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PRECIPITATION IN PURE STATE OF BORRELIA ANSERINA FROM SPIROCHEATOSIS INFECTED FOWL BLOOD BY ULTRACENTRIFUGATION.

By

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Borrelia anserina, the causative agent of fowl spirochetosis (1), can be demonstrated in fowl blood by Giemsa or Nigrosin techniques, or may be seen in wet mounts by dark field illumination. The spirochetes are always seen in appreciable numbers located outside the erthrocytes singly or in clusters, the latter is more frequently observed toward the end of febrile period when the spirochetes become agglutinated as a result of development of agglutinating antibodies.

Many attempts were carried out for the separation and maintenance of B. anserina from the infected fowl blood. El Dardiry (2) maintained B. anserina in virulent form for 30 days in a refrigerator after separation from clotted blood taken from infected chickens. This method maintained virulence better than if maintained in citrated blood (4) McNeil et al (3) found spleen, heart and liver infectious after storage at zero C. for 31 days.

The aim of this communication is to precipitate B. anserina in pure state from spirochetes infected fowl blood by ultracentrifugation.

Materials and Methods

Spirochetes Infected blood.

A pool of infected blood was made from 7 samples of blood collected from 7 spirochetosis-infected Hubbard type, 6 weeks old chickens, which showed the clinical signs of the disease including body temperature 44°C. which were bled by heart puncture. Blood coagulation was prevented by 8% sodium citrate solution (1 ml of anticoagulant to 7 ml of the blood). This pool was examined

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by dark ground microscopy and the active, motile spirochetes were easily seen. This blood was designated SIB.

Centrifugation.

IEC (International Equipments Co., N.Y.,U.S.A.) automatic superspeed refrigerated centrifuge was used in this experiment. The speed used was ranging from 1500 r.p.m. to 20000 r.p.m.

Results

SIB was examined under the dark field microscope and tremendous numbers of spirochetes were seen which were very active and motile (Fig. 1).

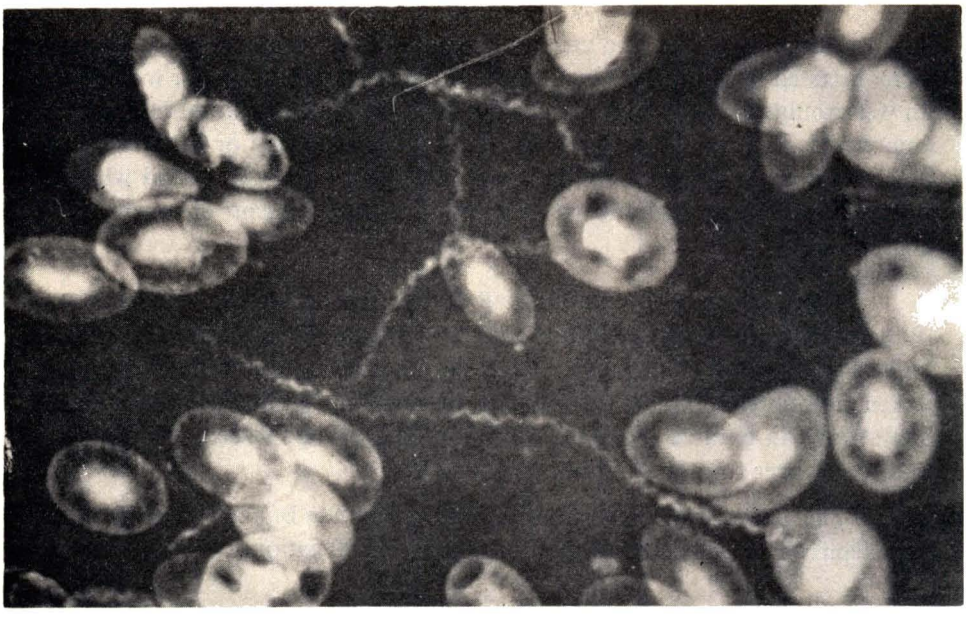

Fig. 1. Borrelia anserina in fowl blood. This smear was made in the early Stages of the disease.

Then this blood was put in the centrifuge at the rate of 1500 r.p.m. for a period of 10 minutes. The deposited red blood cells at the bottom of the centrifuge tube were examined by dark field microscope and no spirochetes could be detected.

The supernatent fluid which constitutes the blood plasma was examined by the same method and the active motile spirochetes were seen clearly in large numbers.

Again this plasma was centrifuged at a speed of 16000 r.p.m. for a period of 20 minutes. A spot of deposition was formed sticking at the bottom of the centrifuge tube with a very clear supernatent fluid.

Very little organisms were seen in the supernatent fluid when examined by dark field microscope (Fig. 2).

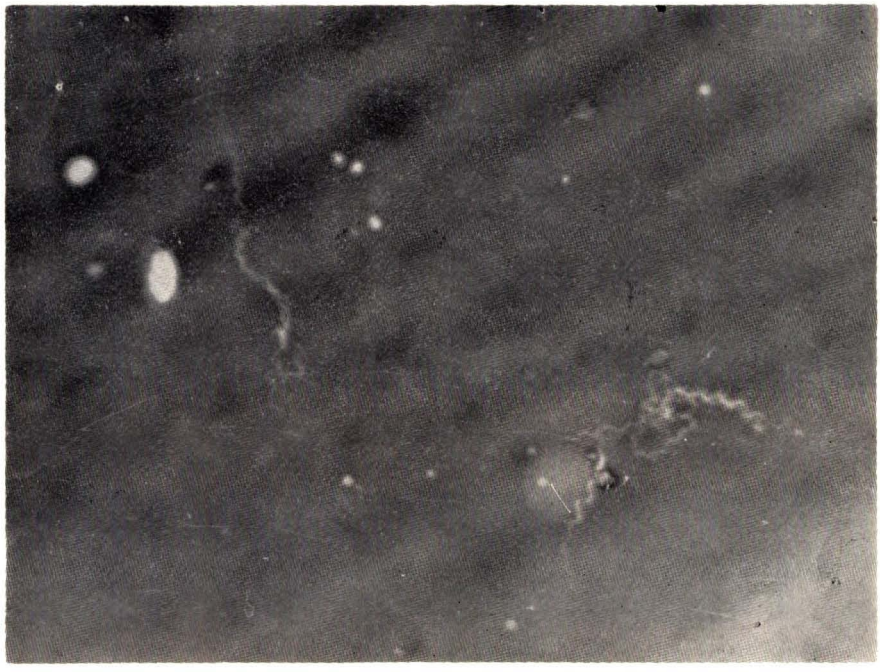

Fig. 2

The deposit was examined by the same method and the spirochetes were seen in pure masses and in very large numbers against a very clear background (Fig. 3a and Fig. 3b).

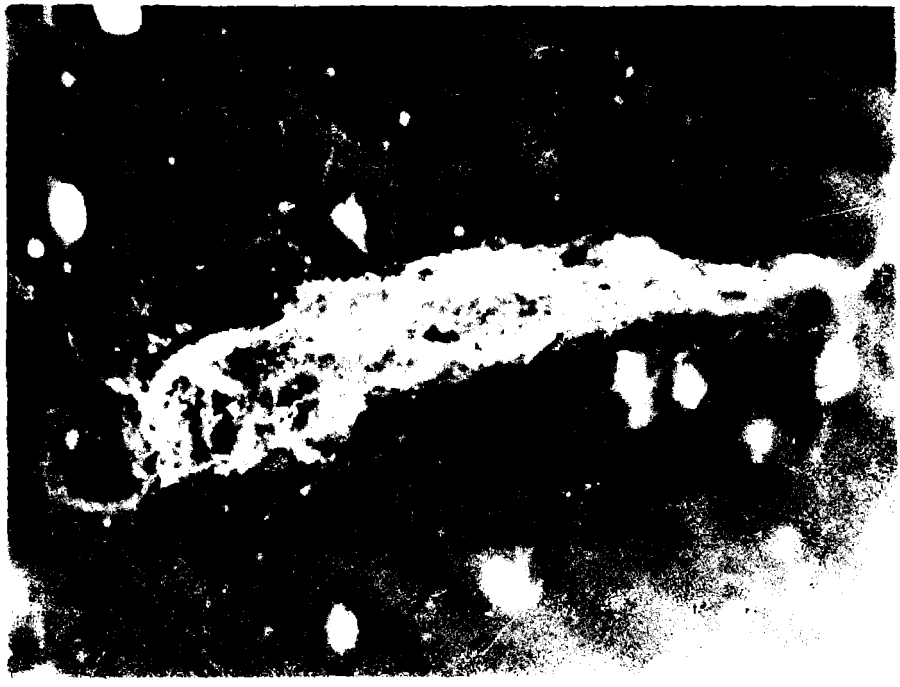

Fig. 3a.

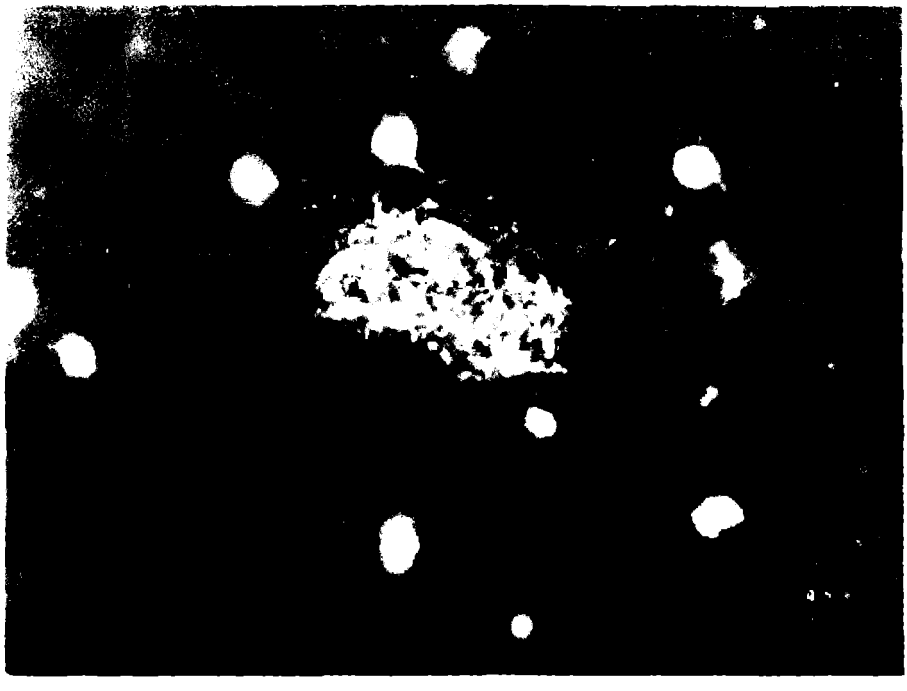

Fig. 3b.

Discussion

The above experiment showed that B. anserina could be precipitated from the infected fowl blood by differential and ultracentrifugation. The most suitable speed was 16000 r.p.m. for 20 minutes. This speed did not adversely effect the organisms and they remained active, motile and virulent. No deposition could be encountered at a speed below this. A speed of 18000 r.p.m. for 20 minutes and more adveresely effected the organisms and they lost activity and motility and many of them were shown destroyed. It is desirable to obtain spirochetes free from blood cells for the followings:

1. It performs a good opportunity for accurate study of morphology and nature motility of the organism.
2. It diminishes contamination with other microorganisms and this useful and essential in chicken embryo inoculation (Vaccine preparation) and in chicken inoculation for pure isolation of the organisms.
3. The free spirochetes could be stored for a longer time than with the blood cells, and in this experiment the free spirochetes remain virulent for chickens for 4 weeks when stored in the refrigerator with some sterile saline, while it must be passaged through chicken every 7 days when stored with blood cells.
4. The immobilization test used in serological dignosis of specific antibodies could be clearly observed and studied when the positive serum is added to the organisms and examined under the dark-field microscope.
5. It helps for attempts to find suitable culture media for the organisms in the future.
6. It performs good chance for the study of the antigenic structure of the organisms.

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