose and cellobiose.

(*d*) *Thirty* P. rettgeri *strains.* All the strains fermented glucose, galactose and inositol. All strains except one fermented mannitol, glycerine and adonitol. Three strains failed to ferment saccharose. Fifteen strains fermented salicin and aesculin. Dulcitol, sorbitol, maltose, raffinose, starch, inulin and cellobiose were not attacked by any strain. Only one strain fermented lactose, two strains fermented dextrin, five strains rhamnose, six strains arabinose and xylose and eight strains trehalose (see Table 1).

Phenylpyruvic reaction

The transformation of phenylalanine into phenylpyruvic acid was tested on the 121 strains investigated and also on 547 different strains of Enterobacteriaceae.

From Table 2 it is apparent that all the 148 urease negative strains, except two urease negative *Proteus* strains, were P.P.A. negative. Also the 209 urease weakly positive strains were negative after 4 hr. All 190 *Proteus* strains, including the two urease negative strains, showed a strong positive reaction. Table 3 shows that all

Table 2. *Urease and phenylpyruvic acid reaction of* 547 *strains of Enterobacteriaceae*

	No. of strains				
Groups and subgroups	Urease − P.P.A. −	Urease + P.P.A. −	Urease − P.P.A. +	Urease + P.P.A. +	No. of strains tested
Escherichia	80	—	—	—	80
Salmonella	30	—	—	—	30
Arizona	6	—	—	—	6
Shigella	30	—	—	—	30
Ballerup	—	4	—	—	4
Bethesda	—	50	—	—	50
Klebsiella	—	120	—	—	120
Paracolobactrum aerogenoides	—	35	—	—	35
Proteus species	—	—	2	—	2
P. morganii	—	—	—	60	60
P. vulgaris	—	—	—	30	30
P. mirabilis	—	—	—	80	80
P. rettgeri	—	—	—	20	20
Total	146	209	2	190	547

Table 3. *Urease and phenylpyruvic acid reaction of* 121 *investigated strains*

	No. of strains			
Group and subgroups	Urease − P.P.A. +	Urease ± P.P.A. +	Urease + P.P.A. +	No. of strains tested
Providence	74	—	—	74
Providence	—	12	—	12
Proteus rettgeri	—	—	30	30
Anaerogenic *Proteus* species	—	—	5	5

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the 74 urease negative Providence strains and the 12 urease weakly positive strains gave the same positive reaction as the *P. rettgeri* and anaerogenic *Proteus* species strains.

DISCUSSION

Stuart *et al.* (1946), studying cultures of anaerogenic paracolon 29911, believed that the strains closely resemble *Proteus* species in their biochemical and IMViC reactions, ability to swarm under proper conditions, and reaction to urea (an occasional strain being weakly positive and one strongly positive in urea medium), and having many minor and occasionally major antigens in common with *Proteus*. Kauffmann (1951) states that 'biochemically the members of the Providence group are closely related to *P. morganii* and *rettgeri*, but are not able to attack urea'. It is for this reason that the Providence group is not incorporated in the *Proteus* group.

A strong O relationship was demonstrated between *Proteus* O group 13 and the strain 1721 of the Providence group by Perch (1950). O antigenic relationship was also found by us between Providence and *P. rettgeri* strains. This will be dealt with elsewhere.

The urease weakly positive strains of Providence are biochemically identical with the urease negative Providence strains except in urease production. Also many strains of the Providence group are biochemically identical with the strains of *P. rettgeri* except in the splitting of urea.

In many families of bacteria urease positive and negative strains are listed together in the same genus, as in the genera *Micrococcus*, *Corynebacterium* and *Bacterium*. The same practice should be applied to the Providence group if the strains are biochemically and antigenically related, and differ only by the urease enzyme.

Henriksen (1950), studying 645 strains, stressed that the faculty to transform phenylalanine rapidly into phenylpyruvic acid coincides with a strong urease activity and is practically limited to members of the *Proteus* group. Testing 668 strains of Enterobacteriaceae with the P.P.A. reaction, we found no parallelism between the breakdown of urea and the P.P.A. reaction: 209 weakly urease positive strains (*Klebsiella*, *Arizona*, *Ballerup*, *Bethesda*) were P.P.A. negative. All the additional 146 urease negative strains were P.P.A. negative, while all our 74 urease negative and 12 weakly urease positive Providence strains gave a strong positive P.P.A. reaction. The P.P.A. reaction can be considered to be a significant test for Providence and *Proteus* group. The test may also be used in routine screening as a means to differentiate strains of Enterobacteriaceae from Providence-*Proteus* group, and also the inagglutinable cultures of *Shigella* from urease negative Providence strains.

Neither the antigenic properties of the Providence group nor those of the *Proteus morganii* and *rettgeri* subgroups have so far been definitely established, and the latter are antigenically related neither to each other nor to *P. vulgaris* and *mirabilis*. *Morganii* and *rettgeri* have been placed in the *Proteus* group on the basis of their biochemical properties only.

All the common physiological characteristics: smell, swarming, IMViC reactions, biochemical properties identical with *Proteus rettgeri*, and the specific phenylpyruvic acid reactions, are sufficient to suggest that the Providence group belongs to the *Proteus* group.

SUMMARY

1. Seventy-four urease negative and twelve urease weakly positive strains were studied which, according to their biochemical properties, had to be considered as belonging to the Providence group. Thirty *Proteus rettgeri* and five anaerogenic *Proteus* species were examined concomitantly.

2. 668 strains of Enterobacteriaceae were tested for the P.P.A. reaction, of which only the 192 *Proteus* and the 86 Providence strains rapidly transformed phenylalanine into phenylpyruvic acid.

3. P.P.A. test, IMViC reactions, fermentation properties, smell, swarming are similar to those of the *Proteus* group, particularly *P. rettgeri*; it is therefore suggested that the Providence strains should be incorporated in the *Proteus* group.

4. For all purposes the P.P.A. reaction appears to be better suited than the urea test for differentiating strains of Enterobacteriaceae from the *Proteus*-Providence group.

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REFERENCES

Bergey, D. H. (1945). *Manual of Determinative Bacteriology.* Baltimore: The Williams and Wilkins Co.

Brooke, M. S. (1951). Biochemical investigation on certain urinary strains of Enterobacteriaceae. (1) *B. cloacae*, (2) 'Providence'. *Acta path. microbiol. scand.* **29**, 1.

Buttiaux, R., Tacquet, A. & Kesteloot, A. (1949). Sur l'action pathogène pour l'homme de bacilles para-coli. *Ann. Inst. Pasteur, Lille*, **1**, 1016.

Christensen, W. B. (1946). Urea decomposition as means of differentiating *Proteus* and paracolon cultures from each other and from *Salmonella* and *Shigella* types. *J. Bact.* **52**, 461.

Edwards, P. R. & Ewing, W. H. (1952). The status of serologic typing in the family Enterobacteriaceae. *Amer. J. publ. Hlth*, **42**, 665.

Ewing, W. H. & Graratti, J. L. (1947). *Shigella* types in Mediterranean area. *J. Bact.* **53**, 191.

Galton, M. M., Hess, M. E. & Collins, P. (1947). The isolation and distribution in Florida of an anaerogenic paracolon type 29911. *J. Bact.* **53**, 649.

Henriksen, S. D. (1950). A comparison of the phenylpyruvic acid reaction and the urease test in the differentiating of *Proteus* from enteric organism. *J. Bact.* **60**, 225.

Kauffmann, F. (1951). *Enterobacteriaceae.* Copenhagen: Munksgaard.

Perch, B. (1950). *Om Proteusgruppens serologi. Nyt Nordisk Forlag.* Copenhagen: Arnold Busck.

Plass, H. F. R. (1947). Outbreak of diarrheal disease associated with paracolon. *J. Lab. clin. Med.* **32**, 886.

Rustigian, R. & Stuart, C. A. (1945). Biochemical and serological relationships of genus *Proteus*. *J. Bact.* **49**, 419.

SACHS, A. (1943). A report of an investigation into the characteristics of new types of non-mannitol fermenting bacilli isolated from cases of bacillary dysentery in India and Egypt. *J. R. Army med. Cps*, **80**, 92.

SINGER, J. (1950). Cultures of Enterobacteriaceae. III. Detection of urease and indol in a single medium. *Amer. J. clin. Path.* **20**, 880.

STUART, C. A., WHEELER, K. M. & MCGANN, V. (1946). Further studies on one anaerogenic paracolon organism type 29911. *J. Bact.* **52**, 431.

STUART, C. A., WHEELER, K. M., RUSTIGIAN, R. & ZIMMERMAN, A. (1943). Biochemical and antigenic relationships of the paracolon bacteria. *J. Bact.* **45**, 101.

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