Zeitschrift: Acta Tropica
Herausgeber: Schweizerisches Tropeninstitut (Basel)
Band: 12 (1955)
Heft: 3

Artikel: Experimental bubonic plague in monkeys : 1. Study of the development of the disease and the peripheral circulatory failure
Autor: Hoessly, G.F. / Walker, D.L. / Larson, A.
DOI: https://doi.org/10.5169/seals-310555

George Williams Hooper Foundation, University of California,
San Francisco, California.

Experimental Bubonic Plague in Monkeys.

I. Study of the Development of the Disease and the Peripheral Circulatory Failure.

By G. F. HOESSLY, D. L. WALKER *, A. LARSON,
and K. F. MEYER.

(Received October 19th, 1954.)

In spite of the long history of plague as a major epidemic disease, relatively little information is available as to the physiologic changes in the host during *Pasteurella pestis* infection or even as to the ultimate cause of death. Because of the conditions under which plague has occurred, the places in which it has been seen, and the risk to those working with patients and materials from patients, observations on natural infections in man have been necessarily limited in scope and method. The study of laboratory infections in lower animals has largely been directed towards epidemiology and immunity.

Most investigators have considered that release of the endotoxin of *P. pestis* is the primary factor leading to illness and eventually to death following infection. How this endotoxin acts or upon what organs or organ systems it acts to result in illness and death is not actually known, but through the literature of several decades repeated reference has been made to the action of plague toxin upon the heart (1-3). Such action has been suggested by histological evidence of cloudy swelling and fatty degeneration in the myocardium of man and lower animals succumbing to the infection and by clinical observations of a rapid, feeble pulse, faint heart sounds and occasionally of gallop rhythm in persons dying of plague. Obvious signs of congestive failure were apparently not part of the clinical pattern, and we are not aware of any electrocardiographic studies which might provide additional evidence of a significant degree of myocarditis. Small extravasations of blood under the skin and mucous membranes and into tissues around lesions are well known phenomena in plague (2) and indicate that the endo-

* Public Health Service Research Fellow, National Institutes of Health.
This work was partly supported by a grant-in-aid from the National Institutes of Health, U.S. Public Health Service.

toxin may affect blood vessels or blood coagulability. Alterations in the function of the central nervous system by the toxin have been surmised (2, 3) from the staggering gait, incoordination and disturbed sensorium of persons severely ill with plague.

To initiate in this laboratory a series of investigations on the pathogenesis of plague infection and on the action of the endotoxin of *P. pestis,* the development of the disease in monkeys and the time relationships between clinical signs of illness and alterations in the values of a series of laboratory tests were carefully observed. Particular attention was given to observations of changes indicative of heart failure, myocarditis or peripheral circulatory failure.

Material and Methods.

Infecting Strain of P. pestis. The highly virulent and quite stable strain Shasta was employed in all experiments. This strain was isolated in 1941 from a rapidly fatal, human, bubonic infection and has been maintained in this laboratory by serial guinea pig passages.

The organism was grown for 48 hours at 37° C. on fresh beef-heart infusion agar and harvested in isotonic saline. Total bacterial cell counts were made for each suspension which was then diluted to yield the desired number of viable cells in 0.5 ml. by considering approximately one-third of the total count to be living bacilli. This estimation of the number of viable cells in the inoculum was always confirmed through plate counts on blood agar.

Method of Study. Fifteen monkeys *(Macacus rhesus),* 3.0 to 5.0 kg. in weight, were held in individual cages. After a period to allow the animals to become adapted to the cage, surroundings and frequent handling, a series of observations to provide baseline "normal" values was made on each monkey prior to inoculation. The animal was then inoculated by injecting, under the skin of the lower part of the abdomen, 0.5 ml. of a freshly prepared suspension of *P. pestis.* Observations were then continued until death or recovery of the animal.

Voluntary activity, vigor, food consumption, rectal temperature, pulse rate, respiratory rate, blood pressure and development of the local lesion and bubo of all animals was observed. The lungs were examined by auscultation for the presence of rales.

Electrocardiographic tracings of the three standard leads were made at frequent intervals employing a portable clinical machine. With the animal supine, blood pressure was measured using the auscultatory method, a mercury manometer and a miniature cuff. Experience had indicated that systolic or diastolic pressures above about 50 mm. of mercury could be measured quite easily by this method. Measurement by palpation rarely could be accomplished at pressure levels below those at which sounds were audible. Pulse rates were usually taken from the electrocardiographs. No reliable methods or criteria were available for evaluation of heart size.

Other observations included total and differential leucocyte counts and erythrocyte counts made by the usual methods. Hematocrit determinations were made with a tube which required only 0.5 ml. instead of the usual 1.0 ml. of blood. Hemoglobin was measured by the Sahli method. Serial blood cultures were made with 0.1 to 0.5 ml. of whole blood on blood agar plates. Bleeding time was measured by the Ivy method and clotting time by the Lee-White

method. Plasma protein levels were determined by the biuret method. Blood was collected under oil for measurement of blood chlorides.

Complete autopsies were performed on all monkeys immediately after death. The gross and histologic findings, characteristic of bubonic plague in monkeys, will be presented elsewhere.

Experimental Data.

Course of the Disease. In monkeys given a dose of virulent *P. pestis* sufficient to initiate a lethal infection the clinical course was strikingly uniform in its major characteristics. The first indication of active infection was seen in edema at the site of injection beginning about 6 to 10 hours after inoculation. Between 12 and 24 hours after infection the temperature began to rise and by 24 hours the inguinal lymph nodes were moderately enlarged.

Further progress of the disease was rapid. The fever was of the sustained type, the temperature usually reaching levels between 40° and 41° C. (the rectal temperature in this series before infection was 38.5° to 39° C.) within the first 36 to 56 hours. Swelling at the site of inoculation and of the regional lymph nodes rapidly increased. Within 48 hours of inoculation there was usually an inguinal bubo 2 to 3 cm. in diameter, and this, during the next 24 hours, occasionally broke down and exuded thin serosanguineous fluid. This fluid, when cultured, invariably contained large numbers of *P. pestis*.

During the 2nd day of the infection the animals frequently ate and drank less, but remained quite vigorous until the 3rd day. They then became quiet and lethargic and soon developed rapidly progressing weakness and prostration. Onset of lethargy and weakness was accompanied by a very noticeable graying of the skin, coolness of the extremities, and moderate cyanosis of mucous membranes, fingers and toes. At no time was there any indication of increased venous pressure. The lung fields were clear of rales except in moribund animals. The rectal temperature remained high until 6 to 10 hours before death when it fell to subnormal levels.

Although, among those ultimately succumbing, 1 monkey died as early as 65 hours after inoculation and 1 as late as the 6th day, with great regularity most animals died during the 4th day after infection. The time of death bore no apparent relationship to the size of the inoculum even though the inoculating dose varied from 10^8 to 10^{10} organisms.

Blood Pressure. When infection was well established (usually after the first 24 hours) and when temperature and leucocyte counts were significantly elevated, the blood pressure (both systolic and diastolic) regularly began gradually to fall. After a

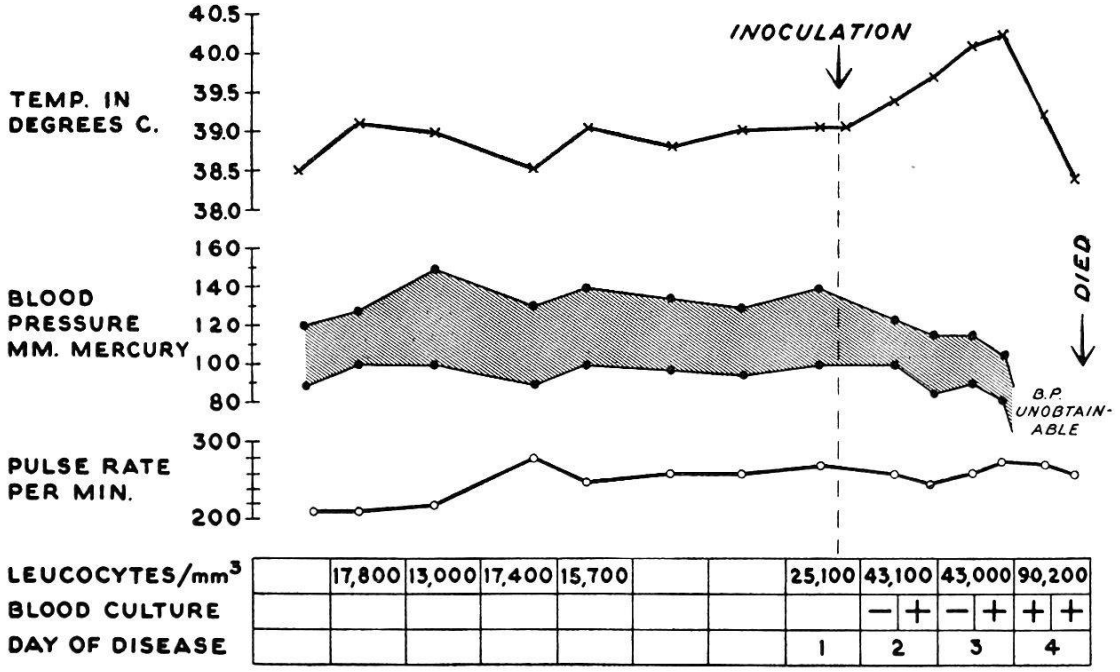


Fig. 1. Record of a fatal bubonic plague infection in a monkey. The course of the blood pressure in this animal illustrates the hypotension seen in all monkeys studied.

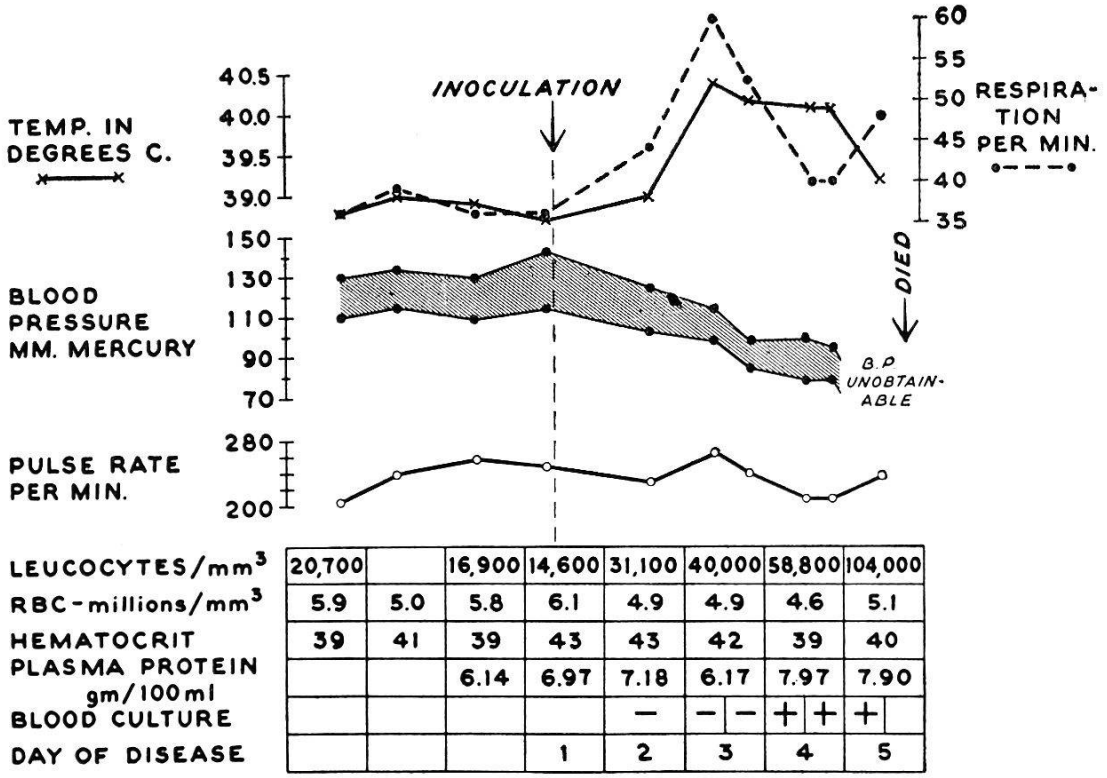
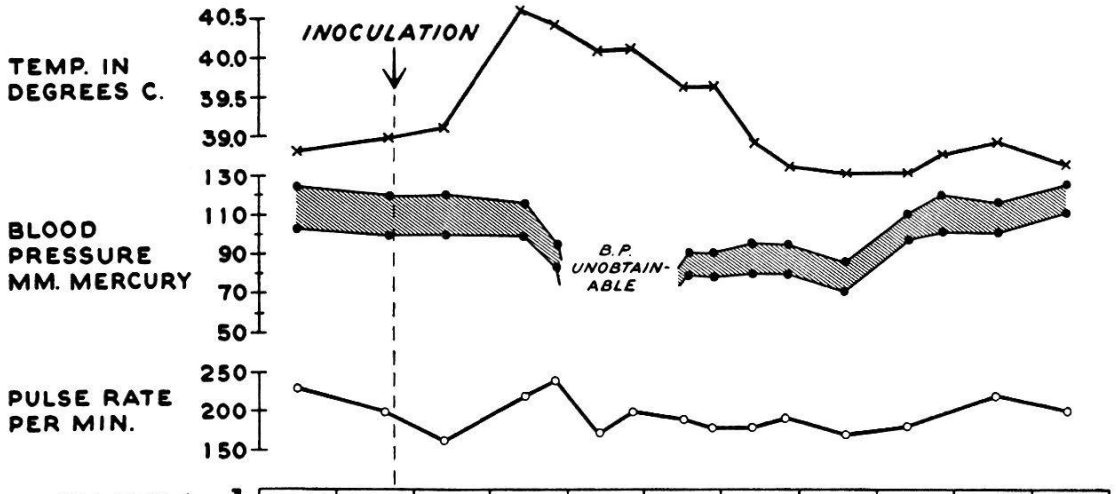


Fig. 2. Record of a typical fatal plague infection with detailed data on blood values prior to and throughout the course of the infection.

period of slow decline lasting about 24 hours there followed a more precipitous drop to very low or immeasurable levels coincident with the onset of severe weakness and prostration.

This drop in blood pressure was accompanied by a collapse of superficial veins, appearance of a gray pallor of the skin, coolness



LEUCOCYTES/mm^3	19,600	18,800	27,800	38,700	27,100	16,700	22,600		21,800	19,100	
RBC - million/mm^3	7.3	6.8	7.4	7.6	6.7	6.8	7.2		6.5		
HB - gms/100 ml	14	13.5	14	13	14	14			14		
BLOOD CULTURE			—	— —	— —	— —	—		—	—	
DAY OF DISEASE			1	2	3	4	5	6	7	8	9

Fig. 3. The course of a non-fatal bubonic plague infection in a monkey. The blood pressure was below auscultatory levels for a 44 hour period.

of the extremities and cyanosis of the tips of the digits. Withdrawal of blood for laboratory studies became increasingly difficult after the first 48 hours and when the peripheral vascular failure became profound, transection of even the brachial vein resulted in only slow oozing of very dark blood. In Figures 1 and 2 data from 2 typical fatal infections in monkeys are charted, and the characteristic course of the blood pressure is well illustrated. This general course was seen in every one of the 15 monkeys in which the blood pressure was measured and followed. It is to be emphasized that this hypotension was not simply a terminal event in moribund animals, but in some instances the blood pressure was below auscultatory levels for as long as 48 hours prior to death. In Figure 3 it can be seen that, although this monkey ultimately recovered, there was a 4 day period of hypotension and during 44 hours the blood pressure was below levels measurable by the auscultatory method used.

Noteworthy in Figures 1 to 3 is the relative lack of response of the heart rate to rise in temperature or to fall in blood pressure. In all monkeys studied, even though the blood pressure was unmeasurable and the picture was that of marked peripheral circulatory collapse, the pulse rate remained approximately at normal baseline levels. The respiratory rate was more responsive (Fig. 2), often rising with elevation of temperature, and during the stage of hypotension and circulatory collapse respirations were usually shallow, weak and rapid.

The Electrocardiogram. Electrocardiograms taken after infection were carefully compared with those made prior to inoculation, looking for the onset of any change in the graph, but particularly for changes in rhythm, in conduction, alterations in RS-T segments and alterations in T waves which might be indicative of myocarditis. At no time were there significant changes in rhythm, conduction or in QRS complexes. The graphs from 2 monkeys showed flattening of T waves in Lead I during the period of severe prostration and the T waves in Lead I became diphasic during the last 2 or 3 hours of life. The lowering of T-I in the tracings from these 2 animals was accompanied by an increase in height and peaking of the T waves in Leads II and III. High, peaked T waves in Leads I, II and III appeared during the last 24 hours before death in the graphs of 2 other monkeys. In Figure 4 this increase in T wave height and peaking can be seen in a graph taken 18 hours before death when it is compared with the preinfection graph. In no instance were these alterations in the T waves accompanied by significant changes in other components of the graph. The S-T segment did not depart from the iso-electric line at any time.

Laboratory Studies. Blood Cultures. With great regularity *P. pestis* could be cultured from venous blood after the 36th hour of

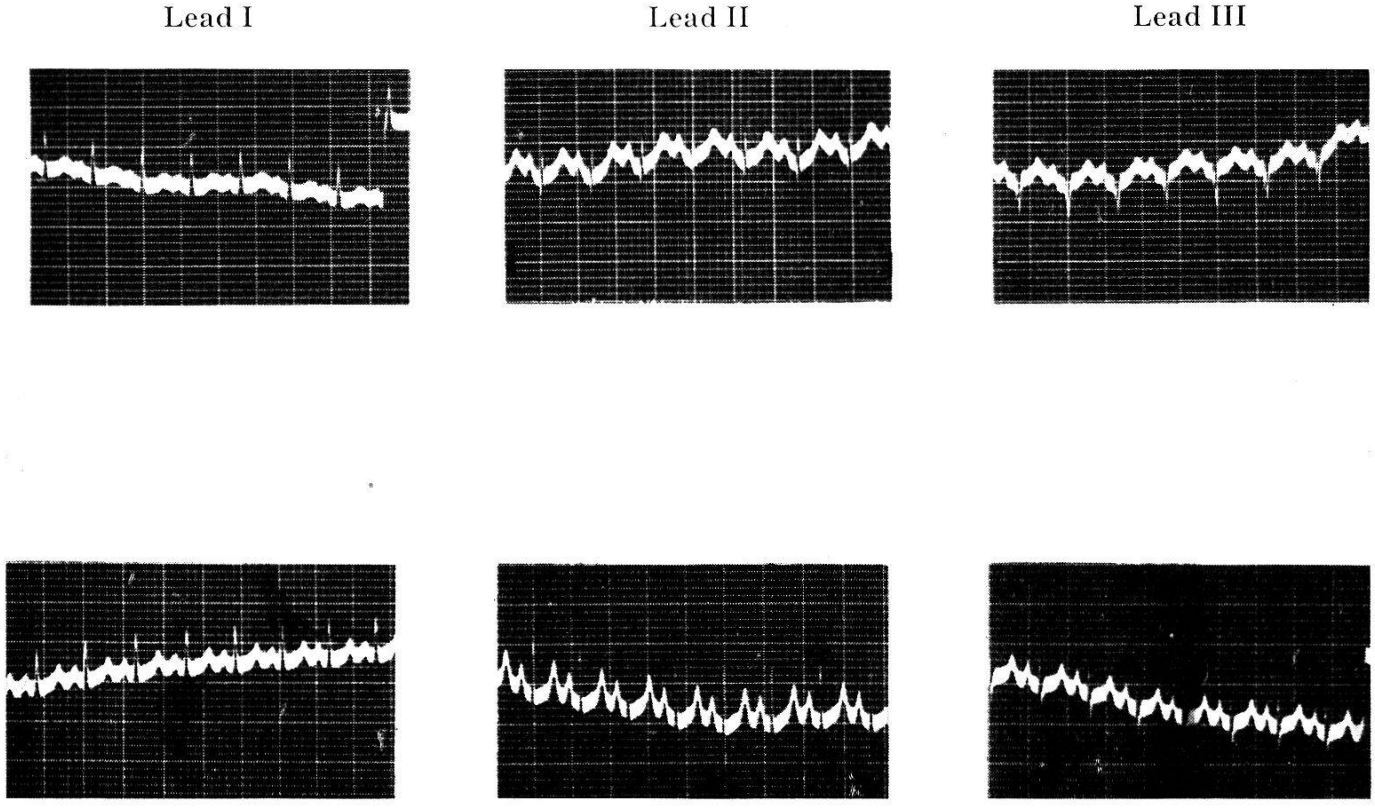


Fig. 4. Electrocardiographs of a monkey with bubonic plague showing increased height and peaking of T waves which appeared in the last 24 hours before death. A = Preinfection graph. B = Graph taken 18 hours before death.

the infection even though the volume of blood taken for culture (0.1 to 0.5 ml.) was relatively small. On a few occasions small numbers of organisms were found as early as 24 hours after inoculation. The number in the peripheral blood rapidly increased when bacteremia was established, and during the 12 to 18 hours before death bacilli often could be seen lying free between blood cells on microscopic examination of smears of peripheral blood.

It is of interest that in the case of the single animal of this study that recovered from plague (Fig. 3) evidence of bacteremia was not found at any time during the disease in a total of 10 blood cultures, though in other respects the animal responded to the infection in a manner similar to that seen in the fatally infected monkeys and though it showed the characteristic blood pressure drop and picture of peripheral circulatory failure.

Leucocyte Count. Within 24 hours after inoculation a significant leucocytosis was regularly found (Figs. 1 to 3). The general course of the total count can be seen in Figure 1. Elevation from the preinfection mean of 16,200 $\pm$ 5,300 to levels of 30,000 to 50,000 cells per cubic millimeter was usual, but counts sometimes exceeded 100,000 during the terminal phase of the illness. The leucocytosis was predominantly due to an increase in the polymorphonuclear series, these cells increasing from a mean of 54 $\pm$ 10 per cent before infection up to 90% or higher during the height of the leucocytosis.

Erythrocyte Count, Hematocrit, Hemoglobin, and Plasma Protein Levels. The relatively constant level of the erythrocyte, hematocrit and plasma protein values during the course of the infection is well illustrated by the data in Figures 2 and 3. No consistent change in erythrocyte count, hemoglobin or hematocrit values accompanied the stage of peripheral circulatory failure, although the plasma protein values rose slightly (Fig. 2, Table 1). Since erythrocyte and hematocrit values were stable in some monkeys from whom no more than 1 to 2 ml. of blood was taken daily, repeated blood loss did not seem to be responsible for the lack of signs of hemoconcentration. In 2 animals albumin-globulin ratios were followed, but they did not change significantly during 4 day periods of survival after inoculation.

In 1 animal values for blood nonprotein nitrogen, chloride and sodium chloride were determined before inoculation and followed at daily intervals through the course of the disease (Table 1). Technical difficulties encountered in the sterilization of blood samples, necessary for safety in carrying out these determinations, prevented further study along these lines during this investigation, but in

TABLE 1.

Blood NPN, Chlorides and Plasma Proteins in Monkey with Bubonic Plague.

Days of Disease	Rectal Temperature, Degrees C.	Blood Pressure	General Status	Blood NPN mgm./100 ml.	Blood NaCl mgm./100 ml.	Plasma Proteins gm./100 ml.
	38.5	140/125	Normal	35.0	462	7.16
1	39.2	110/90	Normal vigor Negative blood culture	30.0	479	7.85
2	40.7	115/95	Moderately ill *P. pestis* in blood culture	35.1	470	8.13
3	40.2	90/65	Very weak Large bubo Positive blood culture	33.6	413	8.23
4	39.5	Unobtainable	Prostrate Sufficient blood for tests not obtainable Died 4 hours later	—	—	—

this single animal, whose infection and course were typical, blood chlorides declined, as was to be expected in an animal in a febrile state, but the blood nonprotein nitrogen remained at preinfection levels. In numerous acute infections the blood nonprotein nitrogen very frequently is quite markedly elevated even though toxic damage to the kidney is not indicated (4). The rapidity of the course in this plague infection may have been a factor in the failure to see such a rise here.

Values for bleeding and clotting times during the disease did not consistently differ from those in the preinfection observation period. It was considered, however, that, in view of the frequent difficulty with which blood was obtained for the determination of clotting time during the acute phase of the disease and the marked change in blood pressure, measurements of bleeding and clotting time could not be relied upon and they were discontinued early in the study.

Discussion.

The most striking abnormality in these monkeys with acute plague was the profound circulatory failure. This was remarkable in its regularity and in its degree. Peripheral circulatory failure has been observed in man in severe infections with a variety of organisms (5-9), and there is indication in descriptions of epidemic plague (1, 2) that peripheral circulatory failure is rather frequent in human infections with *P. pestis*. Even persons dying of infections other than plague, however, very often have quite adequate circulation, and death appears to be caused by other factors. Just how frequently and under what conditions severe circulatory failure occurs in untreated human plague is uncertain, but the many descriptions of the prostration, fading pulse, feeble heart sounds and "failing heart" in persons ill with plague suggest that it is seen in this disease with much greater frequency than in other bacterial infections.

STEAD and EBERT (5) have suggested that the clinical picture of peripheral circulatory failure associated with acute infections is due to increased venous pooling of blood with subsequent decrease in the venous return to the heart and also to a direct effect of the infection on the heart muscle resulting in less efficient heart action. WOOD (6) is skeptical that carditis plays an important role in the cardiovascular disturbances in acute infections (except in rheumatic fever, diphtheria and bacterial endocarditis) and attributes the peripheral collapse to depression of the vasomotor center, to toxic paresis of the peripheral vessel, to suprarenal failure or to diminution of the blood volume from dehydration or loss of plasma into the tissue spaces through damaged vessels. MOON (7) and others (8) have considered this type of shock largely due to toxic injury of peripheral vessels and capillaries with subsequent leakage of plasma into tissues, hemoconcentration and decrease in blood volume. EBERT and STEAD (9), recognizing the clinical similarity between the shock sometimes seen during infections and that following hemorrhage or trauma, studied patients with pneumonia and septicemia in an effort to determine the effect on blood volume and venous pooling. In their cases they found no indication of diminished blood volume or hemoconcentration and found that transfusion was not an effective form of therapy. They concluded that the circulatory failure was not due to failure of any single portion of the cardiovascular system, but that the entire cardiovascular system, including the heart, was damaged.

In the present study of plague infection in monkeys it seems unlikely that the few changes in the electrocardiograms were in-

Fig. 5. Bubonic Plague in Rhesus Monkey No. 739. Immediately below "21" note enlarged left axillary lymph node located above a primary crater-like lesion with sanguineous-purulent discharge; ninth day after infection.

dicative of significant myocarditis, and signs of congestive failure were conspicuously absent. The available evidence renders it difficult to ascribe the circulatory failure seen in these monkeys and their deaths predominantly to action of *P. pestis* toxin on their hearts. A circulatory failure brought about by an effect on peripheral vessels or at least due in the main to an effect on peripheral vessels seems somewhat more likely. As stated before, small extravasations of blood under the skin and mucous membranes and into tissues around lesions are known to be frequent in plague infections (2), and their presence was noted at autopsy of the monkeys dealt with in this study. If, however, large losses of plasma produced a decrease in blood volume, this was not reflected in increases in hemoglobin, erythrocytes, hematocrit or plasma proteins, since all these tended to remain at fairly constant levels, even though no special effort was made to keep the animals hydrated.

The failure of these animals to respond to peripheral circulatory failure with an increase in heart rate might be interpreted as compatible with a toxic effect on the central nervous system and suggests that the peripheral circulatory failure could be due to alteration in the function of the vasomotor center. Central nervous system depression as a cause of circulatory collapse in acute infections has

been considered previously by several investigators, but in the few instances in which efforts have been made to test vasomotor function (9, 10), it has not appeared to be seriously impaired. In the absence of information regarding the heart rate response of monkeys to circulatory failure from causes other than plague infection or of more direct indication of depressed vasomotor activity, such an explanation of the circulatory failure seen in plague is not adequately supported. Further study of this problem would be desirable, because the absence of tachycardia in the monkeys of this series contrasts sharply with observations on cases of severe human plague in which the heart rate usually becomes very rapid and quite often irregular (1, 2).

It was not feasible in this study to measure blood potassium levels, although the appearance of high, peaked T waves in the electrocardiogram of some of the monkeys during the acute circulatory collapse made it desirable to do so since they suggested that blood potassium levels may have become elevated. It is to be emphasized, however, that other components of the electrocardiogram were not altered at the time the high, peaked T-waves were present, nor did they subsequently become altered. It has been shown that blood potassium levels become elevated in shock due to trauma, hemorrhage, anaphylaxis, hyperthermia and histamine toxicity (11). This has been considered to be a result of shock rather than a cause and presumably would have no bearing on the cause of shock in *P. pestis* infection.

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Résumé.

Les auteurs ont observé le développement de la peste bubonique chez des singes Rhésus infectés expérimentalement par une injection subcutanée de *P. pestis*. Les observations cliniques et les tests de laboratoire montrent que le point culminant de la maladie se traduit régulièrement par un collapsus circulatoire périphérique sévère, sans aucun signe d'hémoconcentration et sans évidence d'insuffisance cardiaque ou de myocardite.

Zusammenfassung.

Rhesusaffen wurden subkutan mit *P. pestis* infiziert und der Verlauf der Erkrankung mit klinischen und mit Laboratoriumsmethoden verfolgt. Der Höhepunkt der Erkrankung war stets durch die klinischen Zeichen eines schweren peripheren zirkulatorischen Kollapses ausgezeichnet, ohne irgendwelche Anzeichen von Haemokonzentration und ohne genügende Merkmale von Herzversagen oder Myocarditis.