In young rats fed a pyridoxine deficient diet,[3] the changes in the growth zones were comparable to those of underfed young guinea pigs,[4] kept on a quantitatively restricted but adequately balanced diet. To some extent this is true for the cartilage of mice also. However, the cartilage cells as well as the osteoblasts responded more unfavorably to the pyridoxine deficiency than to general underfeeding, and the formation of bone was more reduced than in any of our previous experiments. This difference might indicate a specific effect of the pyridoxine deficiency, but it may be a manifestation of the more marked sensitivity of the mouse to the deficiency as compared to that of the rat.[5,6]

Feeding a high protein diet accentuated the effects of pyridoxine deficiency inasmuch as growth of cartilage and bone was more inhibited than in pyridoxine deficient mice receiving a diet with regular casein content. These findings are in agreement with previous observations on the greater requirements for pyridoxine in animals fed high protein diets.

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Experimental Fort Bragg Fever (Pretibial Fever) in Chimpanzees.*

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The chimpanzee (*Pan Satyrus*) has been used extensively in experimental work on poliomyelitis[1,2] and to a lesser degree in work on the common cold,[3] but the use of this animal as an experimental host for the reproduction of human infectious disease is a field deserving further exploration. Particularly would this seem to apply to the virus field and to diseases which have readily discernible cutaneous manifestations. We have recently made attempts to induce in these animals 3 human virus diseases: sandfly (Phlebotomus or pappataci) fever, dengue fever, and Fort Bragg (pretibial) fever. This paper will be concerned only with a description of our experiments with the latter disease. Results with the other two diseases will be described in another paper.[4]

Fort Bragg fever was first described by Daniels and Grennan,[5] under the term *pretibial fever*. It is a disease characterized by an acute fever of approximately 5 days' duration, frontal and postorbital aching, splenomegaly, and often with a patchy erythematous rash on the pretibial regions.† Respiratory manifestations are minimal and not persistent. Cases of this disease have appeared in groups during the summer months of 1942, 1943, and 1944 on the military reservation at Fort Bragg, N. C. The manner in which it spreads among humans has not been determined.

* This investigation was conducted by the Commission on Virus and Rickettsial Diseases, Army Epidemiological Board, Office of the Surgeon General, U. S. Army, Washington, D.C.

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† The rash on the shins was such a prevalent feature that Daniels and Grennan[5] have designated this disease as *pretibial fever*.

This disease has been studied by Tatlock[6] who recovered an infectious filtrable agent from an active case. This agent produced a prostrating fatal infection in hamsters and fever in guinea pigs and rabbits. The agent was also maintained for 23 serial passages in embryonated eggs, following which the virus was inoculated into 14 human volunteers. It was possible to induce the clinical picture of Fort Bragg fever including the characteristic skin lesions in some of these subjects, while the majority exhibited only fever for 1 to 3 days. The virus appeared to be unrelated in its properties or immunologically, to the agents of lymphocytic choriomeningitis, Q fever, Rocky Mountain spotted fever, sandfly fever, and dengue fever.

Virus. The strain was received from the Virus and Rickettsial Laboratory of the Army Medical School through the kindness of Dr. Hugh J. Tatlock and Dr. Joseph E. Smadel. Because of difficulties of preserving the infectiousness of the virus on storage, even in sealed glass ampoules at −70°C[6], the virus was best handled by maintaining it in serial passage in hamsters. Only an occasional sample of virus survived storage on dry-ice, a fact which on more than one occasion almost resulted in our losing the virus.

Most consistent results were obtained when young hamsters (3 to 4 weeks of age) were used. Virus was inoculated intracerebrally (0.04 cc) and/or intraperitoneally (0.5 cc), generally as a 10% suspension of brain. Following an incubation period generally from 7 to 18 days, animals were sacrificed and brains harvested for virus on the first day of illness. Prostration and death usually developed within 24 hours of the first signs of disease.

Chimpanzees. Nine young animals were used in these experiments.‡ All of them had been used during the previous one to 3 years for work in poliomyelitis and several of them had developed the non-paralytic form of poliomyelitis. This did not seem to be a contraindication to their use with Fort Bragg fever virus. It should be added, however, that none of these chimpanzees were harboring the virus of poliomyelitis in their intestines at the time of using them for these experiments with Fort Bragg fever virus. All of these animals were under 5 years of age but only 4 of them could be described as being tractable, in other words as being handled easily enough to allow the taking of daily rectal temperatures. All animals, however, were examined daily for signs of illness for a period of 5 to 6 weeks. Animals were bled before inoculation, at the time of fever, and at various intervals after inoculation of virus. Virus was inoculated as indicated, intra- and subcutaneously and sometimes intramuscularly.

Neutralization Tests. These were carried out in hamsters. Suspensions of freshly harvested brains from sick hamsters were made up into varying dilutions ($2x10^{-1}$ to $2x10^{-5}$ concentration of brain). These were mixed with equal amounts of undiluted serum and incubated for one hour at room temperature. The virus-serum mixtures were inoculated into groups of 3 young hamsters either intracerebrally (0.04 cc) or intraperitoneally (0.5 cc) as indicated. Some sera were tested by both the intracerebral and the intraperitoneal routes, in view of the discrepancy which is sometimes found between these two routes in certain virus neutralization tests.[7] In general the intraperitoneal route proved satisfactory,[6] and was most often used. Hamsters were observed for a period of 21 days for signs of illness and death.

Exp. I. Preliminary experiments carried out with 4 chimpanzees in November, 1946 were equivocal. In these animals temperature readings were taken on 2, and fever was recorded in one. But there were no hamster controls to indicate that the sample of virus used was active. Furthermore, the virus was not isolated from the blood of the chimpanzees during the febrile period and no neutralizing antibodies were demonstrable in the convalescent samples (see Table I). The experiments were repeated in March, 1947.

Exp. II. 10 March 1947. Chimpanzee Rosebud and 2 rhesus monkeys were each

[6] Tatlock, H. J., *J. Clin. Invest.*, 1947, **26**, 287.

‡ We are indebted to Dr. Karl S. Lashley of the Yerkes Laboratories of Primate Biology, Orange Park, Florida, for the loan of 6 of these animals.

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TABLE I.
Lack of Development of Neutralizing Antibodies in Chimpanzees Pinta, Webb, and Jent. (Inoculated with Virus Inactivated by Freezing and Thawing.)

Serum of chimpanzee	Date, 1946	Inoculation route in hamsters	Mortality at indicated concentration of infectious hamster brain 10-1	10-2	10-3	10-4	10-5	Result of antibody test
Pinta	12 Nov.	IC		3/3	0/3	0/3	0/3	—
”	11 Dec.	IC	3/3	3/3	1/3			—
”	12 Nov.	IP		3/3	3/3	0/3	0/3	—
”	11 Dec.	IP	4/4	3/4	3/4			
Webb	12 Nov.	IC		3/3	3/3	0/3	0/3	—
”	11 Dec.	IC	3/3	3/3	2/3			—
”	12 Nov.	IP		3/3	3/3	2/3	0/3	—
”	11 Dec.	IP	3/3	3/3	3/3			—
Jent	12 Nov.	IC		3/3	1/3	1/3	0/3	—
”	11 Dec.	IC	3/3	3/3	2/3			—
”	12 Nov.	IP		3/3	3/3	1/3	0/3	—
”	11 Dec.	IP	3/3	3/3	3/3			—

TABLE II.
Development of Neutralizing Antibodies to Fort Bragg Fever Virus in Chimpanzees.

Serum of chimpanzee	Date 1947	Material inoculated	Inoculation route in hamsters	Mortality at indicated concentration of infectious hamster brain 10-1	10-2	10-3	10-4	10-5	Result of antibody test
Rosebud	10 March	Active virus	IP		2/2	3/3	3/3		—
”	23 June	” ”	IP	0/3	0/3	0/3	0/3		+ 1000
”	31 July		IP	0/3	0/3	0/3			+ 1000
Mary-Lou	20 March	Rosebud's febrile blood	IP		3/3	3/3	2/3		—
”	30 April		IP	0/3	0/3	0/3			+ 1000
”	23 June	Active virus	IP	1/3	0/3	0/3	0/3		+ 100
Hickory	29 April	Inactive ”							
”	23 June	Active ”	IP		3/3	1/2	0/3		—
”	31 July		IP	0/3	0/3	0/3			+ 100
Catawba	29 April	Inactive ”	IP		2/2	2/2	1/3		—
”	23 June	Active ”	IP		3/3	2/2	3/3		—
”	31 July		IP	0/3	0/2	0/3			+ 1000
Becky	23 June	” ”	IP		3/3	3/3	2/3	0/3	—
”	31 July	” ”	IP	0/3	0/3	0/3	0/3		+ 1000
”	23 June	” ”	IC		3/3	3/3	1/3	0/3	—
”	31 July	” ”	IC	2/3	0/3	0/3	0/3		+ 100

+ 1000 = Undiluted serum neutralized 1000 lethal doses of virus.

inoculated intra- and subcutaneously (2 cc in several piqures) and intramuscularly (3 cc) and 2 other rhesus monkeys intracerebrally (0.6 cc) with a 20% suspension of infectious hamster brains. These brains were harvested from 5 hamsters ill with the disease on that day, and the suspension was inoculated soon after it was prepared. As controls, 5 new hamsters were inoculated intracerebrally with the virus suspension.

5 Hamsters—10 March. Inoculated with virus.
20 March. One dead, 4 sick with tremors, ataxia, and prostration. Sacrificed for virus harvest.
4 Rhesus Monkeys—10 March. Inoculated with virus.
11-30 March. Normal behavior and temperature.
Chimpanzee Rosebud—10 March. Bled for serological antibody test (Test negative; see Table II). Inoculated with virus.
11-19 March. Normal, temp. between 98° and 99.7°. White blood count between 16,000 and

22,000.

20 March. Temp. 101.7°. White blood count 35,500. Twenty-five cc of blood taken, citrated, and 10 cc inoculated within 5 minutes into Chimpanzee Mary-Lou (7 cc intramuscularly and 3 cc intra- and subcutaneously in 15 piqures). Remainder of citrated blood *frozen* and later after thawing, tested in 6 hamsters with negative results.

22-23 March. One lesion on right shin and one on left forearm, as raised red areas about 1 cm in diameter. Temp. 101°. White blood count 12,800.

24 March. Normal. Temperature 99°. White blood count 17,800.

29-30 March. Normal. Temp. 99.1° to 99.8°.

23 June. Bled for antibody test (Test positive; see Table II).

Chimpanzee Mary Lou--20 March. Bled for serological antibody test (Test negative; see Table II). Inoculated with Rosebud's citrated blood.

21 March-30 April. Normal. Temp. not taken.

30 April. Bled for antibody test (Test positive; see Table II).

Exp. III. 29 April 1947. Chimpanzees Catawba and Hickory were each inoculated intra- and subcutaneously (2 cc) and intramuscularly (1 cc) with 20% suspension of hamster brains. The suspension was made on the day of inoculation from 4 infected hamster brains which had been stored on dry-ice for 5 weeks. As controls 10 new hamsters were inoculated intracerebrally.

10 Hamsters—29 April. Inoculated.

30 April-19 May. Remained well.

Chimpanzee Hickory. 29 April. Bled for antibody test. Inoculated.

30 April-19 May. Normal temp. 98.4° to 99.9°.

23 June. Bled for antibody test (Test negative; see Table II).

Chimpanzee Catawba—29 April. Bled for antibody test. Inoculated.

30 April-19 May. Normal. Temp. not taken.

23 June. Bled for antibody test (Test negative; see Table II).

Exp. IV. 23 June 1947. Five chimpanzees were inoculated with 10% brain suspension freshly prepared from 2 hamsters acutely ill with Fort Bragg fever. Two of these chimpanzees (Rosebud and Mary-Lou) had previously been inoculated with active virus and 2 (Hickory and Catawba) with virus inactivated by storage on dry ice. The fifth animal (Becky) had not been used in Fort Bragg fever work before the present experiment. All 5 chimpanzees were each inoculated intra- and subcutaneously with 2 cc of the virus suspension in 6 to 8 piqures. As controls 9 new hamsters were inoculated intracerebrally with the same suspension.

9 Hamsters—23 June. Inoculated.

3 July. Two animals hyperirritable, tremulous, and ataxic (sacrificed for virus).

4 July. Three animals ataxic progressing to prostration (sacrificed for virus).

5 July. One dead.

6 July. One dead. One sick as those above.

7 July. Two dead.

Chimpanzee Rosebud (previously inoculated with active virus)—23 June. Bled for antibody test (Test positive; see Table II). Inoculated with virus.

24 June-31 July. Normal. Temp. 97° to 99.6°.

31 July. Bled for antibody test (Test positive; see Table II).

Chimpanzee Mary-Lou (previously inoculated with Rosebud's febrile blood)—23 June. Bled for antibody test (Test positive; see Table II). Inoculated with virus.

24 June-31 July. Normal. Temp. not taken.

Chimpanzee Hickory (previously inoculated with inactived virus)—23 June. Bled for antibody test (Test negative; see Table II). Inoculated with virus.

24 June-9 July. Normal. Temp. 98° to 99.6°.

10-12 July. Temp. elevated 100.8° to 101.2°.

14-31 July. Normal. Temp. 97.6° to 99°.

31 July. Bled for antibody test (Test positive; see Table II).

Chimpanzee Catawba (previously inoculated with inactived virus)—23 June. Bled for antibody test (Test negative; see Table II). Inoculated with virus.

24 June-31 July. Normal. Temp. not taken.

31 July. Bled for antibody test (Test positive; see Table II).

Chimpanzee Becky (new animal)—23 June. Bled for antibody test (Test negative; see Table II). Inoculated with virus.

24 June-25 July. Normal. Temp. 97.1° to 98.3°.

3 July. Temp. 100.5°.

4 July. Temp. 100°. Blood taken and 15 cc defibrinated. Inoculated at once by the intracerebral and intraperitoneal routes into 4 young hamsters. All 4 succumbed to the

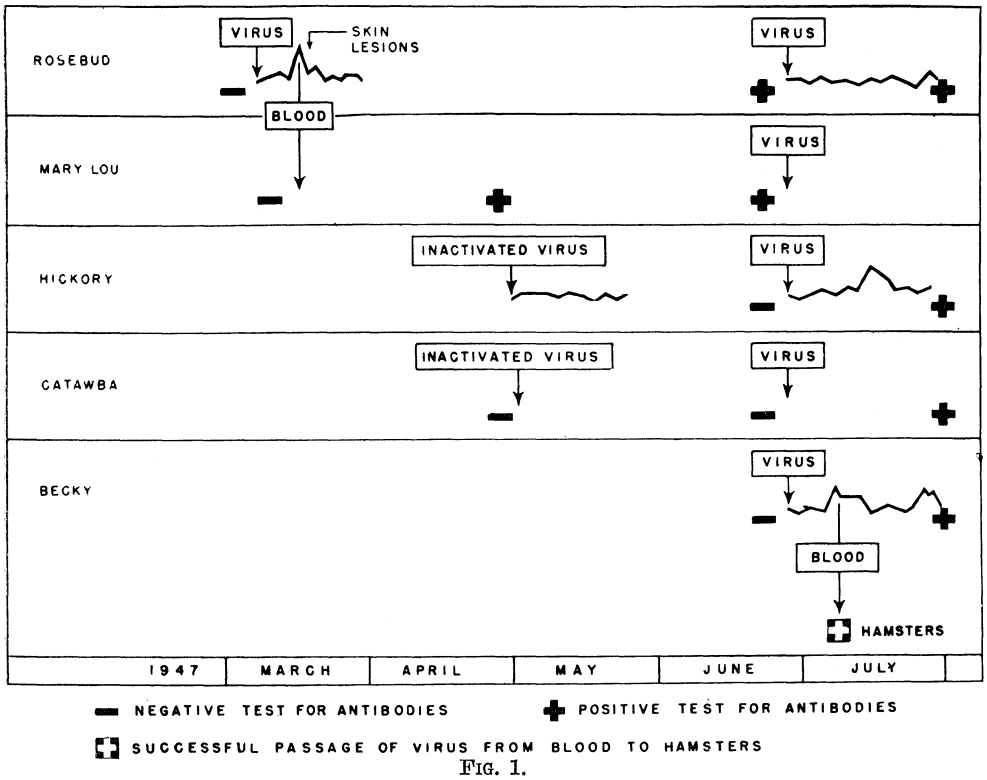

FIG. 1.

Fort Bragg fever in the chimpanzee. The temperature curves of 3 animals are presented together with the results of antibody tests on all 5 animals.

disease (8- to 10-day incubation period) and their brains were shown to contain the virus by another successful subtransfer into new hamsters.

9 July. Temp. back to 99°.

31 July. Bled for antibody test (Test positive; see Table II).

Discussion. The results of this study which are graphically presented in Fig. 1, indicate that chimpanzees are susceptible to the virus of Fort Bragg fever when administered intra- and subcutaneously. The induced disease is similar in many respects to the experimental disease in human beings which has been reported by Tatlock.[6]

A short febrile period developed after 10 to 17 days in the 3 chimpanzees who were tractable enough to have their daily temperatures taken. In the 2 animals in whom the presence of viremia was sought during the febrile period, virus was recovered, in one animal by transfer to another chimpanzee, in the second by transfer to hamsters.

The development of neutralizing antibodies to the virus is further evidence that infection had taken place in these animals. When inactivated virus (inactivated by freezing and thawing whole infected hamster brains) was inoculated into 5 chimpanzees (Hickory, Catawba, Pinta, Webb, and Jent), neutralizing antibodies did not appear in the serum. However, when 2 of these animals (Hickory and Catawba) were later challenged with active virus, both responded by developing antibodies to the virus.

Of considerable importance is the fact that a rash did appear in the appropriate location, the shin and forearm of an inoculated animal. This was not a very constant phenomenon as in the human disease, for out of the 14 subjects inoculated by Tatlock 13 responded with fever and of these, only 5 developed a rash.

Summary. Inoculation of Fort Bragg fever virus into chimpanzees resulted in a mild infection. This appeared to be characterized

by a short febrile period with viremia and by the appearance of neutralizing antibodies in the serum a few weeks after the infection.

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Conversion of Red Muscle to Pale Muscle.*

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It has long been taught that the function of myohemoglobin in red muscle is its function as an oxygen reservoir for the sustained contractions so typical of this kind of muscle.[1] Variations in myohemoglobin content were noted by Whipple[2] who correlated the content of this pigment with the age, state of health, and amount of activity engaged in by dogs. McClintock, Hines, and Jordan[3] observed that increased activity of muscle would lead to an increase in myohemoglobin content. In order to test the validity of the concept that myoglobin content and type of activity are correlated, the experiments described below were carried out.

Methods. Large, mature rabbits were selected for these experiments. Six such animals were used and each was anesthetized with nembutal. Under aseptic conditions, the tendon of the right soleus muscle was severed and the central end of this tendon sutured to the peripheral end of the severed tendon of the synergistic pale tibialis posterior muscle. By causing the red soleus to utilize the insertion of the pale tibialis muscle, it was hoped to convert the soleus to a pale muscle. The ankle was fixed with an orthoplast bandage in such a way that the foot was kept partially flexed for a period of 2 weeks at the end of which time the bandage was removed. The animals were then sacrificed after a period of 6 months. At sacrifice, the animal was placed under nembutal anesthesia and the normal tibialis and soleus of the left leg and the transplanted soleus of the right leg were arranged for recording. All tendon sutures were found to have been successful. The contractions of each of these extensor muscles were elicited as a part of a crossed extensor reflex elicited by stimulation of the contralateral sciatic nerve.

After the recordings were made, the animal was perfused with saline, and the muscle was excised and weighed. Hemoglobin of muscle was determined by the acid hematin method according to Whipple[4] and iron determinations were simultaneously made by the method of Wong.[5]

Results A comparison of reflexly induced tetanic contractions is shown in Fig. 1. This typical result illustrates the fact that excitation with stimuli of equal strength (10 volts), equal frequency (60 c.p.s.), and equal duration (1 sec.) result in a prolonged and maximal degree of contraction, marked by considerable after-discharge in the normal soleus and a very short, lesser degree of tetanus with a conspicuous lack of after-discharge in the case of the normal tibialis posterior. The transplanted soleus, on the other hand, exhibits a reflexly induced contraction which is remarkably similar to the contraction of the pale tibialis muscle. There remain, however, some remnants of red muscle contraction characteristics as evidenced by the greater extent of contraction than with the pale muscle and by the existence of a

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