our riboflavin series, on the other hand it is the Lansing strain which shows the differences between the optimum and the deficient groups while the Theiler's viruses demonstrate no consistent changes. May not these points indicate definite differences in the metabolism of the several viruses?

Summary. Mice fed an adequate diet, save for various levels of riboflavin, showed no consistent differences to infection with Theiler's encephalomyelitis viruses. In four similar series (totaling 234 mice) injected with Lansing strain poliomyelitis virus, the differences were slight but the greater resistance in each series was in the deficient group.

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A Rickettsia-like Organism Recovered from Guinea Pigs.*

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In the summer of 1942 there appeared an unusual febrile disease at Fort Bragg, North Carolina. This mild disease was called "pretibial fever" and was an apparently new clinical entity, as described by Daniels and Grennan.[1] The disease was characterized by 3 to 5 days of fever, splenomegaly, a maculopapular eruption typically limited to the legs, and rapid return to normal health. All of the cases were quartered in the same limited area of the post, a fact of probable epidemiological significance. An army commission which studied the 1942 epidemic was unable to establish the etiology or mode of transmission of the disease. In the summer of 1943, there reappeared a small outbreak of a clinically identical disease in which the cases came from the same area of the reservation as those of the preceding year. A rickettsia-like organism was recovered from 3 of 5 guinea pigs injected with blood from one of these latter patients. Attempts have been made to show that this agent was concerned in the etiology of the disease, but it is not possible at the present time to be sure of its origin. The purpose of this paper is to record the known characteristics of the organism in the hope that future developments may clarify its significance.

Method of Isolation. Defibrinated blood was drawn from the last case of pretibial fever appearing in the 1943 outbreak and was injected intraperitoneally into 5 guinea pigs in 4 to 6 ml amounts. After incubation periods varying from 3 to 5 days, 3 of these guinea pigs became febrile. Three independent passage lines were established by the intraperitoneal transfer of pooled brain and spleen on the third or fourth day of fever and the same organism was recovered from each. After a few transfers, the agent became lethal for guinea pigs; a titration of infected spleen from the fourth passage showed deaths through the 10^{-3} dilution of a 10% emulsion.

The Organism. Poorly staining, gram-negative rods were seen in impression smears of guinea pig tissue or of egg yolk sac. When stained by the Machiavello method,[2] the material was shown to contain numerous bright red, somewhat pleomorphic, small bacillary forms, tending to be paired and often clumped

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1 Daniels, W. B., and Grennan, H. A., *J. A. M. A.*, 1943, **122**, 361.

2 *Virus and Rickettsial Diseases,* Harvard University Press, 1940, p. 896.

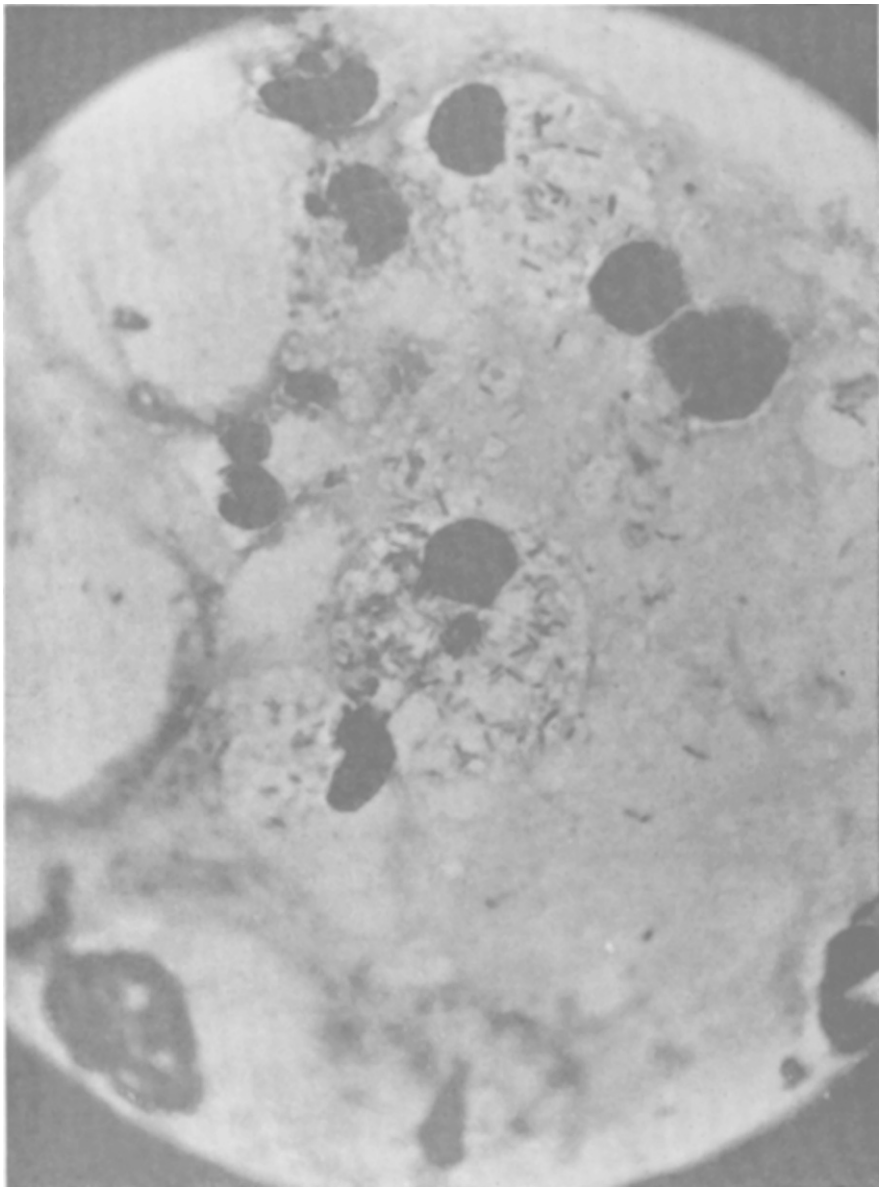

FIG. 1.
Impression smear of guinea pig peritoneal exudate stained by the Machiavello method. Magnification × *ca.* 1500.

together in bundles (Fig. 1). They were larger than *P. tularensis* and the known rickettsiæ. In contrast to the latter, these organisms were easily seen in guinea pig tissue. They were located chiefly in the cytoplasm of the cells in impression smears of the spleen, though in smears of the peritoneal exudate masses of them appeared to be extracellular. They stained purple with Giemsa's stain and were not acid fast.

Unsuccessful attempts were made to grow this organism on a variety of artificial media under both aerobic and anaerobic conditions. In addition to the common bacteriological media containing blood, special media for *P. tularensis, Leptospira, Brucella, B. pleuro-*

pneumoniæ, and *Mycobacteria* were employed without visible growth. Degenerated organisms were still visible in smears after 1 or 2 weeks on slants seeded with yolk sac material, but there was no apparent growth upon transfer to fresh media. Variable growth of the organism occurred, however, when infected guinea pig tissue was injected into the yolk sac of fertile 7-day hens' eggs. There seemed to be a predilection for the yolk sac, since eggs injected by the allantoic sac route showed organisms on smear of yolk sac tissue only. The agent was lethal for the chick embryo, which usually died on the third or fourth day even when a 10^{-6} dilution of the original 10% emulsion was employed as the inoculum. Eleven- to 13-day embryos tended to survive a little longer. After death of the embryo, the organisms became difficult to stain and many stained blue by the Machiavello method.

The pathogenicity of the organism was readily preserved for at least 2 months in the frozen state. Fever was still produced in guinea pigs when a 10^{-6} dilution in 10% serum-saline was incubated for 1 hour at 37°C and then at 4°C overnight.

Disease in the Guinea Pig. Routine passage of guinea pig spleen or peritoneal exudate was carried out by the intraperitoneal route, usually on the third or fourth day of fever. Passage of blood on the second day of fever resulted in a slight febrile response and solid immunity to reinoculation, indicating the presence of the agent in the blood stream. Guinea pigs weighing 250 g or more have appeared almost uniformly susceptible but there have been non-reactors, particularly young animals. The incubation period has varied from 1 to 4 days, depending on the strength of the inoculum. A more or less sustained febrile course has ensued, ranging from 104° to 106°F when early morning recordings were made. The animals became inactive and the fur ruffled; loss of weight occurred but no localizing symptoms developed. There were no neurological signs nor evidences of scrotal reaction. When injections were made intraperitoneally in 0.5 ml quantities, death after 4 to 7 days of fever was the rule through the 10^{-3} dilution of the inoculum. Recovery usually occurred after a week or more of fever for the 10^{-4} to 10^{-6} dilutions; 10^{-7} was the highest dilution producing fever in the injected animal. When virulent material was injected by the subcutaneous route, an extensive area of local induration gradually appeared in 4 or 5 days and the animals ran a febrile course.

Pathology. The pathology of the acute infection with this organism in guinea pigs after intraperitoneal inoculation was characterized by the presence of exudative peritonitis. There was slight redness of the subcutaneous tissue, together with variable but slight enlargement of the peripheral lymph nodes. No definite scrotal reaction was ever present, externally or internally. On opening the peritoneal cavity, from 1 to 5 ml of a thick whitish exudate was found, with an adherent fibrinous reaction over the liver and spleen. The spleen was 3 or 4 times normal size and the surface was finely granular. The adrenals appeared congested but the rest of the abdominal organs were grossly normal. The lungs occasionally showed areas of bronchopneumonia in which the organism could be seen on smear. In one instance, the cerebral meninges were injected and ill-defined collections of small round cells were seen in histological slides of the cerebral cortex. Animals which died or were sacrificed after a subacute course showed only splenic enlargement, without peritoneal exudate.

Microscopic sections of the spleen and subcutaneous tissue showed an inflammatory reaction in which mononuclear cells predominated in the acute stage of the disease. There was much necrosis in the later stages of the disease. The peritoneal exudate contained moderate numbers of polymorphonuclear leukocytes and small numbers of mononuclear cells. The organisms were easily seen in impression smears, often in large numbers, when stained by the Machiavello technic. As mentioned above, they were chiefly intracytoplasmic in the spleen and were mostly extracellular in the peritoneal exudate.

Host Range. It was not possible to transmit the infection to laboratory animals other than guinea pigs unless overwhelming doses were employed. White rats, cotton rats,

hamsters, rabbits, rhesus monkeys, and different strains of mice were tried. The injection of concentrated suspensions of heavily infected yolk sac by the intraperitoneal or intravenous routes resulted in the death of young mice in 2 to 3 days. When the inoculum was diluted slightly, however, the result was asymptomatic survival. A few mice injected with concentrated suspensions developed purpuric areas about the ears and feet before they died, 2 to 3 days after injection. Unsuccessful attempts were made to adapt the agent to mice by the serial passage of liver and spleen at 5- to 7-day intervals.

Immunity. When recovered guinea pigs, including animals with no evidence of disease other than slight febrile reactions, were challenged with fatal doses of passage material, they were solidly immune. Such animals occasionally exhibited an abrupt rise in temperature within 24 hours; the fever usually disappeared in about 48 hours.

Since the organism resembled rickettsia in some respects, cross-protection tests in guinea pigs were set up with the standard strains of epidemic and endemic typhus, Rocky Mountain spotted fever, and tsutsugamushi fever. No cross immunity was shown to be present with any of these known rickettsial strains. There was no immunological relationship to Q fever by the complement-fixation test. Agglutinins for Proteus OX2, OX19, and OXK were not demonstrable in the serum of rabbits at 5, 8, and 10 days after injection with large doses of yolk sac infected with the organism. Sera from guinea pigs immune to the agent were negative in the agglutination test with *B. tularense* and *Br. abortus*.

Infected yolk sac was found to be a satisfactory complement-fixing antigen. Using the technic[3] of 1-hour fixation at 37°C in the presence of 2 full units of complement, complement-fixing antibody levels ranging from 1:16 to 1:64 (initial serum dilutions) were found in serum specimens from many of the recovered guinea pigs at about 3 to 4 weeks. These titers had fallen to less than 1:4 at 8 weeks. Complement-fixation tests were done with sera from the 7 cases of pretibial fever, including the patient from whose blood the primary isolations were made. These tests failed to show any significant rise in antibodies in the convalescent sera.

In addition, agglutination tests were performed. The organisms in infected yolk sac were centrifuged and the clarified resuspended sediment was found to be a satisfactory antigen. With the sera from two hyperimmunized guinea pigs, agglutination titers of 1:32 and 1:128 were obtained. The human sera showed no significant increases in antibody in convalescent specimens by this method.

Using guinea pigs, tests designed to detect the presence of neutralizing antibodies in both the sera of hyperimmunized guinea pigs and of cases of pretibial fever were carried out. Both infected yolk sac and guinea pig spleen were used as sources of the agent. Different methods of incubation of the serum-antigen mixtures were tried. There was no noticeable improvement in neutralization when the time of incubation was increased from 2 hours at room temperature to 37°C for 1 hour, followed by 4°C overnight. Dilutions of the infective material ranging from 10^{-3} to 10^{-6} were used. No protection was demonstrated by the convalescent human sera, but so inconclusive were the results with the immune guinea pig sera that it was felt that no reliance should be placed on these tests.

Attempts to Isolate the Organism from Stock Guinea Pigs. Three serial passages were carried out with guinea pigs from the same lot of animals which were used in the original isolations. The first passage was initiated with blood from a normal individual. There was no febrile response in any of the 6 animals and the organism was not isolated from their tissues. Both of the third passage guinea pigs proved resistant to challenge with the agent, although no complement-fixing antibodies to it were present in their serum prior to challenge. These results suggested the possibility of latency of the organisms in the stock. It should be recorded, however, that their young age (175 g, 195 g) raised the question of non-specific resistance, since non-reactors have often been noted in animals of this size. The sera from 10 normal stock guinea pigs bled 4 months after the original

[3] Bengston, I. A., *Pub. Health Rep.*, 1944, **59**, 402.

isolation contained no complement-fixing antibodies to the agent, though it was not known whether they came from the same source as the original animals.

Discussion. In these experiments a rickettsia-like organism was isolated from guinea pigs injected with blood from a patient with pretibial fever, a recently described disease of unknown etiology. It was not possible to demonstrate the presence of significant titers of complement fixing or agglutinating antibodies to this agent in the human sera. The technics employed were satisfactory for detecting these antibodies in the sera of recovered guinea pigs. Neutralization tests in guinea pigs were unsatisfactory, as is the case with most rickettsial diseases. In view of these negative findings, the origin of the agent is in doubt. Unfortunately, all of the human blood was used up at the time of the original inoculations, and no additional cases of pretibial fever appeared during the 1943 season. It is hoped that more information as to the source of the agent can be obtained in the future.

A search of the literature has failed to show a guinea pig disease similar to the one described. Mochkovski[4] reported the occurrence of a chronic rickettsiosis in the mononuclear cells of guinea pig blood. His paper describes the morphology of the organism and does not refer to the biological characteristics of the agent.

[4] Mochkovski, C., *C. R. Soc. de biol.*, 1937, **126**, 379.

Summary and Conclusions. An agent pathogenic for guinea pigs has been isolated. The organisms appeared as small pleomorphic bacillary forms, staining red by the Machiavello method and gram negative. They were larger than the known rickettsia, as well as *P. tularensis* and *Brucella,* and were immunologically distinct from them. They grew fairly well in the yolk sac of the fertile hen's egg but were not cultivated on artificial media. The host range was limited to the guinea pig except when enormous doses were given.

After a short incubation period, peritonitis was produced in the guinea pig, for which the agent was highly virulent, and a specific immunity resulted in recovered animals. The inflammatory reaction in the tissues was characterized chiefly by the presence of mononuclear cells. The organism appeared predominantly intracytoplasmic in impression smears of the spleen.

The origin of the organism is in doubt. It has not been possible to identify it with any known group of pathogenic agents, but at present it may be termed "rickettsia-like" for descriptive purposes.

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Alleviation by Raw Liver of Anorexia Produced by Sulfapyridine.

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The administration of sulfapyridine to dogs in blacktongue did not interfere with the alleviation of symptoms by nicotinic acid but did prevent weight restoration.[1,2] Raw liver,

[1] West, R., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 369.

[2] Schaeffer, A. E., McKibbin, J. M., and Elvehjem, C. A., *J. Biol. Chem.*, 1942, **144**, 679.