SPOTTED FEVER
II. AN EXPERIMENTAL STUDY OF FIÈVRE BOUTONNEUSE

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There are three principal causes for the inconsistencies which exist among the classifications of the human rickettsial diseases and their etiologic agents. First, many investigators have drawn unwarranted conclusions from data obtained by one or two methods of study. Second, undue emphasis has been placed either upon minor differences among various strains of rickettsiae or slight variations in the diseases which they produce in man and experimental animals. Third, it has been difficult to construct useful classifications in the absence of rigorous standards for judging the significance of certain types of data.

In order to establish uniform methods of experimental procedure and analysis, one of us described several methods for the study of pathogenic rickettsiae (1). The data obtained by application of these methods were evaluated from the point of view of classification. This necessitated the proposal of certain criteria, so designed as to facilitate an analysis of the data. These differential criteria were selected on the basis of experience with several strains of pathogenic rickettsiae studied in this laboratory: European typhus, Breinl strain; Mexican typhus, Mooser strain; North Carolina typhus, Maxcy strain; Rocky Mountain spotted fever, Parker strain; Eastern spotted fever, Rumreich, Dyer, and Badger strain; Minnesota spotted fever, Reimann strain (2-7).

It was stated that a careful analysis of the available data permits the recognition of two genera of rickettsiae pathogenic for man. Furthermore, it was shown that only one species has been discovered in each genus, and that the differences between various strains in each

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species must be regarded as of subspecific value. Consequently, the conclusion was reached that the name, *Rickettsia prowazeki*, originally given to the etiological agent of human typhus by da Rocha-Lima, must be applied to the rickettsiae of all known diseases of the typhus fever group and the name *Dermacentorxenus rickettsi*, originally applied by Wolbach to the etiological agent of Rocky Mountain spotted fever, must be applied to the rickettsiae which cause the diseases of the spotted fever group. The names typhus fever and spotted fever were selected to conform with those of the prototypal diseases, since distinguishing names are necessary for the designation of two such widely separated groups of diseases. There is some evidence that the mite-borne rickettsial diseases may form a third group and that the corresponding rickettsiae may belong to a third genus or species. However, at the present time the available experimental data are not adequate for the support of such distinctions.

We have recently completed a number of experiments with the rickettsiae proved by Caminopetros (8) to be the etiological agent of fièvre boutonneuse. In the present report, the data obtained from our studies have been combined with pertinent information available in the literature. The following aspects of fièvre boutonneuse and its etiological agent will be considered and the data will be subjected to analysis according to the standards previously referred to. 1. Clinical study of the disease in man. 2. Weil-Felix reaction. 3. Clinical study of the disease in guinea pigs. 4. Study of smears of scrotal sac exudate. 5. Histopathology in man and animals. 6. Location and morphology of microorganisms in paraffin sections of tissues of mammalian host. 7. Study of crossed immunity. 8. Study of the microorganisms in the arthropod vector. 9. Study of the microorganisms in tissue culture.

*Source of the Strains.*—Through the courtesy of Dr. Caminopetros of the Pasteur Institute of Athens, three strains of the causative agent of fièvre boutonneuse have been available for our experiments. They have been isolated by the injection of the viscera of the common dog tick (*Rhipicephalus sanguineus*) intraperitoneally into guinea pigs. The ticks had been collected from dogs inhabiting regions of Greece and Morocco where fièvre boutonneuse is endemic. One strain has been isolated in this laboratory. Two strains kindly have been sent
to us by Dr. Parker. All strains have been maintained serially in
guinea pigs by intraperitoneal injection of scrotal sac exudate. Al-
though there has been a trend toward diminished virulence after the
passage of one of these strains through a series of animals, we have
noticed no fundamental differences among the three strains. Within
the limits of the experimental data they must be considered as
identical.

1. Clinical Study of the Disease in Man

Complete clinical descriptions have been made by Olmer (9) and Combiesco
(10). A brief survey has been necessary for our purposes.

Conor and Bruch in 1910 (11) described an endemic disease of Tunis. They
named it fièvre boutonneuse. Since the original account, numerous instances of
diseases with a similar or identical clinical picture have been reported in many
regions of the Mediterranean littoral. New names for the diseases have fre-
quently accompanied these reports. Among them have been the following: fièvre
escharonodulaire, exanthème typhoïde estival, dothiendermie aiguë, exanthème
infectieux épidémique, exanthematosus fever, eruptive fever, Marseilles fever, fever
of Conor and Bruch, typhus-like fever, and typhus fever.

The malady may be defined as a tick-borne, rickettsial disease which usually
has been characterized by a primary lesion at the site of the tick bite, an acute
onset, mild febrile reaction, generalized exanthema, brief clinical course, and low
mortality rate.

The majority of the cases have occurred in adults during the warm summer
months. Rare instances have been reported in the early spring and late autumn.

A primary lesion, the tache noire, has been found in about one-half of the pa-
tients, usually at the site of a tick bite. The typical lesion has been an indurated,
hyperemic, painless, and occasionally ulcerous papule, with regional lymphan-
geitis. In a few instances the primary sore has been in the conjunctiva (12). It
was assumed that the local infection in these patients was probably produced by
introduction of the infectious agent into the eye by rubbing the eye with fingers
contaminated with infectious viscera of crushed ticks. The primary lesion has
been produced experimentally in man by subcutaneous or intracutaneous injec-
tion of blood obtained from human beings with the disease and by a similar
injection of the viscera of infectious ticks (10).

The duration of the disease has usually been 12 to 14 days. During the first
2 to 3 days there have been nonspecific, mild, prodromal symptoms accompanied
by an elevation of temperature to approximately 40°C. Within 3 or 4 days after
the onset of fever, a maculopapular rash has appeared, first on the trunk and
then, after spreading rapidly to the extremities, has involved the soles of the
feet and the palms of the hands. The cutaneous lesions, pale red in color in the
early stages, later have often become hemorrhagic and ulcerous. The fever has
subsided by lysis during the evolution of the rash. Except for a prolonged convalescence, complications have been few and unimportant.

The course of the disease in children has been brief and the symptoms mild. The rare fatalities have occurred in adults whose previous physical state was such that any mild infection would have been serious.

2. Weil-Felix Reaction

There have been numerous reports concerned with the results of the Weil-Felix reaction, conducted with human serum and several strains of *B. proteus*. The results have not been uniform and certain inconsistencies among the data of several investigators have caused some confusion. The discovery that the reaction varies among different patients as well as in the same patient during the course of the disease has aided in explaining some of the inconsistencies. We are inclined to accept the conclusions reached by Felix after he had corroborated Durand's careful studies of the Weil-Felix reaction in fièvre boutonneuse (13). The serum from patients with fièvre boutonneuse usually agglutinated *B. proteus O X 19* in low titre. A lower titre and often no agglutination were obtained when *B. proteus O X 2* and *B. proteus O X K* were used. Felix has interpreted the results as indicative of "group" agglutination, similar to that obtained in the study of Rocky Mountain spotted fever and tick bite fever. The "main" antigen has not been discovered.

Serological studies conducted by Caminopetros and Contos (14) have been concerned with the action of immune serum on the viable rickettsiae. They have found that the serum obtained from monkeys after the animals had been inoculated with nonviable rickettsiae of fièvre boutonneuse and also the serum of patients who were convalescing from fièvre boutonneuse neutralize the action of the viable rickettsiae of fièvre boutonneuse, while the serum of patients who had recovered from typhus fever does not neutralize the action of these microorganisms.

3. Clinical Study of the Disease in Guinea Pigs

The clinical manifestations which have followed the intraperitoneal injection of scrotal sac exudate or the viscera of ticks into guinea pigs have been fairly uniform. Within 2 to 5 days after injection, the temperature usually has reached 104–106°F. and scrotal edema and
hyperemia have developed. During this stage the presence of adhesions between the visceral and parietal layers of the tunica vaginalis may be detected by the mechanical resistance to the manual reduction of the testes into the peritoneal cavity. These adhesions, at first quite easily ruptured, later have become sufficiently organized to prevent an easy reduction of the testes into the peritoneal cavity. Within 3 or 4 days after the onset of edema and hyperemia of the scrotal sac, these manifestations have reached a maximum severity and subsided. The temperature has fallen by lysis and within 10 to 14 days after intraperitoneal injection has reached normal values. The animals have not been prostrated by the infection and the mortality rate has been negligible.

The principal variations from the typical findings have been in the length of the incubation period and the intensity of the scrotal inflammation. Rarely, the incubation period has been prolonged to 7 to 10 days. The severity of the scrotal reaction as produced by two strains has been fairly uniform. The behavior of the third strain has been different in two ways. First, the injection of the viscera of ticks has been followed by the rapid onset of high fever, symptoms of extreme prostration, and death within 2 days. Each animal had an acute generalized peritonitis. The exudate contained, in addition to numerous rickettsiae, a large bacillus. It has been shown that a pure culture of this bacillus causes a sudden fatal peritonitis, not only in normal guinea pigs but also in those which have recovered from fièvre boutonneuse. It has only been through a graded dilution of the emulsions of the viscera of ticks and through reinfection of small amounts of the exudate that the strain of rickettsiae was separated from this unusual contaminant. Secondly, the scrotal reaction produced by this strain of rickettsiae gradually has diminished in intensity after passage through a series of animals. This phenomenon apparently has been encountered by Caminopetros (14). He has stated that infectious cerebral tissue, if injected intraperitoneally, restores the original intensity of the reaction.

There has been a difference, clinically, between those animals injected intraperitoneally and those which have received infectious exudate subcutaneously. Although only a few animals have been injected subcutaneously, none has developed either a high fever or a
scrotal reaction. This type of response has been comparable to that of one animal upon which infectious ticks had fed. This animal had a mild fever and no scrotal reaction. Subsequently, it was found to be immune to an intraperitoneal injection of scrotal sac exudate.

4. Study of Smears of Scrotal Sac Exudate

The methods for microscopic study of scrotal sac exudate have been described in a previous report (3). All stages of the inflammatory reaction have been examined. The most satisfactory preparations have been obtained on the 1st or 2nd day after the formation of adhesions between the two layers of the tunica vaginalis.

The direct smears, stained with Giemsa's solution, have had a variable ratio of polymorphonuclear leucocytes, macrophages, and serosal cells. Polymorphonuclear leucocytes have been numerous during the acute stage of exudation. Macrophages have been predominant in the period of organization and early repair.

Rickettsiae have been found in all preparations. Although they usually have been most numerous in the acute voluminous exudate, there has been no accurate correlation between the number of rickettsiae and the gross appearance of the exudate. Not infrequently a copious exudate obtained from an animal which had a severe scrotal reaction contained few rickettsiae. It has been common to find numerous microorganisms in a scanty exudate obtained from an animal which had a mild scrotal reaction.

The rickettsiae have been found in intracellular and extracellular locations. The majority of the microorganisms have been embedded in the cytoplasm of macrophages and serosal cells. In a few smears the nuclei of several cells have contained clusters of rickettsiae. Rarely, microorganisms have been found in the cytoplasm of polymorphonuclear leucocytes. The scattered extracellular rickettsiae seem to have gained this position principally as the consequence of the crushing of cells during the preparation of smears.

The microorganisms have never occurred in large numbers in the cytoplasm. In the most heavily infected cells, no more than 25 to 30 pairs have been present. As a rule, they have been scattered at
The rickettsiae have had a distinctive morphology. The typical form has been lanceolate and the microorganisms have been associated characteristically in pairs. Spherical diplococcoid or linear diplobacillary forms have represented the usual extremes of morphological range. Isolated single microorganisms and an occasional short chain of 2 to 3 pairs have been found.

There has been a great variation in size. The large forms have been distinguishable under the magnification of a high dry objective. The smallest forms have been so minute that resolution has been difficult to achieve, even with the aid of a 1.3 mm. oil immersion lens and a perfect preparation.

The staining reaction with Giemsa's solution usually has been rather intense. The rickettsiae have been blue and sharply defined, with a narrow clear halo around them.

The intranuclear rickettsiae usually have occurred in one or more small clusters surrounded by a halo and imbedded in a pale blue, homogeneous nucleoplasm. Occasional nuclei have been filled with a compact mass of microorganisms. The average dimensions of intranuclear forms have been less than those of the intracytoplasmic microorganisms, although the extreme ranges have been equivalent.

5. Histopathology in Man and Animals

A few incomplete studies of the histopathology of the disease have been reported. Olmer (9) has examined the cutaneous lesions of Marseilles fever, a disease which is identical with fièvre boutonneuse. He described small accumulations of lymphocytes, monocytes, and polymorphonuclear leucocytes around vessels of precapillary and capillary dimensions. Combesco (10) has found a similar reaction in the subcutaneous tissues. No rickettsiae have been demonstrated in the sections. Troisier and Cattan (15) have described perivascular cellular aggregates in a monkey with fièvre boutonneuse.

Inasmuch as no human tissues have been available for study in this laboratory, our histopathological data have been obtained from an examination of guinea pigs. The tissues have been treated by the Regaud-Giemsa technique, which has been previously described (3).
The brain, scrotal sac, epididymis, testis, and sites of the tick bite have been studied with special care. There have been no cerebral vascular lesions. The scrotal sac and its contents have shown an acute inflammation of the tunica, an exudate over the surface of the tunica, a thromboangitis, and focal necroses. The continuity of the serosal cells frequently has been interrupted by small areas of acute necrosis. From these small abscesses a rather diffuse cellular infiltration has extended for variable distances beneath the partially intact but swollen serosal cells. The musculature and connective tissue of the scrotum have been involved but the testicular substance, except in perivascular regions, usually has been unaffected except for the cessation of spermatogenesis which always accompanies the inflammation with its attendant local elevation in temperature. The reaction has been accompanied by an exudate, distributed rather unevenly over the surface of the tunica. The exudate has been composed of a variable ratio of polymorphonuclear leucocytes, macrophages, and serosal cells. The acute thromboangitis has been restricted to the small arteries and venules, especially those of the scrotal sac and the polar fat of the testis. The more severe lesions of vessels have been characterized by an acute segmental necrosis of the wall of the vessel and a fibrinous thrombus in the lumen. The necrosis usually has extended from the intima through the media into the adventitia. The structure of the intima and media has been replaced by cellular debris and a large number of polymorphonuclear leucocytes and macrophages. The cellular infiltration has been distributed throughout the periadventitial tissues and often has had an eccentric focal zone of concentration.

The microscopic sections of the cutaneous papules which developed at the sites of several tick bites have shown epithelial destruction, inflammation of the corium, extravasation of blood, thromboangitis and rickettsiae. The epithelium over a small area has been replaced by a localized superficial acute inflammatory exudate in which bacteria of various types have been found. Throughout the corium and subcutaneous fat there has been a moderate number of macrophages, polymorphonuclear leucocytes, and extravasated red blood cells. The inflammatory cells have been most numerous around small blood vessels, several of which have shown partial necrosis of their walls and
thrombi in their lumina. The thrombi have been especially common in greatly dilated veins.

6. Location and Morphology of the Microorganisms in Paraffin Sections of Tissues of Mammalian Host

In several instances, the study of the microorganisms in paraffin sections has been more satisfactory than in smears. The general distribution of the organisms in the exudate has been similar to that in smears. Several intranuclear clusters have been found. Only a few rickettsiae have been present in the intact serosal cells. They have been demonstrated with some difficulty in the focal necroses of the tunica and in the endothelial and smooth muscle cells of blood vessels. The blood vessels in the region of the tick bite have contained more rickettsiae in their walls and intimal endothelial cells than those in the scrotum or polar fat of the testis. The morphology of the rickettsiae, whether intracytoplasmic or intranuclear, has been identical with that described in smears.

7. Study of Crossed Immunity

Several experiments concerned with the question of crossed immunity among fièvre boutonneuse and other rickettsial diseases in guinea pigs have been reported. Caminopetros and Contos (14) have found no crossed immunity between fièvre boutonneuse and typhus fever. Two strains of R. prowazeki representing European typhus and Brill's disease were used in their experiments. Brumpt (16) has concluded that fièvre boutonneuse does not protect against Rocky Mountain spotted fever. Badger (17) has obtained evidence of reciprocal crossed immunity between fièvre boutonneuse and Rocky Mountain spotted fever and has concluded that they are immunologically identical. Davis and Parker (18) have reported a positive crossed immunity among São Paulo typhus, Rocky Mountain spotted fever, and fièvre boutonneuse. Parker1 has made the interesting observation that his Rocky Mountain spotted fever vaccine, prepared from infected ticks, does not protect guinea pigs against fièvre boutonneuse.

In order to evaluate the validity of a single contradiction to the generally uniform results, we have conducted a test for crossed immunity between fièvre boutonneuse and Rocky Mountain spotted fever. A strain of Rocky Mountain spotted fever which invariably has been fatal to normal guinea pigs was injected into normal guinea pigs. As soon as the animals had become moribund, blood was aspirated from the heart. Several cubic centimeters of this blood were then injected intraperitoneally into each of six guinea pigs which had recovered from

1 Parker, R.R., personal communication.
fevère boutonneuse several weeks previously. The animals did not react to the injection.

Our experiments with Parker’s vaccine have confirmed his results. Each of twelve guinea pigs was injected with 2 cc. of Rocky Mountain spotted fever vaccine. Six of the vaccinated animals and six control animals were given intraperitoneal injections of 2 cc. of blood from animals moribund with Rocky Mountain spotted fever. The six vaccinated animals exhibited no significant evidence of disease. The control animals died of typical Rocky Mountain spotted fever.

The remaining six vaccinated animals were given, intraperitoneally, graded amounts of scrotal sac exudate and splenic tissue from animals ill with fevère boutonneuse. Six normal unvaccinated controls were injected with the same graded quantities of infectious material. Five animals in each group developed fevère boutonneuse. One vaccinated animal and the corresponding control, each of which had received the smallest amount of infectious tissue, did not have either a scrotal reaction or an elevation of temperature.

8. Study of the Microorganism in the Arthropod Vector

A series of observations and experiments by Caminopetros and others have proved beyond reasonable doubt that the common dog tick (*Rhipicephalus sanguineus*) is a vector of the causative agent of fevère boutonneuse and that the dog is a natural reservoir. The clinical observations have associated the disease with the bite of this tick. It has been shown, in endemic areas, that the viscera of this variety of tick are infectious for several species of animals and that when injected into man, are productive, not only of the primary lesion, but also the typical clinical disease.

These facts have led us to undertake a study of 50 ticks (*Rhipicephalus sanguineus*) which had been collected from dogs owned by a man who had recently acquired fevère boutonneuse. The methods which have been applied to this study are essentially the same as those developed by Wolbach (6).

The ticks were divided arbitrarily into two lots. One lot was used to establish the strain in guinea pigs and to determine by smears the distribution and morphology of the rickettsiae in the vector. For these purposes, each tick was dissected and smears made of portions of several organs. The remnants of the viscera were then crushed in a mixture of serum and Tyrode’s solution. Graded dilutions of the pooled viscera were injected intraperitoneally into twelve guinea pigs. The second lot, composed of twenty-five ticks, was used for histologic study. This lot was divided into two groups. One group (eleven ticks) was dissected without having fed since removal from dogs in Greece, several weeks
previously. The second group (fourteen ticks) was allowed to feed on normal
guinea pigs for 7 days. After incubation at room temperature for 10 to 14 days,
they were dissected. During dissection, smears were made of the hypoderm,
salivary glands, gut, and sexual organs. The viscera of each tick were subsequently fixed in Regaud's solution, embedded in paraffin, sectioned serially, and
stained by the Giemsa method.

The smears of the organs of the ticks used for establishing the strain,
and those of the ticks which also had not refed but which were studied
in serial sections have been essentially identical. A comparison of the
smears of the ticks not refed with those of the ticks refed in this labora-
tory has given evidence that multiplication of the rickettsiae has
followed feeding and incubation. Inasmuch as the smears have given
only a rough approximation to the more precise findings in serial
sections, it is only necessary to present the data obtained from serial
sections.

All of the ticks of the group which had not refed have contained
rickettsiae. The serial sections have shown a great variation in the
number of microorganisms, not only among the individual ticks but
also in the various organs and parts of the organs in the same tick.
In two ticks, almost all organs contained numerous rickettsiae, but
usually the rickettsiae were fairly well localized and sparsely distrib-
uted. The cells of the gut, hypoderm, and ovaries have been the
common sites of predilection. Localization in the cytoplasm has been
constant but no intranuclear rickettsiae have been found in this series.
Diplococcoid and diplobacillary forms frequently have been pre-
dominant. Many other structures, too numerous to mention, have
exhibited such a range in morphology and staining reactions that they
have not been considered as related to the rickettsiae under considera-
tion, although it must be borne in mind that the morphological range
of Dermacentor xenus rickettsi in the tick has not been completely
determined.

A study of fourteen ticks which had refed prior to dissection has
added several interesting facts. The guinea pig upon which they had
fed acquired an immunity to fièvre boutonneuse. Three cutaneous
papules at the site of tick bites showed the characteristic thrombo-
angeitis with rickettsiae in the walls and endothelial lining cells of
blood vessels. The serial sections of these ticks in general have shown
rickettsiae in larger numbers and more diffusely distributed than those of the control series which had not refed. Furthermore, intranuclear rickettsiae have been found in four ticks. The cytoplasm of the cells of the gut, brain, hypoderm, ovaries, smooth muscle, and striated muscle frequently has contained microorganisms in large numbers. Usually they have been either isolated or in small clusters. Compact masses entirely replacing the cytoplasm have not been found. Small diplococcoid and diplobacillary forms have been especially numerous in those ticks in which intranuclear rickettsiae were demonstrable.

The intranuclear rickettsiae have been in several cells of the rectal pouch, the female sexual organs, striated muscle, Malpighian tubules, and hypoderm. These microorganisms have usually occurred in clusters in the center of the nucleus or uniformly distributed throughout the nucleus. In the latter instance, great distension of the nuclear membrane has been a common finding. Chromatin and nucleoli usually have either been wholly indistinguishable or persistent only at the nuclear membrane. When in compact masses the microorganisms usually have been smaller and more coccolid than when in small clusters. However, in both instances lanceolate diplococci and diplobacilli of relatively great size have been found.

9. Study of the Microorganisms in Tissue Culture

The plasma clot tissue culture method (4, 5) and the Maitland technique (19) as applied to the investigation of rickettsiae have previously been described. In this present study, scrotal sac exudate and infectious splenic tissue either singly or in combination with smooth muscle or striated muscle have been explanted. Smooth muscle from the uterus and striated muscle from the thigh of normal guinea pigs have been used. Prior to explantation, the infectious tissues and normal muscle have been incubated together at room temperature for at least 1 hour. The cultures have been maintained at 32-35°C. over a period of from 2 to 4 weeks. Transfers have been made at intervals of from 5 to 14 days. After termination of the period of cultivation, cultures have been selected for tests of infectivity. The remainder have been treated by the Regaud-Giemsa technique. Serial sections have been made of the tissues cultivated in plasma clots, as well as those in Maitland’s medium.

Inasmuch as the cultures in plasma clots have been more successful than those in the fluid medium, the descriptions principally will be restricted to the former. They always have been infectious for
guinea pigs during the period of cultivation (2 to 4 weeks). There
have been no important variations in the microscopic findings which
are attributable to prolongation of the period of cultivation beyond
2 weeks.

The cultures of scrotal sac exudate after growth in vitro for 2 weeks
have contained a few typical macrophages and rare polymorphonuclear
leucocytes. The majority of the cells have been large mesenchymal
cells, frequently in mitosis. These large fusiform or stellate cells have
multiple processes, a reticulated cytoplasm, and prominent ellipsoidal
nuclei.

In the average culture rickettsiae have been present in 80 to 90
per cent of the large mesenchymal cells and macrophages. The nuclei
of approximately 20 per cent of the cells have been partially or com-
pletely filled with rickettsiae. Nuclei undergoing mitosis often have
contained the microorganisms.

The characteristic, lanceolate, diplococcal or diplobacillary forms
of rickettsiae have been encountered most frequently. Isolated, coc-
coid or linear, bacillary forms have shown, as in the smears, the ten-
dency of the organisms to be pleomorphic. Long chains have been
found in many cells.

There has been a considerable range in the dimensions of the or-
ganisms. This has been noted within individual cells, among separate
cells in the same culture, and among the different cultures. The
range in size has occasionally exceeded the limits of that described in
smears and sections of scrotal sac exudate previous to cultivation
in vitro.

The intra cytoplasmic distribution usually has conformed to one or
two general patterns. A common localization has been in the reticu-

lum between unstained spherical spaces which commonly occur in the
cytoplasm of cells cultivated in vitro. These particular spaces repre-
sent the site of dissolved protoplasmic spherules which occur as highly
refractile bodies in the living cell. It has been in this reticulum that
the long chains of rickettsiae often have formed, although isolated
diplococcoid structures have been most frequent. In the cells having
no reticulum of the nature described above, there has been a random
scattering of diplococci and a reduction in the number of chains.
Occasionally the rickettsiae have been present in isolated clusters
surrounded by a narrow clear halo. These clusters frequently have been near the periphery of the cells and have never completely filled the entire cytoplasmic region.

The nuclei, although occasionally distended by a mass of rickettsiae, usually have contained small irregular clusters, one to four in number, centrally located and surrounded by a narrow clear halo. Short chains rarely have been distinguishable. The nucleoli and strands of chromatin have either disappeared or have been retained in scattered fragments among the rickettsiae or along the nuclear membrane. The nuclear membrane often has been thicker and more irregular than normal.

The intranuclear forms have varied greatly in size and shape. As a general rule, the more numerous the rickettsiae, the greater the number of small coccoid forms. There have been no sharp distinctions between the morphological range of the intranuclear forms and that of the intracytoplasmic rickettsiae. It has been a fairly uniform observation that in any single nucleus, all rickettsiae are very similar, while in the cytoplasm of a single cell, great morphologic variations have been found.

There has been no evidence that extensive intranuclear and intracytoplasmic parasitism is detrimental to the cell. On the contrary, rickettsiae, although generally in small numbers, have been found in the mitotic figures and regional protoplasm of dividing cells. It has been common, also, to find certain zones in growing cultures where intranuclear localization is much more prominent than elsewhere.

No additional useful data have been obtained from the cultures of splenic tissue. The significant findings have been identical with those in cultures of scrotal sac exudate.

Several attempts to infect uterine smooth muscle and striated muscle from the thigh have been unsuccessful. The serial sections have shown numerous rickettsiae in cells which had grown between the viable muscle fibers and into the protoplasm of degenerating muscle cells.

The cultures of tissues in Maitland's medium have shown multiplication of rickettsiae. The smears and serial sections of tissue cultivated in this manner have given less uniform and less satisfactory
data than the tissue cultivated in plasma clots, although very heavily
infected cultures have been frequently obtained within a period of
2 weeks. As yet it has been impossible to determine whether there is
an extracellular proliferation of the microorganisms in the Maitland
medium but the weight of the evidence is to the contrary.

Analysis of Data

In this analysis, reference will be made to diseases of the typhus and
spotted fever groups. The diseases of the typhus group include epi-
demic (classical) typhus and endemic (murine) typhus. The diseases
of the spotted fever group include Rocky Mountain spotted fever,
Eastern spotted fever, São Paulo typhus, Reimann's Minnesota dis-
ease, and possibly tick bite fever of South Africa. Our purpose is to
inquire into the relationship of fièvre boutonneuse to these two groups
of diseases.

Past experience has proved that in man, typhus fever cannot always
be differentiated from spotted fever by clinical observations. How-
ever, two clinical facts of differential value have been derived from a
consideration of fièvre boutonneuse. First, the disease often has fol-
lowed the bite of a tick and an initial lesion frequently has formed at
the site of the tick bite. Second, the cutaneous rash has character-
istically involved the palms of the hands and the soles of the feet.

Although the tick may serve as the vector of several diseases, nu-
merous varieties of the tick harbor the rickettsiae of all established
diseases of the spotted fever group. The vectors of the typhus fevers
are the flea and the louse. The problem of apparent vectorial speci-
ficity was approached by Zinsser and Castaneda (20). They proved
that the typhus virus remained viable in the tick for as long as 14 days
but introduced no evidence that the cells of the tick were invaded by
the typhus rickettsiae or that the disease was transmissible by the
bite of the tick.

The local inflammation in the region of the bite of the vector is not
a general characteristic of the spotted fevers. Tick bite fever of
South Africa, an assumed variety of spotted fever, often has a local
lesion. The mite-borne rickettsial diseases usually have a similar but
more severe reaction at the site of the bite of the vector. The exact
relationship between the mite-borne diseases and other rickettsial
diseases has not been determined. No local cutaneous lesion follows
the bite of the flea or louse, vectors of the typhus rickettsiae.

The distribution of the rash over the palms of the hands and the
soles of the feet is characteristic of the spotted fevers and not of dis-
eeases of the typhus group.

The results of the Well-Felix reaction in fièvre boutonneuse conform
with those obtained in all but one disease of the spotted fever group.
The exception is São Paulo typhus. The negative low titre agglutina-
tion of certain strains of *B. proteus* in the presence of serum from
patients with fièvre boutonneuse is in direct contrast to positive high
titre agglutinations which are typical of the typhus fevers.

The experiments concerned with the effect of convalescent sera on
the virulence of the rickettsiae of fièvre boutonneuse also emphasize
the absence of an immunologic relationship between fièvre bouton-
neuse and the typhus fevers.

The clinical aspects of fièvre boutonneuse in the guinea pig are
compatible with those produced by certain strains, either of typhus
fever or spotted fever. This is not surprising because clinical dif-
ferentiation of these two groups of diseases in guinea pigs often is
impossible.

The histopathology of guinea pigs with fièvre boutonneuse is char-
acterized by small focal necroses, an acute thromboangeitis, and the
absence of cerebral lesions. The focal necroses and the severe acute
angeitis with accompanying fibrinous thrombi are always found in
diseases of the spotted fever group. In the typhus fevers a mild
angeitis, insufficient to provoke the formation of fibrinous thrombi,
is a constant finding and no focal necroses are demonstrable. The
absence of cerebral vascular lesions favors a diagnosis of spotted fever,
but no major significance is attached to either the presence or absence
of such lesions.

The histopathologic study of human tissues is not sufficiently com-
plete to warrant comment. The perivascular cellular infiltration in
the subcutaneous tissues in fièvre boutonneuse is compatible with
either the spotted fevers or the typhus fevers.

The tests for crossed immunity between fièvre boutonneuse and
endemic as well as classical typhus are negative. Except for the
results of one investigator, all evidence indicates that there is a positive
crossed immunity between fièvre boutonneuse and at least two diseases
of the spotted fever group, namely Rocky Mountain spotted fever
and São Paulo typhus. If the tests for crossed immunity had been
inconclusive, as was true in the instance of Reimann's strain (7), we
would have evaluated the evidence obtained by application of the
other methods and drawn conclusions accordingly. In dealing with
the puzzling atypical strains, this method of procedure is necessary,
and had it been generally used in the past many errors, made especially
by European investigators, would never have occurred.

The fact that Rocky Mountain spotted fever vaccine, prepared
from ticks, does not protect guinea pigs against fièvre boutonneuse is
of great interest. Further investigation of this problem is indicated
because of its possible bearing on the cause of obvious differences
which exist among various strains of rickettsiae in the same species.

The comparison of the morphology of the rickettsiae of fièvre
boutonneuse with other pathogenic rickettsiae leads to definite con-
clusions. The microorganisms of fièvre boutonneuse characteristically
occur in pairs. They are usually deeply stained with Giemsa's fluid
and are often surrounded by a narrow clear halo. The typical form
is lanceolate, although coccoid and linear bacillary forms may be found.
There is a relatively great range in size but all gradations can be traced.
These characteristics are typical of the rickettsiae of the spotted fevers.
In contrast, the rickettsiae of the typhus fevers are delicate, linear,
bacillary microorganisms which stain lightly with Giemsa and have
only a comparatively small range in size. The common features of
the rickettsiae of typhus and spotted fever are exemplified by the
microorganisms of fièvre boutonneuse, namely the tendency to occur
in pairs and to form chains.

The distribution of the rickettsiae of fièvre boutonneuse conforms
with that of the several established strains of spotted fever. The
distribution, as described in the guinea pig, in the in vitro cultures of
infectious tissues, and in the vector differs greatly from that which is
characteristic of the rickettsiae of the typhus fevers. These differ-
ences in localization are concerned not only with the types of cells
which serve as a satisfactory environment for the specific microor-
ganism but also the absolute partition of intracellular distribution.
The rickettsiae of fièvre boutonneuse and of the spotted fever group of diseases in guinea pigs are found in peritoneal serosal cells, macrophages, endothelial lining cells of blood vessels, smooth muscle cells of blood vessels, and in polymorphonuclear leucocytes (in the latter cell probably only by virtue of phagocytosis). In guinea pigs the rickettsiae of the typhus fevers are restricted to peritoneal serosal cells, endothelial cells of blood vessels, and polymorphonuclear leucocytes. Comparative studies by the method of tissue culture serve to emphasize these constant differences.

The rickettsiae of fièvre boutonneuse in the vector may be distributed throughout the viscera so as to be demonstrable in almost every type of cell. This diffuse parasitization is similar to that described by Wolbach (6) in ticks harboring the rickettsiae of Rocky Mountain spotted fever. This distribution is in contrast to the restricted localization of typhus rickettsiae to the intestinal lining cells of the flea or louse.

In concluding the analysis of data, let us consider the intracellular distribution of the rickettsiae of fièvre boutonneuse and compare it with the findings obtained by the study of several strains of spotted fever and typhus fever. The pattern of intracytoplasmic distribution and the presence or absence of intranuclear localization form the major subjects for inquiry.

The behavior of the rickettsiae of fièvre boutonneuse in the cytoplasm of cells is similar to that which is characteristic of the several varieties of spotted fever. This generalization applies equally to the cells of the vector and the infected guinea pig. The typical pattern is one of a random scattering of the microorganisms throughout the cytoplasm. When an intracytoplasmic reticulum is visible they usually follow its configuration. Occasionally they occur in small compact clusters. They never multiply within the cytoplasm to such an extent as to distend the cellular membrane in the manner which is typical of the rickettsiae of the typhus fevers. These characteristics become more definite after in vitro cultivation of infectious mammalian tissues and the refeeding of parasitized ticks.

Of greater significance than the intracytoplasmic distribution is the presence of rickettsiae within the nuclei of cells. This unique localization is a characteristic of the rickettsiae of the spotted fever group of
diseases and is never observed in typhus. It is a characteristic which is not shared by any microorganisms in mammalian tissues, if the so-called filterable viruses may wholly be excluded from the category of intranuclear organisms. Intranuclear rickettsiae may be demonstrated in certain cells of the infectious scrotal sac exudate or spleen of the guinea pig and in various cells of the arthropod vector.

Fèvre boutonneuse is the first rickettsial disease in which intranuclear parasitism of mammalian cells has been demonstrable prior to the cultivation of tissues. Reimann's Minnesota disease (7), Eastern spotted fever (7), and Rocky Mountain spotted fever have required the application of tissue culture methods before the localization is apparent.

The assumption that the rickettsiae within the nuclei of cells of the arthropod vector are representative of the pathogenic microorganisms causing fèvre boutonneuse may be severely criticized. The experiments were not conducted with sufficient foresight to permit a correlation of infectivity of individual ticks with the presence of intranuclear rickettsiae. Also the possibility that the ticks harbored more than one type of pathogenic rickettsiae was not excluded. Finally, intranuclear rickettsiae were found in only four ticks of the refed group and in none of the group not refed. In an attempt to justify our assumption we cite the following facts.

First, at least one of the fourteen ticks of the refed group contained pathogenic rickettsiae as established by the fact that the guinea pig upon which they fed acquired an immunity to fèvre boutonneuse. Second, refeeding is often essential before intranuclear rickettsiae may be demonstrated in ticks harboring the rickettsiae of Rocky Mountain spotted fever. Third, the diffuse invasion of the viscera of the four ticks in which intranuclear forms were found with rickettsiae indistinguishable from those in the scrotal sac exudate of guinea pigs with fèvre boutonneuse was prominent. Fourth, intranuclear microorganisms may not always be demonstrable, even under ideal conditions. Fifth, intranuclear rickettsiae have never been found in ticks which harbor exclusively nonpathogenic microorganisms.

_even though the generalization is made that the rickettsiae of the

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spotted fevers and fièvre boutonneuse inhabit the nuclei of cells in ticks which serve as vectors, it would be unjustifiable to draw any conclusions of differential value by emphasizing the absence of intranuclear rickettsiae in the flea and louse which serve as the vectors of the typhus fevers. In order to dispense with the obvious errors which might arise from such a comparison, it has been expedient to study in tissue cultures a single type of mammalian cell, susceptible to parasitism by both genera of pathogenic rickettsiae. Thus the interior of this cell is utilized as a common culture medium. The cell so converted to this use is a large mesenchymal cell. In this cell the typhus rickettsiae, regardless of the variety or virulence, multiply freely in the cytoplasm. In this same type of cell under identical conditions, accurately controlled in vitro, the rickettsiae of several diseases of the spotted fever group and of fièvre boutonneuse proliferate, not only in the cytoplasm but also in the nucleus. The selective localization and typical distribution in each instance appear as a fair reproduction of the behavior of the rickettsiae in the cells of their respective vectors. Within the limits of our present methods of study this represents an external manifestation of natural traits and clearly separates the rickettsiae of the typhus fevers from those of the spotted fevers.

DISCUSSION

Our thesis is that a general solution of any rickettsial disease is to be sought at the focus of several methods of approach. These methods cannot be upheld as absolute or even sufficient. They have the advantage of being simple, of being subject to experimental test, and of yielding results which upon analysis give data which are moderately precise.

These methods have been applied to the study of many strains of rickettsiae. An analysis of the results thus far has given no justification for the recognition of more or less than two genera of pathogenic rickettsiae (each genus being composed of a single species), and two corresponding groups of rickettsial diseases in man. One genus and species (Rickettsiae prowazeki) includes the microorganisms which cause diseases of the typhus fever group. The other genus and species (Dermacentroxenus rickettsi) is composed of the rickettsiae of the various members of the spotted fever group. All evidence indicates
that fièvre boutonneuse is a variety of spotted fever and that its etiologic agent belongs to the corresponding genus and species.

With these gross subdivisions as a foundation, some inquiries into the cause of slight variations among the strains of each established genus and species (such as the above mentioned immunological differences found by Parker to exist between the rickettsiae of fièvre boutonneuse and Rocky Mountain spotted fever) may be made in an orderly manner. Such variations, although of subspecific value, are real, and in general have evaded satisfactory explanation.

CONCLUSIONS

Several methods for the experimental study of the rickettsial diseases have been applied to fièvre boutonneuse. An analysis of the results has indicated that fièvre boutonneuse is a variety of spotted fever and that the etiologic agent is a rickettsia which belongs to the genus Dermacentroxenus and to the species rickettsi (Wolbach (6)). The distinctive morphology of the organism and its characteristic intranuclear clustering in ticks and in tissue cultures are the important criteria upon which this conclusion is based. Immunological, histological, and cytological observations of a confirmatory nature are also reported.

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EXPLANATION OF PLATES

PLATE 35

Fig. 1. Photomicrograph of proliferating mesenchymal cells derived from scrotal sac exudate which had been cultivated in vitro for 2 weeks. The region within the nuclear membrane of one cell is largely occupied by rickettsiae and there is a random distribution of the microorganisms throughout the cytoplasm of all cells. Regaud fixation. Giemsa stain. × 2600.

Fig. 2. Photomicrograph of cells of scrotal sac exudate which had been cultivated in vitro for 3 weeks. The nucleus of one cell contains a mass of rickettsiae. The cytoplasm of the adjacent cell shows the maximum degree of parasitization. Regaud fixation. Giemsa stain. × 2800.

Fig. 3. Photomicrograph of a mesenchymal cell which has grown from the margin of explanted scrotal sac exudate, cultivated in vitro for 3 weeks. The nucleus of this cell shows the typical central aggregation of rickettsiae and the random cytoplasmic distribution. Regaud fixation. Giemsa stain. × 2600.

Fig. 4. Photomicrograph of a mesenchymal cell interpreted as a macrophage, derived from scrotal sac exudate cultivated for 2 weeks in vitro. Note the accumulation of rickettsiae in the center of the nucleus, the halo around the mass of microorganisms, and the isolated coccoïd forms in the cytoplasm. Regaud fixation. Giemsa stain. × 3300.

PLATE 36

Fig. 5. Photomicrograph of a segment of a Malpighian tubule of a tick (Rhipicephalus sanguineus) parasitized with rickettsiae of fièvre boutonneuse. One nucleus is distended with rickettsiae. There are a few indistinct organisms in the cytoplasm of each cell. Regaud fixation. Giemsa stain. × 3400.

Fig. 6. Photomicrograph of an artery in the polar fat of the testis of a guinea pig with fièvre boutonneuse. The entire thickness of the vascular wall has been involved in the inflammatory reaction and a mural thrombus has formed. Regaud fixation. Hematoxylin-eosin stain. × 275.

PLATE 37

Fig. 7. Camera lucida drawing of the rickettsiae of fièvre boutonneuse in the endothelial cells of the intima and smooth muscle cells of the media of a vena in the polar fat of the testis of a guinea pig. Regaud fixation. Giemsa stain. × 2000.
Fig. 8. Camera lucida drawings × 2500. Regaud fixation. Giemsa stain.

The upper left hand cell shows rickettsiae of fièvre boutonneuse in the nucleus and cytoplasm. The cell was selected for illustration from scrotal sac exudate cultivated in vitro for 2 weeks. This type of localization is typical of diseases of the spotted fever group.

The upper right hand figure shows rickettsiae of endemic typhus in the cytoplasm of a cell at the proliferating margin of scrotal sac exudate cultivated in vitro for 2 weeks. The localization and degree of intracellular growth are characteristic of diseases of the typhus group.

The lower left hand figure is a drawing of a cell from the hypoderm of a tick, (Rhipicephalus sanguineus) harboring rickettsiae of fièvre boutonneuse. Note the intranuclear and intracytoplasmic parasitism.

The lower right hand cell is from the lining of the gut of a louse infected with rickettsiae of European typhus. The same type of localization of rickettsiae is found in fleas infected with typhus.

Compare the patterns of intracellular parasitism. Note that the rickettsiae of fièvre boutonneuse exhibit characteristics which distinguish them from those of typhus fever and which ally them closely with those of the spotted fevers.