Bacillus serositidis, new species (*)

(Isolated from a human case of a primary inflammation of the serous membranes).

by

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(With plates I—III).

The present paper represents a more detailed account of a bacteria apparently not yet referred to in the litterature, which we have had the opportunity to study. Its main characteristics were published in «Brasil Medico», December 13th, 1930.

On the 25th June, 1930, we received from Dr. Pena de Azevedo material from an autopsy he had just performed with Dr. Oswino Pena. The patient died in Prof. Agenor Porto's ward, Hospital S. Francisco de Assis. Summary of the clinical record:

E. C., brazilian, male, 20 years, old, mulatto workman.

He denies morbid antecedents which might have exerted any influence upon the present disease. His first admission into the hospital was on the 6th December 1929. He was complaining of weakness and weariness, and extensive oedema. Two months previously when playing football he had a sudden attack following which the actual disease begun. The examination revealed a small quantity of free effusion in the peritoneum and pleura, and also scarce stertors of bronchitis. Decayed teeth, with suppurative foci in some of them. Negative Wassermann's reaction in blood. Amount of urea in blood—0.35 per 1,000. Urine examination (24 hours): volume — 500 c.c., density—1020, sodium chloride 2.2 grs. per 1,000, urea—19 grs. per 1,000, albumin—12 grs. Microscopic examination of the sediment—granular and hyalin casts; no erythrocytes. The functional tests of the kidneys demonstrated that there was disturbance of water elimination. The concentration and dilution powers were normal.

The diagnosis of lipoidic nephrosis was established. Several treatments have been made, and after three months he was dismissed, free

(*) Received for publication, July, 21st. 1931.
from infiltrations but with albuminuria. After proper care the condition of the mouth was improved and an amygdalectomy was performed by Dr. Estevão Rezende. Three months later, he again returned to the ward, exhibiting the same symptoms.

A treatment with calcium chloride was made, at that time and afterwards thyroidine was employed. Suddenly, on the 22nd June, in the afternoon, the patient complained of severe pains in the abdomen irradiating over the thorax and followed by diarrhoea and chills. In the morning of the 23rd, his temperature was 40°C. He presented polypnoea, tachycardia, abdominal tension and tympanism. The abdomen was tender at palpation and other symptoms of peritonitis were present. Acute diarrhoea was seen at this time accompanied by vomiting. With these symptoms of a severe and acute infection the patient died at 8 1/2 hours a.m. of the 25th June, 1930.

The present case was reported to the Medical Society of the Hospital S. Francisco de Assis and was published in the paper of the same association, in January 1931, by Dr. Schmidt Mendes to whose care the patient was commended and to whom we are indebted for the clinical records he afforded to us.

On the 7th December 1930, a pathological report was made by Dr. Osvino Pena at the same society.

The autopsy performed 3 hours after death, revealed the presence of a fibrino-purulent inflammation of the peritoneum, pericardium and pleura. Chronic passive congestion of the liver. Nephrosis. Judging from the general aspect, Dr. Osvino Pena and Dr. Pena de Azevedo verified they were dealing with an uncommon case. They, therefore, have taken up some material for bacterial research, and that they did from a small closed blister of the pleura.

Microscopically the contents of the blister showed polymorpho-nuclear leucocytes, most of them containing numerous Gram negative bacilli. Sections from all the serous membranes revealed an acute inflammatory process. As no acute inflammatory process could be found elsewhere (pancreas, appendix vermiformis, biliary ducts, intestine, lungs, etc.) probably, we were dealing in such case with a primary acute inflammation of the serous membranes.

The anatomical diagnosis was: acute fibrino-purulent peritonitis, acute splenitis, lipoidic nephrosis, chronic passive hyperaemia and oedema of the lungs, chronic passive hyperhaemia and fatty degeneration of the liver, oedema of the inferior limbs.

Examining the smears, Friedländer bacillus (K. pneumoniae) was
suspected by us and Dr. O. Pena, and so a mouse was injected with pus from the blister. Cultures were also made in order to isolate bacteria of the colon-typhoid group or dysenteric group. (Plate II fig. 12 and Plate III fig. 1).

Pure culture of a bacteria with the same morphology of that reported from this pleural blister was obtained in culture media.

*Bacillus serositidis*, n. sp.

(*Eubacteriales* — *Bacillaceae*).

**Morphology:** Rods of 1.5 to 3 micra by 0.7 to 1.1 micron; rounded ends; generally isolated. In old cultures elongated forms and some chains are found. They are actively motile.

We have verified always the presence of one polar flagellum in about a hundred smears. There were rare instances in which one could observe two flagella, and very rarely three. It seemed to us we had, then, to deal with phases of division or occasions in which the loosened flagella artificially happened to adhere to the bacillary body. The flagella easily separate themselves from the bacillary body; even in young cultures there are only a few which maintain their connection with the bacillus. (Plate I figs. 3—10).

The bacillus has sub-terminal spores, of a roundish or slightly oval shape. The free spores measure 0.5 to 0.8 and even 1 micron. The spores swell almost always the bacillary body; they are easily stained by the usual dyes, and also easily decolorized by the usual solutions of acids. To put them in evidence it is necessary to use a very diluted acid or absolute alcohol and to proceed rapidly. (Plate II fig. 11 and Plate III fig. 2).

An important and typical character, is the presence of a metachromatic granule, almost always of equal size in all the bacilli of a smear. The granule is situated at one end of the cellular body. This appearance exhibits a great regularity being altered only in old cultures in which the granule appears to be larger or smaller, or sometimes more than one in the same bacillus. The granule is easily brought into evidence by the usual, methods employed in such cases (see Plate I fig. 2).

**Gram's method.** Here it is necessary to emphasize the care which should be taken when performing this staining method. We used the classic techniques as follows: Anilined Gentian-violet solution — 5 minutes; Lugol—2 or 3 minutes; decolorizing with 95% alcohol until complete disappearance of all traces of the dye (1/2 to 2 minutes). Washing in running water and counterstaining with Ziehl's diluted fuchsin (Gram.—Fortschr. d. Med., V. 2—in Zinsser). With this procedure the bacillus appears as Gram
negative. The smears must be thin and homogeneous. Any alteration in this technics results in the appearance of Gram-positive forms, more or less numerous, bearing the aspect of a Gram-doubtful germ, the one and same bacillus exhibiting, at times, Gram-positive and Gram-negatives parts. (Plate I fig. 1).

Summarizing, the main morphological characters are: roundish or oval sub-terminal spore, a polar flagellum, a polar metachromatic granule of the same size in all the bacilli of a film and, finally, the particular staining by Gram's method.

Cultural characters: Plate agar: cultures of 24 hours, 37°C. show colonies, pinhead-like, circular, translucent, shiny, from 0.2 to 0.3 mm., homogeneous, not confluent, with slightly irregular borders under the microscope. After 48 hours, they measure from 0.5 to 1 mm., some smaller ones still remaining. The central part becomes elevated and forms a ring darker than the remaining part of the colony. After 72 hours this aspect grows more accentuated, at times two or more concentric rings being seen. The marginal part of the colony is transparent and its borders are irregular.

Agar slant: In young cultures the slant is translucent and afterwards whitish, slimy and no adherent to the media.

Agar Stab: Growth on the surface and along the puncture near the surface.

Gelatine Plate: After 24 hours, punctiform colonies. After 48 hours, they are bigger, translucent with irregular margins. A dark ring appears at its center as in agar colonies. Liquefaction of the medium begins after 48 to 72 hours.

Gelatine Stab: Crateriform liquefaction followed by a stratiform one.

Broth: Flocculi and streamers into the fluid. No turbidity and no pellicle.

Litmus milk: Very scarce growth, no acidification, no coagulation. Litmus altered becoming dark brown in 8 days.

Potato: Very scarce and no visible growth.

Indol: does not form.

Nitrates: are not reduced to nitrites.

Carbohydrates: No acid or gas in dextrose, lactose, glycerol, mannitol, amygdaline, rhamnose, xylose, salicine, erythritol, adonitol, inulin, mannose, sorbitol, inusitol, dulcitol, aesculin, melecytose and trehalose.

Coagulated serum—No liquefaction.

Optimum temperature: 37°C to 40°C.
**Heat resistance**: It is resistant to 90°C. during 1 hour, the emulsions being made in sealed ampules.

It did not exert any pathogenic activity upon rabbits, Guinea-pigs, rats and mice, when using the usual process of inoculation and passages. Aerobic strict.

**Agglutinines**: Rabbit inoculated with emulsion of living germs produced an agglutinating serum until 1/200. Normal rabbit serum does not agglutinate the germ.

**Complement fixation**: The same serum which had been used in the agglutinines test, strongly fixed the complement in contact with a germ emulsion; no fixation has been observed with serum of normal rabbits.

**SUMMARY AND CONCLUSIONS**

This is a bacteriological report of a case of a primary acute inflammation of the serous membranes (peritoneum, pericardium, pleura). A bacteria apparently not yet described in the litterature was isolated. It is here described as *Bacillus serositidis*, and it was classificated according to the system of the American Bacteriologists Society.

*Bacillus serositidis* exhibits the same morphology either in the pus, smears and sections of the serous membranes or in the culture media.

The patient, evidently, died from a fibrino-purulent inflammation of the serous membranes. This acute inflammatory process was probably caused by the bacillus found in the smears and sections as no other injury could be demonstrated.

**EXPLANATION OF PLATES I—III**

**PLATE I**

Fig. 1—*Bacillus serositidis*. Culture of 24 hours. *Gram’s method*. Counterstaining with Ziehl’s diluted fuchsin (Obj. 1,35 (Winkel), oc. 10 (Zeiss) height of the table).

Fig. 2—*Bacillus serositidis*. Culture of 24 hours. *Neisser-Egge’s Staining method*. metachromatic granules.

Figs. 3-10—*Bacillus serositidis*. Culture of 24 hours. *Casares-Gil’s Staining method*. Flagella found in some microscopical fields.

**PLATE II**

Fig. 11—*Bacillus serositidis*. Culture of 24 hours. *Moeller’s staining method modified*. Sub-terminal spores are seen. The remaining part of the bacillus exhibits a more or less vacuolated protoplasm.

Fig. 12—Peritoneal section. Fixation by *Zenker’s fluid*, embeded in paraffine and sections of 5 micra, stained by *Gram’s method*. Leucocytes are seen phagocytizing, in a great number, the *Bacillus serositidis*. 
PLATE III

Photomicrographs.

Fig. 1—Magnification of 1500 diameters. Peritoneal section, the same which has been used for the drawing represented in Plate II fig. 12. Here also leucocytes are seen phagocytizing the *Bacillus serositidis*.

Fig. 2—*Bacillus serositidis*. Culture of 24 hours. Stained by Gram's method. Photomicrograph of the same slide which has been used for the drawing represented in fig. 1 of Plate I.

Fig. 3—*Bacillus serositidis*. Culture of 48 hours. Demonstration of spores. The same slide which has been used for the drawing represented in fig. 11 of Plate II.
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