Streptococcal epizootic in guinea pigs

by

C. Magarinos Torres, G. Pacheco and Rita A. de Almeida Cardoso

Of a batch of 58 guinea pigs inoculated with Leishmania enriettii, the aetiological agent of spontaneous Leishmaniasis of the guinea pig, from August 1948 until February 1949, 8 animals suffered from an intercurrent disease, at times appearing in animals in which the lesions of Leishmaniasis were already regressing.

I — DESCRIPTION OF THE DISEASE

The disease showed itself by the appearance of purulent cutaneous lesions, localized either in the cervical, axillary or inguinal regions or on the anterior abdominal wall.

These lesions were easily recognized on palpation by the feeling of areas of softness or of slightly mobile nodules under the skin.

After some time epidermis in these regions ulcerated, discharging a creamy pus with a false appearance of caseification.

The autopsy showed that the suppurative inflammation originated from an acute purulent lymphadenitis.

Several animals survived the disease relatively well only having the purulent cutaneous lesions which showed a natural tendency to regression. Other animals, however, died with an acute fibrinopurulent peritonitis and multiple liver abscesses.

In August 1949 an epizootic disease occurred spontaneously among the guinea pigs in the animal house of the Instituto Oswaldo Cruz in the section of guinea pigs imported from the U.S.A. This disease was characterized by a submaxillary lymphadenitis either showing one enlarged lymphatic gland, among others less enlarged, or with the simultaneous enlargement of a group of glands. The lymphadenitis progressed leading to suppuration within 4-5 days, discharging a yellowish pus with a syrupy consistency
causing a permanent fistula until death. Sometimes there was a spontaneous regression of the disease.

Frequently the joints of the anterior, or of all four limbs, were swollen, not uncommonly the joint complications making it difficult or even impossible for the animal to walk. Other times the joint swelling was the only sign present; thus it was purely a polyarthritis.

In the section where the disease occurred 18 guinea pigs died.

The bacterial study we describe below was done on animals belonging to the group kept in the animal house, none of them having been inoculated. The suppurative cutaneous lesions were similar to those we had observed previously in the batch of animals experimentally inoculated with the Leishmania referred to above.

II — AETIOLOGICAL AGENT

The smear of the fluid obtained from the joint did not show any bacteria but on culture there was a pure growth of streptococci showing the characteristics described below.

In the pus from the gland, however, the smear showed a mixed flora, Gram positive cocci and Gram negative bacilli. Culture of the pus, in broth and on blood agar, always gave a growth of bacteria identified as Staphylococcus, Pasteurella, Corynebacterium and numerous colonies with a diameter of less than 1 mm., smooth, round, with regular edges, not adherent to the medium, surrounded by a halo of haemolysis, similar to those grown from joint fluid mentioned above. Smears showed that these were composed of Gram positive cocci in chains of 4-20 or more. We always isolated this coccus in pure culture, either from enlarged lymphatic glands or from the joint fluid.

Animal inoculation with the various bacteria isolated showed that the pathological appearance described above was reproduced only by the streptococcus. Consequently only this was studied thoroughly for its identification.

The use of sulphanilamide added daily to the feeds of all the animals for 30 days, dosis of 10 g. per 3 k. of food, stopeed the epizootic in a short time.

The causal agent was a Gram positive coccus arranged in chains of 5 to 20 or as diplococci with faded opposing faces, non-motile and encapsulated.
Culture in nutrient broth produced turbidity with a viscid deposit which broke up on strong shaking.

Blood agar: Greyish-white colonies non-adherent to the medium, smooth surface, shiny, regular edge, forming round the colony a circle of beta-haemolysis surrounded by another zone of partial haemolysis.

Gelatin: Growth without liquefaction.

Litmus milk: Acidified, slow coagulation (4 days). Some strains only acidified without coagulation.

Bile: Not lysed in 10% bile. Growth on 10% bile agar but not on 40%.

Acetoin not produced, nitrates not reduced to nitrites, neutral red not reduced.

Dextrose, galactose, sorbitol, maltose, mannitol, salicin (slight), aesculin, maltose, lactose and sucrose are fermented without production of gas. Arabinose, ramnose, glycerol, inositol, dulcitol, adonitol and trehalose are not fermented. No hydrolysis of starch.

Coagulated plasma not liquified, showing that there was no production of streptolysin.

Reductase test: negative.

No hydrolysis of sodium hippurate.

Death at exposure to a temperature of 60°C. for 30 minutes.

No growth at 45°C.

Pathogenicity: Guinea pigs inoculated subcutaneously with 0.5 ml of a 24 hour broth culture at 37°C. caused the same disease as that which occurred spontaneously, this being a suppurative lymphadenitis with later formation of an abscess and cutaneous ulceration. The same dose inoculated intraperitoneally caused an acute fibrino-purulent peritonitis extending to the tunica vaginalis.

Doses of 0.25 and 0.5 ml. subcutaneously and intraperitoneally killed mice in less than 24 hours. Filtrates of these cultures in doses of 0.1, 0.2 and 0.3 ml. injected intravenously produced no symptoms or lesions in mice.

The coccus was not found in the circulating blood either in the naturally or artificially infected animals.
Microscopical study of material obtained from the autopsy of a guinea pig showed an acute fibrino-purulent peritonitis, an acute supplicative lymphadenitis, necrosis and abscesses of the liver.

Clumps of bacteria were seen in the lumina of the central lobular vessels and in the adjacent sinusoids of the liver. The whole liver lobule showed coagulative necrosis probably caused by the action of toxins produced by the bacteria lodged in these vessels and which probably spread through the relatively wide zone of the hepatic lobule. The edges of the necrotic area were lined by an infiltration of heterophilic leucocytes. Thus in only a small part nearest the portal spaces were there healthy liver cells.

In the lymphatic glands at the side of small collections of heterophilic leucocytes situated inside the medulla, extensive areas of purulent exudative necrosis were found; no doubt, as in the liver, due to the action of toxins. Macroscopically the creamy pus was suggestive of caseous necrosis. Microscopically it was found to be a purulent inflammatory exudate with almost total necrosis of the pus cells.

The acute inflammatory process found in the lymphatic glands, peritoneum and liver, had the usual characteristics of this type of acute inflammation.

There was a considerable proliferation of fibrocytes in the tissues round the inflammatory process. It was not uncommon to find a densely cellular tissue consisting entirely of young fibrocytes which penetrated and separated the fibres of the adjacent muscles. In a previous phase one could find the active mobilization and multiplication of regional fibrocytes and of the cells of the adventitia of the blood vessels giving rise to macrophages.

This phenomenon is well known and found regularly in acute inflammation. What attracts one's attention, however, is its exaggerated form in the case of the guinea pig compared with what one observes in other animal species and in man.

IV — BACTERIAL IDENTIFICATION

Edwards points out that streptococci found in animals have been not well studied. The differentiation of human strains was attempted by Holth, Anderson, Ayres, et al, Avery and chiefly, according to him, by Ogura.
LANCEFIELD using precipitin tests, managed to divide streptococci into groups designated by letters, including in group C the animal streptococci.

The more significant biochemical properties for differentiating are set out in the table below.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>HYDROLYSIS OF SODIUM HIPPURATE</th>
<th>GROWTH IN BILE</th>
<th>REDUTASE</th>
<th>FERMENTATION OF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10%</td>
<td>40%</td>
<td></td>
<td>sorbitol</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>trehalose</td>
</tr>
<tr>
<td>A.......</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B.......</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C.......</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D.......</td>
<td>+ ou -</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

The hydrolysis of sodium hippurate and the greater acidification of dextrose are considered by Ayres and collaborators to be valuable tests for the distinction of streptococci of human origin from those of animal origin. This has not always been confirmed.

With 173 animal and 75 human strains, Edwards found no difference in the fermentation of salicin and lactose. In his opinion of more value were the fermentation of sorbitol, attacked by streptococci of animal origin and rarely by those of human origin, and the fermentation of trehalose, attacked by those of human origin and rarely by those of animal origin. Glycerol behaves in the same way as trehalose.

Bezeley and Battle, working with 457 strains of streptococci from horses, differentiated very well Streptococcus equi (of strangles) from St. pyogenes, var. equi (from other inflammatory infections of the horse), using chiefly fermentation tests. These agreed with the serological tests (precipitins) of Lancefield and these authors suggested that they could be substituted for them.

In Bergey's Manual the fermentation of sorbitol is accepted as a basic characteristic in the differentiation of St. pyogenes from St. zooepidemicus, the fermentation of lactose being included as a secondary character. None of group C hydrolysed sodium hippurate, a property peculiar to St. agalactiae.

The chief characters of the pyogenic groups in Bergey's Manual are: limits of growth, above 10.9 C. and below 45.9 C. Haemolysis of type beta. Coagulation of milk, rare. Absence of fermentation of mannitol and glycerol.
The causal organism in the epidemic in guinea pigs which we were studying showed these characters besides fermenting lactose and sorbitol and not fermenting trehalose.

As was stressed by LINGELSHEIM, the coagulation of milk depends on the fermentation of lactose, this property having a certain value in his opinion, in the differentiation of the *viridans group*, where there is strong fermentation of this sugar and consequently coagulation of milk.

Considering the characteristics mentioned above, the organism isolated in these epizootic of guinea pigs, belongs to the species *St. zooepidemicus* of FROST and ENGELBRECHT, following the scheme of classification in Bergey's Manual. In any case it would be included in LANCEFIELD'S group C.

**CONCLUSIONS**

At the end of 1948 and 1949 there occurred in Instituto Oswaldo Cruz, epizootics in guinea pigs, the first cases among animals inoculated with *Leishmania enriettii* and the other, in animals of American origin, not inoculated and kept in the animal house of the Institute.

The epizootic was rapidly controlled by the use of sulphanilamide, added to the feeding daily in the dose of 10 g per 3 k of food, given to the whole batch for 30 days.

The causal organism was identified as *St. zooepidemicus* of FROST and ENGELBRECHT, following the classification of Bergey's Manual, and would be included in LANCEFIELD'S group C.

The disease was characterized by cutaneous abscesses following an acute purulent lymphadenitis and polyarthritis.

In some animals the purulent lymphadenitis and the following cutaneous abscesses regressed. In others they persisted until death. In some of these an acute fibrino-purulent peritonitis with necrosis and abscesses in the liver were found.

The considerable proliferation of fibrocytes seen in the regions near the acute inflammatory process was noticed being quite marked as compared with that seen in man and other animals.

This should be taken into account when spontaneous and experimental inflammatory changes are investigated in the guinea pig.