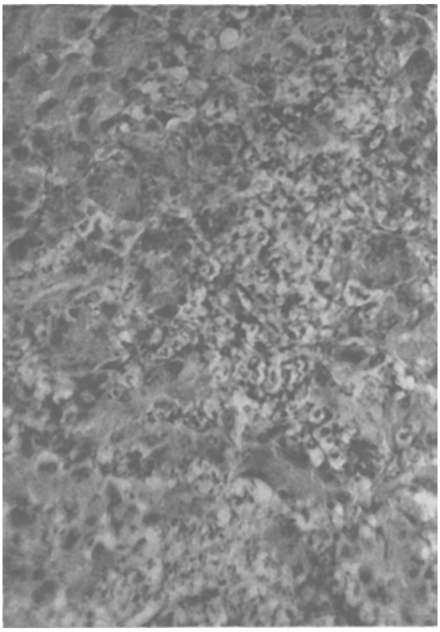

FIG. 4. Degeneration of acinar cells after 15 hr of intraductal pressure of 30 cm H_2O. (Dog J, Fig. 1). ×280.

lation of acinar cytoplasm was limited largely to the group with artificial pressure. In dog J, this progressed to an extreme degree. In small areas the staining properties of the acinar cells were completely abnormal but little or no inflammatory reaction was observed, in association with this change (Fig. 4). In most other areas, however, the cells appeared normal and well stocked with zymogen granules. In most of the dogs subjected to definite pressure, erythrocytes appeared in the interlobular spaces and in one instance infiltrated an acinar area. There was thus a pancreatitis which might be described as hemorrhagic as well as purulent or necrotic.

Summary. Both pancreatic ducts were ligated in 3 dogs. Over a period of 24 hours a gradual rise of serum amylase and a moderate edema with a slight leukocytic infiltration of the interlobular connective tissue of the pancreas occurred. The pancreatic duct systems of five dogs were subjected to a pressure of 30 cm water. Serum amylase rose twice as fast as in the dogs with simple ligation. Histologically, the principal changes in the pancreas were a more intense leukocytic infiltration, including acinar areas, and degeneration of some acinar cells.

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Electron Microscope Study of Anaplasmosis in Bovine Red Blood Cells. (18742)

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In a recent work on the virus host relationships of the foot and mouth disease virus with erythrocytes a technic was developed by which regular blood smears of the infected red cell membranes can be observed under the electron microscope (Epstein, Fonseca, and De Robertis(1). This technic was found to be useful for the study of parasites of erythrocytes. Some preliminary observations on piroplasmosis and especially on anaplasmosis will be reported here.

The *Anaplasma marginalis* Theiler, 1909 is well suited for electron microscope research since it is only about 0.4 to 1 μ in diameter. It is generally described as a round marginal body simply constituted of a single mass of chromatin (Fig. 1a), with no cytoplasm and multiplying by binary division(2). Recently there has been a tendency to consider the

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1. Epstein, B., Fonseca, N. M., De Robertis, E., 1951, in press.

2. Sergent, E., Donatien, A., Parrot, L., and Lestoquard, F., *Études sur les piroplasmpses Bovines,* Institut Pasteur D'Algerie, Alger., 1945.

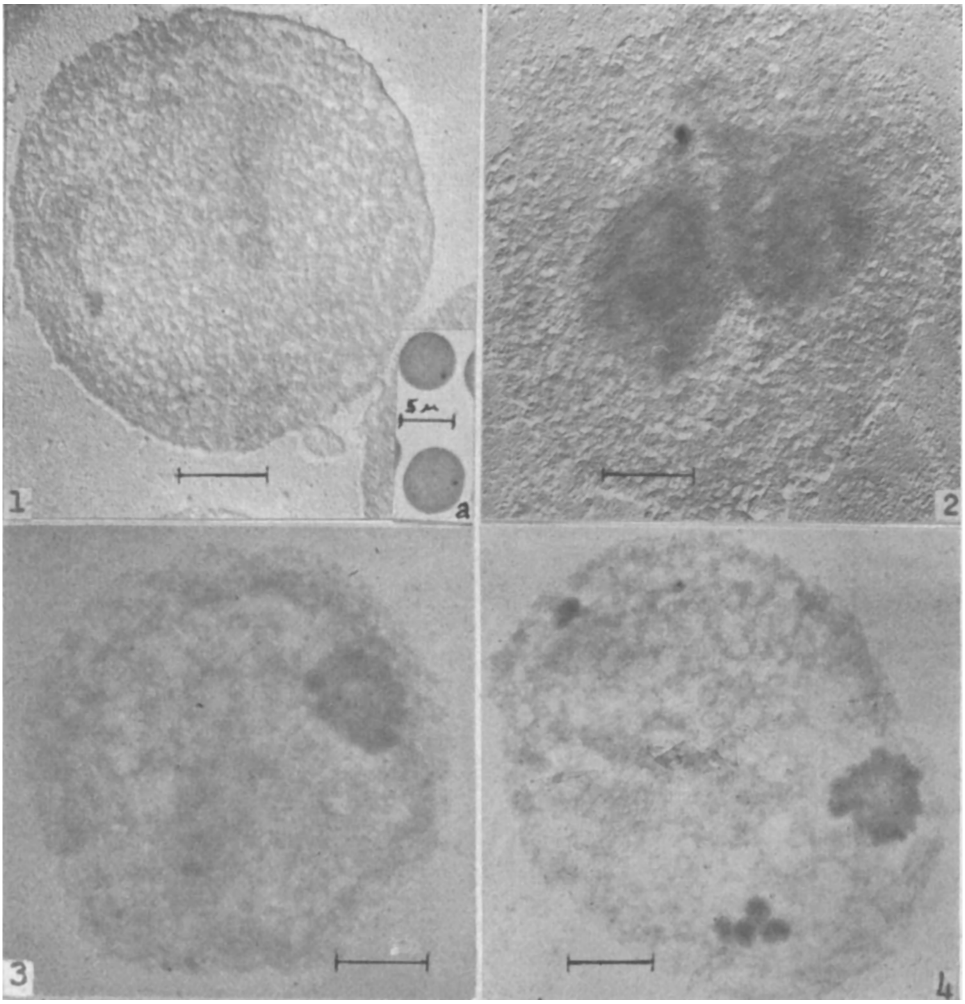

FIG. 1. Normal control red cell membrane from a calf. 11,500×. a. Erythrocytes infected with *Anaplasma marginalis*. Giemsa stain. Optical microscope. 1500×.

FIG. 2. Red cell membrane of a calf infected with *Piroplasma bigeminum*. 11,500×.

FIG. 3. Red cell membrane of a calf infected with *Anaplasma marginalis*. The mass of the parasite appears undivided in the center and divided into round bodies at the periphery. 11,500×.

FIG. 4. The same as Fig. 3. In addition to the marginal parasite 5 elementary bodies are dispersed within the erythrocyte. 11,500×.

anaplasma to be bacterial in nature because of its form, lack of cytoplasm, and the reaction with the Feulgen reagent(2,3). However, the most accepted view is to consider it within the group of other intracellular parasites of protozoan nature(2).

Diagnosis of this disease can be easily made in acute cases by microscopic examination of blood smears, since the marginal bodies are found in a relatively high concentration. However, in chronic cases the diagnosis is frequently questionable because the concentration of marginal bodies is very low and they may resemble other bodies found in normal red blood cells(4).

Experimental. Five calves were inoculated

3. *Bergey's Manual of determinative bacteriology*, 6° ed., Baltimore, 1948.

4. Mott, L. L., Gates, D. W., *Veter. Med.*, 1949, v44, 269.

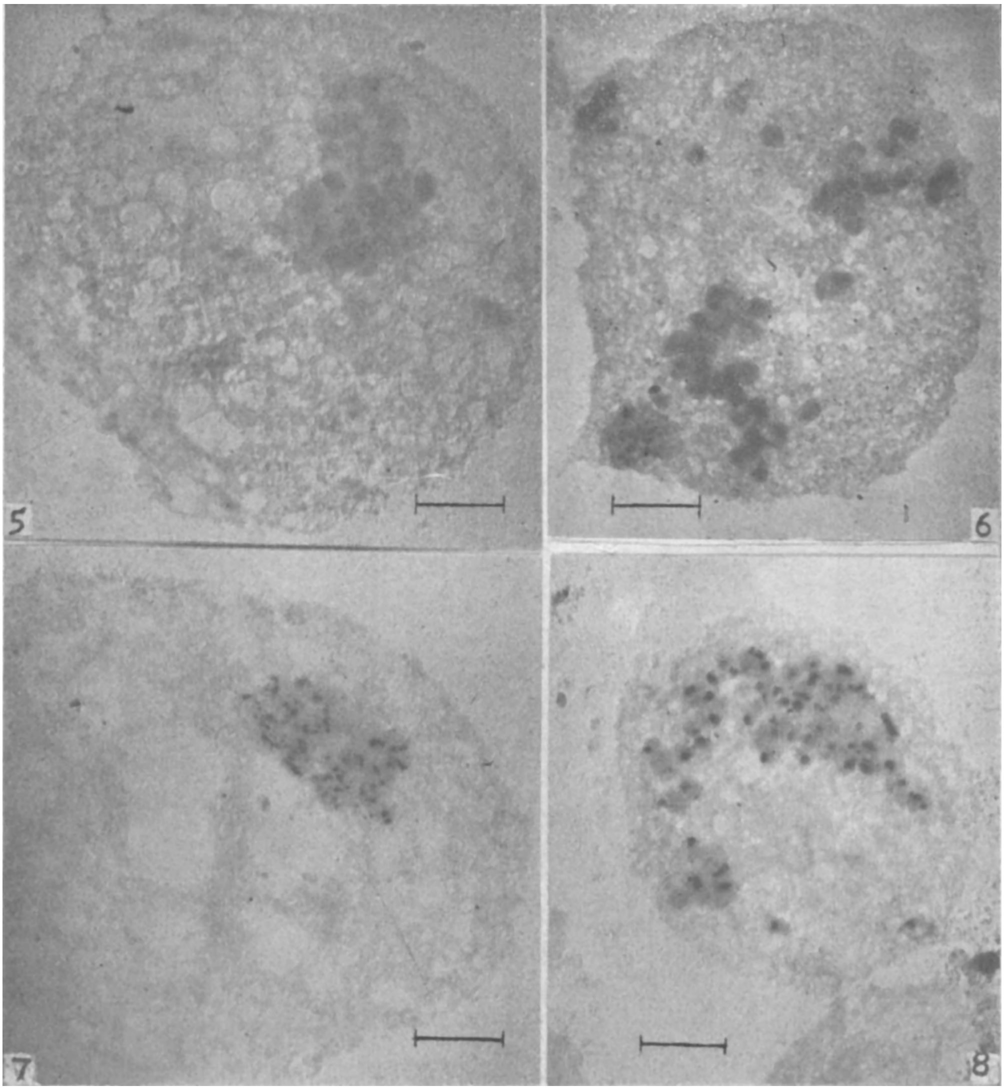

FIG. 5. The same as Fig. 4. The entire mass of the parasite is divided into elementary bodies. 11,500×.

FIG. 6. In addition to an undivided mass (lower left) a large number of bodies are dispersed throughout the erythrocyte. 11,500×.

FIG. 7. Red cell membrane of a calf infected with *Anaplasma marginalis* and treated with "Pirevan." The mass of the parasite has low electron density and the limits become indistinct. Granular material of very high electron density is deposited within the parasite. 11,000×.

FIG. 8. Similar to Fig. 7. Granular dense material deposited within the elementary bodies. 11,000×.

Fig. 1, 2, 5, 7, and 8 are shadowcast with palladium at an angle of 11°.

with virus obtained from the Instituto de Biología Animal of Uruguay, which consisted of citrated blood obtained during the acute stage of the disease. Ten ml of this blood were injected subcutaneously. Typical clinical and hematological symptoms were obtained after an incubation period of 28 days. Blood smears stained with current hematological stains were studied at different times after inoculation. In one case during the acute state the calf was treated with one injection of "Pirevan" (Evans) containing 5% of Dimethylquinolil-methyl-sulfate-urea. A few experiments with *Piroplasma bigeminum* were also carried out on calves. For the electron microscope study, blood smears were hemolyzed with distilled water and the red cell membranes were transferred on standard nickel grids by a parlodion replica technic. Grids were examined directly or after light shadowing with palladium at an angle of 11°. An R.C.A. EMU 2C electron microscope was used.

Results. A normal bovine red cell membrane is shown in Fig. 1. It appears as a flat disc of about 10 mμ in thickness, showing very fine structural details on the surface.

Fig. 2 shows a typical *Piroplasma bigeminum* with a pair of pear shaped masses. Some indications of internal structure are observed but the parasites are too large for detailed electron microscopy.

Typical *Anaplasma marginalis* are illustrated in Fig. 3 and 4. They consist of a round mass of about 1 μ which shows clear indication of an internal structure. The central part of the parasite seems rather undivided but at the periphery there are rounded bodies ranging in diameter from 170 to 220 mμ which protrude on the surface of the parasite. In addition to this composite structure of the *anaplasma* itself the electron microscope reveals bodies dispersed within the red blood cell which are essentially similar to those within the parasite. As a matter of fact observing the sequence of Fig. 4, 5, 6 and many more unpublished electron micrographs, the general impression is obtained that a large number of elementary bodies can be formed and become dispersed throughout the erythrocyte. Fig. 5 is interpreted as probably representing two attached marginal bodies and it seems justified to assume that at the level of the optical microscope they could be interpreted as a parasite dividing by binary fission.

On Fig. 6 in addition to about 40 elementary bodies dispersed throughout the red cell membrane an undivided mass of the parasite of about 0.8 μ can be observed.

The diameter of 119 elementary bodies was measured and the results expressed in a distribution curve (Fig. 9A). The measurements range between 170 to 430 mμ with about 69% of the total ranging between 170 and 220 mμ. These results were also distributed into 2 groups: (1) elementary bodies belonging to the mass of the *anaplasma* (Fig. 9B); (2) elementary bodies distributed throughout the erythrocyte (Fig. 9C). It can be seen that in this last group there is a wider range of distribution with some bodies which are definitely larger than those localized within the *anaplasma*.

Fig. 7 and 8 show some parasites which were found after a single injection of Pirevan together with those of normal appearance. With the available information it is difficult to advance any interpretation of these figures and to establish if they are related to the treatment. On purely morphological bases one can observe a granular material of very high electron density which seems to be precipitated strictly within the limits of the parasites. As seen in Fig. 7 and 8, in addition to this very dense material, the parasites themselves have lost their typical electron density and the boundaries of the elementary bodies fade out and become undistinct.

Discussion. The preliminary results presented here tend to indicate that it would be of considerable interest to make a systematic study with the electron microscope of intracellular parasites, and particularly of those attacking the red blood cells. The limiting factor is the large size of some parasites, such as the *Piroplasma bigeminum*, which makes it very difficult to reveal any internal submicroscopic structure.

The electron microscope study of *Anaplasma marginalis* reveals 2 interesting features. One is the observation that the parasites, which appear at the level of the optical microscope as single masses (Fig. 1a), are generally constituted of a central undivided mass of high electron density and by rounded peripheral bodies of 170 to 220 mμ in diameter. In some cases the whole mass of the parasite appears to be constituted of tightly packed elementary bodies. The second is the fact that in addition to the bodies present within the mass of the parasite, a great number of them are dispersed throughout the erythrocyte. Most of these elementary bodies are definitely submicroscopic (Fig. 9) and

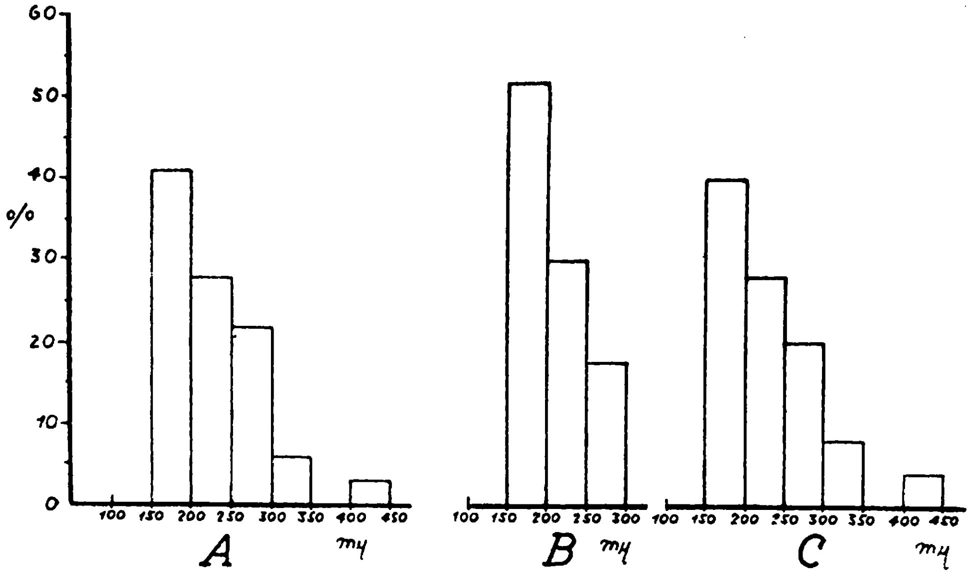

FIG. 9. Distribution curve of the diameters of elementary bodies of *Anaplasma*. A—Distribution of all 119 measurements; B—distribution of bodies within the marginal parasite; C—distribution of bodies dispersed throughout the erythrocyte.

certainly could not be observed with an optical microscope. The fewer larger ones are just at the limit of resolution of the microscope and could easily be mistaken with granular basophile material present in some normal erythrocytes and particularly in cases of hemolytic anemia.

These two facts are suggestive of the presence of smaller units of the parasite in addition to the marginal bodies shown by the optical microscope. The presence of these submicroscopic units might explain some of the clinical features of this disease, such as the persistence of the infective agent in the blood stream after the general symptoms of the disease have disappeared and the fact that the marginal bodies are at very low concentration or can not be ascertained with certainty.

These preliminary observations are also suggestive of a multiple division of the parasite instead of the generally accepted binary fission. The morphological entities of classical marginal bodies and the submicroscopic elementary bodies found with the electron microscope within the parasite or dispersed throughout the erythrocyte may probably represent stages of the life-cycle of the *Anaplasma* within the blood stream.

Summary. 1. Erythrocyte membranes from calves infected with *Anaplasma marginalis* were studied under the electron microscope. The classical single marginal body of the parasite appears to be constituted of a central undivided mass and peripheral elementary bodies of high electron density, 170 to 220 mμ in diameter. In some cases the whole mass of the parasite is divided into these small submicroscopic bodies. In other erythrocytes the elementary bodies are dispersed and have a tendency to grow up. Distribution curves on measurements of 119 elementary bodies are presented. 2. The presence of the submicroscopic forms of the parasite is discussed in the light of the clinical and hematological evolution of the disease, the multiplication and the probable life-cycle of the parasite within the blood stream.

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