ON *PROTEUS PSEUDOVALERIAE* AND ITS OCCURRENCE IN MAN.

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In December 1926, material was sent by the Health Board of the State of Rio de Janeiro to the Department of Biological Diagnosis of the Instituto Vital Brazil for routine examination on the typhoid-paratyphoid group. This material consisted of (*a*) a culture from the blood of a patient (in Therezopolis, State of Rio), in bile-pepton-glycerin medium, and (*b*) the patient's serum for Widal's test. Several other cases of typhoid-like diseases had previously occurred at the patient's home and the suspicion had arisen of a contact infection in this case.

Widal's test, however, proved to be negative in dilutions of 1/100 and 1/200 both against three different strains of typhoid bacillus (*H*/702, *H*/704 and Rawlings) and against *Salmonella paratyphi* (strain No. 13 of the National Collection of Type Cultures, Lister Institute) or *S. schottmuelleri* (No. 15 or Bainbridge's strain of the National Collection of Type Cultures).

By planting some drops of the blooded bile medium upon agar slants, a motile gram-negative bacillus was recovered, which promptly invaded all the surface of the medium and proved to be a gas-producer in dextrose—but not in lactose—media, also developing a strong fluorescence on neutral-red. When tested against specific sera for *Eberthella typhi* (titre 1/10,000), *Salmonella paratyphi* (titre 1/20,000) and *S. schottmuelleri* (titre 1/20,000), no agglutination could be seen in dilutions starting from 1/625 to 1/10,000. Notwithstanding this, a sample of the micro-organism was preserved for further study and, upon our request, material was again carefully collected from the patient about 20 days after the first sample was taken. The second sample gave a negative Widal reaction, although a dilution of but 1/50 was used.

The isolated micro-organism was rapidly and totally agglutinated by the second sample of serum, in dilutions up to 1/500. A series of 30 normal sera was tested in dilutions of 1/25—1/200: 29 sera gave results in all dilutions and only one caused slight agglutination in 1/50 and a partial clumping in 1/25. Therefore, the patient's serum acting in dilution 1/500 seemed to give a specific reaction.

The second blood culture, when transferred from bile to agar slants yielded again a gram-negative motile bacillus with general aspect similar to that described at first.

The laboratory tests for typhoid or paratyphoid having proved negative whilst we obtained an organism which was agglutinated in high dilution of his

serum, the conclusion appeared justified that this organism was the infective agent. This organism showed striking features of the *Proteus* group.

In 1910, Rottkay described a fatal case of a typhoid-like disease from which he was able to recover an organism related to *Pr. vulgaris* Hauser, this bacillus being isolated from faeces and, after the death, from ileon and spleen pulp.

Unfortunately it was impossible to investigate directly our case from a clinical and laboratory aspect. It was known afterwards that the patient recovered; therefore, it was thought necessary to draw attention to similar occurrences in man.

In order to distinguish the micro-organism found in our case from the *Salmonella* group and *Pr. vulgaris*, the description of its general features is as follows (we will call it shortly *Pr.* 55).

DESCRIPTION.

Rods of variable length, from cocciform appearance to long threads covering a remarkable extent of the microscopic field. No buds are seen. Chains absent. Easy and uniform staining with aniline dyes. Frankly gram-negative. Active but not exaggerated motility.

Agar-growth. After 6 hours, the growth becomes easily visible. Round and transparent colonies, with a great tendency to become confluent and presenting a continuous moist appearance On previously moisted agar-slants, the organism being carefully planted at the bottom of the medium, an ascending amoebiform growth develops over the whole agar surface. This growth is transparent and grayish-white, strictly similar to that of *Proteus X* 19 and *Pr. anindologenes* (No. 59 of the National Collection of Type Cultures). The culture emits an unpleasant odour.

Gelatin-growth. Extensive, with general character of that described on agar plates. In gelatin stab the growth becomes visible both in the puncture line and on the surface. No liquefaction occurs.

Peptone-broth and *Peptone-water.* At first, rapid and uniform clouding; after 3 days a whitish collar appears at the surface of the fluid and a distinct deposit appears. Odour very disagreeable.

Potato. Whitish and luxuriant surface growth, not characteristic.

Blooded-broth. In rabbits—or guinea-pig blood-broth, luxuriant growth occurs, a small zone of reduced haemoglobin being seen above the packed blood corpuscles. In 12–15 days the colour changes to a deep greenish brown. No haemolysis occurs in horse's blood, but a similar change in colour occurs.

Milk. After 20 days a very slight coagulation appears only near the bottom of the medium. After 2 months this partial coagulation still persists, without any digestion or peptonisation.

Litmus-milk. After 48 hours, slight fading that increases the medium being colourless on the 5th day. After 12 days, the medium becomes pinkish violet.

Indol. Abundantly formed.

Acetyl-methyl-carbinol. Absent.

Sulphuretted hydrogen. Moderate production.

Neutral-red. Strong fluorescence.

Pigment production. Absent.

Anaerobic cultures. Well grown after 18 hours, both on glucose-agar and on bouillon.

Fermentation of carbohydrates. The fermentation tests were made with Hiss serum-water medium, coloured with 4 per cent. of phenol-red solution (0·04 per cent.), the carbohydrate solution being sterilized by filtration. Chemically pure Pfahnstiehl carbohydrates were used. Table I records the results observed after 1–15 days at 37° C.

Table I.

Time at 37°	Dextrose	Levulose	Galactose	Maltose	Lactose	Sucrose	Mannitol
24 hours	+ + + +	+ + + +	+ + + +	+ + + +	±	−	+ + + +
48 hours	.	.	.	.	+	−	.
4 days	.	.	.	.	+ +	−	.
7 days	.	.	.	.	+ +	−	.
15 days	.	.	.	.	+ +	−	.

Time at 37°	Salicin	Dulcitol	Inulin	Raffinose	Amygdalin	Sorbitol	Erythritol	Arabinose	Dextrin	Glycerin	Rhamnose	Xylose
24 hours	+ + + +	−	−	−	−	−	−	+ + + +	+ +	−	+ + + +	+ + + +
48 hours	.	−	−	−	−	−	−	.	+ +	−	.	.
4 days	.	−	−	−	−	−	−	.	+ +	−	.	.
7 days	.	−	−	−	−	−	−	.	+ + +	−	.	.
15 days	.	−	.	.	.	.	.	.	.	.	.	.

+ + + + = complete coagulation with gas bubbles.
+ + + = complete coagulation without gas.
+ + = acid production without coagulation.
+ = slightly acid (yellowish pink fluid).
− = no change.

Acid and gas were formed from dextrose, levulose, galactose, maltose, *mannitol*, *salicin*, arabinose, xylose and rhamnose; on dextrin there was acid but no gas; *lactose* was slowly attacked with some interesting features, but no gas bubbles were seen; no change occurred in *sucrose*, *dulcitol*, amygdalin, raffinose, erythritol, inulin, sorbitol and glycerin.

Agglutination experiments. As reported above, *Pr.* 55 was not agglutinated by either typhoid or paratyphoid sera. It showed definite agglutinogenic power, both in man and in animals. The patient's serum caused its rapid agglutination in the dilution 1/500. In rabbits, one subcutaneous injection of 1/5 of a living agar slant was followed by agglutinative titre of 1/1000 of the serum, after 5 days. Rabbit's serum, as tested before the immunisation, did not agglutinate in dilutions from 1/25 to 1/200.

By testing the immune serum immediately after the rabbit had been bled, against related micro-organisms, the results recorded in Table II were obtained:

Table II.

Tested strains	*Pr.* 55 anti-serum dilution in 1/50	1/100	1/200	1/500	1/1000	Time of the result
Pr. 55	+ +	+ +	+ +	+ +	+ +	24 hours
Pr. vulg. (*X* 19)	+ +	+	+	±	−	24 hours
Pr. anindologenes	+ +	+ +	+ +	+ +	+ +	1 hour
Pr. mirabilis	+	+ +	+ +	+ +	±	4 hours
Salm. paratyphi	+	±	−	−	−	24 hours
S. schottmuelleri	+ +	+ +	+ +	+ +	+ +	2 hours

A specific agglutination occurred in 1/1000, becoming complete only after 24 hours. Several group clumps were also observed. The agglutination of *Pr. anindologenes* proved to be much more rapid than that of the antigenic strain and a similar but weaker condition was found for *S. schottmuelleri*. On the contrary, *S. paratyphi* proved to be little susceptible to *Pr.* 55 agglutinins.

These group agglutinins were particularly labile in nature, because they disappeared after 4 days in glycerolated serum. Such serum gave results recorded in Table III:

Table III.

Tested strains	*Pr.* 55 anti-serum dilution 1/50	1/100	1/200	1/500	1/1000
Pr. 55	+ +	+ +	+ +	+ +	+ +
Pr. vulg. (*X* 19)	−	−	−	−	−
Pr. anindologenes	−	−	−	−	−
Pr. mirabilis	−	−	−	−	−
Salm. paratyphi	−	−	−	−	−
S. schottmuelleri	−	−	−	−	−

(All readings were made after 24 hours.)

Pathogenicity for animals. Subcutaneous inoculations of 1/5 of a living agar slant were well supported by rabbits and guinea-pigs. The latter proved to be insusceptible to this form of inoculation and to intraperitoneal inoculation. After intracardiac injection the animals died quickly after receiving doses such as 1/5 of an agar slant, the micro-organism being recovered from heart's blood and bile. Smaller doses, viz. 1/10 of an agar slant, caused death more slowly, after 2 days, guinea-pigs showing prostration, loss of appetite and fall of temperature. Even in this instance *Pr.* 55 could be recovered from heart's blood after death.

Rabbits seemed very susceptible to intravenous inoculation, death followed rapidly upon doses such as 1/10 of a living culture on agar slant. White mice proved to be very resistant to subcutaneous and intra-peritoneal administration of living cultures.

Discussion.

The general biological characters of *Pr.* 55 seemed to authorize its inclusion in the genus *Proteus* Hauser, according to the Key of the Committee of American Bacteriologists (see Bergey, 1925, p. 230). Although *Pr.* 55 does not produce acid and gas from sucrose, all its other general features are those of a *Proteus*. Furthermore, in Bergey's Key a bacillus, already described by A. E. Boycott in 1906 as "Valérie 25," is said to belong to the genus *Proteus*, notwithstanding the statement in Boycott's original description that the organism did not attack cane-sugar.

Another feature of *Pr.* 55 that militates against its inclusion among *Proteus* is its behaviour on lactose. It is easy to see, nevertheless, that in the American Key an organism (*Proteus hydrophilus* Sanarelli) of a similar character was described that produces acid but no gas on lactose.

Some features of *Pr.* 55 (motility, no liquefaction of gelatin, fermentation of mannitol and salicin) seemed to secure its place as *Pr. valeriae* Boycott. However, its behaviour on dulcitol, lactose and dextrin, beyond its agglutinative properties and the source of its isolation, indicate that it is a new *species*, which I name *Proteus pseudovaleriae.*

Summary.

This paper deals with a micro-organism recovered from human blood in a case of a typhoid-like infection. No evidence could be produced of either typhoid or paratyphoid bacilli being the cause. Only one germ was recovered from blood cultures on two occasions, it was strongly agglutinated by the patient's serum, suggesting that it probably produced a true infection in man. This study was undertaken because certain features of the organism may lead to a faulty diagnosis of paratyphoid. The author deems it a new species in the light of the tests he applied and refers it to the genus *Proteus* Hauser (*emend.* Amer. Comm.) under the name *Pr. pseudovaleriae.*

REFERENCES.

Bergey, D. H. (1925). *Manual of Determinative Bacteriology*, Baltimore: Williams and Wilkins (2nd ed.).

Boycott, A. E. (1906). *J. of Hygiene*, **6**, 33.

Rottkay, C. von (1910). *Deutsch. med. Wochenschr.* **36**, 750.

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