

IN VITRO STUDIES ON *PROTEUS* ORGANISMS OF ANIMAL ORIGIN

BY J. E. PHILLIPS

Department of Veterinary Bacteriology, Royal (Dick) School of Veterinary Studies, University of Edinburgh

INTRODUCTION

Hauser (1885), who first described the genus *Proteus*, recognized that these organisms could be isolated from animal sources but, although many strains from man have been subjected to detailed examination, a similar survey of strains isolated from animals does not appear to have been made. Choukevitch (1911) recovered them from 25 % of samples of intestinal contents from normal horses, and there are several reports of strains isolated from or associated with disease in animals. In horses *P. vulgaris* has been found associated with abortion and illness in the newborn animal (Miessner & Koser, 1931), and causing funiculitis following castration (Lasserre, Velu & Soulie, 1941) and hoof canker (Velu & Soulie, 1942). *P. vulgaris* may be found in dysentery in pigs, although there is reason to doubt its primary pathogenic role (Andreev, 1940*a, b*; Shanks, 1953). Craige (1948) considered that organisms of the *Proteus* group may be responsible for peritonitis, omphalitis, dysentery and nervous disorders in dogs. Lesbouyries (1942) observed in a rabbit extensive abscesses from which pure cultures of *P. vulgaris* were obtained. In bovines *Proteus* organisms have been incriminated as causing pneumo-enteritis in calves (Braga & Lombardo, 1934), and Habersang (1950) drew attention to their public health importance in animals, and described several instances when *Proteus* bacilli were isolated from the tissues of newly slaughtered cattle which had shown signs of enteritis or metritis *ante mortem*. In turkey poults *Proteus* organisms have been recognized as pathogenic agents (Biester & Schwarte, 1952).

The isolation of *P. morganii* from animals has been seldom recorded, mostly from animals in zoological collections (Scott, 1926; Lovell, 1929). Morgan & Ledingham (1909) isolated this organism from bovine faeces.

It is the purpose of this paper to examine the *in vitro* properties of strains of *Proteus* organisms isolated from animals.

TECHNICAL METHODS

All media were prepared according to the methods of Mackie & MacCartney (1948), and incubated at 37° C. except where otherwise stated. All tissues, etc., were inoculated on horse blood agar plates and glucose broth, except intestinal material for which the selenite F broth-MacConkey technique was used. Strains of *Proteus* which swarmed were readily identified on blood agar plates, but in all cases sub-cultures were made on to MacConkey's medium to obtain discrete colonies which were identified on the basis of the characters described by Bergey (1948), viz. Gram-

negative bacilli failing to ferment lactose and producing rapid decomposition of urea using Christensen's (1946) medium.

Stock cultures were maintained in cooked meat medium.

Each strain was examined for fermentative activity, production of indol, liquefaction of gelatin and coagulated serum, motility, and production of hydrogen sulphide, and was subjected to the methyl-red and Voges-Proskauer tests.

Fermentative activity was tested in peptone water with Andrade's indicator and the following: glucose, lactose, maltose, mannitol, sucrose, salicin, trehalose, xylose and glycerol. The actions of a large number of strains on dulcitol, inositol, sorbitol, raffinose, dextrin, arabinose, inulin and rhamnose were also determined, but as none of these was fermented, their use was discontinued. The cultures were incubated for 14 days, after which fresh indicator was added before they were discarded. Readings were made every day.

The test for indol using Ehrlich's rosindol reagent was performed on 24 hr. peptone water cultures which were also examined for motility, and 3-day-old glucose broth cultures were used for the Voges-Proskauer and methyl-red tests. Hydrogen sulphide production was detected by lead acetate paper.

Stab cultures in nutrient gelatin were maintained at room temperature for 14 days and in deep inspissated bovine serum for a similar period at 37° C.

Each strain was tested for lytic activity against horse and sheep red blood cells. The method employed was similar to that suggested by Mackie & MacCartney (1948, p. 318) for identifying haemolytic streptococci using 6 hr. nutrient broth cultures which were mixed with an equal volume of 3% suspensions of washed red blood cells suspended in sterile Ringer solution. Each series of tests was controlled by sterile nutrient broth in place of culture. After incubation in a water-bath at 37° C. for 90 min., the tubes were centrifuged and the reading made by comparison with haemoglobin standards (corresponding to 100, 75, 50 and 25% haemolysis).

Two strains (37 and 185), which both gave 100% haemolysis with horse and sheep red cells, were used in further experiments in an attempt to determine the nature of the lytic effect. Cultures of these were either filtered through Seitz EK pads or centrifuged at 3000 r.p.m. for 15 min. and tested for lytic activity.

A specific antiserum against strain 37 was prepared in rabbits using formalin-killed broth cultures, whilst that against strain 185 was obtained using centrifuged 6 hr. cultures. Neither antiserum gave cross-agglutination with the heterologous organism.

RESULTS

The origin of the 214 strains examined in this survey is shown in Table 1, which also shows the species distribution of *Proteus* strains in animals. Included in these strains are seventeen recovered from the faeces of 217 dogs with no history of diarrhoea, in an endeavour to assess the incidence of *Proteus* in the normal canine intestine.

All the strains examined fermented glucose, producing acid and gas within 24 hr., with the exception of one strain (no. 103) which was anaerogenic and

formed acid only. Mannitol and lactose were also not fermented by any of the strains; although lactose was used as one of the criteria for the identification of *Proteus* species, it should be noted that in no case was an organism found which gave both characteristic swarming growth and fermented this sugar.

With the other sugars, however, variable results were obtained, some strains causing fermentation of the substrate within 24 hr., some producing a late fermentation and others bringing about no change within the period of incubation. No culture which gave the typical swarming growth failed to decompose urea, and hydrogen sulphide was formed by all strains.

Table 1. *Distribution of Proteus strains in animals*

	Dog	Cat	Bovine	Pig	Bird	Other		Total
						mammals	Reptiles	
<i>Proteus vulgaris</i>	5	1	15	11	10	4	1	47
Atypical <i>P. vulgaris</i>	0	0	1	0	1	0	0	2
<i>P. mirabilis</i>	116	4	14	1	14	4	0	153
Atypical <i>P. mirabilis</i>	2	0	0	0	0	0	0	2
<i>P. morganii</i>	2	0	0	0	3	4	1	10
Total	125	5	30	12	28	12	2	214

It was possible to place all, except four, of the organisms studied into three of the four species named by Bergey (1948), viz. *P. vulgaris*, *P. mirabilis* and *P. morganii*. *P. rettgeri* has not been encountered at any time in this laboratory. The *P. vulgaris* strains, which may be recognized by their prompt fermentation of maltose and sucrose and production of indol, exhibited minor differences in their fermentative activities with other sugars, but the majority fermented salicin and xylose rapidly and gave delayed fermentation of trehalose. It was not possible to correlate the differences with any other characters. Most of the *P. mirabilis* strains, which did not ferment maltose or produce indol and gave late fermentation of sucrose, fermented trehalose and xylose at once and glycerol late, but failed to attack salicin. Although slight differences also existed in these strains, no correlation with other characters could be shown.

Six of the ten *P. morganii* strains were quite typical in failing to ferment any sugars except glucose and glycerol and in forming indol, but these gave a positive reaction in sucrose after several days' incubation and one fermented trehalose.

Three of the four organisms not falling into this classification were 'maltose late positive', one being sucrose and indol positive thus resembling *P. vulgaris*, while the other two did not form indol, thus simulating *P. mirabilis*. The fourth atypical strain resembled *P. vulgaris* in its biochemical reactions with the exception that it did not ferment maltose.

The data shown in Table 2 were derived from Table 1 by grouping and omitting certain species and hosts with low incidence in order to attain a closer approximation to the conditions required for the rigorous application of the χ^2 test, which gave values of $\chi^2 = 76.62$, $n = 3$ and P is less than 0.001, thus showing that *P. mirabilis* occurs more frequently than *P. vulgaris* in dogs and cats, and that in pigs the reverse is true.

Haemolytic tests using horse red blood cells divided the strains into five approximately equal groups giving 0, 25, 50, 75 and 100 % haemolysis respectively, but when sheep red cells were employed 70 % of strains gave 100 % haemolysis.

In every case, whether any lytic action took place or not, the colour of the red cell suspension became bluish red after approximately 5 min. incubation, although the control tubes retained their original bright red colour.

Seitz filtrates of strain 185 produced no haemolysis with horse or sheep red cells, but the supernatant of a centrifuged culture caused complete haemolysis within 90 min.; in this case, however, no alteration of the colour of the blood was seen. Antisera 37 and 185 exerted no inhibitory effect on haemolysis by cultures of these two strains.

Table 2. *Frequency of Proteus species in animals*

	Dog and cat	Bovine	Pig	Bird	Total
<i>Proteus vulgaris</i>	6	15	11	10	42
<i>P. mirabilis</i>	120	14	1	14	149
Total	126	29	12	24	191

DISCUSSION

The findings for *P. vulgaris* and *P. mirabilis* agree with those of Moltke (1927), except that most of his maltose negative strains (from human sources) gave late fermentation of salicin, which similar strains from animals did not ferment.

Strain no. 103 resembles the non-maltose fermenting organisms described by Edwards (1942) and Isaacs (1947) which they isolated from man and which had all the characters of the genus *Proteus*, except the formation of gas from glucose.

Rustigian & Stuart (1945) encountered occasional strains of *P. vulgaris* which fermented maltose feebly, but they made no mention of non-maltose fermenting strains. These workers also recorded strains of *P. mirabilis* which fermented maltose.

It is possible that the more frequent occurrence of *P. mirabilis* in dogs and cats may be related to the close association of these animals with man. Most of the strains examined by Moltke (1927) from human sources corresponded to *P. mirabilis* in their biochemical reactions, a result which was also obtained by Taylor (1928). Levine (1942) recorded that more than 90 % of *Proteus* strains isolated from human stools were *P. mirabilis*, and that this species was the predominant one in wounds and other infections in man. This preponderance of *P. mirabilis* from man was noted also by Damming & Belling (1942), who recorded that 85 % of strains of *Proteus* of human origin were this species. Levine (1942) also found that *P. vulgaris* was the species usually recovered from 'packing house waste' (abattoir effluent), which agrees with the present findings in cattle and pigs.

The haemolytic activities of *Proteus* strains have engaged the attention of several workers. Wenner & Rettger (1919) recorded that none of the strains examined by them were able to lyse red blood cells either in a fluid milieu or on blood agar plates. Taylor (1928), however, obtained lysis of human red cells with all his strains and Yacob (1932), using rabbit blood agar plates, found that all his strains caused haemolysis. Haemolytic strains of *P. morgani* have been examined by Spahr

(1929) and by Sevin & Buttiaux (1939). Rauss (1936) demonstrated the production of a filterable haemolysin by this species. The results of Norton, Verder & Ridgeway (1928) indicated that certain strains of *P. vulgaris* and *P. mirabilis* were able to produce a haemolysin, but they were unable to demonstrate the presence of this lysin in Berkefeld filtrates.

The strains of *Proteus* examined in the present work showed definite differences in their activities towards horse and sheep red cells. Sheep red blood cells were more sensitive to the action of *Proteus* strains than were horse cells. The alteration in colour of the red cell suspension which occurred with every strain of *Proteus* was, presumably, an expression of the respiratory activities of the bacteria, the haemoglobin assuming the reduced form.

More *P. vulgaris* strains were haemolytic than was the case with *P. mirabilis* strains, although the difference was not as marked with sheep cells as with horse cells. The number of strains of *P.morganii* was too small for any comparison with the other species to be made. Most strains from all hosts haemolysed sheep red cells, but with horse cells approximately equal numbers of strains recovered from dogs, cats and bovines were haemolytic and non-haemolytic, whilst in those from pigs and birds the haemolytic predominated over the non-haemolytic.

The nature of the lytic agent produced by *Proteus* bacilli has not been ascertained. The hypothesis of some workers that it is an exotoxin is not upheld by the filtration experiments, although this may have been due to adsorption of the lytic agent on to the filter pad. Supernatant fluid from centrifuged cultures of *Proteus*, however, brought about haemolysis only slightly less rapidly than whole cultures and this suggests that some soluble fraction is involved in the haemolysis. Bach (1921) was unable to show any anti-haemolytic effect with immune sera, and in the present work it was not possible to show any inhibitory effect with specific agglutinating sera. Braun & Shi-Tsing (1923) and Norton *et al.* (1928), however, demonstrated an inhibitory effect on the haemolytic activity of *Proteus* strains using such sera.

Braun & Shi-Tsing (1923) claimed that the haemolytic activity of *Proteus* bacilli is closely associated with the flagellar apparatus and that O forms were non-haemolytic while H forms lysed red cells. The present findings do not agree wholly with this thesis, since only four strains in the whole collection proved to be non-motile, and although three of them gave no change in horse blood the fourth gave 25 % haemolysis. With sheep red cells two were completely non-haemolytic, one gave 50 % and one 100 % haemolysis. Many strains, however, although showing active motility and swarming, failed to produce haemolytic changes.

It was not possible to find any absolute correlation between the biochemical, haemolytic and proteolytic activities.

SUMMARY

1. The *in vitro* properties of 214 strains of *Proteus* organisms isolated from dogs, cats, cattle, pigs, birds and other domestic and captive wild animals were examined.
2. The majority of strains were *P. mirabilis*, 23 % being *P. vulgaris* and 4.7 % *P.morganii*.

3. *P. vulgaris* was found more frequently in pigs and cattle than in other animals.

4. Biochemical varieties of the *Proteus* group occurring in man have been found in animals.

5. The action of *Proteus* strains on red blood cells was examined and strain differences observed.

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REFERENCES

- ANDREEV, P. N. (1940a). *Sovetsk. Vet.*, no. 11/12, 37. Abstracted in *Vet. Bull., Weybridge* (1944), **14**, 202.
- ANDREEV, P. N. (1940b). *Veterinariya, Moscow*, no. 5, 23. Abstracted in *Vet. Bull., Weybridge* (1945), **15**, 391.
- BACH, F. W. (1921). *Zbl. Bakt. (Abt. 1. Orig.)*, **85**, 305.
- BERGEY, D. H. (1948). *Manual of Determinative Bacteriology*, 6th ed. London: Baillière, Tindall and Cox.
- BIESTER, H. E. & SCHWARTE, L. H. (1952). *Diseases of Poultry*, 3rd ed. Ames: Iowa State College Press.
- BRAGA, A. & LOMBARDO, A. (1934). *Rev. Dep. nac. Prod. anim., Rio de J.*, **1**, 41.
- BRAUN, H. & SHI-TSING, C. (1923). *Zbl. Bakt. (Abt. 1. Orig.)*, **89**, 139.
- CHOUKEVITCH, J. (1911). *Ann. Inst. Pasteur*, **25**, 247.
- CHRISTENSEN, W. D. (1946). *J. Bact.* **52**, 461.
- CRAIGE, J. E. (1948). *J. Amer. vet. med. Ass.* **113**, 154.
- DAMMING, G. J. & BELLING, F. T. (1942). *J. Immunol.* **44**, 251.
- EDWARDS, J. L. (1942). *J. Hyg., Camb.*, **42**, 238.
- HABERSANG, H. (1950). *Mh. VetMed.* **5**, 258.
- HAUSER, G. (1885). *Über Faulnissbakterien und deren Beziehung zur Septicämie*. Vogel, Leipzig.
- ISAACS, A. (1947). *J. Path. Bact.* **59**, 318.
- LASSERRE, R., VELU, H. & SOULIE, P. (1941). *Bull. Acad. vét. Fr.* **14**, 83.
- LESBOUYRIES, M. (1942). *Bull. Acad. vét. Fr.* **15**, 278.
- LEVINE, M. (1942). *J. Bact.* **43**, 33.
- LOVELL, R. (1929). *J. Path. Bact.* **32**, 79.
- MACKIE, T. J. & MCCARTNEY, J. E. (1948). *Handbook of Practical Bacteriology*, 8th ed. Edinburgh: Livingstone.
- MIESSNER, H. & KOSER, A. (1931). *Dtsch. tierärztl. Wschr.* **39**, 693.
- MOLTKE, O. (1927). *Contributions of the Characterisation and Systematic Classification of B. proteus vulgaris*. Copenhagen: Levin and Munksgaard.
- MORGAN, H. DE R. & LEDINGHAM, J. C. G. (1909). *Proc. R. Soc. Med. (epid. Sect.)*, **2**, 133.
- NORTON, J. F., VERDER, E. & RIDGEWAY, C. (1928). *J. infect. Dis.* **43**, 458.
- RAUSS, K. F. (1936). *J. Path. Bact.* **42**, 183.
- RUSTIGIAN, R. & STUART, C. A. (1945). *J. Bact.* **49**, 419.
- SCOTT, H. H. (1926). *Proc. zool. Soc. Lond.* p. 231.
- SEVIN, A. & BUTTIAUX, R. (1939). *J. Path. Bact.* **49**, 457.
- SHANKS, P. L. (1953). *Vet. Rec.* **65**, 109.
- SPAHR, M. B. (1929). *Amer. J. Dis. Child.* **37**, 541.
- TAYLOR, J. F. (1928). *J. Path. Bact.* **31**, 897.
- VELU, H. & SOULIE, P. (1942). *Bull. Acad. vét. Fr.* **15**, 177.
- WENNER, J. J. & RETTGER, L. F. (1919). *J. Bact.* **4**, 331.
- YACOB, M. (1932). *Indian J. med. Res.* **19**, 787.

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